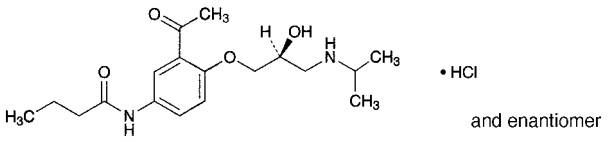
Official Monographs

Acebutolol Hydrochloride

アセブトロール塩酸塩



$C_{18}H_{28}N_2O_4$.HCl: 372.89
N-{3-Acetyl-4-[(2*RS*)-2-hydroxy-3-(1-methylethyl)aminopropyloxy]phenyl}butanamide monohydrochloride
[*34381-68-5*]

Acebutolol Hydrochloride, when dried, contains not less than 98.0% and not more than 102.0% of acebutolol hydrochloride ($C_{18}H_{28}N_2O_4$.HCl).

Description Acebutolol Hydrochloride occurs as white to pale yellowish white, crystals or crystalline powder.

It is freely soluble in water, in methanol, in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

A solution of Acebutolol Hydrochloride (1 in 20) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Acebutolol Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Acebutolol Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Acebutolol Hydrochloride (1 in 100) responds to the Qualitative Tests <*1.09*> for chloride.

Melting point <*2.60*> 141 – 145°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Acebutolol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Acebutolol Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 40 mg of Acebutolol Hydrochloride in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 25 mL, and pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the upper layer of a mixture of water, 1-butanol and acetic acid (100) (5:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 1.0% (0.5 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

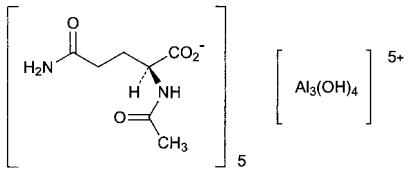
Assay Weigh accurately about 0.25 g of Acebutolol Hydrochloride, previously dried, dissolve in 20 mL of acetic acid (100), add 80 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 37.29 mg of $C_{18}H_{28}N_2O_4$.HCl

Containers and storage Containers—Well-closed containers.

Aceglutamide Aluminum

アセグルタミドアルミニウム



$C_{35}H_{59}Al_3N_{10}O_{24}$: 1084.84
Pentakis[(2*S*)-2-acetylamino-4-carbamoylbutanoato]tetrahydroxotrialuminium
[*12607-92-0*]

Aceglutamide Aluminum contains not less than 85.4% and not more than 87.6% of aceglutamide ($C_7H_{12}N_2O_4$: 188.18), and not less than 7.0% and not more than 8.0% of aluminum (Al: 26.98), calculated on the dried basis.

Description Aceglutamide Aluminum occurs as a white powder, having astringent bitter taste.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

It dissolves in dilute hydrochloric acid.

It is hygroscopic.

Identification **(1)** Dissolve 0.03 g each of Aceglutamide Aluminum and Aceglutamide RS in 5 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, water and acetic acid (100) (16:8:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of bromocresol green in ethanol (95) (1 in 1000), then spray evenly diluted ammonia solution (28) (1 in 100):

the spots from the sample solution and the standard solution show a light yellow and have the same *R*f value.

(2) A solution of Aceglutamide Aluminum in dilute hydrochloric acid (1 in 20) responds to the Qualitative Tests ⟨*1.09*⟩ for aluminum salt.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: -5.5 – $-7.5°$ (2 g calculated on the dried basis, water, 50 mL, 100 mm).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Put 1.0 g of Aceglutamide Aluminum in a porcelain crucible, cover the crucible loosely, and heat gently to carbonize. After cooling, add 2 mL of nitric acid and 1 mL of sulfuric acid, heat gently until the white fumes no more evolve, and heat to incinerate at 500 to 600°C. If the incineration is not accomplished, add 2 mL of nitric acid and 1 mL of sulfuric acid, heat in the same manner as above, then ignite at 500 to 600°C to incinerate. After cooling, add 2 mL of hydrochloric acid, proceed with this solution according to Method 2, and perform the test. Prepare the control solution as follows: proceed in the same manner as the preparation of the test solution with the same amount of the reagents, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Aceglutamide Aluminum according to Method 1, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Aceglutamide Aluminum in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (1). Separately, dissolve 10 mg of 2-acetamidoglutarimide in the mobile phase to make exactly 100 mL. Pipet 3 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (2). Perform the test with exactly 20 μL each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the peak area of 2-acetamidoglutarimide from the sample solution is not larger than that from the standard solution (2), the peak areas other than aceglutamide and 2-acetamidoglutarimide from the sample solution are not larger than 3/10 times the peak area of aceglutamide from the standard solution (1), and the total of the peak areas other than aceglutamide and 2-acetamidoglutarimide from the sample solution is not larger than the peak area of aceglutamide from the standard solution (1).

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of aceglutamide.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay (1).

Test for required detectability: To exactly 5 mL of the standard solution (1) add the mobile phase to make exactly 50 mL. Confirm that the peak area of aceglutamide obtained from 20 μL of this solution is equivalent to 7 to 13% of that of aceglutamide obtained from 20 μL of the standard solution.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak area of aceglutamide is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 5.0% (1 g, 130°C, 5 hours).

Assay **(1)** Aceglutamide—Weigh accurately about 50 mg of Aceglutamide Aluminum, dissolve in a suitable amount of the mobile phase, add exactly 10 mL of the internal standard solution and the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 45 mg of Aceglutamide RS, dissolve in a suitable amount of the mobile phase, add exactly 10 mL of the internal standard solution and the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of aceglutamide to that of the internal standard.

$$\text{Amount (mg) of aceglutamide } (C_7H_{12}N_2O_4) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Aceglutamide RS taken

Internal standard solution—A solution of thymine in methanol (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of diluted perchloric acid (1 in 1000) and methanol (99:1).

Flow rate: Adjust so that the retention time of aceglutamide is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, aceglutamide and the internal standard are eluted in this order with the resolution between these peaks being not less than 11.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of aceglutamide to that of the internal standard is not more than 1.0%.

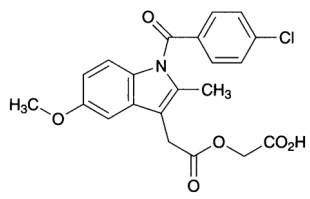
(2) Aluminum—Weigh accurately about 3.0 g of Aceglutamide Aluminum, add 20 mL of dilute hydrochloric acid, and heat on a water bath for 60 minutes. After cooling, add water to make exactly 200 mL. Pipet 20 mL of this solution, add exactly 25 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS and 20 mL of acetic acid-ammonium acetate buffer solution (pH 4.8) and boil for 5 minutes. After cooling, add 50 mL of ethanol (95), and titrate ⟨*2.50*⟩ with 0.05 mol/L zinc acetate VS until the color of the solution changes from light dark green to light red (indicator: 2 mL of dithizone TS). Perform a blank determination in the same manner.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 1.349 mg of Al

Containers and storage Containers—Tight containers.

Acemetacin

アセメタシン



$C_{21}H_{18}ClNO_6$: 415.82
2-{2-[1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetyloxy}acetic acid
[*53164-05-9*]

Acemetacin, when dried, contains not less than 99.0% and not more than 101.0% of acemetacin ($C_{21}H_{18}ClNO_6$).

Description Acemetacin occurs as a light yellow crystalline powder.

It is soluble in acetone, sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

Identification **(1)** To 1 mg of Acemetacin add 1 mL of concentrated chromotropic acid TS, and heat in a water bath for 5 minutes: a red-purple color develops.

(2) Determine the absorption spectrum of a solution of Acemetacin in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Acemetacin as directed in the potassium bromide disk method under Infrared Spectrometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Perform the test with Acemetacin as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Melting point <*2.60*> 151 – 154°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Acemetacin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.40 g of Acemetacin in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetone to make exactly 50 mL. Pipet 1 mL of this solution, add acetone to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin Layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin layer chromatography. Develop the plate with a mixture of hexane, 4-methyl-2-pentanone and acetic acid (100) (3:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): not more than 2 spots other than the principal spot appear from the sample solution, and these spots are not more intense than the spot obtained from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.35 g of Acemetacin, previously dried, dissolve in 20 mL of acetone, add 10 mL of water, and then titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same method, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 41.58 mg of $C_{21}H_{18}ClNO_6$

Containers and storage Containers—Tight containers.

Acemetacin Capsules

アセメタシンカプセル

Acemetacin Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of acemetacin ($C_{21}H_{18}ClNO_6$: 415.82).

Method of preparation Prepare as directed under Capsules, with Acemetacin.

Identification To an amount of powdered contents of Acemetacin Capsules, equivalent to 0.1 g of Acemetacin, add 100 mL of methanol, shake well, and filter. Take 10 mL of the filtrate, and distil the methanol under reduced pressure. To the residue add 1 mL of methanol, shake well, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of acemetacin in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane, 4-methyl-2-pentanone and acetic acid (100) (3:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots obtained from the sample solution and standard solution show the same *R*f value.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take out the contents of 1 capsule of Acemetacin Capsules, add 40 mL of methanol, shake well, and add methanol to make exactly *V* mL so that each mL contains about 0.6 mg of acemetacin ($C_{21}H_{18}ClNO_6$). Filter this solution, discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 2 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of acemetacin ($C_{21}H_{18}ClNO_6$)
$= M_S \times Q_T/Q_S \times V/50$

M_S: Amount (mg) of acemetacin for assay taken

Internal standard solution—A solution of hexyl parahydroxybenzoate in methanol (1 in 1000).

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Acemetacin Capsules is not less than 70%.

Start the test with 1 capsule of Acemetacin Capsules, with-

draw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 33 μg of acemetacin ($C_{21}H_{18}ClNO_6$), and use this solution as the sample solution. Separately, weigh accurately about 17 mg of acemetacin for assay, previously dried at 105°C for 2 hours, dissolve in the dissolution medium to make exactly 100 mL. Pipet 4 mL of this solution, add the dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 319 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Dissolution rate (%) with respect to the labeled amount of acemetacin ($C_{21}H_{18}ClNO_6$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 180$

M_S: Amount (mg) of acemetacin for assay taken
C: Labeled amount (mg) of acemetacin ($C_{21}H_{18}ClNO_6$) in 1 capsule

Assay Take out the contents of not less than 20 Acemetacin Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 30 mg of acemetacin ($C_{21}H_{18}ClNO_6$), add 40 mL of methanol, shake well, and add methanol to make exactly 50 mL. Filter this solution, discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 2 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of acemetacin for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 2 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of acemetacin to that of the internal standard.

Amount (mg) of acemetacin ($C_{21}H_{18}ClNO_6$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of acemetacin for assay taken

Internal standard solution—A solution of hexyl parahydroxybenzoate in methanol (1 in 1000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: To 6 g of acetic acid (100) add water to make 1000 mL, and adjust the pH to 3.2 with a solution of 1.36 g of sodium acetate trihydrate in 100 mL of water. To 200 mL of this solution add 300 mL of acetonitrile.
Flow rate: Adjust so that the retention time of acemetacin is about 7 minutes.
System suitability—
System performance: Dissolve 75 mg of acemetacin and 75 mg of indometacin in 50 mL of methanol. To 2 mL of this solution add 2 mL of the internal standard solution, and add methanol to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, acemetacin, indometacin and the internal standard are eluted in this order with the resolutions between the peaks of acemetacin and indometacin and between the peaks of indometacin and the internal standard being not less than 3, respectively.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of acemetacin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Acemetacin Tablets

アセメタシン錠

Acemetacin Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of acemetacin ($C_{21}H_{18}ClNO_6$: 415.82).

Method of preparation Prepare as directed under Tablets, with Acemetacin.

Identification To a quantity of powdered Acemetacin Tablets, equivalent to 0.1 g of Acemetacin, add 100 mL of methanol, shake well, and filter. Take 10 mL of the filtrate, and distil the methanol under reduced pressure. Dissolve the residue in 1 mL of methanol, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of acemetacin in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane, 4-methyl-2-pentanone and acetic acid (100) (3:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots obtained from the sample solution and standard solution show the same Rf value.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Acemetacin Tablets add 3 mL of water, and shake until the tablet is disintegrated. Add 15 mL of methanol, shake for 20 minutes, and add methanol to make exactly V mL so that each mL contains about 1.2 mg of acemetacin ($C_{21}H_{18}ClNO_6$). Centrifuge this solution, filter the supernatant liquid, discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 1 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of acemetacin ($C_{21}H_{18}ClNO_6$)
$= M_S \times Q_T/Q_S \times V/25$

M_S: Amount (mg) of acemetacin for assay taken

Internal standard solution—A solution of hexyl parahydroxybenzoate in methanol (1 in 250).

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of Acemetacin

Tablets is not less than 80%.

Start the test with 1 tablet of Acemetacin Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 33 μg of acemetacin ($C_{21}H_{18}ClNO_6$), and use this solution as the sample solution. Separately, weigh accurately about 17 mg of acemetacin for assay, previously dried at 105°C for 2 hours, dissolve in the dissolution medium to make exactly 100 mL. Pipet 4 mL of this solution, add the dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 319 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Dissolution rate (%) with respect to the labeled amount of acemetacin ($C_{21}H_{18}ClNO_6$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 180$

M_S: Amount (mg) of acemetacin for assay taken
C: Labeled amount (mg) of acemetacin ($C_{21}H_{18}ClNO_6$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Acemetacin Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.6 g of acemetacin ($C_{21}H_{18}ClNO_6$), add 120 mL of methanol, shake for 20 minutes, and add methanol to make exactly 200 mL. Centrifuge this solution, filter the supernatant liquid, discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add exactly 1 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of acemetacin for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 1 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of acemetacin to that of the internal standard.

Amount (mg) of acemetacin ($C_{21}H_{18}ClNO_6$)
$= M_S \times Q_T/Q_S \times 20$

M_S: Amount (mg) of acemetacin for assay taken

Internal standard solution—A solution of hexyl parahydroxybenzoate in methanol (1 in 250).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 6 g of acetic acid (100) add water to make 1000 mL, and adjust the pH to 3.2 with a solution of 1.36 g of sodium acetate trihydrate in 100 mL of water. To 200 mL of this solution add 300 mL of acetonitrile.

Flow rate: Adjust so that the retention time of acemetacin is about 7 minutes.

System suitability—

System performance: Dissolve 75 mg of acemetacin and 75 mg of indometacin in 50 mL of methanol. To 4 mL of this solution add 1 mL of the internal standard solution, and add methanol to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, acemetacin, indometacin and the internal standard are eluted in this order with the resolutions between the peaks of acemetacin and indometacin and between the peaks of indometacin and the internal standard being not less than 3, respectively.

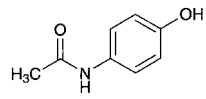
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of acemetacin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Acetaminophen

Paracetamol

アセトアミノフェン



$C_8H_9NO_2$: 151.16
N-(4-Hydroxyphenyl)acetamide
[*103-90-2*]

Acetaminophen, when dried, contains not less than 98.0% of acetaminophen ($C_8H_9NO_2$).

Description Acetaminophen occurs as white, crystals or crystalline powder.

It is freely soluble in methanol and in ethanol (95), sparingly soluble in water, and very slightly, soluble in diethyl ether.

It dissolves in sodium hydroxide TS.

Identification Determine the infrared absorption spectra of Acetaminophen, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Acetaminophen RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 169 – 172°C

Purity **(1)** Chloride <*1.03*>—Dissolve 4.0 g of Acetaminophen in 100 mL of water by heating, cool with shaking in ice water, allow to stand until ordinary temperature is attained, add water to make 100 mL, and filter. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(2) Sulfate <*1.14*>—To 25 mL of the filtrate obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019%).

(3) Heavy metals <*1.07*>—Proceed with 2.0 g of Acetaminophen according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Acetaminophen according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 50 mg of Acetaminophen in 1 mL of methanol, add the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of all peaks other than acetaminophen from the sample solution is not larger than the peak area of acetaminophen from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogenphosphate (pH 4.7) and methanol (4:1).

Flow rate: Adjust so that the retention time of acetaminophen is about 5 minutes.

Selection of column: Dissolve 0.01 g each of Acetaminophen and 4-aminophenol hydrochloride in 1 mL of methanol, add the mobile phase to make 50 mL, to 1 mL of this solution add the mobile phase to make 10 mL. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of 4-aminophenol and acetaminophen in this order with the resolution between these peaks being not less than 7.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of acetaminophen obtained from 10 μL of the standard solution is about 15% of the full scale.

Time span of measurement: About 6 times as long as the retention time of acetaminophen, beginning after the solvent peak.

Loss on drying <*2.41*> Not more than 0.3% (0.5 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 20 mg each of Acetaminophen and Acetaminophen RS, previously dried, dissolve in 2 mL of methanol, and add water to make exactly 100 mL. Pipet 3 mL each of these solutions, add water to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at the wavelength of maximum absorption at about 244 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the blank.

$$\text{Amount (mg) of acetaminophen } (C_8H_9NO_2) = M_S \times A_T/A_S$$

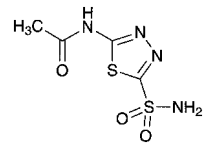
M_S: Amount (mg) of Acetaminophen RS taken

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Acetazolamide

アセタゾラミド



$C_4H_6N_4O_3S_2$: 222.25

N-(5-Sulfamoyl-1,3,4-thiadiazol-2-yl)acetamide

[*59-66-5*]

Acetazolamide contains not less than 98.0% and not more than 102.0% of acetazolamide ($C_4H_6N_4O_3S_2$), calculated on the dried basis.

Description Acetazolamide occurs as a white to pale yellowish white crystalline powder. It is odorless, and has a slight bitter taste.

It is slightly soluble in ethanol (95), very slightly soluble in water, and practically insoluble in diethyl ether.

Melting point: about 255°C (with decomposition).

Identification **(1)** To 0.1 g of Acetazolamide add 5 mL of sodium hydroxide TS, then add 5 mL of a solution of 0.1 g of hydroxylammonium chloride and 0.05 g of copper (II) sulfate pentahydrate in 10 mL of water: a light yellow color develops. Then heat this solution for 5 minutes: a deep yellow color is produced gradually.

(2) To 0.02 g of Acetazolamide add 2 mL of dilute hydrochloric acid, boil for 10 minutes, cool, and add 8 mL of water: this solution responds to the Qualitative Tests <*1.09*> for primary aromatic amines.

(3) To 0.2 g of Acetazolamide add 0.5 g of granulated zinc and 5 mL of diluted hydrochloric acid (1 in 2): the gas evolved darkens moistened lead (II) acetate paper.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Acetazolamide in 10 mL of sodium hydroxide TS: the solution is clear and colorless to pale yellow.

(2) Chloride <*1.03*>—To 1.5 g of Acetazolamide add 75 mL of water, and warm at 70°C for 20 minutes with occasional shaking. After cooling, filter, and to 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(3) Sulfate <*1.14*>—To 25 mL of the filtrate obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(4) Heavy metals <*1.07*>—Proceed with 1.0 g of Acetazolamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Silver-reducing substances—Wet 5 g of Acetazolamide with 5 mL of aldehyde-free ethanol, and add 125 mL of water, 10 mL of nitric acid and exactly 5 mL of 0.1 mol/L silver nitrate VS. Stir for 30 minutes by protecting from light, filter through a glass filter (G3), and wash the residue on the glass filter with two 10-mL portions of water. Combine the filtrate with the washings, to the solution add 5 mL of ferric ammonium sulface TS, and titrate <*2.50*> with 0.1 mol/L ammonium thiocyanate VS: not less than 4.8 mL of 0.1 mol/L ammonium thiocyanate VS is consumed.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (0.5 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.15 g of Acetazolamide, and dissolve in 400 mL of water in a water bath by heating. After cooling, add water to make exactly 1000 mL. Pipet 5 mL of the solution, add 10 mL of 1 mol/L hydrochloric acid TS, and then add water to make exactly 100 mL. Determine the absorbance A of this solution at the wavelength of maximum absorption at about 265 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of acetazolamide ($C_4H_6N_4O_3S_2$)
$= A/474 \times 200{,}000$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Acetic Acid

酢酸

Acetic Acid contains not less than 30.0 w/v% and not more than 32.0 w/v% of acetic acid ($C_2H_4O_2$: 60.05).

Description Acetic Acid is a clear, colorless liquid. It has a pungent, characteristic odor and an acid taste.

It is miscible with water, with ethanol (95) and with glycerin.

Specific gravity d_{20}^{20}: about 1.04

Identification Acetic Acid changes blue litmus paper to red, and responds to the Qualitative Tests ⟨*1.09*⟩ for acetate.

Purity **(1)** Chloride—To 20 mL of Acetic Acid add 40 mL of water, and use this solution as the sample solution. To 10 mL of the sample solution add 5 drops of silver nitrate TS: no opalescence is produced.

(2) Sulfate—To 10 mL of the sample solution obtained in (1) add 1 mL of barium chloride TS: no turbidity is produced.

(3) Heavy metals ⟨*1.07*⟩—Evaporate 10 mL of Acetic Acid on a water bath to dryness, and to the residue add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution with 3.0 mL of Standard Lead Solution by adding 2 mL of dilute acetic acid and water to make 50 mL (not more than 3 ppm).

(4) Potassium permanganate-reducing substances—To 20 mL of the sample solution obtained in (1) add 0.02 mol/L potassium permanganate VS: the red color does not disappear within 30 minutes.

(5) Non-volatile residue—Evaporate 30 mL of Acetic Acid on a water bath to dryness, and dry at 105°C for 1 hour: the mass of the residue is not more than 1.0 mg.

Assay Measure exactly 5 mL of Acetic Acid, add 30 mL of water, and titrate ⟨*2.50*⟩ with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS
= 60.05 mg of $C_2H_4O_2$

Containers and storage Containers—Tight containers.

Glacial Acetic Acid

氷酢酸

H_3C-CO_2H

$C_2H_4O_2$: 60.05
Acetic acid
[*64-19-7*]

Glacial Acetic Acid contains not less than 99.0% of acetic acid ($C_2H_4O_2$).

Description Glacial Acetic Acid is a clear, colorless, volatile liquid, or colorless or white, crystalline masses. It has a pungent, characteristic odor.

It is miscible with water, with ethanol (95) and with diethyl ether.

Boiling point: about 118°C

Specific gravity d_{20}^{20}: about 1.049

Identification A solution of Glacial Acetic Acid (1 in 3) changes blue litmus paper to red, and responds to the Qualitative Tests ⟨*1.09*⟩ for acetate.

Congealing point ⟨*2.42*⟩ Not less than 14.5°C.

Purity **(1)** Chloride—To 10 mL of Glacial Acetic Acid add water to make 100 mL, and use this solution as the sample solution. To 10 mL of the sample solution add 5 drops of silver nitrate TS: no opalescence is produced.

(2) Sulfate—To 10 mL of the sample solution obtained in (1) add 1 mL of barium chloride TS: no turbidity is produced.

(3) Heavy metals ⟨*1.07*⟩—Evaporate 2.0 mL of Glacial Acetic Acid on a water bath to dryness. Dissolve the residue in 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution by adding 2.0 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(4) Potassium permanganate-reducing substances—To 20 mL of the sample solution obtained in (1) add 0.10 mL of 0.1 mol/L potassium permanganate VS: the red color does not disappear within 30 minutes.

(5) Non-volatile residue—Evaporate 10 mL of Glacial Acetic Acid on a water bath to dryness, and dry at 105°C for 1 hour: the mass of the residue is not more than 1.0 mg.

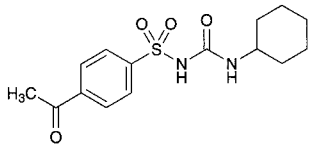
Assay Place 10 mL of water in a glass-stoppered flask, and weigh accurately. Add about 1.5 g of Glacial Acetic Acid, weigh accurately again, then add 30 mL of water, and titrate ⟨*2.50*⟩ with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS
= 60.05 mg of $C_2H_4O_2$

Containers and storage Containers—Tight containers.

Acetohexamide

アセトヘキサミド



$C_{15}H_{20}N_2O_4S$: 324.40
4-Acetyl-*N*-(cyclohexylcarbamoyl)benzenesulfonamide
[*968-81-0*]

Acetohexamide, when dried, contains not less than 98.0% and not more than 101.0% of acetohexamide ($C_{15}H_{20}N_2O_4S$).

Description Acetohexamide occurs as a white to yellowish white powder.

It is freely soluble in *N*,*N*-dimethylformamide, sparingly soluble in acetone, slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Melting point: about 185°C (with decomposition).

Identification **(1)** Dissolve 0.10 g of Acetohexamide in 100 mL of methanol. To 5 mL of the solution add 20 mL of 0.5 mol/L hydrochloric acid TS and 75 mL of methanol, and use the solution as the sample solution (1). Determine the absorption spectrum of the sample solution (1) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using methanol as the blank, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, to exactly 10 mL of the sample solution (1) add methanol to make exactly 50 mL, and use the solution as the sample solution (2). Determine the absorption spectrum of the sample solution (2) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using methanol as the blank, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Acetohexamide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Chloride <*1.03*>—Dissolve 1.5 g of Acetohexamide in 40 mL of *N*,*N*-dimethylformamide, add 6 mL of dilute nitric acid and *N*,*N*-dimethylformamide to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.45 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and *N*,*N*-dimethylformamide to make 50 mL (not more than 0.011%).

(2) Sulfate <*1.14*>—Dissolve 2.0 g of Acetohexamide in 40 mL of *N*,*N*-dimethylformamide, and add 1 mL of dilute hydrochloric acid and *N*,*N*-dimethylformamide to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid and *N*,*N*-dimethylformamide to make 50 mL (not more than 0.010%).

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Acetohexamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substances (i) Cyclohexylamine—Dissolve exactly 1.0 g of Acetohexamide in exactly 30 mL of 0.5 mol/L sodium hydroxide TS, add exactly 5 mL of hexane, shake vigorously for 60 minutes, allow to stand for 5 minutes, and use the upper layer as the sample solution. Separately, dissolve exactly 50 mg of cyclohexylamine in 0.5 mol/L sodium hydroxide TS to make exactly 50 mL. Pipet 2 mL of this solution, and add 0.5 mol/L sodium hydroxide TS to make exactly 300 mL. Pipet 30 mL of this solution, add exactly 5 mL of hexane, shake vigorously for 60 minutes, allow to stand for 5 minutes, and use the upper layer as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following conditions, and determine the peak area of cyclohexylamine by the automatic integration method: the peak area of cyclohexylamine with the sample solution is not more than that with the standard solution.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused-silica column 0.53 mm in inside diameter and 30 m in length, coated the inner surface with methylsilicone polymer for gas chromatography 1.5 μm in thickness.

Column temperature: A constant temperature of about 90°C.

Injection port temperature: A constant temperature of about 150°C.

Detector temperature: A constant temperature of about 210°C.

Carrier gas: Helium.

Flow rate: Adjust so that the retention time of cyclohexylamine is about 4 minutes.

Split ratio: 1:1.

System suitability—

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of cyclohexylamine is not less than 8000.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cyclohexylamine is not more than 5%.

(ii) Dicyclohexylurea—Dissolve exactly 1.0 g of Acetohexamide in exactly 10 mL of 0.5 mol/L sodium hydroxide TS, add exactly 20 mL of methanol, shake, then add exactly 5 mL of diluted hydrochloric acid (1 in 10), shake vigorously for 15 minutes, and centrifuge. Filter 10 mL or more of the supernatant liquid through a membrane filter with pore size of not larger than 0.5 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, dissolve exactly 50 mg of dicyclohexylurea in methanol to make exactly 100 mL. Pipet 2 mL of this solution, and add methanol to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 10 mL of 0.5 mol/L sodium hydroxide TS, shake, then add exactly 5 mL of diluted hydrochloric acid (1 in 10), shake, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak area of dicyclohexylurea by the automatic integration method: the peak area of dicyclohexylurea with the sample solution is not more than that with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 0.5 g of sodium hydroxide in 1000 mL of 0.05 mol/L sodium dihydrogen phosphate TS, and adjust the pH to 6.5 with 0.5 mol/L sodium hydroxide TS. To 500 mL of this solution add 500 mL of acetonitrile.

Flow rate: Adjust so that the retention time of dicyclohexylurea is about 10 minutes.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of dicyclohexylurea is not less than 10,000.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of dicyclohexylurea is not more than 2.0%.

(iii) Other related substances—Dissolve 0.10 g of Acetohexamide in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetone to make exactly 20 mL. Pipet two 1 mL portions of this solution, add acetone to make exactly 10 mL and 25 mL, respectively, and use these solutions as the standard solution (1) and the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol, ammonia solution (28) and cyclohexane (6:2:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than spot from the standard solution (1), and the number of them which are more intense than the spot from the standard solution (2) is not more than 4.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

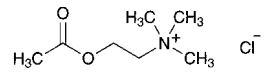
Assay Weigh accurately about 0.3 g of Acetohexamide, previously dried, dissolve in 30 mL of *N*,*N*-dimethylformamide, add 10 mL of water, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination using a solution prepared by adding 19 mL of water to 30 mL of *N*,*N*-dimethylformamide, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 32.44 mg of $C_{15}H_{20}N_2O_4S$

Containers and storage Containers—Well-closed containers.

Acetylcholine Chloride for Injection

注射用アセチルコリン塩化物



$C_7H_{16}ClNO_2$: 181.66
2-Acetoxy-*N*,*N*,*N*-trimethylethylaminium chloride
[*60-31-1*]

Acetylcholine Chloride for Injection is a preparation for injection which is dissolved before use.

It contains not less than 98.0% and not more than 102.0% of acetylcholine chloride ($C_7H_{16}ClNO_2$), and not less than 19.3% and not more than 19.8% of chlorine (Cl: 35.45), calculated on the dried basis.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of acetylcholine chloride ($C_7H_{16}ClNO_2$).

Method of preparation Prepare as directed under Injections.

Description Acetylcholine Chloride for Injection occurs as white, crystals or crystalline powder.

It is very soluble in water, and freely soluble in ethanol (95).

It is extremely hygroscopic.

Identification (1) Determine the infrared absorption spectrum of Acetylcholine Chloride for Injection, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Acetylcholine Chloride for Injection (1 in 10) responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

Melting point ⟨*2.60*⟩ 149 – 152°C. Seal Acetylcholine Chloride for Injection in a capillary tube for melting point immediately after drying both of the sample and the tube at 105°C for 3 hours, and determine the melting point.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Acetylcholine Chloride for Injection in 10 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 0.10 g of Acetylcholine Chloride for Injection in 10 mL of freshly boiled and cooled water, and add 1 drop of bromothymol blue TS, and 0.30 mL of 0.01 mol/L sodium hydroxide VS: the solution is blue in color.

(3) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Acetylcholine Chloride for Injection according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay **(1)** Acetylcholine chloride—Weigh accurately the contents of not less than 10 Acetylcholine Chloride for Injections. Weigh accurately about 0.5 g of the contents, dissolve in 15 mL of water, then add exactly 40 mL of 0.1 mol/L sodium hydroxide VS, stopper loosely, and heat on a water bath for 30 minutes. Cool quickly, and titrate ⟨*2.50*⟩ the excess sodium hydroxide with 0.05 mol/L sulfuric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium hydroxide VS
= 18.17 mg of $C_7H_{16}ClNO_2$

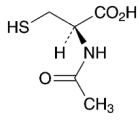
(2) Chlorine—Titrate ⟨*2.50*⟩ the solution, which has been titrated in (1), with 0.1 mol/L silver nitrate VS (indicator: 3 drops of fluorescein sodium TS).

Each mL of 0.1 mol/L silver nitrate VS
= 3.545 mg of Cl

Containers and storage Containers—Hermetic containers.

Acetylcysteine

アセチルシステイン



$C_5H_9NO_3S$: 163.19
(2*R*)-2-Acetylamino-3-sulfanylpropanoic acid
[*616-91-1*]

Acetylcysteine contains not less than 99.0% and not more than 101.0% of acetylcysteine ($C_5H_9NO_3S$), calculated on the dried basis.

Description Acetylcysteine occurs as white, crystals or crystalline powder.

It is freely soluble in water and in ethanol (99.5).

It dissolves in sodium hydroxide TS.

Identification Determine the infrared absorption spectrum of Acetylcysteine as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +21.0 – +27.0° Weigh accurately an amount of Acetylcysteine, equivalent to about 2.5 g calculated on the dried basis, and dissolve with 2 mL of a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (1 in 100) and 15 mL of a solution of sodium hydroxide (1 in 25). To this solution add a solution, prepared by adjusting the pH to 7.0 of 500 mL of a solution of potassium dihydrogen phosphate (17 in 125) with sodium hydroxide TS and adding water to make 1000 mL, to make exactly 50 mL. Determine the optical rotation of this solution using a 100-mm cell.

Melting point ⟨*2.60*⟩ 107 – 111°C

Purity **(1)** Chloride ⟨*1.03*⟩—Dissolve 0.40 g of Acetylcysteine in 25 mL of sodium hydroxide TS, add 4 mL of hydrogen peroxide (30), heat in a water bath for 45 minutes, and cool. Then add 5 mL of nitric acid, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.45 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.040%).

(2) Sulfate ⟨*1.14*⟩—Perform the test with 0.8 g of Acetylcysteine. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.030%).

(3) Ammonium ⟨*1.02*⟩—Perform the test with 0.10 g of Acetylcysteine, using the distillation under reduced pressure. Prepare the control solution with 2.0 mL of Standard Ammonium Solution (not more than 0.02%).

(4) Heavy metals ⟨*1.07*⟩—Dissolve 1.0 g of Acetylcysteine in 40 mL of water, add 3 mL of sodium hydroxide TS, 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: To 1.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(5) Iron ⟨*1.10*⟩—Prepare the test solution with 1.0 g of Acetylcysteine according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(6) Related substances—Dissolve 50 mg of Acetylcysteine in 25 mL of the mobile phase, and use this solution as the sample solution. The sample solution is prepared before using. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, determine each peak area by the automatic integration method, and calculate their amounts by the area percentage method: the area of the peak other than acetylcysteine is not more than 0.3%, and the total area of the peak other than acetylcysteine is not more than 0.6%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 2500) and acetonitrile (19:1).

Flow rate: Adjust so that the retention time of acetylcysteine is about 7 minutes.

Time span of measurement: About 4 times as long as the retention time of acetylcysteine, beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add the mobile phase to make 10 mL. To 1 mL of this solution, add the mobile phase to make 20 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add the mobile phase to make exactly 25 mL. Confirm that the peak area of acetylcysteine obtained with 10 μL of this solution is equivalent to 15 to 25% of that obtained with 10 μL of the solution for system suitability test.

System performance: When the procedure is run with 10 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of acetylcysteine are not less than 15,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of acetylcysteine is not more than

2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 80°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.3% (1 g).

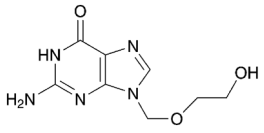
Assay Weigh accurately about 0.2 g of Acetylcysteine, place it in a stoppered flask, dissolve in 20 mL of water, add 4 g of potassium iodide and 5 mL of dilute hydrochloric acid, then add exactly 25 mL of 0.05 mol/L iodine VS, stopper tightly, allow to stand for 20 minutes in an ice cold water in the dark, and titrate ⟨*2.50*⟩ the excess of iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner.

Each mL of 0.05 mol/L iodine VS
= 16.32 mg of $C_5H_9NO_3S$

Containers and storage Containers—Tight containers.

Aciclovir

アシクロビル



$C_8H_{11}N_5O_3$: 225.20
2-Amino-9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-6*H*-purin-6-one
[*59277-89-3*]

Aciclovir contains not less than 98.5% and not more than 101.0% of aciclovir ($C_8H_{11}N_5O_3$), calculated on the anhydrous basis.

Description Aciclovir occurs as a white to pale yellowish white crystalline powder.

It is slightly soluble in water and very slightly soluble in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS and in dilute sodium hydroxide TS.

Identification **(1)** Determine the absorption spectrum of a solution of Aciclovir in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Aciclovir RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Aciclovir as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Aciclovir RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Aciclovir in 20 mL of dilute sodium hydroxide TS: the solution is clear and is not more colored than the following control solution.

Control solution: To 2.5 mL of Matching Fluid F add diluted dilute hydrochloric acid (1 in 10) to make 100 mL.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Aciclovir according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Use the sample solution obtained in the Assay as the sample solution. Separately, weigh accurately about 25 mg of guanine, dissolve in 50 mL of dilute sodium hydroxide TS, and add the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine the peak areas of guanine, A_T and A_S, and calculate the amount of guanine by the following equation: it is not more than 0.7%. Determine each peak area from the sample solution by the automatic integration method, and calculate the amount of each related substance other than aciclovir and guanine by the area percentage method: it is not more than 0.2%. Furthermore, the sum of the amount of guanine calculated above and the amounts of related substances determined by the area percentage method is not more than 1.5%.

$$\text{Amount (\%) of guanine} = M_S/M_T \times A_T/A_S \times 2/5$$

M_S: Amount (mg) of guanine taken
M_T: Amount (mg) of Aciclovir taken

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 8 times as long as the retention time of aciclovir, beginning after the solvent peak.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: To 1 mL of the sample solution add the mobile phase to make 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of aciclovir obtained from 10 μL of this solution is equivalent to 7 to 13% of that obtained from 10 μL of the solution for system suitability test.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of guanine is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 6.0% (50 mg, coulometric titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 20 mg each of Aciclovir and Aciclovir RS (separately determine the water ⟨*2.48*⟩ in the same manner as Aciclovir), dissolve each in 1 mL of dilute sodium hydroxide TS, add the mobile phase to make exactly 20 mL each, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of aciclovir in each solution.

$$\text{Amount (mg) of aciclovir } (C_8H_{11}N_5O_3) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Aciclovir RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-

length: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: Dissolve 1.0 g of sodium 1-decanesulfonate and 6.0 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust the pH to 3.0 with phosphoric acid. To this solution add 40 mL of acetonitrile.

Flow rate: Adjust so that the retention time of aciclovir is about 3 minutes.

System suitability—

System performance: Dissolve 0.1 g of Aciclovir in 5 mL of dilute sodium hydroxide TS, add 2 mL of a solution of guanine in dilute sodium hydroxide TS (1 in 4000), and add the mobile phase to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, aciclovir and guanine are eluted in this order with the resolution between these peaks being not less than 17.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of aciclovir is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Aciclovir Granules

アシクロビル顆粒

Aciclovir Granules contain not less than 93.0% and not more than 107.0% of the labeled amount of aciclovir ($C_8H_{11}N_5O_3$: 225.20).

Method of preparation Prepare as directed under Granules, with Aciclovir.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 254 nm and 258 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: Aciclovir Granules in single-dose packages meet the requirement of the Content uniformity test.

To the total amount of the content of 1 package of Aciclovir Granules, add 100 mL of dilute sodium hydroxide TS, agitate with the aid of ultrasonic waves with occasional shaking, and add dilute sodium hydroxide TS to make exactly 200 mL. Filter this solution, discard the first 20 mL of the filtrate, pipet V mL of the subsequent filtrate, add dilute sodium hydroxide TS to make exactly V' mL so that each mL contains about 1 mg of aciclovir ($C_8H_{11}N_5O_3$). Pipet 15 mL of this solution, add 50 mL of water and 5.8 mL of 2 mol/L hydrochloric acid TS, add water to make exactly 100 mL. Pipet 5 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of acyclovir } (C_8H_{11}N_5O_3) = M_S \times A_T/A_S \times V'/V \times 8$$

M_S: Amount (mg) of Aciclovir RS taken, calculated on the anhydrous basis

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Aciclovir Granules is not less than 85%.

Start the test with an accurately weighed amount of Aciclovir Granules, equivalent to about 0.4 g of aciclovir ($C_8H_{11}N_5O_3$), withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Aciclovir RS (separately determine the water <*2.48*> in the same manner as Aciclovir), and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 252 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

$$\text{Dissolution rate (\%) with respect to the labeled amount of aciclovir } (C_8H_{11}N_5O_3) = M_S/M_T \times A_T/A_S \times 1/C \times 1800$$

M_S: Amount (mg) of aciclovir RS taken, calculated on the anhydrous basis

M_T: Amount (g) of Aciclovir Granules taken

C: Labeled amount (mg) of aciclovir ($C_8H_{11}N_5O_3$) in 1 g

Assay Powder Aciclovir Granules, and weigh accurately a portion of the powder, equivalent to about 0.1 g of aciclovir ($C_8H_{11}N_5O_3$), add 60 mL of dilute sodium hydroxide TS, agitate with the aid of ultrasonic waves for 15 minutes, then add dilute sodium hydroxide TS to make exactly 100 mL, and filter. Discard the first 20 mL of filtrate, pipet 15 mL of the subsequent filtrate, add 50 mL of water and 5.8 mL of 2 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Aciclovir RS (separately determine the water <*2.48*> in the same manner as Aciclovir), and dissolve in dilute sodium hydroxide TS to make exactly 25 mL. Pipet 15 mL of this solution, add 50 mL of water and 5.8 mL of 2 mol/L hydrochloric acid TS, add water to make exactly 100 mL. Pipet 5 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 255 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using 0.1 mol/L hydrochloric acid TS as the blank.

$$\text{Amount (mg) of acyclovir } (C_8H_{11}N_5O_3) = M_S \times A_T/A_S \times 4$$

M_S: Amount (mg) of Aciclovir RS taken, calculated on the anhydrous basis

Containers and storage Containers—Tight containers.

Aciclovir Injection

アシクロビル注射液

Aciclovir Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of aciclovir ($C_8H_{11}N_5O_3$: 225.20).

Method of preparation Prepare as directed under Injections, with Aciclovir.

Description Aciclovir Injection occurs as a colorless or pale yellow, clear liquid.

Identification To a volume of Aciclovir Injection, equivalent to 25 mg of Aciclovir, add 0.5 mol/L hydrochloric acid TS to make 100 mL. To 2 mL of this solution add 0.5 mol/L hydrochloric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 254 nm and 258 nm.

Bacterial endotoxins <*4.01*> Less than 0.5 EU/mg.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay To an exact volume of Aciclovir Injection, equivalent to about 25 mg of aciclovir ($C_8H_{11}N_5O_3$), add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution, then add 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Aciclovir RS (separately determine the water <*2.48*> in the same manner as Aciclovir), and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution, then add 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of aciclovir to that of the internal standard.

$$\text{Amount (mg) of aciclovir } (C_8H_{11}N_5O_3) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Aciclovir RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of nicotinic acid in 0.1 mol/L hydrochloric acid TS (3 in 20,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 1.45 g of phosphoric acid and 25 mL of dilute acetic acid add water to make 900 mL. Adjust this solution to pH 2.5 with 1 mol/L sodium hydroxide TS, and add water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust so that the retention time of aciclovir is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and aciclovir are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of aciclovir to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Aciclovir for Injection

注射用アシクロビル

Aciclovir for Injection is a preparation for injection which is dissolved before use.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of aciclovir ($C_8H_{11}N_5O_3$: 225.20).

Method of preparation Prepare as directed under Injections, with Aciclovir.

Description Aciclovir for Injection occurs as white to pale yellowish white, light masses or powder.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 254 nm and 258 nm.

pH Being specified separately when the drug is granted approval based on the Law.

Purity Clarity and color of solution—Dissolve an amount of Aciclovir for Injection, equivalent to 0.25 g of Aciclovir, in 10 mL of water: the solution is clear and is not more colored than the following control solution.

Control solution: To 2.5 mL of Matching Fluid F add diluted dilute hydrochloric acid (1 in 10) to make 100 mL.

Water <*2.48*> Not more than 7.5% (0.1 g, volumetric titration, direct titration).

Bacterial endotoxins <*4.01*> Less than 0.25 EU/mg.

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test.

Foreign insoluble matter <*6.06*> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 Aciclovir for Injection. Weigh accurately an amount of the contents, equivalent to about 0.1 g of aciclovir ($C_8H_{11}N_5O_3$), and dissolve in dilute sodium hy-

droxide TS to make exactly 100 mL. Pipet 15 mL of this solution, add 70 mL of water and 5 mL of 2 mol/L hydrochloric acid TS, then add water to make exactly 100 mL. Pipet 5 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Aciclovir RS (separately determine the water <2.48> in the same manner as Aciclovir), and dissolve in dilute sodium hydroxide TS to make exactly 20 mL. Pipet 15 mL of this solution, add 70 mL of water and 5 mL of 2 mol/L hydrochloric acid TS, then add water to make exactly 100 mL. Pipet 5 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 255 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using 0.1 mol/L hydrochloric acid TS as the blank.

Amount (mg) of aciclovir ($C_8H_{11}N_5O_3$)
$= M_S \times A_T/A_S \times 5$

M_S: Amount (mg) of Aciclovir RS taken, calculated on the anhydrous basis

Containers and storage Containers—Hermetic containers.

Aciclovir Ointment

アシクロビル軟膏

Aciclovir Ointment contains not less than 95.0% and not more than 105.0% of the labeled amount of aciclovir ($C_8H_{11}N_5O_3$: 225.20).

Method of preparation Prepare as directed under Ointments, with Aciclovir.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 254 nm and 258 nm.

Assay Weigh accurately an amount of Aciclovir Ointment, equivalent to about 10 mg of aciclovir ($C_8H_{11}N_5O_3$), add 25 mL of dilute sodium hydroxide TS, warm if necessary, and dissolve by shaking. After cooling, add water to make exactly 100 mL. Pipet 15 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Aciclovir RS (separately, determine the water <2.48> in the same manner as Aciclovir), and dissolve in dilute sodium hydroxide TS to make exactly 20 mL. Pipet 10 mL of this solution, and add 15 mL of dilute sodium hydroxide TS and water to make exactly 100 mL. Pipet 15 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 255 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using 0.1 mol/L hydrochloric acid TS as the blank.

Amount (mg) of aciclovir ($C_8H_{11}N_5O_3$)
$= M_S \times A_T/A_S \times 1/2$

M_S: Amount (mg) of Aciclovir RS taken, calculated on the anhydrous basis

Containers and storage Containers—Tight containers.

Aciclovir Ophthalmic Ointment

アシクロビル眼軟膏

Aciclovir Ophthalmic Ointment contains not less than 90.0% and not more than 110.0% of the labeled amount of aciclovir ($C_8H_{11}N_5O_3$: 225.20).

Method of preparation Prepare as directed under Ophthalmic Ointments, with Aciclovir.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 254 nm and 258 nm.

Metal Particles <6.01> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Particle diameter Being specified separately when the drug is granted approval based on the Law.

Assay Weigh accurately a portion of Aciclovir Ophthalmic Ointment, equivalent to about 15 mg of aciclovir ($C_8H_{11}N_5O_3$), add exactly 20 mL of hexane and exactly 20 mL of dilute sodium hydroxide TS, and shake vigorously. Centrifuge this mixture, discard the upper layer, pipet 1 mL of the lower layer, add 70 mL of water and 5 mL of 2 mol/L hydrochloric acid TS, then add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 15 mg of Aciclovir RS (separately determine the water <2.48> in the same manner as Aciclovir), and dissolve in dilute sodium hydroxide TS to make exactly 20 mL. Pipet 1 mL of this solution, add 70 mL of water and 5 mL of 2 mol/L hydrochloric acid TS, then add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 255 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

Amount (mg) of acyclovir ($C_8H_{11}N_5O_3$) $= M_S \times A_T/A_S$

M_S: Amount (mg) of Aciclovir RS taken, calculated on the anhydrous basis

Containers and storage Containers—Tight containers.

Aciclovir Syrup

アシクロビルシロップ

Aciclovir Syrup is a suspension syrup.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of aciclovir ($C_8H_{11}N_5O_3$: 225.20).

Method of preparation Prepare as directed under Syrups, with Aciclovir.

Identification To a volume of thoroughly shaked Aciclovir Syrup, equivalent to 80 mg of Aciclovir, add 0.1 mol/L hydrochloric acid TS to make 100 mL. Centrifuge this solution, to 1 mL of the supernatant liquid add 0.1 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a

maximum between 254 nm and 258 nm.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Aciclovir Syrup is not less than 85%.

Start the test with an exact volume of thoroughly shaked Aciclovir Syrup, equivalent to about 0.4 g of aciclovir ($C_8H_{11}N_5O_3$), withdraw not less than 20 mL of the medium at the specified minute after starting the test, and centrifuge. Pipet 2 mL of the supernatant liquid, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Aciclovir RS (separately determine the water ⟨*2.48*⟩ in the same manner as Aciclovir), and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 252 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of aciclovir ($C_8H_{11}N_5O_3$)
$= M_S/V_T \times A_T/A_S \times 1/C \times 1800$

M_S: Amount (mg) of Aciclovir RS taken, calculated on the anhydrous basis
V_T: Amount (mL) of Aciclovir Syrup taken
C: Labeled amount (mg) of aciclovir ($C_8H_{11}N_5O_3$) in 1 mL

Assay Shake thoroughly Aciclovir Syrup. To an exact volume of the syrup, equivalent to about 80 mg of aciclovir ($C_8H_{11}N_5O_3$), add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, then add 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of Aciclovir RS (separately determine the water ⟨*2.48*⟩ in the same manner as Aciclovir), and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution, then add 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of aciclovir to that of the internal standard.

Amount (mg) of aciclovir ($C_8H_{11}N_5O_3$)
$= M_S \times Q_T/Q_S \times 2$

M_S: Amount (mg) of Aciclovir RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of nicotinic acid in 0.1 mol/L hydrochloric acid TS (1 in 2000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: To 1.45 g of phosphoric acid and 25 mL of dilute acetic acid add water to make 900 mL. Adjust this solution to pH 2.5 with 1 mol/L sodium hydroxide TS, and add water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.
Flow rate: Adjust so that the retention time of aciclovir is about 7 minutes.
System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and aciclovir are eluted in this order with the resolution between these peaks being not less than 3.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of aciclovir to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Aciclovir for Syrup

シロップ用アシクロビル

Aciclovir for Syrup is a preparation for syrup, which is suspended before use.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of aciclovir ($C_8H_{11}N_5O_3$: 225.20).

Method of preparation Prepare as directed under Preparations for Syrup, with Aciclovir.

Identification Dissolve an amount of Aciclovir for Syrup, equivalent to 12 mg of Aciclovir, in 0.1 mol/L hydrochloric acid TS to make 50 mL. To 2 mL of this solution add 0.1 mol/L hydrochloric acid TS to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 254 nm and 258 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: Aciclovir for Syrup in single-dose packages meets the requirement of the Content uniformity test.

To the total content of 1 package of Aciclovir for Syrup add $2V/25$ mL of diluted sodium hydroxide TS (1 in 10), and treat with ultrasonic waves to disintegrate, add water to make exactly V mL so that each mL contains about 0.8 mg of aciclovir ($C_8H_{11}N_5O_3$), and filter this solution through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution, then add the mobile phase to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of aciclovir ($C_8H_{11}N_5O_3$)
$= M_S \times Q_T/Q_S \times V/10$

M_S: Amount (mg) of Aciclovir RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of parahydroxybenzoic acid in the mobile phase (1 in 1250).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Aciclovir for Syrup is not less than 85%.

Start the test with an accurately weighed amount of Aciclovir for Syrup, equivalent to about 0.2 g of aciclovir ($C_8H_{11}N_5O_3$), withdraw not less than 5 mL of the medium at

the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 11 mg of Aciclovir RS (separately determine the water <2.48> in the same manner as Aciclovir), and dissolve in water to make exactly 50 mL. Pipet 2 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 254 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of aciclovir ($C_8H_{11}N_5O_3$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 1800$

M_S: Amount (mg) of Aciclovir RS taken, calculated on the anhydrous basis
M_T: Amount (g) of Aciclovir for Syrup taken
C: Labeled amount (mg) of aciclovir ($C_8H_{11}N_5O_3$) in 1 g

Assay Weigh accurately an amount of Aciclovir for Syrup, previously powdered if necessary, equivalent to about 0.2 g of aciclovir ($C_8H_{11}N_5O_3$), add 20 mL of diluted sodium hydroxide TS (1 in 10), treat with ultrasonic waves to disintegrate, then add water to make exactly 200 mL, and filter this solution through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution, then add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Aciclovir RS (separately determine the water <2.48> in the same manner as Aciclovir), add exactly 10 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of aciclovir to that of the internal standard.

Amount (mg) of aciclovir ($C_8H_{11}N_5O_3$)
$= M_S \times Q_T/Q_S \times 20$

M_S: Amount (mg) of Aciclovir RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of parahydroxybenzoic acid in the mobile phase (1 in 1250).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 7.8 g of sodium dihydrogen phosphate dihydrate and 0.85 g of sodium 1-octanesulfonate in 900 mL of water, adjust to pH 3.0 with phosphoric acid, add water to make 950 mL, and add 50 mL of acetonitrile.
Flow rate: Adjust so that the retention time of aciclovir is about 5 minutes.
System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, aciclovir and the internal standard are eluted in this order with the resolution between these peaks being not less than 20.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of aciclovir to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Aciclovir Tablets

アシクロビル錠

Aciclovir Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of aciclovir ($C_8H_{11}N_5O_3$: 225.20).

Method of preparation Prepare as directed under Tablets, with Aciclovir.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 254 nm and 258 nm.

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Aciclovir Tablets is not less than 80%.

Start the test with 1 tablet of Aciclovir Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 8.9 μg of aciclovir ($C_8H_{11}N_5O_3$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of aciclovir RS (separately determine the water <2.48> in the same manner as Aciclovir), and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 252 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of aciclovir ($C_8H_{11}N_5O_3$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of Acyclovir RS taken, calculated on the anhydrous basis
C: Labeled amount (mg) of aciclovir ($C_8H_{11}N_5O_3$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Aciclovir Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of aciclovir ($C_8H_{11}N_5O_3$), add 60 mL of dilute sodium hydroxide TS, and agitate for 15 minutes with the aid of ultrasonic waves, then add dilute sodium hydroxide TS to make exactly 100 mL, and filter. Discard the first 20 mL of filtrate, pipet 15 mL of the subsequent filtrate, add 50 mL of water and 5.8 mL of 2 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solu-

tion as the sample solution. Separately, weigh accurately about 25 mg of Aciclovir RS (separately determine the water ⟨*2.48*⟩ in the same manner as Aciclovir), and dissolve in dilute sodium hydroxide TS to make exactly 25 mL. Pipet 15 mL of this solution, add 50 mL of water and 5.8 mL of 2 mol/L hydrochloric acid TS, add water to make exactly 100 mL. Pipet 5 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 255 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using 0.1 mol/L hydrochloric acid TS as the blank.

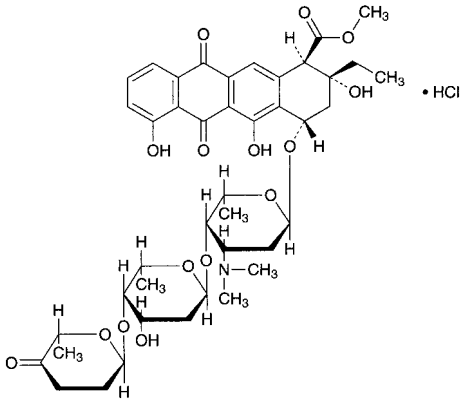
$$\text{Amount (mg) of acyclovir } (C_8H_{11}N_5O_3) = M_S \times A_T/A_S \times 4$$

M_S: Amount (mg) of Aciclovir RS taken, calculated on the anhydrous basis

Containers and storage Containers—Well-closed containers.

Aclarubicin Hydrochloride

アクラルビシン塩酸塩



$C_{42}H_{53}NO_{15}$.HCl: 848.33

Methyl (1*R*,2*R*,4*S*)-4-{2,6-dideoxy-4-*O*-[(2*R*,6*S*)-6-methyl-5-oxo-3,4,5,6-tetrahydro-2*H*-pyran-2-yl]-α-L-*lyxo*-hexopyranosyl-(1→4)-2,3,6-trideoxy-3-dimethylamino-α-L-*lyxo*-hexopyranosyloxy}-2-ethyl-2,5,7-trihydroxy-6,11-dioxo-1,2,3,4-tetrahydrotetracene-1-carboxylate monohydrochloride

[*75443-99-1*]

Aclarubicin Hydrochloride is the hydrochloride of an anthracycline substance having antitumor activity produced by the growth of *Streptomyces galilaeus*.

It contains not less than 920 μg (potency) and not more than 975 μg (potency) per mg, calculated on the anhydrous basis. The potency of Aclarubicin Hydrochloride is expressed as mass (potency) of aclarubicin ($C_{42}H_{53}NO_{15}$: 811.87).

Description Aclarubicin Hydrochloride occurs as a yellow to pale orange-yellow powder.

It is very soluble in chloroform and in methanol, freely soluble in water, and slightly soluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Aclarubicin Hydrochloride in diluted methanol (4 in 5) (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Aclarubicin Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Aclarubicin Hydrochloride in methanol (1 in 200) responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −146 – −162° (50 mg calculated on the anhydrous basis, water, 10 mL, 100 mm).

pH ⟨*2.54*⟩ The pH of a solution obtained by dissolving 0.05 g of Aclarubicin Hydrochloride in 10 mL of water is between 5.5 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Aclarubicin Hydrochloride in 10 mL of water: the solution is clear and yellow to pale orange-yellow.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Aclarubicin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 10 mg of Aclarubicin Hydrochloride in 10 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the related substances by the area percentage method: the amount of aklavinone having the relative retention time of about 0.6 to aclarubicin is not more than 0.2%, aclacinomycin L1 having the relative retention time of about 0.75 to aclarubicin is not more than 0.5%, 1-deoxypyrromycin having the relative retention time of about 1.7 to aclarubicin is not more than 1.5% and aclacinomycin S1 having the relative retention time of about 2.3 to aclarubicin is not more than 0.5%, and the total amount of the peaks other than aclarubicin and the peaks mentioned above is not more than 1.0% of the peak area of aclarubicin.

Operating conditions—

Detector: A visible absorption photometer (wavelength: 436 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of chloroform, methanol, acetic acid (100), water and triethylamine (6800:2000:1000:200:1).

Flow rate: Adjust so that the retention time of aclarubicin is about 5 minutes.

Time span of measurement: As long as about 4 times of the retention time of aclarubicin, beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution, add the mobile phase to make 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of aclarubicin obtained from 20 μL of this solution is equivalent to 7 to 13% of that obtained from 20 μL of the solution for system suitability test.

System performance: Dissolve 5 mg of Aclarubicin Hydrochloride in 10 mL of 0.1 mol/L hydrochloric acid TS,

and allow to stand for 60 minutes. To 1.0 mL of this solution add 1.0 mL of 0.2 mol/L sodium hydroxide TS, 1.0 mL of phosphate buffer solution (pH 8.0) and 1.0 mL of chloroform, shake vigorously, and take the chloroform layer. When the procedure is run with 20 μL of the chloroform under the above operating conditions, aclarubicin and 1-deoxypyrromycin are eluted in this order with the resolution between these peaks being not less than 3.0.

System repeatability: When the test is repeated 5 times with 20 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of aclarubicin is not more than 2.0%.

Water <*2.48*> Not more than 3.5% (0.1 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Aclarubicin Hydrochloride, equivalent to about 20 mg (potency), and dissolve in diluted methanol (4 in 5) to make exactly 100 mL. Pipet 15 mL of this solution, add diluted methanol (4 in 5) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Aclarubicin RS, equivalent to about 20 mg (potency), add 0.6 mL of diluted hydrochloric acid (1 in 250) and diluted methanol (4 in 5) to make exactly 100 mL. Pipet 15 mL of this solution, add diluted methanol (4 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 433 nm.

Amount [μg (potency)] of aclarubicin ($C_{42}H_{53}NO_{15}$) = $M_S \times A_T/A_S \times 1000$

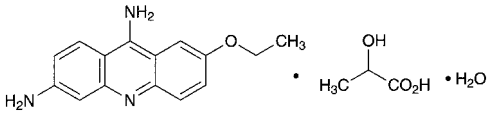
M_S: Amount [mg (potency)] of Aclarubicin RS taken

Containers and storage Containers—Tight containers.
Storage—Light-resistant and at 5°C or below.

Acrinol Hydrate

Ethacridine Lactate

アクリノール水和物



$C_{15}H_{15}N_3O.C_3H_6O_3.H_2O$: 361.39
2-Ethoxy-6,9-diaminoacridine monolactate monohydrate
[*6402-23-9*]

Acrinol Hydrate contains not less than 98.5% and not more than 101.0% of acrinol ($C_{15}H_{15}N_3O.C_3H_6O_3$: 343.38), calculated on the anhydrous basis.

Description Acrinol Hydrate occurs as a yellow crystalline powder.

It is sparingly soluble in water, in methanol and in ethanol (99.5).

Melting point: about 245°C (with decomposition).

The pH of a solution of Acrinol Hydrate (1 in 100) is between 5.5 and 7.0.

Identification (1) Determine the absorption spectrum of a solution of Acrinol Hydrate (3 in 250,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Acrinol Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 5 mL of a solution of Acrinol Hydrate (1 in 100) add 5 mL of dilute sulfuric acid, shake well, allow to stand for about 10 minutes at room temperature, and filter: the filtrate responds to the Qualitative Tests <*1.09*> for lactate.

Purity (1) Chloride <*1.03*>—Dissolve 1.0 g of Acrinol Hydrate in 80 mL of water by warming on a water bath, cool, and add 10 mL of sodium hydroxide TS and water to make 100 mL. Shake well, allow to stand for 30 minutes, filter, to 40 mL of the filtrate add 7 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare 50 mL of the control solution with 4 mL of sodium hydroxide TS, 7 mL of dilute nitric acid, 0.30 mL of 0.01 mol/L hydrochloric acid VS and sufficient water (not more than 0.026%).

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Acrinol Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Volatile fatty acids—Dissolve 0.5 g of Acrinol Hydrate in a mixture of 20 mL of water and 5 mL of dilute sulfuric acid, shake well, filter, and heat the filtrate: no odor of volatile fatty acids is perceptible.

(4) Related substances—Dissolve 10 mg of Acrinol Hydrate in 25 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 1 mL of the standard solution (1), add the mobile phase to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with exactly 10 μL each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than acrinol obtained with the sample solution is not larger than 3 times the peak area of acrinol obtained with the standard solution (2), and the total area of the peaks other than acrinol is not larger than the peak area of acrinol with the standard solution (1).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 268 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 7.8 g of sodium dihydrogen phosphate in 900 mL of water, adjust to pH 2.8 with phosphoric acid, and add water to make 1000 mL. To 700 mL of this solution add 300 mL of acetonitrile for liquid chromatography, and add 1.0 g of sodium 1-octanesulfonate to dissolve.

Flow rate: Adjust so that the retention time of acrinol is about 15 minutes.

Time span of measurement: About 3 times as long as the retention time of acrinol, beginning after the solvent peak.

System suitability—

Test for required detectability: Confirm that the peak area of acrinol obtained with 10 μL of the standard solution (2) is equivalent to 7 to 13% of that obtained with 10 μL of the standard solution (1).

System performance: When the procedure is run with 10 μL of the standard solution (1) under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of acrinol are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak area of acrinol is not more than 1.5%.

Water ⟨*2.48*⟩ 4.5 – 5.5% (0.2 g, volumetric titration, direct titration)

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.27 g of Acrinol Hydrate, dissolve in 5 mL of formic acid, add 60 mL of a mixture of acetic anhydride and acetic acid (100) (1:1), and titrate ⟨*2.50*⟩ immediately with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.34 mg of $C_{15}H_{15}N_3O.C_3H_6O_3$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Acrinol and Zinc Oxide Oil

アクリノール·チンク油

Acrinol and Zinc Oxide Oil contains not less than 44.6% and not more than 54.4% of zinc oxide (ZnO: 81.38).

Method of preparation

Acrinol Hydrate, very finely powdered	10 g
Zinc Oxide Oil	990 g
To make	1000 g

Prepare by mixing the above ingredients. Acrinol Hydrate may be mixed after being dissolved in a little amount of warmed Purified Water or Purified Water in Containers. Instead of Zinc Oxide Oil adequate amounts of Zinc Oxide and vegetable oil may be used, and an adequate amount of Castor Oil or polysorbate 20 may be substituted for a part of the vegetable oil.

Description Acrinol and Zinc Oxide Oil is a yellowish white, slimy substance. Separation of a part of its ingredients occurs on prolonged standing.

Identification **(1)** Shake well 1 g of Acrinol and Zinc Oxide Oil with 10 mL of diethyl ether, 2 mL of acetic acid (100) and 10 mL of water, and separate the water layer. Shake the layer with 5 mL of hydrochloric acid and 2 to 3 drops of sodium nitrite TS, and allow to stand: a dark red color is produced (acrinol).

(2) Place 1 g of Acrinol and Zinc Oxide Oil in a crucible, melt by warming, heat, gradually raising the temperature until the mass is thoroughly charred, and then ignite strongly: a yellow color is produced, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, filter after thorough shaking, and to the filtrate add 2 to 3 drops of potassium hexacyanoferrate (II) TS: a white precipitate is formed (zinc oxide).

(3) Shake well 0.2 g of Acrinol and Zinc Oxide Oil with 20 mL of ethanol (95) and 1 mL of acetic acid (100), centrifuge, filter, and use the filtrate as the sample solution. Separately, dissolve 5 mg of acrinol in 50 mL of ethanol (95) and 2.5 mL of acetic acid (100), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-propanol and acetic acid (100) (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots from the sample solution and standard solution exhibit a blue fluorescence and show the same *R*f value.

Assay Transfer about 0.8 g of well-mixed Acrinol and Zinc Oxide Oil, accurately weighed, to a crucible, heat, gradually raising the temperature until the mass is throughly charred, then strongly heat until the residue becomes yellow. After cooling, dissolve the residue by addition of 1 mL of water and 1.5 mL of hydrochloric acid, and add water to make exactly 100 mL. Pipet 20 mL of this solution, add 80 mL of water, then add sodium hydroxide solution (1 in 50) until slightly precipitates appear, and add 5 mL of ammonia-ammonium chloride buffer solution (pH 10.7). Titrate ⟨*2.50*⟩ with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 4.069 mg of ZnO

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Compound Acrinol and Zinc Oxide Oil

複方アクリノール·チンク油

Method of preparation

Acrinol Hydrate, very finely powdered	10 g
Zinc Oxide Oil	650 g
Ethyl Aminobenzoate, finely powdered	50 g
White Beeswax	20 g
Hydrophilic Petrolatum	270 g
To make	1000 g

Prepare by mixing the above ingredients.

Description Compound Acrinol and Zinc Oxide Oil is light yellow to yellow in color.

Identification **(1)** Shake well 1 g of Compound Acrinol and Zinc Oxide Oil with 10 mL of diethyl ether, 2 mL of acetic acid (100) and 10 mL of water, and separate the water layer. Shake the layer with 5 mL of hydrochloric acid and 2 to 3 drops of sodium nitrite TS, and allow to stand: a dark red color is produced (acrinol).

(2) Place 1 g of Compound Acrinol and Zinc Oxide Oil in a crucible, melt by warming, heat, gradually raising the temperature until the mass is thoroughly charred, and then ignite strongly: a yellow color is produced, and disappears

on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, shake well, and filter. To the filtrate add 2 to 3 drops of potassium hexacyanoferrate (II) TS: a white precipitate is produced (zinc oxide).

(3) Shake well 0.2 g of Compound Acrinol and Zinc Oxide Oil with 20 mL of ethanol (95) and 1 mL of acetic acid (100), centrifuge, filter, and use the filtrate as the sample solution. Separately, dissolve 5 mg of acrinol and 25 mg of ethyl aminobenzoate in 50 mL of ethanol (95) and in 2.5 mL of acetic acid (100), respectively, and use both solutions as the standard solutions (1) and (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-propanol and acetic acid (100) (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots from the sample solution and standard solution (1) exhibit a blue fluorescence, and show the same *R*f value. Also examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution and standard solution (2) exhibit a purple color, and show the same *R*f value.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Acrinol and Zinc Oxide Ointment

アクリノール·亜鉛華軟膏

Method of preparation

Acrinol Hydrate, very finely powdered	10 g
Zinc Oxide Ointment	990 g
To make	1000 g

Prepare as directed under Ointments, with the above ingredients.

Description Acrinol and Zinc oxide Ointment is yellow in color.

Identification **(1)** Shake 0.5 g of Acrinol and Zinc Oxide Ointment with 5 mL of diethyl ether, 5 mL of dilute hydrochloric acid and 2 to 3 drops of sodium nitrite TS, and allow to stand: a dark red color develops in the water layer (acrinol).

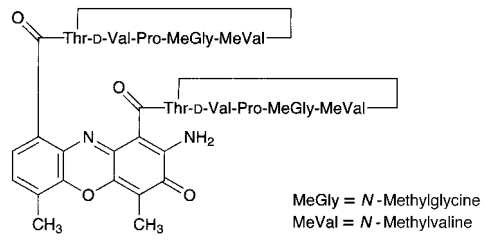
(2) Ignite 0.5 g of Acrinol and Zinc Oxide Ointment to char, and dissolve the residue in 5 mL of dilute hydrochloric acid: the solution responds to the Qualitative Tests <*1.09*> for zinc salt.

(3) Shake 0.5 g of Acrinol and Zinc Oxide Ointment with 5 mL of diethyl ether, 1 mL of acetic acid (100) and 5 mL of water, separate the water layer, and use the water layer as the sample solution. Dissolve 5 mg of acrinol in 1 mL of acetic acid (100) and 5 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether, ethanol (95) and acetic acid (100) (40:10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots from the sample solution and the standard solution exhibit a blue fluorescence and show the same *R*f value.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Actinomycin D

アクチノマイシン D



$C_{62}H_{86}N_{12}O_{16}$: 1255.42
[*50-76-0*]

Actinomycin D is a peptide substance having antitumor activity produced by the growth of *Streptomyces parvulus*.

It, when dried, contains not less than 950 μg (potency) and not more than 1030 μg (potency) per mg. The potency of Actinomycin D is expressed as mass (potency) of actinomycin D ($C_{62}H_{86}N_{12}O_{16}$).

Description Actinomycin D occurs as an orange-red to red crystalline powder.

It is freely soluble in acetone, sparingly soluble in acetonitrile and in methanol, slightly soluble in ethanol (99.5), and very slightly soluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Actinomycin D in methanol (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Actinomycin D RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 0.1 g each of Actinomycin D and Actinomycin D RS in 10 mL of acetone, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and methanol (4:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the *R*f value of the principal spot from the sample solution is the same as that from the standard solution.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -293 – $-329°$ (after drying, 10 mg, methanol, 10 mL, 100 mm).

Loss on drying <*2.41*> Not more than 5.0% (1 g, in vacuum, 60°C, 3 hours).

Assay Weigh accurately an amount of Actinomycin D and Actinomycin D RS, previously dried, equivalent to about 60 mg (potency), dissolve each in the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak area of actinomycin D, A_T and A_S, in each solution.

Amount [μg (potency)] of actinomycin D ($C_{62}H_{86}N_{12}O_{16}$) = $M_S \times A_T/A_S \times 1000$

M_S: Amount [mg (potency)] of Actinomycin D RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.02 mol/L acetic acid-sodium acetate TS and acetonitrile (25:23).

Flow rate: Adjust so that the retention time of actinomycin D is about 23 minutes.

System suitability—

System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of actinomycin D are not less than 2000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 5 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of actinomycin D is not more than 2.0%.

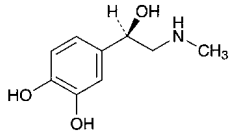
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Adrenaline

Epinephrine

アドレナリン



$C_9H_{13}NO_3$: 183.20

4-[(1*R*)-1-Hydroxy-2-(methylamino)ethyl]benzene-1,2-diol

[*51-43-4*]

Adrenaline, when dried, contains not less than 98.0% and not more than 101.0% of adrenaline ($C_9H_{13}NO_3$).

Description Adrenaline occurs as a white to grayish white crystalline powder.

It is freely soluble in formic acid and in acetic acid (100), very slightly soluble in water, and practically insoluble in methanol and in ethanol (99.5).

It dissolves in dilute hydrochloric acid.

It gradually changes to brown in color by air and by light.

Identification (1) Determine the absorption spectrum of a solution of Adrenaline in 0.01 mol/L hydrochloric acid TS (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Adrenaline as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -50.0 – $-53.5°$ (after drying, 1 g, 1 mol/L hydrochloric acid TS, 25 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Adrenaline in 10 mL of dilute hydrochloric acid: the solution is clear, and is not more colored than Matching Fluid A.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Adrenaline according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Adrenalone—Dissolve 50 mg of Adrenaline in 0.05 mol/L hydrochloric acid TS to make exactly 25 mL, and determine the absorbance of this solution at 310 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it is not more than 0.2.

(4) Noradrenaline—Dissolve 0.20 g of Adrenaline in 1 mL of formic acid, add methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 8.0 mg of Noradrenaline Bitartrate RS in methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and formic acid (7:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Folin's TS on the plate: the spot obtained from the sample solution, corresponding to the spot obtained from the standard solution, is not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 1.0% (1 g, in vacuum, silica gel, 18 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Adrenaline, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 18.32 mg of $C_9H_{13}NO_3$

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and under Nitrogen atmosphere.

Adrenaline Injection

Epinephrine Injection

アドレナリン注射液

Adrenaline Injection is an aqueous injection.

It contains not less than 0.085 w/v% and not more than 0.115 w/v% of adrenaline ($C_9H_{13}NO_3$: 183.20).

Method of preparation Dissolve Adrenaline in diluted Hydrochloric Acid (9 in 10,000), and prepare as directed under Injections.

Description Adrenaline Injection is a colorless, clear liquid.

It changes gradually to pale red and then to brown on exposure to air and light.

pH: 2.3 – 5.0

Identification **(1)** To 1 mL of Adrenaline Injection add 4 mL of water and 1 drop of iron (III) chloride TS: a deep green color is produced, and it gradually changes to red.

(2) Place 1 mL each of Adrenaline Injection in test tubes A and B, and proceed as directed in the Identification (2) under Adrenaline.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Assay Pipet 30 mL of Adrenaline Injection into a separator, add 25 mL of carbon tetrachloride, shake vigorously for 1 minute, allow the liquids to separate, and discard the carbon tetrachloride. Repeat this procedure three times. Rinse the stopper and mouth of the separator with a small amount of water. Add 0.2 mL of starch TS, then while swirling the separator add iodine TS dropwise until a persistent blue color develops, and immediately add sodium thiosulfate TS to discharge the blue color. Add 2.1 g of sodium hydrogen carbonate to the liquid in the separator, preventing it from coming in contact with the mouth of the separator, and shake until most of the sodium hydrogen carbonate dissolves. Rapidly inject 1.0 mL of acetic anhydride into the contents of the separator. Immediately stopper the separator loosely, and allow to stand until the evolution of gas ceases. Shake vigorously, allow to stand for 5 minutes, extract with six 25-mL portions of chloroform, and filter each chloroform extract through a pledget of absorbent cotton. Evaporate the combined chloroform extracts on a water bath in a current of air to 3 mL, completely transfer this residue by means of small portions of chloroform to a tared beaker, and heat again to evaporate to dryness. Dry the residue at 105°C for 30 minutes, cool in a desiccator (silica gel), and accurately measure the mass M (mg) of the dried residue. Dissolve in chloroform to make exactly 5 mL, and determine the optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$ using a 100-mm cell.

$$\text{Amount (mg) of adrenaline } (C_9H_{13}NO_3) = M \times \{0.5 + (0.5 \times |[\alpha]_D^{20}|)/93\} \times 0.592$$

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Adrenaline Solution

Epinephrine Solution

アドレナリン液

Adrenaline Solution contains not less than 0.085 w/v% and not more than 0.115 w/v% of adrenaline ($C_9H_{13}NO_3$: 183.20)

Method of preparation

Adrenaline	1 g
Sodium Chloride	8.5 g
Diluted Hydrochloric Acid (9 in 100)	10 mL
Stabilizer	a suitable quantity
Preservative	a suitable quantity
Purified Water or Purified Water in Containers	a sufficient quantity
	To make 1000 mL

Prepare by mixing the above ingredients.

Description Adrenaline Solution is clear, colorless or slightly reddish liquid.

It changes gradually to pale red and then to brown on exposure to air and light.

pH: 2.3 – 5.0

Identification Proceed as directed in the Identification under Adrenaline Injection.

Assay Proceed as directed in the Assay under Adrenaline Injection.

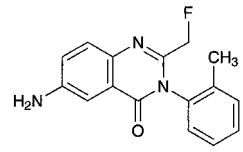
$$\text{Amount (mg) of adrenaline } (C_9H_{13}NO_3) = M \times \{0.5 + (0.5 \times |[\alpha]_D^{20}|)/93\} \times 0.592$$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Afloqualone

アフロクアロン



$C_{16}H_{14}FN_3O$: 283.30

6-Amino-2-fluoromethyl-3-(2-tolyl)-3*H*-quinazolin-4-one

[*56287-74-2*]

Afloqualone, when dried, contains not less than 98.5% of afloqualone ($C_{16}H_{14}FN_3O$).

Description Afloqualone occurs as white to light yellow, crystals or crystalline powder.

It is soluble in acetonitrile, sparingly soluble in ethanol (99.5), and practically insoluble in water.

It is gradually colored by light.

Melting point: about 197°C (with decomposition).

Identification **(1)** Conduct this procedure without exposure to light, using light-resistant containers. Determine the absorption spectrum of a solution of Afloqualone in ethanol (99.5) (1 in 150,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Afloqualone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Acidity or alkalinity—Take 1.0 g of Afloqualone in a light-resistant vessel, add 20 mL of freshly boiled and cooled water, shake well, and filter. To 10 mL of the filtrate add 2 drops of bromothymol blue TS: a yellow color develops. The color changes to blue by adding 0.20 mL of 0.01 mol/L sodium hydroxide TS.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Afloqualone in a platinum crucible according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 10 mg of Afloqualone in 25 mL of the mobile phase, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution.

Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the areas of each peak by the automatic integration method: the total of the peak areas other than afloqualone from the sample solution is not larger than the peak area of afloqualone from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 7.2 g of disodium hydrogen phosphate dodecahydrate in 1000 mL of water, adjust to pH 5.5 with diluted phosphoric acid (1 in 10). To 600 mL of this solution add 400 mL of acetonitrile.

Flow rate: Adjust so that the retention time of afloqualone is about 5.5 minutes.

Time span of measurement: About 4 times as long as the retention time of afloqualone, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, add the mobile phase to make exactly 25 mL, and confirm that the peak area of afloqualone obtained from 20 μL of this solution is equivalent to 15 to 25% of that of afloqualone obtained from 20 μL of the standard solution.

System performance: Dissolve 0.01 g of Afloqualone in a suitable amount of the mobile phase, add 5 mL of a solution of propyl parahydroxybenzoate in the mobile phase (1 in 2000) and the mobile phase to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, afloqualone and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of afloqualone is not more than 5%.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, 60°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1.0 g, platinum crucible).

Assay Weigh accurately about 0.4 g of Afloqualone, previously dried, dissolve in 10 mL of hydrochloric acid and 40 mL of water, and add 10 mL of a solution of potassium bromide (3 in 10). After cooling at 15°C or below, titrate <2.50> with 0.1 mol/L sodium nitrite VS according to the potentiometric titration or amperometric titration under the Electrometric Titration method.

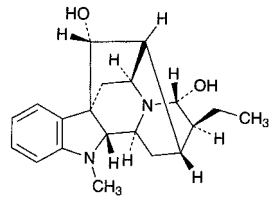
Each mL of 0.1 mol/L sodium nitrite
= 28.33 mg of $C_{16}H_{14}FN_3O$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Ajmaline

アジマリン



$C_{20}H_{26}N_2O_2$: 326.43
(17*R*,21*R*)-Ajmalan-17,21-diol
[*4360-12-7*]

Ajmaline, when dried, contains not less than 96.0% of ajmaline ($C_{20}H_{26}N_2O_2$).

Description Ajmaline occurs as a white to pale yellow crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in acetic anhydride and in chloroform, sparingly soluble in methanol, in ethanol (95), in acetone and in diethyl ether, and very slightly soluble in water.

It dissolves in dilute hydrochloric acid.

Melting point: about 195°C (with decomposition).

Identification **(1)** Dissolve 0.05 g of Ajmaline in 5 mL of methanol, and use this solution as the sample solution. Add 3 mL of nitric acid to 1 mL of the sample solution: a deep red color develops.

(2) Spot the sample solution of (1) on filter paper, and spray Dragendorff's TS: an orange color develops.

Absorbance <2.24> $E^{1\%}_{1\,cm}$ (249 nm): 257 – 271 (after drying, 2 mg, ethanol (95), 100 mL).

$E^{1\%}_{1\,cm}$ (292 nm): 85 – 95 (after drying, 2 mg, ethanol (95), 100 mL).

Optical rotation <2.49> $[\alpha]_D^{20}$: +136 – +151° (after drying, 0.5 g, chloroform, 50 mL, 100 mm).

Purity Related substances—Dissolve 0.10 g of Ajmaline in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and diethylamine (5:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (0.6 g, in vacuum, 80°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (0.5 g).

Assay Weigh accurately about 0.3 g of Ajmaline, previously dried, dissolve in 50 mL of acetic anhydride and 50 mL of acetone for nonaqueous titration, and titrate <2.50> with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 16.32 mg of $C_{20}H_{26}N_2O_2$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Ajmaline Tablets

アジマリン錠

Ajmaline Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of ajmaline ($C_{20}H_{26}N_2O_2$: 326.43).

Method of preparation Prepare as directed under Tablets, with Ajmaline.

Identification **(1)** Shake a quantity of powdered Ajmaline Tablets, equivalent to 0.1 g of Ajmaline, with 30 mL of chloroform, and filter. Evaporate the filtrate on a water bath to dryness. With the residue, proceed as directed in the Identification under Ajmaline.

(2) Dissolve 0.01 g of the residue of (1) in 100 mL of ethanol (95). To 10 mL of this solution add ethanol (95) to make 50 mL, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 247 nm and 251 nm and between 291 nm and 294 nm, and a minimum between 269 nm and 273 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Ajmaline Tablets add 150 mL of 2nd fluid for dissolution test, shake to disintegrate the tablet, then add 2nd fluid for dissolution test to make exactly 200 mL, and filter this solution through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate equivalent to about 0.5 mg of ajmaline ($C_{20}H_{26}N_2O_2$), add 2nd fluid for dissolution test to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of ajmaline for assay, previously dried in vacuum at 80°C for 3 hours, dissolve in 2nd fluid for dissolution test to make exactly 500 mL, and use this solution as the standard solution. Determine the absorbances at 288 nm, A_T and A_S, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

$$\text{Amount (mg) of ajmaline } (C_{20}H_{26}N_2O_2) = M_S \times A_T/A_S \times 1/V \times 4$$

M_S: Amount (mg) of ajmaline for assay taken

Dissolution <*6.10*> When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 60 minutes of Ajmaline Tablets is not less than 75%.

Start the test with 1 tablet of Ajmaline Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 56 μg of ajmaline ($C_{203}H_{26}N_2O_2$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of ajmaline for assay, previously dried in vacuum at 80°C for 3 hours, dissolve in the dissolution medium to make exactly 500 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 288 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

$$\text{Dissolution rate (\%) with respect to the labeled amount of ajmaline } (C_{20}H_{26}N_2O_2) = M_S \times A_T/A_S \times V'/V \times 1/C \times 180$$

M_S: Amount (mg) of ajmaline for assay taken

C: Labeled amount (mg) of ajmaline ($C_{20}H_{26}N_2O_2$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Ajmaline Tablets. Weigh accurately a portion of the powder, equivalent to about 0.3 g of ajmaline ($C_{20}H_{26}N_2O_2$), add 15 mL of ammonia solution (28), and extract with four 25-mL portions of chloroform. Combine the chloroform extracts, wash with 10 mL of water, add 5 g of anhydrous sodium sulfate, shake well, and filter. Wash the container and the residue with two 10-mL portions of chloroform, and filter. Evaporate the combined filtrate on a water bath to dryness, dissolve the residue in 50 mL of acetic anhydride and 50 mL of acetone for nonaqueous titration, and titrate <*2.50*> with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

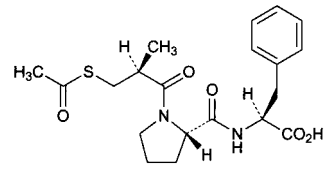
$$\text{Each mL of 0.05 mol/L perchloric acid VS} = 16.32 \text{ mg of } C_{20}H_{26}N_2O_2$$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Alacepril

アラセプリル



$C_{20}H_{26}N_2O_5S$: 406.50

(2*S*)-2-{(2*S*)-1-[(2*S*)-3-(Acetylsulfanyl)-2-methylpropanoyl]pyrrolidine-2-carbonyl}amino-3-phenylpropanoic acid

[*74258-86-9*]

Alacepril, when dried, contains not less than 98.5% and not more than 101.0% of alacepril ($C_{20}H_{26}N_2O_5S$).

Description Alacepril occurs as white, crystals or crystalline powder.

It is freely soluble in methanol, soluble in ethanol (95), and slightly soluble in water.

It dissolves in sodium hydroxide TS.

Identification **(1)** To 20 mg of Alacepril add 0.1 g of sodium hydroxide, and heat gradually to melt: the gas evolved changes the color of a moisten red litmus paper to blue. After cooling, to the melted substance add 2 mL of water, shake, and add 1 mL of lead (II) acetate TS: a brown to black precipitate is formed.

(2) Determine the infrared absorption spectrum of Alacepril, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum:

both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨2.49⟩ $[\alpha]_D^{20}$: −81 – −85° (after drying, 0.25 g, ethanol (95), 25 mL, 100 mm).

Melting point ⟨2.60⟩ 153 – 157°C

Purity **(1)** Chloride ⟨1.03⟩—Dissolve 0.5 g of Alacepril in 30 mL of methanol, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test with this solution as the test solution. Prepare the control solution as follows: to 0.30 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of methanol, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(2) Sulfate ⟨1.14⟩—Dissolve 0.5 g of Alacepril in 30 mL of methanol, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.50 mL of 0.005 mol/L sulfuric acid VS add 30 mL of methanol, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(3) Heavy metals ⟨1.07⟩—Proceed with 1.0 g of Alacepril according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substances—Dissolve 50 mg of Alacepril in 5 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than alacepril from the sample solution is not larger than 2/5 times the peak area of alacepril from the standard solution, and the total area of the peaks other than alacepril from the sample solution is not larger than the peak area of alacepril from the standard solution. For the areas of the peaks, having the relative retention times of about 2.3 and about 2.6 to alacepril, multiply their relative response factors, 1.5 and 1.9, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (100) (1 in 100), acetonitrile, methanol and tetrahydrofuran (6:2:1:1).

Flow rate: Adjust so that the retention time of alacepril is about 5 minutes.

Time span of measurement: About 3 times as long as the retention time of alacepril, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 4 mL of the standard solution add ethanol (95) to make exactly 10 mL. Confirm that the peak area of alacepril obtained with 10 μL of this solution is equivalent to 30 to 50% of that obtained with 10 μL of the standard solution.

System performance: Dissolve 20 mg of Alacepril in 50 mL of a solution of propyl parahydroxybenzoate in ethanol (95) (1 in 80,000). When the procedure is run with 10 μL of this solution under the above operating conditions, alacepril and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of alacepril is not more than 2.0%.

Loss on drying ⟨2.41⟩ Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition ⟨2.44⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Alacepril, previously dried, dissolve in 75 mL of a mixture of methanol and water (2:1), and titrate ⟨2.50⟩ with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 40.65 mg of $C_{20}H_{26}N_2O_5S$

Containers and storage Containers—Tight containers.

Alacepril Tablets

アラセプリル錠

Alacepril Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of alacepril ($C_{20}H_{26}N_2O_5S$: 406.50).

Method of preparation Prepare as directed under Tablets, with Alacepril.

Identification Shake well a quantity of powdered Alacepril Tablets, equivalent to 0.1 g of Alacepril, with 10 mL of ethanol (95), filter, and use the filtrate as the sample solution. Separately, dissolve 10 mg of alacepril in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨2.03⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of ethanol (99.5) and hexane (2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot from the sample solution and the spot from the standard solution show the same color tone and *R*f value.

Uniformity of dosage units ⟨6.02⟩ Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Alacepril Tablets add 2 mL of water, disperse the particle with the aid of ultrasonic wave, and add exactly 2 mL of the internal standard solution for every 10 mg of alacepril ($C_{20}H_{26}N_2O_5S$). To this solution add a suitable amount of methanol, extract for 15 minutes with the aid of ultrasonic wave while occasional shaking, and shake more 15 minutes. Add methanol to make *V* mL so that each mL of the solution contains about 0.5 mg of alacepril ($C_{20}H_{26}N_2O_5S$), centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of alacepril for assay, previously dried at 105°C for 3 hours, add exactly 5 mL of the internal standard solution and methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨2.01⟩ according to the following

conditions, and calculate the ratios, Q_T and Q_S, of the peak area of alacepril to that of the internal standard.

$$\text{Amount (mg) of alacepril } (C_{20}H_{26}N_2O_5S) = M_S \times Q_T/Q_S \times V/50$$

M_S: Amount (mg) of alacepril for assay taken

Internal standard solution—A solution of propyl parahydroxybenzoate in methanol (3 in 20,000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

Proceed as directed in the system suitability in the Assay.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate of a 12.5-mg tablet and a 25-mg tablet in 30 minutes is not less than 75%, and that of a 50-mg tablet in 30 minutes is not less than 70%.

Start the test with 1 tablet of Alacepril Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 14 μg of alacepril ($C_{20}H_{26}N_2O_5S$), and use this solution as the sample solution. Separately, weigh accurately about 14 mg of alacepril for assay, previously dried at 105°C for 3 hours, dissolve in 2 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_{T1} and A_{S1}, at 230 nm, and A_{T2} and A_{S2}, at 300 nm, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using water as the blank.

$$\text{Dissolution rate (\%) with respect to the labeled amount of alacepril } (C_{20}H_{26}N_2O_5S) = M_S \times (A_{T1} - A_{T2})/(A_{S1} - A_{S2}) \times V'/V \times 1/C \times 90$$

M_S: Amount (mg) of alacepril for assay taken

C: Labeled amount (mg) of alacepril ($C_{20}H_{26}N_2O_5S$) in 1 tablet

Assay Weigh accurately, and powder not less than 20 Alacepril Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of alacepril ($C_{20}H_{26}N_2O_5S$), moisten with 2 mL of water, add exactly 3 mL of the internal standard solution and 40 mL of methanol, extract for 15 minutes with the aid of ultrasonic wave, cool, and add methanol to make 50 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of alacepril for assay, previously dried at 105°C for 3 hours, add exactly 3 mL of the internal standard solution, dissolve with methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of alacepril to that of the internal standard.

$$\text{Amount (mg) of alacepril } (C_{20}H_{26}N_2O_5S) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of alacepril for assay taken

Internal standard solution—A solution of propyl parahydroxybenzoate in methanol (1 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (100) (1 in 100), acetonitrile, methanol and tetrahydrofuran (13:5:1:1).

Flow rate: Adjust so that the retention time of alacepril is about 6 minutes.

System suitability—

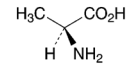
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, alacepril and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of alacepril to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

L-Alanine

L-アラニン



$C_3H_7NO_2$: 89.09

(2*S*)-2-Aminopropanoic acid

[*56-41-7*]

L-Alanine, when dried, contains not less than 98.5% and not more than 101.0% of L-alanine ($C_3H_7NO_2$).

Description L-Alanine occurs as white, crystals or crystalline powder. It has a slightly sweet taste.

It is freely soluble in water and in formic acid, and practically insoluble in ethanol (99.5).

It dissolves in 6 mol/L hydrochloric acid TS.

Identification Determine the infrared absorption spectrum of L-Alanine as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +13.5 – +15.5° (after drying, 2.5 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 1.0 g of L-Alanine in 20 mL of water: the pH of the solution is between 5.7 and 6.7.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of L-Alanine in 10 mL of water: the solution is clear and colorless.

(2) Chloride ⟨*1.03*⟩—Perform the test with 0.5 g of L-Alanine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate ⟨*1.14*⟩—Perform the test with 0.6 g of L-Alanine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium ⟨*1.02*⟩—Perform the test with 0.25 g of

L-Alanine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <*1.07*>—Proceed with 1.0 g of L-Alanine according to Method 1, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Iron <*1.10*>—Prepare the test solution with 1.0 g of L-Alanine according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Weigh accurately about 0.5 g of L-Alanine, dissolve in 0.5 mL of hydrochloric acid and water to make exactly 100 mL. Pipet 10 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the sample solution. Separately, accurately measure 2.5 mmol amounts of L-aspartic acid, L-threonine, L-serine, L-glutamic acid, glycine, L-alanine, L-cystine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-lysine hydrochloride, ammonium chloride, L-histidine and L-arginine, dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 1000 mL, and use this solution as the standard stock solution. Pipet 5 mL of the standard stock solution, add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 4 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Based on the peak heights obtained from the sample solution and standard solution, determine the mass of the amino acids other than alanine contained in 1 mL of the sample solution, and calculate the mass percent: the amount of each amino acid other than alanine is not more than 0.1%.

Operating conditions—

Detector: A visible spectrophotometer (wavelength: 570 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography (Na type) composed with a sulfonated polystyrene copolymer (3 μm in particle diameter).

Column temperature: A constant temperature of about 57°C.

Chemical reaction bath temperature: A constant temperature of about 130°C.

Reaction time: About 1 minute.

Mobile phase: Prepare mobile phases A, B, C, D and E according to the following table, and add 0.1 mL of capric acid to each mobile phase.

	Mobile phase A	Mobile phase B	Mobile phase C	Mobile phase D	Mobile phase E
Citric acid monohydrate	19.80 g	22.00 g	12.80 g	6.10 g	—
Trisodium citrate dihydrate	6.19 g	7.74 g	13.31 g	26.67 g	—
Sodium chloride	5.66 g	7.07 g	3.74 g	54.35 g	—
Sodium hydroxide	—	—	—	—	8.00 g
Ethanol (99.5)	130 mL	20 mL	4 mL	—	100 mL
Thiodiglycol	5 mL	5 mL	5 mL	—	—
Benzyl alcohol	—	—	—	5 mL	—
Lauromacrogol solution (1 in 4)	4 mL	4 mL	4 mL	4 mL	4 mL
Water	Appropriate amount	Appropriate amount	Appropriate amount	Appropriate amount	Appropriate amount
Total volume	1000 mL	1000 mL	1000 mL	1000 mL	1000 mL

Changing mobile phases: When the procedure is run with 20 μL of the standard solution under the above operating conditions, switchover in sequence to mobile phases A, B, C, D and E so that aspartic acid, threonine, serine, glutamic acid, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, ammonia, histidine, and arginine are eluted in this order with the resolution between the peaks of isoleucine and leucine being not less than 1.2.

Reaction reagents: Dissolve 204 g of lithium acetate dihydrate in water, add 123 mL of acetic acid (100) and 401 mL of 1-methoxy-2-propanol, and water to make 1000 mL, introduce nitrogen for 10 minutes, and use this solution as solution (I). Separately, add 39 g of ninhydrin to 979 mL of 1-methoxy-2-propanol, introduce nitrogen for 5 minutes, add 81 mg of sodium borohydride, introduce nitrogen for 30 minutes, and use this solution as solution (II). To 1 volume of solution (I) add 1 volume of solution (II). Prepare before use.

Flow rate of mobile phase: 0.20 mL per minute.

Flow rate of reaction reagent: 0.24 mL per minute.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the resolution between the peaks of glycine and alanine is not less than 1.2.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak height and retention time of each amino acid obtained from the standard solution are not more than 5.0% and not more than 1.0%, respectively.

Loss on drying <*2.41*> Not more than 0.3% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 90 mg of L-Alanine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 8.909 mg of $C_3H_7NO_2$

Containers and storage Containers—Tight containers.

Albumin Tannate

タンニン酸アルブミン

Albumin Tannate is a compound of tannic acid and a protein.

The label states the origin of the protein of Albumin Tannate.

Description Albumin Tannate occurs as a light brown powder. It is odorless, or has a faint, characteristic odor.

It is practically insoluble in water and in ethanol (95).

It dissolves in sodium hydroxide TS with turbidity.

Identification **(1)** To 0.1 g of Albumin Tannate add 10 mL of ethanol (95), and heat in a water bath for 3 minutes with shaking. After cooling, filter, and to 5 mL of the filtrate add 1 drop of iron (III) chloride TS: a blue-purple to bluish black color is produced. On standing, a bluish black precipitate is produced.

(2) To 0.1 g of Albumin Tannate add 5 mL of nitric acid: an orange-yellow color develops.

Purity **(1)** Acidity—Shake 1.0 g of Albumin Tannate with 50 mL of water for 5 minutes, and filter. To 25 mL of the filtrate add 1.0 mL of 0.1 mol/L sodium hydroxide VS and 2 drops of phenolphthalein TS: a red color develops.

(2) Fats—To 2.0 g of Albumin Tannate add 20 mL of petroleum benzine, shake vigorously for 15 minutes, and filter. Evaporate 10 mL of the filtrate on a water bath: the mass of the residue is not more than 50 mg.

Loss on drying ⟨*2.41*⟩ Not more than 6.0% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 1.0% (0.5 g).

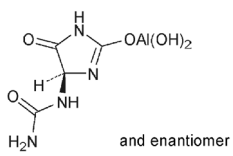
Digestion test To 1.00 g of Albumin Tannate add 0.25 g of saccharated pepsin and 100 mL of water, shake well, and allow to stand for 20 minutes at 40 ± 1°C in a water bath. Add 1.0 mL of dilute hydrochloric acid, shake, and allow to stand for 3 hours at 40 ± 1°C. Cool rapidly to ordinary temperature, and filter. Wash the residue with three 10-mL portions of water, dry in a desiccator (silica gel) for 18 hours, and dry at 105°C for 5 hours: the mass of the residue is 0.50 to 0.58 g.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Aldioxa

アルジオキサ



and enantiomer

Dihydroxo[(4*RS*)-5-oxo-4-ureido-4,5-dihydro-1*H*-imidazol-2-yl]oxoaluminium
[*5579-81-7*]

Aldioxa is a condensation product of allantoin and aluminum hydroxide.

When dried, it contains not less than 65.3% and not more than 74.3% of allantoin ($C_4H_6N_4O_3$: 158.12), and not less than 11.1% and not more than 13.0% of aluminum (Al: 26.98).

Description Aldioxa occurs as a white powder.

It is practically insoluble in water and in ethanol (99.5).

It dissolves in dilute hydrochloric acid.

A solution of Aldioxa in sodium fluoride-hydrochloric acid TS (1 in 100) shows no optical rotation.

Melting point: about 230°C (with decomposition).

Identification **(1)** Determine the infrared absorption spectrum of Aldioxa, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) To 0.2 g of Aldioxa add 10 mL of dilute hydrochloric acid, dissolve by warming, and cool: the solution responds to the Qualitative Tests ⟨*1.09*⟩ for aluminum salt.

Purity **(1)** Chloride ⟨*1.03*⟩—To 0.10 g of Aldioxa add 6 mL of dilute nitric acid, boil to dissolve with shaking for 5 minutes, cool, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.142%).

(2) Heavy metals ⟨*1.07*⟩—To 1.0 g of Aldioxa add 3 mL of hydrochloric acid and 3 mL of water, heat gently to boil with shaking, and evaporate on a water bath to dryness. To the residue add 30 mL of water, shake under warming, cool, filter, and to the filtrate add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 3 mL of hydrochloric acid on a water bath to dryness, and add 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

Loss on drying ⟨*2.41*⟩ Not more than 4.0% (1 g, 105°C, 2 hours).

Assay **(1)** Allantoin—Weigh accurately about 0.1 g of Aldioxa, previously dried, dissolve in 50 mL of dilute sulfuric acid by heating, cool, and add water to make exactly 100 mL. Pipet 10 mL of this solution, and perform the test as directed under Nitrogen Determination ⟨*1.08*⟩.

Each mL of 0.005 mol/L sulfuric acid VS
= 0.3953 mg of $C_4H_6N_4O_3$

(2) Aluminum—Weigh accurately about 0.2 g of Aldioxa, previously dried, dissolve carefully in 50 mL of dilute hydrochloric acid by heating, cool, and add dilute hydrochloric acid to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 25 mL, and use this solution as the sample solution. Separately, pipet a suitable quantity of Standard Aluminum Stock Solution, dilute with water so that each mL of the solution contains not less than 16.0 μg and not more than 64.0 μg of aluminum (Al: 26.98), and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry ⟨*2.23*⟩ according to the following conditions, and calculate the aluminum content of the sample solution from the calibration curve obtained from the absorbance of the standard solution.

Gas: Combustible gas—Acetylene.
Supporting gas—Nitrous oxide.

Lamp: An aluminum hollow cathode lamp.

Wavelength: 309.2 nm.

Containers and storage Containers—Well-closed containers.

Aldioxa Granules

アルジオキサ顆粒

Aldioxa Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of aldioxa ($C_4H_7AlN_4O_5$: 218.10).

Method of preparation Prepare as directed under Granules, with Aldioxa.

Identification **(1)** Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 221 nm and 225 nm.

(2) To a quantity of powdered Aldioxa Granules, equivalent to 0.2 g of Aldioxa, add 10 mL of dilute hydrochloric acid, boil for 5 minutes, and filter: the cooled filtrate responds to the Qualitative Tests ⟨*1.09*⟩ for aluminum salt.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: Aldioxa Granules in single-dose packages meet the requirement of the Content uniformity test.

To the total content of 1 package of Aldioxa Granules add 80 mL of sodium fluoride-hydrochloric acid TS, shake for 20 minutes, add sodium fluoride-hydrochloric acid TS to make exactly 100 mL, and filter. Pipet V mL of the filtrate, add diluted ammonia-ammonium chloride buffer solution (pH 10.0) (1 in 10) to make exactly V' mL so that each mL contains about 20 μg of aldioxa ($C_4H_7AlN_4O_5$), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of aldioxa ($C_4H_7AlN_4O_5$)
$= M_S \times A_T/A_S \times V'/V \times 1/25$

M_S: Amount (mg) of aldioxa for assay taken

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Aldioxa Granules is not less than 85%.

Start the test with an accurately weighed amount of Aldioxa Granules, equivalent to about 0.1 g of aldioxa ($C_4H_7AlN_4O_5$), withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add diluted ammonia-ammonium chloride buffer solution (pH 10.0) (1 in 10) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of aldioxa for assay, previously dried at 105°C for 2 hours, and dissolve in sodium fluoride-hydrochloric acid TS to make exactly 25 mL. Pipet 1 mL of this solution, add diluted ammonia-ammonium chloride buffer solution (pH 10.0) (1 in 10) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 223 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of aldioxa ($C_4H_7AlN_4O_5$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 360$

M_S: Amount (mg) of aldioxa for assay taken
M_T: Amount (g) of Aldioxa Granules taken
C: Labeled amount (mg) of aldioxa ($C_4H_7AlN_4O_5$) in 1 g

Assay Weigh accurately an amount of powdered Aldioxa Granules, equivalent to about 0.1 g of aldioxa ($C_4H_7AlN_4O_5$), add 80 mL of sodium fluoride-hydrochloric acid TS, shake for 20 minutes, add sodium fluoride-hydrochloric acid TS to make exactly 100 mL, and filter. Pipet 2 mL of the filtrate, add diluted ammonia-ammonium chloride buffer solution (pH 10.0) (1 in 10) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of aldioxa for assay, previously dried at 105°C for 2 hours, and dissolve in sodium fluoride-hydrochloric acid TS to make exactly 100 mL. Pipet 4 mL of this solution, add diluted ammonia-ammonium chloride buffer solution (pH 10.0) (1 in 10) to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 223 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of aldioxa ($C_4H_7AlN_4O_5$)
$= M_S \times A_T/A_S \times 2$

M_S: Amount (mg) of aldioxa for assay taken

Containers and storage Containers—Tight containers.

Aldioxa Tablets

アルジオキサ錠

Aldioxa Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of aldioxa ($C_4H_7AlN_4O_5$: 218.10).

Method of preparation Prepare as directed under Tablets, with Aldioxa.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 221 nm and 225 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Aldioxa Tablets add 80 mL of sodium fluoride-hydrochloric acid TS, shake for 20 minutes, add sodium fluoride-hydrochloric acid TS to make exactly 100 mL, and filter. Pipet V mL of the filtrate, add diluted ammonia-ammonium chloride buffer solution (pH 10.0) (1 in 10) to make exactly V' mL so that each mL contains about 20 μg of aldioxa ($C_4H_7AlN_4O_5$), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of aldioxa ($C_4H_7AlN_4O_5$)
$= M_S \times A_T/A_S \times V'/V \times 1/25$

M_S: Amount (mg) of aldioxa for assay taken

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rates in 15 minutes of 50-mg tablet and in 30 minutes of 100-mg tablet are not less than 80% and not less than 70%, respectively.

Start the test with 1 tablet of Aldioxa Tablets, withdraw not less than 20 mL of the medium at the specified minute

after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add diluted ammonia-ammonium chloride buffer solution (pH 10.0) (1 in 10) to make exactly V' mL so that each mL contains about 22 μg of aldioxa ($C_4H_7AlN_4O_5$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of aldioxa for assay, previously dried at 105°C for 2 hours, and dissolve in sodium fluoride-hydrochloric acid TS to make exactly 25 mL. Pipet 1 mL of this solution, add diluted ammonia-ammonium chloride buffer solution (pH 10.0) (1 in 10) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 223 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of aldioxa ($C_4H_7AlN_4O_5$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 72$

M_S: Amount (mg) of aldioxa for assay taken
C: Labeled amount (mg) of aldioxa ($C_4H_7AlN_4O_5$) in 1 tablet

Assay Weigh accurately, and powder not less than 20 Aldioxa Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of aldioxa ($C_4H_7AlN_4O_5$), add 80 mL of sodium fluoride-hydrochloric acid TS, shake for 20 minutes, add sodium fluoride-hydrochloric acid TS to make exactly 100 mL, and filter. Pipet 2 mL of the filtrate, add diluted ammonia-ammonium chloride buffer solution (pH 10.0) (1 in 10) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of aldioxa for assay, previously dried at 105°C for 2 hours, and dissolve in sodium fluoride-hydrochloric acid TS to make exactly 100 mL. Pipet 4 mL of this solution, add diluted ammonia-ammonium chloride buffer solution (pH 10.0) (1 in 10) to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 223 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

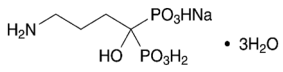
Amount (mg) of aldioxa ($C_4H_7AlN_4O_5$)
$= M_S \times A_T/A_S \times 2$

M_S: Amount (mg) of aldioxa for assay taken

Containers and storage Containers—Tight containers.

Alendronate Sodium Hydrate

アレンドロン酸ナトリウム水和物



$C_4H_{12}NNaO_7P_2.3H_2O$: 325.12
Monosodium trihydrogen 4-amino-1-hydroxybutane-1,1-diyldiphosphonate trihydrate
[*121268-17-5*]

Alendronate Sodium Hydrate contains not less than 99.0% and not more than 101.0% of alendronate sodium ($C_4H_{12}NNaO_7P_2$: 271.08), calculated on the dried basis.

Description Alendronate Sodium Hydrate occurs as a white crystalline powder.

It is sparingly soluble in water, and practically insoluble in ethanol (99.5).

It dissolves in 0.1 mol/L trisodium citrate TS.

Melting point: about 252°C (with decomposition, after drying).

Identification **(1)** To 5 mL of a solution of Alendronate Sodium Hydrate (1 in 50) add 1 mL of ninhydrin TS, and heat: a blue-purple color develops.

(2) Determine the infrared absorption spectrum of Alendronate Sodium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Alendronate Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 0.1 g of Alendronate Sodium Hydrate add 10 mL of a mixture of nitric acid and perchloric acid (1:1). Heat to concentrate to about 1 mL, add about 10 mL of water while hot, and neutralize with a solution of sodium hydroxide (2 in 5): the solution responds to the Qualitative Tests ⟨*1.09*⟩ for phosphate.

(4) A solution of Alendronate Sodium Hydrate (1 in 100) responds to the Qualitative Tests ⟨*1.09*⟩ for sodium salt.

pH ⟨*2.54*⟩ The pH of a solution of 1.0 g of Alendronate Sodium Hydrate in 100 mL of freshly boiled and cooled water is between 4.0 and 5.0.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Put 1.0 g of Alendronate Sodium Hydrate in a Kjeldahl flask, add 9 mL of a mixture of nitric acid and sulfuric acid (5:4), and heat until the solution becomes brown. After cooling, add 9 mL of a mixture of nitric acid and sulfuric acid (5:4), and heat again until the color changes from colorless to brown. After cooling, add 2 mL of nitric acid, strongly heat until brown fumes are no longer evolved, and heat until large amounts of white fumes are evolved. After cooling, add carefully 5 mL of water and 1 mL of hydrogen peroxide (30), heat until white fumes are no longer evolved, and continue heating for more 5 minutes. After cooling, if any yellow color remains, add 2 mL of nitric acid, and repeat the same procedure. After cooling, transfer the solution in the Kjeldahl flask to a beaker, wash out the inside of the flask with 5 mL of water, and add the washing to the beaker. Adjust to pH 3 – 5 with ammonia solution (28), transfer to a Nessler tube, add water to make 50 mL, and perform the test with this solution as the test solution. Prepare the control solution in the same procedure using the same amount of the reagents used for the preparation of the sample solution, add 1.0 mL of Standard Lead Solution and add water to make 50 mL (not more than 10 ppm).

(2) Related substances—Dissolve 15 mg of Alendronate Sodium Hydrate in 25 mL of 0.1 mol/L trisodium citrate TS, and use this solution as the sample stock solution. Pipet 5 mL of the sample stock solution, and add 0.1 mol/L trisodium citrate TS to make exactly 50 mL. Pipet 1 mL of this solution, add 0.1 mol/L trisodium citrate TS to make exactly 100 mL, and use this solution as the standard stock solution. To exactly 5 mL each of the sample stock solution and standard stock solution, add exactly 5 mL each of a solution of sodium tetraborate decahydrate (19 in 1000), acetonitrile and a solution of 9-fluorenylmethyl chloroformate in acetonitrile (1 in 250), shake for 45 seconds, and allow to stand for 30 minutes at room temperature. Then, add 20 mL of dichloromethane to them, shake for 60 seconds, centrifuge, and use the supernatant liquids so obtained as the sample

solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions. Determine each peak area of these solutions by the automatic integration method: each peak area other than alendronic acid obtained from the sample solution is not larger than the peak area of alendronic acid obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 266 nm).

Column: A stainless steel column 4.1 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase A: Dissolve 2.94 g of trisodium citrate dihydrate and 1.42 g of anhydrous disodium hydrogen phosphate in 900 mL of water, adjust to pH 8.0 with phosphoric acid, and add water to make 1000 mL. To 850 mL of this solution add 150 mL of acetonitrile for liquid chromatography.

Mobile phase B: Dissolve 2.94 g of trisodium citrate dihydrate and 1.42 g of anhydrous disodium hydrogen phosphate in 900 mL of water, adjust to pH 8.0 with phosphoric acid, and add water to make 1000 mL. To 300 mL of this solution add 700 mL of acetonitrile for liquid chromatography.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 15	100 → 50	0 → 50
15 – 25	50 → 0	50 → 100

Flow rate: About 1.8 mL per minute.

Time span of measurement: About 5 times as long as the retention time of alendronic acid, beginning after the solvent peak.

System suitability—

System performance: Dissolve 15 mg of Alendronate Sodium Hydrate and 2 mg of 4-aminobutylic acid in 100 mL of 0.1 mol/L trisodium citrate TS. To 5 mL of this solution add 5 mL each of a solution of sodium tetraborate decahydrate (19 in 1000), acetonitrile and a solution of 9-fluorenylmethyl chloroformate in acetonitrile (1 in 250), then, proceed in the same manner as the sample solution. When the procedure is run with 20 μL of this solution under the above operating conditions, alendronic acid and 4-aminobutylic acid are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of alendronic acid is not more than 2.0%.

Loss on drying ⟨2.41⟩ 16.1 – 17.1% (1 g, 140°C, 3 hours).

Assay Weigh accurately about 10 mg each of Alendronate Sodium Hydrate and Alendronate Sodium RS (separately determine the loss on drying ⟨2.41⟩ in the same conditions as Alendronate Sodium Hydrate), dissolve in 0.1 mol/L trisodium citrate TS to make exactly 100 mL, and use these solutions as the sample stock solution and the standard stock solution, respectively. To exactly 5 mL each of these solutions add exactly 5 mL each of a solution of sodium tetraborate decahydrate (19 in 1000) and a solution of 9-fluorenylmethyl chloroformate in acetonitrile (1 in 2000), shake for 30 seconds, and allow to stand for 25 minutes. Then, add 25 mL of dichloromethane, shake for 60 seconds, centrifuge, and use the supernatant liquids so obtained as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of alendronic acid in each solution.

Amount (mg) of alendronate sodium ($C_4H_{12}NNaO_7P_2$) $= M_S \times A_T/A_S$

M_S: Amount (mg) of Alendronate Sodium RS taken, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 266 nm).

Column: A stainless steel column 4.1 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 14.7 g of trisodium citrate dihydrate and 7.1 g of anhydrous disodium hydrogen phosphate in 900 mL of water, adjust to pH 8.0 with phosphoric acid, and add water to make 1000 mL. To 700 mL of this solution add 250 mL of acetonitrile for liquid chromatography and 50 mL of methanol.

Flow rate: Adjust so that the retention time of alendronic acid is about 3 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of alendronic acid are not less than 1500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of alendronic acid is not more than 1.0%.

Containers and storage Containers—Tight containers.

Alendronate Sodium Injection

アレンドロン酸ナトリウム注射液

Alendronate Sodium Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of alendronic acid ($C_4H_{13}NO_7P_2$: 249.10).

Method of preparation Prepare as directed under Injections, with Alendronate Sodium Hydrate.

Description Alendronate Sodium Injection is a clear, colorless liquid.

Identification Use Alendronate Sodium Injection as the sample solution. Separately, dissolve 33 mg of alendronate sodium hydrate in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨2.03⟩. Spot

5 μL each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of water, pyridine, acetic acid (100) and ethyl acetate (1:1:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 100°C for 10 minutes: the principal spots from the sample solution and standard solution show a blue-purple color and the same *R*f value.

pH Being specified separately when the drug is granted approval based on the Law.

Bacterial endotoxins ⟨*4.01*⟩ Less than 119 EU/mg.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to Membrane-filter method: it meets the requirement.

Assay To an exactly measured volume of Alendronate Sodium Injection, equivalent to about 5 mg of alendronic acid ($C_4H_{13}NO_7P_2$), add a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (1 in 500) to make exactly 100 mL, and use this solution as the sample stock solution. Separately, weigh accurately about 33 mg of Alendronate Sodium RS (separately determine the loss on drying ⟨*2.41*⟩ under the same conditions as Alendronate Sodium Hydrate), and dissolve in a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (1 in 500) to make exactly 100 mL. Pipet 10 mL of this solution, add a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (1 in 500) to make exactly 50 mL, and use this solution as the standard stock solution. Pipet 5 mL each of the sample stock solution and standard stock solution, add exactly 5 mL of a solution of sodium tetraborate decahydrate (19 in 500) and exactly 4 mL of a solution of 9-fluorenylmethyl chloroformate in acetonitrile (1 in 1000), shake for 30 seconds, and allow to stand at room temperature for 25 minutes. Then, add 25 mL of dichloromethane to them, shake for 45 seconds, centrifuge, and use the supernatant liquid so obtained as the sample solution and the standard solution, respectively. Perform the test with 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of alendronic acid in each solution.

$$\text{Amount (mg) of alendronic acid } (C_4H_{13}NO_7P_2) = M_S \times A_T/A_S \times 1/5 \times 0.919$$

M_S: Amount (mg) of Alendronate Sodium RS taken, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.1 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 14.7 g of trisodium citrate dihydrate and 8.7 g of dipotassium hydrogen phosphate in 900 mL of water, adjust to pH 8.0 with phosphoric acid, and add water to make 1000 mL. To 750 mL of this solution add 200 mL of acetonitrile for liquid chromatography and 50 mL of methanol.

Flow rate: Adjust so that the retention time of alendronic acid is about 7 minutes.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of alendronic acid are not less than 1500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operations conditions, the relative standard deviation of the peak of alendronic acid is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Alendronate Sodium Tablets

アレンドロン酸ナトリウム錠

Alendronate Sodium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of alendronic acid ($C_4H_{13}NO_7P_2$: 249.10).

Method of preparation Prepare as directed under Tablets, with Alendronate Sodium Hydrate.

Identification To a quantity of powdered Alendronate Sodium Tablets, equivalent to 25 mg of alendronic acid ($C_4H_{13}NO_7P_2$), add 25 mL of water, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh 33 mg of alendronate sodium hydrate, and dissolve in 25 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of water, pyridine, acetic acid (100) and ethyl acetate (1:1:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 100°C for 10 minutes: the principal spots from the sample solution and standard solution show a blue-purple color and the same *R*f value.

Uniformity of dosage units ⟨*6.02*⟩ Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Alendronate Sodium Tablets add 0.1 mol/L trisodium citrate TS to make exactly 100 mL, and stir until the tablet is completely disintegrated. Centrifuge this solution, pipet V mL of the supernatant liquid, and add 0.1 mol/L trisodium citrate TS to make exactly V' mL so that each mL contains about 25 μg of alendronic acid ($C_4H_{13}NO_7P_2$), and use this solution as the sample stock solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of alendronic acid } (C_4H_{13}NO_7P_2) = M_S \times A_T/A_S \times V'/V \times 2/25 \times 0.919$$

M_S: Amount (mg) of Alendronate Sodium RS taken, calculated on the dried basis

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Alendronate Sodium Tablets is not less than 85%.

Start the test with 1 tablet of Alendronate Sodium Tablets,

withdraw not less than 10 mL of the medium at the specified minute after starting the test, and centrifuge. Pipet V mL of the supernatant liquid, add water to make exactly V' mL so that each mL contains about 6 μg of alendronic acid ($C_4H_{13}NO_7P_2$), and use this solution as the sample stock solution. Separately, weigh accurately about 29 mg of Alendronate Sodium RS (separately determine the loss on drying <2.41> under the same conditions as Alendronate Sodium Hydrate), and dissolve in water to make exactly 250 mL. Pipet 3 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard stock solution. Pipet 5 mL each of the sample stock solution and standard stock solution, add exactly 1 mL of trisodium citrate dihydrate solution (22 in 125), exactly 5 mL of a solution obtained by dissolving 6.2 g of boric acid in 950 mL of water, adjusting to pH 9.0 with sodium hydrate TS, and adding water to make 1000 mL, and add exactly 4 mL of a solution of 9-fluorenylmethyl chloroformate in acetonitrile (1 in 2000), shake for 30 seconds, and allow to stand at room temperature for 25 minutes. Add 25 mL of dichloromethane, shake for 45 seconds, then centrifuge, and use the supernatant liquid as the sample solution and the standard solution, respectively. Then, proceed as directed in the Assay.

Dissolution rate (%) with respect to the labeled amount of alendronic acid ($C_4H_{13}NO_7P_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 108/5 \times 0.919$

M_S: Amount (mg) of Alendronate Sodium RS taken, calculated on the dried basis
C: Labeled amount (mg) of alendronic acid ($C_4H_{13}NO_7P_2$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Alendronate Sodium Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of alendronic acid ($C_4H_{13}NO_7P_2$), add 0.1 mol/L trisodium citrate TS to make exactly 1000 mL, stir for 30 minutes, and centrifuge. Pipet 5 mL of the supernatant liquid, add 0.1 mol/L trisodium citrate TS to make exactly 10 mL, and use this solution as the sample stock solution. Separately, weigh accurately about 39 mg of Alendronae Sodium RS (separately determine the loss on drying <2.41> under the same conditions as Alendronae Sodium Hydrate), dissolve in 0.1 mol/L trisodium citrate TS to make exactly 50 mL. Pipet 2 mL of this solution, add 0.1 mol/L trisodium citrate TS to make exactly 50 mL, and use this solution as the standard stock solution. Pipet 5 mL each of the sample stock solution and standard stock solution, add exactly 5 mL of a solution of sodium tetraborate decahydrate (19 in 500) and exactly 4 mL of a solution of 9-fluprenylmethyl chloroformate in acetonitrile (1 in 1000), shake for 30 seconds, and allow to stand at room temperature for 25 minutes. Then, add 25 mL of dichloromethane to them, shake for 45 seconds, centrifuge, and use the supernatant liquid so obtained as the sample solution and the standard solution, respectively. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of alendronic acid in each solution.

Amount (mg) of alendronic acid ($C_4H_{13}NO_7P_2$)
$= M_S \times A_T/A_S \times 8/5 \times 0.919$

M_S: Amount (mg) of Alendronate Sodium RS taken, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 266 nm).

Column: A stainless steel column 4.1 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 14.7 g of trisodium citrate dihydrate and 7.1 g of anhydrous disodium hydrogen phosphate in 900 mL of water, adjust to pH 8.0 with phosphoric acid, and add water to make 1000 mL. To 750 mL of this solution add 200 mL of acetonitrile for liquid chromatography and 50 mL of methanol.

Flow rate: Adjust so that the retention time of alendronic acid is about 7 minutes.

System suitability—

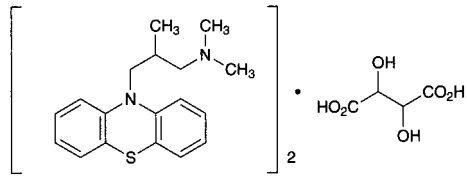
System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of alendronic acid are not less than 1500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operations conditions, the relative standard deviation of the peak of alendronic acid is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Alimemazine Tartrate

アリメマジン酒石酸塩



$(C_{18}H_{22}N_2S)_2.C_4H_6O_6$: 746.98
N,*N*,2-Trimethyl-3-(10*H*-phenothiazin-10-yl)propylamine hemitartrate
[*41375-66-0*]

Alimemazine Tartrate, when dried, contains not less than 98.0% of alimemazine tartrate [$(C_{18}H_{22}N_2S)_2.C_4H_6O_6$].

Description Alimemazine Tartrate occurs as a white powder. It is odorless, and has a bitter taste.

It is freely soluble in water and in acetic acid (100), sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of 1.0 g of Alimemazine Tartrate in 50 mL of water is between 5.0 and 6.5.

It is gradually colored by light.

Identification **(1)** To 2 mL of a solution of Alimemazine Tartrate (1 in 100) add 1 drop of iron (III) chloride TS: a red-brown color is produced, and immediately a yellow precipitate is formed.

(2) Dissolve 1 g of Alimemazine Tartrate in 5 mL of water, add 3 mL of sodium hydroxide TS, extract with two 10-mL portions of diethyl ether [use the aqueous layer obtained in the Identification (4)]. Shake the combined diethyl ether extracts with 3 g of anhydrous sodium sulfate, filter, and evaporate the diethyl ether with the aid of a current of air. Dry the residue in a desiccator (in vacuum, phosphorus

(V) oxide) for 16 hours: it melts ⟨*2.60*⟩ between 66°C and 70°C.

(3) Determine the absorption spectrum of a solution of Alimemazine Tartrate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) The aqueous layer, obtained in the identification (2), when neutralized with dilute acetic acid, responds to the Qualitative Tests ⟨*1.09*⟩ (1) and (2) for tartrate.

Melting point ⟨*2.60*⟩ 159 – 163°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Alimemazine Tartrate in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Alimemazine Tartrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Alimemazine Tartrate according to Method 3, and perform the test. Use a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 5) (not more than 2 ppm).

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

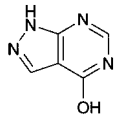
Assay Weigh accurately about 0.8 g of Alimemazine Tartrate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS until the color of the solution changes from red through brown to green-brown (indicator: 2 mL of *p*-naphtholbenzein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 37.35 mg of $(C_{18}H_{22}N_2S)_2.C_4H_6O_6$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Allopurinol

アロプリノール



$C_5H_4N_4O$: 136.11
1*H*-Pyrazolo[3,4-*d*]pyrimidin-4-ol
[*315-30-0*]

Allopurinol, when dried, contains not less than 98.0% and not more than 101.0% of allopurinol ($C_5H_4N_4O$).

Description Allopurinol occurs as white to pale yellowish white, crystals or crystalline powder.

It is slightly soluble in *N*,*N*-dimethylformamide, and very slightly soluble in water and in ethanol (99.5).

It dissolves in ammonia TS.

Identification **(1)** Determine the absorption spectrum of a solution of Allopurinol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Allopurinol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Allopurinol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Allopurinol according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Allopurinol in 10 mL of ammonia TS, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ammonia TS to make exactly 500 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of cellulose with fluorescent indicator for thin-layer chromatography. Develop the plate with ammonia TS-saturated 1-butanol to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.16 g of Allopurinol, previously dried, dissolve in 70 mL of *N*,*N*-dimethylformamide by warming. Cool, and titrate ⟨*2.50*⟩ with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). To 70 mL of *N*,*N*-dimethylformamide add 12 mL of water, perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 13.61 mg of $C_5H_4N_4O$

Containers and storage Containers—Tight containers.

Allopurinol Tablets

アロプリノール錠

Allopurinol Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of allopurinol ($C_5H_4N_4O$: 136.11).

Method of preparation Prepare as directed under Tablets, with Allopurinol.

Identification **(1)** Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 248 nm and 252 nm.

(2) To a quantity of powdered Allopurinol Tablets, equivalent to 0.1 g of Allopurinol, add 5 mL of a solution of diethylamine (1 in 10), shake well, add 5 mL of methanol, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 0.1 g of allopurinol in 5 mL of a

solution of diethylamine (1 in 10), add 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 2.5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-butanone, ammonia solution (28) and 2-methoxyethanol (3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spots obtained from the sample solution and standard solution show the same *R*f value.

Uniformity of dosage units ⟨*6.02*⟩ Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Allopurinol Tablets add *V*/10 mL of 0.05 mol/L sodium hydroxide TS, shake well, and treat with ultrasonic waves for 10 minutes. After cooling, add 0.1 mol/L hydrochloric acid TS to make exactly *V* mL so that each mL contains about 0.5 mg of allopurinol ($C_5H_4N_4O$), and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 0.1 mol/L of hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of allopurinol for assay, previously dried at 105°C for 4 hours, dissolve in 10 mL of 0.05 mol/L sodium hydroxide TS, and add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 2 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 250 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of allopurinol ($C_5H_4N_4O$)
$= M_S \times A_T/A_S \times V/100$

M_S: Amount (mg) of allopurinol for assay taken

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Allopurinol Tablets is not less than 80%.

Start the test with 1 tablet of Allopurinol Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet *V* mL of the subsequent filtrate, add water to make exactly *V′* mL so that each mL contains about 11 μg of allopurinol ($C_5H_4N_4O$), and use this solution as the sample solution. Separately, weigh accurately about 11 mg of allopurinol for assay, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 250 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of allopurinol ($C_5H_4N_4O$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount (mg) of allopurinol for assay taken
C: Labeled amount (mg) of allopurinol ($C_5H_4N_4O$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Allopurinol Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of allopurinol ($C_5H_4N_4O$), add 20 mL of 0.05 mol/L sodium hydroxide TS, shake well, and treat with ultrasonic waves for 10 minutes. After cooling, add 0.1 mol/L hydrochloric acid TS to make exactly 200 mL, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of allopurinol for assay, previously dried at 105°C for 4 hours, dissolve in 20 mL of 0.05 mol/L sodium hydroxide TS, and add 0.1 mol/L hydrochloric acid TS to make exactly 200 mL. Pipet 2 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 250 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

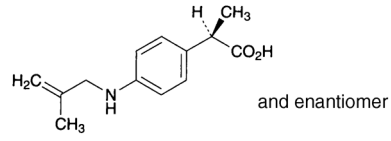
Amount (mg) of allopurinol ($C_5H_4N_4O$) = $M_S \times A_T/A_S$

M_S: Amount (mg) of allopurinol for assay taken

Containers and storage Containers—Well-closed containers.

Alminoprofen

アルミノプロフェン



$C_{13}H_{17}NO_2$: 219.28
(2*RS*)-2-{[4-(2-Methylprop-2-en-1-yl)amino]phenyl}propanoic acid
[*39718-89-3*]

Alminoprofen, when dried, contains not less than 99.0% and not more than 101.0% of alminoprofen ($C_{13}H_{17}NO_2$).

Description Alminoprofen occurs as white to pale yellow, crystals or crystalline powder.

It is freely soluble in ethanol (99.5) and in acetic acid (100), and very slightly soluble in water.

It gradually turns brown on exposure to light.

A solution of Alminoprofen in ethanol (99.5) (1 in 10) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Alminoprofen in ethanol (99.5) (3 in 500,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Alminoprofen as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 106 – 108°C

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of

Alminoprofen according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Alminoprofen according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Conduct this procedure using light-resistant vessels. Dissolve 50 mg of Alminoprofen in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the area of the peak other than alminoprofen obtained from the sample solution is not larger than 1/5 times the peak area of alminoprofen obtained from the standard solution. Furthermore, the total area of the peaks other than alminoprofen from the sample solution is not larger than the peak area of alminoprofen from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 6.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and diluted acetic acid (100) (1 in 1000) (4:1).

Flow rate: Adjust so that the retention time of alminoprofen is about 5 minutes.

Time span of measurement: About 5 times as long as the retention time of alminoprofen, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of alminoprofen obtained from 5 μL of this solution is equivalent to 7 to 13% of that obtained from 5 μL of the standard solution.

System performance: Dissolve 10 mg each of Alminoprofen and butyl parahydroxybenzoate in 100 mL of methanol. Pipet 10 mL of this solution, and add methanol to make exactly 50 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, alminoprofen and butyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of alminoprofen is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 1 hour).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Alminoprofen, previously dried, dissolve in 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 21.93 mg of $C_{13}H_{17}NO_2$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Alminoprofen Tablets

アルミノプロフェン錠

Alminoprofen Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of alminoprofen ($C_{13}H_{17}NO_2$: 219.28).

Method of preparation Prepare as directed under Tablets, with Alminoprofen.

Identification Take an amount of powdered Alminoprofen Tablets, equivalent to 30 mg of Alminoprofen, add ethanol (99.5) to make 100 mL, shake thoroughly, and centrifuge. To 2 mL of the supernatant liquid add ethanol (99.5) to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 253 nm and 257 nm, and between 298 nm and 302 nm.

Purity Related substances—Conduct this procedure using light-resistant vessels. Powder 10 tablets of Alminoprofen Tablets, weigh a portion of the powder equivalent to 50 mg of Alminoprofen, add 50 mL of the mobile phase, shake for 15 minutes, add the mobile phase to make exactly 100 mL, centrifuge, and use the supernatant liquid as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of each solution by the automatic integration method: the area of the peak other than alminoprofen obtained from the sample solution is not larger than 1/2 times the peak area of alminoprofen obtained from the standard solution. Furthermore, the total area of the peaks other than alminoprofen from the sample solution is not larger than 2 times the peak area of alminoprofen from the standard solution.

Operating conditions—

Proceed as directed in the operating conditions in the Purity (3) under Alminoprofen.

System suitability—

Proceed as directed in the system suitability in the Purity (3) in Assay under Alminoprofen.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Alminoprofen Tablets add 5 mL of water, shake until the tablet is disintegrated, add 50 mL of ethanol (99.5), shake for 20 minutes, then add ethanol (99.5) to make exactly 100 mL, and centrifuge. Pipet 3 mL of the supernatant liquid, add ethanol (99.5) to make exactly 50 mL. Pipet V mL of this solution, add ethanol (99.5) to make exactly V' mL so that each mL contains about 6 μg of alminoprofen ($C_{13}H_{17}NO_2$), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of alminoprofen ($C_{13}H_{17}NO_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/3$

M_S: Amount (mg) of alminoprofen for assay taken

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolu-

tions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of Alminoprofen Tablets is not less than 80%.

Start the test with 1 tablet of Alminoprofen Tablets, withdraw not less than 20 mL of the medium at specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add 0.05 mol/L sodium hydroxide TS to make exactly V' mL so that each mL contains about 8.9 μg of alminoprofen ($C_{13}H_{17}NO_2$), and use this solution as the sample solution. Separately, weigh accurately about 30 mg of alminoprofen for assay, previously dried in vacuum for 1 hour using phosphorus (V) oxide as the dessicant, and dissolve in 0.05 mol/L sodium hydroxide TS to make exactly 100 mL. Pipet 3 mL of this solution, add 0.05 mol/L sodium hydroxide TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 245 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Dissolution rate (%) with respect to the labeled amount of alminoprofen ($C_{13}H_{17}NO_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 27$

M_S: Amount (mg) of alminoprofen for assay taken
C: Labeled amount (mg) of alminoprofen ($C_{13}H_{17}NO_2$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 tablets of Alminoprofen Tablets, and powder. Weigh accurately an amount equivalent to about 60 mg of alminoprofen ($C_{13}H_{17}NO_2$), add ethanol (99.5) and shake well, add ethanol (99.5) to make exactly 200 mL, and centrifuge. Pipet 2 mL of the supernatant liquid, add ethanol (99.5) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of alminoprofen for assay, previously dried in vacuum for 1 hour using phosphorus (V) oxide as the dessicant, dissolve in ethanol (99.5) to make exactly 100 mL. Pipet 2 mL of this solution, add ethanol (99.5) to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at the wavelength of maximum absorption at about 255 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

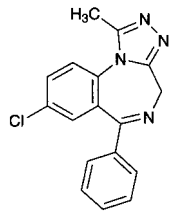
Amount (mg) of alminoprofen ($C_{13}H_{17}NO_2$)
$= M_S \times A_T/A_S \times 2$

M_S: Amount (mg) of alminoprofen for assay taken

Containers and storage Containers—Well-closed containers.

Alprazolam

アルプラゾラム



$C_{17}H_{13}ClN_4$: 308.76
8-Chloro-1-methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine
[*28981-97-7*]

Alprazolam, when dried, contains not less than 98.5% of alprazolam ($C_{17}H_{13}ClN_4$).

Description Alprazolam occurs as white, crystals or crystalline powder.

It is freely soluble in chloroform, soluble in methanol and in ethanol (95), sparingly soluble in acetic anhydride, and practically insoluble in water.

It dissolves in dilute nitric acid.

Identification **(1)** Determine the absorption spectrum of a solution of Alprazolam in ethanol (95) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 0.05 g of Alprazolam in 0.7 mL of deuterochloroform for nuclear magnetic resonance spectroscopy, and determine the spectrum of this solution using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*> (^{1}H): it exhibits a single signal A at around δ 2.6 ppm, doublet signals B and C at around δ 4.0 ppm and δ 5.4 ppm, and a broad signal D between δ 7.1 ppm and 7.9 ppm. The ratio of integrated intensity of each signal, A:B:C:D, is about 3:1:1:8.

(3) Perform the test with Alprazolam as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Melting point <*2.60*> 228 – 232°C

Purity **(1)** Chloride <*1.03*>—Dissolve 0.5 g of Alprazolam in 10 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Alprazolam according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 50 mg of Alprazolam in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, then pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop with a mixture of acetone, hexane, ethyl acetate and ethanol (95) (4:2:2:1) to a distance

of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

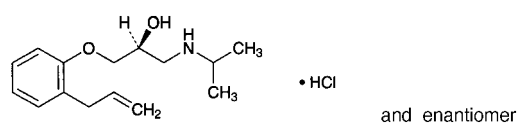
Assay Weigh accurately about 0.25 g of Alprazolam, previously dried, dissolve in 100 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 15.44 mg of $C_{17}H_{13}ClN_4$

Containers and storage Containers—Well-closed containers.

Alprenolol Hydrochloride

アルプレノロール塩酸塩



and enantiomer

$C_{15}H_{23}NO_2$.HCl: 285.81
(*2RS*)-1-(2-Allylphenoxy)-3-[(1-methylethyl)amino]propan-2-ol monohydrochloride
[*13707-88-5*]

Alprenolol Hydrochloride, when dried, contains not less than 99.0% of alprenolol hydrochloride ($C_{15}H_{23}NO_2$.HCl).

Description Alprenolol Hydrochloride occurs as white, crystals or crystalline powder.

It is freely soluble in water, in ethanol (95) and in acetic acid (100), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Identification **(1)** To 2 mL of a solution of Alprenolol Hydrochloride (1 in 100) add 0.05 mL of copper (II) sulfate TS and 2 mL of sodium hydroxide TS: a blue-purple color develops. To this solution add 1 mL of diethyl ether, shake well, and allow to stand: a red-purple color develops in the diethyl ether layer.

(2) Dissolve 0.05 g of Alprenolol Hydrochloride in 5 mL of water, add 1 to 2 drops of bromine TS, and shake: the color of the test solution disappears.

(3) Determine the absorption spectrum of a solution of Alprenolol Hydrochloride in ethanol (95) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Alprenolol Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(5) A solution of Alprenolol Hydrochloride (1 in 50) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

pH ⟨*2.54*⟩ Dissolve 1.0 g of Alprenolol Hydrochloride in 10 mL of water: the pH of this solution is between 4.5 and 6.0.

Melting point ⟨*2.60*⟩ 108 – 112°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Alprenolol Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Alprenolol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Alprenolol Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Alprenolol Hydrochloride in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add ethanol (95) to make exactly 100 mL. Pipet 2.5 mL of this solution, add ethanol (95) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, acetone, acetic acid (100) and water (60:42:5:3) to a distance of about 10 cm, air-dry the plate, and then dry at 80°C for 30 minutes. After cooling, allow the plate to stand in iodine vapor for 30 minutes: the spots other than the principal spot and the spot on the starting point from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Alprenolol Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

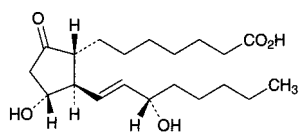
Each mL of 0.1 mol/L perchloric acid VS
= 28.58 mg of $C_{15}H_{23}NO_2$.HCl

Containers and storage Containers—Well-closed containers.

Alprostadil

Prostaglandin E_1

アルプロスタジル



$C_{20}H_{34}O_5$: 354.48
7-{(1*R*,2*R*,3*R*)-3-Hydroxy-2-[(1*E*,3*S*)-3-hydroxyoct-1-en-1-yl]-5-oxocyclopentyl}heptanoic acid
[*745-65-3*]

Alprostadil, when dried, contains not less than

97.0% and not more than 103.0% of alprostadil ($C_{20}H_{34}O_5$).

Description Alprostadil occurs as white, crystals or crystalline powder.

It is freely soluble in ethanol (99.5) and in tetrahydrofuran, slightly soluble in acetonitrile, and practically insoluble in water.

Identification (1) The absorption spectrum of a solution of Alprostadil in ethanol (99.5) (1 in 100,000) determined as directed under Ultraviolet-visible Spectrophotometry <2.24> shows no absorption between 210 nm and 350 nm. Separately, to 10 mL of this solution add 1 mL of potassium hydroxide-ethanol TS, allow to stand for 15 minutes, and determine the absorption spectrum in the same way. Compare the spectrum so obtained with the Reference Spectrum or the spectrum of a solution of Alprostadil RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Alprostadil, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Alprostadil RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: -53 – $-61°$ (after drying, 25 mg, tetrahydrofuran, 5 mL, 100 mm).

Melting point <2.60> 114 – 118°C

Purity Related substances—Dissolve 4 mg of Alprostadil in 2 mL of a mixture of acetonitrile for liquid chromatography and water (9:1), and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, and add the mixture of acetonitrile for liquid chromatography and water (9:1) to make exactly 10 mL. Pipet 2 mL of this solution, add the mixture of acetonitrile for liquid chromatography and water (9:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peaks, having the relative retention time of about 0.70 and 1.26 to alprostadil, is not larger than 1/2 times the peak area of alprostadil with the standard solution, the area of the peaks, having the relative retention time of about 0.88 and 1.18 to alprostadil, is not larger than the peak area of alprostadil with the standard solution, the area of the peaks other than alprostadil and the peaks mentioned above is not larger than 1/10 times the peak area of alprostadil with the standard solution and the total area of the peaks other than alprostadil is not larger than 2 times the peak area of alprostadil with the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 5 times as long as the retention time of alprostadil, beginning after the solvent peak.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Measure exactly 2 mL of the standard solution, add the mixture of acetonitrile for liquid chromatography and water (9:1) to make exactly 20 mL. Confirm that the peak area of alprostadil obtained with 5 μL of this solution is equivalent to 7 to 13% of that obtained with 5 μL of the standard solution.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of alprostadil is not more than 1.5%.

Loss on drying <2.41> Not more than 1.0% (0.1 g, in vacuum, phosphorus (V) oxide, 4 hours).

Assay Weigh accurately about 5 mg each of Alprostadil and Alprostadil RS, previously dried, dissolve in exactly 5 mL of the internal standard solution, add a mixture of acetonitrile for liquid chromatography and water (9:1) to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of alprostadil to that of the internal standard.

Amount (mg) of alprostadil ($C_{20}H_{34}O_5$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of Alprostadil RS taken

Internal standard solution—A solution of dimethyl phthalate in the mixture of acetonitrile for liquid chromatography and water (9:1) (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 196 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 9.07 g of potassium dihydrogen phosphate in water to make 1000 mL. Adjust the pH to 6.3 with a solution prepared by dissolving 9.46 g of disodium hydrogen phosphate in water to make 1000 mL, and dilute to 10 times its volume with water. To 360 mL of this solution add 110 mL of acetonitrile for liquid chromatography and 30 mL of methanol for liquid chromatography.

Flow rate: Adjust so that the retention time of alprostadil is about 10 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, alprostadil and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of alprostadil to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and at a temperature not exceeding 5°C.

Alprostadil Injection

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Alprostadil Injection is an emulsion-type injection.

It contains not less than 80.0% and not more than 125.0% of the labeled amount of alprostadil ($C_{20}H_{34}O_5$: 354.48).

Method of preparation Prepare as directed under Injections, with Alprostadil.

Description Alprostadil Injection occurs as a white emulsion and is slightly viscous. It has a distinctive odor.

Identification To a quantity of Alprostadil Injection, corresponding to 10 μg of Alprostadil, add 2 mL of acetonitrile, shake well, and centrifuge. To 3.5 mL of the supernatant liquid add 7 mL of diluted phosphoric acid (1 in 1000), and then run this solution on a column (prepared by filling a 10 mm inside diameter, 9 mm long chromatography tube with 0.4 g of 70 μm octadecylsilanized silica gel for pretreatment) prewashed with 10 mL of methanol and then 10 mL of water. Wash the column with 10 mL of water and then 20 mL of petroleum ether, followed by elution with 2.5 mL of a mixture of methanol and water (4:1). Remove the solvent from the effluent under reduced pressure, dissolve the residue in 100 μL of ethyl acetate, and use this solution as the sample solution. Separately, dissolve 1 mg of Alprostadil RS in 10 mL of ethyl acetate, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot the entire volume of the sample solution and 100 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Then, develop the plate with a mixture of ethyl acetate, ethanol (99.5) and acetic acid (100) (100:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of phosphomolybdic acid *n*-hydrate in ethanol (99.5) (1 in 10) on the plate, and heat at 100°C for 5 minutes: the color of the spot obtained from the standard solution and the spot corresponding to that location obtained from the sample solution is dark blue.

pH Being specified separately when the drug is granted approval based on the Law.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 4.0 mL of Alprostadil Injection according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 5 ppm).

(2) Prostaglandin A_1—Use the sample solution obtained in the Assay as the sample solution. Separately, weigh accurately about 10 mg of prostaglandin A_1, previously dried for 4 hours in a desiccator (in vacuum, phosphorus (V) oxide), and dissolve in ethanol (99.5) to make exactly 100 mL. Pipet 2.5 mL of this solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add exactly 1 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, calculate the ratios, Q_T and Q_S, of the peak area of prostaglandin A_1 to that of the internal standard, and calculate the amount of prostaglandin A_1 converted to alprostadil using the following equation: not more than 3.0 μg per a volume, equivalent to 5 μg of alprostadil ($C_{20}H_{34}O_5$).

Amount (μg) of prostaglandin A_1 ($C_{20}H_{32}O_4$), converted to alprostadil

$= M_S \times Q_T/Q_S \times 1/2 \times 1.054$

M_S: Amount (mg) of prostaglandin A_1 taken

Internal standard solution—Dissolve 50 mg of 1-naphthol in 20 mL of ethanol (99.5). To 3 mL of this solution add the mobile phase to make 100 mL.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 5 mL. Confirm that the peak area of prostaglandin A_1 obtained with 40 μL of this solution is equivalent to 14 to 26% of that obtained with 40 μL of the standard solution.

(3) Peroxide—Pipet 4 mL of Alprostadil Injection, place in a glass-stoppered flask, add 15 mL of a mixture of acetic acid (100) and isooctane (3:2), previously having undergone a 30 minute nitrogen substitution, and dissolve with gentle shaking. To this solution add 0.5 mL of saturated potassium iodide TS, replace the inside of the vessel with nitrogen, and shake for exactly 5 minutes. Then, add 0.5 mL of starch TS, shake vigorously, add 15 mL of water, and shake vigorously. Under a stream of nitrogen, titrate <*2.50*> with 0.01 mol/L sodium thiosulfate VS until the color of the solution disappears. Separately, perform a blank determination using 4 mL of water, and make any necessary correction. Calculate the amount of peroxides using the following equation: not more than 0.5 meq/L.

Amount (meq/L) of peroxides $= V \times 2.5$

V: Amount (mL) of 0.01 mol/L sodium thiosulfate VS consumed

(4) Free fatty acids—Pipet 3 mL of Alprostadil Injection, add exactly 15 mL of a mixture of 2-propanol, heptane and 0.5 mol/L sulfuric acid TS (40:10:1), and shake for 1 minute. After leaving for 10 minutes, add exactly 9 mL of heptane and exactly 9 mL of water, shake the test tube by inverting 10 times, leave for 15 minutes, and pipet 9 mL of the supernatant liquid. To this solution, add 3 mL of a solution prepared by combining 1 volume of Nile blue solution (1 in 5000) washed 5 times with heptane and 9 volumes of ethanol (99.5), and use this solution as the sample solution. Titrate <*2.50*> this solution with 0.02 mol/L sodium hydroxide VS under a stream of nitrogen. Separately, dissolve 5.65 g of oleic acid in heptane to make exactly 200 mL, and use this solution as the standard solution. Pipet 25 mL of the standard solution, add 2 drops of phenolphthalein TS, titrate <*2.50*> with 0.1 mol/L potassium hydroxide-ethanol VS until a light red color develops, and determine the correction factor f. Pipet 30 mL of the standard solution and add heptane to make exactly 200 mL. Pipet 3 mL of this solution, add exactly 15 mL of a mixture of 2-propanol, heptane and 0.5 mol/L sulfuric acid TS (40:10:1), and shake for 1 minute. After leaving for 10 minutes, add exactly 6 mL of heptane and exactly 12 mL of water, shake the test tube by inverting 10 times, and then titrate <*2.50*> in the same manner as for the sample solution. Determine the volume (mL), V_T and V_S, of 0.02 mol/L sodium hydroxide VS consumed by the sample and standard solutions: the amount of free fatty acid is not more than 12.0 meq/L.

Amount (meq/L) of free fatty acids $= V_T/V_S \times f \times 15$

Bacterial endotoxins ⟨*4.01*⟩ Less than 10 EU/mL.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filter method: it meets the requirement. However, use the sample solution consisting of equal volume of Alprostadil Injection and a solution prepared by adding water to 0.1 g of polysorbate 80 to make 100 mL.

Particle diameter Being specified separately when the drug is granted approval based on the Law.

Assay Measure exactly a volume of Alprostadil Injection corresponding to 5 μg of alprostadil ($C_{20}H_{34}O_5$), add exactly 1 mL of the internal standard solution, shake, and use this solution as the sample solution. Separately, weigh accurately about 5 mg of Alprostadil RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, dissolve in ethanol (99.5) to make exactly 50 mL, and use this solution as standard stock solution. Pipet 2.5 mL of the standard stock solution, add the mobile phase to make exactly 50 mL, pipet 1 mL, add exactly 1 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions using an apparatus equipped with an automatic pretreatment device (using a postcolumn reaction), and calculate the ratios, Q_T and Q_S, of the peak area of alprostadil to that of the internal standard.

Amount (μg) of alprostadil ($C_{20}H_{34}O_5$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of Alprostadil RS taken

Internal standard solution—Dissolve 50 mg of 1-naphthol in 20 mL of ethanol (99.5). To 3 mL of this solution add the mobile phase to make 100 mL.

Operating conditions—

Equipment: Liquid chromatograph consisting of 2 pumps for pumping the mobile phase and the reaction reagent, an automatic pretreatment device, column, reaction coil, detector, and recording apparatus. Use a reaction coil that is maintained at a constant temperature.

Detector: An ultraviolet absorption photometer (wavelength: 278 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 60°C.

Reaction coil: Polytetrafluoroethylene tube 0.5 mm in inside diameter and 10 m in length.

Mobile phase: Dissolve 9.07 g of potassium dihydrogen phosphate in water to make 1000 mL and adjust the pH to 6.3 by adding a solution prepared by dissolving 9.46 g of disodium hydrogen phosphate in water to make 1000 mL. To 1 volume of this solution add 9 volumes of water. To 3 volumes of this solution add 1 volume of acetonitrile for liquid chromatography.

Reaction reagent: Potassium hydroxide TS.

Reaction temperature: A constant temperature of about 60°C.

Mobile phase flow rate: Adjust so that the retention time of alprostadil is about 7 minutes.

Reaction reagent flow rate: 0.5 mL per minute.

Automatic pretreatment device: Composed of a pretreatment column, pump for pumping pretreatment column wash solution, and routing valve for 2 high pressure flow paths.

Pretreatment column: A stainless steel column 4 mm in inside diameter and 2.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Pretreatment column wash solution: Ethanol (99.5).

Flow rate of wash solution: A constant flow rate of about 2.0 mL per minute.

Flow path operating conditions: Change the flow path operating conditions at the times shown in the table below using the valves shown in the figure.

	Time of switchover (minutes)				
Valve	0	9.0	9.1	*1)	*2)
RVA	0	0	1	0	0
RVB	0	1	1	1	0

*1) After the internal standard has completely eluted
*2) 0.1 minutes after *1)

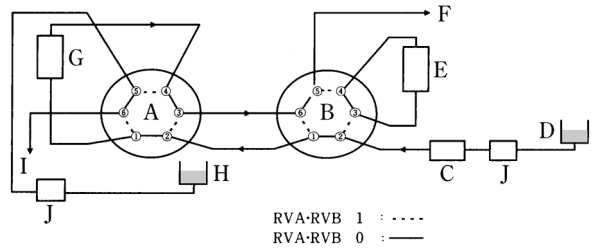
System suitability—

System performance: Dissolve 10 mg of prostaglandin A_1, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, in ethanol (99.5) to make 100 mL. To 2.5 mL of this solution add 2.5 mL of the standard stock solution, and add the mobile phase to make 50 mL. To 1 mL of this solution add 1 mL of the internal standard solution, shake, and perform the test under the above conditions with 40 μL of the solution. Alprostadil, prostaglandin A_1 and the internal standard are eluted in this order, and the resolution between the peaks of alprostadil and prostaglandin A_1 is not less than 10, and that between prostaglandin A_1 and the internal standard is not less than 2.0.

System repeatability: When the test is repeated 6 times with 40 μL of the standard solution under the above conditions, the relative standard deviation of the ratio of the peak area of alprostadil to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant, not exceeding 5°C, avoiding freezing.



A: RVA valve
B: RVB valve
C: Sample injector
D: Mobile phase
E: Column for pressure correction
F: Column
G: Pretreatment column
H: Wash solution
I: Drain
J: Pump

Figure Components of automatic pretreatment system

Alprostadil Alfadex

Prostaglandin E_1 α-Cyclodextrin Clathrate Compound

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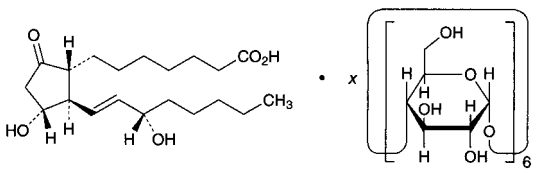

$C_{20}H_{34}O_5.\chi C_{36}H_{60}O_{30}$

7-{(1*R*,2*R*,3*R*)-3-Hydroxy-2-[(1*E*,3*S*)-3-hydroxyoct-1-en-1-yl]-5-oxocyclopentyl}heptanoic acid—α-cyclodextrin [*55648-20-9*]

Alprostadil Alfadex is a α-cyclodextrin clathrate compound of alprostadil.

It contains not less than 2.8% and not more than 3.2% of alprostadil ($C_{20}H_{34}O_5$: 354.48), calculated on the anhydrous basis.

Description Alprostadil Alfadex occurs as a white powder.

It is freely soluble in water, and practically insoluble in ethanol (95), in ethyl acetate and in diethyl ether.

It is hygroscopic.

Identification **(1)** Dissolve 0.02 g of Alprostadil Alfadex in 5 mL of water, add 5 mL of ethyl acetate, shake, and centrifuge. Use the supernatant liquid as the sample solution (1). Separately, to 0.02 g of Alprostadil Alfadex add 5 mL of ethyl acetate, shake, and centrifuge. Use the supernatant liquid as the sample solution (2). Evaporate the solvent from these solutions under reduced pressure, add 2 mL of sulfuric acid to the residue, and shake for 5 minutes: the liquid obtained from the sample solution (1) shows an orange-yellow color, while the liquid obtained from the sample solution (2) does not show that color.

(2) Dissolve 0.02 g of Alprostadil Alfadex in 5 mL of water, add 5 mL of ethyl acetate, shake, centrifuge, and evaporate the solvent from the supernatant liquid under reduced pressure. Dissolve the residue in 2 mL of ethanol (95), add 5 mL of 1,3-dinitrobenzene TS, then add 5 mL of a solution of potassium hydroxide in ethanol (95) (17 in 100) under ice-cooling, and allow to stand for 20 minutes in a dark place under ice-cooling: a purple color develops.

(3) Dissolve 0.05 g of Alprostadil Alfadex in 1 mL of iodine TS, by heating on a water bath, and allow to stand: a dark blue precipitate is formed.

(4) Determine the absorption spectrum of a solution of Alprostadil Alfadex in dilute ethanol (3 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits no absorption between 220 nm and 400 nm. Separately, to 10 mL of the solution add 1 mL of potassium hydroxide-ethanol TS, allow to stand for 15 minutes, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +126 – +138° (0.1 g calculated on the anhydrous basis, dilute ethanol, 20 mL, 100 mm).

pH <*2.54*> Dissolve 0.10 g of Alprostadil Alfadex in 20 mL of water: the pH of this solution is between 4.0 and 5.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Alprostadil Alfadex in 10 mL of water: the solution is colorless. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry within 30 minutes after preparation of the solution: the absorbance at 450 nm is not larger than 0.10.

(2) Prostaglandin A_1—Dissolve 0.10 g of Alprostadil Alfadex in 5 mL of water, add exactly 5 mL of the internal standard solution and ethanol (95) to make 15 mL, and use this solution as the sample solution. Separately, dissolve 1.5 mg of prostaglandin A_1 in ethanol (95) to make exactly 100 mL. Pipet 3 mL of this solution, add exactly 5 mL of the internal standard solution, 2 mL of ethanol (95) and water to make 15 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the operating conditions described in the Assay, and calculate the ratios, Q_T and Q_S, of the peak area of prostaglandin A_1 to that of the internal standard: Q_T is not larger than Q_S.

Internal standard solution—A solution of propyl parahydroxybenzoate in dilute ethanol (1 in 15,000).

(3) Related substances—Dissolve 0.10 g of Alprostadil Alfadex in 3 mL of water, add exactly 3 mL of ethyl acetate, shake, centrifuge, and use the supernatant liquid obtained as the sample solution. Separately, dissolve 1.0 mg of prostaglandin A_1 in ethyl acetate to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of phosphomolybdic acid *n*-hydrate in ethanol (95) (1 in 4) on the plate, and heat at 100°C for 5 minutes: the spots other than the principal spot from the sample solution, and the spots other than the spot corresponding to the spot from the standard solution are all not more intense than the spot from the standard solution.

Water <*2.48*> Not more than 6.0% (0.2 g, direct titration).

Assay Weigh accurately about 0.1 g of Alprostadil Alfadex, dissolve in 5 mL of water, add exactly 5 mL of the internal standard solution and water to make 15 mL, and use this solution as the sample solution. Separately, weigh accurately about 3 mg of Alprostadil RS, dissolve in 5 mL of ethanol (95), add exactly 5 mL of the internal standard solution and water to make 15 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of alprostadil to that of the internal standard.

$$\text{Amount (mg) of alprostadil } (C_{20}H_{34}O_5) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Alprostadil RS taken

Internal standard solution—A solution of propyl parahydroxybenzoate in dilute ethanol (1 in 15,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: A stainless steel column about 5 mm in inside diameter and about 15 cm in length, packed with octadecyl-

silanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.02 mol/L potassium dihydrogenphosphate and acetonitrile (3:2).

Flow rate: Adjust so that the retention time of alprostadil is about 6 minutes.

Selection of column: Dissolve about 0.1 g of Alprostadil Alfadex in 5 mL of water, add 5 mL of a solution of prostaglandin A_1 in ethanol (95) (3 in 200,000) and 5 mL of the internal standard solution. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of alprostadil, the internal standard and prostaglandin A_1 in this order and complete separation of these peaks.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, at a temperature not exceeding 5°C.

Alum Solution

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Alum Solution contains not less than 0.27 w/v% and not more than 0.33 w/v% of aluminum potassium sulfate Hydrate [$AlK(SO_4)_2.12H_2O$: 474.39].

Method of preparation

Aluminum Potassium Sulfate Hydrate	3 g
Mentha Water	50 mL
Water, Purified Water or Purified Water in Containers	a sufficient quantity
To make	1000 mL

Dissolve and mix the above ingredients.

Description Alum Solution is a clear, colorless liquid. It has the odor of the mentha oil and an astringent taste.

Identification **(1)** To 5 mL of Alum Solution add 3 mL of ammonium chloride TS and 1 mL of ammonia TS: a white, gelatinous precipitate is produced, which changes to red upon the addition of 5 drops of alizarin red S TS (aluminum sulfate).

(2) Place 100 mL of Alum Solution in an evaporating dish, evaporate on a water bath to dryness, and dissolve the residue in 5 mL of water: the solution responds to the Qualitative Tests <*1.09*> for potassium salt.

(3) Alum Solution responds to the Qualitative Tests <*1.09*> (1) and (2) for sulfate.

Assay Pipet 50 mL of Alum Solution, add exactly 30 mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, and further add 20 mL of acetic acid-ammonium acetate buffer solution (pH 4.8). Boil for 5 minutes, cool, add 55 mL of ethanol (95), and titrate <*2.50*> with 0.02 mol/L zinc acetate VS (indicator: 2 mL of dithizone TS), until the color of the solution changes from light dark green to light red. Perform a blank determination.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 9.488 mg of $AlK(SO_4)_2.12H_2O$

Containers and storage Containers—Tight containers.

Dried Aluminum Hydroxide Gel

乾燥水酸化アルミニウムゲル

Dried Aluminum Hydroxide Gel contains not less than 50.0% of aluminum oxide (Al_2O_3: 101.96).

Description Dried Aluminum Hydroxide Gel occurs as a white, amorphous powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

Most of it dissolves in dilute hydrochloric acid and in sodium hydroxide TS.

Identification To 0.2 g of Dried Aluminum Hydroxide Gel add 20 mL of dilute hydrochloric acid, warm, and centrifuge: the supernatant liquid responds to the Qualitative Tests <*1.09*> for aluminum salt.

Purity **(1)** Acidity or alkalinity—To 1.0 g of Dried Aluminum Hydroxide Gel add 25 mL of water, shake well, and centrifuge: the supernatant liquid is neutral.

(2) Chloride <*1.03*>—To 1.0 g of Dried Aluminum Hydroxide Gel add 30 mL of dilute nitric acid, heat gently to boil while shaking, cool, add water to make 100 mL, and centrifuge. To 5 mL of the supernatant liquid add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.284%).

(3) Sulfate <*1.14*>—To 1.0 g of Dried Aluminum Hydroxide Gel add 15 mL of dilute hydrochloric acid, heat gently to boil while shaking, cool, add water to make 250 mL, and centrifuge. To 25 mL of the supernatant liquid add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.480%).

(4) Nitrate—To 0.10 g of Dried Aluminum Hydroxide Gel add 5 mL of water, then carefully add 5 mL of sulfuric acid, shake well to dissolve, and cool. Superimpose the solution on 2 mL of iron (II) sulfate TS: no brown-colored ring is produced at the zone of contact.

(5) Heavy metals <*1.07*>—Dissolve 2.0 g of Dried Aluminum Hydroxide Gel in 10 mL of dilute hydrochloric acid by heating, filter if necessary, and add water to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution as follows: evaporate 10 mL of dilute hydrochloric acid to dryness, and add 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(6) Arsenic <*1.11*>—To 0.8 g of Dried Aluminum Hydroxide Gel add 10 mL of dilute sulfuric acid, heat gently to boil while shaking, cool, and filter. Take 5 mL of the filtrate, use this solution as the test solution, and perform the test (not more than 5 ppm).

Acid-consuming capacity Weigh accurately about 0.2 g of Dried Aluminum Hydroxide Gel, and transfer to a glass-stoppered flask. Add exactly 100 mL of 0.1 mol/L hydrochloric acid VS, stopper the flask, shake at 37 ± 2°C for 1 hour, and filter. Measure exactly 50 mL of the filtrate, and titrate <*2.50*> while thoroughly stirring, the excess hydrochloric acid with 0.1 mol/L sodium hydroxide VS until the pH of the solution becomes to 3.5. The volume of 0.1 mol/L hydrochloric acid VS consumed is not less than 250 mL per g of Dried Aluminum Hydroxide Gel.

Assay Weigh accurately about 2 g of Dried Aluminum Hydroxide Gel, add 15 mL of hydrochloric acid, heat on a water bath with shaking for 30 minutes, cool, and add water to make exactly 500 mL. Pipet 20 mL of this solution, add exactly 30 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS and 20 mL of acetic acid (31)-ammonium acetate buffer solution (pH 4.8), boil for 5 minutes, and cool. Add 55 mL of ethanol (95), and titrate <*2.50*> with 0.05 mol/L zinc acetate VS until the color of the solution changes from light dark green to light red. (indicator: 2 mL of dithizone TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.549 mg of Al_2O_3

Containers and storage Containers—Tight containers.

Dried Aluminum Hydroxide Gel Fine Granules

乾燥水酸化アルミニウムゲル細粒

Dried Aluminum Hydroxide Gel Fine Granules contain not less than 47.0% of aluminum oxide (Al_2O_3: 101.96).

Method of preparation Prepare as directed under Granules, with Dried Aluminum Hydroxide Gel.

Identification To 0.2 g of Dried Aluminum Hydroxide Gel Fine Granules add 20 mL of dilute hydrochloric acid, warm and centrifuge: the supernatant liquid responds to the Qualitative Tests <*1.09*> for aluminum salt.

Acid-consuming capacity Proceed as directed for Acid-consuming capacity under Dried Aluminum Hydroxide Gel: the volume of 0.1 mol/L hydrochloric acid VS consumed is not less than 235 mL per g of Dried Aluminum Hydroxide Gel Fine Granules.

Assay Proceed as directed in the Assay under Dried Aluminum Hydroxide Gel.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.549 mg of Al_2O_3

Containers and storage Containers—Tight containers.

Aluminum Monostearate

モノステアリン酸アルミニウム

Aluminum Monostearate is mainly aluminum compounds of stearic acid ($C_{18}H_{36}O_2$: 284.48) and palmitic acid ($C_{16}H_{32}O_2$: 256.42).

Aluminum Monostearate, when dried, contains not less than 7.2% and not more than 8.9% of aluminum (Al: 26.98).

Description Aluminum Monostearate occurs as a white to yellowish white powder. It is odorless or has a faint, characteristic odor.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

Identification **(1)** Heat 3 g of Aluminum Monostearate with 30 mL of hydrochloric acid in a water bath with occasional shaking for 10 minutes. After cooling, shake the mixture vigorously with 50 mL of water and 30 mL of diethyl ether for 3 minutes, and allow to stand. To the separated aqueous layer add sodium hydroxide TS until the solution becomes slightly turbid, and filter: the filtrate responds to the Qualitative Tests <*1.09*> for aluminum salt.

(2) Wash the diethyl ether layer separated in (1) with two 20-mL portions of water, and evaporate the diethyl ether layer on a water bath: the residue melts <*1.13*> at above 54°C.

Acid value for fatty acid <*1.13*> 193 – 210. Weigh accurately about 1 g of fatty acid obtained in the Identification (2), transfer a 250-mL glass-stoppered flask, add 100 mL of a mixture of diethyl ether and ethanol (95) (2:1), warm to dissolve, add several drops of phenolphthalein TS, and proceed as directed under Acid Value.

Purity **(1)** Free fatty acid—Mix 1.0 g of Aluminum Monostearate with about 50 mL of a mixture of neutralized ethanol and diethyl ether (1:1), filter through dry filter paper, wash the vessel and the filter paper with a small amount of a mixture of neutralized ethanol and diethyl ether (1:1), combine the filtrate and the washings, and add 2.1 mL of 0.1 mol/L potassium hydroxide VS: a red color develops.

(2) Water-soluble salts—Heat 2.0 g of Aluminum Monostearate with 80 mL of water in a loosely stoppered conical flask on a water bath for 30 minutes with occasional shaking. After cooling, filter through dry filter paper, wash the residue with a small amount of water, combine the washings with the filtrate, add water to make 100 mL, evaporate 50 mL of this solution on a water bath, and heat strongly at 600°C: the mass of the residue is not more than 10.0 mg.

(3) Heavy metals <*1.07*>—Heat 1.0 g of Aluminum Monostearate over a small flame with caution at the beginning, and continue the heating, gradually raising the temperature, to ash. After cooling, add 10 mL of diluted hydrochloric acid (1 in 2), evaporate on a water bath, and boil the residue with 20 mL of water for 1 minute. Cool, filter, wash the residue with water, combine the filtrate and the washings, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Evaporate 10 mL of diluted hydrochloric acid (1 in 2) on a water bath to dryness, add 2 mL of dilute acetic acid and 5.0 mL of Standard Lead Solution, dilute with water to make 50 mL, and use this solution as the control solution (not more than 50 ppm).

(4) Arsenic <*1.11*>—Mix 1.0 g of Aluminum Monostearate with 2 g of magnesium nitrate hexahydrate, ignite over a small flame, moisten the residue after cooling with 0.5 mL of nitric acid, and heat. Heat again the residue with 10 mL of dilute sulfuric acid until white fumes evolve, add water to make 5 mL, and perform the test with this solution as the test solution (not more than 2 ppm).

Loss on drying <*2.41*> Not more than 3.0% (1 g, 105°C, 3 hours).

Assay Weigh accurately about 1 g of Aluminum Monostearate, previously dried, ignite gently to ash, and cool. Add dropwise 0.5 mL of nitric acid, evaporate on a water bath by heating, and then heat strongly between 900°C and 1100°C to a constant mass. After cooling, weigh rapidly the ignited residue, and designate the mass as aluminum oxide (Al_2O_3: 101.96).

Amount (mg) of aluminum (Al)
= amount (mg) of aluminum oxide (Al_2O_3) × 0.529

Containers and storage Containers—Well-closed containers.

Dried Aluminum Potassium Sulfate

Burnt Alum

乾燥硫酸アルミニウムカリウム

$AlK(SO_4)_2$: 258.21

Dried Aluminum Potassium Sulfate, when dried, contains not less than 98.0% of aluminum potassium sulfate [$AlK(SO_4)_2$].

Description Dried Aluminum Potassium Sulfate occurs as white masses or white powder. It is odorless. It has a slightly sweet, astringent taste.

It is freely soluble in hot water and practically insoluble in ethanol (95).

It dissolves slowly in water.

Identification A solution of Dried Aluminum Potassium Sulfate (1 in 20) responds to the Qualitative Tests <*1.09*> for aluminum salt, to the Qualitative Tests <*1.09*> (1), (3) and (4) for potassium salt, and to the Qualitative Tests <*1.09*> (1) and (3) for sulfate.

Purity **(1)** Water-insoluble substances—To 2.0 g of Dried Aluminum Potassium Sulfate add 40 mL of water, shake frequently, and allow to stand for 48 hours. Collect the insoluble residue on a glass filter (G4), wash with 50 mL of water, and dry at 105°C for 2 hours: the mass of the residue is not more than 50 mg.

(2) Heavy metals <*1.07*>—Dissolve 0.5 g of Dried Aluminum Potassium Sulfate in 45 mL of water, and filter, if necessary. Add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 40 ppm).

(3) Iron <*1.10*>—Prepare the test solution with 0.54 g of Dried Aluminum Potassium Sulfate according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 37 ppm).

(4) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Dried Aluminum Potassium Sulfate, according to Method 1, and perform the test (not more than 5 ppm).

Loss on drying <*2.41*> Not more than 15.0% (2 g, 200°C, 4 hours).

Assay Weigh accurately about 1.2 g of Dried Aluminum Potassium Sulfate, previously dried, add 80 mL of water, and heat on a water bath with occasional shaking for 20 minutes. Cool, add water to make exactly 100 mL, and filter, if necessary. Discard the first 30 mL of the filtrate, take exactly the subsequent 20 mL of the filtrate, and add exactly 30 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS and 20 mL of acetic acid-ammonium acetate buffer solution (pH 4.8), boil for 5 minutes, and cool. Add 55 mL of ethanol (95), and titrate <*2.50*> with 0.05 mol/L zinc acetate VS (indicator: 2 mL of dithizone TS), until the color of the solution changes from light dark green to light red. Perform a blank determination.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 12.91 mg of $AlK(SO_4)_2$

Containers and storage Containers—Tight containers.

Aluminum Potassium Sulfate Hydrate

Alum

硫酸アルミニウムカリウム水和物

$AlK(SO_4)_2.12H_2O$: 474.39

Aluminum Potassium Sulfate Hydrate contains not less than 99.5% of aluminum potassium sulfate hydrate [$AlK(SO_4)_2.12H_2O$].

Description Aluminum Potassium Sulfate Hydrate occurs as colorless or white, crystals or powder. It is odorless. It has a slightly sweet, strongly astringent taste.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

A solution of Aluminum Potassium Sulfate Hydrate (1 in 20) is acid.

Identification A solution of Aluminum Potassium Sulfate Hydrate (1 in 10) responds to the Qualitative Tests <*1.09*> for aluminum salt, to the Qualitative Tests <*1.09*> (1), (3) and (4) for potassium salt, and to the Qualitative Tests <*1.09*> (1) and (3) for sulfate.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Aluminum Potassium Sulfate Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Iron <*1.10*>—Prepare the test solution with 1.0 g of Aluminum Potassium Sulfate Hydrate according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 20 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 0.6 g of Aluminum Potassium Sulfate Hydrate, according to Method 1, and perform the test (not more than 3.3 ppm).

Assay Weigh accurately about 4.5 g of Aluminum Potassium Sulfate Hydrate, and dissolve in water to make exactly 200 mL. Take exactly 20 mL of this solution, and add exactly 30 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS and 20 mL of acetic acid-ammonium acetate buffer solution (pH 4.8), boil for 5 minutes, and cool. Add 55 mL of ethanol (95), and titrate <*2.50*> with 0.05 mol/L zinc acetate VS (indicator: 2 mL of dithizone TS), until the color of the solution changes from light dark green to light red. Perform a blank determination.

Each mL of 0.05 mol/L disodium dihydorgen ethylenediamine tetetraacetate VS
= 23.72 mg of $AlK(SO_4)_2.12H_2O$

Containers and storage Containers—Tight containers.

Natural Aluminum Silicate

天然ケイ酸アルミニウム

Description Natural Aluminum Silicate occurs as a white or slightly colored powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

Natural Aluminum Silicate (1 g) dissolves when heated in 20 mL of a solution of sodium hydroxide (1 in 5), with some decomposition, leaving a large amount of insoluble substance.

Identification **(1)** To 0.5 g of Natural Aluminum Silicate add 3 mL of diluted sulfuric acid (1 in 3), heat until white fumes evolve, cool, add 20 mL of water, and filter. Render the filtrate slightly acid with ammonia TS: the solution responds to the Qualitative Tests <*1.09*> for aluminum salt.

(2) Prepare a bead by fusing ammonium sodium hydrogenphosphate tetrahydrate on a platinum loop. Place the bead in contact with Natural Aluminum Silicate, and fuse again: an infusible material appears in the bead, producing, upon cooling, an opaque bead with a web-like structure.

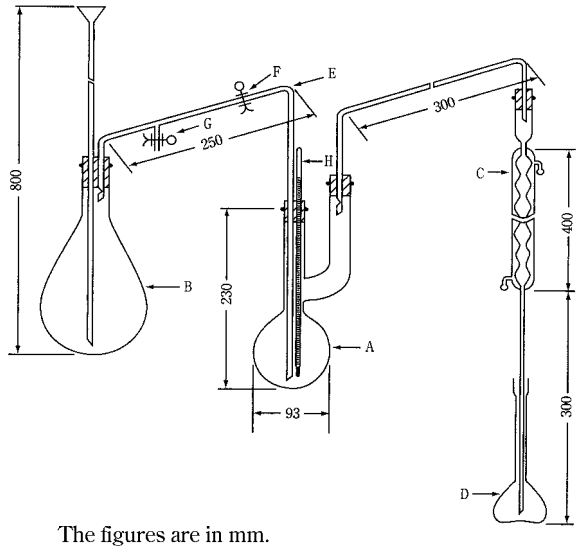
Purity **(1)** Acidity or alkalinity—Shake 5.0 g of Natural Aluminum Silicate with 100 mL of water, and centrifuge: the supernatant liquid so obtained is neutral.

(2) Chloride <*1.03*>—To 5.0 g of Natural Aluminum Silicate add 100 mL of water, boil gently for 15 minutes while shaking, then cool, add water to restore the original volume, and centrifuge. To 10 mL of the supernatant liquid add 6 mL of dilute nitric acid, dilute to 50 mL with water, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <*1.14*>—To the residue obtained in (6) add 3 mL of dilute hydrochloric acid, heat on a water bath for 10 minutes, dilute to 50 mL with water, and filter. To 2.0 mL of the filtrate add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.480%).

(4) Heavy metals <*1.07*>—To 1.5 g of Natural Aluminum Silicate add 50 mL of water and 5 mL of hydrochloric acid, boil gently for 20 minutes while shaking, then cool, centrifuge, remove the supernatant liquid, wash the residue with two 10-mL portions of water, centrifuging each time, combine these washings with the filtrate, and add ammonia solution (28) dropwise, until a precipitate just appears. Add dropwise dilute hydrochloric acid with vigorous shaking and redissolve the precipitate. Heat the mixture with 0.45 g of hydroxylammonium chloride, cool, and add 0.45 g of sodium acetate trihydrate, 6 mL of dilute acetic acid and water to make 150 mL. Perform the test, using 50 mL of this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution, 0.15 g of hydroxylammonium chloride, 0.15 g of sodium acetate trihydrate, 2 mL of dilute acetic acid and water to make 50 mL (not more than 40 ppm).

(5) Arsenic <*1.11*>—To 1.0 g of Natural Aluminum Silicate, add 5 mL of dilute hydrochloric acid, heat gently to boil while shaking well, cool rapidly, and centrifuge. Mix the residue with 5 mL of dilute hydrochloric acid with shaking, centrifuge, then add 10 mL of water to the residue, and repeat the extraction in the same manner. Concentrate the combined extracts on a water bath to 5 mL. Use this solution as the test solution, and perform the test (not more than 2 ppm).



The figures are in mm.

A: Distilling flask of about 300-mL capacity.
B: Steam generator of about 1000-mL capacity, containing a few boiling tips to prevent bumping
C: Condenser
D: Receiver: 200-mL volumetric flask
E: Steam-introducing tube having an internal diameter of about 8 mm
F, G: Rubber tube with a clamp
H: Thermometer

(6) Soluble salts—Evaporate 50 mL of the supernatant liquid obtained in (1) on a water bath to dryness, and ignite the residue at 700°C for 2 hours: the mass of the ignited residue is not more than 40 mg.

(7) Fluoride—(i) Apparatus: Use a hard glass apparatus as illustrated in the figure. Ground-glass joints may be used.

(ii) Procedure: Transfer 5.0 g of Natural Aluminum Silicate to the distilling flask A with the aid of 20 mL of water, add about 1 g of glass wool and 50 mL of diluted purified sulfuric acid (1 in 2), and connect A to the distillation apparatus, previously washed with steam streamed through the steam introducing tube E. Connect the condenser C with the receiver D containing 10 mL of 0.01 mol/L sodium hydroxide VS and 10 mL of water so that the lower end of C is immersed in the solution. Heat A gradually until the temperature of the solution in A reaches 130°C, then open the rubber tube F, close the rubber tube G, boil water in the steam generator B vigorously, and introduce the generated steam into F. Simultaneously, heat A, and maintain the temperature of the solution in A between 135°C and 145°C. Adjust the distilling rate to about 10 mL per minute. Collect about 170 mL of the distillate, then stop the distillation, wash C with a small quantity of water, combine the washings with the distillate, add water to make exactly 200 mL, and use this solution as the test solution. Perform the test with the test solution as directed in the procedure of determination for fluoride under Oxygen Flask Combustion Method <*1.06*>. No corrective solution is used in this procedure. The content of fluoride (F) is not more than 0.01%.

Amount (mg) of fluoride (F: 19.00) in the test solution
= amount (mg) of fluoride in 5 mL of the standard solution
$\times A_T/A_S \times 200/V$

Loss on drying ⟨*2.41*⟩ Not more than 20.0% (1 g, 105°C, 3 hours).

Adsorptive power To 0.10 g of Natural Aluminum Silicate add 20 mL of a solution of methylene blue trihydrate (3 in 2000), shake for 15 minutes, allow to stand for 5 hours at 37 ± 2°C, and centrifuge. Dilute 1.0 mL of the supernatant liquid with water to 200 mL. Place 50 mL of the solution in a Nessler tube and observe horizontally or vertically against a white background: the color of the solution is not deeper than that of the following control solution.

Control solution: Dilute 1.0 mL of a solution of methylene blue trihydrate (3 in 2000) with water to 400 mL, and use 50 mL of this solution.

Containers and storage Containers—Well-closed containers.

Synthetic Aluminum Silicate

合成ケイ酸アルミニウム

Description Synthetic Aluminum Silicate occurs as a white powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

Synthetic Aluminum Silicate (1 g) dissolves when heated in 20 mL of a solution of sodium hydroxide (1 in 5), leaving a small amount of insoluble substance .

Identification **(1)** To 0.5 g of Synthetic Aluminum Silicate add 3 mL of diluted sulfuric acid (1 in 3), heat until white fumes evolve, cool, add 20 mL of water, and filter. Render the filtrate slightly acid with ammonia TS: the solution responds to the Qualitative Tests ⟨*1.09*⟩ for aluminum salt.

(2) Prepare a bead by fusing ammonium sodium hydrogenphosphate tetrahydrate on a platinum loop. Place the bead in contact with Synthetic Aluminum Silicate, and fuse again: an infusible material appears in the bead, producing, upon cooling, an opaque bead with a web-like structure.

Purity **(1)** Acidity or alkalinity—Shake 1.0 g of Synthetic Aluminum Silicate with 20 mL of water, and centrifuge: the supernatant liquid so obtained is neutral.

(2) Chloride ⟨*1.03*⟩—To 5.0 g of Synthetic Aluminum Silicate add 100 mL of water, boil gently for 15 minutes while shaking, then cool, add water to restore the original volume, and centrifuge. To 10 mL of the supernatant liquid add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate ⟨*1.14*⟩—To 2.0 mL of the supernatant liquid obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.480%).

(4) Heavy metals ⟨*1.07*⟩—To 3.0 g of Synthetic Aluminum Silicate add 50 mL of water and 5 mL of hydrochloric acid, boil gently for 20 minutes while shaking, then after cooling, centrifuge, remove the supernatant liquid, wash the precipitate with two 10-mL portions of water, centrifuging each time, combine these washings with the filtrate, and add ammonia solution (28) dropwise until a precipitate just appears. Add dropwise dilute hydrochloric acid with vigorous shaking to redissolve the precipitate. Heat the solution with 0.45 g of hydroxylammonium chloride, and after cooling, add 0.45 g of sodium acetate trihydrate, 6 mL of dilute acetic acid and water to make 150 mL. Perform the test with 50 mL of this solution as the test solution. Prepare the control solution with 3.0 mL of Standard Lead Solution, 0.15 g of hydroxylammonium chloride, 0.15 g of sodium acetate trihydrate, 2 mL of dilute acetic acid and water to make 50 mL (not more than 30 ppm).

(5) Arsenic ⟨*1.11*⟩—To 1.0 g of Synthetic Aluminum Silicate add 10 mL of dilute hydrochloric acid, heat gently to boiling while shaking well, cool rapidly, and centrifuge. Mix the residue with 5 mL of dilute hydrochloric acid with shaking, centrifuge, then add 10 mL of water to the residue, and repeat the extraction in the same manner. Concentrate the combined extracts on a water bath to 5 mL. Use this solution as the test solution, and perform the test (not more than 2 ppm).

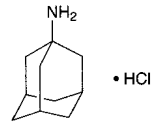
Loss on drying ⟨*2.41*⟩ Not more than 20.0% (1 g, 105°C, 3 hours).

Acid-consuming capacity ⟨*6.04*⟩ Weigh accurately about 1 g of Synthetic Aluminum Silicate, transfer to a glass-stoppered flask, add 200 mL of 0.1 mol/L hydrochloric acid VS, exactly measured, stopper the flask, and shake at 37 ± 2°C for 1 hour. Filter, pipet 50 mL of the filtrate, and titrate ⟨*2.50*⟩ by stirring well the excess hydrochloric acid with 0.1 mol/L sodium hydroxide VS until the pH of the solution changes to 3.5. The volume of 0.1 mol/L hydrochloric acid VS consumed is not less than 50.0 mL per g of Synthetic Aluminum Silicate.

Containers and storage Containers—Well-closed containers.

Amantadine Hydrochloride

アマンタジン塩酸塩



$C_{10}H_{17}N.HCl$: 187.71
Tricyclo[3.3.1.1^{3,7}]dec-1-ylamine monohydrochloride
[*665-66-7*]

Amantadine Hydrochloride, when dried, contains not less than 99.0% of amantadine hydrochloride ($C_{10}H_{17}N.HCl$).

Description Amantadine Hydrochloride occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in formic acid, freely soluble in water, in methanol and in ethanol (95), and practically insoluble in diethyl ether.

Identification **(1)** To 0.1 g of Amantadine Hydrochloride add 1 mL of pyridine and 0.1 mL of acetic anhydride, dissolve by boiling for 1 minute, add 10 mL of dilute hydrochloric acid, and cool in ice water. Filter the crystals separated, wash with water, and dry at 105°C for 1 hour: the

residue melts ⟨*2.60*⟩ between 147°C and 151°C.

(2) Determine the infrared absorption spectrum of Amantadine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Amantadine Hydrochloride (1 in 50) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

pH ⟨*2.54*⟩ Dissolve 1.0 g of Amantadine Hydrochloride in 5 mL of water: the pH of this solution is between 4.0 and 6.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Amantadine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Amantadine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Amantadine Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.50 g of Amantadine Hydrochloride in 10 mL of water, add 10 mL of sodium hydroxide TS and 10 mL of chloroform, and shake. Filter the chloroform layer through absorbent cotton with 3 g of anhydrous sodium sulfate on a funnel, and use the filtrate as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions. Determine each peak area of these solutions by the automatic integration method: each peak area other than amantadine from the sample solution is not larger than 1/3 times the peak area of amantadine from the standard solution, and the total area of each peak is not larger than the peak area of amantadine from the standard solution.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with siliceous earth for gas chromatography (150 to 180 μm in particle diameter) coated with a mixture (L) of branched hydrocarbon of petroleum hexamethyltetracosane group for gas chromatography and potassium hydroxide at the ratios of 2% and 1%, respectively.

Column temperature: Inject at a constant temperature of about 125°C, maintain the temperature for 5 minutes, raise at the rate of 5°C per minute to 150°C, and maintain at a constant temperature of about 150°C for 15 minutes.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the retention time of amantadine is about 11 minutes.

Selection of column: Dissolve 0.15 g of naphthalene in 5 mL of the sample solution, and add chloroform to make 100 mL. Proceed with 2 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of naphthalene and amantadine in this order with the resolution between these peaks being not less than 2.5.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of amantadine obtained from 2 μL of the standard solution composes about 10% of the full scale.

Time span of measurement: About twice as long as the retention time of amantadine, beginning after the solvent peak.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

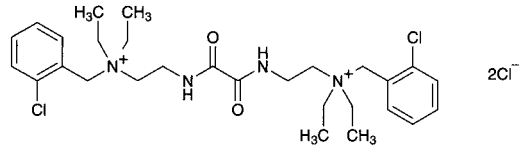
Assay Weigh accurately about 0.2 g of Amantadine Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add exactly 15 mL of 0.1 mol/L perchloric acid VS, and heat on a water bath for 30 minutes. After cooling, add acetic acid (100) to make 70 mL, and titrate ⟨*2.50*⟩ the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 18.77 mg of $C_{10}H_{17}N.HCl$

Containers and storage Containers—Well-closed containers.

Ambenonium Chloride

アンベノニウム塩化物



$C_{28}H_{42}Cl_4N_4O_2$: 608.47

2,2′-[(1,2-Dioxoethane-1,2-diyl)diimino]bis[*N*-(2-chlorobenzyl)-*N*,*N*-diethylethylaminium] dichloride [*115-79-7*]

Ambenonium Chloride contains not less than 98.5% of ambenonium chloride ($C_{28}H_{42}Cl_4N_4O_2$), calculated on the dried basis.

Description Ambenonium Chloride occurs as a white powder.

It is freely soluble in water, in methanol and in acetic acid (100), soluble in ethanol (95), and slightly soluble in acetic anhydride.

It is hygroscopic.

Melting point: about 205°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Ambenonium Chloride in methanol (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ambenonium Chloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ambenonium Chloride (1 in 100) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Ambenonium Chloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Ambenonium Chloride according to Method 4, and perform the test. Use a solution of magnesium nitrate in ethanol (95)

(1 in 5). Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of Ambenonium Chloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 20 mL. Pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, formic acid and water (12:6:5) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 11.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

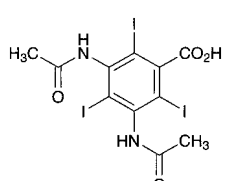
Assay Weigh accurately about 0.3 g of Ambenonium Chloride, and dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3). Titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 30.42 mg of $C_{28}H_{42}Cl_4N_4O_2$

Containers and storage Containers—Tight containers.

Amidotrizoic Acid

アミドトリゾ酸



$C_{11}H_9I_3N_2O_4$: 613.91
3,5-Bis(acetylamino)-2,4,6-triiodobenzoic acid
[*117-96-4*]

Amidotrizoic Acid, calculated on the dried basis, contains not less than 98.0% of amidotrizoic acid ($C_{11}H_9I_3N_2O_4$).

Description Amidotrizoic Acid occurs as a white crystalline powder. It is odorless.

It is slightly soluble in ethanol (95), very slightly soluble in water, and practically insoluble in diethyl ether.

It dissolves in sodium hydroxide TS.

Identification **(1)** Heat 0.1 g of Amidotrizoic Acid over a flame: a purple gas is evolved.

(2) Determine the infrared absorption spectrum of Amidotrizoic Acid as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Amidotrizoic Acid in 10 mL of 0.2 mol/L sodium hydroxide TS: the solution is clear and colorless.

(2) Primary aromatic amines—Dissolve 0.20 g of Amidotrizoic Acid in 5 mL of water and 1 mL of sodium hydroxide TS, add 4 mL of a solution of sodium nitrite (1 in 100) and 10 mL of 1 mol/L hydrochloric acid TS, shake, and allow to stand for 2 minutes. Add 5 mL of ammonium amidosulfate TS, shake well, allow to stand for 1 minute, and add 0.4 mL of a solution of 1-naphthol in ethanol (95) (1 in 10), 15 mL of sodium hydroxide TS and water to make exactly 50 mL. Determine the absorbance of this solution at 485 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*> using a solution, prepared in the same manner, as the blank: the absorbance is not more than 0.15.

(3) Soluble halides—Dissolve 2.5 g of Amidotrizoic Acid in 20 mL of water and 2.5 mL of ammonia TS, add 20 mL of dilute nitric acid and water to make 100 mL, allow to stand for 15 minutes with occasional shaking, and filter. Discard the first 10 mL of the filtrate, transfer the subsequent 25 mL of the filtrate to a Nessler tube, and add ethanol (95) to make 50 mL. Proceed as directed under Chloride Limit Test <*1.03*> using this solution as the test solution. Prepare the control solution as follows: to 0.10 mL of 0.01 mol/L hydrochloric acid VS, add 6 mL of dilute nitric acid and water to make 25 mL, then ethanol (95) to make 50 mL.

(4) Iodine—Dissolve 0.20 g of Amidotrizoic Acid in 2.0 mL of sodium hydroxide TS, add 2.5 mL of 0.5 mol/L sulfuric acid TS, allow to stand for 10 minutes with occasional shaking, add 5 mL of chloroform, shake well, and allow to stand: the solution is colorless in the chloroform layer.

(5) Heavy metals <*1.07*>—Proceed with 2.0 g of Amidotrizoic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Arsenic <*1.11*>—Prepare the test solution with 0.6 g of Amidotrizoic Acid according to Method 3, and perform the test (not more than 3.3 ppm).

Loss on drying <*2.41*> Not more than 7.0% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

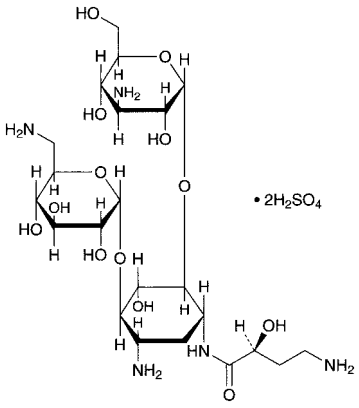
Assay Transfer about 0.5 g of Amidotrizoic Acid, accurately weighed, to a saponification flask, dissolve in 40 mL of sodium hydroxide TS, add 1 g of zinc powder, connect to a reflux condenser, boil for 30 minutes, cool, and filter. Wash the flask and the filter paper with 50 mL of water, and combine the washings and the filtrate. Add 5 mL of acetic acid (100) to this solution, and titrate <*2.50*> with 0.1 mol/L silver nitrate VS until the color of the precipitate changes from yellow to green (indicator: 1 mL of tetrabromophenolphthalein ethyl ester TS).

Each mL of 0.1 mol/L silver nitrate VS
= 20.46 mg of $C_{11}H_9I_3N_2O_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Amikacin Sulfate

アミカシン硫酸塩



$C_{22}H_{43}N_5O_{13}.2H_2SO_4$: 781.76

3-Amino-3-deoxy-α-D-glucopyranosyl-(1→6)-[6-amino-6-deoxy-α-D-glucopyranosyl-(1→4)]-1-*N*-[(2*S*)-4-amino-2-hydroxybutanoyl]-2-deoxy-D-streptamine disulfate

[*39831-55-5*]

Amikacin Sulfate is the sulfate of a derivative of kanamycin.

It contains not less than 691 μg (potency) and not more than 791 μg (potency) per mg, calculated on the dried basis. The potency of Amikacin Sulfate is expressed as mass (potency) of amikacin ($C_{22}H_{43}N_5O_{13}$: 585.60).

Description Amikacin Sulfate occurs as a white to yellowish white powder.

It is very soluble in water, and practically insoluble in ethanol (95).

Identification **(1)** Determine the infrared absorption spectrum of Amikacin Sulfate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Amikacin Sulfate RS previously dried: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 0.1 g each of Amikacin Sulfate and Amikacin Sulfate RS in 4 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of water, ammonia water (28), methanol and tetrahydrofuran (1:1:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin-citric acid-acetic acid TS on the plate, and heat at 100°C for 10 minutes: the principal spot obtained from the sample solution and the spot from the standard solution show a red-purple color and the same *R*f value.

(3) A solution of Amikacin Sulfate (1 in 100) responds to the Qualitative Tests <*1.09*> (1) for sulfate.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +76 – +84° (1 g, water, 100 mL, 100 mm).

pH <*2.54*> Dissolve 1.0 g of Amikacin Sulfate in 100 mL of water: the pH of the solution is between 6.0 and 7.5.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Amikacin Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Amikacin Sulfate in 4 mL of a water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of water, ammonia water (28), methanol and tetrahydrofuran (1:1:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin-citric acid-acetic acid TS on the plate, and heat at 100°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 4.0% (1 g, in vacuum, 60°C, 3 hours).

Assay Weigh accurately an amount of Amikacin Sulfate and Amikacin Sulfate RS, equivalent to about 50 mg (potency), dissolve each in water to make exactly 50 mL. Pipet 200 μL each of these solutions in the test tube with glass stopper, add exactly 3 mL of pyridine and exactly 2 mL of a solution of 2,4,6-trinitrobenzenesulfonic acid (1 in 100), stopper tightly, and heat in a water bath at 70°C for 30 minutes. After cooling, add exactly 2 mL each of acetic aid (100), and use these solutions as the sample solution and standard solution, respectively. Perform the test with exactly 20 μL each of these solutions as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the heights, H_T and H_S, of the peak of amikacin derivative in each solution.

Amount [μg (potency)] of amikacin ($C_{22}H_{43}N_5O_{13}$)
$= M_S \times H_T/H_S \times 1000$

M_S: Amount [mg (potency)] of Amikacin Sulfate RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 2.72 g of potassium dihydrogenphosphate in 800 mL of water, adjust to pH 6.5 with a solution of potassium hydroxide (1 in 40), and add water to make 1000 mL. To 280 mL of this solution add 720 mL of methanol, and mix.

Flow rate: Adjust so that the retention time of amikacin derivative is about 9 minutes.

System suitability—

System performance: Dissolve about 5 mg (potency) of Amikacin Sulfate and about 5 mg (potency) of Kanamycin Sulfate in 5 mL of water. Transfer 200 μL of this solution in a glass-stopperd test tube, add 3 mL of pyridine and 2 mL of a solution of 2,4,6-trinitrobenzenesulfonic acid (1 in 100), stopper tightly, heat in a water bath at 70°C for 30 minutes. After cooling, add 2 mL of acetic acid (100). When the procedure is run with 20 μL of this solution under the above operating conditions, amikacin derivative and kanamycin derivative are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of amikacin derivative is not more than 2.0%.

Containers and storage Containers—Hermetic containers.

Amikacin Sulfate Injection

アミカシン硫酸塩注射液

Amikacin Sulfate Injection is an aqueous injection.

It contains not less than 90.0% and not more than 115.0% of the labeled potency of amikacin ($C_{22}H_{43}N_5O_{13}$: 585.60).

Method of preparation Prepare as directed under Injections, with Amikacin Sulfate.

Description Amikacin Sulfate Injection occurs as a colorless or pale yellow clear liquid.

Identification To a volume of Amikacin Sulfate Injection, equivalent to 0.1 g (potency) of Amikacin Sulfate, add water to make 4 mL, and use this solution as the sample solution. Separately, dissolve 25 mg (potency) of Amikacin Sulfate RS in 1 mL of water, and use this solution as the standard solution. Then, proceed as directed in the Identifiction (2) under Amikacin Sulfate.

Osmotic pressure ratio Being specified separately when the drug is granted approval based on the Law.

pH ⟨*2.54*⟩ 6.0 – 7.5

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.50 EU/mg (potency).

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Take exactly a volume of Amikacin Sulfate Injection, equivalent to about 0.1 g (potency) of Amikacin Sulfate, and add water to make exactly 100 mL. Separately, weigh accurately an amount of Amikacin Sulfate RS, equivalent to about 50 mg (potency), and add water to make exactly 50 mL. Take exactly 200 μL each of these solutions into stoppered test tubes, then proceed as directed in the Assay under Amikacin Sulfate.

Amount [mg (potency)] of amikacin ($C_{22}H_{43}N_5O_{13}$)
$= M_S \times H_T/H_S \times 2$

M_S: Amount [mg (potency)] of Amikacin Sulfate RS taken

Containers and storage Containers—Hermetic containers.

Amikacin Sulfate for Injection

注射用アミカシン硫酸塩

Amikacin Sulfate for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 90.0% and not more than 115.0% of the labeled potency of amikacin ($C_{22}H_{43}N_5O_{13}$: 585.60).

Method of preparation Prepare as directed under Injections, with Amikacin Sulfate.

Description Amikacin Sulfate for Injection occurs as white to yellowish white masses or powder.

Identification Dissolve an amount of Amikacin Sulfate for Injection, equivalent to 25 mg (potency) of Amikacin Sulfate, in 1 mL of water, and use this solution as the sample solution. Separately, dissolve 25 mg (potency) of Amikacin Sulfate RS in 1 mL of water, and use this solution as the standard solution. Then, proceed as directed in the Identification (2) under Amikacin Sulfate.

Osmotic pressure ratio Being specified separately when the drug is granted approval based on the Law.

pH ⟨*2.54*⟩ Dissolve an amount of Amikacin Sulfate for Injection, equivalent to 0.1 g (potency) of Amikacin Sulfate, in 10 mL of water: the pH of this solution is 6.0 to 7.5.

Purity Clarity and color of solution—Dissolve an amount of Amikacin Sulfate for Injection, equivalent to 0.5 g (potency) of Amikacin Sulfate, in 5 mL of water: the solution is clear, and the absorbance at 405 nm of the solution determined as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩ is not more than 0.15.

Loss on drying ⟨*2.41*⟩ Not more than 4.0% (1 g, in vacuum, 60°C, 3 hours).

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.50 EU/mg (potency).

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the content of not less than 10 Amikacin Sulfate for Injection. Weigh accurately a portion of the content, equivalent to about 50 mg (potency) of Amikacin Sulfate, dissolve in water to make exactly 50 mL. Separately, weigh accurately an amount of Amikacin Sulfate RS, equivalent to about 50 mg (potency), and dissolve in water to make exactly 50 mL. Transfer exactly 200 μL each of these solutions to separate glass stoppered tubes, and proceed as directed in the Assay under Amikacin Sulfate.

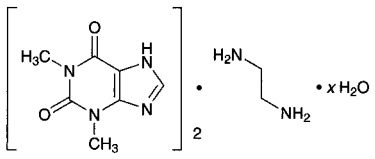
Amount [mg (potency)] of amikacin ($C_{22}H_{43}N_5O_{13}$)
$= M_S \times H_T/H_S$

M_S: Amount [mg (potency)] of Amikacin Sulfate RS taken

Containers and storage Containers—Hermetic containers.

Aminophylline Hydrate

アミノフィリン水和物



$C_{14}H_{16}N_8O_4.C_2H_8N_2.xH_2O$
1,3-Dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione
hemi(ethane-1,2-diamine) hydrate
[*76970-41-7*, monohydrate]

Aminophylline Hydrate contains not less than 84.0% and not more than 86.0% of theophylline ($C_7H_8N_4O_2$: 180.16), and not less than 14.0% and not more than 15.0% of ethylenediamine ($C_2H_8N_2$: 60.10), calculated on the anhydrous basis.

Description Aminophylline Hydrate occurs as white to pale yellow, granules or powder. It is odorless or slightly ammonia-like odor, and has a bitter taste.

It is soluble in water, slightly soluble in methanol, and practically insoluble in ethanol (95) and in diethyl ether.

To 1 g of Aminophylline Hydrate add 5 mL of water, and shake: it dissolves almost completely. Separation of crystals begins in 2 to 3 minutes, and these crystals dissolve on the addition of a small amount of ethylenediamine.

It is gradually affected by light, and gradually loses ethylenediamine in air.

Identification **(1)** Dissolve 0.75 g of Aminophylline Hydrate in 30 mL of water, and use this solution as the sample solution. To 20 mL of the sample solution add 1 mL of dilute hydrochloric acid: a precipitate is gradually formed. Filter the precipitate, recrystallize from water, and dry at 105°C for 1 hour: the crystals so obtained melt ⟨*2.60*⟩ between 271°C and 275°C.

(2) Dissolve 0.1 g of the crystals obtained in (1) in 50 mL of water, and to 2 mL of this solution add tannic acid TS dropwise: a white precipitate is produced, and this precipitate dissolves upon dropwise addition of tannic acid TS.

(3) To 0.01 g of the crystals obtained in (1) add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid, and evaporate on a water bath to dryness: the residue shows a yellow-red color. Invert the dish containing the residue over a vessel containing 2 to 3 drops of ammonia TS: the color of the residue changes to red-purple, which is destroyed on the addition of 2 to 3 drops of sodium hydroxide TS.

(4) Dissolve 0.01 g of the crystals obtained in (1) in 5 mL of water, add 3 mL of ammonia-ammonium chloride buffer solution (pH 8.0) and 1 mL of copper (II) sulfate-pyridine TS, and mix. Add 5 mL of chloroform to the mixture, and shake: the chloroform layer develops a green color.

(5) To 5 mL of the sample solution obtained in (1) add 2 drops of copper (II) sulfate TS: a purple color develops. Add 1 mL of copper (II) sulfate TS: the color changes to blue, and green precipitates are formed on standing.

pH ⟨*2.54*⟩ Dissolve 1.0 g of Aminophylline Hydrate in 25 mL of water: the pH of the solution is between 8.0 and 9.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Aminophylline Hydrate in 10 mL of hot water: the solution is clear and colorless to pale yellow.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Aminophylline Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Water ⟨*2.48*⟩ Not more than 7.9% (0.3 g, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay **(1)** Theophylline—Weigh accurately about 0.25 g of Aminophylline Hydrate, and dissolve in 50 mL of water and 8 mL of ammonia TS by gentle warming on a water bath. Add exactly 20 mL of 0.1 mol/L silver nitrate VS, warm on a water bath for 15 minutes, allow to stand between 5°C and 10°C for 20 minutes, collect the precipitate by suction, and wash with three 10-mL portions of water. Combine the filtrate and washings, and add dilute nitric acid to make neutral. Add 3 mL of dilute nitric acid, and titrate ⟨*2.50*⟩ the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L silver nitrate VS
= 18.02 mg of $C_7H_8N_4O_2$

(2) Ethylenediamine—Weigh accurately about 0.5 g of Aminophylline Hydrate, dissolve in 30 mL of water, and titrate ⟨*2.50*⟩ with 0.1 mol/L hydrochloric acid VS (indicator: 3 drops of bromophenol blue TS).

Each mL of 0.1 mol/L hydrochloric acid VS
= 3.005 mg of $C_2H_8N_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Aminophylline Injection

アミノフィリン注射液

Aminophylline Injection is an aqueous injection.

It contains not less than 75.0% and not more than 86.0% of the labeled amount of theophylline ($C_7H_8N_4O_2$: 180.16), and not less than 13.0% and not more than 20.0% of ethylenediamine ($C_2H_8N_2$: 60.10).

The concentration of Aminophylline Injection is expressed as the quantity of aminophylline dihydrate ($C_{16}H_{24}N_{10}O_4.2H_2O$: 456.46).

Method of preparation Prepare as directed under Injections, with Aminophylline Hydrate. It may be prepared with Theophylline and its equivalent Ethylenediamine, instead of Aminophylline Hydrate.

It may contain not more than 60 mg of Ethylenediamine as a stabilizer for each g of Aminophylline Hydrate.

Description Aminophylline Injection is a clear and colorless liquid. It has a slightly bitter taste.

It gradually changes in color by light.

pH: 8.0 – 10.0

Identification To a volume of Aminophylline Injection, equivalent to 0.75 g of Aminophylline Hydrate, add water to make 30 mL. Proceed with this solution as directed in the Identification under Aminophylline Hydrate.

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.6 EU/mg.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay (1) Theophylline—Pipet a volume of Aminophylline Injection, equivalent to about 39.4 mg of theophylline ($C_7H_8N_4O_2$) (about 50 mg of Aminophylline Hydrate), add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of theophylline for assay, previously dried at 105°C for 4 hours, dissolve in water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of theophylline in each solution.

$$\text{Amount (mg) of theophylline } (C_7H_8N_4O_2) = M_S \times A_T/A_S$$

M_S: Amount (mg) of theophylline for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (100) (1 in 100) and methanol (4:1).

Flow rate: Adjust so that the retention time of theophylline is about 5 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of theophylline are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of theophylline is not more than 1.0%.

(2) Ethylenediamine—To an accurately measured volume of Aminophylline Injection, equivalent to about 30 mg of ethylenediamine ($C_2H_8N_2$) (about 0.2 g of Aminophylline Hydrate), add water to make 30 mL, and titrate ⟨*2.50*⟩ with 0.1 mol/L hydrochloric acid VS (indicator: 2 to 3 drops of bromophenol blue TS).

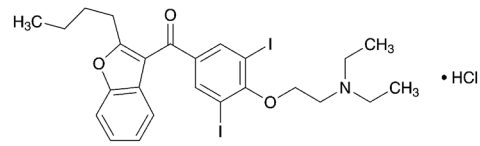
$$\text{Each mL of 0.1 mol/L hydrochloric acid VS} = 3.005 \text{ mg of } C_2H_8N_2$$

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Storage—Light-resistant.

Amiodarone Hydrochloride

アミオダロン塩酸塩



$C_{25}H_{29}I_2NO_3.HCl$: 681.77

(2-Butylbenzofuran-3-yl){4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl}methanone monohydrochloride

[*19774-82-4*]

Amiodarone Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3.HCl$).

Description Amiodarone Hydrochloride occurs as a white to pale yellowish white crystalline powder.

It is very soluble in water at 80°C, freely soluble in dichloromethane, soluble in methanol, sparingly soluble in ethanol (95), and very slightly soluble in water.

Melting point: about 161°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Amiodarone Hydrochloride in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Amiodarone Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 0.1 g of Amiodarone Hydrochloride add 10 mL of water, dissolve by warming at 80°C, and cool: the solution responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

pH ⟨*2.54*⟩ To 1.0 g of Amiodarone Hydrochloride add 20 mL of freshly boiled and cooled water, dissolve by warming at 80°C, and cool: the pH of this solution is between 3.2 and 3.8.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Amiodarone Hydrochloride in 10 mL of methanol: the solution is clear, and is not more colored than the following control solutions (1) and (2).

Control solution (1): To a mixture of 1.0 mL of Cobalt (II) Chloride CS, 2.4 mL of Iron (III) Chloride CS and 0.4 mL of Copper (II) Sulfate CS, add diluted hydrochloric acid (1 in 40) to make 10.0 mL. To 2.5 mL of this solution add diluted hydrochloric acid (1 in 40) to make 20 mL.

Control solution (2): To 3.0 mL of a mixture of 0.2 mL of Cobalt (II) Chloride CS, 9.6 mL of Iron (III) Chloride CS and 0.2 mL of Copper (II) Sulfate CS, add diluted hydrochloric acid (1 in 40) to make 100 mL.

(2) Iodine—To 1.50 g of Amiodarone Hydrochloride add 40 mL of water, dissolve by warming at 80°C, cool, add water to make exactly 50 mL, and use this solution as the sample stock solution. Pipet 15 mL of this solution, add exactly 1 mL of 0.1 mol/L hydrochloric acid TS and exactly 1 mL of a solution of potassium iodate (107 in 10,000), add water to make exactly 20 mL, and use this solution as the sample solution. Separately, pipet 15 mL of the sample stock

solution, add exactly 1 mL of 0.1 mol/L hydrochloric acid TS, exactly 1 mL of a solution of potassium iodide (441 in 5,000,000) and exactly 1 mL of a solution of potassium iodate (107 in 10,000), add water to make exactly 20 mL, and use this solution as the standard solution. Separately, pipet 15 mL of the sample stock solution, add exactly 1 mL of 0.1 mol/L hydrochloric acid TS, add water to make exactly 20 mL, and use this solution as the control solution. Allow the sample solution, standard solution and control solution to stand in a dark place for 4 hours. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using the control solution as the blank: the absorbance of the sample solution at 420 nm is not larger than 1/2 times the absorbance of the standard solution.

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Amiodarone Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substance 1—Dissolve 0.5 g of Amiodarone Hydrochloride in 5 mL of dichloromethane, and use this solution as the sample solution. Separately, dissolve 10 mg of 2-chloroethyl diethylamine hydrochloride in 50 mL of dichloromethane, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and formic acid (17:2:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly bismuth subnitrate TS and then hydrogen peroxide TS: the spot obtained from the sample solution corresponding to the spot from the standard solution is not more intense than the spot from the standard solution.

(5) Related substance 2—Dissolve 0.125 g of Amiodarone Hydrochloride in 25 mL of a mixture of water and acetonitrile for liquid chromatography (1:1), and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add a mixture of water and acetonitrile for liquid chromatography (1:1) to make exactly 50 mL. Pipet 1 mL of this solution, add a mixture of water and acetonitrile for liquid chromatography (1:1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than amiodarone obtained from the sample solution is not larger than the peak area of amiodarone obtained from the standard solution, and the total area of the peaks other than amiodarone from the sample solution is not larger than 2.5 times the peak area of amiodarone from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: To 800 mL of water add 3.0 mL of acetic acid (100), adjust the pH to 4.95 with ammonia solution (28), and add water to make 1000 mL. To 300 mL of this solution add 400 mL of acetonitrile for liquid chromatography and 300 mL of methanol for liquid chromatography.

Flow rate: Adjust so that the retention time of amiodarone is about 24 minutes.

Time span of measurement: About 2 times as long as the retention time of amiodarone.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of water and acetonitrile for liquid chromatography (1:1) to make exactly 25 mL. Confirm that the peak area of amiodarone obtained from 10 μL of this solution is equivalent to 14 to 26% of that obtained from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amiodarone are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amiodarone is not more than 1.0%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, reduced pressure not exceeding 0.3 kPa, 50°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Amiodarone Hydrochloride, previously dried, dissolve in 40 mL of a mixture of acetic anhydride and acetic acid (100) (3:1), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 68.18 mg of $C_{25}H_{29}I_2NO_3$.HCl

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Amiodarone Hydrochloride Tablets

アミオダロン塩酸塩錠

Amiodarone Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3$.HCl: 681.77).

Method of preparation Prepare as directed under Tablets, with Amiodarone Hydrochloride.

Identification To 1 mL of the sample stock solution obtained in the Assay add the mobile phase to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 239 nm and 243 nm.

Uniformity of dosage units <*6.02*> Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Amiodarone Hydrochloride Tablets add 160 mL of the mobile phase, treat with ultrasonic waves for 10 minutes, add the mobile phase to make exactly 200 mL, and centrifuge. Pipet V mL of the supernatant liquid, equivalent to about 1 mg of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3$.HCl), add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of amiodarone hydrochloride for assay, previously dried at 50°C for 4 hours under

reduced pressure not exceeding 0.3 kPa, and dissolve in the mobile phase to make exactly 50 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of amiodarone in each solution.

Amount (mg) of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3$.HCl)
$= M_S \times A_T/A_S \times 8/V$

M_S: Amount (mg) of amiodarone for assay taken

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amiodarone are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amiodarone is not more than 1.0%.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of acetic acid-sodium acetate buffer solution (pH 4.0) as the dissolution medium, the dissolution rate in 30 minutes of Amiodarone Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Amiodarone Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add exactly V mL of methanol, then add a mixture of the dissolution medium and methanol (1:1) to make exactly V' mL so that each mL contains about 11 μg of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of amiodarone hydrochloride for assay, previously dried at 50°C for 4 hours under reduced pressure not exceeding 0.3 kPa, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 2 mL of the dissolution medium, then add a mixture of the dissolution medium and methanol (1:1) to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 241 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a mixture of the dissolution medium and methanol (1:1) as the blank.

Dissolution rate (%) with respect to the labeled amount of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of amiodarone hydrochloride for assay taken

C: Labeled amount (mg) of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3$.HCl) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Amiodarone Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3$.HCl), add 80 mL of the mobile phase, treat with ultrasonic waves for 10 minutes, and add the mobile phase to make exactly 100 mL. Centrifuge this solution, and use the supernatant liquid as the sample stock solution. Pipet 2 mL of the stock solution, add exactly 2 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately 25 mg of amiodarone hydrochloride for assay, previously dried at 50°C for 4 hours under reduced pressure not exceeding 0.3 kPa, and dissolve in the mobile phase to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of amiodarone to that of the internal standard.

Amount (mg) of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3$.HCl)
$= M_S \times Q_T/Q_S \times 2$

M_S: Amount (mg) of amiodarone hydrochloride for assay taken

Internal standard solution—A solution of chlorhexidine hydrochloride in the mobile phase (1 in 2500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 242 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of acetonitrile for liquid chromatography, a solution of sodium laurylsulfate (1 in 50) and phosphoric acid (750:250:1).

Flow rate: Adjust so that the retention time of amiodarone is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and amiodarone are eluted in this order with the resolution between these peaks being not less than 5.

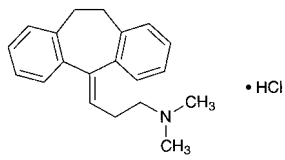
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of amiodarone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Amitriptyline Hydrochloride

アミトリプチリン塩酸塩



$C_{20}H_{23}N.HCl$: 313.86

3-(10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)-*N*,*N*-dimethylpropylamine monohydrochloride [*549-18-8*]

Amitriptyline Hydrochloride, when dried, contains not less than 99.0% of amitriptyline hydrochloride ($C_{20}H_{23}N.HCl$).

Description Amitriptyline Hydrochloride occurs as colorless crystals or a white to pale yellow crystalline powder. It has a bitter taste and a numbing effect.

It is freely soluble in water, in ethanol (95) and in acetic acid (100), soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of 1.0 g of Amitriptyline Hydrochloride in 20 mL of water is between 4.0 and 5.0.

Identification **(1)** Dissolve 5 mg of Amitriptyline Hydrochloride in 3 mL of sulfuric acid: a red color develops. Add 5 drops of potassium dichromate TS to this solution: it turns dark brown.

(2) Acidify 1 mL of a solution of Amitriptyline Hydrochloride (1 in 500) with 0.5 mL of dilute nitric acid, and add 1 drop of silver nitrate TS: a white, opalescent precipitate is produced.

(3) Determine the absorption spectrum of a solution of Amitriptyline Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Amitriptyline Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point <*2.60*> 195 – 198°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Amitriptyline Hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Amitriptyline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Amitriptyline Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 31.39 mg of $C_{20}H_{23}N.HCl$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Amitriptyline Hydrochloride Tablets

アミトリプチリン塩酸塩錠

Amitriptyline Hydrochloride Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of amitriptyline hydrochloride ($C_{20}H_{23}N.HCl$: 313.86).

Method of preparation Prepare as directed under Tablets, with Amitriptyline Hydrochloride.

Identification **(1)** Weigh a quantity of powdered Amitriptyline Hydrochloride Tablets, equivalent to 0.1 g of Amitriptyline Hydrochloride. Add 10 mL of chloroform, shake thoroughly, and filter. Evaporate the filtrate on a water bath to about 2 mL, add diethyl ether until turbidity is produced, and allow to stand. Filter the crystals formed through a glass filter (G4), and proceed as directed in the Identification (1) and (2) under Amitriptyline Hydrochloride.

(2) Determine the absorption spectrum of a solution of the crystals obtained in (1) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 238 nm and 240 nm, and a minimum between 228 nm and 230 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Amitriptyline Hydrochloride Tablets add 50 mL of diluted methanol (1 in 2), shake to disintegrate the tablet, then add diluted methanol (1 in 2) to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet V mL of the subsequent filtrate, add methanol to make exactly V' mL so that each mL contains about 10 μg of amitriptyline hydrochloride ($C_{20}H_{23}N.HCl$), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of amitriptyline hydrochloride ($C_{20}H_{23}N.HCl$)
$= M_S \times A_T/A_S \times V'/V \times 1/20$

M_S: Amount (mg) of Amitriptyline Hydrochloride RS taken

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 60 minutes of Amitriptyline Hydrochloride Tablets is not less than 70%.

Start the test with 1 tablet of Amitriptyline Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet the subsequent V mL of the filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 11 μg of amitriptyline hydrochloride ($C_{20}H_{23}N.HCl$), and use this solution as the sample solution. Separately, weigh accurately about 55 mg of Amitriptyline Hydrochloride RS, previously dried at 105°C for 2 hours, and dissolve in the dissolution medium to make exactly 250 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and stand-

ard solution at 239 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of amitriptyline hydrochloride ($C_{20}H_{23}N.HCl$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of Amitriptyline Hydrochloride RS taken
C: Labeled amount (mg) of amitriptyline hydrochloride ($C_{20}H_{23}N.HCl$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Amitriptyline Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to about 20 mg of amitriptyline hydrochloride ($C_{20}H_{23}N.HCl$), and add 75 mL of diluted methanol (1 in 2). After shaking for 30 minutes, add diluted methanol (1 in 2) to make exactly 100 mL, and filter. Discard the first 20-mL portion of the filtrate, measure exactly the subsequent 5-mL portion, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Amitriptyline Hydrochloride RS, previously dried at 105°C for 2 hours, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Measure exactly 5 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 239 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, respectively.

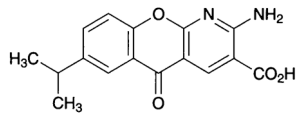
Amount (mg) of amitriptyline hydrochloride ($C_{20}H_{23}N.HCl$)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of Amitriptyline Hydrochloride RS taken

Containers and storage Containers—Tight containers.

Amlexanox

アンレキサノクス



$C_{16}H_{14}N_2O_4$: 298.29
2-Amino-7-(1-methylethyl)-5-oxo-5*H*-[1]benzopyrano[2,3-*b*]pyridine-3-carboxylic acid
[*68302-57-8*]

Amlexanox, when dried, contains not less than 98.0% and not more than 102.0% of amlexanox ($C_{16}H_{14}N_2O_4$).

Description Amlexanox occurs as white to yellowish white, crystals or crystalline powder.

It is very slightly soluble in ethanol (99.5), and practically insoluble in water.

It dissolves in diluted sodium hydroxide TS (1 in 3).

Identification (1) Determine the absorption spectrum of a solution of Amlexanox in ethanol (99.5) (1 in 250,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Amlexanox RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Amlexanox as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Amlexanox RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Chloride <1.03>—Dissolve 1.0 g of Amlexanox in 20 mL of water and 10 mL of sodium hydroxide TS, add 15 mL of dilute nitric acid and water to make 50 mL, centrifuge, and then filter the supernatant liquid. To 25 mL of this filtrate add water to make 50 mL. Perform the test using this solution as the test solution. The control solution consists of 5 mL of sodium hydroxide TS, 7.5 mL of dilute nitric acid, 0.30 mL of 0.01 mol/L hydrochloric acid VS, and water added to make 50 mL (not more than 0.021%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Amlexanox according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—(i) Dissolve 30 mg of Amlexanox in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the area of the peak other than amlexanox obtained from the sample solution is not larger than 2 times the peak area of amlexanox obtained from the standard solution.

Operating conditions—

The detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: Until completion of the elution of amlexanox, beginning after the solvent peak.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 10 mL of the standard solution, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of amlexanox obtained from 10 μL of this solution is equivalent to 7 to 13% of that obtained from 10 μL of the standard solution.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amlexanox is not more than 2.0%.

(ii) Dissolve 30 mg of Amlexanox in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of these solutions by the automatic integration method: the area of the peak other than amlexanox obtained from the sample solution is not larger than 2 times the peak area of amlexanox obtained from the standard solution.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: Dissolve 7.2 g of disodium hydrogen phosphate dodecahydrate in water to make 1000 mL. Adjust the pH of this solution to 8.0 by adding a solution prepared by dissolving 3.1 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water. To 400 mL of this solution add 600 mL of acetonitrile.

Flow rate: To 15 mL of a solution of benzophenone in the mobile phase (3 in 1,000,000) add the mobile phase to make 20 mL. Adjust so that the retention time of benzophenone is about 6.5 minutes when perform the test with 10 μL of this solution under the conditions described above.

Time span of measurement: About 3 times as long as the retention time of benzophenone, beginning after the peak of amlexanox.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of amlexanox obtained from 10 μL of this solution is equivalent to 7 to 13% of that obtained from 10 μL of the standard solution.

System performance: Pipet 1 mL of the sample solution, and add the mobile phase to make 100 mL. To 5 mL of this solution add 15 mL of the solution of benzophenone in the mobile phase (3 in 1,000,000). When perform the test with 10 μL of this solution according to the above conditions, amlexanox and benzophenone are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amlexanox is not more than 2.0%.

(iii) The total amount of related substances, when calculated according to the following formula, is not more than 0.5%.

$$\text{Total amount (\%) of related substances} = \{(A_{T1}/A_{S1}) + (A_{T2}/A_{S2})\} \times 1/10$$

A_{T1}: Total area of the peaks other than amlexanox from the sample solution obtained in (i)

A_{T2}: Total area of the peaks other than amlexanox from the sample solution obtained in (ii)

A_{S1}: Peak area of amlexanox from the standard solution obtained in (i)

A_{S2}: Peak area of amlexanox from the standard solution obtained in (ii)

Loss on drying ⟨*2.41*⟩ Not more than 0.3% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 30 mg each of Amlexanox and Amlexanox RS, both dried, and dissolve them separately in the mobile phase to make exactly 50 mL. Pipet 5 mL each of these solutions, and add exactly 15 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of amlexanox to that of the internal standard, respectively.

$$\text{Amount (mg) of amlexanox } (C_{16}H_{14}N_2O_4) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Amlexanox RS taken

Internal standard solution—A solution of 3-nitroaniline in the mobile phase (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 17.9 g of disodium hydrogen phosphate dodecahydrate in water to make 1000 mL. Adjust the pH of this solution to 8.0 by adding a solution prepared by dissolving 7.8 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water. To 760 mL of this solution add 240 mL of acetonitrile.

Flow rate: Adjust so that the retention time of amlexanox is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution according to the above conditions, amlexanox and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above conditions, the relative standard deviation of the ratio of the peak area of amlexanox to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Amlexanox Tablets

アンレキサノクス錠

Amlexanox Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of amlexanox ($C_{16}H_{14}N_2O_4$: 298.29).

Method of preparation Prepare as directed under Tablets, with Amlexanox.

Identification **(1)** Take an amount of powdered Amlexanox Tablets, equivalent to 10 mg of Amlexanox, add 100 mL of ethanol (99.5), shake vigorously, and filter. Pipet 1 mL of the filtrate, add 25 mL of ethanol (99.5), and use this solution as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits absorption maxima between 240 nm and 244 nm, between 285 nm and 289 nm, and between 341 nm and 352 nm.

(2) Observe the sample solution obtained in (1) under ultraviolet light (main wavelength: 365 nm): the solution shows a bluish-white fluorescence.

Uniformity of dosage units ⟨*6.02*⟩ Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

Take 1 tablet of Amlexanox Tablets, add exactly 0.6 mL of the internal standard solution per 1 mg of amlexanox ($C_{16}H_{14}N_2O_4$), add the mobile phase to make exactly V mL so there is about 167 μg of amlexanox ($C_{16}H_{14}N_2O_4$) per mL, disintegrate the tablet, and then shake vigorously for 5 minutes. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 30 mg of Amlexanox RS, previously dried at 105°C for 2 hours, and dissolve in the mobile phase to make exactly

50 mL. Pipet 25 mL of this solution, add exactly 10 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Amlexanox.

$$\text{Amount (mg) of amlexanox } (C_{16}H_{14}N_2O_4) = M_S \times Q_T/Q_S \times V/200$$

M_S: Amount (mg) of Amlexanox RS taken

Internal standard solution—A solution of 3-nitroaniline in the mobile phase (1 in 500).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of Amlexanox Tablets is not less than 80%.

Start the test with 1 tablet of Amlexanox Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 5.6 μg of amlexanox ($C_{16}H_{14}N_2O_4$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Amlexanox RS, previously dried at 105°C for 2 hours, and dissolve in 2 mL of dilute sodium hydroxide TS, add the dissolution medium to make exactly 50 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 350 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

$$\text{Dissolution rate (\%) with respect to the labeled amount of amlexanox } (C_{16}H_{14}N_2O_4) = M_S \times A_T/A_S \times V'/V \times 1/C \times 18$$

M_S: Amount (mg) of Amlexanox RS taken
C: Labeled amount (mg) of amlexanox ($C_{16}H_{14}N_2O_4$) in 1 tablet

Assay Weigh accurately not less than 20 Amlexanox Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 15 mg of amlexanox ($C_{16}H_{14}N_2O_4$), add exactly 10 mL of the internal standard solution, add 80 mL of the mobile phase, shake vigorously for 5 minutes, and then add the mobile phase to make 100 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 30 mg of Amlexanox RS, previously dried at 105°C for 2 hours, and dissolve in the mobile phase to make exactly 50 mL. Pipet 25 mL of this solution, add exactly 10 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Amlexanox.

$$\text{Amount (mg) of amlexanox } (C_{16}H_{14}N_2O_4) = M_S \times Q_T/Q_S \times 1/2$$

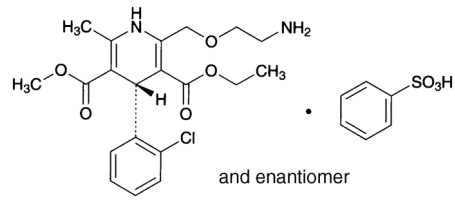
M_S: Amount (mg) of Amlexanox RS taken

Internal standard solution—A solution of 3-nitroaniline in the mobile phase (1 in 500).

Containers and storage Containers—Tight containers.

Amlodipine Besilate

アムロジピンベシル酸塩



$C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$: 567.05
3-Ethyl 5-methyl (4*RS*)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate monobenzenesulfonate
[*111470-99-6*]

Amlodipine Besilate contains not less than 98.0% and not more than 102.0% of amlodipine besilate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$), calculated on the anhydrous basis.

Description Amlodipine Besilate occurs as a white to yellowish white crystalline powder.

It is freely soluble in methanol, sparingly soluble in ethanol (99.5), and slightly soluble in water.

A solution of Amlodipine Besilate in methanol (1 in 100) shows no optical rotation.

Melting point: about 198°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Amlodipine Besilate in 0.01 mol/L hydrochloric acid-methanol TS (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Amlodipine Besilate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Amlodipine Besilate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Amlodipine Besilate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 30 mg of Amlodipine Besilate add 0.1 g of sodium nitrate and 0.1 g of anhydrous sodium carbonate, mix, and gradually ignite. After cooling, dissolve the residue in 2 mL of dilute hydrochloric acid and 10 mL of water, filter if necessary, and add barium chloride TS: a white precipitate is formed.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Amlodipine Besilate according to Method 4, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 25 ppm).

(2) Related substances—Dissolve 0.10 g of Amlodipine Besilate in 50 mL of a mixture of water and acetonitrile (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mixture of water and acetonitrile (1:1) to make exactly 100 mL. Pipet 3 mL of this solution, add the mixture of water and acetonitrile (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak having the relative

retention time of 0.90 to amlodipine, obtained from the sample solution is not larger than the peak area of amlodipine obtained from the standard solution, and the area of the peak other than amlodipine, benzenesulfonic acid having the relative retention time of about 0.15 to amlodipine, and the peak mentioned above from the sample solution is not larger than 1/3 times the peak area of amlodipine from the standard solution. Furthermore, the total area of the peaks other than amlodipine and benzenesulfonic acid from the sample solution is not larger than 2.7 times the peak area of amlodipine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 237 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase A: A mixture of water and trifluoroacetic acid (5000:1).

Mobile phase B: A mixture of acetonitrile and trifluoroacetic acid (5000:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 30	80 → 20	20 → 80
30 – 45	20	80

Flow rate: 1.0 mL per minute.

Time span of measurement: About 3 times as long as the retention time of amlodipine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of water and acetonitrile (1:1) to make exactly 10 mL. Confirm that the peak area of amlodipine obtained with 10 μL of this solution is equivalent to 7 to 13% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amlodipine are not less than 70,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amlodipine is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 0.5% (1 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Assay Weigh accurately about 35 mg each of Amlodipine Besilate and Amlodipine Besilate RS (separately determine the water ⟨*2.48*⟩ using the same manner as Amlodipine Besilate), dissolve them separately in the mobile phase to make exactly 250 mL. Pipet 5 mL each of these solutions, add exactly 5 mL each of the internal standard solution, add the mobile phase to make 25 mL, and use these solutions as the sample solution and standard solution. Perform the test with 20 μL of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of amlodipine to that of the internal standard.

$$\text{Amount (mg) of amlodipine besilate } (C_{20}H_{25}ClN_2O_5.C_6H_6O_3S) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Amlodipine Besilate RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of isobutyl parahydroxybenzoate in the mobile phase (3 in 20,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 237 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and a solution of potassium dihydrogen phosphate (41 in 10,000) (13:7).

Flow rate: Adjust so that the retention time of amlodipine is about 8 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, amlodipine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of amlodipine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Amlodipine Besilate Orally Disintegrating Tablets

アムロジピンベシル酸塩口腔内崩壊錠

Amlodipine Besilate Orally Disintegrating Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of amlodipine besilate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$: 567.05).

Method of preparation Prepare as directed under Tablets, with Amlodipine Besilate.

Identification To an amount of powdered Amlodipine Besilate Orally Disintegrating Tablets, equivalent to 7 mg of Amlodipine Besilate, add 200 mL of 0.01 mol/L hydrochloric acid-methanol TS, treat with ultrasonic waves, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 358 nm and 362 nm.

Purity Related substances—Use the sample solution obtained in the Assay as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and the mobile phase A (3:2) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 30 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the fol-

lowing conditions, and determine each peak area by the automatic integration method: the area of the peak having the relative retention time of about 0.45 to amlodipine obtained from the sample solution is not larger than the peak area of amlodipine from the standard solution, the area of the peak having the relative retention time of about 4.5 to amlodipine from the sample solution is not larger than 1.8 times the peak area of amlodipine from the standard solution, and the area of the peak having the relative retention time of about 0.16 to amlodipine and the peaks other than mentioned above from the sample solution is not larger than 2/5 times the peak area of amlodipine from the standard solution. Furthermore, the total area of the peaks other than amlodipine and the peak having the relative retention time of about 0.16 to amlodipine from the sample solution is not larger than 2.8 times the peak area of amlodipine from the standard solution. For the areas of the peaks, having the relative retention time of about 0.45 and about 4.5 to amlodipine, multiply their relative response factors, 2.0 and 1.9, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 237 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Dissolve 4.1 g of potassium dihydrogen phosphate in 1000 mL of water, adjust to pH 6.0 with a solution of 5.4 g of disodium hydrogen phosphate dodecahydrate in 500 mL of water. To 500 mL of this solution add 500 mL of methanol.

Mobile phase B: Dissolve 4.1 g of potassium dihydrogen phosphate in 1000 mL of water, adjust to pH 6.0 with a solution of 5.4 g of disodium hydrogen phosphate dodecahydrate in 500 mL of water. To 50 mL of this solution add 950 mL of methanol.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 10	80	20
10 – 35	80 → 0	20 → 100
35 – 50	0	100

Flow rate: Adjust so that the retention time of amlodipine is about 10 minutes.

Time span of measurement: About 5 times as long as the retention time of amlodipine.

System suitability—

Test for required detectability: Pipet 10 mL of the standard solution, and add a mixture of methanol and the mobile phase A (3:2) to make exactly 50 mL. Confirm that the peak area of amlodipine obtained with 30 μL of this solution is equivalent to 14 to 26% of that obtained with 30 μL of the standard solution.

System performance: When the procedure is run with 30 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amlodipine are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 30 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amlodipine is not more than 2.0%.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Amlodipine Besilate Orally Disintegrating Tablets add $4V/5$ mL of a mixture of the mobile phase and methanol (1:1), disperse the particles with the aid of ultrasonic waves, add a mixture of the mobile phase and methanol (1:1) to make exactly V mL so that each mL of the solution contains about 0.14 mg of amlodipine besilate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$). Centrifuge this solution, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of amlodipine besilate } (C_{20}H_{25}ClN_2O_5.C_6H_6O_3S) = M_S \times A_T/A_S \times V \times 1/250$$

M_S: Amount (mg) of Amlodipine Besilate RS taken, calculated on the anhydrous basis

Disintegration Being specified separately when the drug is granted approval based on the Law.

Dissolution Being specified separately when the drug is granted approval based on the Law.

Assay Weigh accurately the mass of not less than 20 Amlodipine Besilate Orally Disintegrating Tablets, and powder them. Weigh accurately a portion of this powder, equivalent to about 7 mg of amlodipine besilate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$), add 40 mL of a mixture of the mobile phase and methanol (1:1), disperse the particles with the aid of ultrasonic waves, and add a mixture of the mobile phase and methanol (1:1) to make exactly 50 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 35 mg of Amlodipine Besilate RS (separately, determine the water ⟨*2.48*⟩ in the same manner as Amlodipine Besilate), add 150 mL of a mixture of the mobile phase and methanol (1:1), dissolve with the aid of ultrasonic waves, then add a mixture of the mobile phase and methanol (1:1) to make exactly 250 mL, and use this solution as the standard solution. Perform the test with exactly 30 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩, and determine the peak areas, A_T and A_S, of amlodipine in each solution.

$$\text{Amount (mg) of amlodipine besilate } (C_{20}H_{25}ClN_2O_5.C_6H_6O_3S) = M_S \times A_T/A_S \times 1/5$$

M_S: Amount (mg) of Amlodipine Besilate RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 237 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 4.1 g of potassium dihydrogen phosphate in 1000 mL of water, adjust to pH 6.0 with a solution of 5.4 g of disodium hydrogen phosphate dodecahydrate in 500 mL of water. To 400 mL of this solution add 600 mL of methanol.

Flow rate: Adjust so that the retention time of amlodipine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 30 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amlodipine are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 30 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amlodipine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Amlodipine Besilate Tablets

アムロジピンベシル酸塩錠

Amlodipine Besilate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of amlodipine besilate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$: 567.05).

Method of preparation Prepare as directed under Tablets, with Amlodipine Besilate.

Identification To a quantity of powdered Amlodipine Besilate Tablets, equivalent to 2.5 mg of Amlodipine Besilate, add 100 mL of 0.01 mol/L hydrochloric acid-methanol TS, shake vigorously, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 235 nm and 239 nm, and between 358 nm and 362 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Amlodipine Besilate Tablets add 10 mL of water to disintegrate, disperse with the aid of ultrasonic waves with occasional shaking, add the mobile phase to make exactly V mL so that each mL contains about 69 μg of amlodipine besilate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$), and shake for 60 minutes. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add the mobile phase to make 25 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of amlodipine besilate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$)
$= M_S \times Q_T/Q_S \times V/500$

M_S: Amount (mg) of Amlodipine Besilate RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of isobutyl parahydroxybenzoate in the mobile phase (3 in 20,000).

Dissolution Being specified separately when the drug is granted approval based on the Law.

Assay To 20 Amlodipine Besilate Tablets add 100 mL of water to disintegrate, disperse with the aid of ultrasonic waves with occasional shaking, add the mobile phase to make exactly 1000 mL, and shake for 60 minutes. Centrifuge this solution, pipet a volume of the supernatant liquid, equivalent to about 0.7 mg of amlodipine besilate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$), add exactly 5 mL of the internal standard solution, add the mobile phase to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 35 mg of Amlodipine Besilate RS (separately, determine the water ⟨*2.48*⟩ in the same manner as Amlodipine Besilate), and dissolve in the mobile phase to make exactly 250 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mobile phase to make 25 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of amlodipine to that of the internal standard.

Amount (mg) of amlodipine besilate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$)
$= M_S \times Q_T/Q_S \times 1/50$

M_S: Amount (mg) of Amlodipine Besilate RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of isobutyl parahydroxybenzoate in the mobile phase (3 in 20,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 237 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and potassium dihydrogen phosphate (41 in 10,000) (13:7).

Flow rate: Adjust so that the retention time of amlodipine is about 8 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, amlodipine and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of amlodipine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Ammonia Water

アンモニア水

Ammonia Water contains not less than 9.5 w/v% and not more than 10.5 w/v% of ammonia (NH_3: 17.03).

Description Ammonia Water occurs as a clear, colorless liquid, having a very pungent, characteristic odor.

It is alkaline.

Specific gravity d_{20}^{20}: 0.95 – 0.96

Identification **(1)** Hold a glass rod moistened with hydrochloric acid near the surface of Ammonia Water: dense white fumes are produced.

(2) Hold moistened red litmus paper near the surface of Ammonia Water: it turns blue.

Purity **(1)** Residue on evaporation—Evaporate 10.0 mL of Ammonia Water to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 2.0 mg.

(2) Heavy metals <*1.07*>—Evaporate 5.0 mL of Ammonia Water to dryness on a water bath, add 1 mL of dilute hydrochloric acid to the residue, and evaporate to dryness. Dissolve the residue in 2 mL of dilute acetic acid, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2.5 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 5 ppm).

(3) Potassium permanganate-reducing substances—To 10.0 mL of Ammonia Water add 40 mL of dilute sulfuric acid while cooling, and add 0.10 mL of 0.02 mol/L potassium permanganate VS: the red color of the potassium permanganate does not disappear within 10 minutes.

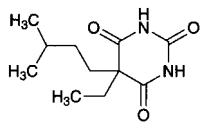
Assay Measure exactly 5 mL of Ammonia Water, add 25 mL of water, and titrate <*2.50*> with 0.5 mol/L sulfuric acid VS (indicator: 2 drops of methyl red TS).

Each mL of 0.5 mol/L sulfuric acid VS
= 17.03 mg of NH_3

Containers and storage Containers—Tight containers.
Storage—Not exceeding 30°C.

Amobarbital

アモバルビタール



$C_{11}H_{18}N_2O_3$: 226.27
5-Ethyl-5-(3-methylbutyl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione
[*57-43-2*]

Amobarbital, when dried, contains not less than 99.0% of amobarbital ($C_{11}H_{18}N_2O_3$).

Description Amobarbital occurs as white, crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in ethanol (95), in acetone and in diethyl ether, sparingly soluble in chloroform, and practically insoluble in water.

It dissolves in sodium hydroxide TS and in sodium carbonate TS.

The pH of a saturated solution of Amobarbital is between 5.0 and 5.6.

Identification **(1)** Boil 0.2 g of Amobarbital with 10 mL of sodium hydroxide TS: the gas evolved changes moistened red litmus paper to blue.

(2) Dissolve 0.05 g of Amobarbital in 2 to 3 drops of ammonia-ammonium chloride buffer solution (pH 10.7) and 5 mL of diluted pyridine (1 in 10). Add 5 mL of chloroform and 0.3 mL of copper (II) sulfate TS to the solution: a red-purple precipitate is produced in the aqueous layer. Shake the mixture: a red-purple color is produced in the chloroform layer.

(3) To 0.4 g of Amobarbital add 0.1 g of anhydrous sodium carbonate and 4 mL of water, shake, and add a solution of 0.3 g of 4-nitrobenzyl chloride in 7 mL of ethanol (95). Heat the mixture on a water bath for 30 minutes under a reflux condenser, and allow to stand for 1 hour. Filter the crystals produced, wash with 7 mL of sodium hydroxide TS and a small portion of water, recrystallize from ethanol, and dry at 105°C for 30 minutes: the crystals so obtained melt <*2.60*> between 168°C and 173°C or between 150°C and 154°C.

Melting point <*2.60*> 157 – 160°C

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Amobarbital in 5 mL of sodium hydroxide TS: the solution is clear and colorless.

(2) Chloride <*1.03*>—Dissolve 0.30 g of Amobarbital in 20 mL of acetone, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: take 0.30 mL of 0.01 mol/L hydrochloric acid VS, 20 mL of acetone and 6 mL of dilute nitric acid, and add water to make 50 mL (not more than 0.035%).

(3) Sulfate <*1.14*>—Dissolve 0.40 g of Amobarbital in 20 mL of acetone, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: take 0.40 mL of 0.005 mol/L sulfuric acid VS, 20 mL of acetone, and 1 mL of dilute hydrochloric acid, and add water to make 50 mL (not more than 0.048%).

(4) Heavy metals <*1.07*>—Proceed with 1.0 g of Amobarbital according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Readily carbonizable substances <*1.15*>—Perform the test with 0.5 g of Amobarbital. The solution is not more colored than Matching Fluid A.

Loss on drying <*2.41*> Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

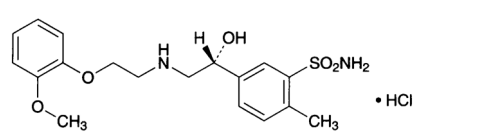
Assay Weigh accurately about 0.5 g of Amobarbital, previously dried, and dissolve in 5 mL of ethanol (95) and 50 mL of chloroform. Titrate <*2.50*> with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution changes from yellow through light blue to purple (indicator: 1 mL of alizarin yellow GG-thymolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 22.63 mg of $C_{11}H_{18}N_2O_3$

Containers and storage Containers—Well-closed containers.

Amosulalol Hydrochloride

アモスラロール塩酸塩



and enantiomer

$C_{18}H_{24}N_2O_5S.HCl$: 416.92
5-((1*RS*)-1-Hydroxy-2-{[2-(2-methoxyphenoxy)ethyl]amino}ethyl)-2-methylbenzenesulfonamide monohydrochloride
[*70958-86-0*]

Amosulalol Hydrochloride contains not less than 98.5% and not more than 101.0% of amosulalol hydrochloride ($C_{18}H_{24}N_2O_5S.HCl$), calculated on the an-

hydrous basis.

Description Amosulalol Hydrochloride occurs as white crystals or a white crystalline powder. It has a bitter taste.

It is very soluble in formic acid, freely soluble in methanol, and sparingly soluble in water and in ethanol (99.5).

It is hygroscopic.

A solution of Amosulalol Hydrochloride in methanol (1 in 100) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Amosulalol Hydrochloride (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Amosulalol Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Amosulalol Hydrochloride (1 in 100) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

Melting point ⟨*2.60*⟩ 158 – 162°C

Purity **(1)** Heavy metals ⟨*1.07*⟩—Place 1.0 g of Amosulalol Hydrochloride in a porcelain crucible, add 1.5 mL of sulfuric acid, cover loosely, and heat gently to carbonize. After cooling, add 2 mL of nitric acid, heat carefully until white fumes no longer are evolved, and then heat intensely to 500 – 600°C to incinerate. After cooling, add 2 mL of hydrochloric acid, proceed according to Method 2, and perform the test. The control solution, processed in the same manner as the test solution using the same amounts of reagents, is prepared by combining 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Amosulalol Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than amosulalol obtained from the sample solution is not larger than 2/5 times the peak area of amosulalol obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 5.7 by adding a solution prepared by dissolving 3.58 g of disodium hydrogen phosphate dodecahydrate in water to make 1000 mL. To 670 mL of this solution add 330 mL of acetonitrile.

Flow rate: Adjust so that the retention time of amosulalol is about 7 minutes.

Time span of measurement: About 2 times as long as the retention time of amosulalol, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of amosulalol obtained with 10 μL of this solution is equivalent to 7 to 13% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amosulalol are not less than 4000 and not more than 1.7, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amosulalol is not more than 1.0%.

Water ⟨*2.48*⟩ Not more than 4.0% (1 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Amosulalol Hydrochloride, dissolve in 3 mL of formic acid, add 80 mL of a mixture of acetic acid (100) and acetic anhydride (3:2), and titrate ⟨*2.50*⟩ within 5 minutes with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination using the same procedure, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 41.69 mg of $C_{18}H_{24}N_2O_5S.HCl$

Containers and storage Containers—Tight containers.

Amosulalol Hydrochloride Tablets

アモスラロール塩酸塩錠

Amosulalol Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of amosulalol hydrochloride ($C_{18}H_{24}N_2O_5S.HCl$: 416.92).

Method of preparation Prepare as directed under Tablets, with Amosulalol Hydrochloride.

Identification To a quantity of powdered Amosulalol Hydrochloride Tablets, equivalent to 50 mg of Amosulalol Hydrochloride, add 25 mL of 0.1 mol/L hydrochloric acid TS, shake well, and then centrifuge. To 2.5 mL of the supernatant liquid add water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 270 nm and 274 nm, and a shoulder between 275 nm and 281 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take 1 tablet of Amosulalol Hydrochloride Tablets, disintegrate by adding 2 mL of 0.1 mol/L hydrochloric acid TS, add 15 mL of methanol, and shake well. Add methanol to make exactly *V* mL so that each mL contains about 0.4 mg of amosulalol hydrochloride ($C_{18}H_{24}N_2O_5S.HCl$), and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of amosulalol

hydrochloride for assay (separately determine the water ⟨2.48⟩ in the same manner as Amosulalol Hydrochloride), and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 2 mL of the internal standard solution, add the mobile phase to make 20 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of amosulalol hydrochloride } (C_{18}H_{24}N_2O_5S.HCl) = M_S \times Q_T/Q_S \times V/50$$

M_S: Amount (mg) of amosulalol hydrochloride for assay taken, calculated on the anhydrous basis

Internal standard solution—A solution of ethyl parahydroxybenzoate in methanol (1 in 6250).

Dissolution ⟨6.10⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Amosulalol Hydrochloride Tablets is not less than 75%.

Start the test with 1 tablet of Amosulalol Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 5.5 μg of amosulalol hydrochloride ($C_{18}H_{24}N_2O_5S.HCl$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of amosulalol hydrochloride for assay (separately determine the water ⟨2.48⟩ in the same manner as Amosulalol Hydrochloride), and dissolve in water to make exactly 200 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions, and determine the amosulalol peak areas, A_T and A_S, in each solution.

$$\text{Dissolution rate (\%) with respect to the labeled amount of amosulalol hydrochloride } (C_{18}H_{24}N_2O_5S.HCl) = M_S \times A_T/A_S \times V'/V \times 1/C \times 45/2$$

M_S: Amount (mg) of amosulalol hydrochloride for assay taken, calculated on the anhydrous basis

C: Labeled amount (mg) of amosulalol hydrochloride ($C_{18}H_{24}N_2O_5S.HCl$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 5.7 by adding a soluion prepared by dissolving 3.58 g of disodium hydrogen phosphate dodecahydrate in water to make 1000 mL. To 670 mL of this solution add 330 mL of acetonitrile.

Flow rate: Adjust so that the retention time of amosulalol is about 5 minutes.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amosulalol are not less than 4000 and not more than 1.7, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amosulalol is not more than 1.0%.

Assay Take 10 Amosulalol Hydrochloride Tablets, add 20 mL of 0.1 mol/L hydrochloric acid TS, and shake well to disintegrate. Add 120 mL of methanol, again shake well, add methanol to make exactly 200 mL, and then centrifuge. Pipet a volume of supernatant liquid corresponding to about 5 mg of amosulalol hydrochloride ($C_{18}H_{24}N_2O_5S.HCl$), add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of amosulalol hydrochloride for assay (separately determine the water ⟨2.48⟩ in the same manner as Amosulalol Hydrochloride), and dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of amosulalol to that of the internal standard.

$$\text{Amount (mg) of amosulalol hydrochloride } (C_{18}H_{24}N_2O_5S.HCl) = M_S \times Q_T/Q_S \times 1/5$$

M_S: Amount (mg) of amosulalol hydrochloride for assay taken, calculated on the anhydrous basis

Internal standard solution—A solution of ethyl parahydroxybenzoate in methanol (1 in 6250).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of diluted acetic acid (100) (1 in 25), acetonitrile and a solution of ammonium acetate (1 in 250) (5:3:2).

Flow rate: Adjust so that the retention time of amosulalol is about 4 minutes.

System suitability—

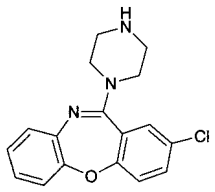
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, amosulalol and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of amosulalol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Amoxapine

アモキサピン



$C_{17}H_{16}ClN_3O$: 313.78
2-Chloro-11-(piperazin-1-yl)dibenzo[*b*,*f*][1,4]oxazepine
[*14028-44-5*]

Amoxapine, when dried, contains not less than 98.5% of amoxapine ($C_{17}H_{16}ClN_3O$).

Description Amoxapine occurs as white to light yellowish white, crystals or crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Amoxapine in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption as the same wavelengths.

(2) Determine the infrared absorption spectrum of Amoxapine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Amoxapine as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Melting point <*2.60*> 178 – 182°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Amoxapine according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 15 ppm).

(2) Related substances—Dissolve 0.5 g of Amoxapine in 10 mL of a mixture of ethanol (95) and acetic acid (100) (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of ethanol (95) and acetic acid (100) (9:1) to make exactly 10 mL. Pipet 1 mL of this solution, add a mixture of ethanol (95) and acetic acid (100) (9:1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethanol (95) and acetic acid (100) (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.4% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Amoxapine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to greenish blue (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 15.69 mg of $C_{17}H_{16}ClN_3O$

Containers and storage Containers—Tight containers.

Amoxicillin Hydrate

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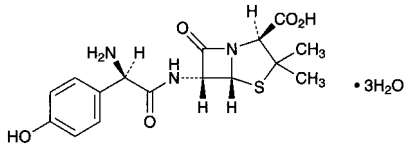


$C_{16}H_{19}N_3O_5S.3H_2O$: 419.45
(2*S*,5*R*,6*R*)-6-[(2*R*)-2-Amino-2-(4-hydroxyphenyl)-acetylamino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate
[*61336-70-7*]

Amoxicillin Hydrate contains not less than 950 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the anhydrous basis. The potency of Amoxicillin Hydrate is expressed as mass (potency) of amoxicillin ($C_{16}H_{19}N_3O_5S$: 365.40).

Description Amoxicillin Hydrate occurs as white to light yellowish white, crystals or crystalline powder.

It is slightly soluble in water and in methanol, and very slightly soluble in ethanol (95).

Identification Determine the infrared absorption spectrum of Amoxicillin Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Amoxicillin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +290 – +315° (0.1 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—To 1.0 g of Amoxicillin Hydrate add 2 mL of a solution of magnesium sulfate heptahydrate (1 in 4), mix, and heat on a water bath to dryness. Carbonize the residue by gently heating. After cooling, add 1 mL of sulfuric acid, heat carefully, then heat at 500 – 600°C to incinerate. After cooling, add 1 mL of hydrochloric acid to the residue, and heat on a water bath to dryness. Then add 10 mL of water to the residue, and heat on a water bath to dissolve. After cooling, add ammonia TS to adjust the pH to 3 – 4, and add 2 mL of dilute acetic acid. If necessary, filter, wash the residue on the filter with 10 mL of water, transfer the filtrate and washings into a Nessler tube, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution as follows: To 2.0 mL of Standard Lead Solution add 2 mL of a solution of magnesium sulfate heptahydrate (1 in 4), then proceed in the same manner as for preparation of the test solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Amoxicillin Hydrate according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Amoxicillin Hydrate in 50 mL of a solution of boric acid (1 in 200), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a solution of boric acid (1 in 200) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than amoxicillin obtained from the sample solution is not larger than the peak area of amoxicillin obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.36 g of sodium acetate trihydrate in 750 mL of water, adjust to pH 4.5 with acetic acid (31), and add water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust so that the retention time of amoxicillin is about 8 minutes.

Time span of measurement: About 4 times as long as the retention time of amoxicillin.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add a solution of boric acid (1 in 200) to make exactly 10 mL. Confirm that the peak area of amoxicillin obtained from 10 μL of this solution is equivalent to 7 to 13% of that obtained from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amoxicillin are not less than 2500 and not nore than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amoxicillin is not more than 1.0%.

Water <2.48> Not less than 11.0% and not more than 15.0% (0.1 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Amoxicillin Hydrate and Amoxicillin RS, equivalent to about 30 mg (potency), dissolve each in a solution of boric acid (1 in 200) to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the peak areas, A_T and A_S, of amoxicillin in each solution.

$$\text{Amount [}\mu\text{g (potency)] of amoxicillin } (C_{16}H_{19}N_3O_5S) = M_S \times A_T/A_S \times 1000$$

M_S: Amount [mg (potency)] of Amoxicillin RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.361 g of sodium acetate trihydrate in 750 mL of water, adjust to pH 4.5 with acetic acid (31), and add water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust so that the retention time of amoxicillin is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of amoxicillin is not less than 2500.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amoxicillin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Amoxicillin Capsules

アモキシシリンカプセル

Amoxicillin Capsules contain not less than 92.0% and not more than 105.0% of the labeled potency of Amoxicillin ($C_{16}H_{19}N_3O_5S$: 365.40).

Method of preparation Prepare as directed under Capsules, with Amoxicillin Hydrate.

Identification Take out the contents of Amoxicillin Capsules, to a quantity of the contents, equivalent to 8 mg (potency) of Amoxicillin Hydrate, add 2 mL of 0.01 mol/L hydrochloric acid TS, shake for 30 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve an amount equivalent to 8 mg (potency) of Amoxicillin RS in 2 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of tetrahydrofuran, water and formic acid (50:5:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in ethanol (95) (1 in 20) on the plate, and heat the plate at 110°C for 15 minutes: the principal spot obtained from the sample solution and the spot obtained from the standard solution show a red-purple color and the same *R*f value.

Purity Related substances—Take out the contents of Amoxicillin Capsules, to a quantity of the contents, equivalent to 0.1 g (potency) of Amoxicillin Hydrate, add 30 mL of a solution of boric acid (1 in 200), shake for 15 minutes, and add a solution of boric acid (1 in 200) to make 50 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Pipet 1 mL of the sample solution, add a solution of boric acid (1 in 200) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than amoxicillin obtained from the sample solution is not larger than the peak area of amoxicillin obtained from the standard solution.

Operating conditions—

Proceed as directed in the operating conditions in the Purity (3) under Amoxicillin Hydrate.

System suitability—

Test for required detectability and system repeatability: Proceed as directed in the system suitability in the Purity (3) under Amoxicillin Hydrate.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amoxicillin is not less than 2500 and not more than 1.5, respectively.

Water ⟨*2.48*⟩ Not more than 15.0% (0.1 g, volumetric titration, direct titration).

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Dissolution ⟨*6.10*⟩ When the test is performed at 100 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 60 minutes of Amoxicillin Capsules is not less than 75%.

Start the test with 1 capsule of Amoxicillin Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 56 μg (potency) of Amoxicillin Hydrate, and use this solution as the sample solution. Separately, weigh accurately an amount equivalent to about 28 mg (potency) of Amoxicillin RS, dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of amoxicillin in each solution.

Dissolution rate (%) with respect to the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 180$

M_S: Amount [mg (potency)] of Amoxicillin RS taken
C: Labeled amount [mg (potency)] of amoxicillin ($C_{16}H_{19}N_3O_5S$) in 1 capsule

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Amoxicillin Hydrate.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amoxicillin are not less than 2500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amoxicillin is not more than 1.5%.

Assay Weigh accurately the mass of not less than 10 Amoxicillin Capsules, take out the contents, and weigh accurately the mass of the emptied shells. Weigh accurately an amount equivalent to about 0.1 g (potency) of Amoxicillin Hydrate, add 70 mL of water, shake for 15 minutes, and add water to make exactly 100 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately an amount equivalent to about 20 mg (potency) of Amoxicillin RS, dissolve in water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of amoxicillin in each solution.

Amount [mg (potency)] of amoxicillin ($C_{16}H_{19}N_3O_5S$)
$= M_S \times A_T/A_S \times 5$

M_S: Amount [mg (potency)] of Amoxicillin RS taken

Operating conditions—

Column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Amoxicillin Hydrate.

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

System suitability—

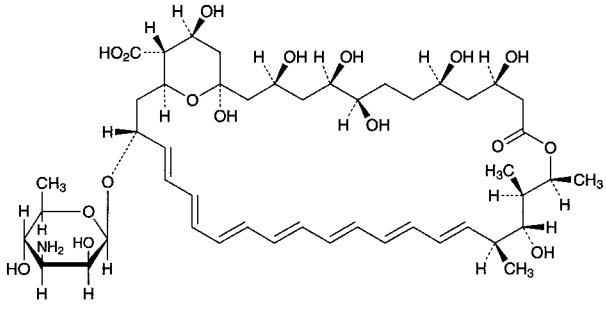
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amoxicillin are not less than 2500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of peak areas of amoxicillin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Amphotericin B

アムホテリシン **B**



$C_{47}H_{73}NO_{17}$: 924.08

(1*R*,3*S*,5*R*,6*R*,9*R*,11*R*,15*S*,16*R*,17*R*,18*S*,19*E*,21*E*,23*E*,25*E*,27*E*,29*E*,31*E*,33*R*,35*S*,36*R*,37*S*)-33-(3-Amino-3,6-dideoxy-β-D-mannopyranosyloxy)-1,3,5,6,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptaene-36-carboxylic acid [*1397-89-3*]

Amphotericin B is a polyene macrolide substance having antifungal activity produced by the growth of *Streptomyces nodosus*.

It contains not less than 840 μg (potency) per mg, calculated on the dried basis. The potency of Amphotericin B is expressed as mass (potency) of amphotericin B ($C_{47}H_{73}NO_{17}$).

Description Amphotericin B occurs as a yellow to orange powder.

It is freely soluble in dimethylsulfoxide and practically insoluble in water and in ethanol (95).

Identification (1) Dissolve 5 mg of Amphotericin B in 10 mL of dimethylsulfoxide. To 1 mL of this solution add 5 mL of phosphoric acid: a blue color develops between the two layers, and the solution becomes blue by shaking. After addition of 15 mL of water it becomes yellow to light yellow-brown by shaking.

(2) Dissolve 25 mg of Amphotericin B in 5 mL of dimethylsulfoxide, and add methanol to make 50 mL. To 1 mL of this solution add methanol to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Amphotericin B RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity Amphotericin A—Weigh accurately about 50 mg each of Amphotericin B and Amphotericin B RS, add exactly 10 mL each of dimethylsulfoxide to dissolve, and add methanol to make exactly 50 mL. Pipet 4 mL each of these solutions, add methanol to make exactly 50 mL, and use these solutions as the sample solution and standard solution (1), respectively. Separately, weigh accurately about 20 mg of Nystatin RS, add exactly 40 mL of dimethylsulfoxide to dissolve, then add methanol to make exactly 200 mL. Pipet 4 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*> using a solution obtained in the same manner as the sample solution as the blank, and determine the absorbances at 282 nm and at 304 nm. Calculate the amount of amphotericin A by the following equation: not more than 5% for Amphotericin B used for injections, and not more than 15% for Amphotericin B not used for injections.

$$\text{Amount (\%) of amphotericin A} = \frac{M_S \times \{(A_{Sa1} \times A_{T2}) - (A_{Sa2} \times A_{T1})\} \times 25}{M_T \times \{(A_{Sa1} \times A_{Sb2}) - (A_{Sa2} \times A_{Sb1})\}}$$

M_S: Amount (mg) of Nystatin RS taken
M_T: Amount (mg) of Amphotericin B taken
A_{Sa1}: Absorbance at 282 nm of the standard solution (1)
A_{Sb1}: Absorbance at 282 nm of the standard solution (2)
A_{Sa2}: Absorbance at 304 nm of the standard solution (1)
A_{Sb2}: Absorbance at 304 nm of the standard solution (2)
A_{T1}: Absorbance at 282 nm of the sample solution
A_{T2}: Absorbance at 304 nm of the sample solution

Loss on drying <*2.41*> Not more than 5.0% (0.1 g, in vacuum, 60°C, 3 hours).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism—*Saccharomyces cerevisiae* ATCC 9763

(ii) Culture medium—Use the medium 2) under (1) Agar media for seed and base layer.

(iii) Preparation of cylinder-agar plate—Proceed as directed in 1.5 Preparation of agar base layer plates under the Cylinder plate method, using Petri dish plates not dispensing the agar medium for base layer and dispensing 8.0 mL of the seeded agar medium.

(iv) Standard solution—Use light-resistant vessels. Weigh accurately an amount of Amphotericin B RS equivalent to about 20 mg (potency), dissolve in dimethylsulfoxide to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 24 hours. Take exactly a suitable amount of the standard stock solution before use, and add dimethylsulfoxide to make solutions so that each mL contains 200 μg (potency) and 50 μg (potency). Pipet 1 mL each of these solutions, add 0.2 mol/L phosphate buffer solution (pH 10.5) to make exactly 20 mL, and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(v) Sample solution—Use light-resistant vessels. Weigh accurately an amount of Amphotericin B equivalent to about 20 mg (potency), dissolve in dimethylsulfoxide to make exactly 20 mL, and use this solution as the sample stock solution. Take exactly a suitable amount of the sample stock solution, add dimethylsulfoxide to make solutions so that each mL contains 200 μg (potency) and 50 μg (potency). Pipet 1 mL each of these solutions, add 0.2 mol/L phosphate buffer solution (pH 10.5) to make exactly 20 mL, and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and in a cold place.

Amphotericin B for Injection

注射用アムホテリシン **B**

Amphotericin B for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 120.0% of the labeled potency of amphotericin B ($C_{47}H_{73}NO_{17}$: 924.08).

Method of preparation Prepare as directed under Injections, with Amphotericin B.

Description Amphotericin B for Injection occurs as yellow to orange, powder or masses.

Identification To an amount of Amphotericin B for Injection, equivalent to 25 mg (potency) of Amphotericin B, add 5 mL of dimethylsulfoxide and 45 mL of methanol, and shake. To 1 mL of this solution add methanol to make 50 mL, and filter if necessary. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 361 nm and 365 nm, between 380 nm and 384 nm and between 403 nm and 407 nm.

pH <*2.54*> Dissolve an amount of Amphotericin B for Injection, equivalent to 50 mg (potency) of Amphotericin B, in 10 mL of water. To 1 mL of this solution add water to make 50 mL: 7.2 – 8.0.

Purity Clarity and color of solution—Dissolve an amount of Amphotericin B for Injection, equivalent to 50 mg (potency) of Amphotericin B, in 10 mL of water: the solution is clear and yellow to orange.

Loss on drying <*2.41*> Not more than 8.0% (0.3 g, in vacuum, 60°C, 3 hours).

Bacterial endotoxins <*4.01*> Less than 3.0 EU/mg (potency).

Uniformity of dosage units <*6.02*> It meets the requirement

of the Mass variation test (*T*: 105.0%).

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism, culture medium, preparation of cylinder-agar plate and standard solutions—Proceed as directed in the Assay under Amphotericin B.

(ii) Sample solutions—Prepare using light-resistant containers. Weigh accurately an amount of Amphotericin B for Injection, equivalent to about 50 mg (potency), dissolve in dimethylsulfoxide to make exactly 50 mL, and use this solution as the sample stock solution. Measure exactly a suitable quantity of the sample stock solution, add dimethylsulfoxide to make solutions so that each mL contains about 200 μg (potency) and 50 μg (potency). Pipet 1 mL each of these solutions, add 0.2 mol/L phosphate buffer solution (pH 10.5) to make exactly 20 mL, and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers. Storage—Light-resistant, at a cold place.

Amphotericin B Syrup

アムホテリシン **B** シロップ

Amphotericin B Syrup contain not less than 90.0% and not more than 115.0% of the labeled potency of amphotericin B ($C_{47}H_{73}NO_{17}$: 924.08).

Method of preparation Prepare as directed under Syrup, with Amphotericin B.

Identification To an amount of Amphotericin B Syrup, equivalent to 25 mg (potency) of Amphotericin B, add 5 mL of dimethylsulfoxide and 45 mL of methanol, and shake. To 1 mL of this solution add methanol to make 50 mL, and filter, if necessary. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 361 nm and 365 nm, between 380 nm and 384 nm and between 403 nm and 407 nm.

pH ⟨*2.54*⟩ 5.0 – 7.0

Microbial limit ⟨*4.05*⟩ The acceptance criteria of TAMC and TYMC are 10^2 CFU/mL and 5×10^1 CFU/mL, respectively.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism, culture medium, preparation of cylinder-agar plate and standard solutions—Proceed as directed in the Assay under Amphotericin B.

(ii) Sample solutions—Prepare using light-resistant containers. Weigh accurately an amount of Amphotericin B Syrup, equivalent to about 0.1 g (potency), add about 70 mL of dimethylsulfoxide, shake, then add dimethylsulfoxide to make exactly 100 mL, and use this solution as the sample stock solution. Measure exactly a suitable amount of the sample stock solution, add dimethylsulfoxide to make solutions so that each mL contains about 200 μg (potency) and 50 μg (potency). Pipet 1 mL each of these solutions, add 0.2 mol/L phosphate buffer solution (pH 10.5) to make exactly 20 mL, and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Amphotericin B Tablets

アムホテリシン **B** 錠

Amphotericin B Tablets contain not less than 90.0% and not more than 120.0% of the labeled potency of amphotericin B ($C_{47}H_{73}NO_{17}$: 924.08).

Method of preparation Prepare as directed under Tablets, with Amphotericin B.

Identification To an amount of pulverized Amphotericin B Tablets, equivalent to 25 mg (potency) of Amphotericin B, add 5 mL of dimethylsulfoxide and 45 mL of methanol, and shake. To 1 mL of this solution add methanol to make 50 mL, and filter, if necessary. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 361 nm and 365 nm, between 380 nm and 384 nm and between 403 nm and 407 nm.

Loss on drying ⟨*2.41*⟩ Not more than 5.0% (0.3 g, in vacuum, 60°C, 3 hours).

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test (*T*: 105.0%).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism, culture medium, preparation of cylinder-agar plate and standard solutions—Proceed as directed in the Assay under Amphotericin B.

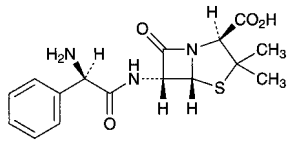
(ii) Sample solutions—Prepare using light-resistant containers. Weigh accurately and powder not less than 20 tablets of Amphotericin B Tablets. Weigh accurately a part of the powder, equivalent to about 0.1 g (potency), add about 70 mL of dimethylsulfoxide, shake, then add dimethylsulfoxide to make exactly 100 mL, centrifuge, and use the supernatant liquid as the sample stock solution. Measure exactly a suitable amount of the sample stock solution, add dimethylsulfoxide to make solutions so that each mL contains 200 μg (potency) and 50 μg (potency). Pipet 1 mL each of these solutions, add 0.2 mol/L phosphate buffer solution (pH 10.5) to make exactly 20 mL, and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Well-closed containers.

Anhydrous Ampicillin

Anhydrous Aminobenzylpenicillin

無水アンピシリン



$C_{16}H_{19}N_3O_4S$: 349.40
(2*S*,5*R*,6*R*)-6-[(2*R*)-2-Amino-2-phenylacetylamino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
[*69-53-4*]

Anhydrous Ampicillin contains not less than 960 μg (potency) and not more than 1005 μg (potency) per mg, calculated on the anhydrous basis. The potency of Anhydrous Ampicillin is expressed as mass (potency) of ampicillin ($C_{16}H_{19}N_3O_4S$).

Description Anhydrous Ampicillin occurs as white to light yellowish white, crystals or crystalline powder.

It is sparingly soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

Identification Determine the infrared absorption spectrum of Anhydrous Ampicillin as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +280 – +305° (0.5 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

pH <*2.54*> The pH of a solution obtained by dissolving 1.0 g of Anhydrous Ampicillin in 100 mL of water is between 4.0 and 5.5.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Anhydrous Ampicillin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Anhydrous Ampicillin according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.05 g of Anhydrous Ampicillin in the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of each peak other than ampicillin from the sample solution is not larger than the peak area of ampicillin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: As long as about 10 times of the retention time of ampicillin.

System suitability—

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of ampicillin obtained from 10 μL of this solution is equivalent to 7 to 13% of that obtained from 10 μL of the standard solution.

Water <*2.48*> Not more than 2.0% (2.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Anhydrous Ampicillin and Ampicillin RS, equivalent to about 50 mg (potency), add exactly 5 mL each of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ampicillin to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of ampicillin } (C_{16}H_{19}N_3O_4S) = M_S \times Q_T/Q_S \times 1000$$

M_S: Amount [mg (potency)] of Ampicillin RS taken

Internal standard solution—A solution of guaifenesin in the mobile phase (1 in 200).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.94 g of diammonium hydrogen phosphate in 850 mL of water, add 100 mL of acetonitrile, adjust the pH to 5.0 with phosphoric acid, and add water to make exactly 1000 mL.

Flow rate: Adjust so that the retention time of ampicillin is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ampicillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 40.

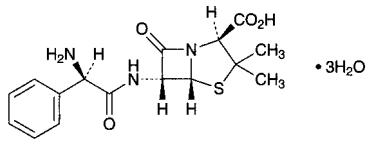
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ampicillin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Ampicillin Hydrate

Aminobenzylpenicillin

アンピシリン水和物



$C_{16}H_{19}N_3O_4S.3H_2O$: 403.45
(2*S*,5*R*,6*R*)-6-[(2*R*)-2-Amino-2-phenylacetylamino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate
[*7177-48-2*]

Ampicillin Hydrate contains not less than 960 μg (potency) and not more than 1005 μg (potency) per mg, calculated on the anhydrous basis. The potency of Ampicillin Hydrate is expressed as mass (potency) of ampicillin ($C_{16}H_{19}N_3O_4S$: 349.40).

Description Ampicillin Hydrate occurs as a white to light yellowish white, crystals or crystalline powder.

It is sparingly soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

Identification Determine the infrared absorption spectrum of Ampicillin Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Ampicillin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +280 – +305° (0.5 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

pH <*2.54*> The pH of a solution obtained by dissolving 1.0 g of Ampicillin Hydrate in 400 mL of water is between 3.5 and 5.5.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Ampicillin Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Ampicillin hydrate according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Ampicillin hydrate in the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than ampicillin obtained from the sample solution is not larger than the peak area of ampicillin obtained from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 10 times as long as the retention time of ampicillin.

System suitability—

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of ampicillin obtained from 10 μL of this solution is equivalent to 7 to 13% of that obtained from 10 μL of the standard solution.

(4) *N*,*N*-Dimethylaniline—Weigh accurately about 1 g of Ampicillin Hydrate, dissolve in 5 mL of sodium hydroxide TS, add exactly 1 mL of the internal standard solution, shake vigorously for 1 minute, and use the upper layer liquid obtained after allowing it to stand as the sample solution. Separately, weigh accurately about 50 mg of *N*,*N*-dimethylaniline, dissolve in 2 mL of hydrochloric acid and 20 mL of water, add water to make exactly 50 mL, and use this solution as the standard stock solution. Pipet 5 mL of the standard stock solution, and add water to make exactly 250 mL. Pipet 1 mL of this solution, add 5 mL of sodium hydroxide TS and exactly 1 mL of the internal standard solution, shake vigorously for 1 minute, and use the upper layer liquid obtained after allowing it to stand as the standard solution. Perform the test with 1 μL each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following conditions, calculate the ratios, Q_T and Q_S, of the peak area of *N*,*N*-dimethylaniline to that of the internal standard, and calculate the amount of *N*,*N*-dimethylaniline by the following equation: not more than 20 ppm.

$$\text{Amount (ppm) of } N,N\text{-dimethylaniline} = M_S/M_T \times Q_T/Q_S \times 400$$

M_S: Amount (g) of *N*,*N*-dimethylaniline taken
M_T: Amount (g) of Ampicillin Hydrate taken

Internal standard solution—A solution of naphthalene in cyclohexane (1 in 20,000).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 2.6 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography (180 – 250 μm in particle diameter) coated with 50% phenyl-50% methyl polysiloxane for gas chromatography at the ratio of 3%.

Column temperature: A constant temperature of about 120°C.

Carrier gas: Helium.

Flow rate: Adjust so that the retention time of *N*,*N*-dimethylaniline is about 5 minutes.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard stock solution, and add water to make exactly 250 mL. Pipet 1 mL of this solution, add 5 mL of sodium hydroxide TS and exactly 1 mL of the internal standard solution, shake vigorously for 1 minute, and use the upper layer liquid obtained after allowing it to stand for the test. Confirm that when the procedure is run with 1 μL of the upper layer liquid under the above operating conditions, the ratio of the peak area of *N*,*N*-dimethylaniline to that of the internal standard is equivalent to 15 to 25% of the ratio of the peak area of *N*,*N*-dimethylaniline to that of the internal standard obtained from the standard solution.

System performance: Dissolve 50 mg of *N*,*N*-dimethylaniline in cyclohexane to make 50 mL. To 1 mL of this solution add the internal standard solution to make 50 mL, and use this solution as the solution for system suitability test. When the procedure is run with 1 μL of the solution for system

suitability test under the above operating conditions, *N*,*N*-dimethylaniline and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 1 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the ratios of the peak area of *N*,*N*-dimethylaniline to that of the internal standard is not more than 2.0%.

Water ⟨*2.48*⟩ 12.0 – 15.0% (0.1 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Ampicillin Hydrate and Ampicillin RS, equivalent to about 50 mg (potency), dissolve in a suitable volume of the mobile phase, add exactly 5 mL each of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ampicillin to that of the internal standard.

Amount [μg (potency)] of ampicillin ($C_{16}H_{19}N_3O_4S$)
$= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Ampicillin RS taken

Internal standard solution—A solution of guaifenesin in the mobile phase (1 in 200).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.94 g of diammonium hydrogenphosphate in 850 mL of water, add 100 mL of acetonitrile, adjust the pH to 5.0 with phosphoric acid, and add water to make exactly 1000 mL.

Flow rate: Adjust so that the retention time of ampicillin is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ampicillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 40.

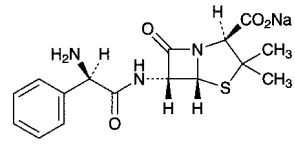
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ampicillin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Ampicillin Sodium

Aminobenzylpenicillin Sodium

アンピシリンナトリウム



$C_{16}H_{18}N_3NaO_4S$: 371.39
Monosodium (2*S*,5*R*,6*R*)-6-[(2*R*)-2-amino-2-phenylacetylamino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate
[*69-52-3*]

Ampicillin Sodium contains not less than 850 μg (potency) and not more than 950 μg (potency) per mg, calculated on the anhydrous basis. The potency of Ampicillin Sodium is expressed as mass (potency) of ampicillin ($C_{16}H_{19}N_3O_4S$: 349.40).

Description Ampicillin Sodium occurs as white to light yellowish white, crystals or crystalline powder.

It is very soluble in water, and sparingly soluble in ethanol (99.5).

Identification **(1)** Determine the infrared absorption spectrum of Ampicillin Sodium, previously dried in a desiccator (reduced pressure not exceeding 0.67 kPa, 60°C) for 3 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Ampicillin Sodium responds to the Qualitative Tests ⟨*1.09*⟩ (1) for sodium salt.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +246 – +272° (1 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

pH ⟨*2.54*⟩ The pH of a solution obtained by dissolving 1.0 g of Ampicillin Sodium in 10 mL of water is between 8.0 and 10.0.

Purity **(1)** Clarity and color of solution—Dissolve 0.25 g (potency) of Ampicillin Sodium in 0.75 mL of water: the solution is clear, and its absorbance at 400 nm, determined as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, is not more than 0.40.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Ampicillin Sodium according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Ampicillin Sodium according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Ampicillin Sodium in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peaks other than ampicillin obtained from the sample solution is not larger than the peak area of ampicillin obtained

from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 10 times as long as the retention time of ampicillin.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of ampicillin obtained from 10 μL of this solution is equivalent to 7 to 13% of that of ampicillin obtained from 10 μL of the standard solution.

System performance: Dissolve 50 mg of Ampicillin RS in a suitable amount of the mobile phase, add 5 mL of a solution of guaifenesin in the mobile phase (1 in 200) and the mobile phase to make 50 mL, and use this solution as the solution for system suitability test. When the procedure is run with 10 μL of the solution for system suitability test under the above operating conditions, ampicillin and guaifenesin are eluted in this order with the resolution between these peaks being not less than 35.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of ampicillin is not more than 1.0%.

Water ⟨*2.48*⟩ Not more than 2.0% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Ampicillin Sodium and Ampicillin RS, equivalent to about 50 mg (potency), dissolve them in a suitable amount of the mobile phase, add exactly 5 mL of the internal standard solution, then add the mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ampicillin to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of ampicillin } (C_{16}H_{19}N_3O_4S) = M_S \times Q_T/Q_S \times 1000$$

M_S: Amount [mg (potency)] of Ampicillin RS taken

Internal standard solution—A solution of guaifenesin in the mobile phase (1 in 200).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.94 g of diammonium hydrogen phosphate in 850 mL of water, add 100 mL of acetonitrile, adjust to pH 5.0 with phosphoric acid, and add water to make 1000 mL.

Flow rate: Adjust so that the retention time of ampicillin is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ampicillin and guaifenesin are eluted in this order with the resolution between these peaks being not less than 35.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ampicillin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Ampicillin Sodium for Injection

注射用アンピシリンナトリウム

Ampicillin Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled potency of ampicillin ($C_{16}H_{19}N_3O_4S$: 349.40).

Method of preparation Prepare as directed under Injections, with Ampicillin Sodium.

Description Ampicillin Sodium for Injection occurs as white to light yellowish white, crystals or crystalline powder.

Identification Proceed as directed in the Identification (1) under Ampicillin Sodium.

Osmotic pressure ratio Being specified separately when the drug is granted approval based on the Law.

pH ⟨*2.54*⟩ The pH of a solution prepared by dissolving an amount of Ampicillin Sodium for Injection, equivalent to 1.0 g (potency) of Ampicillin Sodium, in 10 mL of water is 8.0 to 10.0.

Purity Clarity and color of solution—Dissolve an amount of Ampicillin Sodium for Injection, equivalent to 0.25 g (potency) of Ampicillin Sodium, in 0.75 mL of water: the solution is clear. Perform the test with the solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: the absorbance at 400 nm is not more than 0.40.

Water ⟨*2.48*⟩ Not more than 3.0% (0.2 g, volumetric titration, direct titration).

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.075 EU/mg (potency).

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 containers of Ampicillin Sodium for Injection. Weigh accurately an amount of a portion of the contents, equivalent to about 50 mg (potency) of Ampicillin Sodium, add exactly 5 mL of the internal standard solution and dissolve. Then add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Ampicillin RS, equivalent to about 50 mg (potency), add exactly 5 mL of the internal standard solution and dissolve. Then add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform

the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ampicillin to that of the internal standard.

Amount [mg (potency)] of ampicillin ($C_{16}H_{19}N_3O_4S$) = $M_S \times Q_T/Q_S$

M_S: Amount [mg (potency)] of Ampicillin RS taken

Internal standard solution—A solution of guaifenesin in the mobile phase (1 in 200).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.94 mg of diammonium hydrogen phosphate in 850 mL of water, add 100 mL of acetonitril, add phosphoric acid to adjust the pH to 5.0, then add water to make exactly 1000 mL.

Flow rate: Adjust so that the retention time of ampicillin is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ampicillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 26.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ampicillin to that of the internal standard is not more than1.0%.

Containers and storage Containers—Hermetic containers.

Ampicillin Sodium and Sulbactam Sodium for Injection

注射用アンピシリンナトリウム·スルバクタムナトリウム

Ampicillin Sodium and Sulbactam Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 95.0% and not more than 112.0% of the labeled potency of ampicillin ($C_{16}H_{19}N_3O_4S$: 349.40) and sulbactam ($C_8H_{11}NO_5S$: 233.24).

Method of preparation Prepare as directed under Injections, with Ampicillin Sodium and Sulbactam Sodium.

Description Ampicillin Sodium and Sulbactam Sodium for Injection occurs as a white to yellowish white powder.

Identification (1) The retention times of ampicillin obtained from the sample solution and the standard solution observed in the Assay are the same, and the peak area of ampicillin observed in the Assay obtained from the sample solution is 2.8 to 3.6 times the peak area of ampicillin observed in the test performed with 10 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography <2.01> according to the following conditions.

Operating conditions—

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

(2) The retention times of sulbactam obtained from the sample solution and the standard solution observed in the Assay are the same, and the peak area of sulbactam observed in the Assay obtained from the sample solution is 2.0 to 2.6 times the peak area of sulbactam observed in the test performed with 10 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography <2.01> according to the following conditions.

Operating conditions—

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

pH <2.54> The pH of a solution prepared by dissolving an amount of Ampicillin Sodium and Sulbactam Sodium for Injection, equivalent to 1.0 g (potency) of ampicillin ($C_{16}H_{19}N_3O_4S$), in 10 mL of water is between 8.0 and 10.0.

Purity (1) Clarity and color of solution—Dissolve an amount of Ampicillin Sodium and Sulbactam Sodium for Injection, equivalent to 1.0 g (potency) of ampicillin ($C_{16}H_{19}N_3O_4S$), in 10 mL of water: the solution is clear. Determine the absorption of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance at 425 nm is not more than 0.10.

(2) Total penicilloic acid—Weigh accurately about 25 mg of Ampicillin Sodium and Sulbactam Sodium for Injection, place in a glass-stoppered flask, dissolve in 25 mL of 0.02 mol/L phosphate buffer solution (pH 3.0), add exactly 5 mL of 0.005 mol/L iodine VS, stopper the flask, allow to stand for 5 minutes, and titrate <2.50> with 0.005 mol/L sodium thiosulfate VS (indicator: 1.0 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction: the amount of total penicilloic acid (as $C_{16}H_{21}N_3O_5S$: 367.42) is not more than 3.0%.

Each mL of 0.005 mol/L sodium thiosulfate VS = 0.2064 mg of $C_{16}H_{21}N_3O_5S$

Water <2.48> Not more than 2.0% (0.5 g, volumetric titration, direct titration).

Bacterial endotoxins <4.01> Less than 0.10 EU/mg (potency).

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test (*T*: 105.0%).

Dissolve 1 Ampicillin Sodium and Sulbactam Sodium for Injection in the mobile phase to make exactly *V* mL so that each mL contains 5 mg (potency) of ampicillin ($C_{16}H_{19}N_3O_4S$). Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add the mobile phase to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount [mg (potency)] of ampicillin ($C_{16}H_{19}N_3O_4S$) $= M_{S1} \times Q_{Ta}/Q_{Sa} \times V/10$

Amount [mg (potency)] of sulbactam ($C_8H_{11}NO_5S$) $= M_{S2} \times Q_{Tb}/Q_{Sb} \times V/10$

M_{S1}: Amount [mg (potency)] of Ampicillin RS taken
M_{S2}: Amount [mg (potency)] of Sulbactam RS taken

Internal standard solution—A solution of parahydroxybenzoic acid in the mobile phase (1 in 1000).

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 containers of Ampicillin Sodium and Sulbactam Sodium for Injection. Weigh accurately an amount of a portion of the contents, equivalent to about 0.25 g (potency) of ampicillin ($C_{16}H_{19}N_3O_4S$), and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Ampicillin RS, equivalent to about 50 mg (potency), and an amount of Sulbactam RS, equivalent to about 25 mg (potency), dissolve in the mobile phase, add exactly 10 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_{Ta} and Q_{Tb}, of the peak areas of ampicillin and sulbactam to that of the internal standard obtained from the sample solution, and the ratios, Q_{Sa} and Q_{Sb}, of the peak areas of ampicillin and sulbactam to that of the internal standard obtained from the standard solution.

Amount [mg (potency)] of ampicillin ($C_{16}H_{19}N_3O_4S$) $= M_{S1} \times Q_{Ta}/Q_{Sa} \times 5$

Amount [mg (potency)] of sulbactam ($C_8H_{11}NO_5S$) $= M_{S2} \times Q_{Tb}/Q_{Sb} \times 5$

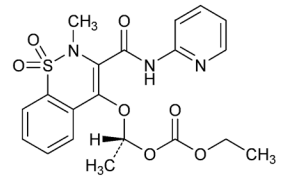
M_{S1}: Amount [mg (potency)] of Ampicillin RS taken
M_{S2}: Amount [mg (potency)] of Sulbactam RS taken

Internal standard solution—A solution of parahydroxybenzoic acid in the mobile phase (1 in 1000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 215 mn).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).
Column temperature: A constant temperature of about 35°C.
Mobile phase: A mixture of 0.02 mol/L phosphate buffer (pH 3.0) and acetonitrile for liquid chromatography (23:2).
Flow rate: Adjust so that the retention time of the internal standard is about 9 minutes.
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, sulbactam, the internal standard and ampicillin are eluted in this order, and either resolution between these peaks is not less than 2.0.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sulbactam is not more than 1.0%.

Containers and storage—Hermetic containers. Plastic containers for aqueous injections may be used.

Ampiroxicam

アンピロキシカム



and enantiomer

$C_{20}H_{21}N_3O_7S$: 447.46
Ethyl (1*RS*)-1-({2-methyl-1,1-dioxido-3-[(pyridin-2-ylamino)carbonyl]-2*H*-1,2-benzothiazin-4-yl}oxy)ethyl carbonate
[*99464-64-9*]

Ampiroxicam, when dried, contains not less than 99.0% and not more than 101.0% of ampiroxicam ($C_{20}H_{21}N_3O_7S$).

Description Ampiroxicam occurs as a white to yellowish white crystalline powder.

It is freely soluble in acetic acid (100), soluble in acetonitrile, very slightly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Ampiroxicam in acetonitrile (1 in 20) shows no optical rotation.

It shows crystal polymorphism.

Identification **(1)** Determine the absorption spectrum of a solution of Ampiroxicam in 0.01 mol/L hydrochloric acid-methanol TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ampiroxicam as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Ampiroxicam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead solution (not more than 20 ppm).

(2) Related substances—Dissolve 20 mg of Ampiroxicam in 50 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having a relative retention time of about 0.17 to ampiroxicam, obtained from the sample solution is not larger than 1/2 times the peak area of ampiroxicam obtained from the standard solution, the area of the peak other than ampiroxicam and the peak mentioned above from the sample solution is not larger than 2/5

times the peak area of ampiroxicam from the standard solution, and the total area of the peaks other than ampiroxicam from the sample solution is not larger than the peak area of ampiroxicam from the standard solution. For the area of the peaks, having the relative retention time of about 0.17 and about 0.46 to ampiroxicam, multiply the relative response factor, 0.37 and 0.60, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of diluted acetic acid (100) (3 in 500), methanol and acetonitrile (5:3:2).

Flow rate: Adjust so that the retention time of ampiroxicam is about 9 minutes.

Time span of measurement: About 2 times as long as the retention time of ampiroxicam, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, add acetonitrile to make exactly 50 mL. Confirm that the peak area of ampiroxicam obtained with 10 μL of this solution is equivalent to 7 to 13% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ampiroxicam are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ampiroxicam is not more than 5%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Assay Weigh accurately about 0.22 g of Ampiroxicam, previously dried, dissolve in 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 44.75 mg of $C_{20}H_{21}N_3O_7S$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Ampiroxicam Capsules

アンピロキシカムカプセル

Ampiroxicam Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of ampiroxicam ($C_{20}H_{21}N_3O_7S$: 447.46).

Method of Preparation Prepare as directed under Capsules, with Ampiroxicam.

Identification Take out the contents of Ampiroxicam Capsules, to a quantity of the contents, equivalent to 10 mg of Ampiroxicam, add 100 mL of 0.01 mol/L hydrochloric acid-methanol TS, shake well, and centrifuge. To 5 mL of the supernatant liquid add 0.01 mol/L hydrochloric acid-methanol TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 318 nm and 322 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take out the contents of 1 capsule of Ampiroxicam Capsules, add acetonitrile to make exactly V mL so that each mL contains about 0.27 mg of ampiroxicam ($C_{20}H_{21}N_3O_7S$). Stir for 30 minutes, then centrifuge, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of ampiroxicam ($C_{20}H_{21}N_3O_7S$)
$= M_S \times A_T/A_S \times V/100$

M_S: Amount (mg) of ampiroxicam for assay taken

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Ampiroxicam Capsules is not less than 70%.

Start the test with 1 capsule of Ampiroxicam Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 15 μg of ampiroxicam ($C_{20}H_{21}N_3O_7S$), and use this solution as the sample solution. Separately, weigh accurately about 30 mg of ampiroxicam for assay, previously dried at 105°C for 3 hours, dissolve in 5 mL of acetonitrile, and add the dissolution medium to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 320 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using the dissolution medium as the blank.

Dissolution rate (%) with respect to the labeled amount of ampiroxicam ($C_{20}H_{21}N_3O_7S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$

M_S: Amount (mg) of ampiroxicam for assay taken
C: Labeled amount (mg) of ampiroxicam ($C_{20}H_{21}N_3O_7S$) in 1 capsule

Assay Take out the contents of not less than 20 Ampiroxicam Capsules, weigh accurately the mass of the contents, and powder if necessary. Weigh accurately a portion of the powder, equivalent to about 13.5 mg of ampiroxicam ($C_{20}H_{21}N_3O_7S$), and add acetonitrile to make exactly 50 mL. Stir for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 27 mg of ampiroxicam for assay, previously dried at 105°C for 3 hours, dissolve in acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following condition, and determine the peak areas, A_T and A_S, of ampiroxicam in each solution.

Amount (mg) of ampiroxicam ($C_{20}H_{21}N_3O_7S$)
$= M_S \times A_T/A_S \times 1/2$

M_S: Amount (mg) of ampiroxicam for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of diluted acetic acid (100) (3 in 500), methanol, and acetonitrile (5:3:2).

Flow rate: Adjust so that the retention time of ampiroxicam is about 9 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ampiroxicam are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ampiroxicam is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Amyl Nitrite

亜硝酸アミル

$C_5H_{11}NO_2$: 117.15

Amyl Nitrite is the nitrous acid ester of 3-methylbutanol-1 and contains a small quantity of 2-methylbutanol-1 and the nitrous acid esters of other homologues.

It contains not less than 90.0% of amyl nitrite ($C_5H_{11}NO_2$).

Description Amyl Nitrite is a clear, light yellowish liquid, and has a characteristic, fruity odor.

It is miscible with ethanol (95), and with diethyl ether.

It is practically insoluble in water.

It is affected by light and by heat.

It is volatile at ordinary temperature and flammable even at a low temperature.

Boiling point: about 97°C

Identification Determine the infrared spectrum of Amyl Nitrite as directed in the liquid film method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: 0.871 – 0.880

Purity **(1)** Acidity—To 5 mL of Amyl Nitrite add a mixture of 1.0 mL of 1 mol/L sodium hydroxide VS, 10 mL of water and 1 drop of phenolphthalein TS, shake, and allow to stand for 1 minute: the light red color of the water layer does not disappear.

(2) Water—Allow 2.0 mL of Amyl Nitrite to stand in ice water: no turbidity is produced.

(3) Aldehyde—To 3 mL of a mixture of equal volumes of silver nitrate TS and aldehyde free-ethanol add ammonia TS dropwise until the precipitate first formed is redissolved. Add 1.0 mL of Amyl Nitrite, and warm between 60°C and 70°C for 1 minute: a brown to black color is not produced.

(4) Residue on evaporation—Evaporate 10.0 mL of Amyl Nitrite on a water bath in a draft chamber, carefully protecting from flame, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 1.0 mg.

Assay Weigh accurately a volumetric flask containing 10 mL of ethanol (95), add about 0.5 g of Amyl Nitrite, and weigh accurately again. Add exactly 25 mL of 0.1 mol/L silver nitrate VS, then add 15 mL of potassium chlorate solution (1 in 20) and 10 mL of dilute nitric acid, stopper the flask immediately, and shake it vigorously for 5 minutes. Dilute with water to make exactly 100 mL, shake, and filter through dry filter paper. Discard the first 20 mL of the filtrate, measure exactly 50 mL of the subsequent filtrate, and titrate ⟨*2.50*⟩ the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L silver nitrate VS
= 35.15 mg of $C_5H_{11}NO_2$

Containers and storage Containers—Hermetic containers not exceeding 10–ml capacity.

Storage—Light-resistant, in a cold place, and remote from fire.

Dental Antiformin

Dental Sodium Hypochlorite Solution

歯科用アンチホルミン

Dental Antiformin contains not less than 3.0 w/v% and not more than 6.0 w/v% of sodium hypochlorite (NaClO: 74.44).

Description Dental Antiformin is a slightly light yellow-green, clear liquid. It has a slight odor of chlorine.

It gradually changes by light.

Identification **(1)** Dental Antiformin changes red litmus paper to blue, and then decolorizes it.

(2) To Dental Antiformin add dilute hydrochloric acid: it evolves the odor of chlorine, and the gas changes potassium iodide starch paper moistened with water to blue.

(3) Dental Antiformin responds to the Qualitative Tests ⟨*1.09*⟩ (1) for sodium salt.

Assay Measure exactly 3 mL of Dental Antiformin in a glass-stoppered flask, add 50 mL of water, 2 g of potassium iodide and 10 mL of acetic acid (31), and titrate ⟨*2.50*⟩ the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS).

Each mL of 0.1 mol/L sodium thiosulfate VS
= 3.722 mg of NaClO

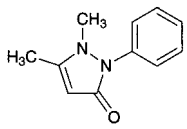
Containers and storage Containers—Tight containers.

Storage—Light-resistant, and not exceeding 10°C.

Antipyrine

Phenazone

アンチピリン



$C_{11}H_{12}N_2O$: 188.23
1,5-Dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one
[*60-80-0*]

Antipyrine, when dried, contains not less than 99.0% of antipyrine ($C_{11}H_{12}N_2O$).

Description Antipyrine occurs as colorless or white crystals, or a white, crystalline powder. It is odorless, and has a slightly bitter taste.

It is very soluble in water, freely soluble in ethanol (95), and sparingly soluble in diethyl ether.

A solution of Antipyrine (1 in 10) is neutral.

Identification **(1)** To 5 mL of a solution of Antipyrine (1 in 100) add 2 drops of sodium nitrite TS and 1 mL of dilute sulfuric acid: a deep green color develops.

(2) To 2 mL of a solution of Antipyrine (1 in 100) add 4 drops of dilute iron (III) chloride TS: a yellow-red color develops. Then add 10 drops of dilute sulfuric acid: the color changes to light yellow.

(3) To 5 mL of a solution of Antipyrine (1 in 100) add 2 to 3 drops of tannic acid TS: a white precipitate is produced.

(4) To 0.1 g of Antipyrine add 0.1 g of vanillin, 5 mL of water and 2 mL of sulfuric acid, boil the mixture, and cool: a yellow-red precipitate is produced.

Melting point ⟨*2.60*⟩ 111 – 113°C

Purity **(1)** Chloride ⟨*1.03*⟩—Perform the test with 1.0 g of Antipyrine. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Antipyrine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Readily carbonizable substances⟨*1.15*⟩—Perform the test with 0.5 g of Antipyrine: the solution remains colorless.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, silica gel, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

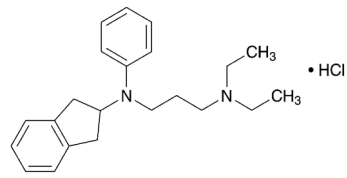
Assay Dissolve about 0.2 g of Antipyrine, previously dried and accurately weighed, in 20 mL of sodium acetate TS, add exactly 30 mL of 0.05 mol/L iodine VS, and allow to stand for 20 minutes with occasional shaking. Dissolve the precipitate in 10 mL of chloroform, and titrate ⟨*2.50*⟩ the excess iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L iodine VS
= 9.412 mg of $C_{11}H_{12}N_2O$

Containers and storage Containers—Well-closed containers.

Aprindine Hydrochloride

アプリンジン塩酸塩



$C_{22}H_{30}N_2$.HCl: 358.95
N-(2,3-Dihydro-1*H*-inden-2-yl)-*N*′,*N*′-diethyl-*N*-phenylpropane-1,3-diamine monohydrochloride
[*33237-74-0*]

Aprindine Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of aprindine hydrochloride ($C_{22}H_{30}N_2$.HCl).

Description Aprindine Hydrochloride occurs as a white to pale yellowish white crystalline powder. It has a bitter taste, numbing the tongue.

It is very soluble in water, in methanol and in acetic acid (100), and freely soluble in ethanol (99.5).

It gradually turns brown on exposure to light.

Identification **(1)** Dissolve 10 mg of Aprindine Hydrochloride in a solution of hydrochloric acid in diluted ethanol (1 in 2) (1 in 125) to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Aprindine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 5 mL of a solution of Aprindine Hydrochloride (1 in 50) add 1 mL of dilute nitric acid: this solution responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

pH ⟨*2.54*⟩ Dissolve 1.0 g of Aprindine Hydrochloride in 50 mL of water: the pH of the solution is between 6.4 and 7.0.

Melting point ⟨*2.60*⟩ 127 – 131°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Aprindine Hydrochloride in 10 mL of methanol: the solution is clear, and its absorbance at 420 nm determined as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩ is not more than 0.10.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Aprindine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 25 mg of Aprindine Hydrochloride in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak other than aprindine obtained from the sample solution is not larger than 1/10 times the peak area

of aprindine obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.40 g of potassium dihydrogen phosphate in 500 mL of water, and adjust the pH to 3.0 with hydrochloric acid. To 500 mL of this solution add 500 mL of acetonitrile.

Flow rate: Adjust so that the retention time of aprindine is about 6 minutes.

Time span of measurement: About 4 times as long as the retention time of aprindine.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of aprindine obtained from 10 μL of this solution is equivalent to 7 to 13% of that obtained from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of aprindine are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of aprindine is not more than 1.5%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, 60°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Aprindine Hydrochloride, previously dried, dissolve in 80 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 35.90 mg of $C_{22}H_{30}N_2$.HCl

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Aprindine Hydrochloride Capsules

アプリンジン塩酸塩カプセル

Aprindine Hydrochloride Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of aprindine hydrochloride ($C_{22}H_{30}N_2$.HCl: 358.95).

Method of preparation Prepare as directed under Capsules, with Aprindine Hydrochloride.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, it exhibits maxima between 264 nm and 268 nm, and between 271 nm and 275 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take out the contents of 1 capsule of Aprindine Hydrochloride Capsules, add 30 mL of a solution of hydrochloric acid in diluted ethanol (1 in 2) (1 in 125), shake vigorously for 20 minutes, add a solution of hydrochloric acid in diluted ethanol (1 in 2) (1 in 125) to make exactly V mL so that each mL contains about 0.2 mg of aprindine hydrochloride ($C_{22}H_{30}N_2$.HCl), and filter. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Proceed as directed in the Assay.

Amount (mg) of aprindine hydrochloride ($C_{22}H_{30}N_2$.HCl)
$= M_S \times A_T/A_S \times V/250$

M_S: Amount (mg) of aprindine hydrochloride for assay taken

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Aprindine Hydrochloride Capsules is not less than 80%.

Start the test with 1 capsule of Aprindine Hydrochloride Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 11 μg of aprindine hydrochloride ($C_{22}H_{30}N_2$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of aprindine hydrochloride for assay, previously dried in vacuum at 60°C for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of aprindine in each solution.

Dissolution rate (%) with respect to the labeled amount of aprindine hydrochloride ($C_{22}H_{30}N_2$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of aprindine hydrochloride for assay taken

C: Labeled amount (mg) of aprindine hydrochloride ($C_{22}H_{30}N_2$.HCl) in 1 capsule

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.40 g of potassium dihydrogen phosphate in 500 mL of water, and adjust the pH to 3.0 with hydrochloric acid. To 500 mL of this solution add 500 mL of acetonitrile.

Flow rate: Adjust so that the retention time of aprindine is about 6 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of aprindine are not less than 3000 and

not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of aprindine is not more than 1.5%.

Assay Take out the contents of not less than 20 Aprindine Hydrochloride Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of aprindine hydrochloride ($C_{22}H_{30}N_2$.HCl), add 60 mL of a solution of hydrochloric acid in diluted ethanol (1 in 2) (1 in 125), shake vigorously for 20 minutes, and add a solution of hydrochloric acid in diluted ethanol (1 in 2) (1 in 125) to make exactly 100 mL. Pipet 10 mL of this solution, add a solution of hydrochloric acid in diluted ethanol (1 in 2) (1 in 125) to make exactly 50 mL, and filter, Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of aprindine hydrochloride for assay, previously dried in vacuum at 60°C for 4 hours, and dissolve in a solution of hydrochloric acid in diluted ethanol (1 in 2) (1 in 125) to make exactly 50 mL. Pipet 10 mL of this solution, add a solution of hydrochloric acid in diluted ethanol (1 in 2) (1 in 125) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 265 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

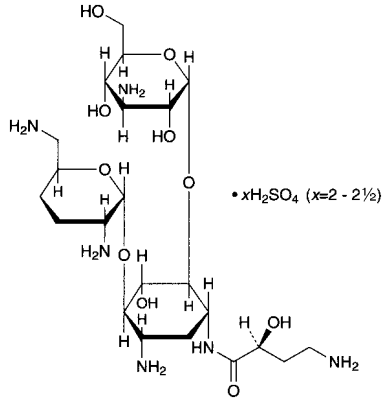
Amount (mg) of aprindine hydrochloride ($C_{22}H_{30}N_2$.HCl) $= M_S \times A_T/A_S \times 2$

M_S: Amount (mg) of aprindine hydrochloride for assay taken

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Arbekacin Sulfate

アルベカシン硫酸塩



$C_{22}H_{44}N_6O_{10}.xH_2SO_4$ ($x = 2 - 2½$)
3-Amino-3-deoxy-α-D-glucopyranosyl-(1→6)-[2,6-diamino-2,3,4,6-tetradeoxy-α-D-*erythro*-hexopyranosyl-(1→4)]-1-*N*-[(2*S*)-4-amino-2-hydroxybutanoyl]-2-deoxy-D-streptamine sulfate
[*51025-85-5*, Arbekacin]

Arbekacin Sulfate is the sulfate of a derivative of dibekacin.

It contains not less than 670 μg (potency) and not more than 750 μg (potency) per mg, calculated on the dried basis. The potency of Arbekacin Sulfate is expressed as mass (potency) of arbekacin ($C_{22}H_{44}N_6O_{10}$: 552.62).

Description Arbekacin Sulfate occurs as a white powder.

It is very soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) Dissolve 10 mg each of Arbekacin Sulfate and Arbekacin Sulfate RS in 1 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia solution (28), methanol, chloroform and ethanol (95) (7:6:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100°C for 10 minutes: the principal spot obtained from the sample solution and the spot obtained from the standard solution are purple-brown in color and their *R*f values are the same.

(2) A solution of Arbekacin Sulfate (1 in 50) responds to the Qualitative Tests <1.09> (1) for sulfate.

Optical rotation <2.49> $[\alpha]_D^{20}$: +69 – +79° (0.25 g after drying, water, 25 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 0.75 g of Arbekacin Sulfate in 10 mL of water is between 6.0 and 8.0.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 1.0 g of Arbekacin Sulfate in 5 mL of water is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Arbekacin Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Dibekacin—Weigh accurately about 20 mg of Arbekacin Sulfate, add exactly 10 mL of the internal standard solution to dissolve, add water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Dibekacin Sulfate RS, equivalent to about 10 mg (potency), and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of dibekacin to that of the internal standard. Calculate the amount of dibekacin by the following equation: not more than 2.0%.

Amount (%) of dibekacin $= M_S/M_T \times Q_T/Q_S \times 1/10 \times 100$

M_S: Amount [mg (potency)] of Dibekacin Sulfate RS taken
M_T: Amount (mg) of Arbekacin Sulfate taken

Internal standard solution—A solution of bekanamycin sulfate (1 in 2000).

Operating conditions—

Detector: Fluorometric detector (excitation wavelength: 340 nm, detection wavelength: 460 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Reaction coil: A column about 0.3 mm in inside diameter and about 3 m in length.

Reaction coil temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 8.70 g of sodium 1-pentane sulfonate and 8.52 g of anhydrous sodium sulfate in 980 mL of water, adjust the pH to 4.0 with acetic acid (100), and add water to make 1000 mL. To 230 mL of this solution add 20 mL of methanol.

Reagent: Dissolve 12.36 g of boric acid in 960 mL of water, add 10 mL of a solution of *o*-phthalaldehyde in ethanol (99.5) (1 in 25), adjust the pH to 10.5 with 8 mol/L potassium hydroxide TS, and add water to make 1000 mL. To this solution add 1 mL of 2-mercaptoethanol.

Reaction temperature: A constant temperature of about 50°C.

Flow rate of mobile phase: 0.5 mL per minute.

Flow rate of reagent: 1 mL per minute.

System suitability—

System performance: Dissolve 20 mg each of Arbekacin Sulfate, becanamycin sulfate and dibekacin sulfate in 200 mL of water. When the procedure is run with 5 μL of this solution under the above operating conditions, becanamycin, arbekacin and dibekacin are eluted in this order, and the resolution between the peaks, becanamycin and arbekacin is not less than 5 and arbekacin and dibekacin is not less than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of dibekacin to that of the internal standard is not more than 2.0%.

(4) Related substances—Dissolve 20 mg of Arbekacin Sulfate in 20 mL of water, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add water to make exactly 250 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the area of each peak by the automatic integration method: the total area of the peaks other than arbekacin and dibekacin obtained from the sample solution is not larger than the peak area of arbekacin obtained from the standard solution.

Operating conditions—

Detector, column, column temperature, reaction coil, reaction coil temperature, mobile phase, reagent, reaction temperature, flow rate of mobile phase, and flow rate of reagent: Proceed as directed in the operating conditions in the Purity (3).

Time span of measurement: About 1.5 times as long as the retention time of arbekacin.

System suitability—

System performance: Dissolve 10 mg each of Arbekacin Sulfate and dibekacin sulfate in 200 mL of water. When the procedure is run with 5 μL of this solution under the above operating conditions, arbekacin and dibekacin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of arbekacin is not more than 5.0%.

Loss on drying ⟨*2.41*⟩ Not more than 5.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer, having pH 7.8 – 8.0 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Arbekacin Sulfate RS, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution (pH 6.0) (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 to 15°C and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Arbekacin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Arbekacin Sulfate Injection

アルベカシン硫酸塩注射液

Arbekacin Sulfate Injection is an aqueous injection.

It contains not less than 90.0% and not more than 110.0% of the labeled potency of arbekacin sulfate ($C_{22}H_{44}N_6O_{10}$: 552.62).

Method of preparation Prepare as directed under Injections, with Arbekacin Sulfate.

Description Arbekacin Sulfate Injection occurs as a clear and colorless liquid.

Identification To 0.2 mL of Arbekacin Sulfate Injection add 1 mL of water, and use this solution as the sample solution. Separately, dissolve 10 mg of Arbekacin Sulfate RS in 1 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of ammonia solution (28), methanol, chloroform and ethanol (95) (7:6:4:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 80°C for 10 minutes: the principal spot with the sample solution and the spot with the standard solution show a purple-brown color and the same *R*f value.

Osmotic pressure ratio ⟨*2.47*⟩ 0.8 – 1.2 (for the preparation intended for intramuscular use).

pH ⟨*2.54*⟩ 6.0 – 8.0

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.50 EU/mg (potency).

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according

to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

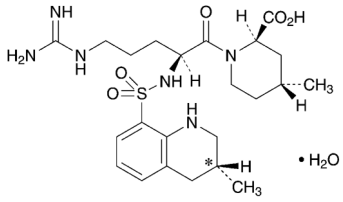
(i) Test organism, Culture medium and Standard solutions: Proceed as directed in the Assay under Arbekacin Sulfate.

(ii) Sample solutions—Take exactly a volume of Arbekacin Sulfate Injection, equivalent to about 20 mg (potency), and add water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers.

Argatroban Hydrate

アルガトロバン水和物



and epimer at C*

$C_{23}H_{36}N_6O_5S.H_2O$: 526.65

(2*R*,4*R*)-4-Methyl-1-((2*S*)-2-{[(3*RS*)-3-methyl-1,2,3,4-tetrahydroquinolin-8-yl]sulfonyl}amino-5-guanidinopentanoyl)piperidine-2-carboxylic acid monohydrate

[*141396-28-3*]

Argatroban Hydrate contains not less than 98.5% and not more than 101.0% of argatroban ($C_{23}H_{36}N_6O_5S$: 508.63), calculated on the anhydrous basis.

Description Argatroban Hydrate occurs as white, crystals or crystalline powder. It has a bitter taste.

It is freely soluble in acetic acid (100), sparingly soluble in methanol, slightly soluble in ethanol (99.5), and very slightly soluble in water.

It is gradually decomposed on exposure to light.

Identification **(1)** Determine the absorption spectrum of a solution of Argatroban Hydrate in ethanol (99.5) (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Argatroban Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +175 – +185° (0.2 g calculated on the anhydrous basis, methanol, 25 mL, 100 mm).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Argatroban Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic ⟨*1.11*⟩—Incinerate 2.0 g of Argatroban Hydrate according to Method 4. After cooling, add 10 mL of dilute hydrochloric acid to the residue, dissolve by warming on a water bath, and perform the test using this solution as the test solution. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 1 ppm).

(3) Related substance 1—Dissolve 50 mg of Argatroban Hydrate in 40 mL of methanol, add water to make 100 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area from the sample solution by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of each peak other than argatroban is not more than 0.1%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase A: To 2.5 mL of acetic acid (100) add water to make 1000 mL, and adjust the pH to 5.0 with ammonia TS. To 500 mL of this solution add 500 mL of methanol.

Mobile phase B: To 2.5 mL of acetic acid (100) add water to make 1000 mL, and adjust the pH to 5.0 with ammonia TS. To 200 mL of this solution add 800 mL of methanol.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 5	100	0
5 – 35	100 → 5	0 → 95

Flow rate: About 1.0 mL per minute.

Time span of measurement: About 1.5 times as long as the retention time of argatroban, beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add the mobile phase A to make 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase A to make exactly 10 mL. Confirm that the peak area of argatroban obtained from 10 μL of this solution is equivalent to 7 to 13% of that obtained from 10 μL of the solution for system suitability test.

System performance: Dissolve 5 mg of Argatroban Hydrate and 5 μL of methyl benzoate in 40 mL of methanol, and add water to make 100 mL. To 5 mL of this solution add 40 mL of methanol and water to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, methyl benzoate and argatroban are eluted in this order with the resolution between these

peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of argatroban is not more than 2.0%.

(4) Related substance 2—Dissolve 0.10 g of Argatroban Hydrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 3 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, ethyl acetate and water (10:10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of spots other than the principal spot obtained from the sample solution is not more than 2, and they are not more intense than the spot obtained from the standard solution.

Water <*2.48*> 2.5 – 4.5% (0.2 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Isomer ratio Dissolve 50 mg of Argatroban Hydrate in 50 mL of methanol, add water to make 100 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the areas of two adjacent peaks, A_a and A_b, having the retention times of about 40 minutes, where A_a is the peak area of shorter retention time and A_b is the peak area of longer retention time: $A_b/(A_a + A_b)$ is between 0.30 and 0.40.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 6.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 500 mL of water add 500 mL of methanol, 13 mL of diluted 40% tetrabutylammonium hydroxide TS (1 in 4) and 0.68 mL of phosphoric acid, and adjust the pH to 6.8 with ammonia TS and diluted ammonia solution (28) (1 in 20).

Flow rate: Adjust so that the retention time of the peak having the shorter retention time of the two peaks of argatroban is about 40 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the sample solution under the above operating conditions, the resolution between the two peaks is not less than 1.2.

System repeatability: When the test is repeated 6 times with 10 μL of the sample solution under the above operating conditions, the relative standard deviation of the total area of the two separate peaks of argatroban is not more than 2.0%.

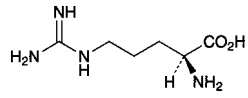
Assay Weigh accurately about 0.5 g of Argatroban Hydrate, dissolve in 20 mL of acetic acid for nonaqueous titration, add 40 mL of acetone for nonaqueous titration, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 50.86 mg of $C_{23}H_{36}N_6O_5S$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

L-Arginine

L-アルギニン



$C_6H_{14}N_4O_2$: 174.20
(2*S*)-2-Amino-5-guanidinopentanoic acid
[*74-79-3*]

L-Arginine, when dried, contains not less than 98.5% and not more than 101.0% of L-arginine ($C_6H_{14}N_4O_2$).

Description L-Arginine occurs as white, crystals or crystalline powder. It has a characteristic odor.

It is freely soluble in water and in formic acid, and practically insoluble in ethanol (99.5).

It dissolves in dilute hydrochloric acid.

It is hygroscopic.

Identification Determine the infrared absorption spectrum of previously dried L-Arginine as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +26.9 – +27.9° (after drying, 2 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <*2.54*> The pH of a solution prepared by dissolving 1.0 g of L-Arginine in 10 mL of water is between 10.5 and 12.0.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 1.0 g of L-Arginine in 10 mL of water is clear and colorless.

(2) Chloride <*1.03*>—Perform the test with 0.5 g of L-Arginine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <*1.14*>—Perform the test with 0.6 g of L-Arginine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <*1.02*>—Perform the test with 0.25 g of L-Arginine, using the distillation under reduced pressure. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <*1.07*>—Dissolve 2.0 g of L-Arginine in 30 mL of water, add 1 drop of phenolphthalein TS, neutralize with dilute hydrochloric acid, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Iron <*1.10*>—Prepare the test solution with 1.0 g of L-Arginine according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Dissolve 0.10 g of L-Arginine in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make

exactly 50 mL. Pipet 2 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-propanol and ammonia solution (28) (7:3) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly ninhydrin-butanol TS on the plate, and heat at 80°C for 10 minutes: the spot other than the principal spot with the sample solution is not more intense than the spot with the standard solution.

Loss on drying <2.41> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

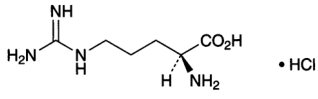
Assay Weigh accurately about 80 mg of L-Arginine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 8.710 mg of $C_6H_{14}N_4O_2$

Containers and storage Containers—Tight containers.

L-Arginine Hydrochloride

L-アルギニン塩酸塩



$C_6H_{14}N_4O_2$.HCl: 210.66
(2*S*)-2-Amino-5-guanidinopentanoic acid monohydrochloride
[*1119-34-2*]

L-Arginine Hydrochloride, when dried, contains not less than 98.5% of L-arginine hydrochloride ($C_6H_{14}N_4O_2$.HCl).

Description L-Arginine Hydrochloride occurs as white, crystals or crystalline powder. It is odorless, and has a slight, characteristic taste.

It is freely soluble in water and in formic acid, and very slightly soluble in ethanol (95).

Identification **(1)** Determine the infrared absorption spectrum of L-Arginine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of L-Arginine Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: +21.5 – +23.5° (after drying, 2 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of L-Arginine Hydrochloride in 10 mL of water: the pH of this solution is between 4.7 and 6.2.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of L-Arginine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test with 0.6 g of L-Arginine Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(3) Ammonium <1.02>—Perform the test with 0.25 g of L-Arginine Hydrochloride, using the distillation under reduced pressure. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of L-Arginine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of L-Arginine Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 0.20 g of L-Arginine Hydrochloride in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 10 mL. Pipet 1 mL of this solution, add water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), water, 1-butanol and ammonia water (28) (2:1:1:1) to a distance of about 10 cm, and dry the plate at 100°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.20% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g of L-Arginine Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add exactly 15 mL of 0.1 mol/L perchloric acid VS, and heat on a water bath for 30 minutes. After cooling, add 45 mL of acetic acid (100), and titrate <2.50> the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 10.53 mg of $C_6H_{14}N_4O_2$.HCl

Containers and storage Containers—Tight containers.

L-Arginine Hydrochloride Injection

L-アルギニン塩酸塩注射液

L-Arginine Hydrochloride Injection is an aqueous injection.

It contains not less than 9.5 w/v% and not more than 10.5 w/v% of L-arginine hydrochloride ($C_6H_{14}N_4O_2$.HCl: 210.66).

Method of preparation

L-Arginine Hydrochloride	100 g
Water for Injection or Sterile Water for Injection in Containers	a sufficient quantity
	To make 1000 mL

Prepare as directed under Injections, with the above ingredients.

No preservative is added.

Description L-Arginine Hydrochloride Injection is a clear, colorless liquid.

Identification **(1)** To 5 mL of a solution of L-Arginine Hydrochloride Injection (1 in 100) add 1 mL of ninhydrin TS, and heat for 3 minutes: a blue-purple color develops.

(2) To 5 mL of a solution of L-Arginine Hydrochloride Injection (1 in 10) add 2 mL of sodium hydroxide TS and 1 to 2 drops of a solution of 1-naphthol in ethanol (95) (1 in 1000), allow to stand for 5 minutes, and add 1 to 2 drops of sodium hypochlorite TS: a red-orange color develops.

pH *<2.54>* 5.0 – 6.0

Bacterial endotoxins *<4.01>* Less than 0.50 EU/mL.

Extractable volume *<6.05>* It meets the requirement.

Foreign insoluble matter *<6.06>* Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter *<6.07>* It meets the requirement.

Sterility *<4.06>* Perform the test according to the Membrane filtration method: it meets the requirement.

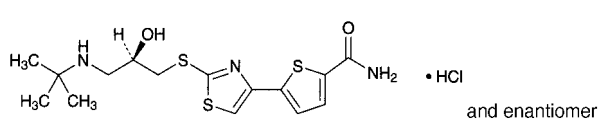
Assay Pipet 20 mL of L-Arginine Hydrochloride Injection, add 7.5 mol/L hydrochloric acid TS to make exactly 100 mL, and determine the optical rotation α_D as directed under Optical Rotation Determination *<2.49>* at 20 ± 1°C in a 100-mm cell.

Amount (mg) of L-arginine hydrochloride ($C_6H_{14}N_4O_2$.HCl) = $\alpha_D \times 4444$

Containers and storage Containers—Hermetic containers.

Arotinolol Hydrochloride

アロチノロール塩酸塩



$C_{15}H_{21}N_3O_2S_3$.HCl: 408.00
5-{2-[(2*RS*)-3-(1,1-Dimethylethyl)amino-2-hydroxypropylsulfanyl]-1,3-thiazol-4-yl}thiophene-2-carboxamide monohydrochloride
[*68377-91-3*]

Arotinolol Hydrochloride, when dried, contains not less than 99.0% of arotinolol hydrochloride ($C_{15}H_{21}N_3O_2S_3$.HCl).

Description Arotinolol Hydrochloride occurs as a white to light yellow crystalline powder.

It is freely soluble in dimethylsulfoxide, slightly soluble in methanol and in water, very slightly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

A solution of Arotinolol Hydrochloride in methanol (1 in 125) does not show optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Arotinolol Hydrochloride in methanol (1 in 75,000) as directed under Ultraviolet-visible Spectrophotometry *<2.24>*, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Arotinolol Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry *<2.25>*, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Arotinolol Hydrochloride (1 in 200) responds to the Qualitative Tests *<1.09>* (2) for chloride.

Purity **(1)** Heavy metals *<1.07>*—Proceed with 1.0 g of Arotinolol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.05 g of Arotinolol Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL. Pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography *<2.03>*. Spot 40 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, acetone and ammonia solution (28) (30:10:10:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying *<2.41>* Not more than 0.20% (1 g, in vacuum, 105°C, 4 hours).

Residue on ignition *<2.44>* Not more than 0.1% (1 g).

Assay Weigh accurately about 1.5 g of Arotinolol Hydrochloride, previously dried, dissolve in dimethylsulfoxide to make exactly 25 mL. Pipet 5 mL of this solution, add 100 mL of water and 5 mL of sodium hydroxide TS, and extract with three 50-mL portions of dichloromethane. Filter each dichloromethane extract through a pledget of absorbent cotton with anhydrous sodium sulfate on it. Evaporate combined filtrate to dryness in vacuum. Dissolve the residue in 70 mL of acetic acid (100), and titrate *<2.50>* with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 20.40 mg of $C_{15}H_{21}N_3O_2S_3$.HCl

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Arsenical Paste

亜ヒ酸パスタ

Arsenical Paste contains not less than 36.0% and not more than 44.0% of arsenic trioxide (As_2O_3: 197.84).

Method of preparation

Arsenic Trioxide, finely powdered	40 g
Procaine Hydrochloride, finely powdered	10 g
Hydrophilic Cream	30 g
Clove Oil	a suitable quantity
Medicinal Carbon	a suitable quantity
To make	100 g

Mix Arsenic Trioxide and Procaine Hydrochloride with Hydrophilic Cream, and add Clove Oil to make a suitably viscous liquid, followed by Medicinal Carbon for coloring.

Description Arsenical Paste is grayish black and has the odor of clove oil.

Identification **(1)** Place 0.1 g of Arsenical Paste in a small flask, add 5 mL of fuming nitric acid and 5 mL of sulfuric acid, and heat over a flame until the reacting liquid becomes colorless and white fumes begin to evolve. After cooling, add the reacting liquid to 20 mL of water cautiously, and add 10 mL of hydrogen sulfide TS while warming: a yellow precipitate is produced (arsenic trioxide).

(2) Shake thoroughly 0.5 g of Arsenical Paste with 25 mL of diethyl ether, 5 mL of dilute hydrochloric acid and 20 mL of water, separate the water layer, and filter: 5 mL of the filtrate responds to the Qualitative Tests <*1.09*> for primary aromatic amines (procaine hydrochloride).

(3) Shake thoroughly 0.5 g of Arsenical Paste with 25 mL of diethyl ether and 25 mL of water, separate the water layer, filter, and use the filtrate as the sample solution. Dissolve 0.01 g of procaine hydrochloride in 5 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and ammonia solution (28) (50:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 mm): the spots from the sample solution and standard solution exhibit the same *R*f value.

Assay Weigh accurately about 0.3 g of Arsenical Paste into a 150-mL Kjeldahl flask, add 5 mL of fuming nitric acid and 10 mL of sulfuric acid, and shake thoroughly. Heat cautiously the mixture, gently at first, and then continue strong heating, until red fumes of nitrogen oxide are sparingly evolved. After cooling, add 5 mL of fuming nitric acid, heat again until red fumes of nitrogen oxide are no longer evolved and the reacting liquid becomes clear, and cool. Add 30 mL of a saturated solution of ammonium oxalate monohydrate, heat again until white fumes of sulfuric acid are evolved, and continue the heating for 10 minutes. Decompose completely oxalic acid, cool, transfer cautiously the colorless reacting liquid to a glass-stoppered flask, containing 40 mL of water. Wash thoroughly the Kjeldahl flask with 60 mL of water, add the washings to the content of the glass-stoppered flask, and cool. Dissolve 3 g of potassium iodide in this solution, allow to stand in a dark place at room temperature for 45 minutes, and titrate <*2.50*> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 5 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 4.946 mg of As_2O_3

Containers and storage Containers—Tight containers.

Arsenic Trioxide

Arsenous Acid

三酸化二ヒ素

As_2O_3: 197.84

Arsenic Trioxide, when dried, contains not less than 99.5% of arsenic trioxide (As_2O_3).

Description Arsenic Trioxide occurs as a white powder.

It is odorless. It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in sodium hydroxide TS.

Identification Dissolve 0.2 g of Arsenic Trioxide in 40 mL of water by heating on a water bath: the solution responds to the Qualitative Tests <*1.09*> for arsenite.

Purity Clarity of solution—To 1.0 g of Arsenic Trioxide add 10 mL of ammonia TS, and heat gently: the solution is clear.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

Assay Weigh accurately about 0.15 g of Arsenic Trioxide, previously dried, dissolve in 20 mL of a solution of sodium hydroxide (1 in 25), by warming, if necessary. Add 40 mL of water and 2 drops of methyl orange TS, then add dilute hydrochloric acid until the color of the solution becomes light red. Add 2 g of sodium hydrogen carbonate and 50 mL of water to this solution, and titrate <*2.50*> with 0.05 mol/L iodine VS (indicator: 3 mL of starch TS).

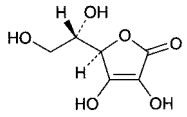
Each mL of 0.05 mol/L iodine VS = 4.946 mg of As_2O_3

Containers and storage Containers—Tight containers.

Ascorbic Acid

Vitamin C

アスコルビン酸



$C_6H_8O_6$: 176.12
L-*threo*-Hex-2-enono-1,4-lactone
[*50-81-7*]

Ascorbic Acid, when dried, contains not less than 99.0% of L-ascorbic acid ($C_6H_8O_6$).

Description Ascorbic Acid occurs as white crystals or a

white crystalline powder. It is odorless, and has an acid taste.

It is freely soluble in water, sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 190°C (with decomposition).

Identification **(1)** To 5 mL each of a solution of Ascorbic Acid (1 in 50) add 1 drop of potassium permanganate TS or 1 to 2 drops of 2,6-dichloroindophenol sodium TS: the color of the solution is discharged immediately in each case.

(2) Dissolve 0.1 g of Ascorbic Acid in 100 mL of a solution of metaphosphoric acid (1 in 50). To 5 mL of the solution add iodine TS until the color of the solution becomes light yellow. Then add 1 drop of a solution of copper (II) sulfate pentahydrate (1 in 1000) and 1 drop of pyrrole, and warm the mixture at 50°C for 5 minutes: a blue color develops.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +20.5 – +21.5° (2.5 g, water, 25 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 1.0 g of Ascorbic Acid in 20 mL of water: the pH of this solution is between 2.2 and 2.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Ascorbic Acid in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals ⟨*1.07*⟩—Perform the test with 1.0 g of Ascorbic Acid according to Method 1. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying ⟨*2.41*⟩ Not more than 0.20% (1 g, silica gel, 24 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Ascorbic Acid, previously dried, dissolve in 50 mL of a solution of metaphosphoric acid (1 in 50), and titrate ⟨*2.50*⟩ with 0.05 mol/L iodine VS (indicator: 1 mL of starch TS).

Each mL of 0.05 mol/L iodine VS
= 8.806 mg of $C_6H_8O_6$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Ascorbic Acid Injection

Vitamin C Injection

アスコルビン酸注射液

Ascorbic Acid Injection is an aqueous injection.

It contains not less than 95.0% and not more than 115.0% of the labeled amount of L-ascorbic acid ($C_6H_8O_6$: 176.12).

Method of preparation Prepare as directed under Injections, with the sodium salt of Ascorbic Acid.

Description Ascorbic Acid Injection occurs as a clear, colorless liquid.

Identification **(1)** Measure a volume of Ascorbic Acid Injection, equivalent to 0.5 g of Ascorbic Acid, and add water to make 25 mL. Proceed with 5 mL each of the solution as directed in the Identification (1) under Ascorbic Acid.

(2) Measure a volume of Ascorbic Acid Injection, equivalent to 5 mg of Ascorbic Acid. Add a solution of metaphosphoric acid (1 in 50) to make 5 mL, and proceed with this solution as directed in the Identification (2) under Ascorbic Acid.

(3) Ascorbic Acid Injection responds to the Qualitative Tests (1) for sodium salt.

pH ⟨*2.54*⟩ 5.6 – 7.4

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.15 EU/mg.

Extractable volume ⟨*6.05*⟩ It meets requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Ascorbic Acid Injection, equivalent to about 0.1 g of L-ascorbic acid ($C_6H_8O_6$), previously diluted with metaphosphoric acid-acetic acid TS, if necessary, and add metaphosphoric acid-acetic acid TS to make exactly 200 mL. Measure exactly 2 mL of the solution, and shake with 8 mL of metaphosphoric acid-acetic acid TS and 2 mL of hydrogen peroxide TS. Titrate ⟨*2.50*⟩ with 2,6-dichloroindophenol sodium TS for titration until a light red color persists for 5 seconds. Perform a blank determination, and make any necessary correction.

Each mL of 2, 6-dichlorophenol-indophenol sodium TS for titration
= *A* mg of $C_6H_8O_6$

A is decided by the following standardization of 2,6-dichloroindophenol sodium TS for titration.

2,6-Dichlorophenol-indophenol sodium TS for titration:

Preparation—Dissolve 42 mg of sodium hydrogen carbonate in 50 mL of water, add 0.5 g of 2,6-dichloroindophenol sodium dihydrate and water to make 200 mL, and filter. Prepare before use.

Standardization—Weigh accurately about 50 mg of Ascorbic Acid RS, previously dried in a desiccator (silica gel) for 24 hours, and dissolve in metaphosphoric acid-acetic acid TS to make exactly 100 mL. Pipet 2 mL of this solution, shake with 8 mL of metaphosphoric acid-acetic acid TS and 2 mL of hydrogen peroxide TS, and titrate ⟨*2.50*⟩ with 2,6-dichloroindophenol sodium TS for titration until a light red color persists for 5 seconds. Perform a blank determination, and make any necessary correction. Calculate the quantity (A mg) of L-ascorbic acid ($C_6H_8O_6$) equivalent to 1 mL of this test solution.

Containers and storage Containers—Hermetic containers.
Storage—Under nitrogen atmosphere.

Ascorbic Acid Powder

Vitamin C Powder

アスコルビン酸散

Ascorbic Acid Powder contains not less than 95.0% and not more than 120.0% of the labeled amount of L-ascorbic acid ($C_6H_8O_6$: 176.12).

Method of preparation Prepare as directed under Granules or Powders, with Ascorbic Acid.

Identification **(1)** Weigh a portion of Ascorbic Acid Powder, equivalent to 0.5 g of Ascorbic Acid, add 30 mL of water, shake for 1 minute, and filter. Proceed with 5 mL each of the filtrate as directed in the Identification (1) under Ascorbic Acid.

(2) Weigh a portion of Ascorbic Acid Powder, equivalent to about 0.01 g of Ascorbic Acid, add 10 mL of a solution of metaphosphoric acid (1 in 50), shake for 1 minute, and filter. Proceed with 5 mL of the filtrate as directed in the Identification (2) under Ascorbic Acid.

Purity Rancidity—Ascorbic Acid Powder is free from any unpleasant or rancid odor and taste.

Assay Weigh accurately a portion of Ascorbic Acid Powder, equivalent to about 0.1 g of L-ascorbic acid ($C_6H_8O_6$), extract with several successive portions of metaphosphoric acid-acetic acid TS, combine the extracts, and filter. Wash the residue with metaphosphoric acid-acetic acid TS. Combine the filtrates and washings, and add metaphosphoric acid-acetic acid to make exactly 200 mL. Pipet 2 mL of the solution, and shake with 8 mL of metaphosphoric acid-acetic acid TS and 2 mL of hydrogen peroxide TS. Titrate ⟨*2.50*⟩ with 2,6-dichloroindophenol sodium TS for titration until a light red color persists for 5 seconds. Perform a blank determination, and make any necessary correction.

Each mL of 2,6-dichlorophenol-indophenol sodium TS for titration
= A mg of $C_6H_8O_6$

A is decided by the following standardization of 2,6-dichloroindophenol sodium TS for titration.

2,6-Dichlorophenol-indophenol sodium TS for titration:

Preparation—Dissolve 42 mg of sodium hydrogen carbonate in 50 mL of water, add 0.05 g of 2,6-dichloroindophenol sodium dihydrate and water to make 200 mL, and filter. Prepare before use.

Standardization—Weigh accurately about 50 mg of Ascorbic Acid RS, previously dried in a desiccator (silica gel) for 24 hours, and dissolve in metaphosphoric acid-acetic acid TS to make exactly 100 mL. Pipet 2 mL of this solution, shake with 8 mL of metaphosphoric acid-acetic acid TS and 2 mL of hydrogen peroxide TS, and titrate ⟨*2.50*⟩ with 2,6-dichloroindophenol sodium TS for titration until a light red color persists for 5 seconds. Perform a blank determination, and make any necessary correction. Calculate the quantity (A mg) of L-ascorbic acid ($C_6H_8O_6$) equivalent to 1 mL of this test solution.

Containers and storage Containers—Tight containers.

Ascorbic Acid and Calcium Pantothenate Tablets

アスコルビン酸·パントテン酸カルシウム錠

Ascorbic Acid and Calcium Pantothenate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of L-ascorbic acid ($C_6H_8O_6$: 176.12) and not less than 93.0% and not more than 107.0% of the labeled amount of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$: 476.53).

Method of preparation Prepare as directed under Tablets, with Ascorbic Acid and Calcium Pantothenate.

Identification **(1)** To a quantity of powdered Ascorbic Acid and Calcium Pantothenate Tablets, equivalent to 0.5 g of Ascorbic Acid, add 30 mL of water, shake for 1 minute, and filter. Proceed as directed in the Identification (1) under Ascorbic Acid using 5 mL each of the filtrate.

(2) To a quantity of powdered Ascorbic Acid and Calcium Pantothenate Tablets, equivalent to 3 mg of Calcium Pantothenate, add 20 mL of ethanol (95), shake vigorously for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 3 mg of calcium pantothenate in 20 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and dilute acetic acid (5:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in ethanol (95) (1 in 200) on the plate, and heat at 120°C for 20 minutes: one of the spot obtained from the sample solution and the spot obtained from the standard solution are purple in color and their Rf value are the same.

Uniformity of dosage units ⟨*6.02*⟩ **(1)** L-Ascorbic acid—Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Ascorbic Acid and Calcium Pantothenate Tablets add 100 mL of a solution of metaphosphoric acid (1 in 50), stir thoroughly, and titrate ⟨*2.50*⟩ with 0.05 mol/L iodine VS (indicator: 1 mL of starch TS).

Each mL of 0.05 mol/L iodine VS = 8.806 mg of $C_6H_8O_6$

(2) Calcium pantothenate—Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Ascorbic Acid and Calcium Pantothenate Tablets add exactly V mL of the internal standard solution so that each mL contains about 0.15 mg of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$), shake vigorously for 15 minutes, filter through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Then, proceed as directed in the Assay (2).

Amount (mg) of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$)
= $M_S \times Q_T/Q_S \times V/200$

M_S: Amount (mg) of Calcium Pantothenate RS taken, calculated on the dried basis

Internal Standard Solution—A solution of acetaminophen (1 in 50,000).

Dissolution ⟨*6.10*⟩ **(1)** L-Ascorbic acid—When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 60 minutes of Ascorbic Acid and Calcium Pantothenate Tablets is not less than 85%.

Start the test with 1 tablet of Ascorbic Acid and Calcium Pantothenate Tablets, withdraw 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add 1st fluid for dissolution test to make exactly V' mL so that each mL contains about 11 μg of L-ascorbic acid ($C_6H_8O_6$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Ascorbic Acid RS, previously dried in a desiccator (silica gel) for 24 hours, dissolve in water to make exactly 100 mL, and warm at 37°C for 1 hour. Pipet 5 mL of this solution, add

1st fluid for dissolution test to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 243 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24> within 1 hour after withdrawing the medium, using 1st fluid for dissolution test as the blank.

Dissolution rate (%) with respect to the labeled amount of L-ascorbic acid ($C_6H_8O_6$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$

M_S: Amount (mg) of Ascorbic Acid RS taken
C: Labeled amount (mg) of L-ascorbic acid ($C_6H_8O_6$) in 1 tablet

(2) Calcium pantothenate—When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 90 minutes of Ascorbic Acid and Calcium Pantothenate Tablets is not less than 75%.

Start the test with 1 tablet of Ascorbic Acid and Calcium Pantothenate Tablets, withdraw 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add 1st fluid for dissolution test to make exactly V' mL so that each mL contains about 3.3 μg of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$), and use this solution as the sample solution. Separately, weigh accurately about 16.5 mg of Calcium Pantothenate RS (separately determine the loss on drying <2.41> under the same conditions as Calcium Pantothenate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of pantothenic acid in each solution.

Dissolution rate (%) with respect to the labeled amount of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of Calcium Pantothenate RS taken, calculated on the dried basis
C: Labeled amount (mg) of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silicone polymer coated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust to pH 2.6 with phosphoric acid, and add water to make 1000 mL. To 970 mL of this solution add 30 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust so that the retention time of pantothenic acid is about 10 minutes.

System suitability—

System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pantothenic acid are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pantothenic acid is not more than 2.0%.

Assay (1) L-Ascorbic acid—Weigh accurately the mass of not less than 20 tablets of Ascorbic Acid and Calcium Pantothenate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of L-ascorbic acid ($C_6H_8O_6$), add 50 mL of a solution of metaphosphoric acid (1 in 50), stir thoroughly, and titrate <2.50> with 0.05 mol/L iodine VS (indicator: 1 mL of starch TS).

Each mL of 0.05 mol/L iodine VS = 8.806 mg of $C_6H_8O_6$

(2) Calcium pantothenate—Weigh accurately the mass of not less than 20 tablets of Ascorbic Acid and Calcium Pantothenate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 3 mg of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$), add exactly 20 mL of the internal standard solution, shake for 15 minutes, filter through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Separately, weigh accurately about 30 mg of Calcium Pantothenate RS (separately determine the loss on drying <2.41> under the same conditions as Calcium Pantothenate), and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 20 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of pantothenic acid to that of the internal standard.

Amount (mg) of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$)
$= M_S \times Q_T/Q_S \times 1/10$

M_S: Amount (mg) of Calcium Pantothenate RS taken, calculated on the dried basis

Internal standard solution—A solution of acetaminophen (1 in 50,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 200 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile for liquid chromatography (97:3).

Flow rate: Adjust so that the retention time of pantothenic acid is about 3 minutes.

System suitability—

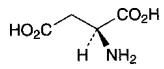
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, pantothenic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pantothenic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

L-Aspartic Acid

L-アスパラギン酸



$C_4H_7NO_4$: 133.10
(2*S*)-2-Aminobutanedioic acid
[*56-84-8*]

L-Aspartic Acid, when dried, contains not less than 98.5% and not more than 101.0% of L-aspartic acid ($C_4H_7NO_4$).

Description L-Aspartic Acid occurs as white, crystals or crystalline powder.

It is sparingly soluble in water, and practically insoluble in ethanol (99.5).

It dissolves in dilute hydrochloric acid and in 0.2 mol/L sodium hydroxide TS.

Identification Determine the infrared absorption spectrum of L-Aspartic Acid as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +24.0 – +26.0° (2 g, after drying, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <*2.54*> Dissolve 0.4 g of L-Aspartic Acid in 100 mL of water by warming, and allow to cool: between 2.5 and 3.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of L-Aspartic Acid in 20 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride <*1.03*>—Dissolve 0.5 g of L-Aspartic Acid in 6 mL of dilute nitric acid and 20 mL of water, add water to make 50 mL, and perform the test with this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <*1.14*>—Dissolve 0.6 g of L-Aspartic Acid in 5 mL of dilute hydrochloric acid and 30 mL of water, add water to make 45 mL, and add 5 mL of barium chloride TS. Perform the test with this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS, add 5 mL of dilute hydrochloric acid and water to make 45 mL, and add 5 mL of barium chloride (not more than 0.028%).

(4) Ammonium <*1.02*>—Perform the test with 0.25 g of L-Aspartic Acid. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <*1.07*>—Proceed with 1.0 g of L-Aspartic Acid according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Iron <*1.10*>—Prepare the test solution with 1.0 g of L-Aspartic Acid according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Dissolve 0.20 g of L-Aspartic Acid in 10 mL of 0.2 mol/L sodium hydroxide TS, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 10 mL. Pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) (1 in 100), and heat at 80°C for 10 minutes: the spot other than the principal spot obtained with the sample solution is not more intense than the spot obtained with the standard solution.

Loss on drying <*2.41*> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.15 g of L-Aspartic Acid, previously dried, dissolve in 50 mL of water by warming. After cooling, titrate <*2.50*> with 0.1 mo/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

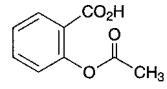
Each mL of 0.1 mol/L sodium hydroxide VS
= 13.31 mg of $C_4H_7NO_4$

Containers and storage Containers—Tight containers.

Aspirin

Acetylsalicylic Acid

アスピリン



$C_9H_8O_4$: 180.16
2-Acetoxybenzoic acid
[*50-78-2*]

Aspirin, when dried, contains not less than 99.5% of aspirin ($C_9H_8O_4$).

Description Aspirin occurs as white crystals, granules or powder. It is odorless, and has a slight acid taste.

It is freely soluble in ethanol (95) and in acetone, soluble in diethyl ether, and slightly soluble in water.

It dissolves in sodium hydroxide TS and in sodium carbonate TS.

In moist air, it gradually hydrolyzes to salicylic acid and acetic acid.

Melting point: about 136°C (bath fluid is heated at 130°C previously).

Identification **(1)** Boil 0.1 g of Aspirin in 5 mL of water for 5 to 6 minutes, cool, and add 1 to 2 drops of iron (III) chloride TS: a red-purple color is produced.

(2) Boil 0.5 g of Aspirin in 10 mL of sodium carbonate TS for 5 minutes, and add 10 mL of dilute sulfuric acid: the odor of acetic acid is perceptible, and a white precipitate is produced. Filter the precipitate, add 3 mL of ethanol (95) and 3 mL of sulfuric acid to the filtrate, and heat: the odor of ethyl acetate is perceptible.

Purity **(1)** Clarity of solution—Dissolve 0.5 g of Aspirin in 10 mL of warm sodium carbonate TS: the solution is clear.

(2) Salicylic acid—Dissolve 2.5 g of Aspirin in 25 mL of ethanol (95), and add 1.0 mL of this solution to a solution which is prepared by transferring 1 mL of a freshly prepared dilute ammonium iron (III) sulfate TS to a Nessler tube and diluting with water to 50 mL. Allow to stand for 30 seconds: the solution has no more color than the following control solution.

Control solution: Dissolve 0.100 g of salicylic acid in water, and add 1 mL of acetic acid (100) and water to make 1000 mL. Add 1.0 mL of this solution to a solution which is prepared by transferring 1 mL of freshly prepared dilute ammonium iron (III) sulfate TS and 1 mL of ethanol (95) to a Nessler tube and diluting with water to 50 mL. Allow to stand for 30 seconds.

(3) Chloride <*1.03*>—Boil 1.8 g of Aspirin in 75 mL of water for 5 minutes, cool, add water to make 75 mL, and filter. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.015%).

(4) Sulfate <*1.14*>—To 25 mL of the filtrate obtained in (3) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.040%).

(5) Heavy metals <*1.07*>—Dissolve 2.5 g of Aspirin in 30 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2.5 mL of Standard Lead Solution, 30 mL of acetone, 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(6) Readily carbonizable substances <*1.15*>—Weigh 0.5 g of Aspirin, and perform the test. The solution has no more color than Matching Fluid Q.

Loss on drying <*2.41*> Not more than 0.5% (3 g, silica gel, 5 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 1.5 g of Aspirin, previously dried, add exactly 50 mL of 0.5 mol/L sodium hydroxide VS, and boil gently for 10 minutes under a reflux condenser with a carbon dioxide-absorbing tube (soda lime). Cool, and titrate <*2.50*> immediately the excess sodium hydroxide with 0.25 mol/L sulfuric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.5 mol/L sodium hydroxide VS
= 45.04 mg of $C_9H_8O_4$

Containers and storage Containers—Well-closed containers.

Aspirin Tablets

Acetylsalicylic Acid Tablets

アスピリン錠

Aspirin Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of aspirin ($C_9H_8O_4$: 180.16).

Method of preparation Prepare as directed under Tablets, with Aspirin.

Identification (1) Weigh a quantity of powdered Aspirin Tablets, equivalent to 0.1 g of Aspirin, add 10 mL of water, and boil for 5 to 6 minutes. After cooling, filter, and add 1 to 2 drops of iron (III) chloride TS to the filtrate: a red-violet color develops.

(2) Weigh a portion of powdered Aspirin Tablets, equivalent to 0.5 g of Aspirin, extract with two 10-mL portions of warm ethanol (95), and filter the combined extracts. Evaporate the filtrate to dryness, and boil the residue with 10 mL of sodium carbonate TS for 5 minutes. Proceed as directed in the Identification (2) under Aspirin.

Purity Salicylic acid—Take a portion of the powdered Aspirin Tablets, equivalent to 1.0 g of Aspirin, shake with 15 mL of ethanol (95) for 5 minutes, filter, discard the first 5 mL of the filtrate, and add 1.0 mL of the subsequent filtrate to a solution which is prepared by transferring 1 mL of freshly prepared dilute ammonium iron (III) sulfate TS to a Nessler tube and diluting with water to make 50 mL. Proceed as directed in the Purity (2) under Aspirin.

Assay Weigh accurately and powder not less than 20 Aspirin Tablets. Weigh accurately a portion of the powder, equivalent to about 1.5 g of aspirin ($C_9H_8O_4$), add exactly 50 mL of 0.5 mol/L sodium hydroxide VS, and proceed as directed in the Assay under Aspirin.

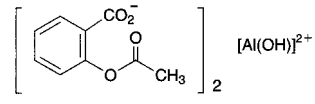
Each mL of 0.5 mol/L sodium hydroxide VS
= 45.04 mg of $C_9H_8O_4$

Containers and storage Containers—Well-closed containers.

Aspirin Aluminum

Aluminum Acetylsalicylate

アスピリンアルミニウム



$C_{18}H_{15}AlO_9$: 402.29
Bis(2-acetoxybenzoato)hydroxoaluminium
[*23413-80-1*]

Aspirin Aluminum contains not less than 83.0% and not more than 90.0% of aspirin ($C_9H_8O_4$: 180.16), and not less than 6.0% and not more than 7.0% of aluminum (Al: 26.98), calculated on the anhydrous basis.

Description Aspirin Aluminum occurs as a white crystalline powder. It is odorless or has a slight, acetic odor.

It is practically insoluble in water, in methanol, in ethanol (95) and in diethyl ether.

It dissolves, with decomposition, in sodium hydroxide TS and in sodium carbonate TS.

Identification (1) Dissolve 0.1 g of Aspirin Aluminum in 10 mL of sodium hydroxide TS by heating, if necessary. Neutralize 2 mL of the solution with hydrochloric acid, and add 1 to 2 drops of iron (III) chloride TS: a red-purple color develops.

(2) Determine the absorption spectrum of the sample solution obtained in the Assay (1) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 277 nm and 279 nm.

(3) Place 2 g of Aspirin Aluminum in a platinum cruci-

ble, and ignite until charred. To the residue add 1 g of anhydrous sodium carbonate, and ignite for 20 minutes. After cooling, to the residue add 15 mL of dilute hydrochloric acid, shake, and filter: the filtrate responds to the Qualitative Tests <*1.09*> for aluminum salt.

Purity **(1)** Salicylate—Using A_{T2} and A_{S2} obtained in the Assay (1), calculate the amount of salicylate [as salicylic acid ($C_7H_6O_3$: 138.12)] by the following equation: salicylate content is not more than 7.5%, calculated on the anhydrous basis.

$$\text{Amount (mg) of salicylic acid } (C_7H_6O_3) = M_S \times A_{T2}/A_{S2} \times 1/4$$

M_S: Amount (mg) of salicylic acid for assay taken

(2) Heavy metals <*1.07*>—Place 2.0 g of Aspirin Aluminum in a porcelain crucible, cover the crucible loosely, and ignite at a low temperature until charred. After cooling, add 2 mL of nitric acid and 1 mL of sulfuric acid to the content of the crucible, heat gently the crucible until white fumes are evolved, and continue the heating until white fumes are no longer evolved, then ignite between 500°C and 600°C until the carbon is incinerated. When the incineration is not completed, add 2 mL of nitric acid and 1 mL of sulfuric acid, and heat gently in the same manner, then ignite between 500°C and 600°C to incinerate completely. After cooling, add 2 mL of hydrochloric acid, and proceed as directed in Method 2, and perform the test. Prepare the control solution by using the same quantities of the same reagents as directed for the preparation of the test solution, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 10 ppm).

(3) Arsenic <*1.11*>—Dissolve 1.0 g of Aspirin Aluminum in 15 mL of sodium hydroxide TS. To this solution add 1 drop of phenolphthalein TS, and with stirring, add dropwise hydrochloric acid until the red color of the solution disappears. Then add 2 mL of hydrochloric acid, cool with occasional shaking for 10 minutes, and filter with a glass filter (G3). Wash the residue with two 5 mL portions of 1 mol/L hydrochloric acid TS, and combine the filtrate and the washings. Use this solution as the test solution, and perform the test (not more than 2 ppm).

Water <*2.48*> Not more than 4.0% (0.15 g, direct titration).

Assay **(1)** Aspirin—Weigh accurately about 0.1 g of Aspirin Aluminum, add 40 mL of sodium fluoride TS, and shake for 5 minutes. Allow the solution to stand for 10 minutes with frequent shaking. Extract the solution with six 20-mL portions of chloroform. Combine all chloroform extracts, and add chloroform to make exactly 200 mL. Measure exactly 10 mL of this solution, add chloroform to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 90 mg of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours, and dissolve in chloroform to make exactly 200 mL. Measure exactly 5 mL of this solution, add chloroform to make exactly 200 mL, and use this solution as the standard solution (1). Then weigh accurately about 90 mg of Aspirin RS, previously dried in a desiccator (silica gel) for 5 hours, and dissolve in chloroform to make exactly 200 mL. Measure exactly 10 mL of this solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*>. Determine the absorbances, A_{T1} and A_{S1}, of the sample solution and standard solution (1) at 278 nm, and absorbances, A_{T2} and A_{S2}, of these solution, at 308 nm, respectively. Then determine the absorbance A_{S3} of the standard solution (2) at 278 nm.

$$\text{Amount (mg) of aspirin } (C_9H_8O_4) = M_S \times \left(\frac{A_{T1} - \dfrac{A_{T2} \times A_{S1}}{A_{S2}}}{A_{S3}} \right)$$

M_S: Amount (mg) of Aspirin RS taken

(2) Aluminum—Weigh accurately about 0.4 g of Aspirin Aluminum, and dissolve in 10 mL of sodium hydroxide TS. Add dropwise 1 mol/L hydrochloric acid TS to adjust the solution to a pH of about 1, add 20 mL of acetic acid-ammonium acetate buffer solution (pH 3.0) and 0.5 mL of Cu-PAN TS, and heat. While boiling, titrate <*2.50*> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red to yellow and persists for 1 minute. Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 1.349 mg of Al

Containers and storage Containers—Well-closed containers.

Aspoxicillin Hydrate

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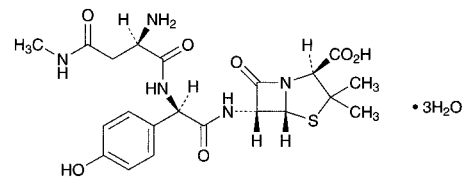

$C_{21}H_{27}N_5O_7S.3H_2O$: 547.58
(2*S*,5*R*,6*R*)-6-[(2*R*)-2-[(2*R*)-2-Amino-3-methylcarbamoylpropanoylamino]-2-(4-hydroxyphenyl)acetylamino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate
[*63358-49-6*, anhydride]

Aspoxicillin Hydrate contains not less than 950 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Aspoxicillin Hydrate is expressed as mass (potency) of aspoxicillin ($C_{21}H_{27}N_5O_7S$: 493.53).

Description Aspoxicillin Hydrate occurs as a white, crystals or crystalline powder.

It is freely soluble in *N*,*N*-dimethylformamide, sparingly soluble in water, and practically insoluble in acetonitrile, in methanol and in ethanol (95).

Identification **(1)** Determine the absorption spectrum of a solution of Aspoxicillin Hydrate (1 in 4000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Aspoxicillin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Aspoxicillin Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and

compare the spectrum with the Reference Spectrum or spectrum of Aspoxicillin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +170 – +185° (0.2 g calculated on the anhydrous bases, water, 20 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 1.0 g of Aspoxicillin Hydrate in 50 mL of water: the pH of the solution is between 4.2 and 5.2.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Aspoxicillin Hydrate in 50 mL of water: the solution is clear and colorless.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Aspoxicillin Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 2.0 g of Aspoxicillin Hydrate according to Method 5, and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 0.05 g of Aspoxicillin Hydrate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of these solutions as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the areas of each peak by the automatic integration method: the area of each peak other than aspoxicillin from the sample solution is not larger than 3/10 times the peak area of aspoxicillin from the standard solution, and the total of peak areas other than aspoxicillin from the sample solution is not larger than the peak area of aspoxicillin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 6 times as long as the retention time of aspoxicillin.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 2 mL of the standard solution, add the mobile phase to make exactly 10 mL. Confirm that the peak area of aspoxicillin obtained from 10 μL of this solution is equivalent to 15 to 25% of that of aspoxicillin obtained from 10 μL of the standard solution.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of aspoxicillin is not more than 5%.

Water ⟨*2.48*⟩ Not less than 9.5% and not more than 13.0% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Aspoxicillin Hydrate and Aspoxicillin RS, equivalent to about 0.1 g (potency), dissolve each in a suitable amount of water, add exactly 10 mL of the internal standard solution, 6.5 mL of acetonitrile and water to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of aspoxicillin to that of the internal standard.

Amount [μg (potency)] of aspoxicillin ($C_{21}H_{27}N_5O_7S$) $= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Aspoxicillin RS taken

Internal standard solution—A solution of *N*-(3-hydroxyphenyl)acetamide (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 130 mL of acetonitrile add potassium dihydrogenphosphate TS (pH 3.0) to make 1000 mL.

Flow rate: Adjust so that the retention time of aspoxicillin is about 3 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, aspoxicillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of aspoxicillin to that of the internal standard is not more than 0.8%.

Containers and storage Containers—Tight containers.

Atenolol

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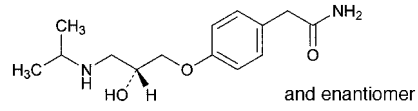

$C_{14}H_{22}N_2O_3$: 266.34

2-(4-{(2*RS*)-2-Hydroxy-3-[(1-methylethyl)amino]propyloxy}phenyl)acetamide

[*29122-68-7*]

Atenolol, when dried, contains not less than 99.0% and not more than 101.0% of atenolol ($C_{14}H_{22}N_2O_3$).

Description Atenolol occurs as a white to pale yellow crystalline powder.

It is freely soluble in methanol and in acetic acid (100), soluble in ethanol (99.5), and slightly soluble in water.

A solution of Atenolol in methanol (1 in 25) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Atenolol in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Atenolol as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting Pint ⟨*2.60*⟩ 152 – 156°C

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Atenolol according to Method 2, and perform the test. Pre-

pare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Atenolol in 25 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than atenolol obtained with the sample solution is not larger than 1/2 times the peak area of atenolol obtained with the standard solution, and the total area of the peaks other than atenolol with the sample solution is not larger than the peak area of atenolol with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 226 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 3.4 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to pH 3.0 with phosphoric acid. To 40 volume of this solution add 9 volume of methanol and 1 volume of tetrahydrofuran. Dissolve 1 g of sodium 1-octanesulfonate and 0.4 g of tetrabutylammonium hydrogensulfate in 1000 mL of this solution.

Flow rate: Adjust so that the retention time of atenolol is about 8 minutes.

Time span of measurement: About 4 times as long as the retention time of atenolol.

System suitability—

Test for required detectability: To exactly 10 mL of the standard solution add the mobile phase to make exactly 50 mL. Confirm that the peak area of atenolol obtained with 10 μL of this solution is equivalent to 14 to 26% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of atenolol are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of atenolol is not more than 1.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.3 g of Atenolol, previously dried, dissolve in 100 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 26.63 mg of $C_{14}H_{22}N_2O_3$

Containers and storage Containers—Tight containers.

Atorvastatin Calcium Hydrate

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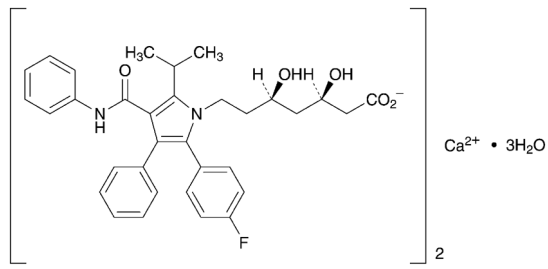


$C_{66}H_{68}CaF_2N_4O_{10}.3H_2O$: 1209.39

Monocalcium bis{(3*R*,5*R*)-7-[2-(4-fluorophenyl)-5-(1-methylethyl)-3-phenyl-4-(phenylcarbamoyl)-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoate} trihydrate

[*344423-98-9*]

Atorvastatin Calcium Hydrate contains not less than 98.0% and not more than 102.0% of atorvastatin calcium ($C_{66}H_{68}CaF_2N_4O_{10}$: 1155.34), calculated on the anhydrous basis.

Description Atorvastatin Calcium Hydrate occurs as a white to pale yellowish white crystalline powder.

It is very soluble in methanol, freely soluble in dimethylsulfoxide, and very slightly soluble in water and in ethanol (99.5).

It gradually turns yellowish white on exposure to light.

It shows crystal polymorphism.

Identification (1) Determine the absorption spectrum of a solution of Atorvastatin Calcium Hydrate in methanol (1 in 62,500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Atorvastatin Calcium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Atorvastatin Calcium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Atorvastatin Calcium RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample and the reference standard according to the method otherwise specified, filter and dry the crystals, and perform the test with the crystals.

(3) A gruel-like liquid of Atorvastatin Calcium Hydrate prepared by adding a small amount of dilute hydrochloric acid responds to the Qualitative Tests <1.09> (1) for calcium salt. A solution of Atorvastatin Calcium Hydrate in a mixture of methanol and water (7:3) (1 in 250) is also responds to the Qualitative Tests <1.09> (3) for calcium salt.

Optical rotation <2.49> $[\alpha]_D^{25}$: -7 – $-10°$ (0.2 g, calculated on the anhydrous basis, dimethylsulfoxide, 20 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Atorvastatin Calcium Hydrate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 20 mg of Atorvastatin Calcium Hydrate in 20 mL of a mixture of water and acetonitrile (1:1), and use this solution as the sample solution.

Pipet 1 mL of the sample solution, add a mixture of water and acetonitrile (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.8 to atorvastatin, obtained from the sample solution is not larger than 3/10 times the peak area of atorvastatin obtained from the standard solution, the area of the peak other than atorvastatin and the peak mentioned above from the sample solution is not larger than 1/10 times the peak area of atorvastatin from the standard solution, and the total area of the peaks other than atorvastatin from the sample solution is not larger than the peak area of atorvastatin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: Dissolve 10.5 g of citric acid monohydrate in 900 mL of water, adjust to pH 5.0 with ammonia solution (28), and add water to make 1000 mL. To 400 mL of this solution add 100 mL of acetonitrile and 100 mL of tetrahydrofuran.

Mobile phase B: A mixture of acetonitrile and tetrahydrofuran (1:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 40	93	7
40 – 80	93 → 60	7 → 40

Flow rate: Adjust so that the retention time of atorvastatin is about 16 minutes.

Time span of measurement: About 5 times as long as the retention time of atorvastatin, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of water and acetonitrile (1:1) to make exactly 100 mL. Confirm that the peak area of atorvastatin obtained with 20 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of atorvastatin are not less than 8000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of atorvastatin is not more than 2.0%.

Water <2.48> 3.5 – 5.5% (50 mg, coulometric titration).

Assay Weigh accurately about 20 mg each of Atorvastatin Calcium Hydrate and Atorvastatin Calcium RS (separately determine the water <2.48> in the same manner as Atorvastatin Calcium Hydrate), dissolve each in an adequate amount of a mixture of water and acetonitrile (1:1), add exactly 10 mL of the internal standard solution, then add a mixture of water and acetonitrile (1:1) to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of atorvastatin to that of the internal standard.

Amount (mg) of atorvastatin calcium ($C_{66}H_{68}CaF_2N_4O_{10}$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of Atorvastatin Calcium RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of water and acetonitrile (1:1) (1 in 1500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 10.5 g of citric acid monohydrate in 900 mL of water, adjust to pH 4.0 with ammonia solution (28), and add water to make 1000 mL. To 530 mL of this solution add 270 mL of acetonitrile and 200 mL of tetrahydrofuran.

Flow rate: Adjust so that the retention time of atorvastatin is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and atorvastatin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of atorvastatin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Atorvastatin Calcium Tablets

アトルバスタチンカルシウム錠

Atorvastatin Calcium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of atorvastatin calcium hydrate ($C_{66}H_{68}CaF_2N_4O_{10}.3H_2O$: 1209.39).

Method of preparation Prepare as directed under Tablets, with Atorvastatin Calcium Hydrate.

Identification To a quantity of powdered Atorvastatin Calcium Tablets, equivalent to 10 mg of Atorvastatin Calcium Hydrate, add 50 mL of methanol, shake thoroughly, and centrifuge. To 2.5 mL of the supernatant liquid add metha-

nol to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 244 nm and 248 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Atorvastatin Calcium Tablets add $3V/5$ mL of a mixture of water and methanol (1:1), and disintegrate the tablet by shaking. Add exactly $V/10$ mL of the internal standard solution, and add a mixture of water and methanol (1:1) to make V mL so that each mL contains about 0.1 mg of atorvastatin calcium hydrate ($C_{66}H_{68}CaF_2N_4O_{10}.3H_2O$). Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 22 mg of Atorvastatin Calcium RS (separately determine the water ⟨*2.48*⟩ in the same manner as Atorvastatin Calcium Hydrate), and dissolve in a mixture of water and methanol (1:1) to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add a mixture of water and methanol (1:1) to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of atorvastatin to that of the internal standard.

Amount (mg) of atorvastatin calcium hydrate ($C_{66}H_{68}CaF_2N_4O_{10}.3H_2O$)
$= M_S \times Q_T/Q_S \times V/200 \times 1.047$

M_S: Amount (mg) of Atorvastatin Calcium RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of 1,3-dinitrobenzene in methanol (1 in 2500).

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and atorvastatin are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of atorvastatin to that of the internal standard is not more than 1.0%.

Dissolution ⟨*6.10*⟩ When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Atorvastatin Calcium Tablets is not less than 80%.

Start the test with 1 tablet of Atorvastatin Calcium Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 6 μg of atorvastatin calcium hydrate ($C_{66}H_{68}CaF_2N_4O_{10}.3H_2O$), and use this solution as the sample solution. Separately, weigh accurately about 60 mg of Atorvastatin Calcium RS (separately determine the water ⟨*2.48*⟩ in the same manner as Atorvastatin Calcium Hydrate), and dissolve in a mixture of water and methanol (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩, and determine the peak areas, A_T and A_S, of atorvastatin in each solution.

Dissolution rate (%) with respect to the labeled amount of atorvastatin calcium hydrate ($C_{66}H_{68}CaF_2N_4O_{10}.3H_2O$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9 \times 1.047$

M_S: Amount (mg) of Atorvastatin Calcium RS taken, calculated on the anhydrous basis

C: Labeled amount (mg) of atorvastatin calcium hydrate ($C_{66}H_{68}CaF_2N_4O_{10}.3H_2O$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of atorvastatin are not less than 6000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of atorvastatin is not more than 2.0%.

Assay To 20 Atorvastatin Calcium Tablets add $3V/5$ mL of a mixture of water and methanol (1:1), and disintegrate the tablet by shaking. Add exactly $V/10$ mL of the internal standard solution, add a mixture of water and methanol (1:1) to make V mL so that each mL contains about 2 mg of atorvastatin calcium hydrate ($C_{66}H_{68}CaF_2N_4O_{10}.3H_2O$), and centrifuge. To 2.5 mL of the supernatant liquid add a mixture of water and methanol (1:1) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 44 mg of Atorvastatin Calcium RS (separately determine the water ⟨*2.48*⟩ in the same manner as Atorvastatin Calcium Hydrate), add exactly 2 mL of the internal standard solution, and add a mixture of water and methanol (1:1) to make 20 mL. Pipet 2.5 mL of this solution, add a mixture of water and methanol (1:1) to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of atorvastatin to that of the internal standard.

Amount (mg) of atorvastatin calcium hydrate ($C_{66}H_{68}CaF_2N_4O_{10}.3H_2O$)
in 1 tablet of Atorvastatin Calcium Tablets
$= M_S \times Q_T/Q_S \times V/400 \times 1.047$

M_S: Amount (mg) of Atorvastatin Calcium RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of 1,3-dinitrobenzene in methanol (1 in 125).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 244 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 10.5 g of citric acid monohydrate in 900 mL of water, adjust to pH 4.0 with ammonia solution (28), and add water to make 1000 mL. To 530 mL of this solution add 270 mL of acetonitrile and 200 mL of tetrahydrofuran.

Flow rate: Adjust so that the retention time of atorvastatin is about 9 minutes.

System suitability—

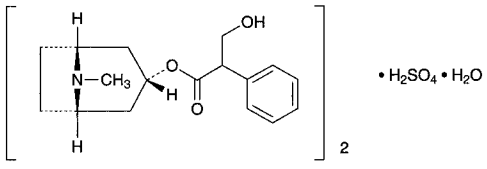
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and atorvastatin are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of atorvastatin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Atropine Sulfate Hydrate

アトロピン硫酸塩水和物



$(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O$: 694.83

(1*R*,3*r*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl [(2*RS*)-3-hydroxy-2-phenyl]propanoate hemisulfate hemihydrate [*5908-99-6*]

Atropine Sulfate Hydrate, when dried, contains not less than 98.0% of atropine sulfate $[(C_{17}H_{23}NO_3)_2.H_2SO_4$: 676.82].

Description Atropine Sulfate Hydrate occurs as colorless crystals or a white crystalline powder. It is odorless.

It is very soluble in water and in acetic acid (100), freely soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: 188 – 194°C (with decomposition). Introduce a capillary tube charged with dried sample into a bath previously heated to 180°C, and continue to heat at a rate of rise of about 3°C per minute.

It is affected by light.

Identification **(1)** To 1 mg of Atropine Sulfate Hydrate add 3 drops of fuming nitric acid, and evaporate the mixture on a water bath to dryness. Dissolve the residue in 1 mL of *N*,*N*-dimethylformamide, and add 5 to 6 drops of tetraethylammonium hydroxide TS: a red-purple color develops.

(2) To 2 mL of a solution of Atropine Sulfate Hydrate (1 in 50) add 4 to 5 drops of hydrogen tetrachloroaurate (III) TS: a lusterless, yellowish white precipitate is formed.

(3) To 5 mL of a solution of Atropine Sulfate Hydrate (1 in 25) add 2 mL of ammonia TS, and allow to stand for 2 to 3 minutes. Collect the precipitate, wash with water, and dry in a desiccator (in vacuum, silica gel) for 4 hours: it melts ⟨*2.60*⟩ between 115°C and 118°C.

(4) A solution of Atropine Sulfate Hydrate (1 in 20) responds to the Qualitative Tests ⟨*1.09*⟩ for sulfate.

Purity **(1)** Clarity and color of solution —Dissolve 0.5 g of Atropine Sulfate Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 1.0 g of Atropine Sulfate Hydrate in 20 mL of water, and add 0.30 mL of 0.02 mol/L sodium hydroxide VS and 1 drop of methyl red-methylene blue TS: a green color develops.

(3) Related substances—Dissolve 0.25 g of Atropine Sulfate Hydrate in 1 mL of diluted hydrochloric acid (1 in 10), add water to make 15 mL, and use this solution as the sample solution.

(i) To 5 mL of the sample solution add 2 to 3 drops of hydrogen hexachloroplatinate (IV) TS: no precipitate is formed.

(ii) To 5 mL of the sample solution add 2 mL of ammonia TS, and shake vigorously: the turbidity of the solution is not greater than that of the following control solution.

Control solution: To 0.30 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL. To this solution add 1 mL of silver nitrate TS, and allow 7 mL of the mixture to stand for 5 minutes.

(4) Hyoscyamine—Weigh accurately about 1 g of Atropine Sulfate Hydrate, previously dried, and dissolve in water to make exactly 10 mL: the specific optical rotation $[\alpha]_D^{20}$ ⟨*2.49*⟩ of this solution in a 100-mm cell is between −0.60° and +0.10°.

(5) Readily carbonizable substances ⟨*1.15*⟩—Take 0.20 g of Atropine Sulfate Hydrate, and perform the test: the solution has no more color than Matching Fluid A.

Loss on drying ⟨*2.41*⟩ Not more than 4.0% (0.5 g, in vacuum, phosphorus (V) oxide, 110°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (0.5 g).

Assay Dissolve about 0.25 g of Atropine Sulfate Hydrate, previously dried and accurately weighed, in 30 mL of acetic acid (100). If necessary, dissolve it by warming, and cool. Titrate ⟨*2.50*⟩ with 0.05 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 33.84 mg of $(C_{17}H_{23}NO_3)_2.H_2SO_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Atropine Sulfate Injection

アトロピン硫酸塩注射液

Atropine Sulfate Injection is an aqueous injection.
It contains not less than 93.0% and not more than 107.0% of the labeled amount of atropine sulfate hydrate $[(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O$: 694.83].

Method of preparation Prepare as directed under Injections, with Atropine Sulfate Hydrate.

Description Atropine Sulfate Injection is a clear, colorless liquid.

pH: 4.0 – 6.0

Identification **(1)** Evaporate a volume of Atropine Sulfate Injection, equivalent to 1 mg of Atropine Sulfate Hydrate, on a water bath to dryness. Proceed with the residue

as directed in the Identification (1) under Atropine Sulfate Hydrate.

(2) Evaporate an exactly measured volume of Atropine Sulfate Injection, equivalent to 5 mg of Atropine Sulfate Hydrate, on a water bath to dryness. After cooling, dissolve the residue in 1 mL of ethanol (95), and use this solution as the sample solution. If insoluble substance remains, crush it, allow to stand, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of Atropine Sulfate RS in 2 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, water and ammonia water (28) (90:7:3) to a distance of about 10 cm, and dry the plate at 80°C for 10 minutes. After cooling, spray evenly Dragendorff's TS for spraying on the plate: the spots obtained from the sample solution and the standard solution show an orange color and the same *R*f value.

(3) Atropine Sulfate Injection responds to the Qualitative Tests <*1.09*> for sulfate.

Bacterial endotoxins <*4.01*> Less than 75 EU/mg.

Extractable volume <*6.05*> It meets the requirements.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test: it meets the requirement.

Assay To an exactly measured volume of Atropine Sulfate Injection, equivalent to about 5 mg of atropine sulfate hydrate $[(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O]$, add exactly 3 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Atropine Sulfate RS (separately determine the loss on drying <*2.41*> under the same conditions as Atropine Sulfate Hydrate), and dissolve in water to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 3 mL of the internal standard solution and water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of atropine to that of the internal standard.

$$\text{Amount (mg) of atropine sulfate hydrate } [(C_{17}H_{23}NO_3)_2.H_2SO_4.H_2O] = M_S \times Q_T/Q_S \times 1/5 \times 1.027$$

M_S: Amount (mg) of Atropine Sulfate RS taken, calculated based on the dried basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 0.4 g of sodium lauryl sulfate add 500 mL of diluted phosphoric acid (1 in 1000) to dissolve, and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust so that the retention time of atropine is about 16 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and atropine are eluted in this order with the resolution between these peaks being not less than 3.

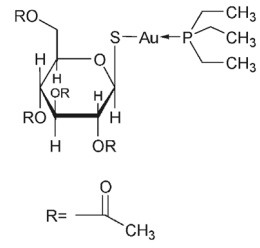
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of atropine to that of the internal standard is not more than 1.5%.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant.

Auranofin

オーラノフィン



$C_{20}H_{34}AuO_9PS$: 678.48

(2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glucopyranosato)(triethylphosphine)gold

[*34031-32-8*]

Auranofin, when dried, contains not less than 98.0% and not more than 102.0% of auranofin ($C_{20}H_{34}AuO_9PS$).

Description Auranofin occurs as a white crystalline powder.

It is very soluble in chloroform, freely soluble in methanol, sparingly soluble in ethanol (99.5), and practically insoluble in water.

It shows crystal polymorphism.

Identification **(1)** To 50 mg of Auranofin add 3 mL of water, 3 mL of nitric acid and 3 mL of sulfuric acid, shake, and allow to stand: golden colored suspended matters are produced.

(2) Determine the infrared absorption spectrum of Auranofin as directed in the paste method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Auranofin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Prepare the test solution with 1 mg of Auranofin as directed under Oxygen Flask Combustion Method <*1.06*>, using 10 mL of water as the absorbing liquid. Wash out the test solution into a Nessler tube with water to make 30 mL. Add 10 mL of dilute sulfuric acid, 3 mL of hexaammonium heptamolybdate-sulfuric acid TS and 0.1 mL of tin (II) chloride TS, shake, and allow to stand for 10 to 15 minutes: a blue color is developed.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -54.0 – $-62.0°$ (after dry-

ing, 0.2 g, methanol, 20 mL, 100 mm).

Melting point ⟨*2.60*⟩ 113 – 116°C

Purity **(1)** Chloride ⟨*1.03*⟩—Put 0.5 g of Auranofin in a porcelain crucible, add 0.25 g of anhydrous sodium carbonate, mix well, and ignite until the carbonized substance is disappeared. After cooling, add 20 mL of water, heat, and filter after cooling. Wash the residue with 20 mL of water, combine the filtrate and the washings, neutralize with dilute nitric acid, then add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: Dissolve 0.25 g of anhydrous sodium carbonate in 20 mL of water, neutralize with dilute nitric acid, add 0.50 mL of 0.01 mol/L hydrochloric acid, 6 mL of dilute nitric acid, and water to make 50 mL (not more than 0.036%).

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Auranofin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic ⟨*1.11*⟩—Put 0.5 g of Auranofin in a Kjeldahl flask, add cautiously 2 mL of sulfuric acid and 5 mL of nitric acid, and heat until the solution becomes almost colorless. After cooling, add 15 mL of a saturated solution of ammonium oxalate monohydrate, heat until white fumes are evolved, and concentrate to 1 to 2 mL. Then, add 3 mL of water and 1 drop of methyl orange TS, neutralize with ammonia solution (28), filter, and perform the test using the filtrate as the test solution: the color is not darker than that of the following control solution.

Control solution: Heat a mixture of 2 mL of sulfuric acid and 5 mL of nitric acid until white fumes are no longer evolved. After cooling, add 15 mL of a saturated solution of ammonium oxalate monohydrate, heat until white fumes are evolved, and concentrate to 1 to 2 mL. Add 3 mL of water and 1 drop of methyl orange TS, neutralize with ammonia solution (28), and filter. To the filtrate add 2.0 mL of Standard Arsenic Solution, then proceed in the same manner as for the test solution (not more than 4 ppm).

(4) Related substances—Dissolve 50 mg of Auranofin in 5 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 100 mL. To exactly 3 mL of this solution add chloroform to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and acetone (4:1) to a distance of about 10 cm, and air-dry the plate. Dry, furthermore, at 80°C for 30 minutes. After cooling, allow the plate to stand in a iodine vapor for 30 minutes: the spots other than the principal spot obtained from the sample solution are not more intense than the spot obtained from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Assay Weigh accurately about 20 mg each of Auranofin and Auranofin RS, both previously dried, dissolve each in 10 mL of a mixture of water and acetonitrile (1:1), and add exactly 5 mL each of the internal standard solution. Then add a mixture of water and acetonitrile (1:1) to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of auranofin to that of the internal standard.

$$\text{Amount (mg) of auranofin } (C_{20}H_{34}AuO_9PS) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Auranofin RS taken

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of water and acetonitrile (1:1) (3 in 1250).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of sodium dihydrogen phosphate dihydrate solution (1 in 100), tetrahydrofuran and acetonitrile (12:5:3).

Flow rate: Adjust so that the retention time of auranofin is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, auranofin and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of auranofin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Auranofin Tablets

オーラノフィン錠

Auranofin Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of auranofin ($C_{20}H_{34}AuO_9PS$: 678.48).

Method of preparation Prepare as directed under Tablets, with Auranofin.

Identification Put an amount of powdered Auranofin Tablets, equivalent to 11 mg of Auranofin, in a porcelain crucible, and heat weakly to carbonize. After cooling, add 2 mL of nitric acid and 5 drops of sulfuric acid, heat cautiously at first then incinerate by ignition. After cooling, add 4 mL of aqua regia to the residue, dissolve by warming, and add 16 mL of water. To 5 mL of this solution add 0.5 mL of tin (II) chloride TS: a purple to red-brown color is developed.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Auranofin Tablets add 2 mL of water, disintegrate the tablet with the aid of ultrasonic waves, add exactly 2 mL of the internal standard solution for every 3 mg of auranofin ($C_{20}H_{34}AuO_9PS$), and add 2 mL of a mixture of water and acetonitrile (1:1). Shake for 15 minutes, then add a mixture of water and acetonitrile (1:1) to make V mL so that each mL contains 0.3 mg of auranofin

($C_{20}H_{34}AuO_9PS$), centrifuge, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of auranofin ($C_{20}H_{34}AuO_9PS$)
$= M_S \times Q_T/Q_S \times V/100$

M_S: Amount (mg) of Auranofin RS taken

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (9 in 10,000).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Auranofin Tablets is not less than 85%.

Start the test with 1 tablet of Auranofin Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 3.3 μg of auranofin ($C_{20}H_{34}AuO_9PS$), and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Auranofin RS, previously dried at 105°C for 3 hours, and dissolve in acetonitrile to make exactly 50 mL. Pipet 5 mL of this solution, and add water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ under the following conditions, and determine the peak areas, A_T and A_S, of auranofin in each solution.

Dissolution rate (%) with respect to the labeled amount of auranofin ($C_{20}H_{34}AuO_9PS$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9$

M_S: Amount (mg) of Auranofin RS taken
C: Labeled amount (mg) of auranofin ($C_{20}H_{34}AuO_9PS$) in 1 tablet

Operating conditions—
Proceed as directed in the operating conditions in the Assay under Auranofin.
System suitability—
System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of auranofin are not less than 5000 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of auranofin is not more than 1.0%.

Assay Accurately weigh the mass of not less than 20 Auranofin Tablets, and powder them. Weigh accurately a portion of the powder, equivalent to about 60 mg of auranofin ($C_{20}H_{34}AuO_9PS$), add 40 mL of water, disperse the particles with the aid of ultrasonic waves, then add exactly 40 mL of the internal standard solution, add 40 mL of a mixture of water and acetonitrile (1:1), and shake for 15 minutes. To this solution add a mixture of water and acetonitrile (1:1) to make 200 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 30 mg of Auranofin RS, previously dried at 105°C for 3 hours, dissolve in 60 mL of a mixture of water and acetonitrile (1:1), add exactly 20 mL of the internal standard solution, then add water to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of auranofin to that of the internal standard.

Amount (mg) of auranofin ($C_{20}H_{34}AuO_9PS$)
$= M_S \times Q_T/Q_S \times 2$

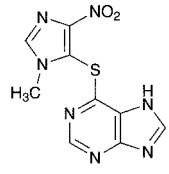
M_S: Amount (mg) of Auranofin RS taken

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (9 in 10,000).
Operating conditions—
Proceed as directed in the operating conditions in the Assay under Auranofin.
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, auranofin and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of auranofin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Azathioprine

アザチオプリン



$C_9H_7N_7O_2S$: 277.26
6-(1-Methyl-4-nitro-1*H*-imidazol-5-ylthio)purine
[*446-86-6*]

Azathioprine, when dried, contains not less than 98.5% of azathioprine ($C_9H_7N_7O_2S$).

Description Azathioprine is light yellow, crystals or crystalline powder. It is odorless.
It is sparingly soluble in *N*,*N*-dimethylformamide and in pyridine, very slightly soluble in water and in ethanol (99.5), and practically insoluble in chloroform and in diethyl ether.
It dissolves in sodium hydroxide TS and in ammonia TS.
It is gradually colored by light.
Melting point: about 240°C (with decomposition).

Identification **(1)** Dissolve 0.01 g of Azathioprine in 50 mL of water by warming. To 5 mL of this solution add 1 mL of dilute hydrochloric acid and 0.01 g of zinc powder, and allow to stand for 5 minutes: a yellow color is produced. Filter this solution: the filtrate responds to the Qualitative Tests ⟨*1.09*⟩ for primary aromatic amines, and a red color is produced.
(2) Dissolve 0.01 g of Azathioprine in 50 mL of water by warming. To 1 mL of this solution add 0.5 mL of phosphotungstic acid TS and 0.5 mL of dilute hydrochloric acid: a white precipitate is formed.

(3) Prepare the test solution by proceeding with 0.03 g of Azathioprine according to the Oxygen Flask Combustion Method <*1.06*>, using 20 mL of water as the absorbing liquid: the test solution responds to the Qualitative Tests <*1.09*> (1) for sulfate.

(4) Dissolve 0.01 g of Azathioprine in 2 mol/L hydrochloric acid TS to make 100 mL. Dilute 5 mL of the solution with water to make 50 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Azathioprine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Azathioprine in 50 mL of *N*,*N*-dimethylformamide: the solution is clear and shows a light yellow color.

(2) Acidity or alkalinity—Add 100 mL of water to 2.0 g of Azathioprine, shake well for 15 minutes, centrifuge for 5 minutes at 10,000 revolutions per minute, and filter. Discard the first 20 mL of the filtrate, add 2 drops of methyl red TS to 40 mL of the subsequent filtrate, and use this solution as the sample solution.

(i) Add 0.10 mL of 0.02 mol/L hydrochloric acid VS to 20 mL of the sample solution: a red color develops.

(ii) Add 0.10 mL of 0.02 mol/L sodium hydroxide VS to 20 mL of the sample solution: a yellow color develops.

(3) Sulfate <*1.14*>—To 25 mL of the filtrate obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(4) Heavy metals <*1.07*>—Proceed with 2.0 g of Azathioprine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Azathioprine, according to Method 3, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 10 mg of Azathioprine in 80 mL of the mobile phase by warming, cool, add the mobile phase to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the total area of the peaks other than that of azathioprine from the sample solution is not larger than 1/2 times the peak area of azathioprine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 296 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Adjust to pH 2.5 of a solution of 0.05 mol/L potassium dihydrogenphosphate TS (1 in 2) with diluted phosphoric acid (3 in 2000). To 800 mL of this solution add 200 mL of methanol.

Flow rate: Adjust so that the retention time of azathioprine is about 8 minutes.

Time span of measurement: About three times as long as the retention time of azathioprine, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add water to make exactly 50 mL. Confirm that the peak area of azathioprine obtained from 20 μL of this solution is equivalent to 8 to 12% of that of azathioprine obtained from 20 μL of the standard solution.

System performance: Dissolve 10 mg of Azathioprine in 80 mL of water by warming, cool, and add water to make 100 mL. To 2 mL of this solution add 2 mL of a solution, separately prepared by dissolving 0.06 g of benzoic acid in 3 mL of methanol and diluting with water to make 10 mL, and add the mobile phase to make 25 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, azathioprine and benzoic acid are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of azathioprine is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 5 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Azathioprine, previously dried, add 80 mL of *N*,*N*-dimethylformamide, and warm to dissolve. After cooling, titrate <*2.50*> with 0.1 mol/L tetramethylammonium hydroxide VS until the color of the solution changes from yellow through yellow-green to blue-green (indicator: 1 mL of thymol blue-dimethylformamide TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS = 27.73 mg of $C_9H_7O_7O_2S$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Azathioprine Tablets

アザチオプリン錠

Azathioprine Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of azathioprine ($C_9H_7N_7O_2S$: 277.26).

Method of preparation Prepare as directed under Tablets, with Azathioprine.

Identification **(1)** Weigh a quantity of powdered Azathioprine Tablets, equivalent to 0.01 g of Azathioprine. Add 50 mL of water, shake well while warming, and filter. Proceed with 5 mL of the filtrate as directed in the Identification (1) under Azathioprine.

(2) Proceed with 1 mL of the filtrate obtained in (1) as directed in the Identification (2) under Azathioprine.

(3) Determine the absorption spectrum of the sample solution in the Assay as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 278 nm and 282 nm.

(4) Weigh a quantity of powdered Azathioprine Tablets, equivalent to 0.1 g of Azathioprine to the labeled amount.

Add 10 mL of a solution of ammonia solution (28) in methanol (1 in 10), shake well, filter, and use the filtrate as the sample solution. Separately, dissolve 0.1 g of Azathioprine RS in 10 mL of a solution of ammonia solution (28) in methanol (1 in 10), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, a solution of ammonia solution (28) in methanol (1 in 10), *n*-butyl formate and 1,2-dichloroethane (15:10:5:2) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution and the standard solution show the same *R*f value.

Uniformity of dosage units ⟨*6.02*⟩ Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Azathioprine Tablets add 1 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry per 5 mg of azathioprine ($C_9H_7N_7O_2S$), shake well, add 0.1 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 0.2 mg of azathioprine ($C_9H_7N_7O_2S$), and filter. Discard the first 20 mL of the filtrate, pipet 3 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of azathioprine ($C_9H_7N_7O_2S$)
$= M_S \times A_T/A_S \times V/500$

M_S: Amount (mg) of Azathioprine RS taken

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Azathioprine Tablets is not less than 80%.

Start the test with 1 tablet of Azathioprine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 11 μg of azathioprine ($C_9H_7N_7O_2S$), and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Azathioprine RS, previously dried at 105°C for 5 hours, and dissolve in water to make exactly 100 mL. Pipet 6 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 280 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of azathioprine ($C_9H_7N_7O_2S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 108$

M_S: Amount (mg) of Azathioprine RS taken
C: Labeled amount (mg) of azathioprine ($C_9H_7N_7O_2S$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Azathioprine Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of azathioprine ($C_9H_7N_7O_2S$), add 20 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, shake well, add 0.1 mol/L hydrochloric acid TS to make exactly 500 mL, and filter. Discard the first 20 mL of the filtrate, measure exactly 3 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Azathioprine RS, previously dried at 105°C for 5 hours, dissolve in 20 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, and add 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Measure exactly 3 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 280 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

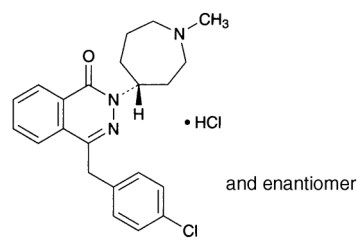
Amount (mg) of azathioprine ($C_9H_7N_7O_2S$)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of Azathioprine RS taken

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Azelastine Hydrochloride

アゼラスチン塩酸塩



$C_{22}H_{24}ClN_3O.HCl$: 418.36
4-[(4-Chlorophenyl)methyl]-2-[(4*RS*)-(1-methylazepan-4-yl)]phthalazin-1(2*H*)-one monohydrochloride
[*79307-93-0*]

Azelastine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of azelastine hydrochloride ($C_{22}H_{24}ClN_3O.HCl$).

Description Azelastine Hydrochloride occurs as a white crystalline powder.

It is freely soluble in formic acid, and slightly soluble in water and in ethanol (99.5).

Melting point: about 225°C (with decomposition).

A solution of Azelastine Hydrochloride (1 in 200) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Azelastine Hydrochloride (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Azelastine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 10 mL of a saturated solution of Azelastine Hydrochloride add 1 mL of dilute nitric acid, and filter to separate formed crystals: the filtrate responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of

Azelastine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Azelastine Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Azelastine Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: each peak area other than azelastine obtained from the sample solution is not larger than 1/10 times the peak area of azelastine obtained from the standard solution, and the total area of the peaks other than the peak of azelastine from the sample solution is not larger than 1/2 times the peak area of azelastine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of water, acetonitrile and perchloric acid (660:340:1).

Flow rate: Adjust so that the retention time of azelastine is about 10 minutes.

Time span of measurement: About 2 times as long as the retention time of azelastine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of azelastine obtained from 20 μL of this solution is equivalent to 7 to 13% of that obtained from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of azelastine is not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of azelastine is not more than 1.0%.

Loss on drying <*2.41*> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of previously dried Azelastine Hydrochloride, dissolve in 5 mL of formic acid, add 70 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 41.84 mg of $C_{22}H_{24}ClN_3O.HCl$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Azelastine Hydrochloride Granules

アゼラスチン塩酸塩顆粒

Azelastine Hydrochloride Granules contain not less than 93.0% and not more than 107.0% of the labeled amount of azelastine hydrochloride ($C_{22}H_{24}ClN_3O.HCl$: 418.36).

Method of preparation Prepare as directed under Granules, with Azelastine Hydrochloride.

Identification To a quantity of Azelastine Hydrochloride Granules, equivalent to 2 mg of Azelastine Hydrochloride, add 30 mL of 0.1 mol/L hydrochloric acid TS, and treat with ultrasonic waves for 30 minutes. After cooling, add 0.1 mol/L hydrochloric acid TS to make 50 mL, and centrifuge. Determine the absorption spectrum of the supernatant liquid as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 283 nm and 287 nm.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 0.05 mol/L acetic acid-sodium acetate buffer solution (pH 4.0) as the dissolution medium, the dissolution rate in 45 minutes of Azelastine Hydrochloride Granules is not less than 80%.

Start the test with accurately weighed amount of Azelastine Hydrochloride Granules, equivalent to about 1 mg of azelastine hydrochloride ($C_{22}H_{24}ClN_3O.HCl$), withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of azelastine hydrochloride for assay, previously dried at 105°C for 2 hours, and dissolve in the dissolution medium to make exactly 250 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of azelastine in each solution.

Dissolution rate (%) with respect to the labeled amount of azelastine hydrochloride ($C_{22}H_{24}ClN_3O.HCl$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 9/5$

M_S: Amount (mg) of azelastine hydrochloride for assay taken

M_T: Amount (g) of Azelastine Hydrochloride Granules taken

C: Labeled amount (mg) of azelastine hydrochloride ($C_{22}H_{24}ClN_3O.HCl$) in 1 g

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of azelastine are not less than 2000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak

area of azelastine is not more than 2.0%.

Assay Weigh accurately an amount of Azelastine Hydrochloride Granules, equivalent to about 2 mg of azelastine hydrochloride ($C_{22}H_{24}ClN_3O.HCl$), add 50 mL of 0.1 mol/L hydrochloric acid TS, treat with ultrasonic waves for 20 minutes, add 40 mL of ethanol (99.5), add exactly 5 mL of the internal standard solution, and add ethanol (99.5) to make 100 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of azelastine hydrochloride for assay, previously dried at 105°C for 2 hours, and dissolve in water to make exactly 50 mL. Pipet 10 mL of this solution, and add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 10 mL of this solution, add 40 mL of 0.1 mol/L hydrochloric acid TS and 40 mL of ethanol (99.5), add exactly 5 mL of the internal standard solution, add ethanol (99.5) to make 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of azelastine to that of the internal standard.

$$\text{Amount (mg) of azelastine hydrochloride } (C_{22}H_{24}ClN_3O.HCl) = M_S \times Q_T/Q_S \times 1/25$$

M_S: Amount (mg) of azelastine hydrochloride for assay taken

Internal standard solution—Dissolve 0.2 g of 2-ethylhexyl parahydroxybenzoate in ethanol (99.5) to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of acetonitrile and a solution of sodium lauryl sulfate in diluted acetic acid (100) (1 in 250) (1 in 500) (11:9).

Flow rate: Adjust so that the retention time of azelastine is about 6 minutes.

System suitability—

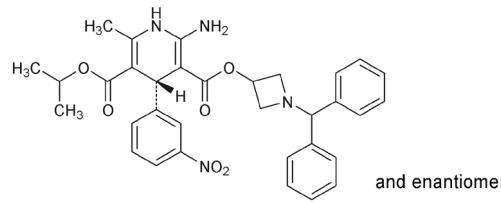
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, azelastine and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of azelastine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Azelnidipine

アゼルニジピン



and enantiomer

$C_{33}H_{34}N_4O_6$: 582.65

3-[1-(Diphenylmethyl)azetidin-3-yl] 5-(1-methylethyl) (4*RS*)-2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate

[*123524-52-7*]

Azelnidipine contains not less than 99.0% and not more than 101.0% of azelnidipine ($C_{33}H_{34}N_4O_6$), calculated on the dried basis.

Description Azelnidipine occurs as a light yellow to yellow, crystalline powder or powder containing masses.

It is freely soluble in ethanol (99.5) and in acetic acid (100), and practically insoluble in water.

A solution of Azelnidipine in ethanol (99.5) (1 in 100) shows no optical rotation.

Azelnidipine shows crystal polymorphism.

Identification **(1)** Determine the absorption spectrum of a solution of Azelnidipine in ethanol (99.5) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Azelnidipine as directed in the paste method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Azelnidipine according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.10 g of Azelnidipine in a mixture of acetonitrile and water (4:1) to make 100 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add a mixture of acetonitrile and water (4:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the areas of the peak, having the relative retention time of about 0.50 and about 1.42 to azelnidipine, obtained from the sample solution are not larger than 1/5 times and 3/10 times the peak area of azelnidipine obtained from the standard solution, respectively, the area of the peak other than azelnidipine and the peaks mentioned above from the sample solution is not larger than 1/10 times the peak area of azelnidipine from the standard solution, and the total area of the peaks other than azelnidipine from the sample solution is not larger than 7/10 times the peak area of azelnidipine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-

length: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.05 g of potassium dihydrogen phosphate in 350 mL of water, add 650 mL of a mixture of acetonitrile and methanol (7:3), and adjust to pH 5.5 with diluted phosphoric acid (1 in 10).

Flow rate: Adjust so that the retention time of azelnidipine is about 36 minutes.

Time span of measurement: About 2 times as long as the retention time of azelnidipine, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add a mixture of acetonitrile and water (4:1) to make exactly 20 mL. Confirm that the peak area of azelnidipine obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of azelnidipine are not less than 15,000 and 0.8 to 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of azelnidipine is not more than 1.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, 70°C, 5 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Azelnidipine, dissolve in 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.13 mg of $C_{33}H_{34}N_4O_6$

Containers and storage Containers—Tight containers.

Azelnidipine Tablets

アゼルニジピン錠

Azelnidipine Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of azelnidipine ($C_{33}H_{34}N_4O_6$: 582.65).

Method of preparation Prepare as directed under Tablets, with Azelnidipine.

Identification Powder Azelnidipine Tablets. Weigh a portion of the powder, equivalent to 4 mg of Azelnidipine, add 150 mL of ethanol (99.5), treat with ultrasonic waves for 15 minutes, then add ethanol (99.5) to make 200 mL. Centrifuge this solution, filter the supernatant liquid through a glass wool filter with a pore size not exceeding 0.7 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 253 nm and 257 nm and between 339 nm and 346 nm.

Purity Related substances—Conduct this procedure using light-resistant vessels. Powder Azelnidipine Tablets. Weigh a portion of the powder, equivalent to 10 mg of Azelnidipine, add 10 mL of a mixture of acetonitrile and water (4:1), agitate gently, then disperse to fine particles with the aid of ultrasonic waves for 15 minutes. Centrifuge this solution, and use the supernatant liquid as the sample solution. Pipet 2 mL of the sample solution, add a mixture of acetonitrile and water (4:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the areas of the peaks, having the relative retention times of about 0.10, about 0.13, about 0.50, and about 1.42 to azelnidipine, obtained from the sample solution, are not larger than 9/20 times, 1/5 times, 2/5 times, and 2/5 times the peak area of azelnidipine obtained from the standard solution, respectively, the area of the peak, other than azelnidipine and the peaks mentioned above, is not larger than 1/10 times the peak area of azelnidipine from the standard solution. Furthermore, the total area of these peaks other than azelnidipine is not larger than 1.75 times the peak area of azelnidipine from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (2) under Azelnidipine.

Time span of measurement: About 2 times as long as the retention time of azelnidipine.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add a mixture of acetonitrile and water (4:1) to make exactly 20 mL. Confirm that the peak area of azelnidipine obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of azelnidipine are not less than 15,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of azelnidipine is not more than 1.0%.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Azelnidipine Tablets add exactly 1 mL of the internal standard solution per 2 mg of azelnidipine ($C_{33}H_{34}N_4O_6$), and add a mixture of acetonitrile and water (4:1) to make 32 mL. Disintegrate the tablet with occasional shaking, and treat with ultrasonic waves for 10 minutes. Centrifuge this solution, pipet V mL of the supernatant liquid, equivalent to 2.5 mg of azelnidipine ($C_{33}H_{34}N_4O_6$), add a mixture of acetonitrile and water (4:1) to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of azelnidipine ($C_{33}H_{34}N_4O_6$)
$= M_S \times Q_T/Q_S \times 8/5V$

M_S: Amount (mg) of azelnidipine for assay taken

Internal standard solution—A solution of 2,2′-dinaphthylether in a mixture of acetonitrile and water (4:1) (1 in 1000).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of Azelnidipine Tablets is not less than 75%.

Start the test with 1 tablet of Azelnidipine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 8.9 μg of azelnidipine ($C_{33}H_{34}N_4O_6$), and use this solution as the sample solution. Separately, weigh accurately about 45 mg of azelnidipine for assay, previously dried in vacuum at 70°C for 5 hours, dissolve in ethanol (99.5) to make exactly 25 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 270 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using the dissolution medium as the blank.

Dissolution rate (%) with respect to the labeled amount of azelnidipine ($C_{33}H_{34}N_4O_6$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of azelnidipine for assay taken
C: Labeled amount (mg) of azelnidipine ($C_{33}H_{34}N_4O_6$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Azelnidipine Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of azelnidipine ($C_{33}H_{34}N_4O_6$), add exactly 25 mL of the internal standard solution, add 50 mL of a mixture of acetonitrile and water (4:1). After treating with ultrasonic waves for 10 minutes, add a mixture of acetonitrile and water (4:1) to make 100 mL. Centrifuge this solution, to 5 mL of the supernatant liquid add a mixture of acetonitrile and water (4:1) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of azelnidipine for assay, previously dried in vacuum at 70°C for 5 hours, dissolve in exactly 25 mL of the internal standard solution, and add a mixture of acetonitrile and water (4:1) to make 100 mL. To 5 mL of this solution add a mixture of acetonitrile and water (4:1) to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of azelnidipine to that of the internal standard.

Amount (mg) of azelnidipine ($C_{33}H_{34}N_4O_6$) $= M_S \times Q_T/Q_S$

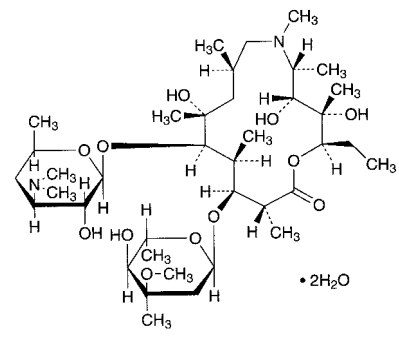
M_S: Amount (mg) of azelnidipine for assay taken

Internal standard solution—2,2′-dinaphthylether in a mixture of acetonitrile and water (4:1) (1 in 1000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 0.9 g of potassium dihydrogen phosphate in 300 mL of water, add 700 mL of acetonitrile, then adjust to pH 6.0 with dilute sodium hydroxide TS.
Flow rate: Adjust so that the retention time of azelnidipine is about 13 minutes.
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, azelnidipine and the internal standard are eluted in this order with the resolution between these peaks being not less than 12.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of azelnidipine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Azithromycin Hydrate

アジスロマイシン水和物



$C_{38}H_{72}N_2O_{12}.2H_2O$: 785.02
(2*R*,3*S*,4*S*,5*R*,6*R*,8*R*,11*R*,12*R*,13*S*,14*R*)-5-(3,4,6-Trideoxy-3-dimethylamino-β-D-*xylo*-hexopyranosyloxy)-3-(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-ribo-hexopyranosyloxy)-10-aza-6,12,13-trihydroxy-2,4,6,8,10,11,13-heptamethylhexadecan-14-olide dihydrate
[*117772-70-0*]

Azithromycin Hydrate is the derivative of erythromycin.

It contains not less than 945 μg (potency) and not more than 1030 μg (potency) per mg, calculated on the anhydrous basis. The potency of Azithromycin Hydrate is expressed as mass (potency) of azithromycin ($C_{38}H_{72}N_2O_{12}$: 748.98).

Description Azithromycin Hydrate occurs as a white crystalline powder.

It is freely soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification Determine the infrared absorption spectrum of Azithromycin Hydrate as directed in the potassium bromide disk method under the Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Azithromycin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −45 – −49° (0.4 g calculated on the anhydrous basis, ethanol (99.5), 20 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Azithromycin Hydrate according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Being specified separately when the drug is granted approval based on the Law.

Water <*2.48*> Not less than 4.0% and not more than 5.0% (0.4 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Azithromycin Hydrate and Azithromycin RS, equivalent to about 50 mg (potency), dissolve each in an adequate amount of a mixture of acetonitrile and water (3:2), add exactly 2 mL of the internal standard solution and the mixture of acetonitrile and water (3:2) to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of azithromycin to that of the internal standard.

Amount [μg (potency)] of azithromycin ($C_{38}H_{72}N_2O_{12}$)
$= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Azithromycin RS taken

Internal standard solution—A solution of 4,4′-bis(diethylamino)benzophenone in acetonitrile (3 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized polyvinyl alcohol gel polymer for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 6.97 g of dipotassium hydrogen phosphate in about 750 mL of water, adjust the pH to 11.0 with potassium hydroxide TS, and add water to make 1000 mL. To 400 mL of this solution add 600 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust so that the retention time of azithromycin is about 10 minutes.

System suitability—

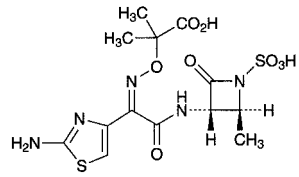
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, azithromycin and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of azithromycin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Aztreonam

アズトレオナム



$C_{13}H_{17}N_5O_8S_2$: 435.43

2-{(*Z*)-(2-Aminothiazol-4-yl)-[(2*S*,3*S*)-2-methyl-4-oxo-1-sulfoazetidin-3-ylcarbamoyl]methyleneaminooxy}-2-methyl-1-propanoic acid

[*78110-38-0*]

Aztreonam contains not less than 920 μg (potency) and not more than 1030 μg (potency) per mg, calculated on the anhydrous basis. The potency of Aztreonam is expressed as mass (potency) of aztreonam ($C_{13}H_{17}N_5O_8S_2$).

Description Aztreonam occurs as a white to yellowish white crystalline powder.

It is freely soluble in dimethylsulfoxide, slightly soluble in water and in methanol, and very slightly soluble in ethanol (95).

Identification **(1)** Determine the absorption spectrum of a solution of Aztreonam (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Aztreonam RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the spectrum of a solution of Aztreonam in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 10), using a light hydrogen substance existing in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy as an internal reference compound and 2.50 ppm for its chemical shift, as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*> (^{1}H): it exhibits a multiple signal at around δ 1.5 ppm, and a single signal at around δ 7.0 ppm. The ratio of integrated intensity of each signal is 9:1.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -26 – $-32°$ (0.25 g calculated on the anhydrous bases, water, 50 mL, 100 mm).

pH <*2.54*> Dissolve 0.05 g of Aztreonam in 10 mL of water: the pH of this solution is between 2.2 and 2.8.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Aztreonam in 20 mL of dimethylsulfoxide: the solution is clear, and its absorbance at 420 nm, determined as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, is not more than 0.06.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Aztreonam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 40 mg of Aztreonam in 100 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following condi-

tions, and determine each peak area of both solutions by the automatic integration method: the area of the peak other than aztreonam obtained from the sample solution is not larger than the peak area of aztreonam from the standard solution, and the total area of peaks other than aztreonam from the sample solution is not larger than 2.5 times the peak area of aztreonam from the standard solution.

Operating conditions—

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Time span of measurement: About 4 times as long as the retention time of aztreonam, beginning after the solvent peak.

System suitability—

Test for required detectability: To 5 mL of the standard solution add water to make 10 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add water to make exactly 10 mL. Confirm that the peak area of aztreonam obtained from 25 μL of this solution is equivalent to 7 to 13% of that obtained from 25 μL of the solution for system suitability test.

System performance: When the procedure is run under the above operating conditions with 25 μL of the standard solution obtained in the Assay, the internal standard and aztreonam are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of aztreonam is not more than 2.0%.

Water <*2.48*> Not more than 2.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Aztreonam and Aztreonam RS, equivalent to about 20 mg (potency), dissolve each in 70 mL of water, add exactly 10 mL of the internal standard solution and water to make 100 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 25 μL each of these solutions as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of aztreonam to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of aztreonam } (C_{13}H_{17}N_5O_8S_2) = M_S \times Q_T/Q_S \times 1000$$

M_S: Amount [mg (potency)] of Aztreonam RS taken

Internal standard solution—A solution of 4-aminobenzoic acid (1 in 6250).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.7 g of tetrabutylammonium hydrogensulfate in 300 mL of water, adjust to pH 3.0 with 0.5 mol/L disodium hydrogenphosphate TS, and add water to make 1000 mL. To 650 mL of this solution add 350 mL of methanol.

Flow rate: Adjust so that the retention time of aztreonam is about 8 minutes.

System suitability—

System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, the internal standard and aztreonam are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of aztreonam to that of the internal standard is not more than 1.5%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Aztreonam for Injection

注射用アズトレオナム

Aztreonam for Injection is a preparation for injection which is dissolved before use.

It contains not less than 93.0% and not more than 107.0% of the labeled potency of aztreonam ($C_{13}H_{17}N_5O_8S_2$: 435.43).

Method of preparation Prepare as directed under Injections, with Aztreonam.

Description Aztreonam for Injection is white to yellowish white masses or powder.

Identification (1) Dissolve an amount of Aztreonam for Injection, equivalent to 6 mg (potency) of Aztreonam, in 1 mL of hydroxylammonium chloride-ethanol TS, allow to stand for 3 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS, and mix: a red-brown color develops.

(2) Dissolve an amount of Aztreonam for Injection, equivalent to 3 mg (potency) of Aztreonam, in 100 mL of water, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 289 nm and 293 nm.

pH <*2.54*> The pH of a solution prepared by dissolving an amount of Aztreonam for Injection, equivalent to 1.0 g (potency) of Aztreonam, in 10 mL of water is 4.5 to 7.0.

Purity Clarity and color of solution—Dissolve an amount of Aztreonam for Injection, equivalent to 1.0 g (potency) of Aztreonam, in 10 mL of water: the solution is clear, and its absorbance <*2.24*> at 450 nm is not more than 0.06.

Water <*2.48*> Not more than 2.0% (0.5 g, volumetric titration, direct titration).

Bacterial endotoxins <*4.01*> Less than 0.10 EU/mg (potency).

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test.

Foreign insoluble matter <*6.06*> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Take an amount of Aztreonam for Injection, equivalent to about 5 g (potency) of Aztreonam, dissolve the contents with a suitable amount of water, and transfer to a 100-mL volumetric flask. Wash each container with water, combine the washings and the solution, and add water to make exactly 100 mL. Pipet 10 mL of this solution, and add water to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Aztreonam RS, equivalent to about 20 mg (potency), dissolve in a suitable amount of water, add exactly 10 mL of the internal standard solution and water to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Aztreonam.

$$\text{Amount [mg (potency)] of aztreonam } (C_{13}H_{17}N_5O_8S_2) = M_S \times Q_T/Q_S \times 250$$

M_S: Amount [mg (potency)] of Aztreonam RS taken

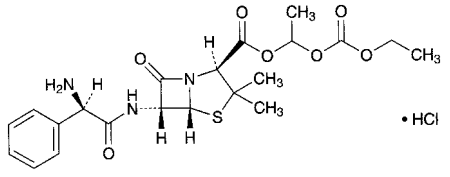
Internal standard solution—A solution of 4-aminobenzoic acid (1 in 6250).

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant.

Bacampicillin Hydrochloride

Ampicillin Ethoxycarbonyloxyethyl Hydrochloride

バカンピシリン塩酸塩



$C_{21}H_{27}N_3O_7S.HCl$: 501.98
1-Ethoxycarbonyloxyethyl (2*S*,5*R*,6*R*)-6-[(2*R*)-2-amino-2-phenylacetylamino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrochloride
[*37661-08-8*]

Bacampicillin Hydrochloride is a hydrochloride of ampicilline ethoxycarbonyloxyethyl ester.

It contains not less than 626 μg (potency) and not more than 710 μg (potency) per mg, calculated on the anhydrous basis. The potency of Bacampicillin Hydrochloride is expressed as mass (potency) of ampicillin ($C_{16}H_{19}N_3O_4S$: 349.40).

Description Bacampicillin Hydrochloride occurs as a white to pale yellow crystalline powder.

It is freely soluble in methanol and in ethanol (95), and soluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Bacampicillin Hydrochloride in methanol (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Bacampicillin Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bacampicillin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Bacampicillin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Bacampicillin Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> for chloride.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +140 – +170° (0.1 g calculated on the anhydrous basis, ethanol (95), 25 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Bacampicillin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Bacampicillin Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(3) Free ampicillin—Carry out the determination immediately after preparing the sample solution. Weigh accurately about 0.1 g of Bacampicillin Hydrochloride, dissolve in exactly 10 mL of the internal standard solution, add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Ampicillin RS, equivalent to about 25 mg (potency), and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add exactly 10 mL of the internal standard solution, add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ampicillin to that of the internal standard in each solution. The amount of ampicillin, calculated by the following equation, is not more than 1.0%.

$$\text{Amount (\%) of ampicillin } (C_{16}H_{19}N_3O_4S) = M_S/M_T \times Q_T/Q_S \times 4$$

M_S: Amount [mg (potency)] of Ampicillin RS taken
M_T: Amount (mg) of Bacampicillin Hydrochloride taken

Internal standard solution—A solution of anhydrous caffeine in the mobile phase (1 in 25,000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 230 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: Dissolve 1.22 g of potassium dihydrogen phosphate in water to make 900 mL, and add 100 mL of acetonitrile.
Flow rate: Adjust so that the retention time of ampicillin is about 7 minutes.
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ampicillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ampicillin to that of the internal standard is not more than 2.0%.

Water ⟨2.48⟩ Not more than 1.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition ⟨2.44⟩ Not more than 1.5% (1 g).

Assay Weigh accurately an amount of Bacampicillin Hydrochloride and Bacampicillin Hydrochloride RS, equivalent to about 40 mg (potency), dissolve each in water to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of bacampicillin in each solution.

Amount [μg (potency)] of ampicillin ($C_{16}H_{19}N_3O_4S$) = $M_S \times A_T/A_S \times 1000$

M_S: Amount [mg (potency)] of Bacampicillin Hydrochloride RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 500 mL of diluted 2 mol/L sodium dihydrogen phosphate TS (1 in 100), add diluted 0.05 mol/L disodium hydrogen phosphate TS (2 in 5) to adjust the pH to 6.8. To 500 mL of this solution add 500 mL of acetonitrile.

Flow rate: Adjust so that the retention time of bacampicillin is about 6.5 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of bacampicillin are not less than 10,000 and not more than 2, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of peak areas of bacampicillin is not more than 2.0%.

Containers and storage Containers—Tight containers.

Bacitracin

バシトラシン

[*1405-87-4*]

Bacitracin is a mixture of peptide substances having antibacterial activity including bacitracin A as the main component produced by the growth of *Bacillus subtilis* or *Bacillus licheniformis*.

It contains not less than 60 Units per mg, calculated on the dried basis. The potency of Bacitracin is expressed as unit calculated from the amount of bacitracin A ($C_{66}H_{103}N_{17}O_{16}S$: 1422.69). One unit of Bacitracin is equivalent to 23.8 μg of bacitracin A ($C_{66}H_{103}N_{17}O_{16}S$).

Description Bacitracin occurs as a white to light brown powder.

It is freely soluble in water, and slightly soluble in ethanol (99.5).

Identification **(1)** To 3 mL of a solution of Bacitracin (1 in 100) add 3 mL of 4-dimethylaminobenzaldehyde TS, shake until red-rosy to red-purple color appears, then add several drops of a solution of sodium nitrite (1 in 100), and shake: a green to dark green color is produced.

(2) Dissolve 60 mg each of Bacitracin and Bacitracin RS in 10 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨2.03⟩. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, acetic acid (100), water, pyridine and ethanol (99.5) (30:15:10:6:5) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin TS on the plate, and heat at 110°C for 5 minutes: the spots obtained from the sample solution and standard solution show the same *R*f value.

Purity **(1)** Heavy metals ⟨1.07⟩—Proceed with 1.0 g of Bacitracin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.15 g of Bacitracin in 0.05 mol/L sulfuric acid TS to make 100 mL. To 2 mL of this solution add 0.05 mol/L sulfuric acid TS to make 10 mL, and determine the absorbances of this solution, A_1 and A_2, at 252 nm and 290 nm as directed under Ultraviolet-visible Spectrophotometry ⟨2.24⟩: A_2/A_1 is not more than 0.20.

Loss on drying ⟨2.41⟩ Not more than 5.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition ⟨2.44⟩ Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨4.02⟩ according to the following conditions.

(i) Test organism—*Micrococcus luteus* ATCC 10240.

(ii) Culture medium—Use the medium iii in 3) under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Bacitracin RS, equivalent to about 400 units, dissolve in phosphate buffer solution (pH 6.0) to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 10°C and use within 2 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution (pH 6.0) to make solutions so that each mL contains 2 units and 0.5 units, and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

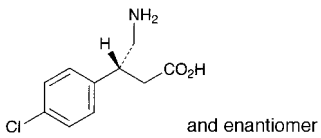
(iv) Sample solutions—Weigh accurately an amount of Bacitracin, equivalent about 400 units, dissolve in phosphate buffer solution (pH 6.0) to make exactly 20 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution (pH 6.0) to make solutions so that each mL contains 2 units and 0.5 units, and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—In a cold place.

Baclofen

バクロフェン



$C_{10}H_{12}ClNO_2$: 213.66
(3*RS*)-4-Amino-3-(4-chlorophenyl)butanoic acid
[*1134-47-0*]

Baclofen contains not less than 98.5% of baclofen ($C_{10}H_{12}ClNO_2$), calculated on the anhydrous basis.

Description Baclofen occurs as a white to pale yellowish white crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in water, very slightly soluble in methanol and in ethanol (95), and practically insoluble in diethyl ether.

It dissolves in dilute hydrochloric acid.

Identification **(1)** To 5 mL of a solution of Baclofen (1 in 1000) add 1 mL of ninhydrin TS, and heat on a water bath for 3 minutes: a blue-purple color develops.

(2) Determine the absorption spectrum of a solution of Baclofen in 0.1 mol/L hydrochloric acid TS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Baclofen RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Perform the test with Baclofen as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Purity **(1)** Chloride <*1.03*>—Dissolve 0.5 g of Baclofen in 50 mL of acetic acid (100), and add water to make 100 mL. To 10 mL of this solution add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.30 mL of 0.01 mol/L hydrochloric acid VS add 5 mL of acetic acid (100), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.21%).

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Baclofen according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Baclofen according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Baclofen in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1.0 mL and 1.5 mL of the sample solution, to each add the mobile phase to make exactly 100 mL, and use these solutions as the standard solutions (1) and (2), respectively. Perform the test with exactly 25 μL each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak height of these solutions: each height of the peaks other than the peak of baclofen from the sample solution is not larger than the peak height of baclofen from the standard solution (1), and the total height of these peaks is not larger than the peak height of baclofen from the standard solution (2).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 268 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and diluted acetic acid (100) (1 in 900) (3:2).

Flow rate: Adjust so that the retention time of baclofen is about 4 minutes.

Time span of measurement: About 3 times as long as the retention time of baclofen, beginning after the solvent peak.

System suitability—

Test for required detectability: Adjust the sensitivity so that the peak height of baclofen obtained from 25 μL of the standard solution (1) is between 5 and 10 mm.

System performance: Dissolve 0.40 g of Baclofen and 5 mg of methyl parahydroxybenzoate in 200 mL of the mobile phase. To 10 mL of this solution add the mobile phase to make 100 mL. When the procedure is run with 25 μL of this solution under the above operating conditions, baclofen and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak heights of baclofen is not more than 3.0%.

Water <*2.48*> Not more than 1.0% (1 g, direct titration).

Residue on ignition <*2.44*> Not more than 0.3% (1 g).

Assay Weigh accurately about 0.5 g of Baclofen, dissolve in 80 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to greenish blue (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 21.37 mg of $C_{10}H_{12}ClNO_2$

Containers and storage Containers—Well-closed containers.

Baclofen Tablets

バクロフェン錠

Baclofen Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of baclofen ($C_{10}H_{12}ClNO_2$: 213.66).

Method of preparation Prepare as directed under Tablets, with Baclofen.

Identification **(1)** To a portion of powdered Baclofen Tablets, equivalent to 0.01 g of Baclofen, add 10 mL of water, shake well, and filter. To 5 mL of the filtrate add 1 mL of ninhydrin TS, and proceed as directed in the Identification (1) under Baclofen.

(2) To a portion of powdered Baclofen Tablets, equivalent to 25 mg of Baclofen, add 50 mL of 0.1 mol/L hydrochloric acid TS, shake for 15 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 257 nm and 261 nm, between 264 nm and 268 nm, and between 272 nm and 276 nm.

(3) To a portion of powdered Baclofen Tablets, equiva-

lent to 0.01 g of Baclofen, add 2 mL of a mixture of methanol and acetic acid (100) (4:1), shake well, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 0.01 g of Baclofen RS in 2 mL of a mixture of methanol and acetic acid (100) (4:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution and that from the standard solution show the same Rf value.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Baclofen Tablets add 5 mL of 0.1 mol/L hydrochloric acid TS, disperse the tablet into small particles with the aid of ultrasonic waves, then shake for 10 minutes, and add 0.1 mol/L hydrochloric acid TS to make exactly V mL so that each mL contains about 0.5 mg of baclofen ($C_{10}H_{12}ClNO_2$). Centrifuge, pipet 5 mL of the supernatant liquid, add 2 drops of phenolphthalein TS, neutralize with dilute sodium hydroxide TS, then add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Baclofen RS (separately determine the water ⟨*2.48*⟩ in the same manner as Baclofen), and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 5 mL of this solution, add 2 drops of phenolphthalein TS, neutralize with dilute sodium hydroxide TS, then add water to make exactly 50 mL, and use this solution as the standard solution. To exactly 2 mL each of the sample solution and standard solution add 4 mL of ninhydrin-tin (II) chloride TS, mix, heat on a water bath for 20 minutes, then immediately shake vigorously for 2 minutes. After cooling, add a mixture of water and 1-propanol (1:1) to make them exactly 25 mL, and determine the absorbances, A_T and A_S, of them at 570 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a solution obtained with 2 mL of water by the same procedure as above as the blank.

Amount (mg) of baclofen ($C_{10}H_{12}ClNO_2$)
$= M_S \times A_T/A_S \times V/50$

M_S: Amount (mg) of Baclofen RS taken, calculated on the anhydrous basis

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 500 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Baclofen Tablets is not less than 70%.

Start the test with 1 tablet of Baclofen Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent, add water to make exactly V' mL so that each mL contains about 10 μg of baclofen ($C_{10}H_{12}ClNO_2$), and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Baclofen RS (separately determine the water ⟨*2.48*⟩ in the same manner as Baclofen), dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and the standard solution at 220 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of baclofen ($C_{10}H_{12}ClNO_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 50$

M_S: Amount (mg) of Baclofen RS taken, calculated on the anhydrous basis
C: Labeled amount (mg) of baclofen ($C_{10}H_{12}ClNO_2$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Baclofen Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of baclofen ($C_{10}H_{12}ClNO_2$), add 130 mL of 0.1 mol/L hydrochloric acid TS, shake for 10 minutes, add 0.1 mol/L hydrochloric acid TS to make exactly 200 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add 2 drops of phenolphthalein TS, neutralize with dilute sodium hydroxide TS, add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.25 g of Baclofen RS (separately determine the water content ⟨*2.48*⟩ in the same manner as Baclofen), and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 10 mL of this solution, and add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 10 mL of this solution, add 2 drops of phenolphthalein TS, neutralize with dilute sodium hydroxide TS, add water to make exactly 50 mL, and use this solution as the standard solution. Pipet 2 mL each of the sample solution and the standard solution, to each add 4 mL of ninhydrin-stannous chloride TS, shake, heat on a water bath for 20 minutes, and shake at once vigorously for 2 minutes. After cooling, to each solution add a mixture of water and 1-propanol (1:1) to make exactly 25 mL. Determine the absorbances, A_T and A_S, of these solutions at 570 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a blank prepared with 2 mL of water in the same manner.

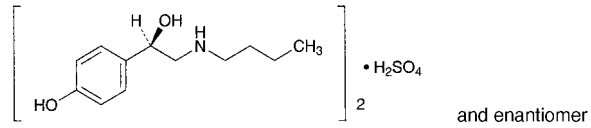
Amount (mg) of baclofen ($C_{10}H_{12}ClNO_2$)
$= M_S \times A_T/A_S \times 1/5$

M_S: Amount (mg) of Baclofen RS taken, calculated on the anhydrous basis

Containers and storage Containers—Well-closed containers.

Bamethan Sulfate

バメタン硫酸塩



and enantiomer

$(C_{12}H_{19}NO_2)_2.H_2SO_4$: 516.65
(1*RS*)-2-Butylamino-1-(4-hydroxyphenyl)ethanol hemisulfate
[*5716-20-1*]

Bamethan Sulfate, when dried, contains not less than 99.0% of bamethan sulfate [$(C_{12}H_{19}NO_2)_2.H_2SO_4$].

Description Bamethan Sulfate occurs as white, crystals or crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in water and in acetic acid (100), soluble in methanol, slightly soluble in ethanol (95), and practically

insoluble in diethyl ether.

Melting point: about 169°C (with decomposition).

Identification **(1)** To 1 mL of a solution of Bamethan Sulfate (1 in 1000) add 5 mL of a solution of 4-nitrobenzenediazonium fluoroborate (1 in 2000) and 10 mL of boric acid-potassium chloride-sodium hydroxide buffer solution (pH 9.2): an orange-red color develops.

(2) Determine the absorption spectrum of a solution of Bamethan Sulfate in 0.01 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Bamethan Sulfate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 1618, 1597, 1518, 1118 and 833 cm^{-1}.

(4) A solution of Bamethan Sulfate (1 in 100) responds to the Qualitative Tests <*1.09*> for sulfate.

pH <*2.54*> Dissolve 1.0 g of Bamethan Sulfate in 10 mL of water: the pH of this solution is between 4.0 and 5.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Bamethan Sulfate in 20 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To 1.5 mL of Matching Fluid O add diluted hydrochloric acid (1 in 40) to make 200 mL.

(2) Chloride <*1.03*>—Perform the test with 3.5 g of Bamethan Sulfate. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.002%).

(3) Heavy metals <*1.07*>—Proceed with 2.0 g of Bamethan Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Bamethan Sulfate according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Bamethan Sulfate in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (7:2) in a developing vessel saturated with ammonia vapor to a distance of about 12 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate, air-dry for 15 minutes, spray Dragendorff's TS for spraying again, then, after 1 minute, spray evenly a solution of sodium nitrite (1 in 20), and immediately put a glass plate on the plate. Examine the plate after 30 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

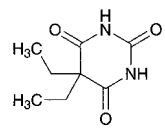
Assay Weigh accurately about 0.75 g of Bamethan Sulfate, previously dried, dissolve in 80 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 51.67 mg of $(C_{12}H_{19}NO_2)_2.H_2SO_4$

Containers and storage Containers—Tight containers.

Barbital

バルビタール



$C_8H_{12}N_2O_3$: 184.19
5,5-Diethylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione
[*57-44-3*]

Barbital, when dried, contains not less than 99.0% of barbital ($C_8H_{12}N_2O_3$).

Description Barbital occurs as colorless or white crystals or a white crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in acetone and in pyridine, soluble in ethanol (95), sparingly soluble in diethyl ether, and slightly soluble in water and in chloroform.

It dissolves in sodium hydroxide TS and in ammonia TS.

The pH of its saturated solution is between 5.0 and 6.0.

Identification **(1)** Boil 0.2 g of Barbital with 10 mL of sodium hydroxide TS: the gas evolved changes moistened red litmus paper to blue.

(2) Dissolve 0.05 g of Barbital in 5 mL of diluted pyridine (1 in 10), add 0.3 mL of copper (II) sulfate TS, shake, and allow to stand for 5 minutes: a red-purple precipitate is formed. Shake the mixture with 5 mL of chloroform: a red-purple color develops in the chloroform layer. Separately, dissolve 0.05 g of Barbital in 2 to 3 drops of ammonia-ammonium chloride buffer solution (pH 10.7) and 5 mL of diluted pyridine (1 in 10). Add 5 mL of chloroform and 0.3 mL of copper (II) sulfate TS to the solution: a red-purple precipitate is produced in the aqueous layer. The red-purple precipitate is not dissolved in the chloroform by shaking.

(3) To 0.4 g of Barbital add 0.1 g of anhydrous sodium carbonate and 4 mL of water, shake, and add a solution of 0.3 g of 4-nitrobenzyl chloride in 7 mL of ethanol (95). Heat the mixture on a water bath under a reflux condenser for 30 minutes, and allow to stand for 1 hour. Collect the separated crystals, wash with 7 mL of sodium hydroxide TS and a small amount of water, recrystallize from a mixture of ethanol (95) and chloroform (1:1), and dry at 105°C for 30 minutes: the crystals melt <*2.60*> between 192°C and 196°C.

Melting point <*2.60*> 189 – 192°C

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Barbital in 5 mL of sodium hydroxide TS: the solution is clear and colorless.

(2) Chloride <*1.03*>—Dissolve 0.30 g of Barbital in 20 mL of acetone, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: take 0.30 mL of 0.01 mol/L hydrochloric acid VS, 20 mL of acetone and 6 mL of dilute nitric acid, and add water to make 50 mL (not more than 0.035%).

(3) Sulfate <*1.14*>—Dissolve 0.40 g of Barbital in 20 mL of acetone, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as

the test solution. Prepare the control solution as follows: take 0.40 mL of 0.005 mol/L sulfuric acid VS, 20 mL of acetone, and 1 mL of dilute hydrochloric acid, and add water to make 50 mL (not more than 0.048%).

(4) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Barbital according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead solution (not more than 20 ppm).

(5) Readily carbonizable substances ⟨*1.15*⟩—Perform the test with 0.5 g of Barbital. The solution is not more colored than Matching Fluid A.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Barbital, previously dried, and dissolve in 5 mL of ethanol (95) and 50 mL of chloroform. Titrate ⟨*2.50*⟩ with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution changes from yellow through light blue to purple (indicator: 1 mL of alizarin yellow GG-thymolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 18.42 mg of $C_8H_{12}N_2O_3$

Containers and storage Containers—Well-closed containers.

Barium Sulfate

硫酸バリウム

$BaSO_4$: 233.39

Description Barium Sulfate occurs as a white powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It does not dissolve in hydrochloric acid, in nitric acid and in sodium hydroxide TS.

Identification **(1)** Mix 0.5 g of Barium Sulfate with 2 g each of anhydrous sodium carbonate and potassium carbonate in a crucible, heat the mixture until fusion is complete, treat the cooled mass with hot water, and filter. The filtrate, acidified with hydrochloric acid, responds to the Qualitative Tests ⟨*1.09*⟩ for sulfate.

(2) Wash the hot water-insoluble residue obtained in (1) with water, dissolve in 2 mL of acetic acid (31), and filter, if necessary: the solution responds to the Qualitative Tests ⟨*1.09*⟩ for barium salt.

Purity **(1)** Acidity or alkalinity—Agitate 1.0 g of Barium Sulfate with 20 mL of water for 5 minutes: the solution is neutral.

(2) Phosphate—Boil 1.0 g of Barium Sulfate with 3 mL of nitric acid and 5 mL of water for 5 minutes, cool, and add water to restore the original volume. Filter through a filter paper, previously washed with dilute nitric acid, to the filtrate add an equal volume of hexaammonium heptamolybdate TS, and allow to stand between 50°C and 60°C for 1 hour: no yellow precipitate is produced.

(3) Sulfide—Place 10 g of Barium Sulfate in a 250-mL conical flask, add 10 mL of dilute hydrochloric acid and water to make 100 mL, and boil for 10 minutes: the gas evolved does not darken moistened lead (II) acetate paper.

(4) Heavy metals ⟨*1.07*⟩—Boil 5.0 g of Barium Sulfate with 2.5 mL of acetic acid (100) and 50 mL of water for 10 minutes, cool, add 0.5 mL of ammonia TS and water to make 100 mL, and filter. Perform the test with a 50-mL portion of this filtrate. Prepare the control solution with 2.5 mL of Standard Lead Solution, 1.25 mL of acetic acid (100), 0.25 mL of ammonia TS and water to make 50 mL (not more than 10 ppm).

(5) Arsenic ⟨*1.11*⟩—Prepare the test solution with 2.0 g of Barium Sulfate according to Method 1, and perform the test (not more than 1 ppm).

(6) Hydrochloric acid-soluble substances and soluble barium salts—Cool the solution obtained in (3), add water to make 100 mL, and filter. Evaporate 50 mL of the filtrate on a water bath to dryness, add 2 drops of hydrochloric acid and 10 mL of warm water, filter through filter paper for assay, and wash with 10 mL of warm water. Evaporate the combined filtrate and washings on a water bath to dryness, and dry the residue at 105°C for 1 hour: the residue weighs not more than 15 mg. Shake the residue, if any, with 10 mL of water, and filter. To the filtrate add 0.5 mL of dilute sulfuric acid, and allow to stand for 30 minutes: no turbidity is produced.

Containers and storage Containers—Well-closed containers.

Freeze-dried BCG Vaccine (for Percutaneous Use)

乾燥 **BCG** ワクチン

Freeze-dried BCG Vaccine (for Percutaneous Use) is a preparation for injection which is dissolved before use.

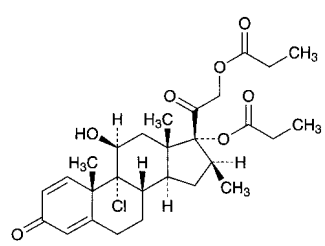
It contains live bacteria derived from a culture of the bacillus of Calmette and Guérin.

It conforms to the requirements of Freeze-dried BCG Vaccine (for Percutaneous Use) in the Minimum Requirements for Biological Products.

Description Freeze-dried BCG Vaccine (for Percutaneous Use) becomes a white to light yellow, turbid liquid on addition of solvent.

Beclometasone Dipropionate

ベクロメタゾンプロピオン酸エステル



$C_{28}H_{37}ClO_7$: 521.04
9-Chloro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17,21-dipropanoate
[*5534-09-8*]

Beclometasone Dipropionate, when dried, contains not less than 97.0% and not more than 103.0% of

beclometasone dipropionate ($C_{28}H_{37}ClO_7$).

Description Beclometasone Dipropionate occurs as a white to pale yellow powder.

It is soluble in methanol, sparingly soluble in ethanol (95) and in 1,4-dioxane, and practically insoluble in water.

Melting point: about 208°C (with decomposition).

It shows crystal polymorphism.

Identification **(1)** Dissolve 2 mg of Beclometasone Dipropionate in 2 mL of sulfuric acid: initially a yellowish color develops, and gradually changes through orange to dark red-brown. To this solution add carefully 10 mL of water: the color changes to bluish green, and a flocculent precipitate is formed.

(2) Dissolve 0.01 g of Beclometasone Dipropionate in 1 mL of methanol, add 1 mL of Fehling's TS, and heat: a red to red-brown precipitate is formed.

(3) Perform the test with 0.02 g of Beclometasone Dipropionate as directed under Oxygen Flask Combustion Method <*1.06*>, using a mixture of 1 mL of sodium hydroxide TS and 20 mL of water as an absorbing liquid: the test solution responds to the Qualitative Tests <*1.09*> for chloride.

(4) Determine the infrared absorption spectrum of Beclometasone Dipropionate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Beclometasone Dipropionate RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Beclometasone Dipropionate and Beclometasone Dipropionate RS in ethanol (95), respectively, then evaporate the ethanol to dryness, and repeat the test on the residues.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +88 – +94° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 0.5 g of Beclometasone Dipropionate according to Method 2, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 20 mg of Beclometasone Dipropionate in 5 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (9:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1,2-dichloroethane, methanol and water (475:25:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly alkaline blue tetrazolium TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (0.5 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 20 mg each of Beclometasone Dipropionate and Beclometasone Dipropionate RS, previously dried, and dissolve each in methanol to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL of the internal standard solution and methanol to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of beclometasone dipropionate to that of the internal standard, respectively.

Amount (mg) of beclometasone dipropionate ($C_{28}H_{37}ClO_7$) $= M_S \times Q_T/Q_S$

M_S: Amount (mg) of Beclometasone Dipropionate RS taken

Internal standard solution—A solution of testosterone propionate in methanol (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetonitrile and water (3:2).

Flow rate: Adjust so that the retention time of beclometasone dipropionate is about 6 minutes.

System suitability—

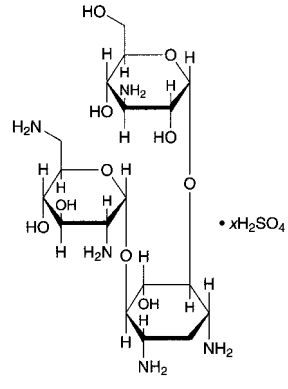
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, beclometasone dipropionate and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of beclometasone dipropionate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Bekanamycin Sulfate

ベカナマイシン硫酸塩



$C_{18}H_{37}N_5O_{10}.xH_2SO_4$

3-Amino-3-deoxy-α-D-glucopyranosyl-(1→6)-[2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl-(1→4)]-2-deoxy-D-streptamine sulfate

[*70550-99-1*]

Bekanamycin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of the mutant of *Streptomyces kanamyceticus*.

It contains not less than 680 μg (potency) and not more than 770 μg (potency) per mg, calculated on the dried basis. The potency of Bekanamycin Sulfate is expressed as mass (potency) of bekanamycin ($C_{18}H_{37}N_5O_{10}$: 483.51).

Description Bekanamycin Sulfate occurs as a white powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) Dissolve 20 mg of Bekanamycin Sulfate in 2 mL of 1/15 mol/L phosphate buffer solution (pH 5.6), add 1 mL of ninhydrin TS, and boil: a blue-purple color develops.

(2) Dissolve 30 mg each of Bekanamycin Sulfate and Bekanamycin Sulfate RS in 5 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS, and heat at 100°C for 10 minutes: the principal spots obtained from the sample solution and the standard solution show a purple-brown color and the same *R*f value.

(3) To a solution of Bekanamycin Sulfate (1 in 5) add 1 drop of barium chloride TS: a white turbidity is produced.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +102 – +116° (after drying, 0.25 g, water, 25 mL, 100 mm).

pH <*2.54*> The pH of a solution obtained by dissolving 0.50 g of Bekanamycin Sulfate in 10 mL of water is between 6.0 and 8.5.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Bekanamycin Sulfate in 5 mL of water: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Bekanamycin Sulfate according to Method 4, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 2.0 g of Bekanamycin Sulfate according to Method 1, and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 60 mg of Bekanamycin Sulfate in 10 mL of water, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot obtained from the standard solution.

Loss on drying <*2.41*> Not more than 5.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.5% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer having pH <*2.54*> 7.8 to 8.0 after sterilization.

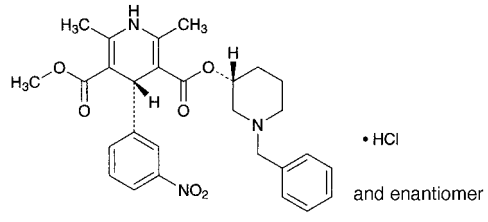
(iii) Standard solutions—Weigh accurately an amount of Bekanamycin Sulfate RS, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution (pH 6.0) (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 to 15°C and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 10 μg (potency) and 2.5 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Bekanamycin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make solutions so that each mL contains 10 μg (potency) and 2.5 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Benidipine Hydrochloride

ベニジピン塩酸塩



$C_{28}H_{31}N_3O_6$.HCl: 542.02

3-[(3*RS*)-1-Benzylpiperidin-3-yl] 5-methyl (4*RS*)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate monohydrochloride

[*91599-74-5*]

Benidipine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of benidipine hydrochloride ($C_{28}H_{31}N_3O_6$.HCl).

Description Benidipine Hydrochloride occurs as a yellow crystalline powder.

It is very soluble in formic acid, soluble in methanol, sparingly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Benidipine Hydrochloride in methanol (1 in 100) shows no optical rotation.

Melting point: about 200°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Benidipine Hydrochloride in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Benidipine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectro-

photometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 5 mL of a solution of Benidipine Hydrochloride (1 in 10) add 5 mL of ammonia TS, heat on a water bath for 5 minutes, cool, and filter. The filtrate, which is acidified with dilute nitric acid, responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Benidipine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 20 mg of Benidipine Hydrochloride in 100 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water and methanol (1:1) to make exactly 500 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of bisbenzylpiperidyl ester having the relative retention time of about 0.35 to benidipine, dehydro derivative having the relative retention time of about 0.75 and other related substances are not larger than 1/2 times the peak area of benidipine with the standard solution, and the total area of the peaks other than benidipine is not larger than the peak area of benidipine with the standard solution. For the areas of the peaks of bisbenzylpiperidyl ester and dehydro derivative, multiply their relative response factor 1.6, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 237 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate TS (pH 3.0), methanol and tetrahydrofuran (65:27:8).

Flow rate: Adjust so that the retention time of benidipine is about 20 minutes.

Time span of measurement: About 2 times as long as the retention time of benidipine, beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 5 mL of the standard solution, and add the mixture of water and methanol (1:1) to make exactly 20 mL. Confirm that the peak area of benidipine obtained with 10 μL of this solution is equivalent to 18 to 32% of that obtained with 10 μL of the standard solution.

System performance: Dissolve 6 mg of Benidipine Hydrochloride and 5 mg of benzoin in 200 mL of the mixture of water and methanol (1:1). When the procedure is run with 10 μL of this solution under the above operating conditions, benzoin and benidipine are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of benidipine is not more than 3.5%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (0.5 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.7 g of Benidipine Hydrochloride, previously dried, dissolve in 10 mL of formic acid, add 70 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 54.20 mg of $C_{28}H_{31}N_3O_6$.HCl

Containers and storage Containers—Tight containers.

Benidipine Hydrochloride Tablets

ベニジピン塩酸塩錠

Benidipine Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of benidipine hydrochloride ($C_{28}H_{31}N_3O_6$.HCl: 542.02).

Method of preparation Prepare as directed under Tablets, with Benidipine Hydrochloride.

Identification Shake well a quantity of powdered Benidipine Hydrochloride Tablets, equivalent to 10 mg of Benidipine Hydrochloride, with 100 mL of methanol, and centrifuge. To 10 mL of the supernatant liquid add methanol to make 100 mL, and use this solution as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 235 nm and 239 nm, and between 350 nm and 360 nm.

Purity Dehydro derivative—Powder Benidipine Hydrochloride Tablets in an agate mortar. To an amount of the powder, equivalent to 20 mg of Benidipine Hydrochloride, add about 80 mL of a mixture of diluted phosphoric acid (1 in 500) and methanol (1:1), shake well, and add the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make exactly 100 mL. Filter through a membrane filter with pore size of 0.45 μm, and use the filtrate as the sample solution. Separately, dissolve 20 mg of benidipine hydrochloride for assay in the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make exactly 100 mL. Pipet 1 mL of this solution, add the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the peak area of dehydro derivative having the relative retention time of about 0.75 to benidipine is not larger than 1/2 times the peak area of benidipine with the standard solution. For the area of the peak of dehydro derivative, multiply the relative response factor 1.6.

Operating conditions—

Perform as directed in the operating conditions in the Assay.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make exactly 20 mL. Confirm that the peak area of benidipine obtained with 10 μL of this solution is equivalent to 7 to 13% of that ob-

tained with 10 μL of the standard solution.

System performance: Dissolve 6 mg of benidipine hydrochloride and 5 mg of benzoin in 200 mL of a mixture of water and methanol (1:1). When the procedure is run with 10 μL of this solution under the above operating conditions, benzoin and benidipine are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of benidipine is not more than 2.0%.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Benidipine Hydrochloride Tablets add 40 mL of a mixture of diluted phosphoric acid (1 in 500) and methanol (1:1), shake to disintegrate, and add a suitable amount of the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make exactly V mL of a solution, containing 40 μg of benidipine hydrochloride ($C_{28}H_{31}N_3O_6$.HCl) per mL. Centrifuge the solution, pipet 20 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of benidipine hydrochloride ($C_{28}H_{31}N_3O_6$.HCl)
$= M_S \times Q_T/Q_S \times V/1000$

M_S: Amount (mg) of benidipine hydrochloride for assay taken

Internal standard solution—A solution of benzoin in a mixture of water and methanol (1:1) (13 in 200,000).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of 1st fluid for dissolution test as the dissolution medium, the dissolution rate of a 2-mg tablet and a 4-mg tablet in 30 minutes is not less than 80%, and that of a 8-mg tablet in 45 minutes is not less than 85%.

Start the test with 1 tablet of Benidipine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate pipet the subsequent V mL, and add the dissolution medium to make exactly V' mL so that each mL contains about 2.2 μg of benidipine hydrochloride ($C_{28}H_{31}N_3O_6$.HCl). Pipet 5 mL of this solution, add exactly 5 mL of the mobile phase, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of benidipine hydrochloride for assay, previously dried at 105°C for 2 hours, and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, and add the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, and add the mobile phase to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the dissolution medium, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of benidipine in each solution.

Dissolution rate (%) of benidipine hydrochloride ($C_{28}H_{31}N_3O_6$.HCl) with respect to the labeled amount
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9$

M_S: Amount (mg) of benidipine hydrochloride for assay taken

C: Labeled amount (mg) of benidipine hydrochloride ($C_{28}H_{31}N_3O_6$.HCl) in 1 tablet.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 237 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate TS (pH 3.0) and acetonitrile (11:9).

Flow rate: Adjust so that the retention time of benidipine is about 5 minutes.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of benidipine are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of benidipine is not more than 1.5%.

Assay Weigh accurately the mass of not less than 20 Benidipine Hydrochloride Tablets, and powder using an agate mortar. Weigh accurately a part of the powder, equivalent to about 8 mg of benidipine hydrochloride ($C_{28}H_{31}N_3O_6$.HCl), add about 150 mL of a mixture of diluted phosphoric acid (1 in 500) and methanol (1:1), shake, then add the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make exactly 200 mL, and centrifuge. Pipet 20 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of benidipine hydrochloride for assay, previously dried at 105°C for 2 hours, and dissolve in the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of benidipine to that of the internal standard.

Amount (mg) of benidipine hydrochloride ($C_{28}H_{31}N_3O_6$.HCl)
$= M_S \times Q_T/Q_S \times 1/5$

M_S: Amount (mg) of benidipine hydrochloride for assay taken

Internal standard solution—A solution of benzoin in a mixture of water and methanol (1:1) (13 in 200,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 237 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about

25°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate TS (pH 3.0), methanol and tetrahydrofuran (65:27:8).

Flow rate: Adjust so that the retention time of benidipine is about 20 minutes.

System suitability—

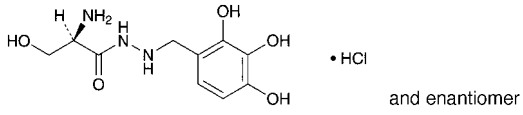
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and benidipine are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of benidipine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Benserazide Hydrochloride

ベンセラジド塩酸塩



and enantiomer

$C_{10}H_{15}N_3O_5$.HCl: 293.70
(2*RS*)-2-Amino-3-hydroxy-*N'*-(2,3,4-trihydroxybenzyl)propanoylhydrazide monohydrochloride
[*14919-77-8*]

Benserazide Hydrochloride contains not less than 98.0% and not more than 101.0% of benserazide hydrochloride ($C_{10}H_{15}N_3O_5$.HCl), calculated on the anhydrous basis.

Description Benserazide Hydrochloride occurs as a white to grayish white crystalline powder.

It is freely soluble in water and in formic acid, soluble in methanol, very slightly soluble in ethanol (95).

It dissolves in 0.1 mol/L hydrochloric acid TS.

The pH of a solution of 1.0 g of Benserazide Hydrochloride in 100 mL of water is between 4.0 and 5.0.

It is hygroscopic.

It is gradually colored by light.

A solution of Benserazide Hydrochloride (1 in 100) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Benserazide Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Benserazide Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 10 mL of a solution of Benserazide Hydrochloride (1 in 30) add silver nitrate TS: a white precipitate is formed. To a portion of this precipitate add dilute nitric acid: the precipitation does not dissolve.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Benserazide Hydrochloride in 10 mL of water, and perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: the absorbance of this solution at 430 nm is not more than 0.10.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Benserazide Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.25 g of Benserazide Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL and 3 mL of the sample solution, add methanol to make exactly 200 mL, and use these solutions as the standard solution (1) and (2), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solutions (1) and (2) on a plate of cellulose for thin-layer chromatography. Develop the plate with a solution of formic acid in sodium chloride TS (1 in 1000) to a distance of about 10 cm, and air-dry the plate. Spray evenly sodium carbonate TS, air-dry, and then spray evenly Folin's TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (2), and the number of the spots which intense more than the spot from the standard solution (1) are not more than 2.

Water <*2.48*> Not more than 2.5% (0.5 g, volumetric titration, direct titration). Use a solution of salicylic acid in methanol for water determination (3 in 20) instead of methanol for water determination.

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Benserazide Hydrochloride, dissolve in 5 mL of formic acid, add 50 mL of acetic acid (100), and titrate <*2.50*> immediately with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.37 mg of $C_{10}H_{15}N_3O_5$.HCl

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Bentonite

ベントナイト

Bentonite is a natural, colloidal, hydrated aluminum silicate.

Description Bentonite occurs as a very fine, white to light yellow-brown powder. It is odorless. It has a slightly earthy taste.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It swells in water.

Identification **(1)** Add 0.5 g of Bentonite to 3 mL of diluted sulfuric acid (1 in 3), and heat until white fumes are evolved. Cool, add 20 mL of water, and filter. To 5 mL of the filtrate add 3 mL of ammonia TS: a white, gelatinous precipitate is produced, which turns red on the addition of 5

drops of alizarin red S TS.

(2) Wash the residue obtained in (1) with water, add 2 mL of a solution of methylene blue trihydrate (1 in 10,000), and wash again with water: the residue is blue in color.

pH ⟨*2.54*⟩ To 1.0 g of Bentonite add 50 mL of water, and shake: the pH of the suspension is between 9.0 and 10.5.

Purity **(1)** Heavy metals ⟨*1.07*⟩—To 1.5 g of Bentonite add 80 mL of water and 5 mL of hydrochloric acid, and boil gently for 20 minutes with thorough stirring. Cool, centrifuge, collect the supernatant liquid, wash the residue with two 10-mL portions of water, and centrifuge each. Combine the supernatant liquid and the washings, and add dropwise ammonia solution (28). When a precipitate is produced, add dropwise dilute hydrochloric acid with vigorous stirring, and dissolve. To the solution add 0.45 g of hydroxylammonium chloride, and heat. Cool, and add 0.45 g of sodium acetate trihydrate, 6 mL of dilute acetic acid and water to make 150 mL. Pipet 50 mL of the solution, and perform the test using this solution as the test solution. Prepare the control solution as follows: mix 2.5 mL of Standard Lead Solution, 0.15 g of hydroxylammonium chloride, 0.15 g of sodium acetate trihydrate, and 2 mL of dilute acetic acid, and add water to make 50 mL (not more than 50 ppm).

(2) Arsenic ⟨*1.11*⟩—To 1.0 g of Bentonite add 5 mL of dilute hydrochloric acid, and gently heat to boil while stirring well. Cool immediately, and centrifuge. To the residue add 5 mL of dilute hydrochloric acid, shake well, and centrifuge. To the residue add 10 mL of water, and perform the same operations. Combine all the extracts, and heat on a water bath to concentrate to 5 mL. Perform the test with this solution as the test solution (not more than 2 ppm).

(3) Foreign matter—Place 2.0 g of Bentonite in a mortar, add 20 mL of water to swell, disperse evenly with a pestle, and dilute with water to 100 mL. Pour the suspension through a No. 200 (74 μm) sieve, and wash the sieve thoroughly with water. No grit is felt when the fingers are rubbed over the wire mesh of the sieve.

Loss on drying ⟨*2.41*⟩ 5.0 – 10.0% (2 g, 105°C, 2 hours).

Gel formation Mix 6.0 g of Bentonite with 0.30 g of magnesium oxide. Add the mixture, in several portions, to 200 mL of water contained in a glass-stoppered 500-mL cylinder. Agitate for 1 hour, transfer 100 mL of the suspension to a 100-mL graduated cylinder, and allow to stand for 24 hours: not more than 2 mL of supernatant appears on the surface.

Swelling power To 100 mL of water in a glass-stoppered 100-mL cylinder add 2.0 g of Bentonite in ten portions, allowing each portion to settle before adding the next, and allow to stand for 24 hours: the apparent volume of the sediment at the bottom is not less than 20 mL.

Containers and storage Containers—Well-closed containers.

Benzalkonium Chloride

ベンザルコニウム塩化物

Benzalkonium Chloride is represented by the for mula $[C_6H_5CH_2N(CH_3)_2R]Cl$, in which R extends from C_8H_{17} to $C_{18}H_{37}$, with $C_{12}H_{25}$ and $C_{14}H_{29}$ comprising the major portion.

It contains not less than 95.0% and not more than 105.0% of benzalkonium chloride (as $C_{22}H_{40}ClN$: 354.01), calculated on the anhydrous basis.

Description Benzalkonium Chloride occurs as a white to yellowish white powder, colorless to light yellow, gelatinous pieces, or jelly-like fluid or mass. It has a characteristic odor.

It is very soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

A solution of Benzalkonium Chloride foams strongly when shaken.

Identification **(1)** Dissolve 0.2 g of Benzalkonium Chloride in 1 mL of sulfuric acid, add 0.1 g of sodium nitrate, and heat for 5 minutes on a water bath. After cooling, add 10 mL of water and 0.5 g of zinc powder, heat for 5 minutes, cool, and filter: the filtrate responds to the Qualitative Tests ⟨*1.09*⟩ for primary aromatic amines. The color of the solution is red.

(2) To 2 mL of a solution of Benzalkonium Chloride (1 in 1000) add a mixture of 0.2 mL of a solution of bromophenol blue (1 in 2000) and 0.5 mL of sodium hydroxide TS: a blue color develops. Add 4 mL of chloroform to this solution, and shake vigorously: the blue color shifts to the chloroform layer. Collect the chloroform layer, and add dropwise, with stirring, a solution of sodium lauryl sulfate (1 in 1000): the chloroform layer turns colorless.

(3) Determine the absorption spectrum of a solution of Benzalkonium Chloride in 0.1 mol/L hydrochloric acid TS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) To 1 mL of a solution of Benzalkonium Chloride (1 in 100) add 2 mL of ethanol (95), 0.5 mL of dilute nitric acid and 1 mL of silver nitrate TS: a white precipitate is produced. This precipitate does not dissolve on the addition of dilute nitric acid, but dissolves on the addition of ammonia TS.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Benzalkonium Chloride in 10 mL of water: the solution is clear and colorless to light yellow.

(2) Petroleum ether-soluble substances—To 3.0 g of Benzalkonium Chloride add water to make 50 mL, then add 50 mL of ethanol (99.5) and 5 mL of 0.5 mol/L sodium hydroxide TS, and extract with three 50-mL portions of petroleum ether. Combine the petroleum ether extracts, and wash with three 50-mL portions of dilute ethanol. After shaking well with 10 g of anhydrous sodium sulfate, filter through a dry filter paper, and wash the filter paper with two 10-mL portions of petroleum ether. Evaporate the petroleum ether on a water bath by heating, and dry the residue at 105°C for 1 hour: the residue is not more than 1.0%.

Water ⟨*2.48*⟩ Not more than 15.0% (volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Assay Weigh accurately about 0.15 g of Benzalkonium Chloride, and dissolve in 75 mL of water. Adjust the pH between 2.6 and 3.4 by adding dropwise diluted dilute hydrochloric acid (1 in 2), add 1 drop of methyl orange TS, and titrate ⟨*2.50*⟩ with 0.02 mol/L sodium tetraphenylboron VS until the color of the solution becomes red.

Each mL of 0.02 mol/L sodium tetraphenylboron VS
= 7.080 mg of $C_{22}H_{40}ClN$

Containers and storage Containers—Tight containers.

Benzalkonium Chloride Solution

ベンザルコニウム塩化物液

Benzalkonium Chloride Solution is an aqueous solution containing not more than 50.0 w/v% of benzalkonium chloride.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of benzalkonium chloride ($C_{22}H_{40}ClN$: 354.01).

Method of preparation Dissolve Benzalkonium Chloride in Water, Purified Water or Purified Water in Containers. It is also prepared by diluting Concentrated Benzalkonium Chloride Solution 50 with Water, Purified Water or Purified Water in Containers.

Description Benzalkonium Chloride Solution is a clear, colorless to light yellow liquid, having a characteristic odor.

It foams strongly on shaking.

Identification **(1)** Evaporate a volume of Benzalkonium Chloride Solution, equivalent to 0.2 g of Benzalkonium Chloride, on a water bath to dryness, and proceed with the residue as directed in the Identification (1) under Benzalkonium Chloride.

(2) To a volume of Benzalkonium Chloride Solution, equivalent to 0.01 g of Benzalkonium Chloride, add water to make 10 mL. Proceed with 2 mL of this solution as directed in the Identification (2) under Benzalkonium Chloride.

(3) To a volume of Benzalkonium Chloride Solution, equivalent to 1 g of Benzalkonium Chloride, add water or concentrate on a water bath, if necessary, to make 10 mL. To 1 mL of this solution add 0.1 mol/L hydrochloric acid VS to make 200 mL, and proceed as directed in the Identification (3) under Benzalkonium Chloride.

(4) To a volume of Benzalkonium Chloride Solution, equivalent to 0.1 g of Benzalkonium Chloride, add water or concentrate on a water bath, if necessary, to make 10 mL. Proceed with 1 mL of this solution as directed in the Identification (4) under Benzalkonium Chloride.

Assay Pipet a volume of Benzalkonium Chloride Solution, equivalent to about 0.15 g of benzalkonium chloride ($C_{22}H_{40}ClN$), dilute with water to make 75 mL, if necessary, and proceed as directed in the Assay under Benzalkonium Chloride.

Each mL of 0.02 mol/L sodium tetraphenylboron VS
= 7.080 mg of $C_{22}H_{40}ClN$

Containers and storage Containers—Tight containers.

Benzalkonium Chloride Concentrated Solution 50

濃ベンザルコニウム塩化物液 **50**

Benzalkonium Chloride Concentrated Solution 50 is an aqueous solution, presented as $[C_6H_5CH_2N(CH_3)_2R]Cl$, where R ranges from C_8H_{17} to $C_{18}H_{37}$, and mainly consisting of $C_{12}H_{25}$ and $C_{14}H_{29}$.

It contains more than 50.0% and not more than 55.0% of benzalkonium chloride ($C_{22}H_{40}ClN$: 354.01).

Description Benzalkonium Chloride Concentrated Solution 50 is a colorless to light yellow liquid or jelly-like fluid, and has a characteristic odor.

It is very soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

A solution prepared by adding water to it vigorously foams when shaken.

Identification **(1)** Dissolve 0.4 g of Benzalkonium Chloride Concentrated Solution 50 in 1 mL of sulfuric acid, add 0.1 g of sodium nitrate, and heat for 5 minutes on a water bath. After cooling, add 10 mL of water and 0.5 g of zinc powder, heat for 5 minutes, cool, and filter: the filtrate responds to the Qualitative Tests <*1.09*> for primary aromatic amines. The color of the solution is red.

(2) To 2 mL of a solution of Benzalkonium Chloride Concentrated Solution 50 (1 in 500) add a mixture of 0.2 mL of a solution of bromophenol blue (1 in 2000) and 0.5 mL of sodium hydroxide TS: a blue color develops. Add 4 mL of chloroform to this solution, and shake vigorously: the blue color shifts to the chloroform layer. Collect the chloroform layer, and add dropwise, with stirring, a solution of sodium lauryl sulfate (1 in 1000): the chloroform layer turns colorless.

(3) Determine the absorption spectrum of a solution of Benzalkonium Chloride Concentrated Solution 50 in 0.1 mol/L hydrochloric acid TS (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum of Benzalkonium Chloride: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) To 1 mL of a solution of Benzalkonium Chloride Concentrated Solution 50 (1 in 50) add 2 mL of ethanol (95), 0.5 mL of dilute nitric acid and 1 mL of silver nitrate TS: a white precipitate is produced. This precipitate does not dissolve on the addition of dilute nitric acid, but dissolves on the addition of ammonia TS.

Purity **(1)** Clarity and color of solution—Dissolve 2.0 g of Benzalkonium Chloride Concentrated Solution 50 in 10 mL of water: the solution is clear and colorless to light yellow.

(2) Petroleum ether-soluble substances—To 6.0 g of Benzalkonium Chloride Concentrated Solution 50 add water to make 50 mL, then add 50 mL of ethanol (99.5) and 5 mL of 0.5 mol/L sodium hydroxide TS, and extract with three 50-mL portions of petroleum ether. Combine the petroleum ether extracts, and wash with three 50-mL portions of dilute ethanol. After shaking well with 10 g of anhydrous sodium sulfate, filter through a dry filter paper, and wash the filter paper with two 10-mL portions of petroleum ether. Evaporate the petroleum ether on a water bath by heating, and dry the residue at 105°C for 1 hour: the residue is not more than 1.0%.

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

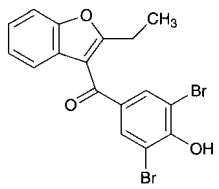
Assay Weigh accurately about 0.3 g of Benzalkonium Chloride Concentrated Solution 50, and dissolve in 75 mL of water. Adjust the pH to between 2.6 and 3.4 by adding dropwise diluted dilute hydrochloric acid (1 in 2), add 1 drop of methyl orange TS, and titrate <*2.50*> with 0.02 mol/L sodium tetraphenylboron VS until the color of the solution becomes red.

Each mL of 0.02 mol/L sodium tetraphenylborate VS
= 7.080 mg of $C_{22}H_{40}ClN$

Containers and storage Containers—Tight containers.

Benzbromarone

ベンズブロマロン



$C_{17}H_{12}Br_2O_3$: 424.08
3,5-Dibromo-4-hydroxyphenyl 2-ethylbenzo[*b*]furan-3-yl ketone
[*3562-84-3*]

Benzbromarone, when dried, contains not less than 98.5% and not more than 101.0% of benzbromarone ($C_{17}H_{12}Br_2O_3$).

Description Benzbromarone occurs as a white to light yellow crystalline powder.

It is very soluble in *N*,*N*-dimethylformamide, freely soluble in acetone, sparingly soluble in ethanol (99.5), and practically insoluble in water.

It dissolves in dilute sodium hydroxide TS.

Identification **(1)** Determine the absorption spectrum of a solution of Benzbromarone in 0.01 mol/L sodium hydroxide TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Benzbromarone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 149 – 153°C

Purity **(1)** Sulfate <*1.14*>—Dissolve 1.0 g of Benzbromarone in 40 mL of acetone, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 40 mL of acetone, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.019%).

(2) Soluble halides—Dissolve 0.5 g of Benzbromarone in 40 mL of acetone, and add 6 mL of dilute nitric acid and water to make 50 mL. Proceed with this solution as directed under Chloride Limit Test <*1.03*>. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 40 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL.

(3) Heavy metals <*1.07*>—Proceed with 2.0 g of Benzbromarone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Iron <*1.10*>—Prepare the test solution with 1.0 g of Benzbromarone according to Method 3, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 20 ppm).

(5) Related substances—Dissolve 0.10 g of Benzbromarone in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, 4-methyl-2-pentanone, ethanol (99.5) and acetic acid (100) (100:20:2:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 50°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

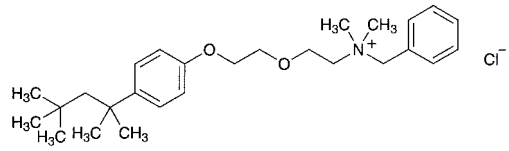
Assay Weigh accurately about 0.6 g of Benzbromarone, previously dried, dissolve in 30 mL of *N*,*N*-dimethylformamide, and titrate <*2.50*> with 0.1 mol/L tetramethylammonium hydroxide VS (indicator: 5 drops of thymol blue-dimethylformamide TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 42.41 mg of $C_{17}H_{12}Br_2O_3$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Benzethonium Chloride

ベンゼトニウム塩化物



$C_{27}H_{42}ClNO_2$: 448.08
N-Benzyl-*N*,*N*-dimethyl-2-{2-[4-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy}ethylaminium chloride
[*121-54-0*]

Benzethonium Chloride, when dried, contains not less than 97.0% of benzethonium chloride ($C_{27}H_{42}ClNO_2$).

Description Benzethonium Chloride occurs as colorless or white crystals. It is odorless.

It is very soluble in ethanol (95), freely soluble in water, and practically insoluble in diethyl ether.

A solution of Benzethonium Chloride foams strongly when shaken.

Identification **(1)** Dissolve 0.2 g of Benzethonium Chloride in 1 mL of sulfuric acid, add 0.1 g of sodium nitrate, and heat for 5 minutes on a water bath. After cooling, add 10 mL of water and 0.5 g of zinc powder, heat for 5 minutes, cool, and filter: the filtrate responds to the Qualitative Tests <*1.09*> for primary aromatic amines, developing a red color.

(2) To 2 mL of a solution of Benzethonium Chloride (1 in 1000) add a mixture of 0.2 mL of a solution of bromophenol blue (1 in 2000) and 0.5 mL of sodium hydroxide TS: a blue color develops. Add 4 mL of chloroform to this solution, and shake vigorously: the blue color shifts to the chlo-

roform layer. Collect the chloroform layer, and add dropwise a solution of sodium lauryl sulfate (1 in 1000) with stirring: the chloroform layer turns colorless.

(3) Determine the absorption spectrum of a solution of Benzethonium Chloride in 0.1 mol/L hydrochloric acid TS (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) To 1 mL of a solution of Benzethonium Chloride (1 in 100) add 2 mL of ethanol (95), 0.5 mL of dilute nitric acid and 1 mL of silver nitrate TS: a white precipitate is produced. This precipitate does not dissolve on addition of dilute nitric acid, but dissolves on addition of ammonia TS.

Melting point <*2.60*> 158 – 164°C (after drying).

Purity Ammonium—Dissolve 0.10 g of Benzethonium Chloride in 5 mL of water, and boil with 3 mL of sodium hydroxide TS: the evolving gas dose not change moistened red litmus paper to blue.

Loss on drying <*2.41*> Not more than 5.0% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Benzethonium Chloride, previously dried, dissolve in 75 mL of water, add diluted dilute hydrochloric acid (1 in 2) dropwise to adjust the pH to 2.6–3.4, then add 1 drop of methyl orange TS, and titrate <*2.50*> with 0.02 mol/L tetraphenylboron VS until the solution develops a red.

Each mL of 0.02 mol/L sodium tetraphenylboron VS
= 8.962 mg of $C_{27}H_{42}ClNO_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Benzethonium Chloride Solution

ベンゼトニウム塩化物液

Benzethonium Chloride Solution contains not less than 93.0% and not more than 107.0% of the labeled amount of benzethonium chloride ($C_{27}H_{42}ClNO_2$: 448.08).

Method of preparation Dissolve Benzethonium Chloride in Water, Purified Water or Purified Water in Containers.

Description Benzethonium Chloride Solution is a clear, colorless liquid. It is odorless.

It foams strongly when shaken.

Identification **(1)** Evaporate a volume of Benzethonium Chloride Solution, equivalent to 0.2 g of Benzethonium Chloride, on a water bath to dryness, and proceed with the residue as directed in the Identification (1) under Benzethonium Chloride.

(2) To a volume of Benzethonium Chloride Solution, equivalent to 0.01 g of Benzethonium Chloride, add water to make 10 mL, proceed with 2 mL of this solution as directed in the Identification (2) under Benzethonium Chloride.

(3) To a volume of Benzethonium Chloride Solution, equivalent to 1 g of Benzethonium Chloride, and add water or concentrate on a water bath to make 10 mL. To 1 mL of this solution add 0.1 mol/L hydrochloric acid TS to make 500 mL, and determine the abosprition spectrum as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 262 nm and 264 nm, between 268 nm and 270 nm, and between 274 nm and 276 nm.

(4) To a volume of Benzethonium Chloride Solution, equivalent to 0.1 g of Benzethonium Chloride, add water, or concentrate on a water bath, if necessary, to make 10 mL, and proceed with 1 mL of this solution as directed in the Identification (4) under Benzethonium Chloride.

Purity **(1)** Nitrite—Add 1.0 mL of Benzethonium Chloride Solution to a mixture of 1 mL of a solution of glycine (1 in 10) and 0.5 mL of acetic acid (31): no gas is evolved.

(2) Oxidizing substances—To 5 mL of Benzethonium Chloride Solution add 0.5 mL of potassium iodide TS and 2 to 3 drops of dilute hydrochloric acid: no yellow color is produced.

Assay Pipet a volume of Benzethonium Chloride Solution, equivalent to about 0.2 g of benzethonium chloride ($C_{27}H_{42}ClNO_2$), dilute with water to make 75 mL, if necessary, and proceed as directed in the Assay under Benzethonium Chloride.

Each mL of 0.02 mol/L sodium tetraphenylboron VS
= 8.962 mg of $C_{27}H_{42}ClNO_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Benzoic Acid

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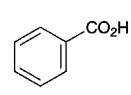

$C_7H_6O_2$: 122.12
Benzoic acid
[*65-85-0*]

Benzoic Acid, when dried, contains not less than 99.5% of benzoic acid ($C_7H_6O_2$).

Description Benzoic Acid occurs as white crystals or crystalline powder. It is odorless, or has a faint, benzaldehyde-like odor.

It is freely soluble in ethanol (95), in acetone and in diethyl ether, soluble in hot water, and slightly soluble in water.

Identification Dissolve 1 g of Benzoic Acid in 8 mL of sodium hydroxide TS, and add water to make 100 mL. This solution responds to the Qualitative Tests <*1.09*> (2) for benzoate.

Melting point <*2.60*> 121 – 124°C

Purity **(1)** Heavy metals <*1.07*>—Dissolve 1.0 g of Benzoic Acid in 25 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid, 25 mL of acetone and water to make 50 mL (not more than 20 ppm).

(2) Chlorinated compounds—Take 0.5 g of Benzoic Acid and 0.7 g of calcium carbonate in a crucible, mix with a small amount of water, and dry. Ignite it at about 600°C, dissolve in 20 mL of dilute nitric acid, and filter. Wash the residue with 15 mL of water, combine the filtrate and the washing, add water to make 50 mL, and add 0.5 mL of silver nitrate TS: this solution has not more turbid than the follow-

ing control solution.

Control solution: Dissolve 0.7 g of calcium carbonate in 20 mL of dilute nitric acid, and filter. Wash the residue with 15 mL of water, combine the filtrate and the washings, add 1.2 mL of 0.01 mol/L hydrochloric acid VS and water to make 50 mL, and add 0.5 mL of silver nitrate TS.

(3) Potassium permanganate-reducing substances—Add 0.02 mol/L potassium permanganate VS dropwise to a boiling mixture of 100 mL of water and 1.5 mL of sulfuric acid, until a red color persists for 30 seconds. Dissolve 1.0 g of Benzoic Acid in this boiling solution, and add 0.50 mL of 0.02 mol/L potassium permanganate VS: a red color persists for at least 15 seconds.

(4) Phthalic acid—To 0.10 g of Benzoic Acid add 1 mL of water and 1 mL of resorcinol-sulfuric acid TS, and heat the mixture in an oil bath heated at a temperature between 120°C and 125°C. After evaporating the water, heat the residue for 90 minutes, cool, and dissolve in 5 mL of water. To 1 mL of the solution add 10 mL of a solution of sodium hydroxide (43 in 500), shake, then examine under light at a wavelength between 470 nm and 490 nm: the green fluorescence of the solution is not more intense than that of the following control solution.

Control solution: Dissolve 61 mg of potassium hydrogen phthalate in water to make exactly 1000 mL. Measure exactly 1 mL of the solution, add 1 mL of resorcinol-sulfuric acid TS, and proceed as directed above.

(5) Readily carbonizable substances ⟨*1.15*⟩—Perform the test with 0.5 g of Benzoic Acid. The solution is not more colored than Matching Fluid Q.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, silica gel, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.05% (1 g).

Assay Weigh accurately about 0.5 g of Benzoic Acid, previously dried, dissolve in 25 mL of neutralized ethanol and 25 mL of water, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 12.21 mg of $C_7H_6O_2$

Containers and storage Containers—Well-closed containers.

Benzyl Alcohol

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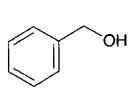

C_7H_8O: 108.14
Benzyl alcohol
[*100-51-6*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Benzyl Alcohol contains not less than 98.0% and not more than 100.5% of benzyl alcohol (C_7H_8O).

◆The label states, where applicable, that it is suitable for use in the manufacture of injection forms.◆

◆**Description** Benzyl Alcohol is a clear, colorless oily liquid.

It is miscible with ethanol (95), with fatty oils and with essential oils.

It is soluble in water.

Specific gravity d_{20}^{20}: 1.043 – 1.049◆

◆**Identification** Determine the infrared absorption spectrum of Benzyl Alcohol as directed in the liquid film method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.◆

Refractive index ⟨*2.45*⟩ n_D^{20}: 1.538 – 1.541

Purity ◆**(1)** Clarity and color of solution—Dissolve 2.0 mL of Benzyl Alcohol in 60 mL of water: the solution is clear and colorless.◆

(2) Acidity—To 10 mL of Benzyl Alcohol add 10 mL of ethanol (95) and 2 drops of phenolphthalein TS, and add dropwise 0.1 mol/L sodium hydroxide VS until the solution acquires a light red color: the amount of 0.1 mol/L sodium hydroxide VS used is not more than 1.0 mL.

(3) Benzaldehyde and other related substances—Use Benzyl Alcohol as the sample solution. Separately, dissolve exactly 0.100 g of ethylbenzene in Benzyl Alcohol to make exactly 10 mL. Pipet 2 mL of this solution, add Benzyl Alcohol to make exactly 20 mL, and use this solution as the ethylbenzene stock solution. Separately, dissolve exactly 2.000 g of dicyclohexyl in Benzyl Alcohol to make exactly 10 mL. Pipet 2 mL of this solution, add Benzyl Alcohol to make exactly 20 mL, and use this solution as the dicyclohexyl stock solution. Separately, weigh exactly 0.750 g of benzaldehyde and 0.500 g of cyclohexylmethanol, and add Benzyl Alcohol to make exactly 25 mL. Pipet 1 mL of this solution, add exactly 2 mL of ethylbenzene stock solution and exactly 3 mL of dicyclohexyl stock solution, then add Benzyl Alcohol to make exactly 20 mL, and use this solution as the standard solution (1). Perform the test with exactly 0.1 μL each of the sample solution and standard solution (1) as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions, and when the peak having the retention time corresponding to ethylbenzene and dicyclohexyl appears on the chromatogram obtained with the sample solution, correct the peak areas of ethylbenzene and dicyclohexyl obtained with the standard solution (1) by deducting the relevant peak area obtained with the sample solution, the peak area of benzaldehyde obtained with the sample solution is not more than the difference between the peak areas of benzaldehyde of the sample solution and the standard solution (1) (0.15%), and the peak area of cyclohexylmethanol with the sample solution is not more than the difference between the peak areas of cyclohexylmethanol of the sample solution and the standard solution (1) (0.10%). The total area of the peaks having smaller retention time than benzyl alcohol and other than benzaldehyde and cyclohexylmethanol obtained with the sample solution is not more than 4 times the peak area or the corrected peak area of ethylbenzene with the standard solution (1) (0.04%). The total area of the peaks having larger retention time than benzyl alcohol obtained with the sample solution is not more than the peak area or the corrected peak area of dicyclohexyl with the standard solution (1) (0.3%). For these calculations the peak areas less than 1/100 times the peak area or the corrected peak area of ethylbenzene with the standard solution (1) are excluded.

Benzyl Alcohol labeled that it is suitable for use in the manufacture of injection forms meets the following requirements.

Use Benzyl Alcohol as the sample solution. Separately, weigh exactly 0.250 g of benzaldehyde and 0.500 g of cyclo-

hexylmethanol, and add Benzyl Alcohol to make exactly 25 mL. Pipet 1 mL of this solution, add exactly 2 mL of the ethylbenzene stock solution and exactly 2 mL of the dicyclohexyl stock solution, then add Benzyl Alcohol to make exactly 20 mL, and use this solution as the standard solution (2). Perform the test with exactly 0.1 μL each of the sample solution and standard solution (2) as directed under Gas Chromatography <*2.02*> according to the following conditions, and when the peak having the retention time corresponding to ethylbenzene and dicyclohexyl appears on the chromatogram obtained with the sample solution, correct the peak areas of ethylbenzene and dicyclohexyl obtained with the standard solution (2) by deducting the relevant peak area obtained with the sample solution, the peak area of benzaldehyde obtained with the sample solution is not more than the difference between the peak areas of benzaldehyde of the sample solution and the standard solution (2) (0.05%), and the peak area of cyclohexylmethanol with the sample solution is not more than the difference between the peak areas of cyclohexylmethanol of the sample solution and the standard solution (2) (0.10%). The total area of the peaks having smaller retention time than benzyl alcohol and other than benzaldehyde and cyclohexylmethanol obtained with the sample solution is not more than 2 times the peak area or the corrected peak area of ethylbenzene with the standard solution (2) (0.02%). The total area of the peaks having larger retention time than benzyl alcohol obtained with the sample solution is not more than the peak area of or the corrected peak area dicyclohexyl with the standard solution (2) (0.2%). For these calculation the peak areas less than 1/100 times the peak area or the corrected peak area of ethylbenzene with the standard solution (2) are excluded.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.32 mm in inside diameter and 30 m in length, coated inside with polyethylene glycol 20 M for gas chromatography in 0.5 μm thickness.

Column temperature: Inject at a constant temperature of about 50°C, raise the temperature at a rate of 5°C per minute to 220°C, and maintain at 220°C for 35 minutes.

Temperature of injection port: A constant temperature of about 200°C.

Temperature of detector: A constant temperature of about 310°C.

Carrier gas: Helium.

Flow rate: 25 cm/second.

Split ratio: Splitless.

Detection sensitivity: When 0.1 μL of the standard solution (1) is injected, adjust the sensitivity of the detector so that the height of the peak of ethylbenzene is not less than 30% of the full scale of the recorder. For Benzyl Alcohol labeled to use for injection, use the standard solution (2) instead of the standard solution (1).

System suitability—

System performance: When the procedure is run with the standard solution (1) under the above operating conditions, the retention time of benzyl alcohol is about 26 minutes, the relative retention times of ethylbenzene, dicyclohexyl, benzaldehyde and cyclohexylmethanol to benzyl alcohol are about 0.28, about 0.59, about 0.68 and about 0.71, respectively, and the resolution between the peaks of benzaldehyde and cyclohexylmethanol is not less than 3.0. In the case of Benzyl Alcohol labeled to use for injection, proceed with the standard solution (2) instead of the standard solution (1).

(4) Peroxide value—Weigh accurately about 5 g of Benzyl Alcohol, and dissolve in 30 mL of a mixture of acetic acid (100) and chloroform (3:2) in a 250-mL glass-stoppered conical flask. Add 0.5 mL of saturated potassium iodide solution, shake for exactly 1 minute, add 30 mL of water, and titrate <*2.50*> with 0.01 mol/L sodium thiosulfate VS, adding the titrant slowly with continuous vigorous shaking, until the blue color of the solution disappears after addition of 5 mL of starch TS near the end point where the solution is a pale yellow color. Perform a blank determination in the same manner. Calculate the amount of peroxide by the following formula: not more than 5. In the determination, the required amount of 0.01 mol/L sodium thiosulfate VS must not exceed 0.1 mL.

$$\text{Amount (mEq/kg) of peroxide} = 10 \times (V_1 - V_0)/M$$

V_1: Volume (mL) of 0.01 mol/L sodium thiosulfate VS consumed in the test

V_0: Volume (mL) of 0.01 mol/L sodium thiosulfate VS consumed in the blank determination

M: Amount (g) of Benzyl Alcohol taken

(5) Residue on evaporation—Perform the test after conformation that the sample meets the requirement of the peroxide value. Transfer 10.0 g of Benzyl Alcohol to a porcelain or quartz crucible or platinum dish, previously weighed accurately, and heat on a hot-plate at not exceeding 200°C, taking care to avoid boiling, to evaporate to dryness. Dry the residue on the hot-plate for 1 hour, and allow to cool in a desiccator: not more than 5 mg.

Assay Weigh accurately about 0.9 g of Benzyl Alcohol, add exactly 15 mL of a freshly prepared mixture of dehydrated pyridine and acetic anhydride (7:1), and heat on a water bath under a reflux condenser for 30 minutes. Cool, add 25 mL of water, and titrate <*2.50*> the excess acetic acid with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS
= 108.1 mg of C_7H_8O

◆**Containers and storage** Containers—Tight containers.
Storage—Light-resistant.◆

Benzyl Benzoate

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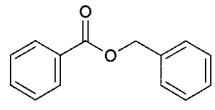

$C_{14}H_{12}O_2$: 212.24
Benzyl benzoate
[*120-51-4*]

Benzyl Benzoate contains not less than 99.0% of benzyl benzoate ($C_{14}H_{12}O_2$).

Description Benzyl Benzoate is a colorless, clear, viscous liquid. It has a faint, aromatic odor and a pungent, burning taste.

It is miscible with ethanol (95) and with diethyl ether.

It is practically insoluble in water.

Congealing point: about 17°C

Specific gravity d_{20}^{20}: about 1.123

Boiling point: about 323°C

Identification **(1)** Heat gently 1 mL of Benzyl Benzoate with 5 mL of sodium carbonate TS and 2 mL of potassium permanganate TS: the odor of benzaldehyde is perceptible.

(2) Warm the titrated mixture obtained in the Assay on a water bath to remove ethanol, and add 0.5 mL of iron (III) chloride TS: a light yellow-red precipitate is produced, which turns white on the addition of dilute hydrochloric acid.

Refractive index ⟨*2.45*⟩ n_D^{20}: 1.568 – 1.570

Purity Acidity—Dissolve 5.0 mL of Benzyl Benzoate in 25 mL of neutralized ethanol, and add 0.50 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

Residue on ignition ⟨*2.44*⟩ Not more than 0.05% (2 g).

Assay Weigh accurately about 2 g of Benzyl Benzoate, add exactly 50 mL of 0.5 mol/L potassium hydroxide-ethanol VS, and boil gently for 1 hour under a reflux condenser with a carbon dioxide-absorbing tube (soda lime). Cool, and titrate ⟨*2.50*⟩ the excess potassium hydroxide with 0.5 mol/L hydrochloric acid VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.5 mol/L potassium hydroxide-ethanol VS
= 106.1 mg of $C_{14}H_{12}O_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Benzylpenicillin Benzathine Hydrate

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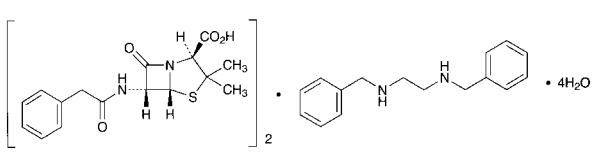

$(C_{16}H_{18}N_2O_4S)_2.C_{16}H_{20}N_2.4H_2O$: 981.18
(2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid hemi(*N*,*N*′-dibenzylethane-1,2-diamine)dihydrate
[*41372-02-5*]

Benzylpenicillin Benzathine Hydrate is the *N*,*N*′-dibenzylethylenediamine salt of a penicillin compound having antibacterial activity produced by the growth of *Penicillium* species.

It contains not less than 1213 Units and not more than 1333 Units per mg, calculated on the anhydrous basis. The potency of Benzylpenicillin Benzathine Hydrate is expressed as unit calculated from the amount of benzylpenicillin sodium ($C_{16}H_{17}N_2NaO_4S$: 356.37). 1 Unit of Benzylpenicillin Benzathine Hydrate is equivalent to 0.6 μg of benzylpenicillin sodium ($C_{16}H_{17}N_2NaO_4S$). It contains not less than 24.0% and not more than 27.0% of *N*,*N*′-dibenzylethylenediamine ($C_{16}H_{20}N_2$: 240.34), calculated on the anhydrous basis.

Description Benzylpenicillin Benzathine Hydrate occurs as a white crystalline powder.

It is slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Benzylpenicillin Benzathine Hydrate in methanol (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Benzylpenicillin Benzathine Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +217 – +233° (0.1 g calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

Purity (1) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Benzylpenicillin Benzathine Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Benzylpenicillin Benzathine Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 70 mg of Benzylpenicillin Benzathine Hydrate in 25 mL of methanol, add a solution prepared by dissolving 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in water to make 1000 mL to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase A to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak having the relative retention time of about 2.4 to benzylpenicillin obtained from the sample solution is not larger than 2 times the total area of the peaks of benzylpenicillin and *N*,*N*′-dibenzylethylenediamine obtained from the standard solution, and the area of the peak other than benzylpenicillin, *N*,*N*′-dibenzylethylenediamine and the peak having the relative retention time of about 2.4 to benzylpenicillin is not larger than the total area of the peaks of benzylpenicillin and *N*,*N*′-dibenzylethylenediamine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: A mixture of water, methanol and 0.25 mol/L potassium dihydrogen phosphate TS (pH 3.5) (6:3:1).

Mobile phase B: A mixture of methanol, water and 0.25 mol/L potassium dihydrogen phosphate TS (pH 3.5) (6:3:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 10	75	25
10 – 20	75 → 0	25 → 100
20 – 55	0	100

Flow rate: 1.0 mL per minute.

Time span of measurement: About 3 times as long as the retention time of benzylpenicillin, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the

standard solution add the mobile phase A to make exactly 20 mL. Confirm that the peak area of benzylpenicillin obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that obtained from the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, *N*,*N*′-dibenzylethylenediamine and benzylpenicillin are eluted in this order with the resolution between these peaks being not less than 25.

System repeatability: When the test is repeated 3 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of benzylpenicillin is not more than 2.0%.

Water ⟨*2.48*⟩ 5.0 – 8.0% (1 g, volumetric titration, direct titration).

Assay (1) Benzylpenicillin—Weigh accurately an amount of Benzylpenicillin Benzathine Hydrate, equivalent to about 85,000 Units, dissolve in 25 mL of methanol, and add a solution containing 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of the solution containing 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water and methanol (1:1) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Benzylpenicillin Potassium RS, equivalent to about 85,000 Units, and about 25 mg of *N*,*N*′-dibenzylethylenediamine diacetate, dissolve in 25 mL of methanol, and add the solution containing 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of the solution containing 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water and methanol (1:1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of benzylpenicillin in each solution.

Amount (unit) of benzylpenicillin sodium ($C_{16}H_{17}N_2NaO_4S$) $= M_S \times A_T/A_S$

M_S: Amount (unit) of Benzylpenicillin Potassium RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, methanol and 0.25 mol/L potassium dihydrogen phosphate TS (pH 3.5) (11:7:2).

Flow rate: Adjust so that the retention time of benzylpenicillin is about 18 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, *N*,*N*′-dibenzylethylenediamine and benzylpenicillin are eluted in this order with the resolution between these peaks being not less than 20.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak areas of *N*,*N*′-dibenzylethylenediamine and benzylpenicillin are not more than 2.0%, respectively.

(2) *N*,*N*′-Dibenzylethylenediamine—Determine the areas, A_T and A_S, of the peak corresponding to *N*,*N*′-dibenzylethylenediamine on the chromatograms obtained in (1) with the sample solution and standard solution.

Amount (%) of *N*,*N*′-dibenzylethylenediamine ($C_{16}H_{20}N_2$) $= M_S/M_T \times A_T/A_S \times 100 \times 0.667$

M_S: Amount (mg) of *N*,*N*′-dibenzylethylenediamine diacetate taken

M_T: Amount (mg) of Benzylpenicillin Benzathine Hydrate taken

0.667: Conversion factor for the molecular mass of *N*,*N*′-dibenzylethylenediamine diacetate ($C_{16}H_{20}N_2 \cdot 2CH_3COOH$) to that of *N*,*N*′-dibenzylethylenediamine (benzathine, $C_{16}H_{20}N_2$)

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Benzylpenicillin Potassium

Penicillin G Potassium

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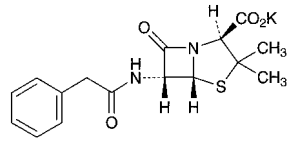


$C_{16}H_{17}KN_2O_4S$: 372.48

Monopotassium (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate

[*113-98-4*]

Benzylpenicillin Potassium is the potassium salt of a penicillin substance having antibacterial activity produced by the growth of *Penicillium* species.

It contains not less than 1430 units and not more than 1630 units per mg, calculated on the dried basis. The potency of Benzylpenicillin Potassium is expressed as mass unit of benzylpenicillin potassium ($C_{16}H_{17}KN_2O_4S$). One unit of Benzylpenicillin Potassium is equivalent to 0.63 μg of benzylpenicillin potassium.

Description Benzylpenicillin Potassium occurs as white, crystals or crystalline powder.

It is very soluble in water, and slightly soluble in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Benzylpenicillin Potassium (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Benzylpenicillin Potassium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Benzylpenicillin Potassium as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and

compare the spectrum with the Reference Spectrum or the spectrum of Benzylpenicillin Potassium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Benzylpenicillin Potassium responds to the Qualitative Tests <*1.09*> (1) for potassium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +270 – +300° (1.0 g calculated on the dried basis, water, 50 mL, 100 mm).

pH <*2.54*> The pH of a solution obtained by dissolving 1.0 g of Benzylpenicillin Potassium in 100 mL of water is between 5.0 and 7.5.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 1.0 g of Benzylpenicillin Potassium in 10 mL of water is clear, and its absorbance at 400 nm determined as directed under Ultraviolet-visible Spectrophotometry <*2.24*> is not more than 0.10.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Benzylpenicillin Potassium according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution by incinerating 1.0 g of Benzylpenicillin Potassium according to Method 4, and perform the test. In the incineration, use a crucible of porcelain, and after addition of 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) add 1 mL of hydrogen peroxide (30), then burn the ethanol (not more than 2 ppm).

(4) Related substances—Dissolve 40 mg of Benzylpenicillin Potassium in 20 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peaks other than benzylpenicillin obtained from the sample solution is not larger than the peak area of benzylpenicillin obtained from the standard solution, and the total area of the peaks other than benzylpenicillin from the sample solution is not larger than 3 times the peak area of benzylpenicillin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 5 times as long as the retention time of benzylpenicillin.

System suitability—

System Performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 10 mL of the standard solution, and add water to make exactly 100 mL. Confirm that the peak area of benzylpenicillin obtained from 20 μL of this solution is equivalent to 7 to 13% of that obtained from 20 μL of the standard solution.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of benzylpenicillin is not more than 2.0%.

Loss on drying <*2.41*> Not more than 1.0% (3 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Weigh accurately amounts of Benzylpenicillin Potassium and Benzylpenicillin Potassium RS, equivalent to about 6×10^4 Units, dissolve each in water to make exactly 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of benzylpenicillin in each solution.

$$\text{Amount (unit) of benzylpenicillin potassium } (C_{16}H_{17}KN_2O_4S) = M_S \times A_T/A_S$$

M_S: Amount (unit) of benzylpenicillin potassium RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of diammonium hydrogen phosphate solution (33 in 5000) and acetonitrile (19:6), adjusted to pH 8.0 with phosphoric acid.

Flow rate: Adjust so that the retention time of benzylpenicillin is about 7.5 minutes.

System suitability—

System performance: Dissolve 40 mg of Benzylpenicillin Potassium in 20 mL of water. Separately, dissolve 10 mg of methyl parahydroxybenzoate in 20 mL of acetonitrile. To 1 mL of this solution add water to make 20 mL. Mix 1 mL each of these solutions, and add water to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, benzylpenicillin and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of benzylpenicillin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Benzylpenicillin Potassium for Injection

注射用ベンジルペニシリンカリウム

Benzylpenicillin Potassium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 93.0% and not more than 107.0% of the labeled potency of benzylpenicillin potassium ($C_{16}H_{17}KN_2O_4S$: 372.48).

Method of preparation Prepare as directed under Injections, with Benzylpenicillin Potassium.

Description Benzylpenicillin Potassium for Injection occurs as white, crystals or crystalline powder.

Identification Proceed as directed in the Identification (2) under Benzylpenicillin Potassium.

Osmotic pressure ratio Being specified separately when the drug is granted approval based on the Law.

pH <*2.54*> The pH of a solution prepared by dissolving an amount of Benzylpenicillin Potassium for Injection, equiva-

lent to 1.0×10^5 Units of Benzylpenicillin Potassium, in 10 mL of water is 5.0 to 7.5.

Purity Clarity and color of solution—A solution prepared by dissolving an amount of Benzylpenicillin Potassium for Injection, equivalent to 1.0×10^6 Units of Benzylpenicillin Potassium, in 10 mL of water is clear. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: the absorbance at 400 nm is not more than 0.10.

Loss on drying <*2.41*> Not more than 1.2% (3 g, in vacuum, below 0.67 kPa, 60°C, 3 hours).

Bacterial endotoxins <*4.01*> Less than 1.25×10^{-4} EU/Unit.

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test.

Foreign insoluble matter <*6.06*> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 containers of Benzylpenicillin Potassium for Injection. Weigh accurately an amount of a portion of the contents, equivalent to about 6×10^4 Units of Benzylpenicillin Potassium, dissolve in water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Benzylpenicillin Potassium RS, equivalent to about 6×10^4 Units, dissolve in water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_T and A_S, of benzylpenicillin in each solution.

$$\text{Amount (unit) of Benzylpenicillin Potassium } (C_{16}H_{17}KN_2O_4S) = M_S \times A_T/A_S$$

M_S: Amount (unit) of Benzylpenicillin Potassium RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To a mixture of diammonium hydrogen phosphate solution (33 in 5000) and acetonitril (19:6), add phosphoric acid to adjust the pH of this solution to 8.0.

Flow rate: Adjust so that the retention time of benzylpenicillin is about 7.5 minutes.

System suitability—

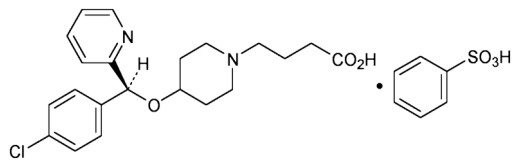
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of benzylpenicillin are not less than 6000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of benzylpenicillin is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Bepotastine Besilate

ベポタスチンベシル酸塩



$C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$: 547.06

(*S*)-4-{4-[(4-Chlorophenyl)(pyridin-2-yl)methoxy]piperidin-1-yl}butanoic acid monobenzenesulfonate

[*190786-44-8*]

Bepotastine Besilate contains not less than 99.0% and not more than 101.0% of bepotastine besilate ($C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$), calculated on the anhydrous and residual solvent-free basis.

Description Bepotastine Besilate occurs as white to pale yellowish white, crystals or crystalline powder.

It is very soluble in acetic acid (100), and sparingly soluble in water and in ethanol (99.5).

The pH of a solution of 1 g of Bepotastine Besilate in 100 mL of water is about 3.8.

Identification **(1)** Determine the absorption spectrum of a solution of Bepotastine Besilate (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bepotastine Besilate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Bepotastine Besilate as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

(4) Mix well 30 mg of Bepotastine Besilate with 0.1 g of sodium nitrate and 0.1 g of anhydrous sodium carbonate, and gradually ignite. After cooling, dissolve the residue in 2 mL of dilute hydrochloric acid and 10 mL of water, filter if necessary, and add barium chloride TS: a white precipitate is produced.

Melting point <*2.60*> 159–163°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Bepotastine Besilate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 10 mg of Bepotastine Besilate in 25 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each

peak area by the automatic integration method: the area of the peak, having a relative retention time of about 2.5 to bepotastine, obtained from the sample solution is not larger than the peak area of bepotastine obtained from the standard solution, and the area of the peak other than bepotastine and the peak mentioned above from the sample solution is not larger than 1/10 times the peak area of bepotastine from the standard solution. Furthermore, the total area of the peaks other than bepotastine from the sample solution is not larger than the peak area of bepotastine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium 1-pentane sulfonate in a mixture of 0.05 mol/L potassium dihydrogen phosphate TS (pH 3.0) and acetonitrile (7:3) to make 1000 mL.

Flow rate: Adjust so that the retention time of bepotastine is about 6 minutes.

Time span of measurement: About 5 times as long as the retention time of bepotastine, beginning after the peak of benzenesulfonic acid.

System suitability—

Test for required detectability: Pipet 2.5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of bepotastine obtained with 20 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of bepotastine are not less than 3000 and 0.8 to 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of bepotastine is not more than 2.0%.

(3) Optical isomer—Dissolve 5.0 mg of Bepotastine Besilate in 25 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the area of each peak by the automatic integration method: the area of the peak, having a relative retention time of about 0.9 to bepotastine obtained from the sample solution, is not larger than the peak area of bepotastine obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 6.0 mm in inside diameter and 15 cm in length, packed with β-cyclodextrin binding silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.02 mol/L potassium dihydrogen phosphate TS and acetonitrile (3:1).

Flow rate: Adjust so that the retention time of bepotastine is about 17 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of bepotastine are not less than 3000 and 0.8 to 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of bepotastine is not more than 5.0%.

Water <2.48> Not more than 0.1% (0.3 g, coulometric titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.8 g of Bepotastine Besilate, dissolve in 60 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 54.71 mg of $C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$

Containers and storage Containers—Tight containers.

Bepotastine Besilate Tablets

ベポタスチンベシル酸塩錠

Bepotastine Besilate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of bepotastine besilate ($C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$: 547.06).

Method of preparation Prepare as directed under Tablets, with Bepotastine Besilate.

Identification To an amount of powdered Bepotastine Besilate Tablets, equivalent to 2 mg of Bepotastine Besilate, add 40 mL of water, shake thoroughly, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 260 nm and 264 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Bepotastine Besilate Tablets add exactly $V/5$ mL of the internal standard solution, then add the mobile phase to make V mL so that each mL contains about 0.4 mg of bepotastine besilate ($C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$), shake vigorously for 10 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, to 2 mL of the subsequent filtrate add the mobile phase to make 10 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of bepotastine besilate ($C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$)
$= M_S \times Q_T/Q_S \times V/50$

M_S: Amount (mg) of bepotastine besilate for assay taken, calculated on the anhydrous and residual solvent-free basis

Internal standard solution—A solution of ethyl parahydroxybenzoate in acetonitrile (1 in 4500).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Bepotastine Besilate Tablets is not less than 85%.

Start the test with 1 tablet of Bepotastine Besilate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the mobile phase to make exactly V' mL so that each mL contains about 2.2 μg of bepotastine besilate ($C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$), and use this solution as the sample solution. Separately, weigh accurately about 0.11 g of bepotastine besilate for assay (separately determine the water ⟨*2.48*⟩ and the residual solvent in the same manner as Bepotastine Besilate), and dissolve in water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of bepotastine in each solution.

Dissolution rate (%) with respect to the labeled amount of bepotastine besilate ($C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/5$

M_S: Amount (mg) of bepotastine besilate for assay taken, calculated on the anhydrous and residual solvent-free basis

C: Labeled amount (mg) of bepotastine besilate ($C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of bepotastine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of bepotastine is not more than 1.5%.

Assay Weigh accurately the mass of not less than 20 tablets of Bepotastine Besilate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of bepotastine besilate ($C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$), add exactly 5 mL of the internal standard solution, then add 20 mL of the mobile phase, shake thoroughly for 10 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, to 2 mL of the subsequent filtrate add the mobile phase to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of bepotastine besilate for assay (separately determine the water ⟨*2.48*⟩ and the residual solvent in the same manner as Bepotastine Besilate), add exactly 10 mL of the internal standard solution, and dissolve in the mobile phase to make 50 mL. To 2 mL of this solution add the mobile phase to make 10 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of bepotastine to that of the internal standard.

Amount (mg) of bepotastine besilate ($C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$)
$= M_S \times Q_T/Q_S \times 1/2$

M_S: Amount (mg) of bepotastine besilate for assay taken, calculated on the anhydrous and residual solvent-free basis

Internal standard solution—A solution of ethyl parahydroxybenzoate in acetonitrile (1 in 4500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A solution of sodium 1-pentanesulfonate in a mixture of 0.05 mol/L potassium dihydrogen phosphate TS (pH 3.0) and acetonitrile (7:3) (1 in 1000).

Flow rate: Adjust so that the retention time of bepotastine is about 6 minutes.

System suitability—

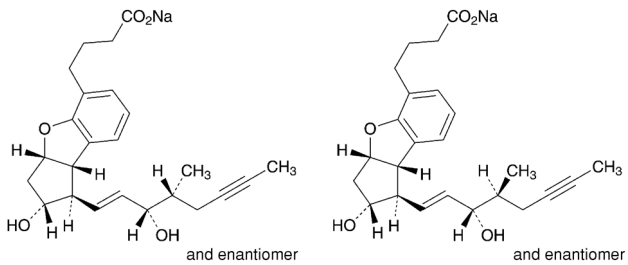
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, bepotastine and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of bepotastine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Beraprost Sodium

ベラプロストナトリウム



$C_{24}H_{29}NaO_5$: 420.47

Monosodium (1*RS*,2*RS*,3a*SR*,8b*SR*)-2,3,3a,8b-tetrahydro-2-hydroxy-1-[(1*E*,3*SR*,4*RS*)-3-hydroxy-4-methyloct-1-en-6-yn-1-yl]-1*H*-cyclopenta[*b*]benzofuran-5-butanoate

Monosodium (1*RS*,2*RS*,3a*SR*,8b*SR*)-2,3,3a,8b-tetrahydro-2-hydroxy-1-[(1*E*,3*SR*,4*SR*)-3-hydroxy-4-methyloct-1-en-6-yn-1-yl]-1*H*-cyclopenta[*b*]benzofuran-5-butanoate

[*88475-69-8*]

Beraprost Sodium, when dried, contains not less than 98.5% and not more than 101.0% of beraprost

sodium ($C_{24}H_{29}NaO_5$).

Description Beraprost Sodium occurs as a white powder.

It is very soluble in methanol, and freely soluble in water and in ethanol (99.5).

It is hygroscopic.

A solution of Beraprost Sodium (1 in 200) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Beraprost Sodium in methanol (3 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of previously dried Beraprost Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Beraprost Sodium in methanol (1 in 1000) responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

Purity Related substances—Dissolve 20 mg of Beraprost Sodium in 2 mL of methanol, and use this solution as the sample solution. Perform the test with 15 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate their amounts by the area percentage method: the amount of the peak having the relative retention time of about 0.5 to the second eluting principal peak of beraprost and the adjacent two peaks having the relative retention time of about 1.7 and another adjacent two peaks having the relative retention time of about 2.0 are not more than 0.2%, respectively, the amount of the peak having the relative retention time of about 1.2 is not more than 0.3%, the amount of the peak, other than the two peaks of beraprost and the peaks mentioned above, is less than 0.1%, and the total amount of the peaks, other than the two peaks of beraprost, is not more than 1.5%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase A: A mixture of water, acetonitrile, methanol and acetic acid (100) (640:330:30:1).

Mobile phase B: A mixture of acetonitrile, water and acetic acid (100) (900:100:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 30	100	0
30 – 45	100 → 56	0 → 44
45 – 60	56	44
60 – 70	56 → 0	44 → 100
70 – 80	0	100

Flow rate: Adjust so that the retention time of the second peak of beraprost is about 23 minutes.

Time span of measurement: For 80 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add methanol to make 20 mL. To 1 mL of this solution add methanol to make 20 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add methanol to make exactly 10 mL. Confirm that the total area of the two peaks of beraprost obtained with 15 μL of this solution is equivalent to 14 to 26% of that with 15 μL of the solution for system suitability test.

System performance: When the procedure is run with 15 μL of the solution for system suitability test under the above operating conditions, the resolution between the two peaks of beraprost is not less than 1.5.

System repeatability: When the test is repeated 6 times with 15 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the total area of the two peaks of beraprost is not more than 2.0%.

Loss on drying <*2.41*> Not more than 3.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, silica gel, 60°C, 5 hours).

Isomer ratio Dissolve 10 mg of Beraprost Sodium in 5 mL of methanol, and use this solution as the sample solution. Perform the test with 15 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the areas, A_a of the peak which appears at the retention time about 25 minutes, and A_b of the peak which appears at about 27 minutes: A_b/A_a is between 0.90 and 1.10.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of methanol, water and acetic acid (100) (600:400:1).

Flow rate: Adjust so that the retention time of the second eluting peak of beraprost is about 27 minutes.

System suitability—

System performance: When the procedure is run with 15 μL of the sample solution under the above operating conditions, the resolution between the two peaks of beraprost is not less than 1.2.

System repeatability: When the test is repeated 6 times with 15 μL of the sample solution under the above operating conditions, the relative standard deviation of the total area of the two peak of beraprost is not more than 2.0%.

Assay Weigh accurately about 0.1 g of Beraprost Sodium, previously dried, dissolve in 30 mL of diluted ethanol with freshly boiled and cooled water (7 in 10), add exactly 2 mL of 0.2 mol/L hydrochloric acid TS, and titrate <*2.50*> with 0.025 mol/L sodium hydroxide-ethanol VS from the first equivalence point to the second equivalence point (potentiometric titration).

Each mL of 0.025 mol/L sodium hydroxide-ethanol VS
= 10.51 mg of $C_{24}H_{29}NaO_5$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Beraprost Sodium Tablets

ベラプロストナトリウム錠

Beraprost Sodium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of beraprost sodium ($C_{24}H_{29}NaO_5$: 420.47).

Method of preparation Prepare as directed under Tablets, with Beraprost Sodium.

Identification Powder Beraprost Sodium Tablets. To a portion of the powder, equivalent to 0.2 mg of Beraprost Sodium, add 10 mL of water, shake, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To the filtrate add 1 mL of 0.1 mol/L hydrochloric acid TS, extract with two 50-mL portions of ethyl acetate, combine the extracts, and evaporate in reduced pressure at 40°C. Dissolve the residue in 1 mL of methanol, use this solution as the sample solution. Separately, dissolve 1 mg of beraprost sodium in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with the upper layer of a mixture of 11 volumes of ethyl acetate, 10 volumes of water, 4 volumes of isooctane and 2 volumes of acetic acid (100) to a distance of about 10 cm, air-dry the plate, and heat at 120°C for 30 minutes. After cooling, spray evenly a mixture of ethanol (99.5), water, sulfuric acid and 4-methoxybenzaldehide (17:2:1:1) on the plate, and heat at 120°C for 3 minutes: the principal spot obtained from the sample solution and the spot obtained from the standard solution show the same *R*f value.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Beraprost Sodium Tablets add exactly *V* mL of the internal standard solution so that each mL contains about 2 μg of beraprost sodium ($C_{24}H_{29}NaO_5$), shake at 30°C for 30 minutes, filter through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of beraprost sodium ($C_{24}H_{29}NaO_5$)
$= M_S \times Q_T/Q_S \times V/10{,}000$

M_S: Amount (mg) of beraprost sodium for assay taken

Internal standard solution—A mixture of water and a solution of 4-isopropylphenol in methanol (1 in 250,000) (1:1).

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Beraprost Sodium Tablets is not less than 85%.

Start the test with 1 tablet of Beraprost Sodium Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet *V* mL of the subsequent filtrate, add water to make exactly *V′* mL so that each mL contains about 22 ng of beraprost sodium ($C_{24}H_{29}NaO_5$), and use this solution as the sample solution. Separately, weigh accurately about 20 mg of beraprost sodium for assay, previously dried in reduced pressure not exceeding 0.67 kPa at 60°C for 5 hours using silica gel as a desiccant, and dissolve in methanol to make exactly 100 mL. Pipet 2 mL of this solution, and add water to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 200 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the total areas, A_T and A_S, of the two peaks of beraprost in each solution.

Dissolution rate (%) with respect to the labeled amount of beraprost sodium ($C_{24}H_{29}NaO_5$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/100$

M_S: Amount (mg) of beraprost sodium for assay taken
C: Labeled amount (mg) of beraprost sodium ($C_{24}H_{29}NaO_5$) in 1 tablet

Operating conditions—

Detector, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay.

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Flow rate: Adjust so that the retention time of the first eluting peak of beraprost is about 10 minutes.

System suitability—

System performance: When the procedure is run with 200 μL of the standard solution under the above operating conditions, the resolution between the two peaks of beraprost is not less than 1.2.

System repeatability: When the test is repeated 6 times with 200 μL of the standard solution under the above operating conditions, the relative standard deviation of the total area of the two peaks of beraprost is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Beraprost Sodium Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 40 μg of beraprost sodium ($C_{24}H_{29}NaO_5$), add exactly 20 mL of the internal standard solution, shake at 30°C for 30 minutes, filter through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Separately, weigh accurately about 20 mg of beraprost sodium for assay, previously dried in reduced pressure not exceeding 0.67 kPa at 60°C for 5 hours using silica gel as a desiccant, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, and add methanol to make exactly 200 mL. Pipet 4 mL of this solution, and evaporate under reduced pressure at 40°C. To the residue add exactly 20 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the total area of the two peaks of beraprost to the peak area of the internal standard.

Amount (mg) of beraprost sodium ($C_{24}H_{29}NaO_5$)
$= M_S \times Q_T/Q_S \times 1/500$

M_S: Amount (mg) of beraprost sodium for assay taken

Internal standard solution—A mixture of water and a solution of 4-isopropylphenol in methanol (1 in 250,000) (1:1).

Operating conditions—

Detector: A fluorophotometer (excitation wavelength: 285 nm, fluorescence wavelength: 614 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel

for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of methanol, water and acetic acid (100) (650:350:1).

Flow rate: Adjust so that the retention time of the first eluting peak of beraprost is about 15 minutes.

System suitability—

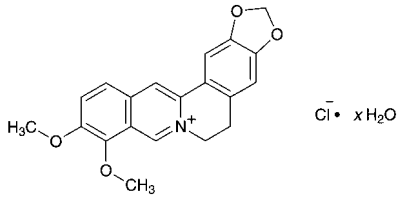
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and beraprost are eluted in this order and the resolution between the internal standard peak and the first eluting peak of beraprost is not less than 11, and the resolution between the two peaks of beraprost is not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the total area of the two peaks of beraprost to the peak area of the internal standard is not more than 2.0%.

Containers and storage Containers—Well-closed containers.

Berberine Chloride Hydrate

ベルベリン塩化物水和物



$C_{20}H_{18}ClNO_4.xH_2O$

9,10-Dimethoxy-5,6-dihydro[1,3]dioxolo[4,5-*g*]isoquino[3,2-*a*]isoquinolin-7-ium chloride hydrate

[*633-65-8*, anhydride]

Berberine Chloride Hydrate contains not less than 95.0% and not more than 102.0% of berberine chloride ($C_{20}H_{18}ClNO_4$: 371.81), calculated on the anhydrous basis.

Description Berberine Chloride Hydrate occurs as yellow, crystals or crystalline powder. It is odorless or has a faint, characteristic odor. It has a very bitter taste.

It is sparingly soluble in methanol, slightly soluble in ethanol (95), and very slightly soluble in water.

Identification (1) Determine the absorption spectrum of a solution of Berberine Chloride Hydrate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Berberine Chloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Berberine Chloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Berberine Chloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.1 g of Berberine Chloride Hydrate in 20 mL of water by warming, add 0.5 mL of nitric acid, cool, and filter after allowing to stand for 10 minutes. To 3 mL of the filtrate add 1 mL of silver nitrate TS, and collect the produced precipitate: the precipitate does not dissolve in dilute nitric acid, but it dissolves in an excess amount of ammonia TS.

Purity (1) Acidity—Shake thoroughly 0.10 g of Berberine Chloride Hydrate with 30 mL of water, and filter. To the filtrate add 2 drops of phenolphthalein TS and 0.10 mL of 0.1 mol/L sodium hydroxide VS: the yellow color changes to an orange to red color.

(2) Sulfate <*1.14*>—Shake 1.0 g of Berberine Chloride Hydrate with 48 mL of water and 2 mL of dilute hydrochloric acid for 1 minute, and filter. Discard the first 5 mL of the filtrate, take the subsequent 25 mL of the filtrate, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS, 1 mL of dilute hydrochloric acid, 5 to 10 drops of bromophenol blue TS and water to make 50 mL (not more than 0.048%).

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Berberine Chloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(4) Related substances—Dissolve 10 mg of Berberine Chloride Hydrate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 4 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than berberine obtained with the sample solution is not larger than the peak area of berberine obtained with the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, flow rate, and selection of column: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of berberine, beginning after the solvent peak.

Detection sensitivity: Adjust so that the peak height of berberine obtained from 10 μL of the standard solution is about 10% of the full scale.

Water <*2.48*> 8 – 12% (0.1 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 10 mg of Berberine Chloride Hydrate, dissolve in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Berberine Chloride RS (separately, determine the water content <*2.48*> in the same manner as Berberine Chloride Hydrate), and dissolve in the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_T and A_S of berberine in each solution.

Amount (mg) of berberine chloride ($C_{20}H_{18}ClNO_4$) $= M_S \times A_T/A_S$

M_S: Amount (mg) of Berberine Chloride RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 345 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.4 g of monobasic potassium phosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of a mixture of water and acetonitrile (1:1).

Flow rate: Adjust so that the retention time of berberine is about 10 minutes.

Selection of column: Dissolve each 1 mg of berberine chloride and palmatin chloride in the mobile phase to make 10 mL. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of palmatin and berberine in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 5 times with the standard solution under the above operating conditions, the relative standard deviation of the peak areas of berberine is not more than 1.5%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Berberine Tannate

タンニン酸ベルベリン

Berberine Tannate is a compound of berberine and tannic acid.

It contains not less than 27.0% and not more than 33.0% of berberine ($C_{20}H_{19}NO_5$: 353.37), calculated on the anhydrous basis.

Description Berberine Tannate occurs as a yellow to light yellow-brown powder. It is odorless or has a faint, characteristic odor, and is tasteless.

It is practically insoluble in water, in acetonitrile, in methanol and in ethanol (95).

Identification **(1)** To 0.1 g of Berberine Tannate add 10 mL of ethanol (95), and heat in a water bath for 3 minutes with shaking. Cool, filter, and to 5 mL of the filtrate add 1 drop of iron (III) chloride TS: a blue-green color is produced, and on allowing to stand, a bluish black precipitate is formed.

(2) Dissolve 0.01 g of Berberine Tannate in 10 mL of methanol and 0.4 mL of 1 mol/L hydrochloric acid TS, and add water to make 200 mL. To 8 mL of the solution add water to make 25 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Berberine Tannate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Acidity—To 0.10 g of Berberine Tannate add 30 mL of water, and filter after shaking well. To the filtrate add 2 drops of phenolphthalein TS and 0.10 mL of 0.1 mol/L sodium hydroxide VS: the color of the solution changes from yellow to orange to red.

(2) Chloride <*1.03*>—Shake 1.0 g of Berberine Tannate with 38 mL of water and 12 mL of dilute nitric acid for 5 minutes, and filter. Discard the first 5 mL of the filtrate, to 25 mL of the subsequent filtrate add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.01 mol/L hydrochloric acid VS by adding 6 mL of dilute nitric acid, 10 to 15 drops of bromophenol blue TS and water to make 50 mL (not more than 0.035%).

(3) Sulfate <*1.14*>—Shake 1.0 g of Berberine Tannate with 48 mL of water and 2 mL of dilute hydrochloric acid for 1 minute, and filter. Discard the first 5 mL of the filtrate, take the subsequent 25 mL of the filtrate, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS, 1 mL of dilute hydrochloric acid, 5 to 10 drops of bromophenol blue TS and water to make 50 mL (not more than 0.048%).

(4) Heavy metals <*1.07*>—Proceed with 1.0 g of Berberine Tannate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(5) Related substances—Dissolve 10 mg of Berberine Tannate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 4 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than berberine obtained with the sample solution is not larger than the peak area of berberine obtained with the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of berberine, beginning after the solvent peak.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of berberine obtained with 10 μL of this solution is equivalent to 7 to 13% of that obtained with 10 μL of the standard solution.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of berberine is not more than 3.0%.

Water <*2.48*> Not more than 6.0% (0.7 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 1.0% (1 g).

Assay Weigh accurately about 30 mg of Berberine Tannate, dissolve in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Berberine Chloride RS (separately, determine the water <*2.48*> in the same manner as Berberine Chloride Hydrate), dissolve in the mobile phase

to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_T and A_S, of berberine in each solution.

$$\text{Amount (mg) of berberine } (C_{20}H_{19}NO_5) = M_S \times A_T/A_S \times 0.950$$

M_S: Amount (mg) of Berberine Chloride RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 345 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.4 g of potassium dihydrogen phosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of a mixture of water and acetonitrile (1:1).

Flow rate: Adjust so that the retention time of berberine is about 10 minutes.

System suitability—

System performance: Dissolve 1 mg each of berberine chloride and palmatin chloride in the mobile phase to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, palmatin and berberine are eluted in this order with the resolution between these peaks being not less than 1.5.

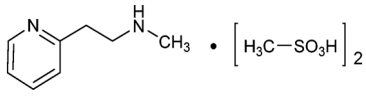
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of berberine is not more than 1.5%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Betahistine Mesilate

ベタヒスチンメシル酸塩



$C_8H_{12}N_2.2CH_4O_3S$: 328.41

N-Methyl-2-pyridin-2-ylethylamine dimethanesulfonate

[*5638-76-6*, Betahistine]

Betahistine Mesilate, when dried, contains not less than 98.0% and not more than 101.0% of betahistine mesilate ($C_8H_{12}N_2.2CH_4O_3S$).

Description Betahistine Mesilate occurs as white, crystals or crystalline powder.

It is very soluble in water, freely soluble in acetic acid (100), and sparingly soluble in ethanol (99.5).

It dissolves in dilute hydrochloric acid.

It is hygroscopic.

Identification (1) Determine the absorption spectrum of a solution of Betahistine Mesilate in 0.1 mol/L hydrochloric acid (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Betahistine Mesilate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A 30 mg portion of Betahistine Mesilate responds to the Qualitative Tests <1.09> (2) for mesilate.

Melting point <2.60> 110 – 114°C (after drying).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Betahistine Mesilate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Betahistine Mesilate in 10 mL of a mixture of water and acetonitrile (63:37), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water and acetonitrile (63:37) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than betahistine with the sample solution is not larger than 1/10 times the peak area of betahistine with the standard solution, and the total area of the peaks other than the peak of betahistine with the sample solution is not larger than 1/2 times the peak area of betahistine with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 261 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: To 5 mL of diethylamine and 20 mL of acetic acid (100) add water to make 1000 mL. Dissolve 2.3 g of sodium lauryl sulfate in 630 mL of this solution, and add 370 mL of acetonitrile.

Flow rate: Adjust so that the retention time of betahistine is about 5 minutes.

Time span of measurement: About 3 times as long as the retention time of betahistine, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add the mixture of water and acetonitrile (63:37) to make exactly 50 mL. Confirm that the peak area of betahistine obtained with 20 μL of this solution is equivalent to 7 to 13% of that obtained with 20 μL of the standard solution.

System performance: Dissolve 10 mg of betahistine mesilate and 10 mg of 2-vinylpyridine in 50 mL of the mixture of water and acetonitrile (63:37). To 2 mL of this solution add the mixture of water and acetonitrile (63:37) to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, 2-vinylpyridine and betahistine are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of betahistine is not more than 1.0%

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, in vacuum, phosphorus (V) oxide, 70°C, 24 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Betahistine Mesilate, previously dried, dissolve in 1 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 16.42 mg of $C_8H_{12}N_2.2CH_4O_3S$

Containers and storage Containers—Tight containers.

Betahistine Mesilate Tablets

ベタヒスチンメシル酸塩錠

Betahistine Mesilate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of betahistine mesilate ($C_8H_{12}N_2.2CH_4O_3S$: 328.41).

Method of preparation Prepare as directed under Tablets, with Betahistine Mesilate.

Identification To 5 mL of the sample solution obtained in the Assay add 0.1 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 259 nm and 263 nm.

Purity Related substances—Powder not less than 20 Betahistine Mesilate Tablets. To a portion of the powder, equivalent to about 50 mg of Betahistine Mesilate, add 10 mL of a mixture of water and acetonitrile (63:37), agitate for 10 minutes with the aid of ultrasonic waves, centrifuge, and use the supernatant liquid as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water and acetonitrile (63:37) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 1.9 to betahistine obtained from the sample solution, is not larger than 3/5 times the peak area of betahistine obtained from the standard solution, and the total area of the peaks other than betahistine from the sample solution is not larger than the peak area of betahistine from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 8 times as long as the retention time of betahistine, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add the mixture of water and acetonitrile (63:37) to make exactly 50 mL. Confirm that the peak area of betahistine obtained with 20 μL of this solution is equivalent to 7 to 13% of that obtained with 20 μL of the standard solution.

System performance: Dissolve 10 mg of betahistine mesilate and 10 mg of 2-vinylpyridine in 50 mL of the mixture of water and acetonitrile (63:37). To 2 mL of this solution add the mixture of water and acetonitrile (63:37) to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, 2-vinylpyridine and betahistine are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of betahistine is not more than 1.0%.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Betahistine Mesilate Tablets add exactly V mL of 0.1 mol/L hydrochloric acid TS so that each mL contains about 0.4 mg of betahistine mesilate ($C_8H_{12}N_2.2CH_4O_3S$), agitate for about 10 minutes with the aid of ultrasonic waves to disintegrate the tablet, then centrifuge, and use the supernatant liquid as the sample solution. Proceed as directed in the Assay.

Amount (mg) of betahistine mesilate ($C_8H_{12}N_2.2CH_4O_3S$)
$= M_S \times A_T/A_S \times V/250$

M_S: Amount (mg) of betahistine mesilate for assay taken

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Betahistine Mesilate Tablets is not less than 85%.

Start the test with 1 tablet of Betahistine Mesilate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 6.7 μg of betahistine mesilate ($C_8H_{12}N_2.2CH_4O_3S$), and use this solution as the sample solution. Separately, weigh accurately about 17 mg of betahistine mesilate for assay, previously dried under reduced pressure with phosphorous (V) oxide at 70°C for 24 hours, and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of betahistine in each solution.

Dissolution rate (%) with respect to the labeled amount of betahistine mesilate ($C_8H_{12}N_2.2CH_4O_3S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of betahistine mesilate for assay taken
C: Labeled amount (mg) of betahistine mesilate ($C_8H_{12}N_2.2CH_4O_3S$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of betahistine are not less than 2000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times

with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of betahistine is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Betahistine Mesilate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of betahistine mesilate ($C_8H_{12}N_2.2CH_4O_3S$), add 40 mL of 0.1 mol/L hydrochloric acid TS, agitate for 10 minutes with the aid of ultrasonic waves, and add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 0.1 g of betahistine mesilate for assay, previously dried under reduced pressure with phosphorous (V) oxide at 70°C for 24 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 10 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*>, according to the following conditions, and determine the peak areas, A_T and A_S, of betahistine in each solution.

Amount (mg) of betahistine mesilate ($C_8H_{12}N_2.2CH_4O_3S$) $= M_S \times A_T/A_S \times 1/5$

M_S: Amount (mg) of betahistine mesilate for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 261 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: To 5 mL of diethylamine and 20 mL of acetic acid (100) add water to make 1000 mL. In 630 mL of this solution dissolve 2.3 g of sodium lauryl sulfate, and add 370 mL of acetonitrile.

Flow rate: Adjust so that the retention time of betahistine is about 5 minutes.

System suitability—

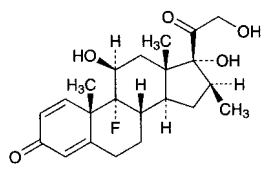
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of betahistine are not less than 2000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of betahistine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Betamethasone

ベタメタゾン



$C_{22}H_{29}FO_5$: 392.46

9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione

[*378-44-9*]

Betamethasone, when dried, contains not less than 96.0% and not more than 103.0% of betamethasone ($C_{22}H_{29}FO_5$).

Description Betamethasone occurs as a white to pale yellowish white crystalline powder.

It is sparingly soluble in methanol, in ethanol (95) and in acetone, and practically insoluble in water.

Melting point: about 240°C (with decomposition).

It shows crystal polymorphism.

Identification **(1)** Proceed with 10 mg of Betamethasone as directed under Oxygen Flask Combustion Method <*1.06*>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the test solution so obtained responds to the Qualitative Tests <*1.09*> for fluoride.

(2) Dissolve 1.0 mg of Betamethasone in 10 mL of ethanol (95). Mix 2.0 mL of the solution with 10 mL of phenylhydrazinium hydrochloride TS, heat in a water bath at 60°C for 20 minutes, and cool the solution. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using as the blank the solution prepared with 2.0 mL of ethanol (95) in the same manner as the former solution, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Betamethasone RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Betamethasone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Betamethasone RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Betamethasone and Betamethasone RS in acetone, respectively, then evaporate the acetone to dryness, and repeat the test on the residues.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +118 – +126° (after drying, 0.1 g, methanol, 20 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 0.5 g of Betamethasone according to Method 2, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 10 mg of Betamethasone in 5 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (9:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions

as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, diethyl ether, methanol and water (385:75:40:6) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <2.44> Not more than 0.5% (0.1 g, platinum crucible).

Assay Dissolve about 20 mg each of Betamethasone and Betamethasone RS, previously dried and accurately weighed, in methanol to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL each of the internal standard solution, then add methanol to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of betamethasone to that of the internal standard.

$$\text{Amount (mg) of betamethasone } (C_{22}H_{29}FO_5) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Betamethasone RS taken

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (1 in 1750).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column about 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (3:2).

Flow rate: Adjust so that the retention time of betamethasone is about 4 minutes.

System suitability—

System performance: When proceed the test with 10 μL of the standard solution under the above operating conditions, betamethasone and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of betamethasone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Betamethasone Tablets

ベタメタゾン錠

Betamethasone Tablets contain not less than 90.0% and not more than 107.0% of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$: 392.46).

Method of preparation Prepare as directed under Tablets, with Betamethasone.

Identification Pulverize Betamethasone Tablets. To a portion of the powder, equivalent to 2 mg of Betamethasone, add 20 mL of methanol, shake for 5 minutes, and filter. Evaporate the filtrate on a water bath to dryness, dissolve the residue after cooling in 2 mL of methanol, filter if necessary, and use this as the sample solution. Separately, dissolve 2 mg of Betamethasone RS in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop with a mixture of 1-butanol, water and acetic anhydride (3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained with the sample solution and the spot obtained with the standard solution show the same *R*f value.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Betamethasone Tablets add *V* mL of water so that each mL contains about 50 μg of betamethasone ($C_{22}H_{29}FO_5$). Add exactly 2*V* mL of the internal standard solution, shake vigorously for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Betamethasone RS, previously dried for 4 hours in a desiccator (in vacuum, phosphorus (V) oxide), dissolve in acetonitrile to make exactly 200 mL. Pipet 5 mL of this solution, add exactly 20 mL of the internal standard solution, add 5 mL of water, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of betamethasone to that of the internal standard.

$$\text{Amount (mg) of betamethasone } (C_{22}H_{29}FO_5) = M_S \times Q_T/Q_S \times V/400$$

M_S: Amount (mg) of Betamethasone RS taken

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 40,000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, betamethasone and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of

the peak area of betamethasone to that of the internal standard is not more than 1.0%.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Betamethasone Tablets is not less than 85%.

Start the test with 1 tablet of Betamethasone Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet the subsequent V mL of the filtrate, add water to make exactly V' mL so that each mL contains about 0.56 μg of betamethasone ($C_{22}H_{29}FO_5$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Betamethasone RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of betamethasone in each solution.

Dissolution rate (%) with respect to the labeled amount of betamethasone ($C_{22}H_{29}FO_5$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/5$

M_S: Amount (mg) of Betamethasone RS taken
C: Labeled amount (mg) of betamethasone ($C_{22}H_{29}FO_5$) in 1 tablet

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 241 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of methanol and water (3:2).
Flow rate: Adjust so that the retention time of betamethasone is about 7 minutes.
System suitability—
System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of betamethasone are not less than 3000 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of betamethasone is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Betamethasone Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 5 mg of betamethasone ($C_{22}H_{29}FO_5$), add 25 mL of water, then add exactly 50 mL of the internal standard solution, and shake vigorously for 10 minutes. Filter through a membrane filter with pore size not exceeding 0.5 μm, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of Betamethasone RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in acetonitrile to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 20 mL of the internal standard solution and 5 mL of water, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of betamethasone to that of the internal standard.

Amount (mg) of betamethasone ($C_{22}H_{29}FO_5$)
$= M_S \times Q_T/Q_S \times 1/4$

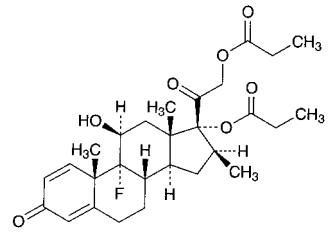
M_S: Amount (mg) of Betamethasone RS taken

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 10,000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 240 nm).
Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of water and acetonitrile (3:2).
Flow rate: Adjust so that the retention time of betamethasone is about 4 minutes.
System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, betamethasone and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of betamethasone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Betamethasone Dipropionate

ベタメタゾンジプロピオン酸エステル



$C_{28}H_{37}FO_7$: 504.59
9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17,21-dipropanoate
[*5593-20-4*]

Betamethasone Dipropionate, when dried, contains not less than 97.0% and not more than 103.0% of betamethasone dipropionate ($C_{28}H_{37}FO_7$), and not less than 3.4% and not more than 4.1% of fluorine (F:19.00).

Description Betamethasone Dipropionate occurs as a white to pale yellowish white crystalline powder. It is odorless.
It is freely soluble in acetone, in 1,4-dioxane and in chlo-

roform, soluble in methanol, sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water and in hexane.

It is affected gradually by light.

Identification **(1)** To 1 mL of a solution of Betamethasone Dipropionate in methanol (1 in 10,000) add 4 mL of isoniazid TS, and heat on a water bath for 2 minutes: a yellow color develops.

(2) Proceed with 0.01 g of Betamethasone Dipropionate as directed under Oxygen Flask Combustion Method ⟨*1.06*⟩, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the test solution so obtained responds to the Qualitative Tests ⟨*1.09*⟩ for fluoride.

(3) Determine the absorption spectrum of a solution of Betamethasone Dipropionate in methanol (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Betamethasone Dipropionate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +63 – +70° (after drying, 50 mg, 1,4-dioxane, 10 mL, 100 mm).

Melting point ⟨*2.60*⟩ 176 – 180°C

Purity **(1)** Fluoride—To 0.10 g of Betamethasone Dipropionate add 10.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), shake for 10 minutes, and filter through a membrane filter (0.4-μm pore size). Place 5.0 mL of the filtrate in a 20-mL volumetric flask, and add 10 mL of a mixture of alizalin complexone TS, acetic acid-potassium acetate buffer solution (pH 4.3) and cerium (III) nitrate TS (1:1:1), add water to make 20 mL, allow to stand for 1 hour, and use this solution as the sample solution. Separately, place 1.0 mL of Standard Fluorine Solution in a 20-mL volumetric flask, add 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), then 10 mL of a mixture of alizalin complexone TS, acetic acid-potassium acetate buffer solution (pH 4.3) and cerium (III) nitrate TS (1:1:1), proceed in the same manner as the preparation of the sample solution, and use this solution as the standard solution. Place 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) in a 20-mL volumetric flask, and proceed in the same manner as the preparation of the sample solution. Using this solution as the blank, determine the absorbances of the sample solution and standard solution at 600 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: the absorbance of the sample solution is not more than that of the standard solution (not more than 0.012%).

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Betamethasone Dipropionate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 10 mg of Betamethasone Dipropionate in 10 mL of chloroform, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography ⟨*2.03*⟩ with these solutions. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (0.5 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (0.5 g, platinum crucible).

Assay **(1)** Betamethasone dipropionate—Weigh accurately about 15 mg of Betamethasone Dipropionate, previously dried, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, and dilute with methanol to exactly 50 mL. Determine the absorbance A of this solution at the wavelength of maximum absorption at about 239 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of betamethasone dipropionate ($C_{28}H_{37}FO_7$) = $A/312 \times 10{,}000$

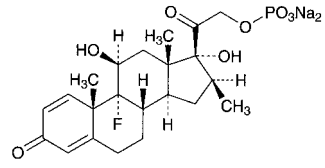
(2) Fluorine—Weigh accurately about 10 mg of Betamethasone Dipropionate, previously dried, and proceed as directed in the procedure of determination for fluorine under Oxygen Flask Combustion Method ⟨*1.06*⟩, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Betamethasone Sodium Phosphate

ベタメタゾンリン酸エステルナトリウム



$C_{22}H_{28}FNa_2O_8P$: 516.40

9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 21-(disodium phosphate)

[*151-73-5*]

Betamethasone Sodium Phosphate contains not less than 97.0% and not more than 103.0% of betamethasone sodium phosphate ($C_{22}H_{28}FNa_2O_8P$), calculated on the anhydrous basis.

Description Betamethasone Sodium Phosphate occurs as white to pale yellowish white, crystalline powder or masses. It is odorless.

It is freely soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

It is hygroscopic.

Melting point: about 213°C (with decomposition).

Identification **(1)** Dissolve 2 mg of Betamethasone Sodium Phosphate in 2 mL of sulfuric acid: a brown color develops, and gradually changes to blackish brown.

(2) Prepare the test solution with 0.01 g of Betamethasone Sodium Phosphate as directed under Oxygen Flask

Combustion Method ⟨*1.06*⟩, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid: the test solution responds to the Qualitative Tests ⟨*1.09*⟩ (2) for fluoride.

(3) Take 40 mg of Betamethasone Sodium Phosphate in a platinum crucible, and carbonize by heating. After cooling, add 5 drops of nitric acid, and incinerate by heating. To the residue add 10 mL of diluted nitric acid (1 in 50), and boil for several minutes. After cooling, filter if necessary, and use this solution as the sample solution. The sample solution responds to the Qualitative Tests ⟨*1.09*⟩ (2) for phosphate. The sample solution neutralized with ammonia TS responds to the Qualitative Tests ⟨*1.09*⟩ for sodium salt, and to the Qualitative Tests ⟨*1.09*⟩ (1) and (3) for phosphate.

(4) Determine the infrared absorption spectrum of Betamethasone Sodium Phosphate, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Betamethasone Sodium Phosphate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +99 – +105° (0.1 g calculated on the anhydrous basis, water, 10 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 0.10 g of Betamethasone Sodium Phosphate in 20 mL of water: the pH of this solution is between 7.5 and 9.0.

Purity **(1)** Clarity and color of solution—Dissolve 0.25 g of Betamethasone Sodium Phosphate in 10 mL of water: the solution is clear and colorless.

(2) Free phosphoric acid—Weigh accurately about 20 mg of Betamethasone Sodium Phosphate, dissolve in 20 mL of water, and use this solution as the sample solution. Separately, pipet 4 mL of Standard Phosphoric Acid Solution, add 20 mL of water, and use this solution as the standard solution. To each of the sample solution and the standard solution add exactly 7 mL of dilute sulfuric acid, exactly 2 mL of hexaammonium heptamolybdate-sulfuric acid TS and exactly 2 mL of *p*-methylaminophenol sulfate TS, shake well, and allow to stand at 20 ± 1°C for 15 minutes. To each add water to make exactly 50 mL, and allow to stand at 20 ± 1°C for 15 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a solution prepared with 20 mL of water in the same manner as the blank. Determine the absorbances, A_T and A_S, of each solution from the sample solution and standard solution at 730 nm: the amount of free phosphoric acid is not more than 0.5%.

$$\text{Amount (\%) of free phosphoric acid } (H_3PO_4) = A_T/A_S \times 1/M \times 10.32$$

M: Amount (mg) of Betamethasone Sodium Phosphate taken, calculated on the anhydrous basis

(3) Betamethasone—Dissolve 20 mg of Betamethasone Sodium Phosphate in exactly 2 mL of methanol, and use this solution as the sample solution. Separately, dissolve 20 mg of Betamethasone RS in exactly 10 mL of methanol. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a freshly prepared mixture of 1-butanol, water and acetic anhydride (3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution corresponding to the spot from the standard solution is not more intense than the spot from the standard solution.

Water ⟨*2.48*⟩ Not more than 10.0% (0.2 g, volumetric titration, back titration).

Assay Weigh accurately about 20 mg each of Betamethasone Sodium Phosphate and Betamethasone Sodium Phosphate RS (separately, determine the water ⟨*2.48*⟩ in the same manner as Betamethasone Sodium Phosphate), and dissolve each in methanol to make exactly 20 mL. Pipet 5 mL each of these solutions, and exactly 5 mL of the internal standard solution, then add methanol to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of betamethasone phosphate to that of the internal standard.

$$\text{Amount (mg) of betamethasone sodium phosphate } (C_{22}H_{28}FNa_2O_8P) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Betamethasone Sodium Phosphate RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (1 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.6 g of tetra-*n*-butylammonium bromide, 3.2 g of disodium hydrogen phosphate dodecahydrate and 6.9 g of potassium dihydrogen phosphate in 1000 mL of water, and add 1500 mL of methanol.

Flow rate: Adjust so that the retention time of betamethasone phosphate is about 5 minutes.

System suitability—

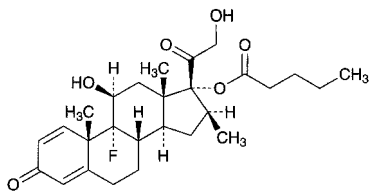
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, betamethasone phosphate and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of betamethasone phosphate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Betamethasone Valerate

ベタメタゾン吉草酸エステル



$C_{27}H_{37}FO_6$: 476.58
9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-pentanoate
[*2152-44-5*]

Betamethasone Valerate, when dried, contains not less than 97.0% and not more than 103.0% of betamethasone valerate ($C_{27}H_{37}FO_6$).

Description Betamethasone Valerate occurs as a white crystalline powder. It is odorless.

It is freely soluble in chloroform, soluble in ethanol (95), sparingly soluble in methanol, slightly soluble in diethyl ether, and practically insoluble in water.

Melting point: about 190°C (with decomposition).

Identification **(1)** Proceed with 0.01 g of Betamethasone Valerate as directed under Oxygen Flask Combustion Method <*1.06*>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the test solution so obtained responds to the Qualitative Tests <*1.09*> for fluoride.

(2) Determine the infrared absorption spectrum of Betamethasone Valerate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Betamethasone Valerate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +77 – +83° (after drying, 0.1 g, methanol, 20 mL, 100 mm).

Purity Related substances—Conduct this procedure without exposure to daylight. Dissolve 0.02 g of Betamethasone Valerate in 5 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (9:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (9:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly alkaline blue tetrazolium TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.2% (0.5 g, platinum crucible).

Assay Dissolve about 10 mg each of Betamethasone Valerate and Betamethasone Valerate RS, previously dried and accurately weighed, in methanol to make exactly 100 mL. Pipet 10 mL each of these solutions, add 10 mL each of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of betamethasone valerate to that of the internal standard.

Amount (mg) of betamethasone valerate ($C_{27}H_{37}FO_6$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of Betamethasone Valerate RS taken

Internal standard solution—A solution of isoamyl benzoate in methanol (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and water (7:3).

Flow rate: Adjust so that the retention time of betamethasone valerate is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, betamethasone valerate and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of betamethasone valerate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Betamethasone Valerate and Gentamicin Sulfate Cream

ベタメタゾン吉草酸エステル·ゲンタマイシン硫酸塩クリーム

Betamethasone Valerate and Gentamicin Sulfate Cream contains not less than 90.0% and not more than 110.0% of the labeled amount of betamethasone valerate ($C_{27}H_{37}FO_6$: 476.58) and not less than 90.0% and not more than 115.0% of the labeled amount of gentamicin C_1($C_{21}H_{43}N_5O_7$: 477.60).

Method of preparation Prepare as directed under Creams, with Betamethasone Valerate and Gentamicin Sulfate.

Identification **(1)** To a quantity of Betamethasone Valerate and Gentamicin Sulfate Cream, equivalent to about 1.2 mg of Betamethasone Valerate, add 20 mL of methanol and 20 mL of hexane, shake vigorously for 10 minutes, and allow to stand. Take 15 mL of the lower layer, evaporate the layer to dryness on a water bath under a current of nitrogen. To the residue add 1 mL of ethyl acetate, mix, and use as the sample solution. Separately, dissolve about 18 mg of Betamethasone Valerate RS in 20 mL of ethyl acetate, and use this solution as the standard solution. Perform the test with

these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with ethyl acetate to a distance of about 10 cm, and air-dry the plate. Spray evenly alkaline blue tetrazolium TS on the plate, and heat at 100°C: the principal spot with the sample solution and the spot with the standard solution are purple in color, and their *R*f values are the same.

(2) To a quantity of Betamethasone Valerate and Gentamicin Sulfate Cream, equivalent to about 2 mg (potency) of Gentamicin Sulfate, add 20 mL of ethyl acetate and 10 mL of water, shake vigorously for 10 minutes, and centrifuge. To 3 mL of the lower layer add 1 mL of dilute sodium hydroxide TS and 2 mL of ninhydrin TS, and heat in a water bath at 90 – 95°C for 10 minutes: a purple to dark purple color develops.

pH <2.54> To a quantity of Betamethasone Valerate and Gentamicin Sulfate Cream, equivalent to 6 mg of Betamethasone Valerate, add 15 mL of water, and mix while warming on a water bath to make a milky liquid: the pH of the cooled liquid is between 4.0 and 6.0.

Purity Related substances—Weigh accurately an amount of Betamethasone Valerate and Gentamicin Sulfate Cream, equivalent to about 1 mg of Betamethasone Valerate, and add 10 mL of a mixture of methanol and water (7:3). Warm in a water bath at 60°C for 5 minutes, and shake vigorously for 20 minutes. Repeat this procedure 2 times. After cooling for 15 minutes with ice, centrifuge for 5 minutes, take away the bubbles from the upper surface, and filter the remaining liquid. Discard first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Perform the test with 150 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, determine each peak area by the automatic integration method, and calculate these amounts by the area percentage method: the amount of the substance other than betamethasone valerate is not more than 3.5%, and the total amount of them is not more than 7.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of water, acetonitrile and methanol (12:7:1).

Flow rate: Adjust so that the retention time of betamethasone valerate is about 16 minutes.

Time span of measurement: About 2.5 times as long as the retention time of betamethasone valerate beginning after the solvent peak. The peaks of the compounding ingredients are not determined.

System suitability—

Test for required detectability: Dissolve 20 mg of Betamethasone Valerate in 100 mL of a mixture of methanol and water (7:3). To exactly 1 mL of this solution add the mixture of methanol and water (7:3) to make exactly 100 mL, and use this solution as the solution for system suitability test. To exactly 2.5 mL of the solution for system suitability test add the mixture of methanol and water (7:3) to make exactly 50 mL. Confirm that the peak area of betamethasone valerate obtained with 150 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 150 μL of the solution for system suitability test.

System performance: When the procedure is run with 150 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of betamethasone valerate are not less than 4000 and 0.8 to 1.3, respectively.

System repeatability: When the test is repeated 6 times with 150 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of betamethasone valerate is not more than 2.0%.

Assay (1) Betamethasone valerate—Weigh accurately an amount of Betamethasone Valerate and Gentamicin Sulfate Cream, equivqlent to about 1 mg of betamethasone valerate ($C_{27}H_{37}FO_6$), add 10 mL of a mixture of methanol and water (7:3), and add exactly 10 mL of the internal standard solution. After warming in a water bath at 60°C for 5 minutes, shake vigorously for 20 minutes. Repeat this procedure twice, cool with ice for 15 minutes, centrifuge for 5 minutes, then filter the supernatant liquid, discard the first 5 mL of filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Betamethasone Valerate RS, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, and add the mixture of methanol and water (7:3) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, mix, and use this solution as the standard solution. Perform the test with 3 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of betamethasone valerate to that of the internal standard.

$$\text{Amount (mg) of betamethasone valerate } (C_{27}H_{37}FO_6) = M_S \times Q_T/Q_S \times 1/25$$

M_S: Amount (mg) of Betamethasone Valerate RS taken

Internal standard solution—Dissolve 20 mg of beclometasone dipropionate in 10 mL of methanol, and add the mixture of methanol and water (7:3) to make 200 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 2.1 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3.5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and water (13:7).

Flow rate: Adjust so that the retention time of betamethasone valerate is about 16 minutes.

System suitability—

System performance: When the procedure is run with 3 μL of the standard solution under the above operating conditions, betamethasone valerate and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 3 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of betamethasone valerate to that of the internal standard is not more than 1.0%.

(2) Gentamicin sulfate—Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism, agar media for base layer and seed

layer, agar medium for transferring test organisms, and standard solutions—Proceed as directed in the Assay under Gentamicin Sulfate.

(ii) Sample solutions—Weigh accurately an amount of Betamethasone Valerate and Gentamicin Sulfate Cream, equivalent to about 1 mg (potency) of Gentamicin Sulfate, add 100 mL of 0.1 mol/L phosphate buffer solution (pH 8.0) previously warmed to about 85°C, and shake well to dissolve. After cooling, add 0.1 mol/L phosphate buffer solution (pH 8.0) to make exactly 250 mL to make the high concentration sample solution, which contains 4 μg (potency) per mL. Pipet a suitable amount of the high concentration sample solution, add 0.1 mol/L phosphate buffer solution (pH 8.0) so that each mL contains 1 μg (potency), and use this solution as the low concentration sample solution.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Betamethasone Valerate and Gentamicin Sulfate Ointment

ベタメタゾン吉草酸エステル·ゲンタマイシン硫酸塩軟膏

Betamethasone Valerate and Gentamicin Sulfate Ointment contains not less than 95.0% and not more than 110.0% of the labeled amount of betamethasone valerate ($C_{27}H_{37}FO_6$: 476.58) and not less than 90.0% and not more than 115.0% of the labeled potency of gentamicin C_1 ($C_{21}H_{43}N_5O_7$: 477.60).

Method of preparation Prepare as directed under Ointment, with Betamethasone Valerate and Gentamicin Sulfate.

Identification (1) To a quantity of Betamethasone Valerate and Gentamicin Sulfate Ointment, equivalent to 1.2 mg of Betamethasone Valerate, add 20 mL of methanol and 20 mL of hexane, and disperse the ointment with the aid of ultrasonic. Shake vigorously for 5 minutes, centrifuge for 5 minutes, cool for 15 minutes with ice, and take 15 mL of the lower layer. Evaporate the layer to dryness on a water bath under a current of nitrogen. To the residue add 1 mL of ethyl acetate, apply ultrasonic waves, filter, if necessary, and use the filtrate as the sample solution. Separately, dissolve 18 mg of Betamethasone Valerate RS in 20 mL of ethyl acetate, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with ethyl acetate to a distance of about 10 cm, and air-dry the plate. Spray evenly alkaline blue tetrazolium TS on the plate, and heat at 100°C: the principal spot from the sample solution and the spot from the standard solution are purple in color, and their *R*f values are the same.

(2) To a quantity of Betamethasone Valerate and Gentamicin Sulfate Ointment, equivalent to 2 mg (potency) of Gentamicin Sulfate, add 20 mL of hexane and 10 mL of water, shake vigorously for 10 minutes, and centrifuge. To 3 mL of the lower layer add 1 mL of dilute sodium hydroxide TS and 2 mL of ninhydrin TS, and heat in a water bath at 90 – 95°C for 10 minutes: a red-brown color develops.

pH <*2.54*> To a quantity of Betamethasone Valerate and Gentamicin Sulfate Ointment, equivalent to 6 mg of Betamethasone Valerate, add 15 mL of water, and warm on a water bath to dissolve. After cooling, separate the water layer: the pH of the layer is between 4.0 and 7.0.

Assay (1) Betamethasone valerate—Weigh accurately an amount of Betamethasone Valerate and Gentamicin Sulfate Ointment, equivalent to about 1 mg of betamethasone valerate ($C_{27}H_{37}FO_6$), add 10 mL of a mixture of methanol and water (7:3), and add exactly 10 mL of the internal standard solution. After warming in a water bath at 75°C for 5 minutes, shake vigorously for 10 minutes. Repeat this procedure once more, cool with ice for 15 minutes, filter, discard the first 5 mL of filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Betamethasone Valerate RS, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, and add the mixture of methanol and water (7:3) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, mix, and use this solution as the standard solution. Perform the test with 3 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of betamethasone valerate to that of the internal standard.

$$\text{Amount (mg) of betamethasone valerate } (C_{27}H_{37}FO_6) = M_S \times Q_T/Q_S \times 1/25$$

M_S: Amount (mg) of Betamethasone Valerate RS taken

Internal standard solution—Dissolve 20 mg of beclometasone dipropionate in 10 mL of methanol, and add the mixture of methanol and water (7:3) to make 200 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 2.1 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3.5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and water (13:7).

Flow rate: Adjust so that the retention time of betamethasone valerate is about 16 minutes.

System suitability—

System performance: When the procedure is run with 3 μL of the standard solution under the above operating conditions, betamethasone valerate and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 3 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of betamethasone valerate to that of the internal standard is not more than 1.0%.

(2) Gentamicin sulfate—Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism, agar media for base layer and seed layer, agar medium for transferring test organisms, and standard solutions—Proceed as directed in the Assay under Gentamicin Sulfate.

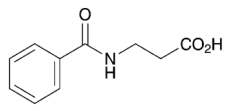
(ii) Sample solutions—Weigh accurately an amount of Betamethasone Valerate and Gentamicin Sulfate Ointment, equivalent to about 1 mg (potency) of Gentamicin Sulfate, transfer to a separator, add 50 mL of petroleum ether and exactly 100 mL of 0.1 mol/L phosphate buffer solution (pH 8.0), shake for 10 minutes, and allow to stand. Pipet a suitable amount of the water layer, add 0.1 mol/L phosphate

buffer solution (pH 8.0) to make solutions so that each mL contains 4 μg (potency) and 1 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Betamipron

ベタミプロン



$C_{10}H_{11}NO_3$: 193.20
3-Benzoylaminopropanoic acid
[*3440-28-6*]

Betamipron contains not less than 99.0% and not more than 101.0% of betamipron ($C_{10}H_{11}NO_3$), calculated on the anhydrous basis.

Description Betamipron occurs as white, crystals or crystalline powder.

It is freely soluble in methanol, soluble in ethanol (99.5), and slightly soluble in water.

It dissolves in sodium hydroxide TS.

Identification (1) Determine the absorption spectrum of a solution of Betamipron in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Betamipron as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <*2.54*> Dissolve 0.25 g of Betamipron in 100 mL of water by warming, and cool: the pH of this solution is between 3.0 and 3.4.

Melting point <*2.60*> 132 – 135°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Betamipron in 10 mL of sodium hydroxide TS: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Betamipron according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) *β*-Alanine—Dissolve 0.25 g of Betamipron in 10 mL of methanol, and use this solution as the sample solution. Separately, dissolve 50 mg of *β*-alanine in methanol to make exactly 100 mL. Pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of methanol, ethyl acetate, ammonia solution (28) and water (200:200:63:37) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin-butanol TS on the plate, and heat at 105°C for 5 minutes: the spot obtained from the sample solution corresponding to the spot obtained from the standard solution is not more intense than the spot from the standard solution.

(4) Related substances—Dissolve 20 mg of Betamipron in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than betamipron from the sample solution is not larger than 2/5 times the peak area of betamipron from the standard solution, and the total area of the peaks other than betamipron from the sample solution is not larger than the peak area of betamipron from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.12 g of sodium dihydrogen phosphate dihydrate in 800 mL of water, adjust to pH 7.0 with dilute sodium hydroxide TS, and add water to make 1000 mL. To 900 mL of this solution add 100 mL of acetonitrile.

Flow rate: Adjust so that the retention time of betamipron is about 6 minutes.

Time span of measurement: About 2 times as long as the retention time of betamipron, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of betamipron obtained with 10 μL of this solution is equivalent to 7 to 13% of that obtained with 10 μL of the standard solution.

System performance: Dissolve 5 mg of Betamipron and 5 mg of benzoic acid in 200 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, benzoic acid and betamipron are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of betamipron is not more than 2.0%.

Water <*2.48*> Not more than 0.5% (1 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

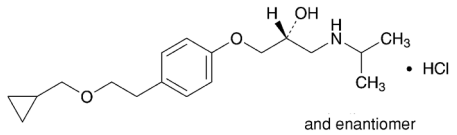
Assay Weigh accurately about 0.25 g of Betamipron, dissolve in 25 mL of ethanol (99.5), add 25 mL of water, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform the blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 19.32 mg of $C_{10}H_{11}NO_3$

Containers and storage Containers—Tight containers.

Betaxolol Hydrochloride

ベタキソロール塩酸塩



$C_{18}H_{29}NO_3$.HCl: 343.89
(2*RS*)-1-{4-[2-(Cyclopropylmethoxy)ethyl]phenoxy}-3-[(1-methylethyl)amino]propan-2-ol monohydrochloride
[*63659-19-8*]

Betaxolol Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of betaxolol hydrochloride ($C_{18}H_{29}NO_3$.HCl).

Description Betaxolol Hydrochloride occurs as white, crystals or crystalline powder.

It is very soluble in water, and freely soluble in methanol, in ethanol (99.5) and in acetic acid (100).

Dissolve 1.0 g of Betaxolol Hydrochloride in 50 mL of water: the pH of the solution is between 4.5 and 6.5.

A solution of Betaxolol Hydrochloride (1 in 100) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Betaxolol Hydrochloride in ethanol (99.5) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Betaxolol Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Betaxolol Hydrochloride (1 in 10) responds to the Qualitative Tests <*1.09*> (2) for chloride.

Melting point <*2.60*> 114 – 117°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Betaxolol Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Betaxolol Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 2.0 g of Betaxolol Hydrochloride according to Method 3, and perform the test (not more than 1 ppm).

(4) Related substance I—Dissolve 0.10 g of Betaxolol Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 3 mL of the sample solution, and add methanol to make exactly 50 mL. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and acetic acid (100) (10:3:3) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 1 hour: the number of the spots other than the principal spot obtained from the sample solution is not more than 3, and they are not more intense than the spot obtained from the standard solution.

(5) Related substance II—Dissolve 0.10 g of Betaxolol Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak other than betaxolol obtained from the sample solution is not larger than the peak area of betaxolol from the standard solution, and the total area of the peaks other than the peak of betaxolol from the sample solution is not larger than 2 times the peak area of betaxolol from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 273 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of diluted 0.05 mol/L potassium dihydrogen phosphate TS (1 in 2) with the pH adjusted to 3.0 with 1 mol/L hydrochloric acid TS, acetonitrile and methanol (26:7:7).

Flow rate: Adjust so that the retention time of betaxolol is about 9 minutes.

Time span of measurement: About 2 times as long as the retention time of betaxolol, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 4 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of betaxolol obtained from 10 μL of this solution is equivalent to 14 to 26% of that obtained from 10 μL of the standard solution.

System performance: Dissolve 50 mg of Betaxolol Hydrochloride and 5 mg of 2-naphthol in 200 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, betaxolol and 2-naphthol are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of betaxolol is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

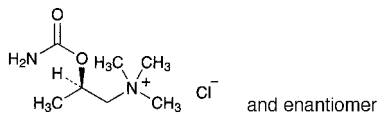
Assay Weigh accurately about 0.3 g of Betaxolol Hydrochloride, previously dried, dissolve in 30 mL of acetic acid (100), add 30 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.39 mg of $C_{18}H_{29}NO_3$.HCl

Containers and storage Containers—Tight containers.

Bethanechol Chloride

ベタネコール塩化物



$C_7H_{17}ClN_2O_2$: 196.68
(2*RS*)-2-Carbamoyloxy-*N*,*N*,*N*-trimethylpropylaminium chloride
[*590-63-6*]

Bethanechol Chloride, when dried, contains not less than 98.0% and not more than 101.0% of bethanechol chloride ($C_7H_{17}ClN_2O_2$).

Description Bethanechol Chloride occurs as colorless or white crystals or a white, crystalline powder.

It is very soluble in water, freely soluble in acetic acid (100), and sparingly soluble in ethanol (99.5).

It is hygroscopic.

A solution of Bethanechol Chloride (1 in 10) shows no optical rotation.

Identification **(1)** To 2 mL of a solution of Bethanechol Chloride (1 in 40) add 0.1 mL of a solution of cobalt (II) chloride hexahydrate (1 in 100), then add 0.1 mL of potassium hexacyanoferrate (II) TS: A green color is produced, and almost entirely fades within 10 minutes.

(2) To 1 mL of a solution of Bethanechol Chloride (1 in 100) add 0.1 mL of iodine TS: a brown precipitate is produced, and the solution shows a greenish brown color.

(3) Determine the infrared absorption spectrum of Bethanechol Chloride as directed in the paste method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Bethanechol Chloride (1 in 100) responds to the Qualitative Tests <*1.09*> for chloride.

Melting point <*2.60*> 217 – 221°C (after drying).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Bethanechol Chloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 1.0 g of Bethanechol Chloride in 2.5 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 1 μL each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of a solution of ammonium acetate (1 in 100), acetone, 1-butanol and formic acid (20:20:20:1) to a distance of about 10 cm, and dry the plate at 105°C for 15 minutes. Spray evenly hydrogen hexachloroplatinate (IV)-potassium iodide TS on the plate, and allow to stand for 30 minutes: the spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

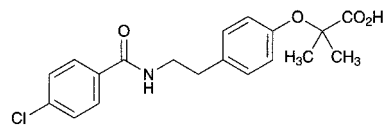
Assay Weigh accurately about 0.4 g of Bethanechol Chloride, previously dried, dissolve in 2 mL of acetic acid (100), add 40 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 19.67 mg of $C_7H_{17}ClN_2O_2$

Containers and storage Containers—Tight containers.

Bezafibrate

ベザフィブラート



$C_{19}H_{20}ClNO_4$: 361.82
2-(4-{2-[(4-Chlorobenzoyl)amino]ethyl}phenoxy)-2-methylpropanoic acid
[*41859-67-0*]

Bezafibrate, when dried, contains not less than 98.5% and not more than 101.0% of bezafibrate ($C_{19}H_{20}ClNO_4$).

Description Bezafibrate occurs as a white crystalline powder.

It is freely soluble in *N*,*N*-dimethylformamide, soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Bezafibrate in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bezafibrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Bezafibrate as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Melting point <*2.60*> 181 – 186°C

Purity **(1)** Chloride <*1.03*>—Dissolve 3.0 g of Bezafibrate in 15 mL of *N*,*N*-dimethylformamide, add water to make 60 mL, shake well, allow to stand for more than 12 hours, and filter. To 40 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.70 mL of 0.01 mol/L hydrochloric acid VS add 10 mL of *N*,*N*-dimethylformamide, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.012%).

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Bezafibrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.10 g of Bezafibrate in 35 mL of methanol, add diluted 0.5 mol/L ammonium acetate TS (1 in 50) to make 50 mL, and use this solution as the

sample solution. Pipet 1 mL of the sample solution, add 70 mL of methanol and diluted 0.5 mol/L ammonium acetate TS (1 in 50) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the areas of the peaks having the relative retention times of about 0.65 and 1.86 to bezafibrate obtained from the sample solution are not larger than 1/2 times the peak area of bezafibrate obtained from the standard solution, the area of the peak other than those and other than bezafibrate from the sample solution is not larger than 1/5 times the peak area of bezafibrate from the standard solution, and the total area of the peaks other than the peak of bezafibrate from the sample solution is not larger than 3/4 times the peak area of bezafibrate from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and diluted acetic acid (100) (1 in 100) (9:4).

Flow rate: Adjust so that the retention time of bezafibrate is about 6 minutes.

Time span of measurement: About 2.5 times as long as the retention time of bezafibrate, beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 5 mL of the standard solution, and add a mixture of methanol and diluted 0.5 mol/L ammonium acetate TS (1 in 50) (7:3) to make exactly 50 mL. Confirm that the peak area of bezafibrate obtained with 5 μL of this solution is equivalent to 7 to 13% of that obtained with 5 μL of the standard solution.

System performance: Dissolve 20 mg of Bezafibrate and 10 mg of 4-chlorobenzoate in 70 mL of methanol, and add diluted 0.5 mol/L ammonium acetate TS (1 in 50) to make 100 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, 4-chlorobenzoate and bezafibrate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of bezafibrate is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.7 g of Bezafibrate, previously dried, dissolve in 50 mL of ethanol (99.5), and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 36.18 mg of $C_{19}H_{20}ClNO_4$

Containers and storage Containers—Tight containers.

Bezafibrate Extended-release Tablets

ベザフィブラート徐放錠

Bezafibrate Extended-release Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of bezafibrate ($C_{19}H_{20}ClNO_4$: 361.82).

Method of preparation Prepare as directed under Tablets, with Bezafibrate.

Identification Mix well an amount of powdered Bezafibrate Extended-release Tablets, equivalent to 0.1 g of Bezafibrate, with 100 mL of methanol, and filter. To 1 mL of the filtrate add methanol to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 227 nm and 231 nm.

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of disodium hydrogen phosphate-citric acid buffer solution (pH 7.2) as the dissolution medium, the dissolution rates of a 100-mg tablet in 1.5 hours, in 2.5 hours and in 8 hours are 15 – 45%, 35 – 65% and not less than 80%, respectively, and those of a 200-mg tablet in 1.5 hours, in 2.5 hours and in 8 hours are 15 – 45%, 30 – 60% and not less than 75%, respectively.

Start the test with 1 tablet of Bezafibrate Extended-release Tablets, withdraw exactly 20 mL of the medium at the specified minutes after starting the test, and immediately fill up the dissolution medium each time with exactly 20 mL of fresh dissolution medium, previously warmed to 37 ± 0.5°C. Filter these media through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add the dissolution medium to make exactly V' mL so that each mL contains about 13 μg of bezafibrate ($C_{19}H_{20}ClNO_4$), and use these solutions as the sample solutions. Separately, weigh accurately about 66 mg of bezafibrate for assay, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, $A_{T(n)}$ (n = 1,2,3) and A_S, of the sample solutions and standard solution at 228 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using the dissolution medium as the blank.

Dissolution rate (%) in each case of n with respect to the labeled amount of bezafibrate ($C_{19}H_{20}ClNO_4$)

$$= M_S \times \left\{ \frac{A_{T(n)}}{A_S} + \sum_{i=1}^{n-1} \left(\frac{A_{T(i)}}{A_S} \times \frac{1}{45} \right) \right\} \times \frac{V'}{V} \times \frac{1}{C} \times 18$$

M_S: Amount (mg) of bezafibrate for assay taken

C: Labeled amount (mg) of bezafibrate ($C_{19}H_{20}ClNO_4$) in 1 tablet

Assay Weigh accurately, and powder not less than 20 Bezafibrate Extended-release Tablets. Weigh accurately a portion of the powder, equivalent to about 20 mg of bezafibrate ($C_{19}H_{20}ClNO_4$), add 60 mL of methanol and exactly 10 mL of the internal standard solution, and shake for 20 minutes. Add diluted 0.5 mol/L ammonium acetate

TS (1 in 50) to make 100 mL, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 20 mg of bezafibrate for assay, previously dried at 105°C for 3 hours, dissolve in 60 mL of methanol, add exactly 10 mL of the internal standard solution and diluted 0.5 mol/L ammonium acetate TS (1 in 50) to make 100 mL, and use this solution as the standard solution. Perform the test with 2 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of bezafibrate to that of the internal standard.

Amount (mg) of bezafibrate ($C_{19}H_{20}ClNO_4$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of bezafibrate for assay taken

Internal standard solution—A solution of 4-nitrophenol in methanol (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and diluted acetic acid (100) (1 in 100) (9:4).

Flow rate: Adjust so that the retention time of bezafibrate is about 6 minutes.

System suitability—

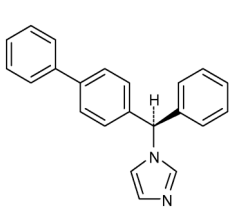
System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the internal standard and bezafibrate are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of bezafibrate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Bifonazole

ビホナゾール



and enantiomer

$C_{22}H_{18}N_2$: 310.39

1-[(*RS*)-(Biphenyl-4-yl)(phenyl)methyl]-1*H*-imidazole

[*60628-96-8*]

Bifonazole, when dried, contains not less than 98.5% of bifonazole ($C_{22}H_{18}N_2$).

Description Bifonazole occurs as a white to pale yellow powder. It is odorless and tasteless.

It is freely soluble in dichloromethane, soluble in methanol, sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

A solution of Bifonazole in methanol (1 in 100) does not show optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Bifonazole in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bifonazole, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 147 – 151°C

Purity **(1)** Chloride <*1.03*>—To 2.0 g of Bifonazole add 40 mL of water, warm for 5 minutes, and after cooling, filter. To 10 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(2) Sulfate <*1.14*>—To 10 mL of the filtrate obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (0.048%).

(3) Heavy metals <*1.07*>—Proceed with 2.0 g of Bifonazole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.10 g of Bifonazole in 10 mL of methanol, and use this solution as the sample solution. Pipet 3 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 25 mL and 5 mL of this solution, add methanol to make exactly 50 mL each, and use these solutions as the standard solutions (1) and (2), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and ammonia solution (28) (49:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot with *R*f value of about 0.20 from the sample solution is not more intense than the spot from the standard solution (1). And the spots other than the spot mentioned above and the principal spot from the sample solution are not more intense than the spot from the standard solution (2).

Loss on drying <*2.41*> Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.15 g of Bifonazole, previously dried, and dissolve in dichloromethane to make exactly 50 mL. Pipet 5 mL of this solution in a glass-stoppered conical flask, add 10 mL of water, 5 mL of dilute sulfuric acid and 25 mL of dichloromethane, and add 2 to 3 drops of a solution of methyl yellow in dichloromethane (1 in 500) as indicator, and titrate <*2.50*>, while shaking vigorously, with 0.01 mol/L sodium lauryl sulfate VS by a buret with 0.02-mL minimum graduation. The end point is reached when the color of the dichloromethane layer changes from yellow to orange-red after dropwise addition of 0.01 mol/L

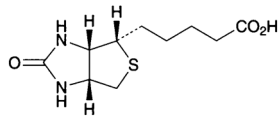
sodium lauryl sulfate VS, strong shaking, and standing for a while.

Each mL of 0.01 mol/L sodium lauryl sulfate VS
= 3.104 mg of $C_{22}H_{18}N_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Biotin

ビオチン



$C_{10}H_{16}N_2O_3S$: 244.31
5-[(3a*S*,4*S*,6a*R*)-2-Oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoic acid
[*58-85-5*]

Biotin, when dried, contains not less than 98.5% and not more than 101.0% of biotin ($C_{10}H_{16}N_2O_3S$).

Description Biotin occurs as white crystals or a white crystalline powder.
It is very slightly soluble in water and in ethanol (99.5).
It dissolves in dilute sodium hydroxide TS.
Melting point: about 231°C (with decomposition).

Identification Determine the infrared absorption spectrum of Biotin as directed in the potassium bromide disc method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +89 – +93° (after drying, 0.4 g, dilute sodium hydroxide TS, 20 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Biotin in 10 mL of 0.5 mol/L sodium hydroxide TS: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Biotin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <*1.11*>—Place 0.7 g of Biotin in a Kjeldahl flask, add 5 mL of nitric acid and 2 mL of sulfuric acid, place a small funnel on the mouth of the flask, and carefully heat until white fumes are evolved. After cooling, add 2 mL of nitric acid twice, heat, add 2 mL of hydrogen peroxide (30) several times, and heat until the color of the solution becomes colorless or pale yellow. After cooling, add 2 mL of saturated ammonium oxalate solution, and heat to concentrate until white fumes are evolved again. After cooling, add water to make 5 mL, and perform the test using this solution as the test solution (not more than 2.8 ppm).

(4) Related substances—Dissolve 0.10 g of Biotin in 10 mL of diluted ammonia solution (28) (7 in 100), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add diluted ammonia solution (28) (7 in 100) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted ammonia solution (28) (7 in 100) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, and acetic acid (100) (5:2:1) to a distance of about 10 cm, air-dry the plate, and then dry for 30 minutes at 105°C. Spray the plate evenly with a mixture of a solution of 4-dimethylaminocinnamaldehyde in ethanol (99.5) (1 in 500) and a solution of sulfuric acid in ethanol (99.5) (1 in 50) (1:1): the spots other than the principal spot obtained from the sample solution are not more intense than the spot obtained from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (0.5 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

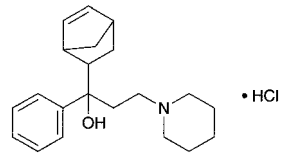
Assay Weigh accurately about 0.25 g of Biotin, previously dried, dissolve by adding exactly 20 mL of 0.1 mol/L sodium hydroxide VS, and titrate <*2.50*> the excess sodium hydroxide with 0.1 mol/L hydrochloric acid VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L sodium hydroxide VS
= 24.43 mg of $C_{10}H_{16}N_2O_3S$

Containers and storage Containers—Tight containers.

Biperiden Hydrochloride

ビペリデン塩酸塩



$C_{21}H_{29}NO.HCl$: 347.92
1-(Bicyclo[2.2.1]hept-5-en-2-yl)-1-phenyl-3-(piperidin-1-yl)propan-1-ol monohydrochloride
[*1235-82-1*]

Biperiden Hydrochloride, when dried, contains not less than 99.0% of biperiden hydrochloride ($C_{21}H_{29}NO.HCl$).

Description Biperiden Hydrochloride occurs as a white to brownish and yellowish white crystalline powder.
It is freely soluble in formic acid, slightly soluble in water, in methanol and in ethanol (95), and practically insoluble in diethyl ether.
Melting point: about 270°C (with decomposition).

Identification **(1)** Dissolve 0.02 g of Biperiden Hydrochloride in 5 mL of phosphoric acid: a green color develops.

(2) Dissolve 0.01 g of Biperiden Hydrochloride in 5 mL of water by heating, cool, and add 5 to 6 drops of bromine TS: a yellow precipitate is formed.

(3) Determine the absorption spectrum of a solution of Biperiden Hydrochloride (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Biperiden Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(5) Dissolve 0.02 g of Biperiden Hydrochloride in 10 mL of water by heating, and cool: the solution responds to the Qualitative Tests <*1.09*> for chloride.

Purity **(1)** Acidity or alkalinity—To 1.0 g of Biperiden Hydrochloride add 50 mL of water, shake vigorously, filter, and to 20 mL of the filtrate add 1 drop of methyl red TS: no red to yellow color develops.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Biperiden Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Biperiden Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Biperiden Hydrochloride in 20 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 50 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (80:15:2) to a distance of about 15 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Biperiden Hydrochloride, previously dried, dissolve in 5 mL of formic acid, add 60 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

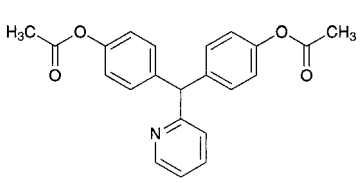
Each mL of 0.1 mol/L perchloric acid VS
= 34.79 mg of $C_{21}H_{29}NO.HCl$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Bisacodyl

ビサコジル



$C_{22}H_{19}NO_4$: 361.39
4,4′-(Pyridin-2-ylmethylene)bis(phenyl acetate)
[*603-50-9*]

Bisacodyl, when dried, contains not less than 98.5% of bisacodyl ($C_{22}H_{19}NO_4$).

Description Bisacodyl occurs as a white crystalline powder.

It is freely soluble in acetic acid (100), soluble in acetone, slightly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

Identification **(1)** Determine the absorption spectrum of a solution of Bisacodyl in ethanol (95) (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Bisacodyl RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bisacodyl, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Bisacodyl RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 132 – 136°C

Purity **(1)** Chloride <*1.03*>—Dissolve 1.0 g of Bisacodyl in 30 mL of acetone, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.35 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.012%).

(2) Sulfate <*1.14*>—Dissolve 1.0 g of Bisacodyl in 2 mL of dilute hydrochloric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.35 mL of 0.005 mol/L sulfuric acid VS add 2 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.017%).

(3) Heavy metals <*1.07*>—Proceed with 2.0 g of Bisacodyl according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 0.20 g of Bisacodyl in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-butanone, chloroform and xylene (1:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Bisacodyl, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from orange-yellow to green (indicator: 0.5 mL of *p*-naphtholbenzein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 36.14 mg of $C_{22}H_{19}NO_4$

Containers and storage Containers—Well-closed containers.

Bisacodyl Suppositories

ビサコジル坐剤

Bisacodyl Suppositories contain not less than 90.0% and not more than 110.0% of the labeled amount of bisacodyl ($C_{22}H_{19}NO_4$: 361.39).

Method of preparation Prepare as directed under Suppositories, with Bisacodyl.

Identification **(1)** To a quantity of Bisacodyl Suppositories, equivalent to 6 mg of Bisacodyl, add 20 mL of ethanol (95), warm on a water bath for 10 minutes, shake vigorously for 10 minutes, and allow to stand in ice water for 1 hour. Centrifuge the solution, filter the supernatant liquid, and to 2 mL of the filtrate add ethanol (95) to make 20 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 261 nm and 265 nm.

(2) Use the filtrate obtained in (1) as the sample solution. Separately, dissolve 6 mg of Bisacodyl RS in 20 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-butanone, chloroform and xylene (1:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution and that from the standard solution show the same *R*f value.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 suppository of Bisacodyl Suppositories add a suitable amount of tetrahydrofuran, warm to 40°C, and shake to dissolve. After cooling, add tetrahydrofuran to make exactly *V* mL so that each mL contains about 0.2 mg of bisacodyl ($C_{22}H_{19}NO_4$). Pipet 5 mL of this solution, and proceed as directed in the Assay.

$$\text{Amount (mg) of bisacodyl } (C_{22}H_{19}NO_4) = M_S \times Q_T/Q_S \times V/50$$

M_S: Amount (mg) of Bisacodyl RS taken

Internal standard solution—A solution of ethyl parahydroxybenzoate in acetonitrile (3 in 100,000).

Assay Weigh accurately not less than 20 Bisacodyl Suppositories, make them fine fragments carefully, and mix uniformly. Weigh accurately a portion of the fragments, equivalent to about 10 mg of bisacodyl ($C_{22}H_{19}NO_4$), add 40 mL of tetrahydrofuran, warm to 40°C, dissolve by shaking, cool, and add tetrahydrofuran to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and add the mobile phase to make 100 mL. Cool this solution in ice for 30 minutes, centrifuge, filter the supernatant liquid through a membrane filter with pore size of 0.5 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 10 mg of Bisacodyl RS, previously dried at 105°C for 2 hours, and dissolve in tetrahydrofuran to make exactly 50 mL. Pipet 5 mL of this solution, proceed in the same manner as the sample solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of bisacodyl to that of the internal standard.

$$\text{Amount (mg) of bisacodyl } (C_{22}H_{19}NO_4) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Bisacodyl RS taken

Internal standard solution—A solution of ethyl parahydroxybenzoate in acetonitrile (3 in 100,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.01 mol/L citric acid TS, acetonitrile and methanol (2:1:1).

Flow rate: Adjust so that the retention time of bisacodyl is about 8 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and bisacodyl are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of bisacodyl to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Bismuth Subgallate

Dermatol

次没食子酸ビスマス

Bismuth Subgallate, when dried, contains not less than 47.0% and not more than 51.0% of bismuth (Bi: 208.98).

Description Bismuth Subgallate occurs as a yellow powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid, in dilute nitric acid and in dilute sulfuric acid on warming. It dissolves in sodium hydroxide TS, forming a clear, yellow solution, which turns red immediately.

It is affected by light.

Identification **(1)** Ignite 0.5 g of Bismuth Subgallate: it chars at first, and leaves finally a yellow residue. The residue responds to the Qualitative Tests <*1.09*> for bismuth salt.

(2) To 0.5 g of Bismuth Subgallate add 25 mL of water and 20 mL of hydrogen sulfide TS, and shake well. Filter off the blackish brown precipitate, and add 1 drop of iron (III) chloride TS to the filtrate: a blue-black color is produced.

Purity **(1)** Clarity of solution—Dissolve 1.0 g of Bismuth Subgallate in 40 mL of diluted sodium hydroxide TS (1 in 8): the solution is clear.

(2) Sulfate—Ignite 3.0 g of Bismuth Subgallate in a porcelain crucible, and cautiously dissolve the residue in 2.5 mL

of nitric acid by warming. Pour the solution into 100 mL of water, shake, and filter. Evaporate 50 mL of the filtrate on a water bath to 15 mL. Add water to make 20 mL, filter again, and use the filtrate as the sample solution. To 5 mL of the sample solution add 2 to 3 drops of barium nitrate TS: no turbidity is produced.

(3) Nitrate—To 0.5 g of Bismuth Subgallate add 5 mL of dilute sulfuric acid and 25 mL of iron (II) sulfate TS, shake well, and filter. Superimpose carefully 5 mL of the filtrate on sulfuric acid: no red-brown color develops at the zone of contact.

(4) Ammonium—Dissolve 1.0 g of Bismuth Subgallate in 5 mL of sodium hydroxide TS, and heat: the gas evolved does not change moistened red litmus paper to blue.

(5) Copper—To 5 mL of the sample solution obtained in (2) add 1 mL of ammonia TS, and filter: no blue color develops in the filtrate.

(6) Lead—Ignite 1.0 g of Bismuth Subgallate at about 500°C in a porcelain crucible, dissolve the residue in a smallest possible amount of nitric acid added dropwise, evaporate over a low flame to dryness , and cool. Add 5 mL of a solution of potassium hydroxide (1 in 6) to the residue, boil carefully for 2 minutes, cool, and centrifuge. Take the supernatant liquid in a test tube, add 10 drops of potassium chromate TS, and acidify the solution by adding acetic acid (100) dropwise: neither turbidity nor a yellow precipitate is produced.

(7) Silver—To 5 mL of the sample solution obtained in (2) add 0.5 mL of nitric acid and 2 to 3 drops of dilute hydrochloric acid: no turbidity is produced.

(8) Alkaline earth metals and alkali metals—Boil 1.0 g of Bismuth Subgallate with 40 mL of diluted acetic acid (31) (1 in 2) for 2 minutes, cool, add water to make 40 mL, and filter. To 20 mL of the filtrate add 2 mL of dilute hydrochloric acid, boil, immediately pass hydrogen sulfide thoroughly through the solution, filter the precipitate produced, and wash with water. Combine the filtrate and the washings, add 5 drops of sulfuric acid, and evaporate to dryness. Ignite as directed under Residue on Ignition <*2.44*>: the mass of the residue is not more than 5.0 mg.

(9) Arsenic <*1.11*>—Mix well 0.20 g of Bismuth Subgallate with 0.20 g of calcium hydroxide, and ignite the mixture. Dissolve the residue in 5 mL of dilute hydrochloric acid, use this solution as the test solution, and perform the test (not more than 10 ppm).

(10) Gallic acid—To 1.0 g of Bismuth Subgallate add 20 mL of ethanol (95), shake for 1 minute, and filter. Evaporate the filtrate on a water bath to dryness: the mass of the residue is not more than 5.0 mg.

Loss on drying <*2.41*> Not more than 6.0% (1 g, 105°C, 3 hours).

Assay Weigh accurately about 0.5 g of Bismuth Subgallate, previously dried, ignite at about 500°C for 30 minutes, and cool. Dissolve the residue in 5 mL of diluted nitric acid (2 in 5) by warming, and add water to make exactly 100 mL. Measure exactly 30 mL of this solution, add 200 mL of water, and titrate <*2.50*> with 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to yellow (indicator: 2 to 3 drops of xylenol orange TS).

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 4.180 mg of Bi

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Bismuth Subnitrate

次硝酸ビスマス

Bismuth Subnitrate, when dried, contains not less than 71.5% and not more than 74.5% of bismuth (Bi: 208.98).

Description Bismuth Subnitrate occurs as a white powder.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It readily dissolves in hydrochloric acid and in nitric acid without effervescence.

It is slightly hygroscopic, and changes moistened blue litmus paper to red.

Identification Bismuth Subnitrate responds to the Qualitative Tests <*1.09*> for bismuth salt and nitrate.

Purity **(1)** Chloride <*1.03*>—Dissolve 0.7 g of Bismuth Subnitrate in 2 mL of water and 2 mL of nitric acid, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 2 mL of nitric acid on a water bath to dryness, add 0.70 mL of 0.01 mol/L hydrochloric acid VS, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.035%).

(2) Sulfate—Dissolve 3.0 g of Bismuth Subnitrate in 3.0 mL of warmed nitric acid, pour this solution into 100 mL of water, shake, and filter. Concentrate the filtrate on a water bath to 30 mL, filter, and use this filtrate as the sample solution. To 5 mL of the sample solution add 2 to 3 drops of barium nitrate TS: no turbidity is produced.

(3) Ammonium—Boil 0.10 g of bismuth Subnitrate with 5 mL of sodium hydroxide TS: the gas evolved does not change moistened red litmus paper to blue.

(4) Copper—To 5 mL of the sample solution obtained in (2) add 2 mL of ammonia TS, and filter: no blue color develops.

(5) Lead—To 1.0 g of Bismuth Subnitrate add 5 mL of a solution of sodium hydroxide (1 in 6), boil carefully for 2 minutes, cool and centrifuge. Transfer the supernatant liquid to a test tube, add 10 drops of potassium chromate TS, and add dropwise acetic acid (31) to render the solution acid: no turbidity or yellow precipitate is produced.

(6) Silver—To 5 mL of the sample solution obtained in (2) add 0.5 mL of nitric acid and 2 to 3 drops of dilute hydrochloric acid: no turbidity is produced.

(7) Alkaline earth metals and alkali metals—Boil 2.0 g of Bismuth Subnitrate with 40 mL of diluted acetic acid (31) (1 in 2) for 2 minutes, cool, add water to make 40 mL, and filter. To 20 mL of the filtrate add 2 mL of dilute hydrochloric acid, boil, immediately pass hydrogen sulfide thoroughly through the solution, filter, and wash the residue with water. Combine the filtrate and the washings, add 5 drops of sulfuric acid, evaporate to dryness, and ignite as directed under Residue on Ignition <*2.44*>: the residue is not exceed 5.0 mg

(8) Arsenic <*1.11*>—To 0.20 g of Bismuth Subnitrate add 2 mL of sulfuric acid, heat until white fumes evolve, dilute cautiously with water to 5 mL, use this solution as the test solution, and perform the test (not more than 10 ppm).

Loss on drying <*2.41*> Not more than 3.0% (2 g, 105°C, 2 hours).

Assay Weigh accurately about 0.4 g of Bismuth Subnitrate,

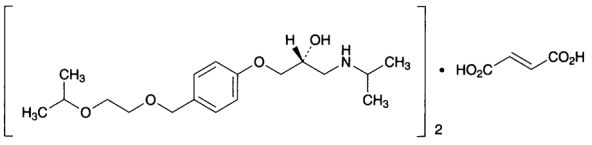
previously dried, dissolve in 5 mL of diluted nitric acid (2 in 5) by warming, and add water to make exactly 100 mL. Pipet 25 mL of the solution, add 200 mL of water and titrate ⟨*2.50*⟩ with 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to yellow (indicator: 5 drops of xylenol orange TS)

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 4.180 mg of Bi

Containers and storage Containers—Well-closed containers.

Bisoprolol Fumarate

ビソプロロールフマル酸塩



and enantiomer

$(C_{18}H_{31}NO_4)_2.C_4H_4O_4$: 766.96
(2*RS*)-1-(4-{[2-(1-Methylethoxy)ethoxy]methyl}phenoxy)-3-[(1-methylethyl)amino]propan-2-ol hemifumarate
[*104344-23-2*]

Bisoprolol Fumarate, when dried, contains not less than 98.5% and not more than 101.0% of bisoprolol fumarate $[(C_{18}H_{31}NO_4)_2.C_4H_4O_4]$.

Description Bisoprolol Fumarate occurs as white crystals or a white crystalline powder.

It is very soluble in water and in methanol, and freely soluble in ethanol (99.5) and in acetic acid (100).

A solution of Bisoprolol Fumarate (1 in 10) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Bisoprolol Fumarate in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bisoprolol Fumarate as directed in the potassium bromide disc method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 101 – 105°C

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Bisoprolol Fumarate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Bisoprolol Fumarate in 100 mL of a mixture of water and acetonitrile (4:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water and acetonitrile (4:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peaks other than bisoprolol obtained from the sample solution is not larger than 1/2 times the peak area of bisoprolol obtained from the standard solution. Furthermore, the total of the areas of peaks other than bisoprolol from the sample solution is not larger than the peak area of bisoprolol from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 4.08 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to pH 2.5 with phosphoric acid. To 800 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust so that the retention time of bisoprolol is about 8 minutes.

Time span of measurement: About 2 times as long as the retention time of bisoprolol, beginning after the fumaric acid peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add a mixture of water and acetonitrile (4:1) to make exactly 20 mL. Confirm that the peak area of bisoprolol obtained from 20 μL of this solution is equivalent to 7 to 13% of that obtained from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of bisoprolol are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of bisoprolol is not more than 1.5%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 80°C, 5 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Bisoprolol Fumarate, previously dried, dissolve in 70 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). The endpoint of titration is when the purple color of the solution turns blue and then blue-green. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 38.35 mg of $(C_{18}H_{31}NO_4)_2.C_4H_4O_4$

Containers and storage Containers—Tight containers.

Bisoprolol Fumarate Tablets

ビソプロロールフマル酸塩錠

Bisoprolol Fumarate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of bisoprolol fumarate [$(C_{18}H_{31}NO_4)_2.C_4H_4O_4$: 766.96].

Method of preparation Prepare as directed under Tablets, with Bisoprolol Fumarate.

Identification To a quantity of powdered Bisoprolol Fumarate Tablets, equivalent to 10 mg of Bisoprolol Fumarate, add 60 mL of methanol, shake vigorously for 10 minutes, add methanol to make 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 271 nm and 275 nm.

Purity Related substances—This is applied to 0.625-mg tablets. Shake vigorously for 10 minutes a portion of powdered Bisoprolol Fumarate Tablets, equivalent to 5 mg of Bisoprolol Fumarate, with exactly 20 mL of a mixture of water and acetonitrile (3:1), filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of the peak other than bisoprolol and the peak having the relative retention time of about 0.8 to bisoprolol by the area percentage method: the amount of the two peaks, having relative retention time of about 1.2 and about 3.8 to bisoprolol, are not more than 1.0%, respectively, the amount of the peak other than the peaks mentioned above is not more than 0.2%, and the total amount of the peaks other than bisoprolol is not more than 2.5%. For the area of the peak, having the relative retention time of about 1.2 to bisoprolol, multiply the relative response factor 5.

Operating conditions—

Detector, column, column temperature, and flow rate: Proceed as directed in the operating conditions in the Assay.

Mobile phase: Dissolve 4.08 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 2.5 with phosphoric acid. To 750 mL of this solution add 250 mL of acetonitrile.

Time span of measurement: About 5 times as long as the retention time of bisoprolol, beginning after the peak of fumaric acid.

System suitability—

Test for required detectability: To 1 mL of the sample solution add a mixture of water and acetonitrile (3:1) to make 100 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add a mixture of water and acetonitrile (3:1) to make exactly 20 mL. Confirm that the peak area of bisoprolol obtained with 20 μL of this solution is equivalent to 7 to 13% of that obtained with 20 μL of the solution for system suitability test.

System performance: When the procedure is run with 20 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of bisoprolol are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of bisoprolol is not more than 1.5%.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take 1 tablet of Bisoprolol Fumarate Tablets, disintegrate by adding 8 mL of water, and add water to make exactly 10 mL, and then filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 62.5 μg of bisoprolol fumarate [$(C_{18}H_{31}NO_4)_2.C_4H_4O_4$], and use this solution as the sample solution. Separately, weigh accurately about 20 mg of bisoprolol fumarate for assay, previously dried under reduced pressure at 80°C for 5 hours, using phosphorus (V) oxide as a dessicant, and dissolve in water to make exactly 200 mL. Pipet 15 mL of this solution, add water to make exactly 25 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 271.5 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of bisoprolol fumarate [$(C_{18}H_{31}NO_4)_2.C_4H_4O_4$]
$= M_S \times A_T/A_S \times V'/V \times 3/100$

M_S: Amount (mg) of bisoprolol fumarate for assay taken

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Bisoprolol Fumarate Tablets is not less than 85%.

Start the test with 1 tablet of Bisoprolol Fumarate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 0.7 μg of bisoprolol fumarate [$(C_{18}H_{31}NO_4)_2.C_4H_4O_4$], and use this solution as the sample solution. Separately, weigh accurately about 14 mg of bisoprolol fumarate for assay, previously dried in vacuum at 80°C for 5 hours, using phosphorus (V) oxide as a dessicant, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01>, and determine the peak areas, A_T and A_S, of bisoprolol in each solution.

Dissolution rate (%) with respect to the labeled amount of bisoprolol fumarate [$(C_{18}H_{31}NO_4)_2.C_4H_4O_4$]
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/2$

M_S: Amount (mg) of bisoprolol fumarate for assay taken
C: Labeled amount (mg) of bisoprolol fumarate [$(C_{18}H_{31}NO_4)_2.C_4H_4O_4$] in 1 tablet

Operating conditions—

Detector, column, column temperature, and flow rate: Proceed as directed in the operating conditions in the Assay.

Mobile phase: Dissolve 4.08 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to pH 2.5 with phosphoric acid. To 750 mL of this solution add 250 mL of acetonitrile.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of bisoprolol are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of bisoprolol is not more than 2.0%.

Assay Weigh accurately not less than 20 Bisoprolol Fumarate Tablets and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of bisoprolol fumarate [$(C_{18}H_{31}NO_4)_2.C_4H_4O_4$], add 70 mL of a mixture of water and acetonitrile (3:1) and exactly 10 mL of the internal standard solution, shake vigorously for 10 minutes, and add the mixture of water and acetonitrile (3:1) to make 100 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of bisoprolol fumarate for assay, previously dried in vacuum at 80°C for 5 hours using phosphorus (V) oxide as the dessicant, add exactly 10 mL of the internal standard solution, dissolve in the mixture of water and acetonitrile (3:1) to make 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of bisoprolol to that of the internal standard.

Amount (mg) of bisoprolol fumarate [$(C_{18}H_{31}NO_4)_2.C_4H_4O_4$]
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of bisoprolol fumarate for assay taken

Internal standard solution—A solution of isopropyl parahydroxybenzoate in the mixture of water and acetonitrile (3:1) (1 in 250).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 4.08 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to pH 2.5 with phosphoric acid. To 800 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust so that the retention time of bisoprolol is about 8 minutes.

System suitability—

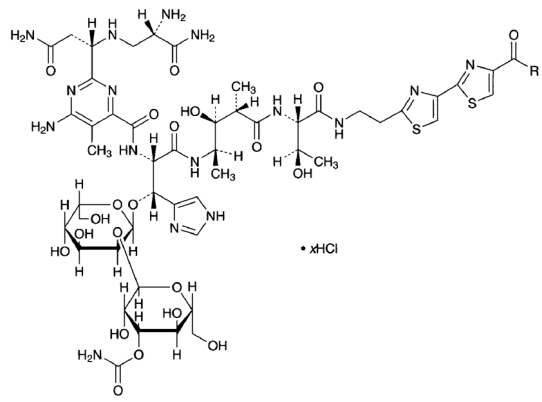
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, fumaric acid, bisoprolol and the internal standard are eluted in this order with the resolution between the peaks of bisoprolol and the internal standard being not less than 12.

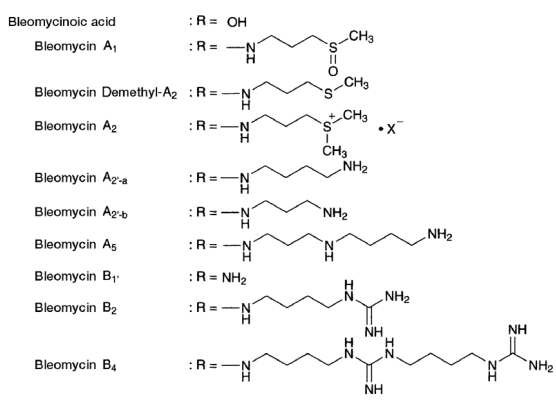
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of bisoprolol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Bleomycin Hydrochloride

ブレオマイシン塩酸塩





Bleomycinoic Acid
1-Bleomycinoic acid hydrochloride
Bleomycin A_1
N^1-[3-(Methylsulfinyl)propyl]bleomycinamide hydrochloride
Bleomycin Demethyl-A_2
N^1-[3-(Methylsulfanyl)propyl]bleomycinamide hydrochloride
Bleomycin A_2
N^1-[3-(Dimethylsulfonio)propyl]bleomycinamide hydrochloride
Bleomycin $A_{2'\text{-a}}$
N^1-(4-Aminobutyl)bleomycinamide hydrochloride
Bleomycin $A_{2'\text{-b}}$
N^1-(3-Aminopropyl)bleomycinamide hydrochloride
Bleomycin A_5
N^1-{3-[(4-Aminobutyl)amino]propyl}bleomycinamide hydrochloride
Bleomycin $B_{1'}$
Bleomycinamide hydrochloride
Bleomycin B_2
N^1-(4-Guanidinobutyl)bleomycinamide hydrochloride
Bleomycin B_4
N^1-{4-[3-(4-Guanidinobutyl)guanidino]butyl}-bleomycinamide hydrochloride
[*11056-06-7*, Bleomycin]

Bleomycin Hydrochloride is the hydrochloride of a mixture of substances having antitumor activity produced by the growth of *Streptomyces verticillus*.

It contains not less than 1400 μg (potency) and not more than 2000 μg (potency) per mg, calculated on

the dried basis. The potency of Bleomycin Hydrochloride is expressed as mass (potency) of bleomycin A_2 ($C_{55}H_{84}ClN_{17}O_{21}S_3$: 1451.00).

Description Bleomycin Hydrochloride occurs as a white to yellowish white powder.

It is freely soluble in water, and slightly soluble in ethanol (95).

It is hygroscopic.

Identification **(1)** To 4 mg of Bleomycin Hydrochloride add 5 μL of copper (II) sulfate TS, and dissolve in water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bleomycin Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Bleomycin Hydrochloride (1 in 100) responds to the Qualitative Tests <*1.09*> (2) for chloride.

pH <*2.54*> The pH of a solution obtained by dissolving 0.10 g of Bleomycin Hydrochloride in 20 mL of water is between 4.5 and 6.0.

Content ratio of the active principle Dissolve 10 mg of Bleomycin Hydrochloride in 20 mL of water, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method, and calculate their amounts by the area percentage method: the amount of the peak of bleomycin A_2 (the first principal peak) is between 55% and 70%, that of bleomycin B_2 (the second principal peak) is between 25% and 32%, the total amount of the peak of bleomycin A_2 and bleomycin B_2 is not less than 85%, the amount of the peak of demethylbleomycin A_2 (a peak having the relative retention time of 1.5 – 2.5 to bleomycin A_2) is not more than 5.5%, and the total amount of the rest peaks is not more than 9.5%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase stock solution: Dissolve 0.96 g of sodium 1-pentanesulfonate and 1.86 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 1000 mL of water and 5 mL of acetic acid (100), and adjust the pH to 4.3 with ammonia TS.

Mobile phase A: A mixture of the mobile phase stock solution and methanol (9:1).

Mobile phase B: A mixture of the mobile phase stock solution and methanol (3:2).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 60	100 → 0	0 → 100
60 – 75	0	100

Flow rate: About 1.2 mL per minute.

Time span of measurement: 20 minutes after elution of the peak of demethylbreomycin A_2, beginning after the solvent peak.

System suitability—

System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, bleomycin A_2 and bleomycin B_2 are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of bleomycin A_2 is not more than 2.0%.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 80 mg of Bleomycin Hydrochloride in 4 mL of water is clear and colorless.

(2) Copper—Dissolve exactly 75 mg of Bleomycin Hydrochloride in exactly 10 mL of diluted nitric acid (1 in 100), and use this solution as the sample solution. Separately, to exactly 15 mL of Standard Copper Solution add diluted nitric acid (1 in 100) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions: the absorbance of the sample solution is not more than that of the standard solution (not more than 200 ppm).

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Copper hollow-cathode lamp.
Wavelength: 324.8 nm.

Loss on drying <*2.41*> Not more than 5.0% (60 mg, in vacuum, phosphorus (V) oxide, 60°C, 3 hours. Take the sample to be tested while avoiding moisture absorption).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism—*Mycobacterium smegmatis* ATCC 607

(ii) Agar medium for seed, base layer and transferring the test organism

Glycerin	10.0 g
Peptone	10.0 g
Meat extract	10.0 g
Sodium chloride	3.0 g
Agar	15.0 g
Water	1000 mL

Mix all the components, and sterilize. Adjust the pH after sterilization to 6.9 – 7.1 with sodium hydroxide TS.

(iii) Liquid media for suspending the test organism

Glycerin	10.0 g
Peptone	10.0 g
Meat extract	10.0 g
Sodium chloride	3.0 g
Water	1000 mL

Mix all the components, and sterilize. Adjust the pH after sterilization to 6.9 – 7.1 with sodium hydroxide TS.

(iv) Preparation of seeded agar layer—Cultivate the test

organism on the slant of the agar medium for transferring the test organism at 27°C for 40 to 48 hours, then inoculate the test organism thus obtained in 100 mL of the liquid media for suspending the test organism, cultivate with shaking at between 25°C and 27°C for 5 days, and use this as the suspension of test organism. Store the suspension of test organism at a temperature not exceeding 5°C, and use within 14 days. Add 0.5 mL of the suspension of test organism in 100 mL of the agar medium for seed previously kept at 48°C, mix thoroughly, and use as the seeded agar layer.

(v) Preparation of cylinder-agar plate—Proceed as directed in 1.7. Preparation of cylinder-agar plates under the Microbial Assay for Antibiotics, dispensing 5.0 mL of agar medium for base layer and 8.0 mL of the agar medium for seed into the Petri dish.

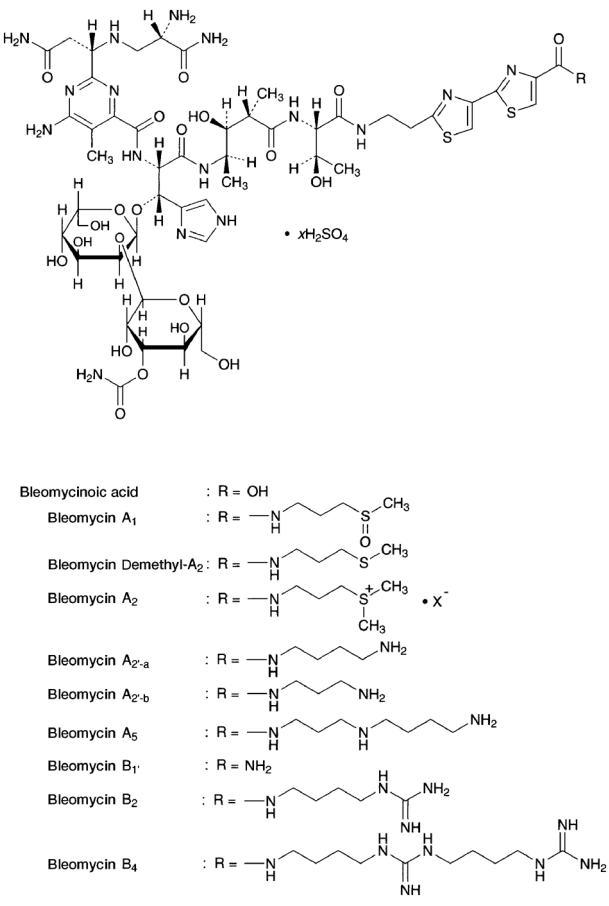
(vi) Standard solutions—Weigh accurately an amount of Bleomycin A_2 Hydrochloride RS, previously dried under reduced pressure not exceeding 0.67 kPa at an ordinary temperature for 3 hours, equivalent to about 15 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution (pH 6.8) to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution (pH 6.8) to make solutions so that each mL contains 30 μg (potency) and 15 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(vii) Sample solutions—Weigh accurately an amount of Bleomycin Hydrochloride, equivalent to about 15 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution (pH 6.8) to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution (pH 6.8) to make solutions so that each mL contains 30 μg (potency) and 15 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Bleomycin Sulfate

ブレオマイシン硫酸塩



Bleomycinoic Acid
1-Bleomycinoic acid sulfate
Bleomycin A_1
N^1-[3-(Methylsulfinyl)propyl]bleomycinamide sulfate
Bleomycin Demethyl-A_2
N^1-[3-(Methylsulfanyl)propyl]bleomycinamide sulfate
Bleomycin A_2
N^1-[3-(Dimethylsulfonium)propyl]bleomycinamide sulfate
Bleomycin $A_{2'\text{-a}}$
N^1-(4-Aminobutyl)bleomycinamide sulfate
Bleomycin $A_{2'\text{-b}}$
N^1-(3-Aminopropyl)bleomycinamide sulfate
Bleomycin A_5
N^1-{3-[(4-Aminobutyl)amino]propyl}bleomycinamide sulfate
Bleomycin B_1'
Bleomycinamide sulfate
Bleomycin B_2
N^1-(4-Guanidinobutyl)bleomycinamide sulfate
Bleomycin B_4
N^1-{4-[3-(4-Guanidinobutyl)guanidino]butyl}-bleomycinamide sulfate
[*9041-93-4*, Bleomycin Sulfate]

Bleomycin Sulfate is the sulfate of a mixture of substances having antitumor activity produced by the growth of *Streptomyces verticillus*.

It contains not less than 1400 μg (potency) and not more than 2000 μg (potency) per mg, calculated on the dried basis. The potency of Bleomycin Sulfate

is expressed as mass (potency) of bleomycin A_2 ($C_{55}H_{84}ClN_{17}O_{21}S_3$: 1451.00).

Description Bleomycin Sulfate occurs as a white to yellowish white powder.

It is freely soluble in water, and slightly soluble in ethanol (95).

It is hygroscopic.

Identification **(1)** To 4 mg of Bleomycin Sulfate add 5 μL of copper (II) sulfate TS, and dissolve in water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bleomycin Sulfate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Bleomycin Sulfate (1 in 200) responds to the Qualitative Tests <*1.09*> (1) and (2) for sulfate.

pH <*2.54*> The pH of a solution obtained by dissolving 10 mg of Bleomycin Sulfate in 20 mL of water is between 4.5 and 6.0.

Content ratio of the active principle Dissolve 10 mg of Bleomycin Sulfate in 20 mL of water, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method, and calculate their amounts by the area percentage method: the amount of the peak of bleomycin A_2 (the first principal peak) is between 55% and 70%, that of bleomycin B_2 (the second principal peak) is between 25% and 32%, the total amount of the peak of bleomycin A_2 and bleomycin B_2 is not less than 85%, the amount of the peak of demethylbleomycin A_2 (a peak having the relative retention time of 1.5 – 2.5 to bleomycin A_2) is not more than 5.5%, and the total amount of the rest peaks is not more than 9.5%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase stock solution: Dissolve 0.96 g of sodium 1-pentanesulfonate and 1.86 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 1000 mL of water and 5 mL of acetic acid (100), and adjust the pH to 4.3 with ammonia TS.

Mobile phase A: A mixture of the mobile phase stock solution and methanol (9:1).

Mobile phase B: A mixture of the mobile phase stock solution and methanol (3:2).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 60	100 → 0	0 → 100
60 – 75	0	100

Flow rate: About 1.2 mL per minute.

Time span of measurement: Twenty minutes after elution of the peak of demethylbleomycin A_2, beginning after the solvent peak.

System suitability—

System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, bleomycin A_2 and bleomycin B_2 are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of bleomycin A_2 is not more than 2.0%.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 80 mg of Bleomycin Sulfate in 4 mL of water is clear and colorless.

(2) Copper—Dissolve exactly 75 mg of Bleomycin Sulfate in 10 mL of diluted nitric acid (1 in 100), and use this solution as the sample solution. Separately, to exactly 15 mL of Standard Copper Solution add diluted nitric acid (1 in 100) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions: the absorbance of the sample solution is not more than that of the standard solution (not more than 200 ppm).

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Copper hollow-cathode lamp.
Wavelength: 324.8 nm.

Loss on drying <*2.41*> Not more than 3.0% (60 mg, in vacuum, phosphorus (V) oxide, 60°C, 3 hours. Take the sample to be tested while avoiding moisture absorption).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism—*Mycrobacterium smegmatis* ATCC 607

(ii) Agar medium for seed, base layer and transferring the test organism

Glycerin	10.0 g
Peptone	10.0 g
Meat extract	10.0 g
Sodium chloride	3.0 g
Agar	15.0 g
Water	1000 mL

Mix all the components and sterilize. Adjust the pH after sterilization to 6.9 – 7.1 with sodium hydroxide TS.

(iii) Liquid media for suspending the test organism

Glycerin	10.0 g
Peptone	10.0 g
Meat extract	10.0 g
Sodium chloride	3.0 g
Water	1000 mL

Mix all the components and sterilize. Adjust the pH after sterilization to 6.9 – 7.1 with sodium hydroxide TS.

(iv) Preparation of seeded agar layer—Cultivate the test organism on the slant of the agar medium for transferring

the test organism at 27°C for 40 to 48 hours, then inoculate the test organism thus obtained in 100 mL of the liquid media for suspending the test organism, cultivate with shaking at between 25°C and 27°C for 5 days, and use this as the suspension of test organism. Store the suspension of test organism at a temperature not exceeding 5°C, and use within 14 days. Add 0.5 mL of the suspension of test organism in 100 mL of the agar medium for seed previously kept at 48°C, mix thoroughly, and use as the seeded agar layer.

(v) Preparation of cylinder-agar plate—Proceed as directed in 1.7. Preparation of cylinder-agar plates under the Microbial Assay for Antibiotics, dispensing 5.0 mL of agar medium for base layer and 8.0 mL of the agar medium for seed into the Petri dish.

(vi) Standard solutions—Weigh accurately an amount of Bleomycin A_2 Hydrochloride RS, previously dried under reduced pressure not exceeding 0.67 kPa at an ordinary temperature for 3 hours, equivalent to about 15 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution (pH 6.8) to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution (pH 6.8) to make solutions so that each mL contains 30 μg (potency) and 15 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(vii) Sample solutions—Weigh accurately an amount of Bleomycin Sulfate, equivalent to about 15 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution (pH 6.8) to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution (pH 6.8) to make solutions so that each mL contains 30 μg (potency) and 15 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Boric Acid

ホウ酸

H_3BO_3: 61.83

Boric Acid, when dried, contains not less than 99.5% of boric acid (H_3BO_3).

Description Boric Acid occurs as colorless or white, crystals or crystalline powder. It is odorless, and has a slight, characteristic taste.

It is freely soluble in warm water, in hot ethanol (95) and in glycerin, soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of 1.0 g of Boric Acid in 20 mL of water is between 3.5 and 4.1.

Identification A solution of Boric Acid (1 in 20) responds to the Qualitative Tests <*1.09*> for borate.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Boric Acid in 25 mL of water or in 10 mL of hot ethanol (95): the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Boric Acid according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Boric Acid according to Method 1, and perform the test (not more than 5 ppm).

Loss on drying <*2.41*> Not more than 0.5% (2 g, silica gel, 5 hours).

Assay Weigh accurately about 1.5 g of Boric Acid, previously dried, add 15 g of D-sorbitol and 50 mL of water, and dissolve by warming. After cooling, titrate <*2.50*> with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS
= 61.83 mg of H_3BO_3

Containers and storage Containers—Well-closed containers.

Freeze-dried Botulism Antitoxin, Equine

乾燥ボツリヌスウマ抗毒素

Freeze-dried Botulism Antitoxin, Equine, is a preparation for injection which is dissolved before use.

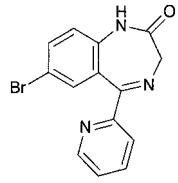
It contains botulism antitoxin type A, botulism antitoxin type B, botulism antitoxin type E and botulism antitoxin type F in immunoglobulin of horse origin. It may contain one, two or three of these four antitoxins.

It conforms to the requirements of Freeze-dried Botulism Antitoxin, Equine, in the Minimum Requirements for Biological Products.

Description Freeze-dried Botulism Antitoxin, Equine, becomes a colorless or yellow-brown, clear liquid or a slightly white-turbid liquid on the addition of solvent.

Bromazepam

ブロマゼパム



$C_{14}H_{10}BrN_3O$: 316.15
7-Bromo-5-(pyridin-2-yl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one
[*1812-30-2*]

Bromazepam, when dried, contains not less than 99.0% and not more than 101.0% of bromazepam ($C_{14}H_{10}BrN_3O$).

Description Bromazepam occurs as white to light yellowish white, crystals or crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in methanol, in ethanol (99.5) and in acetone, and practically insoluble in water.

Melting point: about 245°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Bromazepam in ethanol (99.5) (1 in 200,000) as

directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bromazepam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals <1.07>—Proceed with 1.0 g of Bromazepam in a platinum crucible according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Bromazepam in 5 mL of a mixture of acetone and methanol (3:2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mixture of acetone and methanol (3:2) to make exactly 50 mL. Pipet 5 mL of this solution, add the mixture of acetone and methanol (3:2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ammonia solution (28) and ethanol (99.5) (38:1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution and the spot of the starting point are not more than 2, and not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.20% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

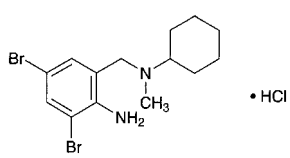
Assay Weigh accurately about 0.4 g of Bromazepam, previously dried, dissolve in 80 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 31.62 mg of $C_{14}H_{10}BrN_3O$

Containers and storage Containers—Well-closed containers.

Bromhexine Hydrochloride

ブロムヘキシン塩酸塩



$C_{14}H_{20}Br_2N_2.HCl$: 412.59
2-Amino-3,5-dibromo-*N*-cyclohexyl-*N*-methylbenzylamine monohydrochloride
[*611-75-6*]

Bromhexine Hydrochloride, when dried, contains not less than 98.5% of bromhexine hydrochloride ($C_{14}H_{20}Br_2N_2.HCl$).

Description Bromhexine Hydrochloride occurs as white, crystals or crystalline powder.

It is freely soluble in formic acid, sparingly soluble in methanol, and slightly soluble in water and in ethanol (95).

The pH of its saturated solution is between 3.0 and 5.0.

Melting point: about 239°C (with decomposition).

Identification **(1)** Dissolve 3 mg of Bromhexine Hydrochloride in 0.01 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bromhexine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Add 20 mL of water to 1 g of Bromhexine Hydrochloride. After thorough shaking, add 3 mL of sodium hydroxide TS, and extract with four 20-mL portions of diethyl ether. Neutralize the water layer with dilute nitric acid: the solution responds to the Qualitative Tests <1.09> (2) for chloride.

Purity **(1)** Heavy metals <1.07>—Proceed with 2.0 g of Bromhexine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 50 mg of Bromhexine Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 20 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: each peak area other than bromhexine obtained with the sample solution is not larger than the peak area of bromhexine obtained with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Column: A stainless steel column about 5 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of potassium dihydrogen phosphate in 900 mL of water, adjust the pH to 7.0 with 0.5 mol/L sodium hydroxide TS, and add water to make 1000 mL. To 200 mL of this solution add 800 mL of acetonitrile.

Flow rate: Adjust so that the retention time of bromhexine is about 6 minutes.

Selection of column: To 0.05 g of bamethane sulfate add 0.5 mL of the sample solution, and add the mobile phase to make 10 mL. Proceed with 5 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of bamethane and bromhexine in this order with the resolution between these peaks being not less than 7.

Detection sensitivity: Adjust the detection sensitivity so

that the peak height of bromhexine from 5 μL of the standard solution is between 5 mm and 15 mm.

Time span of measurement: About 2 times as long as the retention time of bromhexine, beginning after the solvent peak.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Bromhexine Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add 60 mL of acetic anhydride, and warm in a water bath at 50°C for 15 minutes. After cooling, titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-green to yellow-green (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

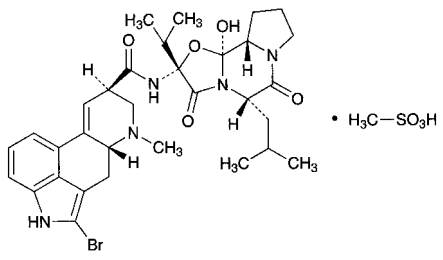
Each mL of 0.1 mol/L perchloric acid VS
= 41.26 mg of $C_{14}H_{20}Br_2N_2.HCl$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Bromocriptine Mesilate

ブロモクリプチンメシル酸塩



$C_{32}H_{40}BrN_5O_5.CH_4O_3S$: 750.70
(5′*S*)-2-Bromo-12′-hydroxy-2′-(1-methylethyl)-5′-(2-methylpropyl)ergotaman-3′,6′,18-trione monomethanesulfonate
[*22260-51-1*]

Bromocriptine Mesilate contains not less than 98.0% of bromocriptine mesilate ($C_{32}H_{40}BrN_5O_5.CH_4O_3S$), calculated on the dried basis.

Description Bromocriptine Mesilate occurs as a white to pale yellowish white or pale brownish white crystalline powder. It is odorless, or has a faint characteristic odor.

It is very soluble in acetic acid (100), freely soluble in methanol, sparingly soluble in ethanol (95), very slightly soluble in acetic anhydride, in dichloromethane and in chloroform, and practically insoluble in water and in diethyl ether.

It is gradually colored by light.

Identification **(1)** Dissolve 2 mg of Bromocriptine Mesilate in 1 mL of methanol, add 2 mL of 4-dimethylaminobenzaldehyde-ferric chloride TS, and shake: a purplish blue color develops.

(2) Determine the absorption spectrum of a solution of Bromocriptine Mesilate in methanol (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Bromocriptine Mesilate as directed in the paste method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Perform the test with Bromocriptine Mesilate as directed under Flame Coloration Test ⟨*1.04*⟩ (2): a green color appears.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +95 – +105° [0.1 g, calculated on the dried basis, a mixture of methanol and dichloromethane (1:1), 10 mL, 100 mm].

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Bromocriptine Mesilate in 10 mL of methanol: the solution is clear, and has no more color than the following control solution.

Control solution: To 2.5 mL of Cobalt (II) Chloride CS, 6.0 mL of Iron (III) Chloride CS and 1.0 mL of Copper (II) Sulfate CS add diluted hydrochloric acid (1 in 40) to make exactly 100 mL.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Bromocriptine Mesilate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.10 g of Bromocriptine Mesilate in 10 mL of a mixture of methanol and chloroform (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and chloroform (1:1) to make exactly 200 mL, and use this solution as the standard solution (1). Pipet 10 mL of the standard solution (1), add a mixture of methanol and chloroform (1:1) to make exactly 20 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solutions (1) and (2), as a band with 1 cm in width, on a plate of silica gel for thin-layer chromatography. Develop the plate immediately with a mixture of dichloromethane, 1,4-dioxane, ethanol (95) and ammonia solution (28) (1800:150:50:1) to a distance of about 10 cm, and dry the plate under reduced pressure for 30 minutes. Spray evenly Dragendorff's TS for spraying on the plate, then spray evenly hydrogen peroxide TS, cover the plate with a glass plate, and examine: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (1), and the spot other than the principal spot, which is more intense than the spot from the standard solution (2), is not more than one.

Loss on drying ⟨*2.41*⟩ Not more than 3.0% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, 80°C, 5 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Bromocriptine Mesilate, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (7:1), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

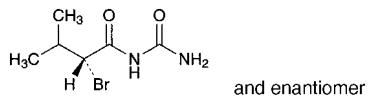
Each mL of 0.1 mol/L perchloric acid VS
= 75.07 mg of $C_{32}H_{40}BrN_5O_5.CH_4O_3S$

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and not exceeding −18°C.

Bromovalerylurea

ブロモバレリル尿素



and enantiomer

$C_6H_{11}BrN_2O_2$: 223.07
(2*RS*)-(2-Bromo-3-methylbutanoyl)urea
[*496-67-3*]

Bromovalerylurea, when dried, contains not less than 98.0% of bromovalerylurea ($C_6H_{11}BrN_2O_2$).

Description Bromovalerylurea occurs as colorless or white, crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is soluble in ethanol (95), sparingly soluble in diethyl ether, and very slightly soluble in water.

It dissolves in sulfuric acid, in nitric acid and in hydrochloric acid, and precipitates are produced on the addition of water.

It dissolves in sodium hydroxide TS.

Identification **(1)** Boil 0.2 g of Bromovalerylurea with 5 mL of a solution of sodium hydroxide (1 in 10): the gas evolved changes moistened red litmus paper to blue. Boil this solution with an excess of dilute sulfuric acid: the odor of valeric acid is perceptible.

(2) To 0.1 g of Bromovalerylurea add 0.5 g of anhydrous sodium carbonate, and decompose thoroughly by gentle heating. Dissolve the residue in 5 mL of hot water, cool, acidify with acetic acid (31), and filter: the filtrate responds to the Qualitative Tests <*1.09*> (2) for bromide.

Melting point <*2.60*> 151 – 155°C

Purity **(1)** Acidity or alkalinity—To 1.5 g of Bromovalerylurea add 30 mL of water, shake for 5 minutes, and filter: the filtrate is neutral.

(2) Chloride <*1.03*>—Perform the test with a 10-mL portion of the filtrate obtained in (1). Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.028%).

(3) Sulfate <*1.14*>—Perform the test with 10 mL of the filtrate obtained in (1). Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(4) Heavy metals <*1.07*>—Proceed with 2.0 g of Bromovalerylurea according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic <*1.11*>—Dissolve 0.5 g of Bromovalerylurea in 5 mL of sodium hydroxide TS, use this solution as the test solution, and perform the test (not more than 4 ppm).

(6) Readily carbonizable substances <*1.15*>—Perform the test with 0.5 g of Bromovalerylurea: the solution is not more colored than Matching Fluid A.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 80°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

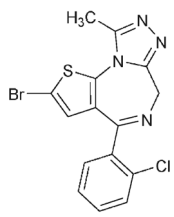
Assay Weigh accurately about 0.4 g of Bromovalerylurea, previously dried, in a 300-mL conical flask, add 40 mL of sodium hydroxide TS, and boil gently for 20 minutes under a reflux condenser. Cool, wash the lower part of the reflux condenser and the mouth of the flask with 30 mL of water, and combine the washings with the solution in the conical flask. Add 5 mL of nitric acid and exactly 30 mL of 0.1 mol/L silver nitrate VS, and titrate <*2.50*> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L silver nitrate VS
= 22.31 mg of $C_6H_{11}BrN_2O_2$

Containers and storage Containers—Well-closed containers.

Brotizolam

ブロチゾラム



$C_{15}H_{10}BrClN_4S$: 393.69
2-Bromo-4-(2-chlorophenyl)-9-methyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine
[*57801-81-7*]

Brotizolam, when dried, contains not less than 98.5% and not more than 101.0% of brotizolam ($C_{15}H_{10}BrClN_4S$).

Description Brotizolam occurs as a white or pale yellowish crystalline powder.

It is sparingly soluble in methanol, slightly soluble in acetonitrile and in ethanol (99.5), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Brotizolam in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Brotizolam as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 208 – 212°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Brotizolam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Brotizolam in 50 mL of acetonitrile, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add acetonitrile to make exactly 100 mL. Pipet 1 mL of this solution, add acetonitrile to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: each peak area other than

brotizolam from the sample solution is not larger than 1/2 times the peak area of brotizolam from the standard solution, and the total area of the peaks other than the peak of brotizolam from the sample solution is not larger than the peak area of brotizolam from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 242 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: Dissolve 1.84 g of sodium 1-heptanesulfonate in 1000 mL of water.

Mobile phase B: Dissolve 0.46 g of sodium 1-heptanesulfonate in 250 mL of water and 750 mL of acetonitrile.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 4	63	37
4 – 15	63 → 12	37 → 88

Flow rate: About 2 mL per minute.

Time span of measurement: About 2 times as long as the retention time of brotizolam, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of brotizolam obtained with 5 μL of this solution is equivalent to 18 to 32% of that with 5 μL of the standard solution.

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of brotizolam are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of brotizolam is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.15 g of Brotizolam, previously dried, dissolve in 75 mL of a mixture of acetic anhydride and acetic acid (100) (2:1), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 19.68 mg of $C_{15}H_{10}BrClN_4S$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Brotizolam Tablets

ブロチゾラム錠

Brotizolam Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of brotizolam ($C_{15}H_{10}BrClN_4S$: 393.69).

Method of preparation Prepare as directed under Tablets, with Brotizolam.

Identification Shake a quantity of powdered Brotizolam Tablets, equivalent to 0.1 mg of Brotizolam, with 10 mL of methanol, and centrifuge. Determine the absorption spectrum of the supernatant liquid as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 239 nm and 243 nm.

Purity Related substances—Use the sample solution obtained in the Assay as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than brotizolam obtained from the sample solution is not larger than 1.5 times the peak area of brotizolam obtained from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of brotizolam, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 10 mL of the standard solution add the mobile phase to make exactly 100 mL. Confirm that the peak area of brotizolam obtained with 40 μL of this solution is equivalent to 7 to 13% of that obtained with 40 μL of the standard solution.

System performance: When the procedure is run with 40 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of brotizolam are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of brotizolam is not more than 2.0%.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Brotizolam Tablets add exactly V mL of the mobile phase so that each mL contains about 25 μg of brotizolam ($C_{15}H_{10}BrClN_4S$), shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of brotizolam ($C_{15}H_{10}BrClN_4S$)
$= M_S \times A_T/A_S \times V/1000$

M_S: Amount (mg) of brotizolam for assay taken

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate

in 15 minutes of Brotizolam Tablets is not less than 85%.

Start the test with 1 tablet of Brotizolam Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 0.14 μg of brotizolam ($C_{15}H_{10}BrClN_4S$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of brotizolam for assay, previously dried at 105°C for 3 hours, dissolve in 10 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 200 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of brotizolam in each solution.

Dissolution rate (%) with respect to the labeled amount of brotizolam ($C_{15}H_{10}BrClN_4S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/20$

M_S: Amount (mg) of brotizolam for assay taken
C: Labeled amount (mg) of brotizolam ($C_{15}H_{10}BrClN_4S$) in 1 tablet

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: A mixture of water and acetonitrile (63:37).

Flow rate: Adjust so that the retention time of brotizolam is about 7 minutes.

System suitability—

System performance: When the procedure is run with 200 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of brotizolam are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 200 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of brotizolam is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Brotizolam Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.25 mg of brotizolam ($C_{15}H_{10}BrClN_4S$), add exactly 10 mL of the mobile phase, and shake for 15 minutes. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of brotizolam for assay, previously dried at 105°C for 3 hours, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of brotizolam in each solution.

Amount (mg) of brotizolam ($C_{15}H_{10}BrClN_4S$)
$= M_S \times A_T/A_S \times 1/100$

M_S: Amount (mg) of brotizolam for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 1.1 g of ammonium carbonate in 1000 mL of water. To 600 mL of this solution add 500 mL of acetonitrile.

Flow rate: Adjust so that the retention time of brotizolam is about 3 minutes.

System suitability—

System performance: When the procedure is run with 40 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of brotizolam are not less than 3000 and not more than 2.0, respectively.

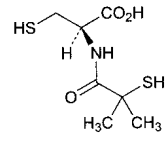
System repeatability: When the test is repeated 6 times with 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of brotizolam is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Bucillamine

ブシラミン



$C_7H_{13}NO_3S_2$: 223.31
(2*R*)-2-(2-Methyl-2-sulfanylpropanoylamino)-3-sulfanylpropanoic acid
[*65002-17-7*]

Bucillamine, when dried, contains not less than 98.5% and not more than 101.0% of bucillamine ($C_7H_{13}NO_3S_2$).

Description Bucillamine occurs as white, crystals or crystalline powder.

It is freely soluble in methanol and in ethanol (95), and slightly soluble in water.

Identification **(1)** To 5 mL of a solution of Bucillamine (1 in 250) add 2 mL of sodium hydroxide TS and 2 drops of sodium pentacyanonitrosylferrate (III) TS: the solution reveals a red-purple color.

(2) Determine the infrared absorption spectrum of Bucillamine as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +33.0 – +36.5° (after drying, 2 g, ethanol (95), 50 mL, 100 mm).

Melting point <*2.60*> 136 – 140°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Bucillamine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Bucillamine according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 60 mg of Bucillamine

in 20 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mixture of water and methanol (1:1) to make exactly 200 mL, and use this solution as the standard solution. Immediately perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of related substances, having the relative retention time of about 2.3 and about 3.1 to bucillamine, obtained from the sample solution are not larger than 8/15 times and 2/5 times the peak area of bucillamine obtained from the standard solution, respectively, and the area of the peak other than the bucillamine and the peaks mentioned above from the sample solution is not larger than 1/5 times the peak area of bucillamine from the standard solution. The total area of the peaks other than bucillamine from the sample solution is not larger than the peak area of bucillamine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 6.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.01 mol/L citric acid TS and methanol (1:1).

Flow rate: Adjust so that the retention time of bucillamine is about 5 minutes.

Time span of measurement: About 7 times as long as the retention time of bucillamine, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mixture of water and methanol (1:1) to make exactly 10 mL. Confirm that the peak area of bucillamine obtained with 20 μL of this solution is equivalent to 7 to 13% of that obtained with 20 μL of the standard solution.

System performance: Dissolve 0.10 g of bucillamine and 10 mg of 4-fluorobenzoic acid in 100 mL of methanol. To 10 mL of this solution add water to make exactly 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, bucillamine and 4-fluorobenzoic acid are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of bucillamine is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 6 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of Bucillamine, dissolve in 35 mL of methanol, add 15 mL of water, and titrate <*2.50*> with 0.05 mol/L iodine VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L iodine VS
= 11.17 mg of $C_7H_{13}NO_3S_2$

Containers and storage Containers—Tight containers.

Bucillamine Tablets

ブシラミン錠

Bucillamine Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of bucillamine ($C_7H_{13}NO_3S_2$: 223.31).

Method of preparation Prepare as directed under Tablets, with Bucillamine.

Identification **(1)** To a quantity of powdered Bucillamine Tablets, equivalent to 0.1 g of Bucillamine, add 0.1 g of sodium hydrogen carbonate and 10 mL of water, shake well, filter, and add 1 or 2 drops of ninhydrin TS to the filtrate: it exhibits a red-brown color.

(2) To a quantity of powdered Bucillamine Tablets, equivalent to 0.1 g of Bucillamine, add 25 mL of water, shake well, and filter. To 5 mL of the filtrate, add 2 mL of dilute sodium hydroxide TS and 1 or 2 drops of sodium pentacyanonitrosylferrate (III) TS: it exhibits a red-purple color.

Uniformity of dosage units <*6.02*>—Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

Store the sample solution and standard solution in a cold place until performing the measurements. Take 1 tablet of Bucillamine Tablets, add exactly 1 mL of the internal standard solution per 0.1 g of bucillamine ($C_7H_{13}NO_3S_2$), then add 3 mL of water and 6 mL of methanol per 0.1 g of bucillamine ($C_7H_{13}NO_3S_2$), and stir well until the tablet completely disintegrated. To 1 mL of this solution add the mobile phase to make 25 mL, filter through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of bucillamine ($C_7H_{13}NO_3S_2$)
$= M_S \times Q_T/Q_S \times C \times 1/200$

M_S: Amount (mg) of bucillamine for assay taken
C: Labeled amount (mg) of bucillamine ($C_7H_{13}NO_3S_2$) in 1 tablet

Internal standard solution—A solution of 4-fluorobenzoic acid in methanol (1 in 100).

Dissolution <*6.10*>—When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Bucillamine Tablets is not less than 80%.

Store the sample solution and standard solution in a cold place until performing the measurements. Start the test with 1 tablet of Bucillamine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of bucillamine for assay equivalent to the labeled amount of the tablet, previously dried in vacuum at 60°C for 6 hours using phosphorus (V) oxide as a dessicant, and dissolve in methanol to make exactly 10 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of bucillamine in each solution.

Dissolution rate (%) with respect to the labeled amount of bucillamine ($C_7H_{13}NO_3S_2$)
$= M_S \times A_T/A_S \times 1/C \times 90$

M_S: Amount (mg) of bucillamine for assay taken
C: Labeled amount (mg) of bucillamine ($C_7H_{13}NO_3S_2$) in 1 tablet

Operating conditions—
Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.
Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and methanol (11:9).
Flow rate: Adjust so that the retention time of bucillamine is about 4 minutes.
System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of bucillamine are not less than 3000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of bucillamine is not more than 2.0%.

Assay Store the sample solution and standard solution in a cold place until performing the measurements. Take 10 tablets of Bucillamine Tablets, add exactly 1 mL of the internal standard solution per 0.1 g of bucillamine ($C_7H_{13}NO_3S_2$), add 3 mL of water and 6 mL of methanol, and stir well until the tablets completely disintegrated. To 1 mL of this solution add the mobile phase to make 25 mL, filter through a membrane filter with a pore size not exceeding 0.45 μm, and use this solution as the sample solution. Separately, weigh accurately about 0.2 g of bucillamine for assay, previously dried in vacuum at 60°C for 6 hours using phosphorus (V) oxide as a dessicant, add exactly 2 mL of the internal standard solution, and add 6 mL of water and 12 mL of methanol. To 1 mL of this solution add the mobile phase to make 25 mL, filter through a membrane filter with a pore size not exceeding 0.45 μm, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of bucillamine to that of the internal standard.

Amount (mg) of bucillamine ($C_7H_{13}NO_3S_2$)
$= M_S \times Q_T/Q_S \times C \times 1/200$

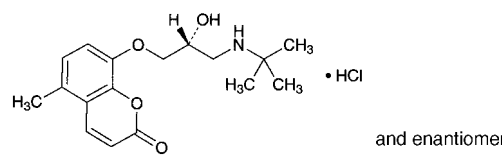
M_S: Amount (mg) of bucillamine for assay taken
C: Labeled amount (mg) of bucillamine ($C_7H_{13}NO_3S_2$) in 1 tablet

Internal standard solution—A solution of 4-fluorobenzoic acid in methanol (1 in 100).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and methanol (3:2).
Flow rate: Adjust so that the retention time of bucillamine is about 5 minutes.
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, bucillamine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of bucillamine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Bucumolol Hydrochloride

ブクモロール塩酸塩



and enantiomer

$C_{17}H_{23}NO_4.HCl$: 341.83
8-{(2*RS*)-3-[(1,1-Dimethylethyl)amino]-2-hydroxypropyloxy}-5-methylchromen-2-one monohydrochloride
[*36556-75-9*]

Bucumolol Hydrochloride, when dried, contains not less than 99.0% of bucumolol hydrochloride ($C_{17}H_{23}NO_4.HCl$).

Description Bucumolol Hydrochloride occurs as white, crystals or crystalline powder.
It is freely soluble in water, sparingly soluble in methanol and in ethanol (95), slightly soluble in acetic acid (100), and practically insoluble in diethyl ether.
Melting point: about 228°C (with decomposition).

Identification **(1)** Dissolve 0.01 g of Bucumolol Hydrochloride in 10 mL of diluted ethanol (95) (1 in 2), and observe under ultraviolet light (main wavelength: 365 nm): the solution shows a yellow-green fluorescence. Render this solution alkaline by adding sodium hydroxide TS: the fluorescence disappears. Acidify the solution by adding dilute hydrochloric acid: the fluorescence reappears.
(2) Dissolve 0.1 g of Bucumolol Hydrochloride in 5 mL of water, and add 5 drops of Reinecke salt TS: a light red precipitate is formed.
(3) Determine the absorption spectrum of a solution of Bucumolol Hydrochloride (1 in 60,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.
(4) Determine the infrared absorption spectrum of Bucumolol Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.
(5) A solution of Bucumolol Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> for chloride.

Absorbance <*2.24*> $E^{1\%}_{1\,cm}$ (296 nm): 330 – 360 (after drying, 40 mg, water, 2500 mL).

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g

of Bucumolol Hydrochloride in 20 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Bucumolol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Bucumolol Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Bucumolol Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol to make exactly 25 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia-ammonium chloride buffer solution (pH 10.7) (30:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 5 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

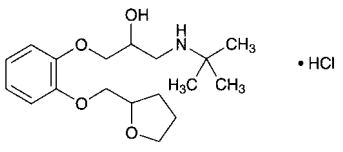
Assay Weigh accurately about 0.4 g of Bucumolol Hydrochloride, previously dried, add 45 mL of acetic acid (100), dissolve by warming at 60°C, and cool. Add 105 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.18 mg of $C_{17}H_{23}NO_4.HCl$

Containers and storage Containers—Well-closed containers.

Bufetolol Hydrochloride

ブフェトロール塩酸塩



$C_{18}H_{29}NO_4.HCl$: 359.89
1-(1,1-Dimethylethyl)amino-3-[2-(tetrahydrofuran-2-ylmethoxy)phenoxy]propan-2-ol monohydrochloride
[*35108-88-4*]

Bufetolol Hydrochloride, when dried, contains not less than 98.5% of bufetolol hydrochloride ($C_{18}H_{29}NO_4.HCl$).

Description Bufetolol Hydrochloride occurs as white, crystals or crystalline powder.

It is freely soluble in water and in methanol, soluble in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

A solution of Bufetolol Hydrochloride (1 in 10) shows no optical rotation.

Identification **(1)** To 5 mL of a solution of Bufetolol Hydrochloride (1 in 100) add 5 drops of Reinecke salt TS: a light red precipitate is formed.

(2) Determine the absorption spectrum of a solution of Bufetolol Hydrochloride (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Bufetolol Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Bufetolol Hydrochloride (1 in 50) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

Melting point ⟨*2.60*⟩ 153 – 157°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Bufetolol Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate ⟨*1.14*⟩—Perform the test with 0.5 g of Bufetolol Hydrochloride. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(3) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Bufetolol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 0.20 g of Bufetolol Hydrochloride in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone, ethanol (95) and ammonia solution (28) (40:20:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

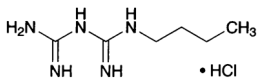
Assay Weigh accurately about 0.4 g of Bufetolol Hydrochloride, previously dried, dissolve in 10 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 35.99 mg of $C_{18}H_{29}NO_4.HCl$

Containers and storage Containers—Tight containers.

Buformin Hydrochloride

ブホルミン塩酸塩



$C_6H_{15}N_5.HCl$: 193.68
1-Butylbiguanide hydrochloride
[*1190-53-0*]

Buformin Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of buformin hydrochloride ($C_6H_{15}N_5.HCl$).

Description Buformin Hydrochloride occurs as a white crystalline powder.

It is freely soluble in water and in ethanol (99.5).

Identification **(1)** To 5 mL of a solution of Buformin Hydrochloride (1 in 2000) add 1 mL of dilute sodium pentacyanonitrosylferrate (III)-potassium hexacyanoferrate (III) TS: a red-brown color develops.

(2) Determine the absorption spectrum of a solution of Buformin Hydrochloride (1 in 125,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Buformin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Buformin Hydrochloride (1 in 20) responds to the Qualitative Tests <*1.09*> for chlorides.

Melting point <*2.60*> 175 – 180°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Buformin Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Buformin Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Buformin Hydrochloride in 200 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the area of the peak other than buformin obtained from the sample solution is not larger than 1/5 times the peak area of buformin obtained from the standard solution. Furthermore, the total of the areas of all peaks other than the buformin peak from the sample solution is not larger than 1/2 times the peak area of buformin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of a solution of sodium perchlorate monohydrate in diluted phosphoric acid (1 in 1000) (7 in 250) and acetonitrile (7:1).

Flow rate: Adjust so that the retention time of buformin is about 6 minutes.

Time span of measurement: About 2 times as long as the retention time of buformin, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of buformin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of buformin are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of buformin is not more than 1.0%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.15 g of Buformin Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and immediately titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 9.684 mg of $C_6H_{15}N_5.HCl$

Containers and storage Containers—Tight containers.

Buformin Hydrochloride Delayed-release Tablets

ブホルミン塩酸塩腸溶錠

Buformin Hydrochloride Delayed-release Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of buformin hydrochloride ($C_6H_{15}N_5.HCl$: 193.68).

Method of preparation Prepare as directed under Tablets, with Buformin Hydrochloride.

Identification To a quantity of powdered Buformin Hydrochloride Delayed-release Tablets, equivalent to 0.1 g of Buformin Hydrochloride, add 10 mL of water, shake well, and then filter. To 4 mL of the filtrate add 1 mL of a mixture of hydrogen peroxide TS, sodium pentacyanonitrosylferrate (III) TS and a solution of sodium hydroxide (1 in 10) (2:1:1): the solution exhibits a red to red-purple color.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Buformin Hydrochloride Delayed-release Tablets add 5 mL of a mixture of ethanol (99.5) and acetone

(1:1), disperse the pellicle to smaller using ultrasonic waves, add exactly 10 mL of the internal standard solution per 50 mg of buformin hydrochloride ($C_6H_{15}N_5$.HCl), and then add diluted acetonitrile (1 in 2) to make $13V/20$ mL. Disintegrate the tablet using ultrasonic waves, then shake for 20 minutes, and add diluted acetonitrile (1 in 2) to make V mL so that each mL contains about 0.5 mg of buformin hydrochloride ($C_6H_{15}N_5$.HCl) per mL. Centrifuge this solution, to 1 mL of the supernatant liquid, add the mobile phase to make 50 mL. If necessary, filter this solution through a membrane filter with a pore size not exceeding 0.5 μm, and use the filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of buformin hydrochloride ($C_6H_{15}N_5$.HCl) $= M_S \times Q_T/Q_S \times V/50$

M_S: Amount (mg) of buformin hydrochloride for assay taken

Internal standard solution—A solution of *p*-acetanisidide in diluted acetonitrile (1 in 2) (1 in 150).

Dissolution ⟨*6.10*⟩ When the tests are performed at 50 revolutions per minute according to the Paddle method, using 900 mL each of 1st fluid for dissolution test and 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 120 minutes of Buformin Hydrochloride Delayed-release Tablets using 1st fluid is not more than 5%, and that in 90 minutes of Buformin Hydrochloride Delayed-release Tablets using 2nd fluid is not less than 80%.

Start the test with 1 tablet of Buformin Hydrochloride Delayed-release Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the relevant dissolution medium to make exactly V' mL so that each mL contains about 56 μg of buformin hydrochloride ($C_6H_{15}N_5$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of buformin hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in the relevant dissolution medium to make exactly 100 mL. Pipet 4 mL of this solution, add the relevant dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of buformin in each solution.

Dissolution rate (%) with respect to the labeled amount of buformin hydrochloride ($C_6H_{15}N_5$.HCl) $= M_S \times A_T/A_S \times V'/V \times 1/C \times 180$

M_S: Amount (mg) of buformin hydrochloride for assay taken

C: Labeled amount (mg) of buformin hydrochloride ($C_6H_{15}N_5$.HCl) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of a solution of sodium perchlorate in diluted phosphoric acid (1 in 1000) (7 in 500) and acetonitrile (7:1).

Flow rate: Adjust so that the retention time of buformin is about 6 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of buformin are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of buformin is not more than 2.0%.

Assay Add 20 mL of a mixture of ethanol (99.5) and acetone (1:1) to an amount of Buformin Hydrochloride Delayed-release Tablets equivalent to 0.5 g of buformin hydrochloride ($C_6H_{15}N_5$.HCl), disperse the pellicles to smaller using ultrasonic waves, and then add 100 mL of diluted acetonitrile (1 in 2). Disintegrate the tablets with the aid of ultrasonic waves, shake for 20 minutes, and then add diluted acetonitrile (1 in 2) to make exactly 200 mL. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and then add diluted acetonitrile (1 in 2) to make 50 mL. Pipet 1 mL of this solution, and add the mobile phase to make 50 mL. If necessary, filter this solution through a membrane filter with a pore size not exceeding 0.5 μm, and use the filtrate as the sample solution. Separately, weigh accurately about 25 mg of buformin hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in an adequate amount of diluted acetonitrile (1 in 2), add exactly 5 mL of the internal standard solution, and then add diluted acetonitrile (1 in 2) to make 50 mL. To 1 mL of this solution add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of buformin to that of the internal standard.

Amount (mg) of buformin hydrochloride ($C_6H_{15}N_5$.HCl) $= M_S \times Q_T/Q_S \times 20$

M_S: Amount (mg) of buformin hydrochloride for assay taken

Internal standard solution—A solution of *p*-acetanisidide in diluted acetonitrile (1 in 2) (1 in 150).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 233 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of a solution of sodium perchlorate (7 in 250) and acetonitrile (7:1).

Flow rate: Adjust so that the retention time of buformin is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, buformin and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of

the peak area of buformin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Buformin Hydrochloride Tablets

ブホルミン塩酸塩錠

Buformin Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of buformin hydrochloride ($C_6H_{15}N_5$.HCl: 193.68).

Method of preparation Prepare as directed under Tablets, with Buformin Hydrochloride.

Identification To a quantity of powdered Buformin Hydrochloride Tablets, equivalent to 1 g of Buformin Hydrochloride, add 100 mL of water, shake well, and then filter. To 4 mL of the filtrate add 1 mL of dilute sodium pentacyanonitrosylferrate (III)-potassium hexacyanoferrate (III) TS: the solution exhibits a red-brown color.

Uniformity of dosage units ⟨*6.02*⟩ Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

Take 1 tablet of Buformin Hydrochloride Tablets, add water to make exactly 200 mL, and then treat with ultrasonic waves for 5 minutes. Take 40 mL of this solution and centrifuge. Pipet V mL of the supernatant liquid equivalent to about 0.5 mg of buformin hydrochloride ($C_6H_{15}N_5$.HCl), add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of buformin hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 233 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of buformin hydrochloride ($C_6H_{15}N_5$.HCl) $= M_S \times A_T/A_S \times 2/V$

M_S: Amount (mg) of buformin hydrochloride for assay taken

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Buformin Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Buformin Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, and add water to make exactly V' mL so that each mL contains about 5.6 μg of buformin hydrochloride ($C_6H_{15}N_5$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of buformin hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and determine the absorbances, A_T and A_S, at 233 nm.

Dissolution rate (%) with respect to the labeled amount of buformin hydrochloride ($C_6H_{15}N_5$.HCl) $= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of buformin hydrochloride for assay taken

C: Labeled amount (mg) of buformin hydrochloride ($C_6H_{15}N_5$.HCl) in 1 tablet

Assay Weigh accurately not less than 20 Buformin Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 60 mg of buformin hydrochloride ($C_6H_{15}N_5$.HCl), add water to make exactly 200 mL, and treat with ultrasonic waves for 5 minutes. Take 40 mL of this solution, centrifuge, pipet 2 mL of the supernatant liquid, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of buformin hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and determine the absorbances, A_T and A_S, at 233 nm.

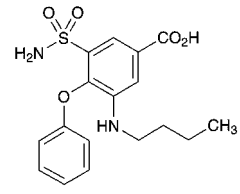
Amount (mg) of buformin hydrochloride ($C_6H_{15}N_5$.HCl) $= M_S \times A_T/A_S$

M_S: Amount (mg) of buformin hydrochloride for assay taken

Containers and storage Containers—Well-closed containers.

Bumetanide

ブメタニド



$C_{17}H_{20}N_2O_5S$: 364.42
3-Butylamino-4-phenoxy-5-sulfamoylbenzoic acid
[*28395-03-1*]

Bumetanide, when dried, contains not less than 98.5% of bumetanide ($C_{17}H_{20}N_2O_5S$).

Description Bumetanide occurs as white, crystals or crystalline powder.

It is freely soluble in pyridine, soluble in methanol and in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in potassium hydroxide TS.

It is gradually colored by light.

Identification **(1)** Dissolve 0.01 g of Bumetanide in 1 mL of pyridine, add 2 drops of copper (II) sulfate TS, shake, add 3 mL of water and 5 mL of chloroform, shake, and allow to stand: a light blue color develops in the chloroform layer.

(2) Dissolve 0.04 g of Bumetanide in 100 mL of phosphate buffer solution (pH 7.0) and dilute 10 mL of the solu-

tion with water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Bumetanide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 232 – 237°C

Purity **(1)** Clarity and color of solution—Dissolve 50 mg of Bumetanide in 2 mL of a solution of potassium hydroxide (1 in 30) and 8 mL of water: the solution is clear, and is not more colored than the following control solution.

Control solution: Pipet 0.5 mL each of Cobalt (II) Chloride CS, Iron (III) Chloride CS and Copper (II) Sulfate CS, mix them, and add diluted hydrochloric acid (1 in 40) to make exactly 100 mL.

(2) Chloride <*1.03*>—Mix well 0.5 g of Bumetanide with 0.7 g of potassium nitrate and 1.2 g of anhydrous sodium carbonate, transfer, in small portions, to a red-hot platinum crucible, and heat to red-hot until the reaction is complete. After cooling, to the residue add 14 mL of dilute sulfuric acid and 6 mL of water, boil for 5 minutes, filter, wash the residue with 10 mL of water, combine the filtrate and the washing, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Heavy metals <*1.07*>—Proceed with 2.0 g of Bumetanide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Bumetanide according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.10 g of Bumetanide in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetic acid (100), cyclohexane and methanol (32:4:4:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

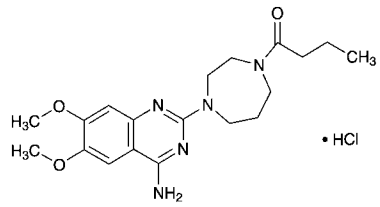
Assay Weigh accurately about 0.5 g of Bumetanide, previously dried, dissolve in 50 mL of ethanol (95), and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 36.44 mg of $C_{17}H_{20}N_2O_5S$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Bunazosin Hydrochloride

ブナゾシン塩酸塩



$C_{19}H_{27}N_5O_3.HCl$: 409.91
4-Amino-2-(4-butanoyl-1,4-diazepan-1-yl)-6,7-dimethoxyquinazoline monohydrochloride
[*52712-76-2*]

Bunazosin Hydrochloride, when dried, contains not less than 98.0% of bunazosin hydrochloride ($C_{19}H_{27}N_5O_3.HCl$).

Description Bunazosin Hydrochloride occurs as a white crystalline powder.

It is very soluble in formic acid, slightly soluble in water and in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

Melting point: about 273°C (with decomposition).

Identification **(1)** Dissolve 0.1 g of Bunazosin Hydrochloride in 10 mL of 0.2 mol/L hydrochloric acid TS, and boil for 3 minutes over a flame: butylic acid like odor is perceptible.

(2) Determine the infrared absorption spectrum of Bunazosin Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Bunazosin Hydrochloride (1 in 100) responds to the Qualitative Tests <*1.09*> for chloride.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Bunazosin Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.05 g of Bunazosin Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. To exactly 1 mL of the sample solution add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than bunazosin from the sample solution is not larger than the peak area of bunazosin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecyl-

silanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 1.44 g of sodium lauryl sulfate in a suitable amount of water, add 10 mL of acetic acid (100), 500 mL of acetonitrile and water to make 1000 mL.

Flow rate: Adjust so that the retention time of bunazosin is about 5 minutes.

Selection of column: Proceed with 20 μL of a mixture of the standard solution and a solution of procaine hydrochloride in the mobile phase (1 in 20,000) (1:1) under the above operating conditions, and calculate the resolution. Use a column giving elution of procaine and bunazosin in this order with the resolution between these peaks being not less than 3.0.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of bunazosin obtained from 20 μL of the standard solution is 20 to 60% of the full-scale.

Time span of measurement: About 6 times of the retention time of bunazosin.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Bunazosin Hydrochloride, previously dried, dissolve in 6 mL of formic acid, add exactly 15 mL of 0.1 mol/L perchloric acid, and heat for 20 minutes on a water bath. After cooling, add 20 mL of acetic acid (100), and titrate ⟨*2.50*⟩ the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination.

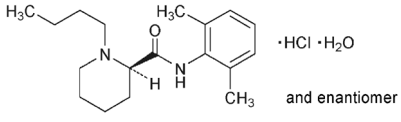
Each mL of 0.1 mol/L perchloric acid VS
= 40.99 mg of $C_{19}H_{27}N_5O_3.HCl$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Bupivacaine Hydrochloride Hydrate

ブピバカイン塩酸塩水和物



$C_{18}H_{28}N_2O.HCl.H_2O$: 342.90

(2*RS*)-1-Butyl-*N*-(2,6-dimethylphenyl)piperidine-2-carboxamide monohydrochloride monohydrate

[*14252-80-3*]

Bupivacaine Hydrochloride Hydrate contains not less than 98.5% and not more than 101.0% of bupivacaine hydrochloride ($C_{18}H_{28}N_2O.HCl$: 324.89), calculated on the anhydrous basis.

Description Bupivacaine Hydrochloride Hydrate occurs as a white crystalline powder.

It is freely soluble in acetic acid (100), and soluble in water, in methanol and in ethanol (99.5).

It dissolves in 0.01 mol/L hydrochloric acid TS.

A solution of 0.5 g of Bupivacaine Hydrochloride Hydrate in 50 mL of a mixture of ethanol (99.5), water and 5 mol/L sodium hydroxide TS (34:15:1) shows no optical rotation.

Melting point: about 252°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Bupivacaine Hydrochloride Hydrate in 0.01 mol/L hydrochloric acid TS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bupivacaine Hydrochloride Hydrate as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Bupivacaine Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

pH ⟨*2.54*⟩ The pH of a solution obtained by dissolving 1.0 g of Bupivacaine Hydrochloride Hydrate in 100 mL of freshly boiled and cooled water is between 4.5 to 6.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Bupivacaine Hydrochloride Hydrate in 50 mL of water: the solution is clear and colorless.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Bupivacaine Hydrochloride Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) 2,6-Dimethylaniline—Dissolve exactly 0.50 g of Bupivacaine Hydrochloride Hydrate in 10 mL of methanol. To 2 mL of this solution add 1 mL of a freshly prepared solution of 4-dimethylaminobenzaldehyde in methanol (1 in 100) and 2 mL of acetic acid (100), and allow to stand for 10 minutes: the color of the solution is not more colored than the following control solution.

Control solution: Prepare by proceeding in the same manner as above, using 2 mL of a solution of 2,6-dimethylaniline in methanol (1 in 200,000).

(4) Related substances—Dissolve 50 mg of Bupivacaine Hydrochloride Hydrate in 2.5 mL of water, add 2.5 mL of 2 mol/L sodium hydroxide TS and 5 mL of the internal standard solution, shake, collect the lower layer, filter, and use the filtrate as the sample solution. Pipet 1 mL of the sample solution, and add the internal standard solution to make exactly 100 mL. Pipet 1 mL of this solution, add the internal standard solution to make exactly 10 mL, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the ratio of the area of the peak other than bupivacaine to the peak area of the internal standard obtained from the sample solution is not larger than the ratio of the peak area of bupivacaine to that of the internal standard obtained from the standard solution.

Internal standard solution—A solution of methyl behenate in dichloromethane (1 in 20,000).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A quartz tube 0.32 mm in inside diameter and 30 m in length, coated the inside surface with 5% diphenyl-95% dimethylpolysiloxane for gas chromatography 0.25 μm in thickness.

Column temperature: Rise the temperature from 180°C to 230°C at the rate of 5°C per minute, and maintain at 230°C for 5 minutes.

Injection port temperature: A constant temperature of

about 250°C.

Detector temperature: A constant temperature of about 250°C.

Carrier gas: Helium.

Flow rate: Adjust so that the retention time of bupivacaine is about 10 minutes.

Split ratio: 1:12.

Time span of measurement: About 1.5 times as long as the retention time of bupivacaine.

System suitability—

System performance: To 1 mL of the sample solution add the internal standard solution to make 100 mL, and use this solution as the solution for system suitability test. When the procedure is run with 1 μL of the solution for system suitability test under the above operating conditions, bupivacaine and the internal standard are eluted in this order with the resolution between these peaks being not less than 20.

System repeatability: When the test is repeated 6 times with 1 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the ratio of the peak area of bupivacaine to that of the internal standard is not more than 2.0%.

Water ⟨*2.48*⟩ 4.0 – 6.0% (0.25 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

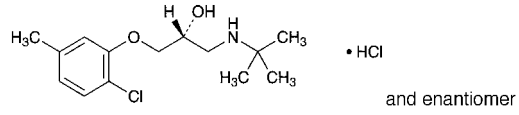
Assay Weigh accurately about 0.5 g of Bupivacaine Hydrochloride Hydrate, dissolve in 20 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.49 mg of $C_{18}H_{28}N_2O.HCl$

Containers and storage Containers—Tight containers.

Bupranolol Hydrochloride

ブプラノロール塩酸塩



and enantiomer

$C_{14}H_{22}ClNO_2.HCl$: 308.24

(2*RS*)-3-(2-Chloro-5-methylphenoxy)-1-(1,1-dimethylethyl)aminopropan-2-ol monohydrochloride [*15148-80-8*]

Bupranolol Hydrochloride, when dried, contains not less than 98.0% of bupranolol hydrochloride ($C_{14}H_{22}ClNO_2.HCl$).

Description Bupranolol Hydrochloride occurs as a white crystalline powder.

It is sparingly soluble in methanol, slightly soluble in water, in ethanol (95) and in acetic acid (100), very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of 1.0 g of Bupranolol Hydrochloride in 1000 mL of water is between 5.2 and 6.2.

Identification **(1)** Take 0.01 g of Bupranolol Hydrochloride in a test tube, mix with 25 mg of potassium iodide and 25 mg of oxalic acid dihydrate, cover the mouth of the test tube with filter paper moistened with a solution of 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine in ethanol (95) (1 in 100), and heat gently for several minutes. Expose the filter paper to ammonia gas: the filter paper acquires a blue color.

(2) Determine the absorption spectrum of a solution of Bupranolol Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Bupranolol Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Bupranolol Hydrochloride (1 in 200) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

Absorbance ⟨*2.24*⟩ $E^{1\%}_{1\,cm}$ (275 nm): 57 – 60 (after drying, 50 mg, 0.1 mol/L hydrochloric acid TS, 500 mL).

Melting point ⟨*2.60*⟩ 223 – 226°C

Purity **(1)** Clarity and color of solution—Dissolve 0.10 g of Bupranolol Hydrochloride in 15 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 0.10 g of Bupranolol Hydrochloride in 15 mL of freshly boiled and cooled water, and add 1 drop of methyl red TS: a light red color develops. To this solution add 0.05 mL of 0.01 mol/L sodium hydroxide VS: the color changes to yellow.

(3) Sulfate ⟨*1.14*⟩—Perform the test with 0.10 g of Bupranolol Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.168%).

(4) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Bupranolol Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Bupranolol Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 0.30 g of Bupranolol Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of polyamide with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, ammonia solution (28) and water (16:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (0.5 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

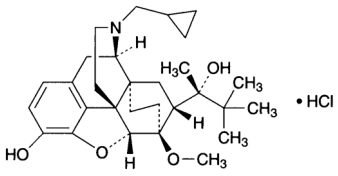
Assay Weigh accurately about 0.18 g of Bupranolol Hydrochloride, previously dried, dissolve in 60 mL of a mixture of acetic anhydride and acetic acid (100) (2:1) by warming, cool, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 30.82 mg of $C_{14}H_{22}ClNO_2.HCl$

Containers and storage Containers—Well-closed containers.

Buprenorphine Hydrochloride

ブプレノルフィン塩酸塩



$C_{29}H_{41}NO_4.HCl$: 504.10
(2*S*)-2-[(5*R*,6*R*,7*R*,14*S*)-17-(Cyclopropylmethyl)-4,5-epoxy-3-hydroxy-6-methoxy-6,14-ethanomorphinan-7-yl]-3,3-dimethylbutan-2-ol monohydrochloride
[*53152-21-9*]

Buprenorphine Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of buprenorphine hydrochloride ($C_{29}H_{41}NO_4.HCl$).

Description Buprenorphine Hydrochloride occurs as white to yellowish white, crystals or a crystalline powder.

It is freely soluble in methanol and in acetic acid (100), and sparingly soluble in water and in ethanol (99.5).

Melting point: about 268°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Buprenorphine Hydrochloride (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Buprenorphine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Buprenorphine Hydrochloride (1 in 100) responds to the Qualitative Tests <*1.09*> for chloride.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −92 – −98° (after drying, 0.4 g, methanol, 20 mL, 100 mm).

pH <*2.54*> The pH of a solution prepared by dissolving 1.0 g of Buprenorphine Hydrochloride in 200 mL of water is between 4.0 and 6.0.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 0.1 g of Buprenorphine Hydrochloride in 10 mL of water is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Buprenorphine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.10 g of Buprenorphine Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than buprenorphine obtained from the sample solution is not larger than 1/4 times the peak area of buprenorphine obtained from the standard solution. Furthermore, the total area of the peaks other than buprenorphine from the sample solution is not larger than 13/20 times the peak area of buprenorphine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 288 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of methanol, ammonium acetate solution (1 in 100), and acetic acid (100) (6000:1000:1).

Flow rate: Adjust so that the retention time of buprenorphine is about 17 minutes.

Time span of measurement: About 2.5 times as long as the retention time of buprenorphine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of buprenorphine obtained from 20 μL of this solution is equivalent to 7 to 13% of that obtained from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of buprenorphine are not less than 6500 and not more than 1.2, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of buprenorphine is not more than 2.0%.

Loss on drying <*2.41*> Not more than 1.0% (1 g, 115°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

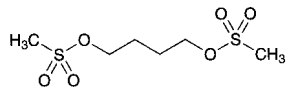
Assay Weigh accurately about 0.5 g of Buprenorphine Hydrochloride, previously dried, dissolve in 5 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 50.41 mg of $C_{29}H_{41}NO_4.HCl$

Containers and storage Containers—Well-closed containers.

Busulfan

ブスルファン



$C_6H_{14}O_6S_2$: 246.30
Tetramethylenedimethanesulfonate
[*55-98-1*]

Busulfan contains not less than 98.5% of busulfan ($C_6H_{14}O_6S_2$), calculated on the dried basis.

Description Busulfan occurs as a white crystalline powder.

It is slightly soluble in diethyl ether, very slightly soluble in ethanol (95), and practically insoluble in water.

Identification **(1)** To 0.1 g of Busulfan add 10 mL of water and 5 mL of sodium hydroxide TS, dissolve by heating, and use this solution as the sample solution.

(i) To 7 mL of the sample solution add 1 drop of potassium permanganate TS: the red-purple color of potassium permanganate TS changes from blue-purple through blue to green.

(ii) Acidify 7 mL of the sample solution with dilute sulfuric acid, and add 1 drop of potassium permanganate TS: the color of potassium permanganate TS remains.

(2) Determine the infrared absorption spectrum of Busulfan, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 115 – 118°C

Purity **(1)** Sulfate <*1.14*>—To 1.0 g of Busulfan add 40 mL of water, and dissolve by heating. Cool in ice for 15 minutes, and filter. Wash the residue with 5 mL of water, combine the washings with the filtrate, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019%).

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Busulfan according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <*2.41*> Not more than 2.0% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Busulfan, add 40 mL of water, and boil gently under a reflux condenser for 30 minutes. Cool, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

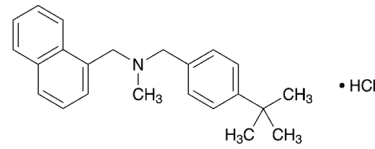
Each mL of 0.1 mol/L sodium hydroxide VS
= 12.32 mg of $C_6H_{14}O_6S_2$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Butenafine Hydrochloride

ブテナフィン塩酸塩



$C_{23}H_{27}N.HCl$: 353.93
N-[4-(1,1-Dimethylethyl)benzyl]-*N*-methyl-1-(naphthalen-1-yl)methylamine monohydrochloride
[*101827-46-7*]

Butenafine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of butenafine hydrochloride ($C_{23}H_{27}N.HCl$).

Description Butenafine Hydrochloride occurs as white, crystals or crystalline powder.

It is very soluble in formic acid, freely soluble in methanol and in ethanol (99.5), and slightly soluble in water.

The pH of a solution dissolved 0.20 g of Butenafine Hydrochloride in 100 mL of water by warming and cooled is 3.0 to 4.0.

Melting point: about 214°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Butenafine Hydrochloride in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Butenafine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Butenafine Hydrochloride in dilute ethanol (1 in 200) responds to the Qualitative Tests <*1.09*> (1) for chloride.

Purity **(1)** Heavy metals <*1.07*>—Dissolve 2.0 g of Butenafine Hydrochloride in 20 mL of ethanol (99.5), add 2 mL of dilute acetic acid and ethanol (99.5) to make 50 mL, and perform the test using this solution as the test solution. The control solution: To 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid, and add ethanol (99.5) to make 50 mL (not more than 10 ppm).

(2) Related substances—Dissolve 30 mg of Butenafine Hydrochloride in 50 mL of a mixture of water and acetonitrile for liquid chromatography (3:2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and acetonitrile for liquid chromatography (3:2) to make exactly 50 mL. Pipet 1 mL of this solution, add a mixture of water and acetonitrile for liquid chromatography (3:2) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the area of the peak, having the relative retention time of about 0.16 to butenafine, obtained from the sample solution is not larger than 3/10 times the peak area of butenafine obtained from the standard solution, and the

area of the peak other than butenafine and the peak mentioned above from the sample solution is not larger than the peak area of butenafine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 217 nm).

Column: A stainless steel column 3.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: Diluted 0.5 mol/L ammonium acetate TS (1 in 1000).

Mobile phase B: Acetonitrile for liquid chromatography.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 10	60 → 20	40 → 80
10 – 60	20	80

Flow rate: 0.4 mL per minute.

Time span of measurement: For 60 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add a mixture of water and acetonitrile for liquid chromatography (3:2) to make exactly 10 mL. Confirm that the peak area of butenafine obtained with 10 μL of this solution is equivalent to 14 to 26% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of butenafine are not less than 20,000 and 0.9 to 1.2, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of butenafine is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.1% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Butenafine Hydrochloride, previously dried, dissolve in 5 mL of formic acid, add 80 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 35.39 mg of $C_{23}H_{27}N.HCl$

Containers and storage Containers—Tight containers.

Butenafine Hydrochloride Cream

ブテナフィン塩酸塩クリーム

Butenafine Hydrochloride Cream contains not less than 95.0% and not more than 105.0% of the labeled amount of butenafine hydrochloride ($C_{23}H_{27}N.HCl$: 353.93).

Method of preparation Prepare as directed under Creams, with Butenafine Hydrochloride.

Identification To an amount of Butenafine Hydrochloride Cream, equivalent to 20 mg of Butenafine Hydrochloride, add 20 mL of acetonitrile, and warm on a water bath to melt the bases. Shake thoroughly, add an appropriate amount of sodium chloride, and allow to stand for 30 minutes in an ice cold water keeping not exceeding 0°C to separate out the bases. Centrifuge, collect the supernatant liquid, add an appropriate amount of sodium chloride to the liquid, allow to stand for 1 hour in an ice cold water keeping not exceeding 0°C, and filter while cooling. To 1 mL of the filtrate add methanol to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 272 nm and 276 nm, between 281 nm and 285 nm, between 311 nm and 315 nm, and between 316 nm and 320 nm, and a shoulder between 289 nm and 299 nm.

Assay Weigh accurately a quantity of Butenafine Hydrochloride Cream, equivalent to about 5 mg of butenafine hydrochloride ($C_{23}H_{27}N.HCl$), add 20 mL of methanol, and add exactly 10 mL of the internal standard solution. Warm this in a water bath for 5 minutes, and shake vigorously for 20 minutes. Then, cool in an ice bath for 15 minutes, centrifuge, and filter the supernatant liquid with a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of butenafine hydrochloride for assay, previously dried in vacuum at 60°C for 3 hours using phosphorus (V) oxide as desiccant, and dissolve in methanol to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of butenafine to that of the internal standard.

Amount (mg) of butenafine hydrochloride ($C_{23}H_{27}N.HCl$)
$= M_S \times Q_T/Q_S \times 1/5$

M_S: Amount (mg) of butenafine hydrochloride for assay taken

Internal standard solution—A solution of diphenyl in methanol (3 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 282 nm).

Column: A stainless steel column 3.0 mm in inside diameter and 5 cm in length, packed with octylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of acetonitrile and diluted 0.5 mol/L ammonium acetate TS (1 in 500) (4:1).

Flow rate: Adjust so that the retention time of butenafine is about 2.5 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard and butenafine are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of butenafine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Butenafine Hydrochloride Solution

ブテナフィン塩酸塩液

Butenafine Hydrochloride Solution is a liquid for external use.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of butenafine hydrochloride ($C_{23}H_{27}N.HCl$: 353.93).

Method of preparation Prepare as directed under Liquids and Solutions for Cutaneous Application, with Butenafine Hydrochloride.

Identification To an amount of Butenafine Hydrochloride Solution, equivalent to 10 mg of Butenafine Hydrochloride, add methanol to make 200 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 272 nm and 276 nm, between 281 nm and 285 nm, between 311 nm and 315 nm, and between 316 nm and 320 nm, and a shoulder between 289 nm and 299 nm.

Assay To an exact volume of Butenafine Hydrochloride Solution, equivalent to about 20 mg of butenafine hydrochloride ($C_{23}H_{27}N.HCl$), add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 4 mL of the internal standard solution, then add methanol to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of butenafine hydrochloride for assay, previously dried in vacuum at 60°C for 3 hours using phosphorus (V) oxide as desiccant, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 4 mL of the internal standard solution, then add methanol to make 25 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of butenafine to that of the internal standard.

Amount (mg) of butenafine hydrochloride ($C_{23}H_{27}N.HCl$) $= M_S \times Q_T/Q_S$

M_S: Amount (mg) of butenafine hydrochloride for assay taken

Internal standard solution—A solution of diphenyl in methanol (3 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 282 nm).

Column: A stainless steel column 3.0 mm in inside diameter and 5 cm in length, packed with octylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of acetonitrile and diluted 0.5 mol/L ammonium acetate TS (1 in 500) (4:1).

Flow rate: Adjust so that the retention time of butenafine is about 2.5 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard and butenafine are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of butenafine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Butenafine Hydrochloride Spray

ブテナフィン塩酸塩スプレー

Butenafine Hydrochloride Spray contains not less than 95.0% and not more than 105.0% of the labeled amount of butenafine hydrochloride ($C_{23}H_{27}N.HCl$: 353.93).

Method of preparation Prepare as directed under Pump Sprays for Cutaneous Application, with Butenafine Hydrochloride.

Identification To an amount of Butenafine Hydrochloride Spray, equivalent to 10 mg of Butenafine Hydrochloride, add methanol to make 200 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 272 nm and 276 nm, between 281 nm and 285 nm, between 311 nm and 315 nm, and between 316 nm and 320 nm, and a shoulder between 289 nm and 299 nm.

Assay To an exact volume of Butenafine Hydrochloride Spray, equivalent to about 20 mg of butenafine hydrochloride ($C_{23}H_{27}N.HCl$), add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 4 mL of the internal standard solution, then add methanol to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of butenafine hydrochloride for assay, previously dried in vacuum at 60°C for 3 hours using phosphorus (V) oxide as desiccant, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 4 mL of the internal standard solution, then add methanol to make 25 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of butenafine to that of the internal standard.

Amount (mg) of butenafine hydrochloride ($C_{23}H_{27}N.HCl$) $= M_S \times Q_T/Q_S$

M_S: Amount (mg) of butenafine hydrochloride for assay

taken

Internal standard solution—A solution of diphenyl in methanol (3 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 282 nm).

Column: A stainless steel column 3.0 mm in inside diameter and 5 cm in length, packed with octylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of acetonitrile and diluted 0.5 mol/L ammonium acetate TS (1 in 500) (4:1).

Flow rate: Adjust so that the retention time of butenafine is about 2.5 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard and butenafine are eluted in this order with the resolution between these peaks being not less than 6.

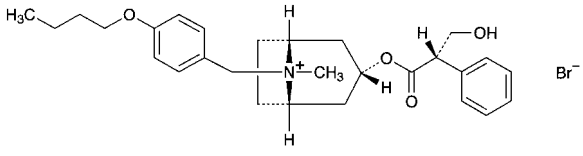
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of butenafine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Butropium Bromide

ブトロピウム臭化物



$C_{28}H_{38}BrNO_4$: 532.51

(1*R*,3*r*,5*S*)-8-(4-Butoxybenzyl)-3-[(2*S*)-hydroxy-2-phenylpropanoyloxy]-8-methyl-8-azoniabicyclo[3.2.1]octane bromide

[*29025-14-7*]

Butropium Bromide, when dried, contains not less than 98.0% of butropium bromide ($C_{28}H_{38}BrNO_4$).

Description Butropium Bromide occurs as white, crystals or crystalline powder.

It is very soluble in formic acid, freely soluble in methanol, soluble in ethanol (95), slightly soluble in water, and practically insoluble in diethyl ether and in acetic anhydride.

Identification **(1)** To 1 mg of Butropium Bromide add 3 drops of fuming nitric acid, and evaporate on a water bath to dryness. Dissolve the residue in 1 mL of *N*,*N*-dimethylformamide, and add 5 to 6 drops of tetraethylammonium hydroxide TS: a red-purple color develops.

(2) Determine the absorption spectrum of a solution of Butropium Bromide in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Butropium Bromide in methanol (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) A solution of Butropium Bromide in methanol (1 in 20) responds to the Qualitative Tests <*1.09*> (1) for bromide.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −14.0 – −17.0° (after drying, 0.5 g, methanol, 20 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Dissolve 1.0 g of Butropium Bromide in 40 mL of ethanol (95), add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test, using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Butropium Bromide in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the peak area, having the relative retention time about 0.5 to butropium from the sample solution is not larger than 1/4 times the peak area from the standard solution, and the total area of all peaks other than the peak eluted first, the peak, having the relative retention time to butropium about 0.5 and butropium peak from the sample solution is not larger than the peak area from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column about 5 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.15 g of sodium lauryl sulfate in 1000 mL of a mixture of acetonitrile and 0.005 mol/L sulfuric acid (3:2).

Flow rate: Adjust so that the retention time of butropium is about 5 minutes.

Selection of column: Dissolve 0.50 g of Butropium Bromide in 9 mL of ethanol (99.5) and 1 mL of 0.1 mol/L potassium hydroxide-ethanol TS, and heat at 70°C for 15 minutes. After cooling, to 1 mL of this solution add the mobile phase to make 100 mL. Proceed with 5 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of the peak of butropium and the peak having a ratio of the retention time about 0.7 to butropium with the resolution between these peaks being not less than 2.5.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of the butropium obtained from 5 μL of the standard solution is between 10 mm and 30 mm.

Time span of measurement: About twice as long as the retention time of butropium.

Loss on drying <*2.41*> Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.8 g of Butropium Bromide, previously dried, dissolve in 5 mL of formic acid,

add 100 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid-dioxane VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

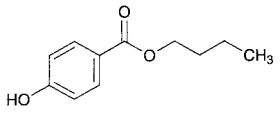
Each mL of 0.1 mol/L perchloric acid-dioxane VS
= 53.25 mg of $C_{28}H_{38}BrNO_4$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Butyl Parahydroxybenzoate

パラオキシ安息香酸ブチル



$C_{11}H_{14}O_3$: 194.23
Butyl 4-hydroxybenzoate
[*94-26-8*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆)

Butyl Parahydroxybenzoate contains not less than 98.0% and not more than 102.0% of butyl parahydroxybenzoate ($C_{11}H_{14}O_3$).

◆**Description** Butyl Parahydroxybenzoate occurs as colorless crystals or white crystalline powder.

It is very soluble in methanol, freely soluble in ethanol (95) and in acetone, and practically insoluble in water.◆

Identification Determine the infrared absorption spectrum of Butyl Parahydroxybenzoate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Butyl Parahydroxybenzoate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 68 – 71°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Butyl Parahydroxybenzoate in ethanol (95) to make 10 mL: the solution is clear and not more intensely colored than the following control solution.

Control solution: To 5.0 mL of Cobalt (II) Chloride CS, 12.0 mL of Iron (III) Chloride CS and 2.0 mL of Copper (II) Sulfate CS add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

(2) Acidity—To 2 mL of the solution of Butyl Parahydroxybenzoate obtained in (1) add 3 mL of ethanol (95), add 5 mL of freshly boiled and cooled water and 0.1 mL of bromocresol green-sodium hydroxide-ethanol TS, then add 0.1 mol/L sodium hydroxide VS until the solution shows a blue color: the volume of 0.1 mol/L sodium hydroxide VS used does not exceed 0.1 mL.

◆**(3)** Heavy metals <1.07>—Dissolve 1.0 g of Butyl Parahydroxybenzoate in 25 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 25 mL of acetone, 2 mL of dilute acetic acid, and water to make 50 mL (not more than 20 ppm).◆

(4) Related substances—Dissolve 50 mg of Butyl Parahydroxybenzoate in 2.5 mL of methanol, and add the mobile phase to make exactly 50 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 20 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of parahydroxybenzoic acid having a relative retention time of about 0.1 to butyl parahydroxybenzoate obtained from the sample solution is not larger than the peak area of butyl parahydroxybenzoate obtained from the standard solution (0.5%). For the area of the peak of parahydroxybenzoic acid multiply the relative response factor, 1.4. Furthermore, the area of the peak other than butyl parahydroxybenzoate and parahydroxybenzoic acid from the sample solution is not larger than the peak area of butyl parahydroxybenzoate from the standard solution (0.5%), and the total area of the peaks other than butyl parahydroxybenzoate is not larger than 2 times the peak area of butyl parahydroxybenzoate from the standard solution (1.0%). For this calculation the peak area not larger than 1/5 times the peak area of butyl parahydroxybenzoate from the standard solution is excluded (0.1%).

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 1.5 times as long as the retention time of butyl parahydroxybenzoate.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

◆Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of butyl parahydroxybenzoate obtained with 10 μL of this solution is equivalent to 14 to 26% of that obtained with 10 μL of the standard solution.◆

◆System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of butyl parahydroxybenzoate is not more than 2.0%.◆

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Butyl Parahydroxybenzoate and Butyl Parahydroxybenzoate RS, dissolve separately in 2.5 mL each of methanol, and add the mobile phase to make exactly 50 mL. Pipet 10 mL each of these solutions, add the mobile phase to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of butyl parahydroxybenzoate in each solution.

Amount (mg) of butyl parahydroxybenzoate ($C_{11}H_{14}O_3$)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of Butyl Parahydroxybenzoate RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-

length: 272 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of methanol and potassium dihydrogen phosphate solution (17 in 2500) (1:1).

Flow rate: 1.3 mL per minute.

System suitability—

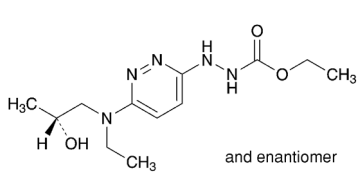
System performance: Dissolve 5 mg each of Butyl Parahydroxybenzoate, propyl parahydroxybenzoate and parahydroxybenzoic acid in the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the solution for system suitability test (1). Separately, dissolve 5 mg of isobutyl parahydroxybenzoate in the mobile phase to make exactly 100 mL. Pipet 0.5 mL of this solution, add the standard solution to make exactly 50 mL, and use this solution as the solution for system suitability test (2). When the procedure is run with 10 μL each of the solution for system suitability test (1) and (2) under the above operating conditions, parahydroxybenzoic acid, propyl parahydroxybenzoate, isobutyl parahydroxybenzoate and butyl parahydroxybenzoate are eluted in this order, the relative retention times of parahydroxybenzoic acid, propyl parahydroxybenzoate and isobutyl parahydoxybenzoate to butyl parahydroxybenzoate are about 0.1, about 0.5 and about 0.9, respectively, the resolution between the peaks of propyl parahydroxybenzoate and butyl parahydroxybenzoate is not less than 5.0, and the resolution between the peaks of isobutyl parahydroxybenzoate and butyl parahydroxybenzoate is not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of butyl parahydroxybenzoate is not more than 0.85%.

◆**Containers and storage** Containers—Well-closed containers.◆

Cadralazine

カドララジン



$C_{12}H_{21}N_5O_3$: 283.33

Ethyl 3-(6-{ethyl[(2*RS*)-2-hydroxypropyl]amino}pyridazin-3-yl)carbazate

[*64241-34-5*]

Cadralazine, when dried, contains not less than 98.5% and not more than 101.0% of cadralazine ($C_{12}H_{21}N_5O_3$).

Description Cadralazine occurs as a pale yellow to light yellow crystalline powder.

It is freely soluble in acetic acid (100), soluble in methanol, sparingly soluble in ethanol (99.5), and slightly soluble in water.

It dissolves in 0.05 mol/L sulfuric acid TS.

A solution of Cadralazine in methanol (1 in 40) shows no optical rotation.

Melting point: about 165°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Cadralazine in 0.05 mol/L sulfuric acid TS (1 in 125,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cadralazine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Chloride <*1.03*>—Dissolve 0.40 g of Cadralazine in 15 mL of methanol, add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS by adding 15 mL of methanol, 6 mL of dilute nitric acid, and water to make 50 mL (not more than 0.036%).

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Cadralazine according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 50 mg of Cadralazine in 20 mL of 0.05 mol/L sulfuric acid TS, add water to 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak, having the relative retention time of about 2.1 to cadralazine, obtained from the sample solution is not larger than the peak area of cadralazine obtained from the standard solution, and the area of the peak other than cadralazine and the peak mentioned above is not larger than 2/5 times the peak area of cadralazine from the standard solution. Furthermore, the total area of the peaks other than cadralazine from the sample solution is not larger than 2 times the peak area of cadralazine from the standard solution. For the areas of the peaks, having the relative retention time of about 0.49 and about 2.1 to cadralazine, multiply their relative response factors, 0.65 and 1.25, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 250 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 13.6 g of sodium acetate trihydrate in 800 mL of water, adjust the pH to 5.8 with dilute acetic acid, and add water to make 1000 mL. To 860 mL of this solution add 140 mL of acetonitrile.

Flow rate: Adjust so that the retention time of cadralazine is about 10 minutes.

Time span of measurement: About 3 times as long as the retention time of cadralazine.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add water to make exactly 25 mL. Confirm that the peak area of cadralazine obtained from 10 μL of this

solution is equivalent to 15 to 25% of that obtained from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cadralazine are not less than 4000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cadralazine is not more than 4.0%.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Cadralazine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 28.33 mg of $C_{12}H_{21}N_5O_3$

Containers and storage Containers—Well-closed containers.

Cadralazine Tablets

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Cadralazine Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of cadralazine ($C_{12}H_{21}N_5O_3$: 283.33).

Method of preparation Prepare as directed under Tablets, with Cadralazine.

Identification To a quantity of powdered Cadralazine Tablets, equivalent to 20 mg of Cadralazine, add 50 mL of 0.05 mol/L sulfuric acid TS, shake well, and centrifuge. To 1 mL of the supernatant liquid add 0.05 mol/L sulfuric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 247 nm and 251 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Cadralazine Tablets add 30 mL of 0.05 mol/L sulfuric acid TS, shake well to disintegrate, and add 0.05 mol/L sulfuric acid TS to make exactly 50 mL. Centrifuge this solution, pipet 3 mL of the supernatant liquid, add 0.05 mol/L sulfuric acid TS to make exactly V mL so that each mL contains about 6 μg of cadralazine ($C_{12}H_{21}N_5O_3$), and use this solution as the sample solution. Separately, weigh accurately about 20 mg of cadralazine for assay, previously dried at 105°C for 3 hours, and dissolve in 0.05 mol/L sulfuric acid TS to make exactly 100 mL. Pipet 3 mL of this solution, add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 249 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of cadralazine ($C_{12}H_{21}N_5O_3$)
$= M_S \times A_T/A_S \times V/200$

M_S: Amount (mg) of cadralazine for assay taken

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Cadralazine Tablets is not less than 80%.

Start the test with 1 tablet of Cadralazine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 5.6 μg of cadralazine ($C_{12}H_{21}N_5O_3$), and use this solution as the sample solution. Separately, weigh accurately about 30 mg of cadralazine for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 200 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 254 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of cadralazine ($C_{12}H_{21}N_5O_3$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of cadralazine for assay taken
C: Labeled amount (mg) of cadralazine ($C_{12}H_{21}N_5O_3$) in 1 tablet

Assay To 10 Cadralazine Tablets add 70 mL of 0.05 mol/L sulfuric acid TS, shake well to disintegrate, add 0.05 mol/L sulfuric acid TS to make exactly 200 mL. Centrifuge this solution, pipet a volume of the supernatant liquid, equivalent to about 2.5 mg of cadralazine ($C_{12}H_{21}N_5O_3$), add exactly 5 mL of the internal standard solution, add water to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of cadralazine for assay, previously dried at 105°C for 3 hours, and dissolve in 0.05 mol/L of sulfuric acid TS to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add water to make 25 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cadralazine to that of the internal standard.

Amount (mg) of cadralazine ($C_{12}H_{21}N_5O_3$)
$= M_S \times Q_T/Q_S \times 1/10$

M_S: Amount (mg) of cadralazine for assay taken

Internal standard solution—A solution of *p*-toluenesulfonamide in acetonitrile (1 in 50).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 250 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 13.6 g of sodium acetate trihydrate in 800 mL of water, adjust the pH to 5.8 with dilute acetic acid, and add water to make 1000 mL. To 860 mL of this solution add 140 mL of acetonitrile.

Flow rate: Adjust so that the retention time of cadralazine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, cadralazine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cadralazine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Anhydrous Caffeine

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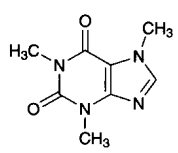

$C_8H_{10}N_4O_2$: 194.19
1,3,7-Trimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione
[*58-08-2*]

Anhydrous Caffeine, when dried, contains not less than 98.5% of caffeine ($C_8H_{10}N_4O_2$).

Description Anhydrous Caffeine occurs as white, crystals or powder. It is odorless, and has a bitter taste.

It is freely soluble in chloroform, sparingly soluble in water, in acetic acid (100) and in acetic anhydride, and slightly soluble in ethanol (95) and in diethyl ether.

The pH of a solution of 1.0 g of Anhydrous Caffeine in 100 mL of water is between 5.5 and 6.5.

Identification **(1)** To 2 mL of a solution of Anhydrous Caffeine (1 in 500) add tannic acid TS dropwise: a white precipitate, which dissolves upon the dropwise addition of tannic acid TS, is produced.

(2) To 0.01 g of Anhydrous Caffeine add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid, and evaporate on a water bath to dryness: the residue acquires a yellow-red color. Invert the residue over a vessel containing 2 to 3 drops of ammonia TS: the color turns red-purple, and disappears upon the addition of 2 to 3 drops of sodium hydroxide TS.

(3) Dissolve 0.01 g of Anhydrous Caffeine in water to make 50 mL. To 5 mL of this solution add 3 mL of diluted acetic acid (31) (3 in 100) and 5 mL of pyridine (1 in 10), mix, add 2 mL of diluted sodium hypochlorite TS (1 in 5), and allow to stand for 1 minute. Add 2 mL of sodium thiosulfate TS and 5 mL of sodium hydroxide TS to the solution: a yellow color develops.

Melting point ⟨*2.60*⟩ 235 – 238°C

Purity **(1)** Chloride ⟨*1.03*⟩—Dissolve 2.0 g of Anhydrous Caffeine in 80 mL of hot water, cool rapidly to 20°C, add water to make 100 mL, and use this solution as the sample solution. To 40 mL of the sample solution add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(2) Sulfate ⟨*1.14*⟩—To 40 mL of the sample solution obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(3) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Anhydrous Caffeine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 0.10 g of Anhydrous Caffeine in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 100 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95) (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(5) Readily carbonizable substances ⟨*1.15*⟩—Perform the test using 0.5 g of Anhydrous Caffeine: the solution is not more colored than Matching Fluid D.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 80°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.4 g of Anhydrous Caffeine, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (6:1), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS until the solution changes from purple through green to yellow (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 19.42 mg of $C_8H_{10}N_4O_2$

Containers and storage Containers—Tight containers.

Caffeine Hydrate

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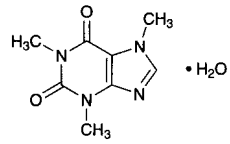


$C_8H_{10}N_4O_2.H_2O$: 212.21
1,3,7-Trimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione
monohydrate
[*5743-12-4*]

Caffeine Hydrate, when dried, contains not less than 98.5% of caffeine ($C_8H_{10}N_4O_2$: 194.19).

Description Caffeine Hydrate occurs as white, soft crystals or powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in chloroform, sparingly soluble in water, in acetic acid (100) and in acetic anhydride, slightly soluble in ethanol (95), and very slightly soluble in diethyl

ether.

The pH of a solution of 1.0 g of Caffeine Hydrate in 100 mL of water is between 5.5 and 6.5.

It effloresces in dry air.

Identification **(1)** To 2 mL of a solution of Caffeine Hydrate (1 in 500) add tannic acid TS dropwise: a white precipitate, which dissolves upon the dropwise addition of tannic acid TS, is produced.

(2) To 0.01 g of Caffeine Hydrate add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid, and evaporate to dryness on a water bath: the residue acquires a yellow-red color. Invert the residue over a vessel containing 2 to 3 drops of ammonia TS: the color turns red-purple, and disappears upon the addition of 2 to 3 drops of sodium hydroxide TS.

(3) Dissolve 0.01 g of Caffeine Hydrate in water to make 50 mL. To 5 mL of this solution add 3 mL of diluted acetic acid (31) (3 in 100) and 5 mL of a solution of pyridine (1 in 10), mix, add 2 mL of diluted sodium hypochlorite TS (1 in 5), and allow to stand for 1 minute. Add 2 mL of sodium thiosulfate TS and 5 mL of sodium hydroxide TS to the solution: a yellow color develops.

Melting point ⟨*2.60*⟩ 235 – 238°C (after drying).

Purity **(1)** Chloride ⟨*1.03*⟩—Dissolve 2.0 g of Caffeine Hydrate in 80 mL of hot water, cool rapidly to 20°C, add water to make 100 mL, and use this solution as the sample solution. To 40 mL of the sample solution add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(2) Sulfate ⟨*1.14*⟩—To 40 mL of the sample solution obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(3) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Caffeine Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 0.10 g of Caffeine Hydrate in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 100 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL, and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95) (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(5) Readily carbonizable substances ⟨*1.15*⟩—Perform the test using 0.5 g of Caffeine Hydrate: the solution is not more colored than Matching Fluid D.

Loss on drying ⟨*2.41*⟩ 0.5 – 8.5% (1 g, 80°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.4 g of Caffeine Hydrate, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (6:1), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS until the solution changes from purple through green to yellow (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 19.42 mg of $C_8H_{10}N_4O_2$

Containers and storage Containers—Tight containers.

Caffeine and Sodium Benzoate

安息香酸ナトリウムカフェイン

Caffeine and Sodium Benzoate, when dried, contains not less than 48.0% and not more than 50.0% of caffeine ($C_8H_{10}N_4O_2$: 194.19), and not less than 50.0% and not more than 52.0% of sodium benzoate ($C_7H_5NaO_2$: 144.10).

Description Caffeine and Sodium Benzoate occurs as a white powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in water, soluble in acetic acid (100) and in acetic anhydride, sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification **(1)** Dissolve 1 g of Caffeine and Sodium Benzoate in 10 mL of water in a separator, add 1 drop of phenolphthalein TS, and add carefully 0.01 mol/L sodium hydroxide VS dropwise until a faint red color develops. Extract with three 20-mL portions of chloroform by thorough shaking, and separate the chloroform layer from the water layer. [Use the water layer for test (2).] Filter the combined chloroform extracts, evaporate the filtrate to dryness on a water bath, and proceed the following tests with the residue:

(i) To 2 mL of a solution of the residue (1 in 500) add tannic acid TS dropwise: a white precipitate, which dissolves upon the dropwise addition of tannic acid TS, is produced.

(ii) To 0.01 g of the residue add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid, evaporate to dryness on a water bath: the residue acquires a yellow-red color. Invert the residue over a vessel containing 2 to 3 drops of ammonia TS: the color turns red-purple, and disappears upon the addition of 2 to 3 drops of sodium hydroxide TS.

(iii) Dissolve 0.01 g of the residue in water to make 50 mL. To 5 mL of this solution add 3 mL of diluted acetic acid (31) (3 in 100) and 5 mL of a solution of pyridine (1 in 10), mix, add 2 mL of diluted sodium hypochlorite TS (1 in 5), and allow to stand for 1 minute. Add 2 mL of sodium thiosulfate TS and 5 mL of sodium hydroxide TS to the solution: a yellow color develops.

(2) To 5 mL of the water layer obtained in (1) add 5 mL of water: the solution responds to the Qualitative Tests ⟨*1.09*⟩ (2) for benzoate.

(3) Heat Caffeine and Sodium Benzoate: white fumes are evolved. Ignite furthermore, and to the residue add hydrochloric acid: bubbles are produced, and the solution responds to the Qualitative Tests ⟨*1.09*⟩ (1) for sodium salt.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Caffeine and Sodium Benzoate in 5 mL of water: the solution is clear and colorless.

(2) Alkalinity—Dissolve 1.0 g of Caffeine and Sodium Benzoate in 20 mL of water, and add 1 or 2 drops of phenolphthalein TS: no red color develops.

(3) Chloride ⟨*1.03*⟩—Dissolve 0.5 g of Caffeine and Sodium Benzoate in 10 mL of water, and add 30 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Pre-

pare the control solution with 0.70 mL of 0.01 mol/L hydrochloric acid VS, 30 mL of ethanol (95) and water to make 50 mL (not more than 0.050%).

(4) Chlorinated compounds—Dissolve 1.0 g of Caffeine and Sodium Benzoate in 40 mL of water, add 10 mL of dilute sulfuric acid, and extract with two 20-mL portions of diethyl ether. Allow the combined diethyl ether extracts to evaporate at room temperature to dryness. Place this residue and 0.7 g of calcium carbonate in a crucible, mix with a small amount of water, and dry. Ignite at about 600°C, dissolve the residue in 20 mL of dilute nitric acid, and filter. Wash the residue with 15 mL of water, combine the filtrate and the washings, and add water to make 50 mL. To this solution add 0.5 mL of silver nitrate TS: the solution is not more turbid than the following control solution to which 0.5 mL of silver nitrate TS has been added.

Control solution: Dissolve 0.7 g of calcium carbonate in 20 mL of dilute nitric acid, and filter. Wash the residue with 15 mL of water, combine the filtrate and the washings, and add 1.2 mL of 0.01 mol/L hydrochloric acid VS and water to make 50 mL.

(5) Heavy metals <*1.07*>—Dissolve 2.0 g of Caffeine and Sodium Benzoate in 47 mL of water, add slowly, with vigorous stirring, 3 mL of dilute hydrochloric acid, and filter. Discard the first 5 mL of the filtrate, neutralize the subsequent 25 mL of the filtrate with ammonia TS, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution by adding 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(6) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Caffeine and Sodium Benzoate according to Method 1, and perform the test (not more than 2 ppm).

(7) Phthalic acid—To 0.10 g of Caffeine and Sodium Benzoate add 1 mL of water and 1 mL of resorcinol-sulfuric acid TS, and heat the mixture in an oil bath heated at a temperature between 120°C and 125°C to evaporate the water, then heat the residue for further 90 minutes, cool, and dissolve in 5 mL of water. To 1 mL of the solution add 10 mL of a solution of sodium hydroxide (43 in 500), shake, then examine under light at a wavelength between 470 nm and 490 nm: the green fluorescence of the solution is not more intense than that of the following control solution.

Control solution: Dissolve 61 mg of potassium hydrogen phthalate in water to make exactly 1000 mL. Pipet exactly 1 mL of the solution, add 1 mL of resorcinol-sulfuric acid TS, and proceed as directed above.

(8) Readily carbonizable substances <*1.15*>—Proceed with 0.5 g of Caffeine and Sodium Benzoate, and perform the test: the solution is not more colored than Matching Fluid A.

Loss on drying <*2.41*> Not more than 3.0% (2 g, 80°C, 4 hours).

Assay (1) Sodium benzoate—Weigh accurately about 0.2 g of Caffeine and Sodium Benzoate, previously dried, dissolve by warming in 50 mL of a mixture of acetic anhydride and acetic acid (100) (6:1), cool, and titrate <*2.50*> with 0.1 mol/L perchloric acid-dioxane VS to the first equivalence point (potentiometric titration). Perform a blank determination, and make any nccessary correction.

Each mL of 0.1 mol/L perchloric acid-dioxane VS
= 14.41 mg of $C_7H_5NaO_2$

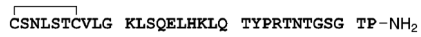
(2) Caffeine—Continue the titration <*2.50*> in (1) with 0.1 mol/L perchloric acid-dioxane VS from the first equivalence point to the second equivalence point (potentiometric titration).

Each mL of 0.1 mol/L perchloric acid-dioxane VS
= 19.42 mg of $C_8H_{10}N_4O_2$

Containers and storage Containers—Well-closed containers.

Calcitonin Salmon

カルシトニン　サケ



$C_{145}H_{240}N_{44}O_{48}S_2$: 3431.85
[*47931-85-1*]

Calcitonin Salmon is a synthetic polypeptide consisting of 32 amino acid residues. It is a hormone with a blood calcium lowering effect.

It contains not less than 4000 Units of calcitonin salmon per 1 mg of peptide.

Description Calcitonin Salmon occurs as a white powder.

It is freely soluble in water.

It dissolves in dilute acetic acid.

Dissolve 20 mg of Calcitonin Salmon in 2 mL of water: the pH of the solution is between 5.0 and 7.0.

It is hygroscopic.

Identification Dissolve 1 mg of Calcitonin Salmon in 1 mL of dilute acetic acid. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Absorbance <*2.24*> $E^{1\%}_{1\,cm}$ (275 nm): 3.3 – 4.0 (1 mg, dilute acetic acid, 1 mL).

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −24 – −32° (25 mg, diluted acetic acid (100) (1 in 2), 10 mL, 100 mm).

Constituent amino acids Weigh accurately about 1 mg of Calcitonin Salmon, put in a test tube for hydrolysis, dissolve in 0.5 mL of diluted hydrochloric acid (1 in 2), freeze in a dry ice-acetone bath, seal the tube under reduced pressure, and heat at 110 ± 2°C for 24 hours. After cooling, open the tube, evaporate the hydrolyzate to dryness under reduced pressure, dissolve the residue in exactly 5 mL of 0.02 mol/L hydrochloric acid TS, and use this solution as the sample solution. Separately, weigh accurately about 27 mg of L-aspartic acid, about 24 mg of L-threonine, about 21 mg of L-serine, about 29 mg of L-glutamic acid, about 23 mg of L-proline, about 15 mg of glycine, about 18 mg of L-alanine, about 23 mg of L-valine, about 48 mg of L-cystine, about 30 mg of methionine, about 26 mg of L-isoleucine, about 26 mg of L-leucine, about 36 mg of L-tyrosine, about 33 mg of phenylalanine, about 37 mg of L-lysine hydrochloride, about 42 mg of L-histidine hydrochloride monohydrate and about 42 mg of L-arginine hydrochloride, dissolve them in 10 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions: 13 peaks of amino acids appear on

the chromatogram obtained from the sample solution, and their respective molar ratios with respect to leucine (=5) are 1.9 – 2.3 for lysine, 0.8 – 1.1 for histidine, 0.9 – 1.1 for arginine, 1.9 – 2.1 for aspartic acid, 4.5 – 4.9 for threonine, 3.2 – 3.8 for serine, 2.8 – 3.1 for glutamic acid, 1.9 – 2.4 for proline, 2.7 – 3.3 for glycine, 1.5 – 2.5 for 1/2 cystine, 0.9 – 1.0 for valine, and 0.8 – 1.0 for tyrosine.

Operating conditions—

Detector: A visible spectrophotometer (wavelength: 440 nm and 570 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 6 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography (Na type) composed with a sulfonated polystyrene copolymer (3 μm in particle diameter).

Column temperature: A constant temperature of about 57°C.

Chemical reaction bath temperature: A constant temperature of about 130°C.

Color developing time: About 1 minute.

Mobile phase: Prepare mobile phases A, B, C, D and E according to the following table.

	Mobile phase A	Mobile phase B	Mobile phase C	Mobile phase D	Mobile phase E
Citric acid monohydrate	19.80 g	22.00 g	12.80 g	6.10 g	—
Trisodium citrate dihydrate	6.19 g	7.74 g	13.31 g	26.67 g	—
Sodium hydroxide	—	—	—	—	8.00 g
Sodium chloride	5.66 g	7.07 g	3.74 g	54.35 g	—
Ethanol (99.5)	130.0 mL	20.0 mL	4.0 mL	—	100.0 mL
Benzyl alcohol	—	—	—	5.0 mL	—
Thiodiglycol	5.0 mL	5.0 mL	5.0 mL	—	—
Lauromacrogol solution (1 in 4)	4.0 mL	4.0 mL	4.0 mL	4.0 mL	4.0 mL
Caprylic acid	0.1 mL	0.1 mL	0.1 mL	0.1 mL	0.1 mL
Water	a sufficient amount	a sufficient amount	a sufficient amount	a sufficient amount	a sufficient amount
Total volume	1000 mL	1000 mL	1000 mL	1000 mL	1000 mL

Flowing of mobile phase: Control the gradient by mixing the mobile phases A, B, C, D and E as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)	Mobile phase C (vol%)	Mobile phase D (vol%)	Mobile phase E (vol%)
0 – 1.5	100	0	0	0	0
1.5 – 4	0	100	0	0	0
4 – 12	0	0	100	0	0
12 – 26	0	0	0	100	0
26 – 30	0	0	0	0	100

Reaction reagent: Mix 407 g of lithium acetate dihydrate, 245 mL of acetic acid (100) and 801 mL of 1-methoxy-2-propanol, add water to make 2000 mL, stir for 10 minutes while passing nitrogen, and use this solution as solution A. Separately, to 1957 mL of 1-methoxy-2-propanol add 77 g of ninhydrin and 0.134 g of sodium borohydride, stir for 30 minutes while passing nitrogen, and use this solution as solution B. Mix solution A and solution B before use.

Flow rate of mobile phase: About 0.4 mL per minute.

Flow rate of reaction reagent: About 0.35 mL per minute.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine are eluted in this order with the resolutions between the peaks of threonine and serine, glycine and alanine, and isoleucine and leucine being not less than 1.2, 1.0 and 1.2, respectively.

System repeatability: When the test is repeated 3 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak areas of aspartic acid, proline, valine and arginine are not more than 2.0%, respectively.

Peptide content Calculate the peptide content in Calcitonin Salmon by the following equation using amino acid analysis values (μmol/mL) obtained in the Constituent amino acids: it is not less than 80.0%.

$$\text{Peptide content (\%)} = 3431.85 \times 5/M \times A/11 \times 100$$

A: Total (μmol/mL) of the amino acid analysis values of valine, leucine, glycine and proline

M: Amount (μg) of Calcitonin Salmon taken

11: Total of the theoretical residue numbers of valine, leucine, glycine and proline per one mole of calcitonin salmon

Purity (1) Acetic acid—Weigh accurately about 10 mg of Calcitonin Salmon, dissolve in water to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 1 g of acetic acid (100), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_T and A_S, of acetic acid in each solution, and calculate the amount of acetic acid by the following equation: the amount of acetic acid is not more than 7.0%.

$$\text{Amount (\%) of acetic acid } (CH_3COOH) = M_S/M_T \times A_T/A_S \times 1/10$$

M_S: Amount (mg) of acetic acid (100) taken

M_T: Amount (mg) of Calcitonin Salmon taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: To 0.7 mL of phosphoric acid add 900 mL of water, adjust the pH to 3.0 with 8 mol/L sodium hydroxide TS, and add water to make 1000 mL.

Mobile phase B: Methanol.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 5	95	5
5 – 10	95 → 50	5 → 50
10 – 20	50	50
20 – 22	50 → 95	50 → 5
22 – 30	95	5

Flow rate: Adjust so that the retention time of acetic acid is about 4 minutes.

System suitability—

System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of acetic acid are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of acetic acid is not more than 2.0%.

(2) Related substances—Dissolve 2 mg of Calcitonin Salmon in 2 mL of dilute acetic acid, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area from the sample solution by the automatic integration method, and calculate the amount of them by the area percentage method: the total amount of the peaks other than calcitonin salmon is not more than 3%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 1% trimethylamine-phosphate buffer solution (pH 3.0) and acetonitrile (27:13).

Flow rate: Adjust so that the retention time of calcitonin salmon is about 9 minutes.

Time span of measurement: About 2 times as long as the retention time of calcitonin salmon, beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add the mobile phase to make 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of calcitonin salmon obtained from 20 μL of this solution is equivalent to 5 to 15% of that obtained from 20 μL of the solution for system suitability test.

System performance: Dissolve 5 mg of methyl parahydroxybenzoate and 7 mg of ethyl parahydroxybenzoate in 100 mL of acetonitrile. When the procedure is run with 20 μL of this solution under the above operating conditions methyl parahydroxybenzoate and ethyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of calcitonin salmon is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 10.0% (5 mg, coulometric titration).

Assay (i) Test animals: Select healthy albino rats weighing between 55 and 180 g, fasted for 24 hours before the test but allowed to drink water ad libitum.

(ii) Standard solutions: Dissolve a quantity of Calcitonin Salmon RS in acetic acid buffer solution containing 0.1% bovine serum albumin, and prepare a high-dose standard solution S_H and a low-dose standard solution S_L containing exactly 0.050 and 0.025 Units per mL, respectively.

(iii) Sample solutions: According to the labeled units, weigh accurately a suitable amount of Calcitonin Salmon, and dissolve in acetic acid buffer solution containing 0.1% bovine serum albumin, and prepare a high-dose sample solution T_H and the low-dose sample solution T_L having Units equal to the standard solutions in equal volumes, respectively.

(iv) Dose for injection: Inject 0.3 mL per animal.

(v) Procedure: Divide the test animals at random into 4 groups, A, B, C and D, with not less than 8 animals and equal numbers in each group. Inject S_H, S_L, T_H and T_L into the tail vein or subcutaneously into the neck of each animal of the respective groups. At 1 hour after the injection, collect blood from the abdominal aorta in a way that minimizes the suffering of the animals, allow the blood samples to stand at room temperature for about 30 minutes, and centrifuge at 3000 revolutions per minute for 10 minutes to separate serum.

(vi) Serum calcium determination: Pipet 0.1 mL of the serum, add exactly 6.9 mL of strontium TS, mix well, and use this solution as the sample solution for calcium determination. Separately, pipet a suitable volume of Standard Calcium Solution for Atomic Absorption Spectrophotometry, dissolve in strontium TS to make a solution so that each mL contains 0.2 to 3 μg of calcium (Ca: 40.08), and use this solution as the standard solution for calcium determination. Perform the test as directed under Atomic Absorption Spectrometry ⟨*2.23*⟩ according to the following conditions, and calculate the calcium content of the sample solution for calcium determination from the calibration curve obtained from the absorbance of the standard solution for calcium determination.

Amount (mg) of Calcium (Ca) in 100 mL of the serum = Calcium content (ppm) in the sample solution for calcium determination × 7

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Calcium hollow-cathode lamp.
Wavelength: 422.7 nm.

(vii) Calculation: Amounts of calcium in the serum obtained with S_H, S_L, T_H and T_L are symbolized as y_1, y_2, y_3 and y_4, respectively. Sum up y_1, y_2, y_3 and y_4 on each set to obtain Y_1, Y_2, Y_3 and Y_4, respectively.

$$\text{Units per mg of peptide} = \text{antilog } M \times b/a \times 1/c \times 5$$

$$M = 0.3010 \times (Y_a/Y_b)$$
$$Y_a = -Y_1 - Y_2 + Y_3 + Y_4$$
$$Y_b = Y_1 - Y_2 + Y_3 - Y_4$$

a: Amount (mg) of Calcitonin Salmon taken
b: Total volume (mL) of the high-dose sample solution prepared by dissolving Calcitonin Salmon in acetic acid buffer solution containing 0.1% bovine serum albumin.
c: Peptide content (%)

F' computed by the following equation should be smaller

than F_1 shown in the table against n with which s^2 is calculated. Calculate L (P = 0.95) by use of the following equation: L should be not more than 0.20. If F' exceeds F_1, or if L exceeds 0.20, repeat the test, increasing the number of animals or arranging the assay conditions so that F' is not more than F_1 and L is not more than 0.20.

$F' = (-Y_1 + Y_2 + Y_3 - Y_4)^2/4fs^2$
f: Number of the test animals of each group.
$s^2 = \{\Sigma y^2 - (Y/f)\}/n$
Σy^2: The sum of squares of y_1, y_2, y_3 and y_4 in each group.
$Y = Y_1^2 + Y_2^2 + Y_3^2 + Y_4^2$
$n = 4(f - 1)$
$L = 2\sqrt{(C - 1)(CM^2 + 0.09062)}$
$C = Y_b^2/(Y_b^2 - 4fs^2t^2)$
t^2: Value shown in the following table against n used to calculate s^2.

n	$t^2 = F_1$	n	$t^2 = F_1$	n	$t^2 = F_1$
1	161.45	13	4.667	25	4.242
2	18.51	14	4.600	26	4.225
3	10.129	15	4.543	27	4.210
4	7.709	16	4.494	28	4.196
5	6.608	17	4.451	29	4.183
6	5.987	18	4.414	30	4.171
7	5.591	19	4.381	40	4.085
8	5.318	20	4.351	60	4.001
9	5.117	21	4.325	120	3.920
10	4.965	22	4.301	∞	3.841
11	4.844	23	4.279		
12	4.747	24	4.260		

Containers and storage Containers—Hermetic containers. Storage—Light-resistant, not exceeding 10°C.

Precipitated Calcium Carbonate

沈降炭酸カルシウム

$CaCO_3$: 100.09

Precipitated Calcium Carbonate, when dried, contains not less than 98.5% of calcium carbonate ($CaCO_3$).

Description Precipitated Calcium Carbonate occurs as a white, fine crystalline powder. It is odorless and tasteless.

It is practically insoluble in water, but its solubility in water is increased in the presence of carbon dioxide.

It is practically insoluble in ethanol (95) and in diethyl ether.

It dissolves with effervescence in dilute acetic acid, in dilute hydrochloric acid and in dilute nitric acid.

Identification **(1)** Dissolve 0.5 g of Precipitated Calcium Carbonate in 10 mL of dilute hydrochloric acid, boil, then cool, and neutralize with ammonia TS: the solution responds to the Qualitative Tests <*1.09*> for calcium salt.

(2) Precipitated Calcium Carbonate responds to the Qualitative Tests <*1.09*> (1) for carbonate.

Purity **(1)** Acid-insoluble substances—To 5.0 g of Precipitated Calcium Carbonate add 50 mL of water, then add 20 mL of hydrochloric acid dropwise with stirring, boil for 5 minutes, cool, add water to make 200 mL, and filter through filter paper for quantitative analysis. Wash the residue until the last washing shows no turbidity with silver nitrate TS, and ignite the residue together with the filter paper: the mass of the residue is not more than 10.0 mg.

(2) Heavy metals <*1.07*>—Mix 2.0 g of Precipitated Calcium Carbonate with 5 mL of water, add slowly 6 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 50 mL of water, and filter. To 25 mL of the filtrate add 2 mL of dilute acetic acid, 1 drop of ammonia TS and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 3 mL of hydrochloric acid on a water bath to dryness, and add 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(3) Barium—Mix 1.0 g of Precipitated Calcium Carbonate with 10 mL of water, add dropwise 4 mL of hydrochloric acid with stirring, boil for 5 minutes, cool, add water to make 40 mL, and filter. With the filtrate, perform the test as directed under Flame Coloration Test <*1.04*> (1): no green color appears.

(4) Magnesium and alkali metals—Dissolve 1.0 g of Precipitated Calcium Carbonate in a mixture of 20 mL of water and 10 mL of dilute hydrochloric acid, boil, neutralize with ammonia TS, and add ammonium oxalate TS until precipitation of calcium oxalate is completed. Heat the mixture on a water bath for 1 hour, cool, dilute with water to 100 mL, shake well, and filter. To 50 mL of the filtrate add 0.5 mL of sulfuric acid, evaporate to dryness, and ignite at 600°C to constant mass: the mass of the residue is not more than 5.0 mg.

(5) Arsenic <*1.11*>—Moisten 0.40 g of Precipitated Calcium Carbonate with 1 mL of water, then dissolve in 4 mL of dilute hydrochloric acid, use this solution as the test solution, and perform the test (not more than 5 ppm).

Loss on drying <*2.41*> Not more than 1.0% (1 g, 180°C, 4 hours).

Assay Weigh accurately about 0.12 g of Precipitated Calcium Carbonate, previously dried, and dissolve in 20 mL of water and 3 mL of dilute hydrochloric acid. Add 80 mL of water, 15 mL of a solution of potassium hydroxide (1 in 10) and 0.05 g of NN indicator, and titrate <*2.50*> immediately with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 5.005 mg of $CaCO_3$

Containers and storage Containers—Tight containers.

Precipitated Calcium Carbonate Fine Granules

沈降炭酸カルシウム細粒

Precipitated Calcium Carbonate Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of calcium carbonate ($CaCO_3$: 100.09).

Method of preparation Prepare as directed under Granules, with Precipitated Calcium Carbonate.

Identification **(1)** To a quantity of powdered Precipitated Calcium Carbonate Fine Granules, equivalent to 0.5 g of Precipitated Calcium Carbonate, add 10 mL of dilute hydro-

chloric acid, shake thoroughly, and filter. Boil the filtrate, then cool, and neutralize with ammonia TS: the solution responds to the Qualitative Tests <1.09> (1), (2) and (3) for calcium salt.

(2) Powdered Precipitated Calcium Carbonate Fine Granules responds to the Qualitative Tests <1.09> (1) for carbonate.

Uniformity of dosage units <6.02> The granules in single-dose packages meet the requirement of the Mass variation test.

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 10 minutes of Precipitated Calcium Carbonate Fine Granules is not less than 80%.

Start the test with an accurately weighed amount of Precipitated Calcium Carbonate Fine Granules, equivalent to about 0.5 g of calcium carbonate ($CaCO_3$), withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of calcium carbonate for assay, previously dried at 180°C for 4 hours, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of calcium in each solution.

Dissolution rate (%) with respect to the labeled amount of calcium carbonate ($CaCO_3$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 1800$

M_S: Amount (mg) of calcium carbonate for assay taken
M_T: Amount (mg) of Precipitated Calcium Carbonate Fine Granules taken
C: Labeled amount (mg) of calcium carbonate ($CaCO_3$) in 1 g

Operating conditions—

Detector: An electric conductivity detector.

Column: A polyether ether ketone tube 4.6 mm in inside diameter and 10 cm in length, packed with slightly acidic ion-exchange silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of a solution of tartaric acid (3 in 2000) and a solution of dipicolinic acid (1 in 3000) (1:1).

Flow rate: Adjust so that the retention time of calcium is about 8 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, sodium and calcium are eluted in this order with the resolution between these peaks being not less than 4.5.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of calcium is not more than 2.0%.

Assay Weigh accurately a quantity of powdered Precipitated Calcium Carbonate Fine Granules, equivalent to about 0.12 g of calcium carbonate ($CaCO_3$), add 20 mL of water and 3 mL of dilute hydrochloric acid, and agitate for 15 minutes with the aid of ultrasonic waves. Then, add 80 mL of water, 15 mL of a solution of potassium hydroxide (1 in 10) and 50 mg of NN indicator, and titrate <2.50> immediately with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
$= 5.005$ mg of $CaCO_3$

Containers and storage Containers—Well-closed containers.

Precipitated Calcium Carbonate Tablets

沈降炭酸カルシウム錠

Precipitated Calcium Carbonate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of calcium carbonate ($CaCO_3$: 100.09).

Method of preparation Prepare as directed under Tablets, with Precipitated Calcium Carbonate.

Identification (1) To a quantity of powdered Precipitated Calcium Carbonate Tablets, equivalent to 0.5 g of Precipitated Calcium Carbonate, add 10 mL of dilute hydrochloric acid, shake throughly, and filter, if necessary. Boil, then cool, and neutralize with ammonia TS: the solution responds to the Qualitative Tests <1.09> (1), (2) and (3) for calcium salt.

(2) Powdered Precipitated Calcium Carbonate Tablets responds to the Qualitative Tests <1.09> (1) for carbonate.

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Disintegration <6.09> Apply to the preparation intended to be used as antacid.

Perform the test using the disk: it meets the requirement.

Dissolution <6.10> Apply to the preparation intended to be used as hyperphosphatemia.

When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 10 minutes of Precipitated Calcium Carbonate Tablets is not less than 80%.

Start the test with 1 tablet of Precipitated Calcium Carbonate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the mobile phase to make exactly V' mL so that each mL contains about 56 μg of calcium carbonate ($CaCo_3$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of calcium carbonate for assay, previously dried at 180°C for 4 hours, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liq-

uid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of calcium in each solution.

Dissolution rate (%) with respect to the labeled amount of calcium carbonate ($CaCO_3$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 180$

M_S: Amount (mg) of calcium carbonate for assay taken
C: Labeled amount (mg) of calcium carbonate ($CaCO_3$) in 1 tablet

Operating conditions—

Detector: An electric conductivity detector.

Column: A polyether ether ketone tube 4.6 mm in inside diameter and 10 cm in length, packed with slightly acidic ion-exchange silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of a solution of tartaric acid (3 in 2000) and a solution of dipicolinic acid (1 in 3000) (1:1).

Flow rate: Adjust so that the retention time of calcium is about 8 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, sodium and calcium are eluted in this order with the resolution between these peaks being not less than 4.5.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of calcium is not more than 2.0%.

Acid-neutralizing capacity <*6.04*> Apply to the preparation intended to be used as antacid.

Weigh accurately and powder not less than 40 Precipitated Calcium Carbonate Tablets. Perform the test with an accurately weighed amount of the powder, equivalent to about 0.25 g of Calcium Carbonate: the amount of 0.1 mol/L hydrochloric acid VS consumed per 1 g of Precipitated Calcium Carbonate is not less than 190 mL.

Assay Weigh accurately and powder not less than 20 Precipitated Calcium Carbonate Tablets. To an accurately weighed portion of the powder, equivalent to about 0.12 g of calcium carbonate ($CaCO_3$), add 20 mL of water, 3 mL of dilute hydrochloric acid, and agitate, if necessary, for 15 minutes with the aid of ultrasonic waves. Then, add 80 mL of water, 15 mL of a solution of potassium hydroxide (1 in 10) and 50 mg of NN indicator, and titrate <*2.50*> immediately with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
$= 5.005$ mg of $CaCO_3$

Containers and storage Containers—Tight containers.

Calcium Chloride Hydrate

塩化カルシウム水和物

$CaCl_2.2H_2O$: 147.01

Calcium Chloride Hydrate contains not less than 96.7% and not more than 103.3% of calcium chloride hydrate ($CaCl_2.2H_2O$).

Description Calcium Chloride Hydrate occurs as white, granules or masses. It is odorless.

It is very soluble in water, and soluble in ethanol (95), and practically insoluble in diethyl ether.

It is deliquescent.

Identification A solution of Calcium Chloride Hydrate (1 in 10) responds to the Qualitative Tests <*1.09*> for calcium salt and for chloride.

pH <*2.54*> The pH of a solution of 1.0 g of Calcium Chloride Hydrate in 20 mL of freshly boiled and cooled water is between 4.5 and 9.2.

Purity **(1)** Clarity and color of solution—A solution of 1.0 g of Calcium Chloride Hydrate in 20 mL of water is clear and colorless.

(2) Sulfate <*1.14*>—Take 1.0 g of Calcium Chloride Hydrate, and perform the test. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(3) Hypochlorite—Dissolve 0.5 g of Calcium Chloride Hydrate in 5 mL of water, add 2 to 3 drops of dilute hydrochloric acid and 2 to 3 drops of zinc iodide-starch TS: no blue color develops immediately.

(4) Heavy metals <*1.07*>—Proceed with 2.0 g of Calcium Chloride Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Iron, aluminum or phosphate—Dissolve, in a Nessler tube, 1.0 g of Calcium Chloride Hydrate in 20 mL of water and 1 drop of dilute hydrochloric acid, boil, then cool, add 3 drops of ammonia TS, and heat the solution to boil: no turbidity or precipitate is produced.

(6) Barium—Dissolve 0.5 g of Calcium Chloride Hydrate in 5 mL of water, add 2 drops of dilute hydrochloric acid and 2 mL of potassium sulfate TS, and allow to stand for 10 minutes: no turbidity is produced.

(7) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Calcium Chloride Hydrate according to Method 1, and perform the test (not more than 2 ppm).

Assay Weigh accurately about 0.4 g of Calcium Chloride Hydrate, and dissolve in water to make exactly 200 mL. Measure exactly 20 mL of this solution, add 40 mL of water, 2 mL of 8 mol/L potassium hydroxide TS and 0.1 g of NN indicator, and titrate <*2.50*> immediately with 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
$= 2.940$ mg of $CaCl_2.2H_2O$

Containers and storage Containers—Tight containers.

Calcium Chloride Injection

塩化カルシウム注射液

Calcium Chloride Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of calcium chloride ($CaCl_2$: 110.98).

The concentration of Calcium Chloride Injection is expressed as the quantity of calcium chloride ($CaCl_2$).

Method of preparation Prepare as directed under Injection, with Calcium Chloride Hydrate.

Description Calcium Chloride Injection is a clear, colorless liquid.

Identification Calcium Chloride Injection responds to the Qualitative Tests <*1.09*> for calcium salt and for chloride.

pH <*2.54*> 4.5 – 7.5

Bacterial endotoxins <*4.01*> Less than 0.30 EU/mg.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Calcium Chloride Injection, equivalent to about 0.4 g of calcium chloride ($CaCl_2$), and proceed as directed in the Assay under Calcium Chloride Hydrate.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.220 mg of $CaCl_2$

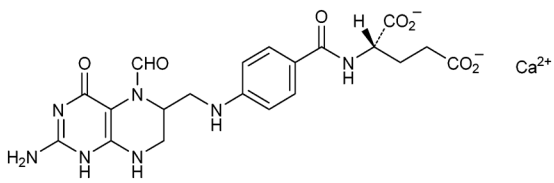
Containers and storage Containers—Hermetic containers.

Plastic containers for aqueous injections may be used.

Calcium Folinate

Calcium Leucovorin

ホリナートカルシウム



$C_{20}H_{21}CaN_7O_7$: 511.50

Monocalcium *N*-(4-{[(2-amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl)methyl]amino}benzoyl)-L-glutamate [*1492-18-8*]

Calcium Folinate contains not less than 95.0% and not more than 102.0% of calcium folinate ($C_{20}H_{21}CaN_7O_7$), calculated on the anhydrous basis.

Description Calcium Folinate occurs as a white to light yellow crystalline powder.

It is sparingly soluble in water, and practically insoluble in methanol and in ethanol (99.5).

Identification **(1)** Determine the absorption spectrum of a solution of Calcium Folinate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Calcium Folinate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Calcium Folinate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Calcium Folinate (1 in 100) responds to the Qualitative Tests <*1.09*> (2) and (3) for calcium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +14 – +19° (0.1 g calculated on the anhydrous basis, water, 10 mL, 100 mm).

pH <*2.54*> To 1.25 g of Calcium Folinate add 50 mL of freshly boiled and cooled water, and warm to 40°C, if necessary, to dissolve: the pH of this solution is between 6.8 and 8.0.

Purity **(1)** Clarity and color of solution—To 1.25 g of Calcium Folinate add 50 mL of freshly boiled and cooled water, and warm to 40°C, if necessary, to dissolve: the solution is clear, and the absorbance at 420 nm of it, determined as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, is not more than 0.25.

(2) Heavy metals <*1.07*>—Proceed with 0.40 g of Calcium Folinate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 50 ppm).

(3) Related substances—Dissolve 10 mg of Calcium Folinate in 25 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than folinate obtained from the sample solution is not larger than the peak area of folinate obtained from the standard solution, and the total area of the peaks other than folinate from the sample solution is not larger than 5 times the peak area of folinate from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of folinate, beginning after the solvent peak.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 5 mL of the standard solution, and add water to make exactly 50 mL. Confirm that the peak area of folinate obtained from 20 μL of this solution is equivalent to 7 to 13% of that obtained from 20 μL of the standard solution.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of folinate is not more than 2.0%.

Water ⟨*2.48*⟩ Not less than 7.0% and not more than 17.0% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately about 10 mg each of Calcium Folinate and Calcium Folinate RS (separately determine the water ⟨*2.48*⟩ in the same manner as Calcium Folinate), dissolve in water to make them exactly 25 mL. Pipet 5 mL each of these solutions, add the mobile phase to make exactly 25 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of folinate in each solution.

$$\text{Amount (mg) of calcium folinate } (C_{20}H_{21}CaN_7O_7) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Calcium Folinate RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of disodium hydrogen phosphate dodecahydrate solution (287 in 100,000), methanol and tetrabutylammonium hydroxide TS (385:110:4), adjusted to pH7.5 with phosphoric acid.

Flow rate: Adjust so that the retention time of folinate is about 10 minutes.

System suitability—

System performance: Dissolve 10 mg each of Calcium Folinate and folic acid in 100 mL of the mobile phase. When the procedure is run with 20 μL of this solution under the above operating conditions, folinate and folic acid are eluted in this order with the resolution between these peaks being not less than 10.

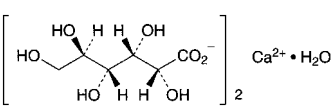
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of folinate is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Calcium Gluconate Hydrate

グルコン酸カルシウム水和物



$C_{12}H_{22}CaO_{14}.H_2O$: 448.39

Monocalcium di-D-gluconate monohydrate

[*299-28-5*]

Calcium Gluconate Hydrate, when dried, contains not less than 99.0% and not more than 104.0% of calcium gluconate hydrate ($C_{12}H_{22}CaO_{14}.H_2O$).

Description Calcium Gluconate Hydrate occurs as a white, crystalline powder or granules.

It is soluble in water, and practically insoluble in ethanol (99.5).

Identification **(1)** To separately 10 mg each of Calcium Gluconate Hydrate and calcium gluconate for thin-layer chromatography add 1 mL of water, dissolve by warming, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (95), water, ammonia solution (28) and ethyl acetate (5:3:1:1) to a distance of about 10 cm, air-dry the plate, and heat the plate at 110°C for 20 minutes. After cooling, spray evenly hexaammonium heptamolybdate-cerium (IV) sulfate TS on the plate, air-dry, and heat at 110°C for 10 minutes: the spots with the sample solution and the standard solution are the same in the *R*f value and color tone.

(2) A solution of Calcium Gluconate Hydrate (1 in 40) responds to the Qualitative Tests ⟨*1.09*⟩ (1), (2) and (3) for calcium salt.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +6 – +11° (after drying, 0.5 g, water, warming, after cooling, 25 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 1.0 g of Calcium Gluconate Hydrate in 20 mL of water by warming: the pH of the solution is between 6.0 and 8.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Calcium Gluconate Hydrate in 50 mL of water by warming: the solution is clear and colorless.

(2) Chloride ⟨*1.03*⟩—Take 0.40 g of Calcium Gluconate Hydrate, and perform the test. Prepare the control solution with 0.80 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.071%).

(3) Sulfate ⟨*1.14*⟩—Take 1.0 g of Calcium Gluconate Hydrate, and perform the test. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(4) Heavy metals ⟨*1.07*⟩—Dissolve 1.0 g of Calcium Gluconate Hydrate in 30 mL of water and 2 mL of dilute acetic acid by warming, cool, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid, and water to make 50 mL (not more than 20 ppm).

(5) Arsenic ⟨*1.11*⟩—Dissolve 0.6 g of Calcium Gluconate Hydrate in 5 mL of water by warming, add 5 mL of dilute sulfuric acid and 1 mL of bromine TS, and concentrate on a water bath to 5 mL. Perform the test with this solution as the test solution (not more than 3.3 ppm).

(6) Sucrose and reducing sugars—To 0.5 g of Calcium Gluconate Hydrate add 10 mL of water and 2 mL of dilute hydrochloric acid, and boil the solution for 2 minutes. After cooling, add 5 mL of sodium carbonate TS, allow to stand for 5 minutes, add water to make 20 mL, and filter. To 5 mL of the filtrate add 2 mL of Fehling's TS, and boil for 1 minute: no orange-yellow to red precipitate is formed immediately.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, 80°C, 2 hours).

Assay Weigh accurately about 0.4 g of Calcium Gluconate Hydrate, previously dried, dissolve in 100 mL of water, add 2 mL of 8 mol/L potassium hydroxide TS and 0.1 g of NN indicator, and titrate ⟨*2.50*⟩ immediately with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 22.42 mg of $C_{12}H_{22}CaO_{14}.H_2O$

Containers and storage Containers—Well-closed containers.

Calcium Hydroxide

Slaked Lime

水酸化カルシウム

$Ca(OH)_2$: 74.09

Calcium Hydroxide contains not less than 90.0% of calcium hydrate [$Ca(OH)_2$].

Description Calcium Hydroxide occurs as a white powder. It has a slightly bitter taste.

It is slightly soluble in water, very slightly soluble in boiling water, and practically insoluble in ethanol (95) and in diethyl ether.

It dissolves in dilute acetic acid, in dilute hydrochloric acid and in dilute nitric acid.

It absorbs carbon dioxide from air.

Identification **(1)** Mix Calcium Hydroxide with 3 to 4 times its mass of water: the mixture is slushy and is alkaline.

(2) Dissolve 1 g of Calcium Hydroxide in 30 mL of dilute acetic acid, and boil. After cooling, neutralize with ammonia TS: the solution responds to the Qualitative tests <*1.09*> (2) and (3) for calcium salt.

Purity **(1)** Acid-insoluble substances—To 5 g of Calcium Hydroxide add 100 mL of water, add hydrochloric acid dropwise with strring until the solution becomes acidic, and further add 1 mL of hydrochloric acid. Boil this solution for 5 minutes, cool, and filter through a tared glass filter (G4). Wash the residue with boiling water until the last washing exhibits no turbidity upon addition of silver nitrate TS, and dry at 105°C to constant mass: the mass is not more than 25 mg.

(2) Heavy metals <*1.07*>—Dissolve 1.0 g of Calcium Hydroxide in 10 mL of dilute hydrochloric acid, evaporate on a water bath to dryness, dissolve the residue in 40 mL of water, and filter. To 20 mL of the filtrate add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 5 mL of dilute hydrochloric acid on a water bath to dryness, and add 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 40 ppm).

(3) Magnesium and alkali metals—Dissolve 1.0 g of Calcium Hydroxide in a mixture of 20 mL of water and 10 mL of dilute hydrochloric acid, boil, neutralize with ammonia TS, and precipitate calcium oxalate completely by adding dropwise ammonium oxalate TS. Heat the mixture on a water bath for 1 hour, cool, dilute with water to 100 mL, shake, and filter. To 50 mL of the filtrate add 0.5 mL of sulfuric acid, evaporate to dryness, and ignite at 600°C to constant mass: the mass of the residue does not exceed 24 mg.

(4) Arsenic <*1.11*>—Dissolve 0.5 g of Calcium Hydroxide in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 4 ppm).

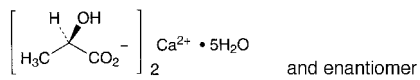
Assay Weigh accurately about 1 g of Calcium Hydroxide, dissolve by adding 10 mL of dilute hydrochloric acid, and add water to make exactly 100 mL. Measure exactly 10 mL of this solution, add 90 mL of water and 1.5 mL of 8 mol/L potassium hydroxide TS, shake, allow to stand for 3 to 5 minutes, and then add 0.1 g of NN indicator. Titrate <*2.50*> immediately with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, until the red-purple color of the solution changes to blue.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 3.705 mg of $Ca(OH)_2$

Containers and storage Containers—Tight containers.

Calcium Lactate Hydrate

乳酸カルシウム水和物



$C_6H_{10}CaO_6.5H_2O$: 308.29
Monocalcium bis[(2*RS*)-2-hydroxypropanonate] pentahydrate
[*63690-56-2*]

Calcium Lactate Hydrate, when dried, contains not less than 97.0% of calcium lactate ($C_6H_{10}CaO_6$: 218.22).

Description Calcium Lactate Hydrate occurs as white, powder or granules. It is odorless, and has a slightly acid taste.

A 1 g portion of it dissolves gradually in 20 mL of water, and it is slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

It is partly efflorescent at ordinary temperature, and yields the anhydride at 120°C.

Identification A solution of Calcium Lactate Hydrate (1 in 20) responds to the Qualitative Tests <*1.09*> for calcium salt and for lactate.

Purity **(1)** Clarity of solution—Dissolve 1.0 g of Calcium Lactate Hydrate in 20 mL of water by warming: the solution is clear.

(2) Acidity or alkalinity—To the solution obtained in (1) add 2 drops of phenolphthalein TS: no red color is produced. Then add 0.50 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3) Heavy metals <*1.07*>—Dissolve 1.0 g of Calcium Lactate Hydrate in 30 mL of water and 5 mL of dilute acetic acid by warming, cool, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution from 2.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid, and dilute with water to 50 mL (not more than 20 ppm).

(4) Magnesium or alkali metals—Dissolve 1.0 g of Calcium Lactate Hydrate in 40 mL of water, add 0.5 g of ammonium chloride, boil, then add 20 mL of ammonium oxalate TS. Heat the mixture on a water bath for 1 hour, cool, dilute with water to 100 mL, and filter. To 50 mL of the filtrate add 0.5 mL of sulfuric acid, evaporate to dryness, and ignite between 450°C and 550°C to constant mass: the mass of the residue is not more than 5 mg.

(5) Arsenic <*1.11*>—Dissolve 0.5 g of Calcium Lactate Hydrate in 2 mL of water and 3 mL of hydrochloric acid, and perform the test with this solution as the test solution

(not more than 4 ppm).

(6) Volatile fatty acid—Warm 1.0 g of Calcium Lactate Hydrate with 2 mL of sulfuric acid: an odor of acetic acid or butyric acid is not perceptible.

Loss on drying ⟨*2.41*⟩ 25.0 – 30.0% (1 g, 80°C, 1 hour at first, then 120°C, 4 hours).

Assay Weigh accurately about 0.5 g of Calcium Lactate Hydrate, previously dried, add water, dissolve by heating on a water bath, cool, and add water to make exactly 100 mL. Pipet 20 mL of this solution, then 80 mL of water and 1.5 mL of 8 mol/L potassium hydroxide TS, and allow to stand for 3 to 5 minutes. Add 0.1 g of NN indicator, and titrate ⟨*2.50*⟩ immediately with 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red to blue.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 4.364 mg of $C_6H_{10}CaO_6$

Containers and storage Containers—Tight containers.

Calcium Oxide

Quick Lime

酸化カルシウム

CaO: 56.08

Calcium Oxide, when incinerated, contains not less than 98.0% of calcium oxide (CaO).

Description Calcium Oxide occurs as hard, white masses, containing a powder. It is odorless.

It is very slightly soluble in boiling water, and practically insoluble in ethanol (95).

One gram of Calcium Oxide dissolves almost completely in 2500 mL of water.

It slowly absorbs moisture and carbon dioxide from air.

Identification (1) Moisten Calcium Oxide with water: heat is generated and a white powder is obtained. Mix the powder with about 5 times its mass of water: the mixture is alkaline.

(2) Dissolve 1 g of Calcium Oxide in 20 mL of water by adding a few drops of acetic acid (31): the solution responds to the Qualitative Tests ⟨*1.09*⟩ for calcium salt.

Purity (1) Acid-insoluble substances—Disintegrate 5.0 g of Calcium Oxide with a small amount of water, add 100 mL of water, add dropwise hydrochloric acid with stirring until the solution becomes acidic, and further add 1 mL of hydrochloric acid. Boil the solution for 5 minutes, cool, filter through a glass filter (G4), wash the residue with boiling water until no turbidity is produced when silver nitrate TS is added to the last washing, and dry at 105°C to constant mass: the mass of the residue is not more than 10.0 mg.

(2) Carbonate—Disintegrate 1.0 g of Calcium Oxide with a small amount of water, mix thoroughly with 50 mL of water, allow to stand for a while, remove most of the supernatant milky liquid by decantation, and add an excess of dilute hydrochloric acid to the residue: no vigorous effervescence is produced.

(3) Magnesium and alkali metals—Dissolve 1.0 g of Calcium Oxide in 75 mL of water by adding dropwise hydrochloric acid, and further add 1 mL of hydrochloric acid. Boil for 1 to 2 minutes, neutralize with ammonia TS, add dropwise an excess of hot ammonium oxalate TS, heat the mixture on a water bath for 2 hours, cool, add water to make 200 mL, mix thoroughly, and filter. Evaporate 50 mL of the filtrate with 0.5 mL of sulfuric acid to dryness, and heat the residue strongly at 600°C to constant mass: the mass of the residue is not more than 15 mg.

Loss on ignition ⟨*2.43*⟩ Not more than 10.0% (1 g, 900°C, constant mass).

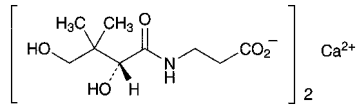
Assay Weigh accurately about 0.7 g of Calcium Oxide, previously incinerated at 900°C to constant mass and cooled in a desiccator (silica gel), and dissolve in 50 mL of water and 8 mL of diluted hydrochloric acid (1 in 3) by heating. Cool, and add water to make exactly 250 mL. Pipet 10 mL of the solution, add 50 mL of water, 2 mL of 8 mol/L potassium hydroxide TS and 0.1 g of NN indicator, and titrate ⟨*2.50*⟩ with 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, until the red-purple color of the solution changes to blue.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 1.122 mg of CaO

Containers and storage Containers—Tight containers.

Calcium Pantothenate

パントテン酸カルシウム



$C_{18}H_{32}CaN_2O_{10}$: 476.53
Monocalcium bis{3-[(2*R*)-2,4-dihydroxy-3,3-dimethylbutanoylamino]propanoate}
[*137-08-6*]

Calcium Pantothenate contains not less than 98.0% and not more than 102.0% of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$), calculated on the dried basis.

Description Calcium Pantothenate occurs as a white powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

The pH of a solution prepared by dissolving 1.0 g of Calcium Pantothenate in 20 mL of water is between 7.0 and 9.0.

It is hygroscopic.

It shows crystal polymorphism.

Identification (1) Determine the infrared absorption spectrum of previously dried Calcium Pantothenate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of dried Calcium Pantothenate RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve the sample and the Reference Standard separately in water, evaporate water, dry the residues in vacuum for 24 hours using silica gel as a desiccant, and perform the test using these residues.

(2) A solution of Calcium Pantothenate (1 in 10) responds to the Qualitative Tests ⟨*1.09*⟩ (1), (2) and (3) for calcium salt.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +25.0 – +28.5° (1 g calculated on the dried basis, water, 20 mL, 100 mm).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Calcium Pantothenate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.30 g of Calcium Pantothenate in 20 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.6 to pantothenic acid obtained from the sample solution is not larger than 1.2 times the peak area of pantothenic acid obtained from the standard solution, the area of the peak, having the relative retention time of about 0.8 is not larger than the peak area of pantothenic acid from the standard solution, the area of the peak, having the relative retention time of about 1.5 is not larger than 3/5 times the peak area of pantothenic acid from the standard solution, and the area of the peak other than pantothenic acid and the peaks mentioned above is not larger than 3/10 times the peak area of pantothenic acid from the standard solution. Additionally, the total area of the peaks other than pantothenic acid from the sample solution is not larger than 2.4 times the peak area of pantothenic acid from the standard solution. For the areas of the peaks, having the relative retention time of about 0.6 and about 0.8 to pantothenic acid, multiply their relative response factors, 19 and 13, respectively.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of pantothenic acid, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add water to make exactly 10 mL. Confirm that the peak area of pantothenic acid obtained with 10 μL of this solution is equivalent to 14 to 26% of that with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pantothenic acid are not less than 10,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pantothenic acid is not more than 2.0%.

(3) Alkaloids—Dissolve 50 mg of Calcium Pantothenate in 5 mL of water, add 0.5 mL of hexaammonium heptamolybdate TS and 0.5 mL of a solution of phosphoric acid (1 in 10): no white turbidity is produced.

Loss on drying ⟨*2.41*⟩ Not more than 5.0% (1 g, 105°C, 4 hours).

Assay Weigh accurately about 20 mg each of Calcium Pantothenate and Calcium Pantothenate RS (separately determine the loss on drying ⟨*2.41*⟩ in the same conditions as Calcium Pantothenate), dissolve each in water to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of pantothenic acid in each solution.

Amount (mg) of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$) $= M_S \times A_T/A_S$

M_S: Amount (mg) of Calcium Pantothenate RS taken, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 0.81 g of sodium 1-heptanesulfonate and 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 2.1 with phosphoric acid. To 980 mL of this solution add 10 mL of acetonitrile and 10 mL of methanol.

Flow rate: Adjust so that the retention time of pantothenic acid is about 17 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pantothenic acid are not less than 10,000 and not more than 1.5, respectively.

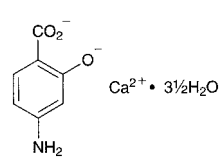
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pantothenic acid is not more than 1.0%.

Containers and storage Containers—Tight containers.

Calcium Paraaminosalicylate Hydrate

Pas-calcium Hydrate

パラアミノサリチル酸カルシウム水和物



$C_7H_5CaNO_3 \cdot 3\frac{1}{2}H_2O$: 254.25

Monocalcium 4-amino-2-oxidobenzoate hemiheptahydrate [*133-15-3*, anhydride]

Calcium Paraaminosalicylate Hydrate contains not less than 97.0% and not more than 103.0% of calcium paraaminosalicylic acid ($C_7H_5CaNO_3$: 191.20), calculated on the anhydrous basis.

Description Calcium Paraaminosalicylate Hydrate occurs as a white to slightly colored powder. It has a slightly bitter taste.

It is very slightly soluble in water, and practically insoluble in methanol and in ethanol (99.5).

It is gradually colored to brown by light.

Identification **(1)** To 50 mg of Calcium Paraaminosalicylate Hydrate add 100 mL of water, shake well, and filter. To 10 mL of the filtrate add 1 mL of 1 mol/L hydrochloric acid TS, shake, and add 1 drop of iron (III) chloride TS: a red-purple color develops.

(2) Determine the infrared absorption spectrum of Calcium Paraaminosalicylate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 3 g of Calcium Paraaminosalicylate Hydrate add 15 mL of ammonium chloride TS and 15 mL of water, heat on a water bath until almost dissolved, and filter after cooling: the filtrate responds to the Qualitative Tests ⟨*1.09*⟩ (1), (2) and (3) for calcium salt.

Purity **(1)** Chloride ⟨*1.03*⟩—Dissolve 1.0 g of Calcium Paraaminosalicylate Hydrate in 15 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.70 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.025%).

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Calcium Paraaminosalicylate Hydrate according to method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic ⟨*1.11*⟩—Dissolve 0.40 g of Calcium Paraaminosalicylate Hydrate in 20 mL of 0.1 mol/L hydrochloric acid TS by warming on a water bath, use this solution as the test solution, and perform the test (not more than 5 ppm).

(4) 3-Aminophenol—To 0.10 g of Calcium Paraaminosalicylate Hydrate add 5 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS, previously cooled in ice-water, and dissolve by shaking vigorously. Add immediately 3 mL of ammonia-ammonium chloride buffer solution (pH 11.0) previously cooled in ice water, and shake. Add 2 mL of 4-amino-*N*,*N*-diethylaniline sulfate TS, shake, add 10.0 mL of cyclohexane and 4 mL of diluted potassium hexacyanoferrate (III) TS (1 in 10), and shake immediately for 20 seconds. Centrifuge this solution, wash the separated cyclohexane layer with two 5-mL portions of diluted ammonia TS (1 in 14), add 1 g of anhydrous sodium sulfate, shake, and allow to stand for 5 minutes: the clear cyclohexane layer is not more colored than the following control solution.

Control solution: Dissolve 50 mg of 3-aminophenol in water, and dilute with water to exactly 500 mL. Measure exactly 20 mL of this solution, and add water to make exactly 100 mL. Take 5.0 mL of this solution, add 3 mL of ammonia-ammonium chloride buffer solution (pH 11.0) previously cooled in ice-water, and treat this solution in the same manner as the sample.

Water ⟨*2.48*⟩ 23.3 – 26.3% (0.1 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.2 g of Calcium Paraaminosalicylate Hydrate, dissolve in 60 mL of water and 0.75 mL of dilute hydrochloric acid by warming on a water bath. After cooling, add water to make exactly 100 mL, and use this solution as the sample solution. Measure exactly 30 mL of the sample solution, transfer to an iodine flask, and add exactly 25 mL of 0.05 mol/L bromine VS and 20 mL of a solution of potassium bromide (1 in 4). Add immediately 14 mL of a mixture of acetic acid (100) and hydrochloric acid (5:2), stopper the flask immediately, and allow to stand for 10 minutes with occasional shaking. Add cautiously 6 mL of potassium iodide TS, and shake gently. After 5 minutes, titrate ⟨*2.50*⟩ the produced iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS
= 3.187 mg of $C_7H_5CaNO_3$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Calcium Paraaminosalicylate Granules

Pas-calcium Granules

パラアミノサリチル酸カルシウム顆粒

Calcium Paraaminosalicylate Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of calcium paraaminosalicylate hydrate ($C_7H_5CaNO_3.3½H_2O$: 254.25).

Method of preparation Prepare as directed under Granules, with Calcium Paraaminosalicylate Hydrate.

Identification Powder Calcium Paraaminosalicylate Granules, weigh a portion of the powder, equivalent to 50 mg of Calcium Paraaminosalicylate Hydrate, add 100 mL of water, shake, and filter. To 10 mL of the filtrate add 1 mL of 1 mol/L hydrochloric acid TS, shake, and add 1 drop of iron (III) chloride TS: a red-purple color develops.

Dissolution ⟨*6.10*⟩ When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 60 minutes of Calcium Paraaminosalicylate Granules is not less than 75%.

Start the test with an accurately weighed amount of Calcium Paraaminosalicylate Granules, equivalent to about 0.25 g of calcium paraaminosalicylate hydrate ($C_7H_5CaNO_3.3½H_2O$), withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of calcium paraaminosalicylate hydrate for assay (separately determine the water ⟨*2.48*⟩ in the same manner as Calcium Paraaminosalicylate Hydrate), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 300 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of calcium paraaminosalicylate hydrate ($C_7H_5CaNO_3.3½H_2O$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 900 \times 1.330$

M_S: Amount (mg) of calcium paraaminosalicylate hydrate for assay taken, calculated on the anhydrous basis
M_T: Amount (g) of Calcium Paraaminosalicylate Granules taken
C: Labeled amount (mg) of calcium paraaminosalicylate hydrate ($C_7H_5CaNO_3.3½H_2O$) in 1 g

Assay Powder Calcium Paraaminosalicylate Granules, weigh accurately a portion of the powder, equivalent to about 0.2 g of calcium paraaminosalicylate hydrate ($C_7H_5CaNO_3.3½H_2O$), add 60 mL of water and 0.75 mL of dilute hydrochloric acid, and dissolve by heating on a water bath. After cooling, add water to make exactly 100 mL, and filter. Pipet 30 mL of the filtrate, transfer to an iodine flask, and proceed as directed in the Assay under Calcium Paraaminosalicylate Hydrate.

Each mL of 0.05 mol/L bromine VS
= 4.238 mg of $C_7H_5CaNO_3.3½H_2O$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Anhydrous Dibasic Calcium Phosphate

無水リン酸水素カルシウム

$CaHPO_4$: 136.06
[*7757-93-9*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Anhydrous Dibasic Calcium Phosphate contains not less than 98.0% and not more than 103.0% of dibasic calcium phosphate ($CaHPO_4$).

◆**Description** Anhydrous Dibasic Calcium Phosphate occurs as white, crystalline powder or granules.

It is practically insoluble in water and in ethanol (99.5).

It dissolves in dilute hydrochloric acid and in dilute nitric acid.◆

Identification **(1)** Dissolve 0.1 g of Anhydrous Dibasic Calcium Phosphate in 10 mL of 2 mol/L hydrochloric acid TS by warming, add 2.5 mL of ammonia TS dropwise with shaking, and add 5 mL of ammonium oxalate TS: a white precipitate is produced.

(2) Dissolve 0.1 g of Anhydrous Dibasic Calcium Phosphate in 5 mL of dilute nitric acid, and add 2 mL of hexaammonium heptamolybdate TS after warming at 70°C for 1 to 2 minutes: a yellow precipitate is produced.

Purity **(1)** Acid-insoluble substances—Dissolve 5.0 g of Anhydrous Dibasic Calcium Phosphate in 40 mL of water and 10 mL of hydrochloric acid, and boil gently for 5 minutes. After cooling, collect the insoluble substance using a filter paper for assay. Wash with water until no more turbidity of the washings is produced when silver nitrate TS is added. Ignite to incinerate the residue and the filter paper at 600 ± 50°C: the mass is not more than 10 mg (not more than 0.2%).

(2) Chloride—To 0.20 g of Anhydrous Dibasic Calcium Phosphate add 20 mL of water and 13 mL of dilute nitric acid, dissolve by warming, if necessary, add water to make 100 mL, and filter, if necessary. Put 50 mL of this solution in a Nessler tube, and use this as the test solution. Transfer 0.70 mL of 0.01 mol/L hydrochloric acid VS to another Nessler tube, add 6 mL of dilute nitric acid and water to make 50 mL, and use this solution as the control solution. Add 1 mL of silver nitrate TS to the test solution and the control solution, mix well, and allow to stand for 5 minutes protecting from light. Compare the opalescence developed in both solutions against a black background by viewing downward or transversely. The opalescence developed in the test solution is not more than that of the control solution. (not more than 0.25%)

(3) Sulfate—Dissolve 0.50 g of Anhydrous Dibasic Calcium Phosphate in 5 mL of water and 5 mL of dilute hydrochloric acid, add water to make 100 mL, and filter, if necessary. Put 20 mL of this solution in a Nessler tube, add 1 mL of dilute hydrochloric acid, and add water to make 50 mL, and use this as the test solution. Transfer 1.0 mL of 0.005 mol/L sulfuric acid VS to another Nessler tube, add 1 mL of dilute hydrochloride acid and water to make 50 mL, and use this solution as the control solution. Add 2 mL of barium chloride TS to the test solution and the control solution, mix well, and allow to stand for 10 minutes. Compare the white turbidity produced in both solutions against a black background by viewing downward or transversely. The turbidity produced in the test solution is not thicker than that of the control solution. (not more than 0.48%)

(4) Carbonate—Mix 1.0 g of Anhydrous Dibasic Calcium Phosphate with 5 mL of freshly boiled and cooled water, and add immediately 2 mL of hydrochloric acid: no effervescence occurs.

◆**(5)** Heavy metals <*1.07*>—Dissolve 0.65 g of Anhydrous Dibasic Calcium Phosphate in a mixture of 5 mL of water and 5 mL of dilute hydrochloric acid by warming, cool, and add ammonia TS until precipitates begin to form in the solution. Dissolve the precipitates by adding a small amount of dilute hydrochloric acid dropwise, add 10 mL of hydrochloric acid-ammonium acetate buffer solution (pH 3.5) and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 10 mL of hydrochloric acid-ammonium acetate buffer solution (pH 3.5) add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 31 ppm).◆

(6) Barium—Heat 0.5 g of Anhydrous Dibasic Calcium Phosphate with 10 mL of water, add 1 mL of hydrochloric acid dropwise with stirring, and filter after cooling, if necessary. Add 2 mL of potassium sulfate TS to this solution, and allow to stand for 10 minutes: no turbidity forms.

◆**(7)** Arsenic <*1.11*>—Dissolve 1.0 g of Anhydrous Dibasic Calcium Phosphate in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 2 ppm).◆

Loss on ignition <*2.43*> Not less than 6.6% and not more than 8.5% (1 g, 800 – 825°C, constant mass).

Assay Weigh accurately about 0.4 g of Anhydrous Dibasic Calcium Phosphate, dissolve in 12 mL of dilute hydrochloric acid by warming on a water bath, if necessary, and add water to make exactly 200 mL. Pipet 20 mL of this solution, add exactly 25 mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution (pH 10.7), and titrate <*2.50*> the excess disodium dihydrogen ethylenediamine tetraacetate with 0.02 mol/L zinc sulfate VS (indicator: 25 mg of eriochrome black T-sodium chloride indicator). Perform a blank determination in the same manner.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.721 mg of $CaHPO_4$

◆**Containers and storage** Containers—Well-closed containers.◆

Dibasic Calcium Phosphate Hydrate

リン酸水素カルシウム水和物

$CaHPO_4.2H_2O$: 172.09
[*7789-77-7*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Dibasic Calcium Phosphate Hydrate contains not less than 98.0% and not more than 105.0% of dibasic calcium phosphate hydrate ($CaHPO_4.2H_2O$).

◆**Description** Dibasic Calcium Phosphate Hydrate occurs as a white crystalline powder.

It is practically insoluble in water and in ethanol (99.5).

It dissolves in dilute hydrochloric acid and in dilute nitric acid.◆

Identification **(1)** Dissolve 0.1 g of Dibasic Calcium Phosphate Hydrate in 10 mL of 2 mol/L hydrochloric acid TS by warming, add 2.5 mL of ammonia TS dropwise with shaking, and add 5 mL of ammonium oxalate TS: a white precipitate is produced.

(2) Dissolve 0.1 g of Dibasic Calcium Phosphate Hydrate in 5 mL of dilute nitric acid, and add 2 mL of hexaammonium heptamolybdate TS after warming at 70°C for 1 to 2 minutes: a yellow precipitate is produced.

Purity **(1)** Acid-insoluble substance—Dissolve 5.0 g of Dibasic Calcium Phosphate Hydrate in 40 mL of water and 10 mL of hydrochloric acid, and boil gently for 5 minutes. After cooling, collect the insoluble substance using a filter paper for assay. Wash with water until no more turbidity of the washing is produced when silver nitrate TS is added. Ignite to incinerate the residue and filter paper at 600 ± 50°C: the mass is not more than 10 mg (not more than 0.2%).

(2) Chloride—To 0.20 g of Dibasic Calcium Phosphate Hydrate add 20 mL of water and 13 mL of dilute nitric acid, dissolve by warming, if necessary, add water to make 100 mL, and filter, if necessary. Put 50 mL of this solution in a Nessler tube, and use this as the test solution. Transfer 0.70 mL of 0.01 mol/L hydrochloric acid VS to another Nessler tube, add 6 mL of dilute nitric acid and water to make 50 mL, and use this solution as the control solution. Add 1 mL of silver nitrate TS to the test solution and the control solution, mix well, and allow to stand for 5 minutes protecting from light. Compare the opalescence developed in both solutions against a black background by viewing downward or transversely. The opalescence developed in the test solution is not more than that of the control solution. (not more than 0.25%)

(3) Sulfate—Dissolve 0.50 g of Dibasic Calcium Phosphate Hydrate in 5 mL of water and 5 mL of dilute hydrochloric acid, add water to make 100 mL, and filter, if necessary. Put 20 mL of this solution in a Nessler tube, add 1 mL of dilute hydrochloric acid, and add water to make 50 mL, and use this as the test solution. Transfer 1.0 mL of 0.005 mol/L sulfuric acid VS to another Nessler tube, add 1 mL of dilute hydrochloride acid and water to make 50 mL, and use this solution as the control solution. Add 2 mL of barium chloride TS to the test solution and the control solution, mix well, and allow to stand for 10 minutes. Compare the white turbidity produced in both solutions against a black background by viewing downward or transversely. The turbidity produced in the test solution is not thicker than that of the control solution. (not more than 0.48%)

(4) Carbonate—Mix 1.0 g of Dibasic Calcium Phosphate Hydrate with 5 mL of freshly boiled and cooled water, and add immediately 2 mL of hydrochloric acid: no effervescence occurs.

◆**(5)** Heavy metals <*1.07*>—Dissolve 0.65 g of Dibasic Calcium Phosphate Hydrate in a mixture of 5 mL of water and 5 mL of dilute hydrochloric acid by warming, cool, and add ammonia TS until precipitates begin to form in the solution. Dissolve the precipitates by adding a small amount of dilute hydrochloric acid dropwise, add 10 mL of hydrochloric acid-ammonium acetate buffer solution (pH 3.5) and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 10 mL of hydrochloric acid-ammonium acetate buffer solution (pH 3.5) add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 31 ppm).◆

(6) Barium—Heat 0.5 g of Dibasic Calcium Phosphate Hydrate with 10 mL of water, add 1 mL of hydrochloric acid dropwise with stirring, and filter after cooling, if necessary. Add 2 mL of potassium sulfate TS to this solution, and allow to stand for 10 minutes: no turbidity forms.

◆**(7)** Arsenic <*1.11*>—Dissolve 1.0 g of Dibasic Calcium Phosphate Hydrate in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 2 ppm).◆

Loss on ignition <*2.43*> Not less than 24.5% and not more than 26.5% (1 g, 800 – 825°C, constant mass).

Assay Weigh accurately about 0.4 g of Dibasic Calcium Phosphate Hydrate, dissolve in 12 mL of dilute hydrochloric acid by warming on a water bath, if necessary, and add water to make exactly 200 mL. Pipet 20 mL of this solution, add exactly 25 mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution (pH 10.7), and titrate <*2.50*> the excess disodium dihydrogen ethylenediamine tetraacetate with 0.02 mol/L zinc sulfate VS (indicator: 25 mg of eriochrome black T-sodium chloride indicator). Perform a blank determination in the same manner.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 3.442 mg of $CaHPO_4.2H_2O$

◆**Containers and storage** Containers—Well-closed containers.◆

Monobasic Calcium Phosphate Hydrate

リン酸二水素カルシウム水和物

$Ca(H_2PO_4)_2.H_2O$: 252.07

Monobasic Calcium Phosphate Hydrate, when dried, contains not less than 90.0% of monobasic calcium phosphate hydrate [$Ca(H_2PO_4)_2.H_2O$].

Description Monobasic Calcium Phosphate Hydrate occurs as white, crystals or crystalline powder. It is odorless and has an acid taste.

It is sparingly soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid and in dilute nitric acid.

It is slightly deliquescent.

Identification **(1)** Dissolve 0.1 g of Monobasic Calcium Phosphate Hydrate in 10 mL of diluted hydrochloric acid (1 in 6) by warming, add 2.5 mL of ammonia TS dropwise with shaking, and add 5 mL of ammonium oxalate TS: a white precipitate is produced.

(2) Dissolve 0.1 g of Monobasic Calcium Phosphate Hydrate in 5 mL of dilute nitric acid, and add 2 mL of hexaammonium heptamolybdate TS after warming for 1 to 2 minutes at 70°C: a yellow precipitate is produced.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Monobasic Calcium Phosphate Hydrate in 19 mL of water and 2 mL of diluted hydrochloric acid (3 in 4), and heat on a water bath for 5 minutes with occasional shaking: the solution is clear and colorless.

(2) Dibasic phosphate and acid—Triturate 1.0 g of Monobasic Calcium Phosphate Hydrate with 3 mL of water, and add 100 mL of water and 1 drop of methyl orange TS: a red color develops. Then add 1.0 mL of 1 mol/L sodium hydroxide VS: the color changes to yellow.

(3) Chloride <*1.03*>—Dissolve 1.0 g of Monobasic Calcium Phosphate Hydrate in 20 mL of water and 12 mL of dilute nitric acid, add water to make 100 mL, and filter, if necessary. Perform the test using 50 mL of this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(4) Sulfate <*1.14*>—Dissolve 1.0 g of Monobasic Calcium Phosphate Hydrate in 20 mL of water and 1 mL of hydrochloric acid, add water to make 100 mL, and filter, if necessary. Perform the test using 50 mL of this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(5) Heavy metals <*1.07*>—Dissolve 0.65 g of Monobasic Calcium Phosphate Hydrate in a mixture of 5 mL of water and 5 mL of dilute hydrochloric acid by warming, cool, and add ammonia TS until precipitates begin to form in the solution. Dissolve the precipitates by adding a small amount of dilute hydrochloric acid dropwise, add 10 mL of hydrochloric acid-ammonium acetate buffer solution (pH 3.5) and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 10 mL of hydrochloric acid-ammonium acetate buffer solution (pH 3.5) add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 31 ppm).

(6) Arsenic <*1.11*>—Dissolve 1.0 g of Monobasic Calcium Phosphate Hydrate in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 2 ppm).

Loss on drying <*2.41*> Not more than 3.0% (1 g, silica gel, 24 hours).

Assay Weigh accurately about 0.4 g of Monobasic Calcium Phosphate Hydrate, previously dried, dissolve in 3 mL of dilute hydrochloric acid, and add water to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 25 mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution (pH 10.7), and titrate <*2.50*> the excess disodium dihydrogen ethylenediamine tetraacetate with 0.02 mol/L zinc acetate VS (indicator: 25 mg of eriochrome black T-sodium chloride indicator). Perform a blank determination.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 5.041 mg of $Ca(H_2PO_4)_2.H_2O$

Containers and storage Containers—Tight containers.

Calcium Polystyrene Sulfonate

ポリスチレンスルホン酸カルシウム

Calcium Polystyrene Sulfonate is a cation exchange resin prepared as the calcium form of the sulfonated styrene divinylbenzene copolymer.

When dried, it contains not less than 7.0% and not more than 9.0% of calcium (Ca: 40.08).

Each g of Calcium Polystyrene Sulfonate, when dried, exchanges with 53 to 71 mg of potassium (K: 39.10).

Description Calcium Polystyrene Sulfonate occurs as a pale yellowish white to light yellow powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

Identification **(1)** Determine the infrared absorption spectrum of Calcium Polystyrene Sulfonate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Mix 0.5 g of Calcium Polystyrene Sulfonate with 10 mL of dilute hydrochloric acid, filter, and neutralize the filtrate with ammonia TS: the solution responds to the Qualitative Tests <*1.09*> for calcium salt.

Purity **(1)** Ammonium—Place 1.0 g of Calcium Polystyrene Sulfonate in a flask, add 5 mL of sodium hydroxide TS, cover the flask with a watch glass having a moistened strip of red litmus paper on the underside, and boil for 15 minutes: the gas evolved does not change the red litmus paper to blue (not more than 5 ppm).

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Calcium Polystyrene Sulfonate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Calcium Polystyrene Sulfonate according to Method 3, and perform the test (not more than 2 ppm).

(4) Styrene—To 10.0 g of Calcium Polystyrene Sulfonate add 10 mL of acetone, shake for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of styrene in acetone to make exactly 100 mL. Pipet 1 mL of this solution, dilute with acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following conditions. Determine the peak heights, H_T and H_S, of styrene in each solution: H_T is not larger than H_S.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A stainless steel column 3 mm in inside diameter and 2 m in length, having polyethylene glycol 20 M coated at the ratio of 15% on siliceous earth for gas chromatography (150 to 180 μm in particle diameter).

Column temperature: A constant temperature of about

90°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the retention time of styrene is about 9 minutes.

System suitability—

System performance: Mix 10 mg of styrene with 1000 mL of acetone. When the procedure is run with 5 μL of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of styrene are not less than 800 and 0.8 to 1.2, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak heights of styrene is not more than 5%.

(5) Sodium—Pipet 2 mL of the 50-mL solution obtained in the Assay (1), add 0.02 mol/L hydrochloric acid TS to make exactly 500 mL, and use this solution as the sample solution. Separately, weigh accurately 0.2542 g of sodium chloride, previously dried at 130°C for 2 hours, and dissolve in 0.02 mol/L hydrochloric acid TS to make exactly 1000 mL. Pipet a suitable volume of this solution, and dilute with 0.02 mol/L hydrochloric acid TS to make a solution containing 1 to 3 μg of sodium (Na: 22.99) per mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions according to the Atomic Absorption Spectrophotometry <*2.23*> under the following conditions, and determine the amount of sodium in the sample solution using the calibration curve obtained from the standard solutions: the amount of sodium is not more than 1%.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.

Lamp: A sodium hollow-cathode lamp.

Wavelength: 589.0 nm.

Loss on drying <*2.41*> Not more than 10.0% (1 g, in vacuum, 80°C, 5 hours).

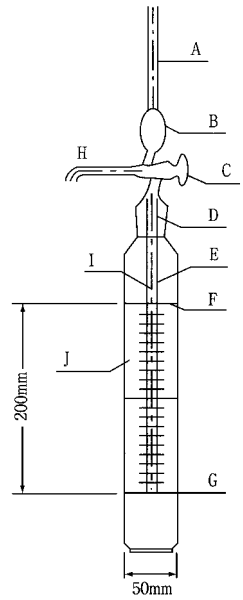
Microparticles (i) Apparatus: Use an apparatus as shown in the illustration.

(ii) Procedure: Weigh accurately about 5.5 g of Calcium Polystyrene Sulfonate, previously dried, add 300 mL of water of 25°C, and mix for 5 minutes. Transfer this turbid solution to the sedimentation tube J, keeping a temperature at 25°C, add water of 25°C to 2 mm below the mark F of 20 cm of the sedimentation tube J, and then insert the pipet. Open the two-way stopcock C, exhaust air, add exactly water from the vent-hole D to the mark F of 20 cm, and close the two-way stopcock C. Shake the apparatus well vertically and horizontally, disperse Calcium Polystyrene Sulfonate in water, and then open the two-way stopcock, and allow to stand at 25 ± 1°C for 5 hours and 15 minutes.

Then, draw exactly the meniscus of the turbid solution in sedimentation tube J up to the mark of pipet bulb A by suction, open the two-way stopcock C to the outlet of pipet H, and transfer exactly measured 20 mL of the turbid solution to a weighing bottle. Repeat the procedure, and combine exactly measured 20 mL of the turbid solution. Evaporate 20 mL of this turbid solution on a water bath to dryness, dry to constant mass at 105°C, and weigh the residue as M_S (g). Pipet 20 mL of used water, and weigh the residue in the same manner as M_B (g). Calculate the difference *mi* (g) between M_S and M_B, and calculate the amount of microparticles (S) by the following equation: the amount of microparticles is not more than 0.1%.

$$S\ (\%) = (mi \times V)/(20 \times M_T) \times 100$$

M_T: Amount (g) of Calcium Polystyrene Sulfonate taken



Actual volume to the mark of 20 cm at which the sedimentation tube is inserted: 550 mL
Single suction volume: 10 mL

A: Mark of pipet bulb
B: Pipet bulb for suction
C: Two-way stopcock
D: Vent-hole
E: Suction part of pipet
F: Mark of 20 cm
G: Base line of 0 cm
H: Outlet of pipet
I: Capillary tube of pipet
J: Sedimentation tube

Fig. Andreasen pipet

V: Actual volume (mL) to the mark of 20 cm at which the suction part of pipet is inserted

Assay (1) Calcium—Weigh accurately about 1 g of Calcium Polystyrene Sulfonate, previously dried, and disperse in 5 mL of 3 mol/L hydrochloric acid TS. Transfer this mixture, and wash out completely with the aid of a small quantity of 3 mol/L hydrochloric acid TS to a column 12 mm in inside diameter and 70 mm in length, packed with a pledged of fine glass wool in the bottom of it, placing a 50-mL volumetric flask as a receiver under the column. Then collect about 45 mL of eluate, adding 3 mol/L hydrochloric acid TS to the column, and add water to make exactly 50 mL. Pipet 20 mL of this solution, adjust with ammonia TS to a pH of exactly 10. Titrate <*2.50*> immediately with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the red-purple color of the solution disappears, and a blue color develops (indicator: 0.04 g eriochrome black T-sodium chloride indicator). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.004 mg of Ca

(2) Potassium exchange capacity—Pipet 50 mL of Standard Potassium Stock Solution into a glass-stoppered flask containing about 1 g of dried Calcium Polystyrene Sulfonate, accurately weighed, stir for 120 minutes, filter, and

discard the first 20 mL of the filtrate. Pipet 5 mL of the subsequent filtrate, and add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 10 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 1000 mL, and use this solution as the sample solution. Separately, measure exactly a suitable volume of Standard Potassium Stock Solution, dilute with 0.02 mol/L hydrochloric acid TS to make solutions containing 0.5 to 2.5 μg of potassium (K: 39.10) per mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions, and determine the amount, Y (mg), of potassium in 1000 mL of the sample solution, using the calibration curve obtained from the standard solutions. The exchange quantity for potassium per g of dried Calcium Polystyrene Sulfonate is 53 to 71 mg, calculating by the following equation.

Exchange quantity (mg) for potassium (K) per g of dried Calcium Polystyrene Sulfonate
$= (X - 100\ Y)/M$

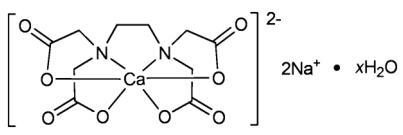
X: The amount (mg) of potassium in 50 mL of Standard Potassium Stock Solution before exchange
M: The amount (g) of dried Calcium Polystyrene Sulfonate taken

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: A potassium hollow-cathode lamp.
Wavelength: 766.5 nm.

Containers and storage Containers—Tight containers.

Calcium Sodium Edetate Hydrate

エデト酸カルシウムナトリウム水和物



$C_{10}H_{12}CaN_2Na_2O_8.xH_2O$
Disodium [{*N*,*N*′-ethane-1,2-diylbis[*N*-(carboxymethyl)glycinato]}(4-)-*N*,*N*′,*O*,*O*′,O^N,$O^{N'}$]calciate(2-) hydrate
[*23411-34-9*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Calcium Sodium Edetate Hydrate contains not less than 98.0% and not more than 102.0% of calcium disodium edetate ($C_{10}H_{12}CaN_2Na_2O_8$: 374.27), calculated on the anhydrous basis.

◆**Description** Calcium Sodium Edetate Hydrate occurs as white, powder or particles.

It is freely soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (99.5).

It is hygroscopic.◆

Identification (1) Dissolve 2 g of Calcium Sodium Edetate Hydrate in 10 mL of water, add 6 mL of a solution of lead (II) nitrate (33 in 1000), shake, and add 3 mL of potassium iodide TS: no yellow precipitate is formed. Make this solution alkaline by the addition of diluted ammonia solution (28) (7 in 50), and add 3 mL of ammonium oxalate TS: a white precipitate is formed.

◆**(2)** Determine the infrared absorption spectrum of Calcium Sodium Edetate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.◆

(3) A solution of Calcium Sodium Edetate Hydrate (1 in 20) responds to the Qualitative Tests <1.09> (2) for sodium salt.

pH <2.54> The pH of a solution of 2.0 g of Calcium Sodium Edetate Hydrate in 10 mL of water is 6.5 to 8.0.

Purity ◆**(1)** Clarity and color of solution—Dissolve 0.25 g of Calcium Sodium Edetate Hydrate in 10 mL of water: the solution is clear and colorless.◆

(2) Chloride <1.03>—Dissolve 0.70 g of Calcium Sodium Edetate Hydrate in water to make 20 mL. To this solution add 30 mL of dilute nitric acid, allow to stand for 30 minutes, and filter. To 10 mL of the filtrate add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.10%).

◆**(3)** Heavy metals <1.07>—Proceed with 1.0 g of Calcium Sodium Edetate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).◆

(4) Disodium edetate—Dissolve 1.00 g of Calcium Sodium Edetate Hydrate in 50 mL of water, add 5 mL of ammonia-ammonium chloride buffer solution (pH 10.7), and titrate <2.50> with 0.01 mol/L magnesium chloride VS until the color of the solution changes from blue to red-purple (indicator: 40 mg of eriochrome black T-sodium chloride indicator): the amount of 0.01 mol/L magnesium chloride VS consumed is not more than 3.0 mL (not more than 1.0%).

◆**(5)** Nitrilotriacetic acid—Conduct this procedure using light-resistant vessels. Dissolve 0.100 g of Calcium Sodium Edetate Hydrate in diluting solution to make exactly 25 mL, and use this solution as the sample solution. Separately, dissolve 40.0 mg of nitrilotriacetic acid in diluting solution to make exactly 100 mL. Pipet 1 mL of this solution, add 0.1 mL of the sample solution, then add diluting solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of nitrilotriacetic acid in each solution: A_T is not larger than A_S (not more than 0.1%).

Diluting solution: Dissolve 10.0 g of iron (III) sulfate *n*-hydrate in 20 mL of 0.5 mol/L sulfuric acid TS and 780 mL of water, adjust to pH 2.0 with sodium hydroxide TS, and add water to make 1000 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 273 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with graphite carbon for liquid chromatography (mean pore size: 25 nm, specific surface: 120 m^2/g, 5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 50.0 mg of iron (III) sulfate *n*-hydrate in 50 mL of 0.5 mol/L sulfuric acid TS, add 750 mL of water, adjust to pH 1.5 with 0.5 mol/L sulfuric acid TS or sodium hydroxide TS, and add 20 mL of ethylene glycol and

water to make 1000 mL.

Flow rate: 1.0 mL per minute (the retention time of nitrilotriacetic acid is about 5 minutes).

System suitability—

Test for required detectability: When perform the test with 20 μL of the standard solution under the above operating conditions, the SN ratio of the peak of nitrilotriacetic acid is not less than 50.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, nitrilotriacetic acid and edetic acid are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nitrilotriacetic acid is not more than 1.0%.◆

Water ⟨*2.48*⟩ 5.0 – 13.0% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.5 g of Calcium Sodium Edetate Hydrate, and dissolve in water to make exactly 200 mL. Pipet 20 mL of this solution, add 80 mL of water, adjust to pH 2 – 3 with dilute nitric acid, and titrate ⟨*2.50*⟩ with 0.01 mol/L bismuth nitrate VS until the color of the solution changes from yellow to red (indicator: 2 drops of xylenol orange TS).

Each mL of 0.01 mol/L bismuth nitrate VS
= 3.743 mg of $C_{10}H_{12}CaN_2Na_2O_8$

◆**Containers and storage** Containers—Tight containers.◆

Calcium Stearate

ステアリン酸カルシウム

Calcium Stearate mainly consists of calcium salts of stearic acid ($C_{18}H_{36}O_2$: 284.48) and palmitic acid ($C_{16}H_{32}O_2$: 256.42).

Calcium Stearate, when dried, contains not less than 6.4% and not more than 7.1% of calcium (Ca: 40.08).

Description Calcium Stearate occurs as a white, light, bulky powder. It feels smooth when touched, and is adhesive to the skin. It is odorless or has a faint, characteristic odor.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

Identification **(1)** Shake vigorously 3 g of Calcium Stearate with 20 mL of diluted hydrochloric acid (1 in 2) and 30 mL of diethyl ether for 3 minutes, and allow to stand: the separated aqueous layer responds to the Qualitative Tests ⟨*1.09*⟩ (1), (2) and (4) for calcium salt.

(2) Wash the diethyl ether layer obtained in (1) with 20 mL and 10 mL of dilute hydrochloric acid and 20 mL of water successively, and evaporate the diethyl ether on a water bath: the residue melts ⟨*1.13*⟩ at a temperature not below 54°C.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Heat gently 1.0 g of Calcium Stearate with caution at the beginning, and heat further, gradually raising the temperature, to incineration. After cooling, add 2 mL of hydrochloric acid, evaporate on a water bath to dryness, warm the residue with 20 mL of water and 2 mL of dilute acetic acid for 2 minutes, cool, filter, and wash the residue with 15 mL of water. Combine the filtrate and the washings, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution by evaporating 2 mL of hydrochloric acid on a water bath to dryness and by adding 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(2) Arsenic ⟨*1.11*⟩—To 1.0 g of Calcium Stearate add 5 mL of diluted hydrochloric acid (1 in 2) and 20 mL of chloroform, shake vigorously for 3 minutes, allow to stand, and separate the water layer. Perform the test with the water layer as the test solution (not more than 2 ppm).

Loss on drying ⟨*2.41*⟩ Not more than 4.0% (1 g, 105°C, 3 hours).

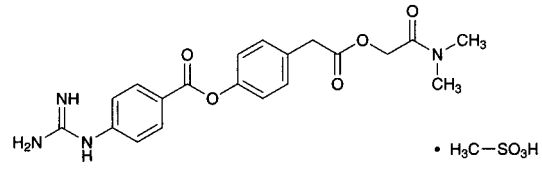
Assay Weigh accurately about 0.5 g of Calcium Stearate, previously dried, heat gently with caution at first, and then ignite gradually to ash. Cool, add 10 mL of dilute hydrochloric acid to the residue, warm for 10 minutes on a water bath, and transfer the contents to a flask with the aid of 10-mL, 10-mL, and 5-mL portions of hot water. Add sodium hydroxide TS until the solution becomes slightly turbid, and then add 25 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, 10 mL of ammonia-ammonium chloride buffer solution (pH 10.7), 4 drops of eriochrome black T TS and 5 drops of methyl yellow TS, and titrate ⟨*2.50*⟩ rapidly the excess disodium dihydrogen ethylenediamine tetraacetate with 0.05 mol/L magnesium chloride VS, until the green color of the solution disappears and a red color develops. Perform a blank determination.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.004 mg of Ca

Containers and storage Containers—Well-closed containers.

Camostat Mesilate

カモスタットメシル酸塩



$C_{20}H_{22}N_4O_5.CH_4O_3S$: 494.52
Dimethylcarbamoylmethyl
4-(4-guanidinobenzoyloxy)phenylacetate
monomethanesulfonate
[*59721-29-8*]

Camostat Mesilate, when dried, contains not less than 98.5% of camostat mesilate ($C_{20}H_{22}N_4O_5.CH_4O_3S$).

Description Camostat Mesilate occurs as white, crystals or crystalline powder.

It is sparingly soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification **(1)** To 4 mL of a solution of Camostat Mesilate (1 in 2000) add 2 mL of 1-naphthol TS and 1 mL of diacetyl TS, and allow to stand for 10 minutes: a red color develops.

(2) Determine the absorption spectrum of a solution of Camostat Mesilate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spec-

trum with the Reference Spectrum or the spectrum of a solution of Camostat Mesilate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) A 0.1 g portion of Camostat Mesilate responds to the Qualitative Tests <*1.09*> (1) for mesilate.

Melting point <*2.60*> 194 – 198°C

Purity **(1)** Heavy metals <*1.07*>—Dissolve 1.0 g of Camostat Mesilate in 40 mL of water by warming, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid (not more than 20 ppm).

(2) Arsenic <*1.11*>—Dissolve 2.0 g of Camostat Mesilate in 20 mL of 2 mol/L hydrochloric acid TS by heating in a water bath, and continue to heat for 20 minutes. After cooling, centrifuge, take 10 mL of the supernatant liquid, and use this solution as the test solution. Perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 30 mg of Camostat Mesilate in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand overnight in iodine vapor: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 1.0% (1 g, silica gel, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately about 50 mg each of Camostat Mesilate and Camostat Mesilate RS, previously dried, and dissolve each in water to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 2 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of camostat to that of the internal standard.

Amount (mg) of camostat mesilate ($C_{20}H_{22}N_4O_5.CH_4O_3S$) $= M_S \times Q_T/Q_S$

M_S: Amount (mg) of Camostat Mesilate RS taken

Internal standard solution—A solution of butyl parahydroxybenzoate in ethanol (95) (1 in 1500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol, a solution of sodium 1-heptane sulfonate (1 in 500), a solution of sodium lauryl sulfate (1 in 1000) and acetic acid (100) (200:100:50:1).

Flow rate: Adjust so that the retention time of camostat is about 10 minutes.

System suitability—

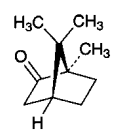
System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, camostat and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of camostat to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

d-Camphor

d-カンフル



$C_{10}H_{16}O$: 152.23
(1*R*,4*R*)-1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one
[*464-49-3*]

d-Camphor contains not less than 96.0% of *d*-camphor ($C_{10}H_{16}O$).

Description *d*-Camphor occurs as colorless or white, translucent crystals, crystalline powder or masses. It has a characteristic, agreeable odor, and a slightly bitter taste, followed by a pleasant, cooling sensation.

It is freely soluble in ethanol (95), in diethyl ether and in carbon disulfide, and slightly soluble in water.

It slowly volatilizes at room temperature.

Identification Dissolve 0.1 g of *d*-Camphor in 2 mL of methanol, add 1 mL of 2,4-dinitrophenylhydradine TS, and heat for 5 minutes on a water bath: an orange-red precipitate is formed.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +41.0 – +43.0° (5 g, ethanol (95), 50 mL, 100 mm).

Melting point <*2.60*> 177 – 182°C

Purity **(1)** Water—Shake 1.0 g of *d*-Camphor with 10 mL of carbon disulfide: the solution is clear.

(2) Chlorinated compounds—Mix 0.20 g of finely powdered *d*-Camphor with 0.4 g of sodium peroxide in a dried porcelain crucible. Heat the crucible gently by the open flame until the incineration is complete. Dissolve the residue in 20 mL of warm water, acidify with 12 mL of dilute nitric acid, and filter the solution into a Nessler tube. Wash the filter paper with three 5-mL portions of hot water, adding the washings to the filtrate. After cooling, add water to make 50 mL, then add 1 mL of silver nitrate TS, mix well, and allow to stand for 5 minutes: the turbidity of the solution does not exceed that of the following control solution.

Control solution: Prepare in the same manner as described above, using 0.20 mL of 0.01 mol/L hydrochloric acid VS.

(3) Non-volatile residue—Heat 2.0 g of *d*-Camphor on a water bath until sublimation is complete, then dry the residue at 105°C for 3 hours: the mass of the residue does not exceed 1.0 mg.

Assay Weigh accurately about 0.1 g each of *d*-Camphor and *d*-Camphor RS, add exactly 5 mL each of the internal standard solution, dissolve in ethanol (99.5) to make 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of *d*-camphor to that of the internal standard.

Amount (mg) of *d*-camphor ($C_{10}H_{16}O$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of *d*-Camphor RS taken

Internal standard solution—A solution of methyl salicylate in ethanol (99.5) (1 in 25).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 3 m in length, which is packed with 10% of polyethylene glycol 20 M for gas chromatography supported on 180 to 250 μm mesh silanized siliceous earth for gas chromatography.

Column temperature: A constant temperature of about 160°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the retention time of *d*-camphor is about 6 minutes.

System suitability—

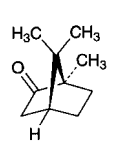
System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, *d*-camphor and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of *d*-camphor to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

dl-Camphor

dl-カンフル



and enantiomer

$C_{10}H_{16}O$: 152.23

(1*RS*,4*RS*)-1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one

[*76-22-2*]

dl-Camphor contains not less than 96.0% of *dl*-camphor ($C_{10}H_{16}O$).

Description *dl*-Camphor occurs as colorless or white, translucent crystals, crystalline powder or masses. It has a characteristic, agreeable odor, and has a slightly bitter taste followed by a pleasant, cooling sensation.

It is freely soluble in ethanol (95), in diethyl ether and in carbon disulfide, and slightly soluble in water.

It slowly volatilizes at room temperature.

Identification Dissolve 0.1 g of *dl*-Camphor in 2 mL of methanol, add 1 mL of 2,4-dinitrophenylhydradine TS, and heat for 5 minutes on a water bath: an orange-red precipitate is formed.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -1.5 – $+1.5°$ (5 g, ethanol (95), 50 mL, 100 mm).

Melting point <*2.60*> 175 – 180°C

Purity **(1)** Water—Shake 1.0 g of *dl*-Camphor with 10 mL of carbon disulfide: the solution is clear.

(2) Chlorinated compounds—Mix 0.20 g of finely powdered *dl*-Camphor with 0.4 g of sodium peroxide in a dried porcelain crucible. Heat the crucible gently by the open flame until the incineration is complete. Dissolve the residue in 20 mL of warm water, acidify with 12 mL of dilute nitric acid, and filter the solution into a Nessler tube. Wash the filter paper with three 5-mL portions of hot water, adding the washings to the filtrate. After cooling, add water to make 50 mL, then add 1 mL of silver nitrate TS, mix well, and allow to stand for 5 minutes: the turbidity of the solution does not exceed that of the following control solution.

Control solution: Prepare in the same manner as described above, using 0.20 mL of 0.01 mol/L hydrochloric acid VS.

(3) Non-volatile residue—Heat 2.0 g of *dl*-Camphor on a water bath until sublimation is complete, then dry the residue at 105°C for 3 hours: the mass of the residue does not exceed 1.0 mg.

Assay Weigh accurately about 0.1 g each of *dl*-Camphor and *dl*-Camphor RS, add exactly 5 mL each of the internal standard solution, dissolve in ethanol (99.5) to make 100 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of *dl*-camphor to that of the internal standard.

Amount (mg) of *dl*-camphor ($C_{10}H_{16}O$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of *dl*-Camphor RS taken

Internal standard solution—A solution of methyl salicylate in ethanol (99.5) (1 in 25).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 3 m in length, which is packed with 10% of polyethylene glycol 20 M for gas chromatography supported on 180 to 250 μm mesh silanized siliceous earth for gas chromatography.

Column temperature: A constant temperature of about 160°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the retention time of *dl*-camphor is about 6 minutes.

System suitability—

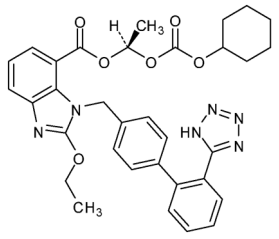
System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, *dl*-camphor and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of *dl*-camphor to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Candesartan Cilexetil

カンデサルタン シレキセチル



$C_{33}H_{34}N_6O_6$: 610.66
(1*RS*)-1-(Cyclohexyloxycarbonyloxy)ethyl-2-ethoxy-1-{[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-1*H*-benzimidazole-7-carboxylate
[*145040-37-5*]

Candesartan Cilexetil contains not less than 99.0% and not more than 101.0% of candesartan cilexetil ($C_{33}H_{34}N_6O_6$), calculated on the anhydrous basis.

Description Candesartan Cilexetil occurs as white crystals or a white crystalline powder.

It is soluble in acetic acid (100), sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Candesartan Cilexetil in methanol (1 in 100) shows no optical rotation.

Candesartan Cilexetil shows crystal polymorphism.

Identification **(1)** Determine the absorption spectrum of a solution of Candesartan Cilexetil in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Candesartan Cilexetil as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample and the RS according to the method otherwise specified, filter and dry the crystals, and perform the test with the crystals.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Candesartan Cilexetil according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 20 mg of Candesartan Cilexetil in 50 mL of a mixture of acetonitrile and water (3:2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetonitrile and water (3:2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peaks, having the relative retention time of about 0.4 and about 2.0 to candesartan cilexetil, obtained from the sample solution is not larger than 1/5 times the peak area of candesartan cilexetil obtained from the standard solution, the area of the peak, having the relative retention time of about 0.5 to candesartan cilexetil, from the sample solution is not larger than 3/10 times the peak area of candesartan cilexetil from the standard solution, the area of the peak other than candesartan cilexetil and the peaks mentioned above from the sample solution is not smaller than 1/10 times the peak area of candesartan cilexetil from the standard solution, and the total area of the peaks other than candesartan cilexetil from the sample solution is not larger than 3/5 times the peak area of candesartan cilexetil from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of acetonitrile, water and acetic acid (100) (57:43:1).

Mobile phase B: A mixture of acetonitrile, water and acetic acid (100) (90:10:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 30	100 → 0	0 → 100

Flow rate: 0.8 mL per minute.

Time span of measurement: For 30 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add a mixture of acetonitrile and water (3:2) to make exactly 20 mL. Confirm that the peak area of candesartan cilexetil obtained with 10 μL of this solution is equivalent to 7 to 13% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of candesartan cilexetil are not less than 12,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of candesartan cilexetil is not more than 2.0%.

Water <*2.48*> Not more than 0.3% (0.5 g, coulometric titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Candesartan Cilexetil, dissolve in 60 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 61.07 mg of $C_{33}H_{34}N_6O_6$

Containers and storage Containers—Well-closed containers.

Candesartan Cilexetil Tablets

カンデサルタン　シレキセチル錠

Candesartan Cilexetil Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of candesartan cilexetil ($C_{33}H_{34}N_6O_6$: 610.66).

Method of preparation Prepare as directed under Tablets, with Candesartan Cilexetil.

Identification Powder Candesartan Cilexetil Tablets. To a portion of the powder, equivalent to 1 mg of Candesartan Cilexetil, add 50 mL of methanol, shake vigorously for 10 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits absorption maxima between 252 nm and 256 nm and between 302 nm and 307 nm.

Purity Related substances—Powder not less than 10 Candesartan Cilexetil Tablets. To a portion of the powder, equivalent to 6 mg of Candesartan Cilexetil, add 15 mL of a mixture of acetonitrile and water (3:2), shake vigorously for 10 minutes, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetonitrile and water (3:2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak having the relative retention time of about 0.5 to candesartan cilexetil obtained from the sample solution is not larger than 1.5 times the peak area of candesartan cilexetil obtained from the standard solution, the area of the peak having the relative retention time of about 0.8, about 1.1 and about 1.5 to candesartan cilexetil from the sample solution is not larger than 1/2 times the peak area of candesartan cilexetil from the standard solution, the area of the peak having the relative retention time of about 2.0 to candesartan cilexetil from the sample solution is not larger than the peak area of candesartan cilexetil from the standard solution, the area of the peak other than candesartan cilexetil, the peak having the relative retention time of about 0.4 to candesartan cilexetil and the peaks mentioned above from the sample solution is smaller than 1/10 times the peak area of candesartan cilexetil from the standard solution, and the total area of the peaks other than candesartan cilexetil from the sample solution is not larger than 4 times the peak area of candesartan cilexetil from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of acetonitrile, water and acetic acid (100) (57:43:1).

Mobile phase B: A mixture of acetonitrile, water and acetic acid (100) (90:10:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 30	100 → 0	0 → 100

Flow rate: 0.8 mL per minute.

Time span of measurement: For 30 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add a mixture of acetonitrile and water (3:2) to make exactly 20 mL. Confirm that the peak area of candesartan cilexetil obtained with 10 μL of this solution is equivalent to 7 to 13% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of candesartan cilexetil are not less than 12,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of candesartan cilexetil is not more than 2.0%.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Candesartan Cilexetil Tablets add 30 mL of a mixture of acetonitrile and water (3:2), shake vigorously for 20 minutes, then add a mixture of acetonitrile and water (3:2) to make exactly V mL so that each mL contains about 40 μg of candesartan cilexetil ($C_{33}H_{34}N_6O_6$), centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of candesartan cilexetil for assay (separately determine the water <*2.48*> in the same manner as Candesartan Cilexetil), and dissolve in acetonitrile to make exactly 50 mL. Pipet 4 mL of this solution, add a mixture of acetonitrile and water (3:2) to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 305 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

$$\text{Amount (mg) of candesartan cilexetil } (C_{33}H_{34}N_6O_6) = M_S \times A_T/A_S \times V/1250$$

M_S: Amount (mg) of candesartan cilexetil for assay taken, calculated on the anhydrous basis

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of a solution of polysorbate 20 (1 in 100) as the dissolution medium, the dissolution rate in 45 minutes of Candesartan Cilexetil Tablets is not less than 75%.

Start the test with 1 tablet of Candesartan Cilexetil Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 2.2 μg of candesartan cilexetil ($C_{33}H_{34}N_6O_6$), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of candesartan cilexetil for assay (separately determine the water <*2.48*> in the same manner as Candesartan Cilexetil), and dissolve in acetonitrile to make exactly 50 mL. Pipet 5 mL of this solution, add acetonitrile to make exactly 50 mL. Pipet 1

mL of this solution, add the dissolution medium to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01>, and determine the peak areas, A_T and A_S, of candesartan cilexetil in each solution.

Dissolution rate (%) with respect to the labeled amount of candesartan cilexetil ($C_{33}H_{34}N_6O_6$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18/5$

M_S: Amount (mg) of candesartan cilexetil for assay taken, calculated on the anhydrous basis
C: Labeled amount (mg) of candesartan cilexetil ($C_{33}H_{34}N_6O_6$) in 1 tablet

Operating conditions—
Proceed as directed in the operating conditions in the Assay.
System suitability—
System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of candesartan cilexetil are not less than 7000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of candesartan cilexetil is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Candesartan Cilexetil Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 6 mg of candesartan cilexetil ($C_{33}H_{34}N_6O_6$), add exactly 15 mL of the internal standard solution, then add a mixture of acetonitrile and water (3:2) to make 150 mL, shake vigorously for 10 minutes, and allow to stand. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of candesartan cilexetil for assay (separately determine the water <2.48> in the same manner as Candesartan Cilexetil), dissolve in acetonitrile to make exactly 50 mL. Pipet 4 mL of this solution, add exactly 10 mL of the internal standard solution, then add a mixture of acetonitrile and water (3:2) to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of candesartan cilexetil to that of the internal standard.

Amount (mg) of candesartan cilexetil ($C_{33}H_{34}N_6O_6$)
$= M_S \times Q_T/Q_S \times 3/25$

M_S: Amount (mg) of candesartan cilexetil for assay taken, calculated on the anhydrous basis

Internal standard solution—A solution of acenaphthene in acetonitrile (1 in 800).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 3.9 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of acetonitrile, water and acetic acid (100) (57:43:1).
Flow rate: Adjust so that the retention time of candesartan cilexetil is about 13 minutes.
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and candesartan cilexetil are eluted in this order with the resolution between these peaks being not less than 5.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of candesartan cilexetil to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Candesartan Cilexetil and Amlodipine Besylate Tablets

カンデサルタン シレキセチル·アムロジピンベシル酸塩錠

Candesartan Cilexetil and Amlodipine Besylate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of candesartan cilexetil ($C_{33}H_{34}N_6O_6$: 610.66) and amlodipine besylate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$: 567.05).

Method of preparation Prepare as directed under Tablets, with Candesartan Cilexetil and Amlodipine Besylate.

Identification (1) Shake thoroughly a quantity of powdered Candesartan Cilexetil and Amlodipine Besylate Tablets, equivalent to 8 mg of Candesartan Cilexetil, with 20 mL of 0.01 mol/L hydrochloric acid TS, and centrifuge. Remove the supernatant liquid, to the residue add 20 mL of 0.01 mol/L hydrochloric acid TS, shake thoroughly, and centrifuge. Remove the supernatant liquid, to the residue add 40 mL of methanol, shake thoroughly, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To 5 mL of the filtrate add methanol to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 252 nm and 256 nm, and between 302 nm and 307 nm.
(2) Shake thoroughly a quantity of powdered Candesartan Cilexetil and Amlodipine Besylate Tablets, equivalent to 2.5 mg of Amlodipine Besylate, with 20 mL of 0.01 mol/L hydrochloric acid TS, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. To 5 mL of the filtrate add methanol to make 25 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 236 nm and 240 nm, and between 360 nm and 364 nm.

Purity Related substances—Shake vigorously for 20 minutes a quantity of powdered Candesartan Cilexetil and Amlodipine Besylate Tablets, equivalent to 8 mg of Candesartan Cilexetil, with 20 mL of diluting solution, and filter this solution through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 1 mL of the sample solution, add diluting solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chroma-

tography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.8 to candesartan cilexetil, obtained from the sample solution is not larger than 1.5 times the peak area of candesartan cilexetil obtained from the standard solution, the area of the peaks, having a relative retention time of about 0.9, about 1.1 and about 1.2 from the sample solution is not larger than 1/2 times the peak area of candesartan cilexetil from the standard solution, the area of the peak, having a relative retention time of about 1.4 from the sample solution, is not larger than the peak area of candesartan cilexetil from the standard solution, and the area of the peak other than candesartan cilexetil and the peaks mentioned above from the sample solution is smaller than 1/10 times the peak area of candesartan cilexetil from the standard solution. Furthermore, the total area of the peaks other than candesartan cilexetil from the sample solution is not larger than 4 times the peak area of candesartan cilexetil from the standard solution.

Diluting solution: To 3.5 mL of triethylamine add water to make 500 mL, and adjust to pH 3.0 with phosphoric acid. To 400 mL of this solution add 600 mL of acetonitrile.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 253 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of water, acetonitrile and trifluoroacetic acid (4000:1000:1).

Mobile phase B: A mixture of acetonitrile, water and trifluoroacetic acid (4000:1000:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 15	100 → 50	0 → 50
15 – 50	50 → 0	50 → 100
50 – 60	0	100

Flow rate: 1.0 mL per minute.

Time span of measurement: For 60 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add diluting solution to make exactly 50 mL. Confirm that the peak area of candesartan cilexetil obtained with 20 μL of this solution is equivalent to 1.4 to 2.6% of that obtained with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of candesartan cilexetil are not less than 100,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of candesartan cilexetil is not more than 2.0%.

Uniformity of dosage units <6.02> Perform the test according to the following methods: it meets the requirements of the Content uniformity test.

(1) Candesartan cilexetil—To 1 tablet of Candesartan Cilexetil and Amlodipine Besylate Tablets add exactly 20 mL of diluting solution, shake for 20 minutes to disintegrate the tablet, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet V mL of the subsequent filtrate, add exactly $V'/5$ mL of the internal standard solution, then add diluting solution to make V' mL so that each mL contains about 0.16 mg of candesartan cilexetil ($C_{33}H_{34}N_6O_6$), and use this solution as the sample solution. Then, proceed as directed in the Assay (1).

$$\text{Amount (mg) of candesartan cilexetil } (C_{33}H_{34}N_6O_6) = M_S \times Q_T/Q_S \times V'/V \times 2/25$$

M_S: Amount (mg) of candesartan cilexetil for assay taken, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in diluting solution (1 in 2500).

Diluting solution: To 3.5 mL of triethylamine add water to make 500 mL, and adjust to pH 3.0 with phosphoric acid. To 400 mL of this solution add 600 mL of acetonitrile.

(2) Amlodipine besylate—To 1 tablet of Candesartan Cilexetil and Amlodipine Besylate Tablets add exactly 20 mL of diluting solution, shake for 20 minutes to disintegrate the tablet, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet V mL of the subsequent filtrate, add exactly $V'/5$ mL of the internal standard solution, then add diluting solution to make V' mL so that each mL contains about 70 μg of amlodipine besylate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$), and use this solution as the sample solution. Then, proceed as directed in the Assay (2).

$$\text{Amount (mg) of amlodipine besylate } (C_{20}H_{25}ClN_2O_5.C_6H_6O_3S) = M_S \times Q_T/Q_S \times V'/V \times 1/25$$

M_S: Amount (mg) of Amlodipine Besylate RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in diluting solution (1 in 2500).

Diluting solution: To 3.5 mL of triethylamine add water to make 500 mL, and adjust to pH 3.0 with phosphoric acid. To 400 mL of this solution add 600 mL of acetonitrile.

Dissolution <6.10> **(1)** Candesartan cilexetil—When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of a solution, prepared by dissolving 1 g of polysorbate 80 in 2nd fluid for dissolution test to make 1000 mL, as the dissolution medium, the dissolution rate in 45 minutes of Candesartan Cilexetil and Amlodipine Besylate Tablets is not less than 80%.

Start the test with 1 tablet of Candesartan Cilexetil and Amlodipine Besylate Tablets, withdraw not less than 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 8.9 μg of candesartan cilexetil ($C_{33}H_{34}N_6O_6$), and use this solution as the sample solution. Separately, weigh accurately about 45 mg of candesartan cilexetil for assay (separately, determine the water <2.48> in the same manner as Candesartan Cilexetil), and dissolve in acetonitrile to make exactly 50 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly

20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of candesartan cilexetil in each solution.

Dissolution rate (%) with respect to the labeled amount of candesartan cilexetil ($C_{33}H_{34}N_6O_6$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of candesartan cilexetil for assay taken, calculated on the anhydrous basis

C: Labeled amount (mg) of candesartan cilexetil ($C_{33}H_{34}N_6O_6$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetonitrile, water and acetic acid (100) (57:43:1).

Flow rate: Adjust so that the retention time of candesartan cilexetil is about 6.5 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of candesartan cilexetil are not less than 2000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of candesartan cilexetil is not more than 1.0%.

(2) Amlodipine besylate—When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 0.05 mol/L acetic acid-sodium acetate buffer solution (pH 4.0) as the dissolution medium, the dissolution rate in 30 minutes of Candesartan Cilexetil and Amlodipine Besylate Tablets is not less than 80%.

Start the test with 1 tablet of Candesartan Cilexetil and Amlodipine Besylate Tablets, withdraw not less than 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 3.9 μg of amlodipine besylate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$), and use this solution as the sample solution. Separately, weigh accurately about 39 mg of Amlodipine Besylate RS (separately, determine the water <2.48> in the same manner as Amlodipine Besylate), and dissolve in acetonitrile to make exactly 50 mL. Pipet 5 mL of this solution, add acetonitrile to make exactly 50 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of amlodipine in each solution.

Dissolution rate (%) with respect to the labeled amount of amlodipine besylate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9$

M_S: Amount (mg) of Amlodipine Besylate RS taken, calculated on the anhydrous basis

C: Labeled amount (mg) of amlodipine besylate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$) in 1 tablet

Operating conditions—

Detector, column, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay (1).

Flow rate: Adjust so that the retention time of amlodipine is about 4 minutes.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amlodipine are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amlodipine is not more than 1.0%.

Assay (1) Candesartan cilexetil—Weigh accurately the mass of not less than 20 Candesartan Cilexetil and Amlodipine Besylate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 8 mg of candesartan cilexetil ($C_{33}H_{34}N_6O_6$), add exactly 20 mL of diluting solution, shake vigorously for 20 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution, then add diluting solution to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of candesartan cilexetil for assay (separately, determine the water <2.48> in the same manner as Candesartan Cilexetil), dissolve in diluting solution to make exactly 100 mL, and use this solution as the candesartan cilexetil standard stock solution. Pipet 10 mL of the candesartan cilexetil standard stock solution, add exactly 5 mL of the internal standard solution, then add diluting solution to make 25 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of candesartan cilexetil to that of the internal standard.

Amount (mg) of candesartan cilexetil ($C_{33}H_{34}N_6O_6$)
$= M_S \times Q_T/Q_S \times 1/5$

M_S: Amount (mg) of candesartan cilexetil for assay taken, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in diluting solution (1 in 2500).

Diluting solution: To 3.5 mL of triethylamine add water to make 500 mL, and adjust to pH 3.0 with phosphoric acid. To 400 mL of this solution add 600 mL of acetonitrile.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 238 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 7 mL of triethylamine add water to make 1000 mL, and adjust to pH 6.5 with phosphoric acid. To 800 mL of this solution add 500 mL of acetonitrile.

Flow rate: Adjust so that the retention time of candesartan cilexetil is about 31 minutes.

System suitability—

System performance: Mix 10 mL of the candesartan cilexetil standard stock solution and 5 mL of the amlodipine besylate standard stock solution prepared in the Assay (2), add 5 mL of the internal standard solution, then add diluting solution to make 25 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, amlodipine, the internal standard and candesartan cilexetil are eluted in this order and the resolution between the peaks of the internal standard and candesartan cilexetil is not less than 15.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of candesartan cilexetil to that of the internal standard is not more than 1.0%.

(2) Amlodipine besylate—Weigh accurately the mass of not less than 20 Candesartan Cilexetil and Amlodipine Besylate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 3.5 mg of amlodipine besylate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$), add exactly 20 mL of diluting solution, shake vigorously for 20 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution, then add diluting solution to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 35 mg of Amlodipine Besylate RS (separately, determine the water <2.48> in the same manner as Amlodipine Besylate), dissolve in diluting solution to make exactly 100 mL, and use this solution as the amlodipine besylate standard stock solution. Pipet 5 mL of the amlodipine besylate standard stock solution, add exactly 5 mL of the internal standard solution, then add diluting solution to make 25 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of amlodipine to that of the internal standard.

$$\text{Amount (mg) of amlodipine besylate } (C_{20}H_{25}ClN_2O_5.C_6H_6O_3S) = M_S \times Q_T/Q_S \times 1/10$$

M_S: Amount (mg) of Amlodipine Besylate RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in diluting solution (1 in 2500).

Diluting solution: To 3.5 mL of triethylamine add water to make 500 mL, and adjust to pH 3.0 with phosphoric acid. To 400 mL of this solution add 600 mL of acetonitrile.

Operating conditions—

Detector, column, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay (1).

Flow rate: Adjust so that the retention time of amlodipine is about 2.5 minutes.

System suitability—

System performance: Mix 10 mL of the candesartan cilexetil standard stock solution prepared in the Assay (1) and 5 mL of the amlodipine besylate standard stock solution, add 5 mL of the internal standard solution, then add diluting solution to make 25 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, amlodipine, the internal standard and candesartan cilexetil are eluted in this order and the resolution between the peaks of amlodipine and the internal standard is not less than 15.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of amlodipine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Candesartan Cilexetil and Hydrochlorothiazide Tablets

カンデサルタン シレキセチル·ヒドロクロロチアジド錠

Candesartan Cilexetil and Hydrochlorothiazide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of candesartan cilexetil ($C_{33}H_{34}N_6O_6$: 610.66) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$: 297.74).

Method of preparation Prepare as directed under Tablets, with Candesartan Cilexetil and Hydrochlorothiazide.

Identification **(1)** To an amount of powdered Candesartan Cilexetil and Hydrochlorothiazide Tablets, equivalent to 4 mg of Candesartan Cilexetil, add 5 mL of acetone, shake thoroughly, centrifuge, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Evaporate the filtrate to dryness with the aid of a current of nitrogen. Dissolve the residue in 0.5 mL of acetone, and use this solution as the sample solution. Separately, dissolve 40 mg of candesartan cilexetil in 5 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and acetic acid (100) (10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the *R*f value of the spot having a larger *R*f value among the spots obtained from the sample solution is the same with that of the spot obtained from the standard solution.

(2) To an amount of powdered Candesartan Cilexetil and Hydrochlorothiazide Tablets, equivalent to 6.25 mg of Hydrochlorothiazide, add 5 mL of acetone, shake thoroughly, centrifuge, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Evaporate the filtrate to dryness with the aid of a current of nitrogen. Dissolve the residue in 0.5 mL of acetone, and use this solution as the sample solution. Separately, dissolve 50 mg of hydrochlorothiazide in 4 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and acetic acid (100) (10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the *R*f value of the spot having a smaller *R*f value among the spots obtained from the sample solution is the same with that of the spot obtained from the standard solution.

Purity Related substances—(i) To an amount of powdered Candesartan Cilexetil and Hydrochlorothiazide Tablets, equivalent to 4 mg of Candesartan Cilexetil, add 10 mL of a

mixture of acetonitrile and water (3:2), shake vigorously for 10 minutes, centrifuge, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetonitrile and water (3:2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having a relative retention time of about 0.5 to candesartan cilexetil, obtained from the sample solution is not larger than 1.5 times the peak area of candesartan cilexetil obtained from the standard solution, the area of the peak, having a relative retention time of about 0.8, about 1.1 and about 1.5, from the sample solution is not larger than 1/2 times the peak area of candesartan cilexetil from the standard solution, the area of the peak, having a relative retention time of about 2.0, from the sample solution is not larger than the peak area of candesartan cilexetil from the standard solution, and the area of the peak, other than candesartan cilexetil and the peaks mentioned above, from the sample solution is smaller than 1/10 times the peak area of candesartan cilexetil from the standard solution. Furthermore, the total area of the peaks other than candesartan cilexetil from the sample solution is not larger than 4 times the peak area of candesartan cilexetil from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phases A and B, flowing of mobile phase, and time span of measurement: Proceed as directed in the operating conditions in the Purity (2) under Candesartan Cilexetil.

Flow rate: 0.6 mL per minute.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add a mixture of acetonitrile and water (3:2) to make exactly 50 mL. Confirm that the peak area of candesartan cilexetil obtained with 10 μL of this solution is equivalent to 1.4% to 2.6% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of candesartan cilexetil are not less than 12,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of candesartan cilexetil is not more than 2.0%.

(ii) To an amount of powdered Candesartan Cilexetil and Hydrochlorothiazide Tablets, equivalent to 6.25 mg of Hydrochlorothiazide, add 10 mL of a mixture of 0.05 mol/L sodium dihydrogen phosphate TS (pH 3.0) and acetonitrile (3:1), shake vigorously for 10 minutes, centrifuge, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 1 mL of the sample solution, add a mixture of 0.05 mol/L sodium dihydrogen phosphate TS (pH 3.0) and acetonitrile (3:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having a relative retention time of about 0.9 and about 3.2 to hydrochlorothiazide, obtained from the sample solution is not larger than the peak area of hydrochlorothiazide obtained from the standard solution, and the area of the peak, other than hydrochlorothiazide and the peaks mentioned above, from the sample solution is not larger than 1/5 times the peak area of hydrochlorothiazide from the standard solution. Furthermore, the total area of the peaks other than hydrochlorothiazide from the sample solution is not larger than 2 times the peak area of hydrochlorothiazide from the standard solution. For the area of the peak, having a relative retention time of about 0.8 and about 0.9 to hydrochlorothiazide, multiply their relative response factors, 1.4 and 0.5, respectively.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (2).

Time span of measurement: For 30 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add a mixture of 0.05 mol/L sodium dihydrogen phosphate TS (pH 3.0) and acetonitrile (3:1) to make exactly 50 mL. Confirm that the peak area of hydrochlorothiazide obtained with 10 μL of this solution is equivalent to 1.4% to 2.6% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of hydrochlorothiazide are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of hydrochlorothiazide is not more than 2.0%.

Uniformity of dosage units <6.02> Perform the test according to the following methods: it meets the requirements of the Content uniformity test.

(1) Candesartan cilexetil—To 1 tablet of Candesartan Cilexetil and Hydrochlorothiazide Tablets add exactly $V/10$ mL of the internal standard solution, add a mixture of acetonitrile and 0.05 mol/L sodium dihydrogen phosphate TS (pH 3.0) (3:2) to make V mL so that each mL contains about 40 μg of candesartan cilexetil ($C_{33}H_{34}N_6O_6$). Shake for 20 minutes to disintegrate the tablet, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of candesartan cilexetil for assay (separately determine the water <2.48> in the same manner as Candesartan Cilexetil), dissolve in acetonitrile to make exactly 50 mL, and use this solution as the candesartan cilexetil standard stock solution. Pipet 4 mL of the candesartan cilexetil standard stock solution, add exactly 10 mL of the internal standard solution, add a mixture of acetonitrile and 0.05 mol/L sodium dihydrogen phosphate TS (pH 3.0) (3:2) to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of candesartan cilexetil to that of the internal standard.

$$\text{Amount (mg) of candesartan cilexetil } (C_{33}H_{34}N_6O_6) = M_S \times Q_T/Q_S \times V \times 1/1250$$

M_S: Amount (mg) of candesartan cilexetil for assay taken, calculated on the anhydrous basis.

Internal standard solution—A solution of benzophenone in acetonitrile (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetonitrile and 0.05 mol/L sodium dihydrogen phosphate TS (pH 5.5) (11:9).

Flow rate: Adjust so that the retention time of candesartan cilexetil is about 7 minutes.

System suitability—

System performance: Mix 4 mL of the candesartan cilexetil standard stock solution and 10 mL of the hydrochlorothiazide standard stock solution obtained in (2), add 10 mL of the internal standard solution, and add a mixture of acetonitrile and 0.05 mol/L sodium dihydrogen phosphate TS (pH 3.0) (3:2) to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, hydrochlorothiazide, candesartan cilexetil and the internal standard are eluted in this order, and the resolution between the peaks of hydrochlorothiazide and candesartan cilexetil is not less than 7, and the resolution between the peaks of candesartan cilexetil and the internal standard is not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of candesartan cilexetil to that of the internal standard is not more than 1.0%.

(2) Hydrochlorothiazide—To 1 tablet of Candesartan Cilexetil and Hydrochlorothiazide Tablets add exactly $V/10$ mL of the internal standard solution, add a mixture of acetonitrile and 0.05 mol/L sodium dihydrogen phosphate TS (pH 3.0) (3:2) to make V mL so that each mL contains about 63 μg of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$). Shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 31 mg of Hydrochlorothiazide RS (separately determine the loss on drying <2.41> under the same conditions as Hydrochlorothiazide), dissolve in acetonitrile to make exactly 50 mL, and use this solution as the hydrochlorothiazide standard stock solution. Pipet 10 mL of the hydrochlorothiazide standard stock solution, add exactly 10 mL of the internal standard solution, add a mixture of acetonitrile and 0.05 mol/L sodium dihydrogen phosphate TS (pH 3.0) (3:2) to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of hydrochlorothiazide to that of the internal standard.

Amount (mg) of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$)
$= M_S \times Q_T/Q_S \times V \times 1/500$

M_S: Amount (mg) of Hydrochlorothiazide RS taken, calculated on the dried basis.

Internal standard solution—A solution of benzophenone in acetonitrile (1 in 10,000).

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay (2).

Mobile phase: A mixture of acetonitrile and 0.05 mol/L sodium dihydrogen phosphate TS (pH 5.5) (11:9).

Flow rate: Adjust so that the retention time of hydrochlorothiazide is about 3.5 minutes.

System suitability—

System performance: Mix 4 mL of the candesartan cilexetil standard stock solution obtained in (1) and 10 mL of the hydrochlorothiazide standard stock solution, add 10 mL of the internal standard solution, and add a mixture of acetonitrile and 0.05 mol/L sodium dihydrogen phosphate TS (pH 3.0) (3:2) to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, hydrochlorothiazide, candesartan cilexetil and the internal standard are eluted in this order, and the resolution between the peaks of hydrochlorothiazide and candesartan cilexetil is not less than 7, and the resolution between the peaks of candesartan cilexetil and the internal standard is not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of hydrochlorothiazide to that of the internal standard is not more than 1.0%.

Dissolution <6.10> **(1)** Candesartan cilexetil—When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of a solution, prepared by dissolving 1 g of polysorbate 80 in 2nd fluid for dissolution test to make 1000 mL, as the dissolution medium, the dissolution rate in 45 minutes of Candesartan Cilexetil and Hydrochlorothiazide Tablets is not less than 75%.

Start the test with 1 tablet of Candesartan Cilexetil and Hydrochlorothiazide Tablets, withdraw not less than 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet V mL of the subsequent filtrate, add 0.05 mol/L sodium dihydrogen phosphate TS (pH 3.0) to make exactly V' mL so that each mL contains about 2.2 μg of candesartan cilexetil ($C_{33}H_{34}N_6O_6$), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of candesartan cilexetil for assay (separately determine the water <2.48> in the same manner as Candesartan Cilexetil), dissolve in acetonitrile to make exactly 100 mL, and use this solution as the candesartan cilexetil standard stock solution. Pipet 2 mL of the candesartan cilexetil standard stock solution, add dissolution medium to make exactly 100 mL. Pipet 10 mL of this solution, add 0.05 mol/L sodium dihydrogen phosphate TS (pH 3.0) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of candesartan cilexetil in each solution.

Dissolution rate (%) with respect to the labeled amount of candesartan cilexetil ($C_{33}H_{34}N_6O_6$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9$

M_S: Amount (mg) of candesartan cilexetil for assay taken, calculated on the anhydrous basis

C: Labeled amount (mg) of candesartan cilexetil ($C_{33}H_{34}N_6O_6$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Uniformity of dosage units (1).

System suitability—

System performance: Mix 2 mL each of the candesartan cilexetil standard stock solution and the hydrochlorothiazide

standard stock solution obtained in (2), and add the dissolution medium to make 100 mL. To 10 mL of this solution add 10 mL of 0.05 mol/L sodium dihydrogen phosphate TS (pH 3.0). When the procedure is run with 40 μL of this solution under the above operating conditions, hydrochlorothiazide and candesartan cilexetil are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of candesartan cilexetil is not more than 1.0%.

(2) Hydrochlorothiazide—When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of a solution, prepared by dissolving 1 g of polysorbate 80 in 2nd fluid for dissolution test to make 1000 mL, as the dissolution medium, the dissolution rate in 45 minutes of Candesartan Cilexetil and Hydrochlorothiazide Tablets is not less than 80%.

Start the test with 1 tablet of Candesartan Cilexetil and Hydrochlorothiazide Tablets, withdraw not less than 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet V mL of the subsequent filtrate, add 0.05 mol/L sodium dihydrogen phosphate TS (pH 3.0) to make exactly V' mL so that each mL contains about 3.5 μg of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$), and use this solution as the sample solution. Separately, weigh accurately about 38 mg of Hydrochlorothiazide RS (separately determine the loss on drying <2.41> under the same conditions as Hydrochlorothiazide), dissolve in acetonitrile to make exactly 100 mL, and use this solution as the hydrochlorothiazide standard stock solution. Pipet 2 mL of the hydrochlorothiazide standard stock solution, add dissolution medium to make exactly 100 mL. Pipet 10 mL of this solution, add 0.05 mol/L sodium dihydrogen phosphate TS (pH 3.0) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of hydrochlorothiazide in each solution.

Dissolution rate (%) with respect to the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9$

M_S: Amount (mg) of Hydrochlorothiazide RS taken, calculated on the dried basis
C: Labeled amount (mg) of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in 1 tablet

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay (2).

Mobile phase: A mixture of acetonitrile and 0.05 mol/L sodium dihydrogen phosphate TS (pH 5.5) (11:9).

Flow rate: Adjust so that the retention time of hydrochlorothiazide is about 3.5 minutes.

System suitability—

System performance: Mix 2 mL each of the candesartan cilexetil standard stock solution obtained in (1) and the hydrochlorothiazide standard stock solution, and add the dissolution medium to make 100 mL. To 10 mL of this solution add 10 mL of 0.05 mol/L sodium dihydrogen phosphate TS (pH 3.0). When the procedure is run with 40 μL of this solution under the above operating conditions, hydrochlorothiazide and candesartan cilexetil are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of hydrochlorothiazide is not more than 1.0%.

Assay (1) Candesartan cilexetil—Weigh accurately the mass of not less than 20 Candesartan Cilexetil and Hydrochlorothiazide Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 4 mg of candesartan cilexetil ($C_{33}H_{34}N_6O_6$), add exactly 10 mL of the internal standard solution, add a mixture of acetonitrile and water (3:2) to make 100 mL, and shake vigorously for 10 minutes. Allow to stand for 5 minutes, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of candesartan cilexetil for assay (separately determine the water <2.48> in the same manner as Candesartan Cilexetil), dissolve in acetonitrile to make exactly 50 mL. Pipet 4 mL of this solution, add exactly 10 mL of the internal standard solution, add a mixture of acetonitrile and water (3:2) to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of candesartan cilexetil to that of the internal standard.

Amount (mg) of candesartan cilexetil ($C_{33}H_{34}N_6O_6$)
$= M_S \times Q_T/Q_S \times 2/25$

M_S: Amount (mg) of candesartan cilexetil for assay taken, calculated on the anhydrous basis

Internal standard solution—A solution of acenaphthene in acetonitrile (1 in 800).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetonitrile, water and acetic acid (100) (57:43:1).

Flow rate: Adjust so that the retention time of candesartan cilexetil is about 13 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and candesartan cilexetil are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of candesartan cilexetil to that of the internal standard is not more than 1.0%.

(2) Hydrochlorothiazide—Weigh accurately the mass of not less than 20 Candesartan Cilexetil and Hydrochlorothiazide Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 6.25 mg of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$), add exactly 10 mL of the internal standard solution, add a mixture of 0.05 mol/L sodium dihydrogen phosphate TS (pH 3.0) and acetonitrile (3:1) to make 100 mL, and shake vigorously for 10 minutes. Allow to stand for 5 minutes, and filter the supernatant liquid

through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 31 mg of Hydrochlorothiazide RS (separately determine the loss on drying <2.41> under the same conditions as Hydrochlorothiazide), dissolve in acetonitrile to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add a mixture of 0.05 mol/L sodium dihydrogen phosphate TS (pH 3.0) and acetonitrile (3:1) to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of hydrochlorothiazide to that of the internal standard.

Amount (mg) of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$)
$= M_S \times Q_T/Q_S \times 1/5$

M_S: Amount (mg) of Hydrochlorothiazide RS taken, calculated on the dried basis

Internal standard solution—A solution of *m*-hydroxyacetophenone in acetonitrile (1 in 6500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS (pH 5.5) and acetonitrile (3:1).

Flow rate: Adjust so that the retention time of hydrochlorothiazide is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, hydrochlorothiazide and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of hydrochlorothiazide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Capsules

カプセル

Capsules are made of Gelatin, and their shape is a pair of cylinders with one end closed which can be overlapped on each other.

Method of preparation Dissolve Gelatin in water by warming, add Glycerin or D-Sorbitol, Macrogol 4000, emulsifier, dispersing agent, preservatives, coloring substances and so forth, if necessary, to make a viscous liquid, and form into capsules while warm.

Capsules may be coated with a lubricant, if necessary.

Solubility and acidity or alkalinity Place, without overlapping of the parts, 1 piece (1 pair) of Capsules in a 100–mL conical flask, add 50 mL of water, and shake often, keeping the temperature at 37 ± 2°C. Perform this test 5 times: they all dissolve within 10 minutes. All these solutions are odorless, and neutral or slightly acidic.

Loss on drying <2.41> 13 – 16% (1 g, 105°C, 2 hours).

Microbial limit <4.05> The acceptance criteria of TAMC and TYMC are 10^3 CFU/g and 10^2 CFU/g, respectively.

Containers and storage Containers—Well-closed containers.

Hypromellose Capsules

ヒプロメロースカプセル

Hypromellose Capsules are made of Hypromellose as the base material, and their shape is a pair of cylinders with one end closed which can be overlapped on each other.

The label states the use or nonuse of the gelling agent and its name.

Method of preparation Dissolve Hypromellose in water by warming, add, if necessary, Glycerin or D-Sorbitol, emulsifiers, dispersing agents, preservatives, coloring agents, gelling agents, and gelling aid, etc. to make a viscous liquid, and form into a certain shape while warming.

They may be coated with a lubricant as necessary.

Solubility and acidity or alkalinity Place one pair of Hypromellose Capsules without snapping in a 100-mL conical flask, add 50 mL of water, and shake occasionally at 37 ± 2°C. When perform this test 5 times, either capsule dissolves within 15 minutes and their solutions are neutral or slightly acidic.

Loss on drying <2.41> 2 – 7% (1 g, 105°C, 2 hours).

Microbial limit <4.05> The acceptance criteria of TAMC and TYMC are 10^3 CFU/g and 10^2 CFU/g, respectively.

Containers and storage Containers—Well-closed containers.

Pullulan Capsules

プルランカプセル

Pullulan Capsules are made of Pullulan as the base material, and their shape is a pair of cylinders with one end closed which can be overlapped on each other.

The label states the use or nonuse of the gelling agent and its name.

Method of preparation Dissolve Pullulan in water by warming, add, if necessary, emulsifiers, dispersing agents, preservatives, coloring agents, gelling agents, and gelling aid, etc. to make a viscous liquid, and form into a certain shape while warming.

They may be coated with a lubricant as necessary.

Solubility and acidity or alkalinity Place one pair of Pullulan Capsules without snapping in a 100-mL conical flask, add 50 mL of water, and shake occasionally at 37 ± 2°C. When perform this test 5 times, either capsule dissolves within 10 minutes and these solutions are neutral or slightly acidic.

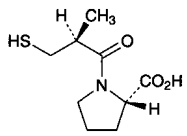
Loss on drying ⟨*2.41*⟩ 10 – 14% (1 g, 105°C, 6 hours).

Microbial limit ⟨*4.05*⟩ The acceptance criteria of TAMC and TYMC are 10^3 CFU/g and 10^2 CFU/g, respectively.

Containers and storage Containers—Well-closed containers.

Captopril

カプトプリル



$C_9H_{15}NO_3S$: 217.29
(2*S*)-1-[(2*S*)-2-Methyl-3-sulfanylpropanoyl]pyrrolidine-2-carboxylic acid
[*62571-86-2*]

Captopril contains not less than 98.0% of captopril ($C_9H_{15}NO_3S$), calculated on the dried basis.

Description Captopril occurs as white, crystals or crystalline powder.

It is very soluble in methanol, freely soluble in ethanol (99.5), and soluble in water.

Identification Determine the infrared absorption spectrum of Captopril as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{25}$: −125 – −134° (after drying, 0.1 g, ethanol (99.5), 10 mL, 100 mm).

Melting point ⟨*2.60*⟩ 105 – 110°C

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Captopril according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Captopril according to Method 1, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.20 g of Captopril in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 15 mg of 1,1′-[3,3′-dithiobis(2-methyl-1-oxopropyl)]-L-diproline in methanol to make exactly 250 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of toluene and acetic acid (100) (13:7) to a distance of about 15 cm, and air-dry the plate. Place the plate in a chamber filled with iodine vapor, and allow to stand for 30 minutes: the number of the spots other than the spot corresponding to that from the standard solution and the principal spot from the sample solution is not more than two, and they are not more intense than the spot from the standard solution.

(4) 1,1′-[3,3′-Dithiobis(2-methyl-1-oxopropyl)]-L-diproline—Dissolive 0.10 g of Captopril in methanol to make exactly 20 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of 1,1′-[3,3′-dithiobis(2-methyl-1-oxopropyl)]-L-diproline in methanol to make exactly 250 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of 1,1′-[3,3′-dithiobis(2-methyl-1-oxopropyl)]-L-diproline in each solution: A_T is not larger than A_S.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, methanol and phosphoric acid (1000:1000:1).

Flow rate: Adjust so that the retention time of 1,1′-[3,3′-dithiobis(2-methyl-1-oxopropyl)]-L-diproline is about 10 minutes.

System suitability—

System performance: Dissolve 25 mg each of Captopril and 1,1′-[3,3′-dithiobis(2-methyl-1-oxopropyl)]-L-diproline in 200 mL of methanol. When the procedure is run with 20 μL of this solution under the above operating conditions, captopril and 1,1′-[3,3′-dithiobis(2-methyl-1-oxopropyl)]-L-diproline are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of 1,1′-[3,3′-dithiobis(2-methyl-1-oxopropyl)]-L-diproline is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, in vacuum, 80°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

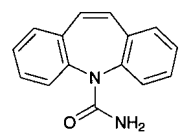
Assay Weigh accurately about 0.3 g of Captopril, dissolve in 100 mL of water, add 20 mL of dilute sulfuric acid and 1 g of potassium iodide, and shake. Titrate ⟨*2.50*⟩ with 1/60 mol/L potassium iodate VS (indicator: 2 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 1/60 mol/L potassium iodate VS
= 21.73 mg of $C_9H_{15}NO_3S$

Containers and storage Containers—Tight containers.

Carbamazepine

カルバマゼピン



$C_{15}H_{12}N_2O$: 236.27
5*H*-Dibenz[*b*,*f*]azepine-5-carboxamide
[*298-46-4*]

Carbamazepine, when dried, contains not less than 97.0% and not more than 103.0% of carbamazepine

($C_{15}H_{12}N_2O$).

Description Carbamazepine occurs as a white to slightly yellowish white powder. It is odorless and tasteless at first, and leaves a slightly bitter aftertaste.

It is freely soluble in chloroform, sparingly soluble in ethanol (95) and in acetone, and very slightly soluble in water and in diethyl ether.

Identification **(1)** To 0.1 g of Carbamazepine add 2 mL of nitric acid, and heat on a water bath for 3 minutes: an orange-red color is produced.

(2) To 0.1 g of Carbamazepine add 2 mL of sulfuric acid, and heat on a water bath for 3 minutes: a yellow color is produced with a green fluorescence.

(3) Examine Carbamazepine under ultraviolet light: the solution shows an intense blue fluorescence.

(4) Determine the absorption spectrum of the solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point <*2.60*> 189 – 193°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Carbamazepine in 10 mL of chloroform: the solution is clear and colorless to pale yellow.

(2) Acidity—To 2.0 g of Carbamazepine add exactly 40 mL of water, stir well for 15 minutes, and filter through a glass filter (G3). To 10 mL of this filtrate add 1 drop of phenolphthalein TS and 0.50 mL of 0.01 mol/L sodium hydroxide VS: a red color is produced.

(3) Alkalinity—To 10 mL of the filtrate obtained in (2) add 1 drop of methyl red TS and 0.50 mL of 0.01 mol/L hydrochloric acid VS: a red color is produced.

(4) Chloride <*1.03*>—Dissolve 0.25 g of Carbamazepine in 30 mL of acetone, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.20 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.028%).

(5) Heavy metals <*1.07*>—Proceed with 2.0 g of Carbamazepine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Related substances—Dissolve 0.25 g of Carbamazepine in 10 mL of chloroform, and use this solution as the sample solution. Separately, dissolve 5.0 mg of iminodibenzyl in chloroform to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of toluene and methanol (19:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly potassium dichromate-sulfuric acid TS on the plate: the spots other than the principal spot obtained from the sample solution is not more intense than the spot obtained from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

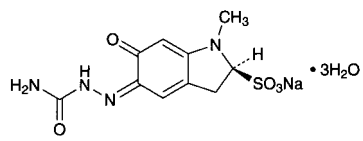
Assay Dissolve about 50 mg of Carbamazepine, previously dried and accurately weighed, in ethanol (95) to make exactly 250 mL. Pipet 5 mL of this solution and add ethanol (95) to make exactly 100 mL. Perform the test as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbance A of this solution at the wavelength of maximum absorption at about 285 nm.

$$\text{Amount (mg) of carbamazepine } (C_{15}H_{12}N_2O) = A/490 \times 50{,}000$$

Containers and storage Containers—Tight containers.

Carbazochrome Sodium Sulfonate Hydrate

カルバゾクロムスルホン酸ナトリウム水和物



and enantiomer

$C_{10}H_{11}N_4NaO_5S.3H_2O$: 376.32
Monosodium (2*RS*)-1-methyl-6-oxo-5-semicarbazono-2,3,5,6-tetrahydroindole-2-sulfonate trihydrate
[*51460-26-5*, anhydride]

Carbazochrome Sodium Sulfonate Hydrate contains not less than 98.0% and not more than 102.0% of carbazochrome sodium sulfonate ($C_{10}H_{11}N_4NaO_5S$: 322.27), calculated on the anhydrous basis.

Description Carbazochrome Sodium Sulfonate Hydrate occurs as orange-yellow, crystals or crystalline powder.

It is sparingly soluble in water, very slightly soluble in methanol and in ethanol (95), and practically insoluble in diethyl ether.

A solution of Carbazochrome Sodium Sulfonate Hydrate (1 in 100) shows no optical rotation.

Melting point: about 210°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Carbazochrome Sodium Sulfonate Hydrate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Carbazochrome Sodium Sulfonate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Carbazochrome Sodium Sulfonate Hydrate (1 in 100) responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

pH <*2.54*> Dissolve 0.8 g of Carbazochrome Sodium Sulfonate Hydrate in 50 mL of water by warming, and cool: the pH of this solution is between 5.0 and 6.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Carbazochrome Sodium Sulfonate Hydrate in 50 mL of water by warming, and allow to cool: the solution is clear. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: the absorbance at 590 nm is not more than 0.070.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Carbazochrome Sodium Sulfonate Hydrate according to Method 2, and perform the test. Prepare the control solution

with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 50 mg of Carbazochrome Sodium Sulfonate Hydrate in 100 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the total area of the peaks other than carbazochrome sulfonate from the sample solution is not larger than the peak area of carbazochrome sulfonate from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 360 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.2 g of ammonium dihydrogen phosphate in 1000 mL of water, and filter through a membrane filter (0.4 μm in pore size) if necessary. To 925 mL of this solution add 75 mL of ethanol (95), shake, and adjust the pH to 3 with phosphoric acid.

Flow rate: Adjust so that the retention time of carbazochrome sulfonate is about 7 minutes.

Time span of measurement: About 3 times as long as the retention time of carbazochrome sulfonate, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of carbazochrome sulfonate obtained from 10 μL of this solution is equivalent to 7 to 13% of that of carbazochrome sulfonate obtained from 10 μL of the standard solution.

System performance: Dissolve 10 mg each of Carbazochrome Sodium Sulfonate Hydrate and carbazochrome in 100 mL of water by warming. When the procedure is run with 10 μL of this solution under the above operating conditions, carbazochrome sulfonate and carbazochrome are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of carbazochrome sulfonate is not more than 2.0%.

Water <2.48> 13.0 – 16.0% (0.3 g, volumetric titration, direct titration).

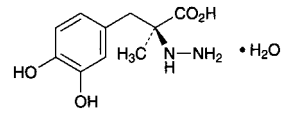
Assay Weigh accurately about 0.25 g of Carbazochrome Sodium Sulfonate Hydrate, dissolve in 50 mL of water, apply to a chromatographic column, 10 mm in diameter, previously prepared with 20 mL of strongly acidic ion exchange resin for column chromatography (type H), and allow to flow at a rate of 4 mL per minute. Wash the column with 150 mL of water, combine the washing and the former effluent solution, and titrate <2.50> with 0.05 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L sodium hydroxide VS
= 16.11 mg of $C_{10}H_{11}N_4NaO_5S$

Containers and storage Containers—Well-closed containers.

Carbidopa Hydrate

カルビドパ水和物



$C_{10}H_{14}N_2O_4.H_2O$: 244.24

(2*S*)-2-(3,4-Dihydroxybenzyl)-2-hydrazinopropanoic acid monohydrate

[*38821-49-7*]

Carbidopa Hydrate contains not less than 98.0% of carbidopa hydrate ($C_{10}H_{14}N_2O_4.H_2O$).

Description Carbidopa Hydrate occurs as a white to yellowish white powder.

It is sparingly soluble in methanol, slightly soluble in water, very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 197°C (with decomposition).

Identification **(1)** Dissolve 0.01 g of Carbidopa Hydrate in 250 mL of a solution of hydrochloric acid in methanol (9 in 1000). Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Carbidopa RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Carbidopa Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Carbidopa RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: −21.0 – −23.5° (1 g, aluminum (III) chloride TS, 100 mL, 100 mm).

Purity **(1)** Heavy metals <1.07>—Proceed with 2.0 g of Carbidopa Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Carbidopa Hydrate in 70 mL of the mobile phase, by warming and using ultrasonication, if necessary. After cooling, add the mobile phase to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area from both solutions by the automatic integration method: the total area of the peaks other than carbidopa from the sample solution is not larger than the peak area of carbidopa from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of carbidopa.

System suitability—

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of carbidopa obtained from 20 μL of this solution is equivalent to 7 to 13% of that of carbidopa obtained from 20 μL of the standard solution.

Loss on drying ⟨*2.41*⟩ 6.9 – 7.9% (1 g, in vacuum not exceeding 0.67 kPa, 100°C, 6 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Carbidopa Hydrate and Carbidopa RS (separately determine the loss on drying ⟨*2.41*⟩ under the same conditions as Carbidopa Hydrate), and dissolve each in 70 mL of the mobile phase, by warming and using ultrasonication if necessary. After cooling, add the mobile phase to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of carbidopa in each solution.

Amount (mg) of carbidopa hydrate ($C_{10}H_{14}N_2O_4.H_2O$) $= M_S \times A_T/A_S \times 1.080$

M_S: Amount (mg) of Carbidopa RS taken, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 950 mL of 0.05 mol/L sodium dihydrogen phosphate TS add 50 mL of ethanol (95), and adjust the pH to 2.7 with phosphoric acid.

Flow rate: Adjust so that the retention time of carbidopa is about 6 minutes.

System suitability—

System performance: Dissolve 50 mg each of Carbidopa Hydrate and methyldopa in 100 mL of the mobile phase. When the procedure is run with 20 μL of this solution under the above operating conditions, methyldopa and carbidopa are eluted in this order with the resolution between these peaks being not less than 0.9.

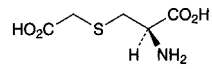
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of carbidopa is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

L-Carbocisteine

L-カルボシステイン



$C_5H_9NO_4S$: 179.19

(2*R*)-2-Amino-3-carboxymethylsulfanylpropanoic acid

[*638-23-3*]

L-Carbocisteine, when dried, contains not less than 98.5% of L-carbocisteine ($C_5H_9NO_4S$).

Description L-Carbocisteine occurs as a white crystalline powder. It is odorless, and has a slightly acid taste.

It is very slightly soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid or in sodium hydroxide TS.

Melting point: about 186°C (with decomposition).

Identification **(1)** To 0.2 g of L-Carbocisteine add 1 mL of lead acetate TS and 3 mL of water, shake, add 0.2 g of sodium hydroxide, and heat over a flame for 1 minute: a dark brown to black precipitate is formed.

(2) Determine the infrared absorption spectrum of L-Carbocisteine as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: -33.5 – $-36.5°$ Weigh accurately about 5 g of L-Carbocisteine, previously dried, dissolve in 20 mL of water and a suitable amount of a solution of sodium hydroxide (13 in 100), and adjust the pH with 1 mol/L hydrochloric acid TS or 0.1 mol/L hydrochloric acid TS to 6.0, and add water to make exactly 50 mL. Determine the optical rotation of this solution in a 100-mm cell.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of L-Carbocisteine in 10 mL of sodium hydroxide TS: the solution is clear and colorless.

(2) Chloride ⟨*1.03*⟩—Dissolve 0.20 g of L-Carbocisteine in 10 mL of water and 20 mL of nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows. To 0.40 mL of 0.01 mol/L hydrochloric acid VS add 20 mL of nitric acid and water to make 50 mL (not more than 0.071%).

(3) Ammonium ⟨*1.02*⟩—Perform the test with 0.25 g of L-Carbocisteine using the distillation under reduced pressure. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(4) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of L-Carbocisteine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of L-Carbocisteine according to Method 3, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 0.30 g of L-Carbocisteine in 10 mL of 0.2 mol/L sodium hydroxide TS, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add 0.2 mol/L sodium hydroxide TS to make exactly 100 mL. Pipet 1 mL of this solution, and add 0.2 mol/L sodium hydroxide TS to make exactly 10 mL, and use this solution as the standard solution. Perform the test

with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution, in 15 mm length along the starting line on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.30% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of L-Carbocisteine, previously dried, dissolve in exactly 20 mL of 0.1 mol/L perchloric acid VS and 50 mL of acetic acid (100), and titrate <2.50> the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 17.92 mg of $C_5H_9NO_4S$

Containers and storage Containers—Tight containers.

L-Carbocisteine Tablets

L-カルボシステイン錠

L-Carbocisteine Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of L-carbocisteine ($C_5H_9NO_4S$: 179.19).

Method of Preparation Prepare as directed under Tablets, with L-Carbocisteine.

Identification Powder L-Carbocisteine Tablets. To a portion of the powder, equivalent to 0.18 g of L-Carbocisteine, add 50 mL of water, stir for 10 minutes, and filter. To 5 mL of the filtrate add 1 mL of ninhydrin TS, and heat in a water bath for 3 minutes: a purple color develops.

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Dissolution <6.10> When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rates in 15 minutes of 250-mg tablet and in 30 minutes of 500-mg tablet are not less than 80% and not less than 85%, respectively.

Start the test with 1 tablet of L-Carbocisteine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the mobile phase to make exactly V' mL so that each mL contains about 0.14 mg of L-carbocisteine ($C_5H_9NO_4S$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of L-carbocisteine for assay, previously dried at 105°C for 2 hours, and dissolve in the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following condition, and determine the peak areas, A_T and A_S, of L-carbocisteine in each solution.

Dissolution rate (%) with respect to the labeled amount of L-carbocisteine ($C_5H_9NO_4S$)

$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 450$$

M_S: Amount (mg) of L-carbocisteine for assay taken
C: Labeled amount (mg) of L-carbocisteine ($C_5H_9NO_4S$) in 1 tablet

Operating conditions—
Proceed as directed in the operating conditions in the Assay.
System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of L-carbocisteine are not less than 1500 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of L-carbocisteine is not more than 1.0%.

Assay To 10 L-Carbocisteine Tablets add 220 mL of 0.5 mol/L hydrochloric acid TS, stir for 30 minutes, add 0.5 mol/L hydrochloric acid TS to make exactly 250 mL, and stir additionally for 30 minutes. Filter this solution, discard the first 20 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add (V—50)/25 mL of 0.5 mol/L hydrochloric acid TS, then add exactly V/25 mL of the internal standard solution, add water to make V mL so that each mL contains about 0.4 mg of L-carbocisteine ($C_5H_9NO_4S$), and use this solution as the sample solution. Separately, weigh accurately about 20 mg of L-carbocisteine for assay, previously dried at 105°C for 2 hours, add 2 mL of 0.5 mol/L hydrochloric acid TS, and exactly 2 mL of the internal standard solution. Then add water to dissolve to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of L-carbocisteine to that of the internal standard.

Amount (mg) of L-carbocisteine ($C_5H_9NO_4S$) in 1 tablet
$= M_S \times Q_T/Q_S \times V/4$

M_S: Amount of L-carbocisteine for assay taken

Internal standard solution—A solution of nicotinic acid (9 in 10,000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 240 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 20°C.
Mobile phase: Diluted trifluoroacetic acid (1 in 1000).
Flow rate: Adjust so that the retention time of L-carbocisteine is about 2 minutes.
System suitability—
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, L-carbocisteine and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating

conditions, the relative standard deviation of the ratio of the peak area of L-carbocisteine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Carbon Dioxide

二酸化炭素

CO_2: 44.01
[*124-38-9*]

Carbon Dioxide contains not less than 99.5 vol% of carbon dioxide (CO_2).

Description Carbon Dioxide is a colorless gas at room temperature and under atmospheric pressure. It is odorless.

A 1 mL volume of Carbon Dioxide dissolves in 1 mL of water, and the solution is slightly acid.

1000 mL of Carbon Dioxide at 0°C and under a pressure of 101.3 kPa weighs 1.978 g.

Identification **(1)** Pass 100 mL of Carbon Dioxide through a carbon dioxide measuring detector tube: the detector tube is changed to a stipulated color tone by each detector tube, provided that the detector tube with a upper limit of measurement of not less than 10% is used.

(2) Pass Carbon Dioxide into calcium hydroxide TS: a white precipitate is produced. Collect the precipitate, and add acetic acid (31): it dissolves with effervescence.

Purity **(1)** Acidity—Place 50 mL of freshly boiled and cooled water in a Nessler tube, and pass 1000 mL of Carbon Dioxide into it for 15 minutes through an introducing tube about 1 mm in diameter extending to 2 mm from the bottom of the Nessler tube, then add 0.10 mL of methyl orange TS: the solution is not more colored than the following control solution.

Control solution: To 50 mL of freshly boiled and cooled water in a Nessler tube add 0.10 mL of methyl orange TS and 1.0 mL of 0.01 mol/L hydrochloric acid VS.

(2) Hydrogen phosphide, hydrogen sulfide or reducing organic substances—Place 25 mL of silver nitrate-ammonia TS and 3 mL of ammonia TS in each of two Nessler tubes A and B, and designate the solution in each tube as solution A and solution B, respectively. Pass 1000 mL of Carbon Dioxide into solution A in the same manner as directed in (1): the turbidity and color of this solution are the same as that of solution B.

(3) Carbon monoxide—Pass a specified amount of Carbon Dioxide through a carbon monoxide measuring detector tube: the concentration of carbon monoxide is less than 15 ppm, provided that the passing amount (mL) of Carbon Dioxide is stipulated according to each detector tube.

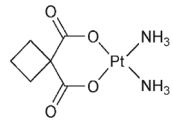
Assay Place 125 mL of a solution of potassium hydroxide (1 in 2) in a gas pipet of suitable capacity. Measure exactly about 100 mL of Carbon Dioxide in a 100-mL gas buret filled with water. Force the entire volume of gas into the gas pipet, and shake for 5 minutes. Draw some of the unabsorbed gas into the gas buret, measure the volume, force the residual back upon the surface of the liquid in the gas pipet, and repeat this procedure until a constant volume of the residual reading is obtained. Determine the volume V (mL) of the residual gas. Calculate the volume of the sample and V on the basis of the gas volume at 20°C and at 101.3 kPa.

Volume (mL) of carbon dioxide (CO_2)
= volume (mL) of the sample − V (mL)

Containers and storage Containers—Cylinders.
Storage—Not exceeding 40°C.

Carboplatin

カルボプラチン



$C_6H_{12}N_2O_4Pt$: 371.25
(*SP*-4-2)-Diammine[cyclobutan-1,1-dicarboxylato(2-)-*O*,*O*′]platinum
[*41575-94-4*]

Carboplatin contains not less than 98.5% and not more than 101.0% of carboplatin ($C_6H_{12}N_2O_4Pt$), calculated on the dried basis.

Description Carboplatin occurs as white, crystals or crystalline powder.

It is sparingly soluble in water, and very slightly soluble in ethanol (99.5).

Melting point: about 200°C (with decomposition).

Identification **(1)** To 2 mL of a solution of Carboplatin (1 in 100) add 2 to 3 drops of diluted tin (II) chloride TS (1 in 15), and allow to stand for 30 minutes: a yellowish brown precipitate is formed.

(2) Determine the infrared absorption spectrum of Carboplatin as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Carboplatin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH ⟨*2.54*⟩ Dissolve 0.10 g of Carboplatin in 10 mL of water: the pH of this solution is 5.0 to 7.0.

Purity **(1)** 1,1-Cyclobutanedicarboxylic acid—Weigh accurately about 40 mg of Carboplatin, dissolve in the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of 1,1-cyclobutanedicarboxylic acid, and dissolve in the mobile phase to make exactly 100 mL. Pipet 4 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine the peak areas, A_T and A_S, of 1,1-cyclobutanedicarboxylic acid in each solution, and calculate the amount of 1,1-cyclobutanedicarboxylic acid by the following formula: it is not more than 0.2%.

Amount (%) of 1,1-cyclobutanedicarboxylic acid
= $M_S/M_T \times A_T/A_S \times 8/5$

M_S: Amount (mg) of 1,1-cyclobutanedicarboxylic acid taken
M_T: Amount (mg) of Carboplatin taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.0 mm in inside diame-

ter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 8.5 g of tetrabutylammonium hydrogensulfate in 80 mL of water, add 3.4 mL of phosphoric acid, and adjust to pH 7.5 with a solution of sodium hydroxide (43 in 100). To 10 mL of this solution add 430 mL of water and 60 mL of acetonitrile.

Flow rate: Adjust so that the retention time of 1,1-cyclobutanedicarboxylic acid is about 5 minutes.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of 1,1-cyclobutanedicarboxylic acid obtained with 25 μL of this solution is equivalent to 14 to 26% of that obtained with 25 μL of the standard solution.

System performance: Dissolve 25 mg each of 1,1-cyclobutanedicarboxylic acid and cyclobutanecarboxylic acid in 100 mL of water. To 10 mL of this solution add the mobile phase to make 25 mL. When the procedure is run with 25 μL of this solution under the above operating conditions, cyclobutanecarboxylic acid and 1,1-cyclobutanedicarboxylic acid are eluded in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of 1,1-cyclobutanedicarboxylic acid is not more than 2.0%.

(2) Related substances—Dissolve 25 mg of Carboplatin in 25 mL of water, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the peaks by the area percentage method: the amount of the peak, having the relative retention time of about 0.8 to carboplatin, is not more than 0.25%, the amount of the peak other than carboplatin and the peak mentioned above is not more than 0.1%, and the total amount of the peaks other than carboplatin is not more than 0.5%.

Operating conditions—

Detector, column, column temperature, mobile phases A and B, and flow rate: Proceed as directed in the operating conditions in the Assay.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 15	100	0
15 – 35	100 → 0	0 → 100
35 – 50	0	100

Time span of measurement: About 2.5 times as long as the retention time of carboplatin, beginning after the solvent peak.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: To 1 mL of the sample solution add water to make 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add water to make exactly 20 mL. Confirm that the peak area of carboplatin obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 10 μL of the solution for system suitability test.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of carboplatin is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.1% (0.5 g, 105°C, 4 hours).

Assay Weigh accurately about 25 mg each of Carboplatin and Carboplatin RS (separately determine the loss on drying <*2.41*> under the same conditions as Carboplatin), dissolve separately in water to make exactly 25 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of carboplatin in each solution.

$$\text{Amount (mg) of carboplatin } (C_6H_{12}N_2O_4Pt) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Carboplatin RS taken, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with phenylhexylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 27°C.

Mobile phase A: Dissolve 8.5 g of tetrabutylammonium hydrogensulfate in 80 mL of water, add 3.4 mL of phosphoric acid, and adjust to pH 7.5 with a solution of sodium hydroxide (43 in 100). To 20 mL of this solution add water to make 1000 mL.

Mobile phase B: Dissolve 8.5 g of tetrabutylammonium hydrogensulfate in 80 mL of water, add 3.4 mL of phosphoric acid, and adjust to pH 7.5 with a solution of sodium hydroxide (43 in 100). To 20 mL of this solution add water to make 800 mL, and add 200 mL of acetonitrile.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 15	100	0
15 – 35	100 → 0	0 → 100

Flow rate: 0.5 mL per minute.

System suitability—

System performance: To 9 mL of the standard solution add 1 mL of diluted hydrogen peroxide TS (1 in 60), and allow to stand at room temperature for not less than 1 hour. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak of carboplatin and the peak having the relative retention time about 0.93 to carboplatin is not less than 1.2.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operat-

ing conditions, the relative standard deviation of the peak area of carboplatin is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Carboplatin Injection

カルボプラチン注射液

Carboplatin Injection is an aqueous injection.
It contains not less than 95.0% and not more than 105.0% of the labeled amount of carboplatin ($C_6H_{12}N_2O_4Pt$: 371.25).

Method of preparation Prepare as directed under Injections, with Carboplatin.

Description Carboplatin Injection is a clear, colorless to pale yellow liquid.

Identification (1) To an amount of Carboplatin Injection, equivalent to 20 mg of Carboplatin, add 2 to 3 drops of diluted tin (II) chloride TS (1 in 15), and allow to stand for 30 minutes: a yellowish brown precipitate is formed.

(2) Evaporate to dryness a volume of Carboplatin Injection, equivalent to 10 mg of Carboplatin, in a water bath at not exceeding 30°C under vacuum. Determine the infrared absorption spectrum of the residue as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩: it exhibits absorption at the wave numbers of about 3270 cm^{-1}, 2990 cm^{-1}, 2960 cm^{-1}, 1645 cm^{-1}, 1610 cm^{-1}, 1381 cm^{-1} and 1348 cm^{-1}.

pH Being specified separately when the drug is granted approval based on the Law.

Purity (1) 1,1-Cyclobutanedicarboxylic acid—To an exact volume of Carboplatin Injection, equivalent to 20 mg of Carboplatin, add the mobile phase to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of 1,1-cyclobutanedicarboxylic acid, and dissolve in the mobile phase to make exactly 100 mL. Pipet 4 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine the peak areas, A_T and A_S, of 1,1-cyclobutanedicarboxylic acid in each solution, and calculate the amount of 1,1-cyclobutanedicarboxylic acid by the following formula: it is not more than 0.7%.

$$\text{Amount (\%) of 1,1-cyclobutanedicarboxylic acid} = M_S \times A_T/A_S \times 1/25$$

M_S: Amount (mg) of 1,1-cyclobutanedicarboxylic acid taken

Operating conditions—
Proceed as directed in the operating conditions in the Purity (1) under Carboplatin.
System suitability—
Proceed as directed in the system suitability in the Purity (1) under Carboplatin.

(2) Related substances—To a volume of Carboplatin Injection, equivalent to 10 mg of Carboplatin, add water to make 10 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of these peaks by the area percentage method: the total amount of the peaks other than carboplatin is not more than 2.0%.
Operating conditions—
Detector, column, column temperature, mobile phases A and B, and flow rate: Proceed as directed in the operating conditions in the Assay under Carboplatin.
Flowing of mobile phase, and time span of measurement: Proceed as directed in the operating conditions in the Purity (2) under Carboplatin.
System suitability—
System performance: Proceed as directed in the system suitability in the Assay under Carboplatin.
Test for required detectability, and system repeatability: Proceed as directed in the system suitability in the Purity (2) under Carboplatin.

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.2 EU/mg.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay To an exact volume of Carboplatin Injection, equivalent to about 20 mg of carboplatin ($C_6H_{12}N_2O_4Pt$), add water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Carboplatin RS (separately determine the loss on drying ⟨*2.41*⟩ under the same conditions as Carboplatine), dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of carboplatin in each solution.

$$\text{Amount (mg) of carboplatin } (C_6H_{12}N_2O_4Pt) = M_S \times A_T/A_S \times 4/5$$

M_S: Amount (mg) of Carboplatin RS taken, calculated on the dried basis

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 230 nm).
Column: A stainless steel column 4.0 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).
Column temperature: A constant temperature of about 35°C.
Mobile phase: Dissolve 8.5 g of tetrabutylammonium hydrogensulfate in 80 mL of water, add 3.4 mL of phosphoric acid, and adjust to pH 7.5 with a solution of sodium hydroxide (43 in 100). To 10 mL of this solution add 880 mL of water and 10 mL of acetonitrile.
Flow rate: Adjust so that the retention time of carboplatin is about 4 minutes.
System suitability—
System performance: To a solution of 25 mg of carboplatin in 20 mL of water add 2.5 mL of a solution of 65 mg of 1,3-phenylenediamine hydrochloride in 50 mL of water, and add water to make 25 mL. When the procedure is run with 10 μL of this solution under the above operating conditions,

carboplatin and 1,3-phenylenediamine are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of carboplatin is not more than 1.0%.

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant.

Shelf life 24 months after preparation.

Carmellose

Carboxymethylcellulose

カルメロース

[*9000-11-7*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Carmellose is partly *O*-carboxymethylated cellulose.

◆**Description** Carmellose occurs as a white powder.
It is practically insoluble in ethanol (95).
It swells with water to form suspension.
It becomes viscid in sodium hydroxide TS.
It is hygroscopic.◆

Identification **(1)** Determine the infrared absorption spectrum of Carmellose as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) The pH <*2.54*> of a suspension, obtained by shaking 1 g of Carmellose with 100 mL of water, is between 3.5 and 5.0.

Purity **(1)** Chloride—Shake well 0.8 g of Carmellose with 50 mL of water, add 10 mL of sodium hydroxide TS to dissolve, and add water to make 100 mL. Heat 20 mL of this solution with 10 mL of dilute nitric acid in a water bath until a flocculent precipitate is produced, cool, centrifuge, and take out the supernatant liquid. Wash the precipitate with three 10-mL portions of water by centrifuge each time, combine the supernatant liquid and the washings, and add water to make 100 mL. Take 25 mL of this solution in a Nessler tube, add 6 mL of dilute nitric acid and water to make 50 mL, and use this solution as the test solution. Separately, to 0.40 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL, and use this solution as the control solution. To the test solution and the control solution add 1 mL each of silver nitrate TS, ◆mix,◆ and allow to stand protected from light for 5 minutes. Compare the opalescence developed in both solutions ◆against a black background by viewing downward or transversely◆. The opalescence in the test solution is not more intense than that in the control solution (not more than 0.36%).

(2) Sulfate—Shake well 0.40 g of Carmellose with 25 mL of water, add 5 mL of sodium hydroxide TS to dissolve, and add 20 mL of water. Heat this solution with 2.5 mL of hydrochloric acid in a water bath until a flocculent precipitate is produced, cool, centrifuge, and take out the supernatant liquid. Wash the precipitate with three 10-mL portions of water by centrifuge each time, combine the supernatant liquid and the washings, and add water to make 100 mL. Filter this solution, discard the first 5 mL of the filtrate, take 25 mL of the subsequent filtrate in a Nessler tube, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and use this solution as the test solution. Separately, to 1.5 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid and water to make 50 mL, and use this solution as the control solution. To the test solution and the control solution add 2 mL each of barium chloride TS, mix, and allow to stand for 10 minutes. Compare the opalescence developed in both solutions ◆against a black background by viewing downward or transversely◆. The opalescence in the test solution is not more intense than that in the control solution (not more than 0.72%).

◆**(3)** Heavy metals <*1.07*>—Proceed with 1.0 g of Carmellose according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).◆

Loss on drying <*2.41*> Not more than 8.0% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 1.5% (after drying, 1 g).

◆**Containers and storage** Containers—Tight containers.◆

Carmellose Calcium

Carboxymethylcellulose Calcium

カルメロースカルシウム

[*9050-04-8*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Carmellose Calcium is the calcium salt of a polycarboxymethylether of cellulose.

◆**Description** Carmellose Calcium occurs as a white to yellowish white powder.
It is practically insoluble in ethanol (95) and in diethyl ether.
It swells with water to form a suspension.
The pH of a suspension, obtained by shaking 1.0 g of Carmellose Calcium with 100 mL of water, is between 4.5 and 6.0.
It is hygroscopic.◆

Identification **(1)** Shake thoroughly 0.1 g of Carmellose Calcium with 10 mL of water, followed by 2 mL of sodium hydroxide TS, allow to stand for 10 minutes, and use this solution as the sample solution. To 1 mL of the sample solution add water to make 5 mL. To 1 drop of this solution add 0.5 mL of chromotropic acid TS, and heat in a water bath for 10 minutes: a red-purple color develops.

(2) Shake 5 mL of the sample solution obtained in (1) with 10 mL of acetone: a white, flocculent precipitate is produced.

(3) Shake 5 mL of the sample solution obtained in (1) with 1 mL of iron (III) chloride TS: a brown, flocculent precipitate is produced.

(4) Ignite 1 g of Carmellose Calcium to ash, dissolve the

residue in 10 mL of water and 6 mL of acetic acid (31), and filter, if necessary. Boil the filtrate, cool, and neutralize with ammonia TS: the solution responds to the Qualitative Tests <*1.09*> (1) and (3) for calcium salt.

Purity **(1)** Alkalinity—Shake thoroughly 1.0 g of Carmellose Calcium with 50 mL of freshly boiled and cooled water, and add 2 drops of phenolphthalein TS: no red color develops.

(2) Chloride <*1.03*>—Shake thoroughly 0.80 g of Carmellose Calcium with 50 mL of water, add 10 mL of sodium hydroxide TS to dissolved, add water to make 100 mL, and use this solution as the sample solution. Heat 20 mL of the sample solution with 10 mL of 2 mol/L nitric acid TS on a water bath until a flocculent precipitate is produced. After cooling, centrifuge, and take out the supernatant liquid. Wash the precipitate with three 10-mL portions of water by centrifuging each time, combine the supernatant and the washings, and add water to make 100 mL. Take 25 mL of this solution, and add 1 mL of nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.36%).

(3) Sulfate <*1.14*>—Heat 10 mL of the sample solution obtained in (2) with 1 mL of hydrochloric acid in a water bath until a flocculent precipitate is produced. Cool, centrifuge, and take out the supernatant liquid. Wash the precipitate with three 10-mL portions of water by centrifuging each time, combine the supernatant and the washings, and add water to make 100 mL. Perform the test with 25 mL this solution as the test solution. Prepare the control solution with 0.42 mL of 0.005 mol/L sulfuric acid VS. To the test solution and the control solution add 1 mL of 3 mol/L hydrochloric acid TS and 3 mL of barium chloride TS, then add water to make 50 mL, and mix. Allow to stand for 10 minutes, and compare the turbidity of these solutions: the turbidity obtained with the test solution is not more than that obtained with the control solution (not more than 1.0%).

◆**(4)** Heavy metals <*1.07*>—Proceed with 1.0 g of Carmellose Calcium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).◆

Loss on drying <*2.41*> Not more than 10.0% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> 10 – 20% (after drying 1 g).

◆**Containers and storage** Containers—Tight containers.◆

Carmellose Sodium

Carboxymethylcellulose Sodium

カルメロースナトリウム

[*9004-32-4*]

Carmellose Sodium is the sodium salt of a polycarboxymethylether of cellulose.

It, when dried, contains not less than 6.5% and not more than 8.5% of sodium (Na: 22.99).

Description Carmellose Sodium occurs as a white to yellowish white, powder or granules. It has no taste.

It is practically insoluble in methanol, in ethanol (95), in acetic acid (100) and in diethyl ether.

It forms a viscid solution in water and in warm water.

It is hygroscopic.

Identification **(1)** Dissolve 0.2 g of Carmellose Sodium in 20 mL of warm water with stirring, cool, and use this solution as the sample solution. To 1 mL of the sample solution add water to make 5 mL. To 1 drop of this solution add 0.5 mL of concentrated chromotropic acid TS, and heat in a water bath for 10 minutes: a red-purple color develops.

(2) To 10 mL of the sample solution obtained in test (1) add 1 mL of copper (II) sulfate TS: a blue flocculent precipitate is produced.

(3) To 3 g of Carmellose Sodium add 20 mL of methanol and 2 mL of dilute hydrochloric acid, boil gently on a water bath for 5 minutes, and filter. Evaporate the filtrate to dryness, and add 20 mL of water to the residue: the solution responds to the Qualitative Tests <*1.09*> for sodium salt.

pH <*2.54*> Add 1.0 g of Carmellose Sodium in small portions to 100 mL of warm water with stirring, dissolve, and cool: the pH of this solution is between 6.0 and 8.0.

Purity **(1)** Clarity and color of solution—Firmly attach a glass plate of good quality 2 mm in thickness, to the bottom of a glass column 250 mm in height, 25 mm in inner diameter and 2 mm in thickness. This is used as an outer tube. Similarly prepare an inner tube by attaching a glass plate of good quality 2 mm in thickness to the bottom of a glass column 300 mm in height, 15 mm in inner diameter and 2 mm in thickness. Dissolve 1.0 g of Carmellose Sodium in 100 mL of water, pour this solution into the outer tube, and place on a piece of white paper on which 15 parallel black lines 1 mm in width and 1 mm in interval are drawn. Moving the inner tube up and down and observing from the upper part, determine the height of the solution up to the lower edge of the inner tube when the distinction of the lines becomes impossible. Repeat the operation 3 times, and calculate the mean value: it is larger than that calculated from the similar operation, using the following control solution.

Control solution: To 5.50 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid, 5 mL of ethanol (95) and water to make 50 mL. Add 2 mL of barium chloride TS, mix well, and allow to stand for 10 minutes. Shake well this solution before use.

(2) Chloride <*1.03*>—Dissolve 0.5 g of Carmellose Sodium in 50 mL of water, and use this solution as the sample solution. Shake 10 mL of the sample solution with 10 mL of dilute nitric acid, heat to produce a flocculent precipitate in a water bath, cool, and centrifuge. Separate the supernatant liquid, wash the precipitate with three 10-mL portions of water, centrifuging each time, combine the supernatant liquid with the washings, and dilute with water to 200 mL. Perform the test using 50 mL of this solution as the test solution. Prepare the control solution with 0.45 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.640%).

(3) Sulfate <*1.14*>—Add 1 mL of hydrochloric acid to 10 mL of the sample solution obtained in (2), shake well, heat to produce a flocculent precipitate in a water bath, cool, and centrifuge. Separate the supernatant liquid, wash the precipitate with three 10-mL portions of water, centrifuging each time, combine the washings with the supernatant liquid mentioned above, and dilute to 50 mL with water. Take 10 mL of this solution, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.960%).

(4) Silicate—Weigh accurately about 1 g of Carmellose Sodium, ignite in a platinum dish, add 20 mL of dilute hy-

drochloric acid, cover with a watch glass, and boil gently for 30 minutes. Remove the watch glass, and evaporate on a water bath to dryness with the aid of a current of air. Continue heating for further 1 hour, add 10 mL of hot water, stir well, and filter through a filter paper for quantitative analysis. Wash the residue with hot water, dry together with the filter paper after no turbidity is produced on the addition of silver nitrate TS to the last washing, and then ignite to constant mass: the mass of the residue is not more than 0.5%.

(5) Heavy metals <*1.07*>—Proceed with 1.0 g of Carmellose Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic <*1.11*>—To 1.0 g of Carmellose Sodium add 20 mL of nitric acid, heat gently until it becomes fluid, cool, add 5 mL of sulfuric acid, and heat until white fumes are evolved. Add, if necessary, 5 mL of nitric acid after cooling, and heat again. Repeat this operation until the solution becomes colorless or slightly yellow. After cooling, add 15 mL of a saturated solution of ammonium oxalate monohydrate, and heat until white fumes are evolved again, cool, and dilute with water to 25 mL. Take 5 mL of this solution as the test solution, and perform the test. The solution has no more color than the following color standard.

Color standard: Without using Carmellose Sodium, proceed in the same manner, then transfer 5 mL of this solution to a generator bottle, add exactly 2 mL of Standard Arsenic Solution, and proceed as directed for the test with the test solution (not more than 10 ppm).

(7) Starch—Add 2 drops of iodine TS to 10 mL of the sample solution obtained in (2): no blue color develops.

Loss on drying <*2.41*> Not more than 10.0% (1 g, 105°C, 4 hours).

Assay Weigh accurately about 0.5 g of Carmellose Sodium, previously dried, add 80 mL of acetic acid (100), connect with a reflux condenser, and heat in an oil bath maintained at 130°C for 2 hours. Cool, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 2.299 mg of Na

Containers and storage Containers—Tight containers.

Croscarmellose Sodium

クロスカルメロースナトリウム

[*74811-65-7*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbol (◆ ◆).

Croscarmellose Sodium is the sodium salt of a crosslinked poly carboxymethylether of cellulose.

◆Description Croscarmellose Sodium occurs as a white to yellowish white powder.

It is practically insoluble in ethanol (99.5) and in diethyl ether.

It swells with water and becomes a suspension.

It is hygroscopic.◆

Identification (1) To 1 g of Croscarmellose Sodium add 100 mL of a solution of methylene blue (1 in 250,000), stir well, and allow to stand: blue cotton-like precipitates appear.

(2) To 1 g of Croscarmellose Sodium add 50 mL of water, and stir well to make a suspension. To 1 mL of this suspension add 1 mL of water and 5 drops of fleshly prepared solution of 1-naphtol in methanol (1 in 25), and gently add 2 mL of sulfuric acid along a wall of the vessel: a red-purple color appears at the zone of contact.

(3) The suspension obtained in (2) responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

pH <*2.54*> To 1.0 g of Croscarmellose Sodium add 100 mL of water, and stir for 5 minutes: the pH of the supernatant liquid is between 5.0 and 7.0.

Purity ◆**(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Croscarmellose Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).◆

◆**(2)** Sodium chloride and sodium glycolate—The total amount of sodium chloride and sodium glycolate is not more than 0.5%, calculated on the dried basis.

(i) Sodium chloride: Weigh accurately about 5 g of Croscarmellose Sodium, add 50 mL of water and 5 mL of hydrogen peroxide (30), and heat on a water bath for 20 minutes with occasional stirring. After cooling, add 100 mL of water and 10 mL of nitric acid, and titrate <*2.50*> with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank determination in the same manner and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS
= 5.844 mg of NaCl

(ii) Sodium glycolate: Weigh accurately about 0.5 g of Croscarmellose Sodium, add 2 mL of acetic acid (100) and 5 mL of water, and stir for 15 minutes. Add gradually 50 mL of acetone with stirring, then add 1 g of sodium chloride, stir for 3 minutes, and filter through a filter paper moistened with acetone. Wash the residue thoroughly with 30 mL of acetone, combine the filtrate and washings, add acetone to make exactly 100 mL, and use this solution as the sample stock solution. Separately, dissolve 0.100 g of glycolic acid in water to make 200 mL. Pipet 0.5 mL, 1 mL, 2 mL, 3 mL and 4 mL of this solution, add water to make them exactly 5 mL, then add 5 mL of acetic acid (100) and acetone to make exactly 100 mL, and designate them standard stock solution (1), standard stock solution (2), standard stock solution (3), standard stock solution (4) and standard stock solution (5), respectively. Pipet 2 mL each of the sample stock solution and the standard stock solutions (1), (2), (3), (4) and (5), and heat them in a water bath for 20 minutes to evaporate acetone. After cooling, add exactly 5 mL of 2,7-dihydroxynaphthalene TS, mix, then add 15 mL of 2,7-dihydroxynaphthalene TS, mix, cover the mouth of the vessels with aluminum foil, and heat in a water bath for 20 minutes. After cooling, add sulfuric acid to make exactly 25 mL, mix, and designate them sample solution, standard solution (1), standard solution (2), standard solution (3), standard solution (4) and standard solution (5), respectively. Separately, to 10 mL of a mixture of water and acetic acid (100) (1:1) add acetone to make exactly 100 mL, and proceed with exactly 2 mL of this solution in the same manner for preparation of the sample solution, and use the solution so obtained as the blank solution. Determine the absorbances, A_T, A_{S1}, A_{S2}, A_{S3}, A_{S4} and A_{S5}, of the sample solution and the standard solutions (1), (2), (3), (4) and (5), respectively, at 540 nm as directed under Ultraviolet-visible Spectrophotometry

<2.24>, using the blank solution as the control. Determine the amount (g) of glycolic acid, X, in 100 ml of the sample solution from the calibration curve obtained with the standard solutions, and calculate the amount of sodium glycolate by the following formula.

$$\text{Amount (\%) of sodium glycolate} = X/M \times 100 \times 1.289$$

M: Amount (g) of sample taken, calculated on the dried basis◆

◆**(3)** Water-soluble substance—Weigh accurately about 10 g of Croscarmellose Sodium, disperse in 800 mL of water by stirring for 1 minute every 10 minutes during 30 minutes, and allow to stand for at most 1 hour to precipitate. Filter by suction or centrifuge the clear upper portion, and weigh accurately the mass of about 150 mL of the filtrate or supernatant liquid. Heat to concentrate this liquid avoiding to dryness, then dry at 105°C for 4 hours, and weigh the mass of the residue accurately. Calculate the amount of the water-soluble substance by the following formula: not less than 1.0% and not more than 10.0%.

$$\text{Amount (\%) of water-soluble substance} = 100\ M_3\ (800 + M_1)/M_1M_2$$

M_1: Amount (g) of sample taken, calculated on the dried basis

M_2: Amount (g) of the filtrate or supernatant liquid of about 150 mL

M_3: Amount (g) of the residue◆

Precipitation test Put 75 mL of water in a 100-mL glass-stoppered graduated cylinder, and add portion by portion with 1.5 g of Croscarmellose Sodium divided into three portions while shaking vigorously at each time. Then, add water to make 100 mL, shake until to get a homogenous dispersion, and allow to stand for 4 hours: the volume of the settled layer is not less than 10.0 mL and not more than 30.0 mL.

Degree of substitution Weigh accurately about 1 g of Croscarmellose Sodium, put in a 500-mL glass-stoppered conical flask, add 300 mL of sodium chloride TS, then add 25.0 mL of 0.1 mol/L sodium hydroxide VS, stopper, and allow to stand for 5 minutes with occasional shaking. Add 5 drops of *m*-cresol purple TS, then add exactly 15 mL of 0.1 mol/L hydrochloric acid VS using a buret, stopper the flask, and shake. If the color of the solution is purple, add exactly 1-mL portions of 0.1 mol/L hydrochloric acid VS using the buret, with shaking each time, until the color of the solution changes to yellow, then titrate <2.50> with 0.1 mol/L sodium hydroxide VS until the color changes from yellow to purple. Perform a blank determination in the same manner. Calculate the degrees of substitution of acid-carboxymethyl group and sodium-carboxymethyl group, A and S: $A + S$ is not less than 0.60 and not more than 0.85.

$$A = 1150M/(7102 - 412M - 80C)$$
$$S = (162 + 58A)C/(7102—80C)$$

M: Amount (mmol) of sodium hydroxide needed to neutralize 1 g of sample taken, calculated on the dried basis

C: The value (%) obtained in Residue on ignition

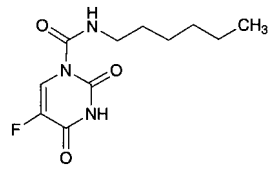
Loss on drying <2.41> Not more than 10.0% (1 g, 105°C, 6 hours).

Residue on ignition <2.44> 14.0 – 28.0% (after drying, 1 g).

Containers and storage Containers—Tight containers.

Carmofur

カルモフール



$C_{11}H_{16}FN_3O_3$: 257.26
5-Fluoro-1-(hexylaminocarbonyl)uracil
[*61422-45-5*]

Carmofur, when dried, contains not less than 98.0% of carmofur ($C_{11}H_{16}FN_3O_3$).

Description Carmofur occurs as a white crystalline powder.

It is very soluble in *N*,*N*-dimethylformamide, freely soluble in acetic acid (100), soluble in diethyl ether, sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Melting point: about 111°C (with decomposition).

Identification **(1)** Proceed with 5 mg of Carmofur as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid, and prepare the test solution: the test solution responds to the Qualitative Tests <1.09> (2) for fluoride.

(2) Determine the absorption spectrum of a solution of Carmofur in a mixture of methanol and phosphoric acid-acetic acid-boric acid buffer solution (pH 2.0) (9:1) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Carmofur, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals <1.07>—Proceed with 2.0 g of Carmofur according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.20 g of Carmofur in 10 mL of a mixture of methanol and acetic acid (100) (99:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and acetic acid (100) (99:1) to make exactly 500 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 15 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of toluene and acetone (5:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. After exposure of the plate to bromine vapor for 30 second, spray evenly a solution of fluorescein in ethanol (95) (1 in 2500): the spots other than the principal spot from the sample solution are not more in-

tense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, 50°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

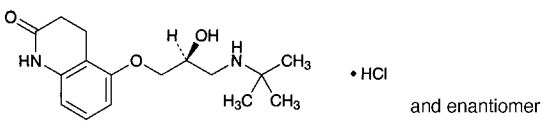
Assay Weigh accurately about 0.5 g of Carmofur, previously dried, dissolve in 20 mL of *N*,*N*-dimethylformamide, and titrate ⟨*2.50*⟩ with 0.1 mol/L tetramethylammonium hydroxide-methanol VS until the color of the solution changes from yellow through blue-green to blue (indicator: 3 drops of thymol blue-dimethylformamide TS).

Each mL of 0.1 mol/L tetramethylammonium hydroxide-methanol VS
= 25.73 mg of $C_{11}H_{16}FN_3O_3$

Containers and storage Containers—Tight containers.

Carteolol Hydrochloride

カルテオロール塩酸塩



$C_{16}H_{24}N_2O_3$.HCl: 328.83
5-[(2*RS*)-3-(1,1-Dimethylethyl)amino-2-hydroxypropyloxy]-3,4-dihydroquinolin-2(1*H*)-one monohydrochloride
[*51781-21-6*]

Carteolol Hydrochloride, when dried, contains not less than 99.0% of carteolol hydrochloride ($C_{16}H_{24}N_2O_3$.HCl).

Description Carteolol Hydrochloride occurs as white, crystals or crystalline powder.

It is soluble in water, sparingly soluble in methanol, very slightly soluble in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

The pH of a solution of 1.0 g of Carteolol Hydrochloride in 100 mL of water is between 5.0 and 6.0.

The solution of Carteolol Hydrochloride (1 in 20) shows no optical rotation.

Melting point: about 277°C (with decomposition).

Identification **(1)** Dissolve 0.1 g of Carteolol Hydrochloride in 5 mL of water, and add 5 drops of Reinecke salt TS: a light red precipitate is formed.

(2) Determine the absorption spectrum of a solution of Carteolol Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Carteolol Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Carteolol Hydrochloride (1 in 50) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Carteolol Hydrochloride in 30 mL of water: the solution is clear and colorless.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Carteolol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Carteolol Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Carteolol Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (50:20:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

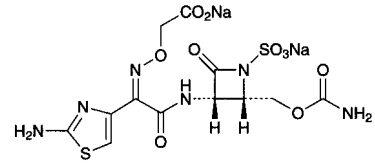
Assay Weigh accurately about 0.5 g of Carteolol Hydrochloride, previously dried, add 30 mL of acetic acid (100), dissolve by heating on a water bath, and cool. After adding 70 mL of acetic anhydride, titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.88 mg of $C_{16}H_{24}N_2O_3$.HCl

Containers and storage Containers—Well-closed containers.

Carumonam Sodium

カルモナムナトリウム



$C_{12}H_{12}N_6Na_2O_{10}S_2$: 510.37
Disodium (*Z*)-{(2-aminothiazol-4-yl)[(2*S*,3*S*)-2-carbamoyloxymethyl-4-oxo-1-sulfonatoazetidin-3-ylcarbamoyl]methyleneaminooxy}acetate
[*86832-68-0*]

Carumonam Sodium contains not less than 850 μg (potency) and not more than 920 μg (potency) per mg, calculated on the anhydrous basis. The potency of Carumonam Sodium is expressed as mass (potency) of carumonam ($C_{12}H_{14}N_6O_{10}S_2$: 466.40).

Description Carumonam Sodium occurs as a white to yellowish white, crystals or crystalline powder.

It is freely soluble in water, soluble in formamide, very slightly soluble in methanol, and practically insoluble in ethanol (99.5) and in acetic acid (100).

Identification **(1)** Determine the absorption spectrum of a solution of Carumonam Sodium (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Carumonam Sodium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Carumonam Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Carumonam Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Carumonam Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>, using sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a double signal A at around δ 5.5 ppm, and a single signal B at around δ 7.0 ppm. The ratio of the integrated intensity of these signals, A:B, is about 1:1.

(4) Carumonam Sodium responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +18.5 – +21.0° (0.1 g calculated on the anhydrous basis, water, 10 mL, 100 mm).

pH <*2.54*> The pH of a solution obtained by dissolving 1.0 g of Carumonam Sodium in 10 mL of water is between 5.0 and 6.5.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Carumonam Sodium in 5 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Carumonam Sodium according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 15 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 2.0 g of Carumonam Sodium according to Method 4, and perform the test (not more than 1 ppm).

(4) Related substance 1—Weigh accurately about 0.1 g of Carumonam Sodium, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Carumonam Sodium RS, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, and add the mobile phase to make exactly 25 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the related substances by the following equation: the amount of the related substance having the relative retention time of about 0.7 to carumonam is not more than 4.0%, and each amount of the related substances other than the related substance having the relative retention time of about 0.7 to carumonam is not more than 1.0%.

$$\text{Amount (\%) of related substance} = M_S/M_T \times A_T/A_S$$

M_S: Amount (g) of Carumonam Sodium RS taken
M_T: Amount (g) of Carumonam Sodium taken
A_S: Peak area of carumonam from the standard solution
A_T: Each peak area other than carumonam from the sample solution

Operating conditions—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.
Time span of measurement: About 3 times as long as the retention time of carumonam.
System suitability—
Test for required detectability: Measure exactly 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of carumonam obtained from 10 μL of this solution is equivalent to 7 to 13% of that obtained from 10 μL of the standard solution.
System performance: Dissolve 40 mg of Carumonam Sodium in 20 mL of the mobile phase. To 5 mL of this solution add 5 mL of a solution of resorcinol in the mobile phase (9 in 1000) and the mobile phase to make 25 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, resorcinol and carumonam are eluted in this order with the resolution between these peaks being not less than 2.5.
System repeatability: When the test is repeated 3 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of carumonam is not more than 2.0%.

(5) Related substance 2—Weigh accurately about 0.1 g of Carumonam Sodium, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Carumonam Sodium RS, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, and add the mobile phase to make exactly 25 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the related substances by the following equation: the amount of each related substance is not more than 1.0%.

$$\text{Amount (\%) of related substance} = M_S/M_T \times A_T/A_S$$

M_S: Amount (g) of Carumonam Sodium RS taken
M_T: Amount (g) of Carumonam Sodium taken
A_S: Peak area of carumonam from the standard solution
A_T: Each area of the peaks appeared after the peak of carumonam from the sample solution

Operating conditions—
Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.
Mobile phase: A mixture of a solution of ammonium sulfate (1 in 10,000), methanol and acetic acid (100) (74:25:1).
Flow rate: Dissolve 0.01 g of phthalic acid in the mobile phase to make 100 mL. Adjust so that the retention time of phthalic acid is about 6.5 minutes when the procedure is run with 10 μL of this solution.
Time span of measurement: About 10 times as long as the retention time of carumonam.
System suitability—
Test for required detectability: Measure exactly 5 mL of the standard solution, and add the mobile phase to make ex-

actly 50 mL. Confirm that the peak area of carumonam obtained from 10 μL of this solution is equivalent to 7 to 13% of that obtained from 10 μL of the standard solution.

System performance: Dissolve 40 mg of Carumonam Sodium in 20 mL of the mobile phase. To 5 mL of this solution add 5 mL of a solution of resorcinol in the mobile phase (9 in 1000) and the mobile phase to make 25 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, resorcinol and carumonam are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 3 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of carumonam is not more than 2.0%.

(6) Total amount of related substances—The total of the amounts of the related substances obtained in the Related substance 1 and the Related substance 2 is not more than 6.0%.

Water ⟨*2.48*⟩ Not more than 2.0% (0.2 g, volumetric titration, direct titration; Use a mixture of formamide for water determination and methanol for water determination (3:1) instead of methanol for water determination).

Assay Weigh accurately an amount of Carumonam Sodium and Carumonam Sodium RS, equivalent to about 40 mg (potency), and dissolve each in the mobile phase to make exactly 20 mL. Measure exactly 5 mL each of these solutions, add exactly 5 mL of the internal standard solution and the mobile phase to make 25 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of carumonam to that of the internal standard.

Amount [μg (potency)] of carumonam ($C_{12}H_{14}N_6O_{10}S_2$) = $M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Carumonam Sodium RS taken

Internal standard solution—A solution of resorcinol in the mobile phase (9 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of ammonium sulfate (1 in 10,000), methanol and acetic acid (100) (97:2:1).

Flow rate: Adjust so that the retention time of carumonam is about 10 minutes.

System suitability—

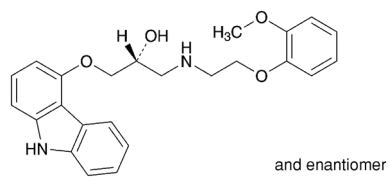
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and carumonam are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of carumonam to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

Carvedilol

カルベジロール



$C_{24}H_{26}N_2O_4$: 406.47

(2*RS*)-1-(9*H*-Carbazol-4-yloxy)-3-{[2-(2-methoxyphenoxy)ethyl]amino}propan-2-ol

[*72956-09-3*]

Carvedilol, when dried, contains not less than 99.0% and not more than 101.0% of carvedilol ($C_{24}H_{26}N_2O_4$).

Description Carvedilol occurs as white to pale yellowish white, crystals or crystalline powder.

It is freely soluble in acetic acid (100), sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Carvedilol in methanol (1 in 100) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Carvedilol in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Carvedilol as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 114 – 119°C

Purity (1) Heavy metals ⟨*1.07*⟩—Wrap 2.0 g of Carvedilol with a filter paper for quantitative analysis, then proceed according to Method 4, and perform the test. Prepare the control solution as follows: Put a filter paper for quantitative analysis in a crucible, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), then proceed as directed for the test solution, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 10 ppm).

(2) Related substances—Dissolve 65 mg of Carvedilol in 100 mL of the mobile phase. To 1 mL of this solution add the mobile phase to make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than carvedilol obtained from the sample solution is not larger than 3/20 times the peak area of carvedilol obtained from the standard solution, and the total area of the peaks other than carvedilol from the sample solution is not larger than 1/2 times the peak area of carvedilol from the standard solu-

tion.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 55°C.

Mobile phase: Dissolve 2.72 g of potassium dihydrogen phosphate in 900 mL of water, adjust to pH 2.0 with phosphoric acid, and add water to make 1000 mL. To 650 mL of this solution add 350 mL of acetonitrile.

Flow rate: Adjust so that the retention time of carvedilol is about 4 minutes.

Time span of measurement: About 9 times as long as the retention time of carvedilol, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, add the mobile phase to make exactly 20 mL. Confirm that the peak area of carvedilol obtained with 20 μL of this solution is equivalent to 7 to 13% of that obtained with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of carvedilol are not less than 6000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of carvedilol is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Carvedilol, previously dried, dissolve in 60 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform the blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 40.65 mg of $C_{24}H_{26}N_2O_4$

Containers and storage Containers—Tight containers.

Carvedilol Tablets

カルベジロール錠

Carvedilol Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of carvedilol ($C_{24}H_{26}N_2O_4$: 406.47).

Method of preparation Prepare as directed under Tablets, with Carvedilol.

Identification Powder Carvedilol Tablets. To a portion of the powder, equivalent to 20 mg of Carvedilol, add 10 mL of methanol, shake well, and filter. To 0.5 mL of the filtrate add methanol to make 200 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectophotometry ⟨*2.24*⟩: it exhibits maxima between 222 nm and 226 nm, between 241 nm and 245 nm, between 284 nm and 288 nm, between 317 nm and 321 nm and between 330 nm and 334 nm.

Purity Related substances—In this procedure the sample solution should be stored not exceeding 5°C and used within 24 hours after preparation. Powder Carvedilol Tablets. Dissolve a portion of the powder, equivalent to 12.5 mg of Carvedilol, add an adequate amount of the mobile phase and disperse the particles with the aid of ultrasonic waves, if necessary, add the mobile phase to make 100 mL, and shake for 30 minutes. Filter through a membrane filter with a pore size not exceeding 0.22 μm, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the two peaks, having the relative retention time between 1.7 and 1.9 and between 2.0 and 3.1 to carvedilol, obtained from the sample solution of 1.25-mg or 2.5-mg tablet is not larger than 3/10 times and 1.6 times the peak area of carvedilol obtained from the standard solution, respectively, the area of the peak other than carvedilol and the peaks mentioned above from the sample solution is not larger than 1/5 times the peak area of carvedilol from the standard solution, and the total area of the peaks other than carvedilol from the sample solution is not larger than 2.2 times the peak area of carvedilol from the standard solution. The area of the two peaks, having the relative retention time between 1.7 and 1.9 and between 2.0 and 3.1 to carvedilol, obtained from the sample solution of 10-mg or 20-mg tablet is not larger than 1/10 times and 2/5 times the peak area of carvedilol from the standard solution, respectively, the area of the peak other than carvedilol and the peak mentioned above from the sample solution is not larger than 1/10 times the peak area of carvedilol from the standard solution, and the total area of the peaks other than carvedilol from the sample solution is not larger than 3/5 times the peak area of carvedilol from the standard solution. For the area of the peak, having the relative retention time between 1.7 and 1.9 to carvedilol, multiply the relative response factor 1.25.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 10 times as long as the retention time of carvedilol, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add the mobile phase to make exactly 100 mL. Confirm that the peak area of carvedilol obtained with 50 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 50 μL of the standard solution.

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of carvedilol are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of carvedilol is not more than 1.0%.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the

Content uniformity test.

To 1 tablet of Carvedilol Tablets add 70 mL of a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1), shake until the tablet is completely disintegrated, then add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make exactly 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make exactly V' mL so that each mL contains about 5 μg of carvedilol ($C_{24}H_{26}N_2O_4$), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of carvedilol for assay, previously dried at 105°C for 2 hours, and dissolve in a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make exactly 100 mL. Pipet 2 mL of this solution, add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 240 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of carvedilol ($C_{24}H_{26}N_2O_4$)
$= M_S \times A_T/A_S \times V'/V \times 1/50$

M_S: Amount (mg) of carvedilol for assay taken

Dissolution <6.10> **(1)** 10-mg tablet and 20-mg tablet

When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of 0.05 mol/L acetic acid-sodium acetate buffer solution (pH 4.0) as the dissolution medium, the dissolution rate in 30 minutes of Carvedilol Tablets is not less than 80%.

Start the test with 1 tablet of Carvedilol Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 11 μg of carvedilol ($C_{24}H_{26}N_2O_4$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of carvedilol for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 285 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using the dissolution medium as the blank.

Dissolution rate (%) with respect to the labeled amount of carvedilol ($C_{24}H_{26}N_2O_4$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of carvedilol for assay taken
C: Labeled amount (mg) of carvedilol ($C_{24}H_{26}N_2O_4$) in 1 tablet

(2) 1.25-mg tablet and 2.5-mg tablet When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 0.05 mol/L acetic acid-sodium acetate buffer solution (pH 4.0) as the dissolution medium, the dissolution rate in 20 minutes is not less than 75%.

Start the test with 1 tablet of Carvedilol Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 1.4 μg of carvedilol ($C_{24}H_{26}N_2O_4$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of carvedilol for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 200 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 240 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using the dissolution medium as the blank.

Dissolution rate (%) with respect to the labeled amount of carvedilol ($C_{24}H_{26}N_2O_4$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/2$

M_S: Amount (mg) of carvedilol for assay taken
C: Labeled amount (mg) of carvedilol ($C_{24}H_{26}N_2O_4$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Carvedilol Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 25 mg of carvedilol ($C_{24}H_{26}N_2O_4$), add exactly 5 mL of the internal standard solution, add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make 250 mL, and shake for 30 minutes. To 2 mL of this solution, add the mobile phase to make 20 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of carvedilol for assay, previously dried at 105°C for 2 hours, add exactly 5 mL of the internal standard solution, and add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make 250 mL. To 2 mL of this solution add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> under the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of carvedilol to that of the internal standard.

Amount (mg) of carvedilol ($C_{24}H_{26}N_2O_4$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of carvedilol for assay taken

Internal standard solution—A solution of isoamyl parahydroxybenzoate in the mobile phase (1 in 70).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.7 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 5.0 with a solution prepared by dissolving 0.7 g of dipotassium hydrogen phosphate in water to make 200 mL. To 450 mL of this solution add 550 mL of methanol.

Flow rate: Adjust so that the retention time of carvedilol is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, carvedilol and the internal standard are eluted in this order with the resolution between these peaks being not less than 20.

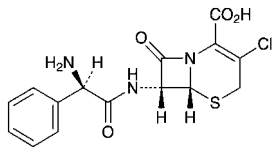
System repeatability: When the test is repeated 6 times

with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of carvedilol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cefaclor

セファクロル



$C_{15}H_{14}ClN_3O_4S$: 367.81
(6*R*,7*R*)-7-[(2*R*)-2-Amino-2-phenylacetylamino]-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
[*53994-73-3*]

Cefaclor contains not less than 950 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefaclor is expressed as mass (potency) of cefaclor ($C_{15}H_{14}ClN_3O_4S$).

Description Cefaclor occurs as a white to yellowish white crystalline powder.

It is slightly soluble in water and in methanol, and practically insoluble in *N*,*N*-dimethylformamide and in ethanol (99.5).

Identification **(1)** Determine the absorption spectrum of a solution of Cefaclor (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefaclor as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 40 mg of Cefaclor in 0.5 mL of heavy water for nuclear magnetic resonance spectroscopy and 1 drop of deuterated hydrochloric acid for nuclear magnetic resonance spectroscopy, and determine the ^1H spectrum of this solution as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits an AB type quartet signal A at around δ 3.7 ppm, and a single signal or a sharp multiple signal B at around δ 7.6 ppm. The ratio of the integrated intensity of each signal, A:B, is about 2:5.

(4) Perform the test with Cefaclor as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +105 – +120° (0.1 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Cefaclor according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution by suspending 1.0 g of Cefaclor in 10 mL of *N*,*N*-dimethylformamide, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Cefaclor in 10 mL of sodium dihydrogen phosphate TS (pH 2.5), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add sodium dihydrogen phosphate TS (pH 2.5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peaks other than cefaclor from the sample solution are not larger than 1/2 times the peak area of cefaclor from the standard solution, and the total area of the peaks other than cefaclor from the sample solution is not larger than 2 times of the peak area of cefaclor from the standard solution. If necessary, proceed with 20 μL of sodium dihydrogen phosphate TS (pH 2.5) in the same manner as above to compensate the base line.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Dissolve 7.8 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust the pH to 4.0 with phosphoric acid.

Mobile phase B: To 550 mL of the mobile phase A add 450 mL of acetonitrile for liquid chromatography.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 30	95 → 75	5 → 25
30 – 45	75 → 0	25 → 100
45 – 55	0	100

Flow rate: 1.0 mL per minute.

Time span of measurement: About 2.5 times as long as the retention time of cefaclor, beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add sodium dihydrogen phosphate TS, pH 2.5 to make exactly 20 mL. Confirm that the peak area of cefaclor obtained from 20 μL of this solution is equivalent to 4 to 6% of that obtained from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefaclor are not less than 40,000 and 0.8 to 1.3, respectively.

System repeatability: When the test is repeated 3 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak areas and the retention times of cefaclor are not more than 2.0%, respectively.

Water <*2.48*> Not more than 6.5% (0.2 g, volumetric titration, back titration).

Assay Weigh accurately an amount of Cefaclor and Cefaclor RS, equivalent to about 50 mg (potency), and dissolve each in 0.1 mol/L phosphate buffer solution (pH 4.5)

to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefaclor to that of the internal standard.

Amount [μg (potency)] of cefaclor ($C_{15}H_{14}ClN_3O_4S$)
$= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Cefaclor RS taken

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L phosphate buffer solution (pH 4.5) (1 in 700).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.8 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 3.4 with diluted phosphoric acid (3 in 500). To 940 mL of this solution add 60 mL of acetonitrile.

Flow rate: Adjust so that the retention time of cefaclor is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefaclor and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefaclor to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cefaclor Capsules

セファクロルカプセル

Cefaclor Capsules contain not less than 90.0% and not more than 110.0% of the labeled potency of cefaclor ($C_{15}H_{14}ClN_3O_4S$: 367.81).

Method of preparation Prepare as directed under Capsules, with Cefaclor.

Identification Shake vigorously a quantity of the contents of Cefaclor Capsules, equivalent to 20 mg (potency) of Cefaclor, with 10 mL of water, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 20 mg of Cefaclor RS in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of acetonitrile, water, ethyl acetate and formic acid (30:10:10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot from the sample solution and the spot from the standard solution show the same *R*f value.

Purity Related substances—Weigh accurately not less than 5 Cefaclor Capsules, open the capsules and carefully take out the contents, mix well, and powder, if necessary. Wash the empty capsules with a little amount of diethyl ether, if necessary, allow the capsules to stand at room temperature to vaporize adhering diethyl ether, and weigh accurately the capsules to calculate the mass of the contents. Weigh accurately a quantity of the contents, equivalent to about 0.25 g (potency) of Cefaclor, shake with 40 mL of 0.1 mol/L phosphate buffer solution (pH 4.5) for 10 minutes, add the same buffer solution to make exactly 50 mL, and filter through a 0.45-μm pore-size membrane filter. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Cefaclor RS, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 20 mL. Pipet 2.5 mL of this solution, add the same buffer solution to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas by the automatic integration method. Calculate the amount of each related substance by the following equation: the amount of each related substance is not more than 0.5%, and the total amount of the related substances is not more than 2.5%. If necessary, correct the fluctuation of the base line by performing the test in the same way with 20 μL of 0.1 mol/L phosphate buffer solution (pH 4.5).

Amount (%) of each related substance
$= M_S/M_T \times A_{Ti}/A_S \times M_M/C \times 25/2$

Total amount (%) of the related substances
$= M_S/M_T \times \Sigma A_{Tn}/A_S \times M_M/C \times 25/2$

M_S: Amount [mg (potency)] of Cefaclor RS taken
M_T: Amount (mg) of the contents of Cefaclor Capsules taken
M_M: Average mass (mg) of the contents in 1 capsule
A_{Ti}: Area of each peak other than cefaclor and solvent from the sample solution
A_S: Peak area of cefaclor from the standard solution
C: Labeled potency [mg (potency)] of Cefaclor in 1 capsule

Operating conditions—

Proceed as directed in the operating conditions in the Purity (3) under Cefaclor.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 20 mL. Confirm that the peak area of cefaclor obtained with 20 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefaclor are not less than 40,000 and 0.8 to 1.3, respectively.

System repeatability: When the test is repeated 3 times with 20 μL of the standard solution under the above operat-

ing conditions, the relative standard deviation of the peak area of cefaclor is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 8.0% (0.2 g, volumetric titration, back titration).

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Cefaclor Capsules is not less than 80%.

Start the test with 1 capsule of Cefaclor Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 20 μg (potency) of Cefaclor, and use this solution as the sample solution. Separately, weigh accurately about 20 mg (potency) of Cefaclor RS, and dissolve in water to make exactly 20 mL. Pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 265 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of cefaclor ($C_{15}H_{14}ClN_3O_4S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount [mg (potency)] of Cefaclor RS taken
C: Labeled amount [mg (potency)] of Cefaclor in 1 capsule

Assay Weigh accurately not less than 5 Cefaclor Capsules, open the capsules and carefully take out the contents, mix well, and powder, if necessary. Wash the empty capsules with a little amount of diethyl ether, if necessary, allow the capsules to stand at room temperature to vaporize adhering diethyl ether, and weigh accurately the capsules to calculate the mass of the contents. Weigh accurately a quantity of the contents, equivalent to about 0.1 g (potency) of Cefaclor, shake vigorously with 60 mL of 0.1 mol/L phosphate buffer solution (pH 4.5) for 10 minutes, add the same buffer solution to make exactly 100 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefaclor RS, equivalent to about 50 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefaclor.

Amount [mg (potency)] of cefaclor ($C_{15}H_{14}ClN_3O_4S$)
$= M_S \times Q_T/Q_S \times 2$

M_S: Amount [mg (potency)] of Cefaclor RS taken

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L phosphate buffer solution (pH 4.5) (1 in 700).

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Cefaclor Combination Granules

セファクロル複合顆粒

Cefaclor Combination Granules contain gastric-soluble granules and enteric-soluble granules in one package.

It contains cefaclor ($C_{15}H_{14}ClN_3O_4S$: 367.81) equivalent to not less than 90.0% and not more than 110.0% of the labeled total potency and the labeled potency of gastric-soluble granule, respectively.

Method of preparation Prepare as directed under Granules, with Cefaclor, and divide into single-dose packages.

Identification Shake vigorously a quantity of Cefaclor Combination Granules, equivalent to 20 mg (potency) of Cefaclor according to the labeled total potency, with 10 mL of water, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 20 mg of Cefaclor RS in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of acetonitrile, water, ethyl acetate and formic acid (30:10:10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot from the sample solution and the spot from the standard solution show the same Rf value.

Purity Related substances—Take out the total contents of not less than 5 packages of Cefaclor Combination Granules, add a small amount of 0.1 mol/L phosphate buffer solution (pH 4.5), grind, add 0.1 mol/L phosphate buffer solution (pH 4.5), shake vigorously for 10 minutes, and add 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly V mL so that each mL contains about 5 mg (potency) of Cefaclor according to the labeled total potency. Pipet 10 mL of this solution, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 25 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Cefaclor RS, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 20 mL. Pipet 2 mL of this solution, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area in each solution by the automatic integration method. Calculate the amount of each related substance by the following equation: the amount of each related substance is not more than 0.6%, and the total amount of the related substances is not more than 2.8%. If necessary, correct the fluctuation of the base line by performing the test in the same way with 50 μL of 0.1 mol/L phosphate buffer solution (pH 4.5).

Amount (%) of each related substance
$= M_S \times A_T/A_S \times V/4 \times \{1/(C \times T)\}$

Total amount (%) of the related substances
$= M_S \times \Sigma A_T/A_S \times V/4 \times \{1/(C \times T)\}$

M_S: Amount [mg (potency)] of Cefaclor RS taken
A_T: Area of each peak other than cefaclor, solvent and excipient from the sample solution
ΣA_T: Total area of the peaks other than cefaclor, solvent and excipient from the sample solution
A_S: Peak area of cefaclor from the standard solution
C: Labeled total potency [mg (potency)] of Cefaclor in 1 package
T: Number (pack) of Cefaclor Combination Granules

Operating conditions—

Proceed as directed in the operating conditions in the Purity (3) under Cefaclor.

System suitability—

Test for required detectability: Pipet 1 mL of standard solution, and add 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 20 mL. Confirm that the peak area of cefaclor obtained from 50 μL of this solution is equivalent to 3.5 to 6.5% of that obtained from 50 μL of the standard solution.

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefaclor are not less than 40,000 and between 0.8 and 1.3, respectively.

System repeatability: When the test is repeated 3 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefaclor is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 5.5% (0.3 g, volumetric titration, back titration).

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

(1) Total potency—Take out the total contents of 1 Cefaclor Combination Granules, add a little amount of 0.1 mol/L phosphate buffer solution (pH 4.5), grind well, add the same buffer solutions to make exactly V mL so that each mL contains about 3.8 mg (potency) of Cefaclor according to the labeled total potency after shaking vigorously for 10 minutes, and centrifuge. Pipet 3 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefaclor RS, equivalent to about 50 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefaclor.

$$\text{Amount [mg (potency)] of cefaclor } (C_{15}H_{14}ClN_3O_4S) = M_S \times Q_T/Q_S \times V/15$$

M_S: Amount [mg (potency)] of Cefaclor RS taken

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L phosphate buffer solution (pH 4.5) (1 in 700).

(2) Potency of gastric-soluble granule—Take out the total contents of 1 Cefaclor Combination Granules stir gently with 60 mL of 0.1 mol/L phosphate buffer solution (pH 4.5) for 5 minutes, add the same buffer solution to make exactly V mL so that each mL contains about 1.5 mg (potency) of Cefaclor according to the labeled potency of gastric-soluble granule, and centrifuge. Pipet 7 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefaclor RS, equivalent to about 50 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefaclor.

$$\text{Amount [mg (potency)] of cefaclor } (C_{15}H_{14}ClN_3O_4S) = M_S \times Q_T/Q_S \times V/35$$

M_S: Amount [mg (potency)] of Cefaclor RS taken

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L phosphate buffer solution (pH 4.5) (1 in 700).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 60 minutes of Cefaclor Combination Granules is between 35% and 45%.

Start the test with the total content of 1 package of Cefaclor Combination Granules, withdraw not less than about 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 20 μg (potency) of Cefaclor according to the labeled potency of gastric-soluble granule, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefaclor RS, equivalent to about 20 mg (potency), and dissolve in the dissolution medium to make exactly 20 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 265 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

$$\text{Dissolution rate (\%) of cefaclor } (C_{15}H_{14}ClN_3O_4S) \text{ with respect to the labeled potency} = M_S \times A_T/A_S \times V'/V \times 1/C \times 90$$

M_S: Amount [mg (potency)] of Cefaclor RS taken
C: Labeled total potency [mg (potency)] of Cefaclor in 1 pack

Separately, when the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 60 minutes of Cefaclor Combination Granules is not less than 70%.

Start the test with the total content of 1 package of Cefaclor Combination Granules, withdraw not less than about 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add 0.01 mol/L hydrochloric acid TS to make exactly V' mL so that each mL contains about 20 μg (potency) of Cefaclor according to the labeled total potency, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefaclor RS, equivalent to about 20 mg (potency), dissolve in the dissolution medium to make exactly 100 mL, and warm at 37°C for 60 minutes. Pipet 2 mL of this solution, add 0.01 mol/L hydrochloric acid TS to make exactly 20 mL, and use this solution as the standard solution. Deter-

mine the absorbances, A_T and A_S, at 265 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using 0.01 mol/L hydrochloric acid TS as the blank.

Dissolution rate (%) of cefaclor ($C_{15}H_{14}ClN_3O_4S$) with respect to the labeled potency
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount [mg (potency)] of Cefaclor RS taken
C: Labeled total potency [mg (potency)] of Cefaclor in 1 package

Assay (1) Total potency—Take out the total contents of not less than 5 Cefaclor Combination Granules, add a small amount of 0.1 mol/L phosphate buffer solution (pH 4.5), grind well, add the same buffer solution so that each mL containing about 5 mg (potency) of Cefaclor according to the labeled total potency after shaking vigorously for 10 minutes, and centrifuge. Pipet 2 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefaclor RS, equivalent to about 50 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefaclor.

Amount [mg (potency)] of cefaclor ($C_{15}H_{14}ClN_3O_4S$)
$= M_S \times Q_T/Q_S \times 1/5$

M_S: Amount [mg (potency)] of Cefaclor RS taken

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L phosphate buffer solution (pH 4.5) (1 in 700).

(2) Potency of gastric-soluble granule—Stir gentry the total contents of not less than 5 Cefaclor Combination Granules with about 100 mL of 0.1 mol/L phosphate buffer solution (pH 4.5) for 5 minutes, add the same buffer solution so that each mL containing about 2 mg (potency) of Cefaclor according to the labeled potency of gastric-soluble granule, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefaclor RS, equivalent to about 50 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefaclor.

Amount [mg (potency)] of cefaclor ($C_{15}H_{14}ClN_3O_4S$)
$= M_S \times Q_T/Q_S \times 1/5$

M_S: Amount [mg (potency)] of Cefaclor RS taken

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L phosphate buffer solution (pH 4.5) (1 in 700).

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Cefaclor Fine Granules

セファクロル細粒

Cefaclor Fine Granules contain not less than 90.0% and not more than 110.0% of the labeled potency of cefaclor ($C_{15}H_{14}ClN_3O_4S$: 367.81).

Method of preparation Prepare as directed under Granules, with Cefaclor.

Identification Shake vigorously a quantity of Cefaclor Fine Granules, equivalent to 20 mg (potency) of Cefaclor, with 10 mL of water, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 20 mg (potency) of Cefaclor RS in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of acetonitrile, water, ethyl acetate and formic acid (30:10:10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot from the sample solution and the spot from the standard solution show the same Rf value.

Purity Related substances—Weigh accurately a quantity of Cefaclor Fine Granules after powdered if necessary, equivalent to about 0.1 g (potency) of Cefaclor, shake with 40 mL of 0.1 mol/L phosphate buffer solution (pH 4.5) for 10 minutes, add the same buffer solution to make exactly 50 mL, and filter through a 0.45-μm pore-size membrane filter. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Cefaclor RS, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 20 mL. Pipet 2 mL of this solution, add the same buffer solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas by the automatic integration method. Calculate the amount of each related substance by the following equation: the amount of each related substance is not more than 0.5%, and the total amount of the related substances is not more than 3.0%. If necessary, correct the fluctuation of the base line by performing the test in the same way with 50 μL of 0.1 mol/L phosphate buffer solution (pH 4.5).

Amount (%) of each related substance
$= M_S/M_T \times A_T/A_S \times 1/C \times 5$

Total amount (%) of the related substances
$= M_S/M_T \times \Sigma A_T/A_S \times 1/C \times 5$

M_S: Amount [mg (potency)] of Cefaclor RS taken
M_T: Amount (g) of Cefaclor Fine Granules taken
A_T: Area of the peak other than cefaclor and the solvent from the sample solution
A_S: Peak area of cefaclor from the standard solution
C: Labeled potency [mg (potency)] of cefaclor ($C_{15}H_{14}ClN_3O_4S$) in 1 g

Operating conditions—
Proceed as directed in the operating conditions in the Purity (3) under Cefaclor.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 20 mL. Confirm that the peak area of cefaclor obtained with 50 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 50 μL of the standard solution.

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefaclor are not less than 40,000 and between 0.8 and 1.3, respectively.

System repeatability: When the test is repeated 3 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefaclor is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 1.5% (1 g, volumetric titration, back titration).

Uniformity of dosage units ⟨*6.02*⟩ The granules in single-dose packages meet the requirement of the Mass variation test.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Cefaclor Fine Granules is not less than 85%.

Start the test with an accurately weighed amount of Cefaclor Fine Granules, equivalent to about 0.25 g (potency) of Cefaclor, withdraw not less than about 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 20 μg (potency) of Cefaclor, and use this solution as the sample solution. Separately, weigh accurately about 20 mg (potency) of Cefaclor RS, and dissolve in water to make exactly 20 mL. Pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 265 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of cefaclor ($C_{15}H_{14}ClN_3O_4S$)
$= M_S/M_T \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount [mg (potency)] of Cefaclor RS taken
M_T: Amount [mg (potency)] of Cefaclor Fine Granules taken
C: Labeled amount [mg (potency)] of cefaclor ($C_{15}H_{14}ClN_3O_4S$) in 1 g

Assay Weigh accurately a quantity of Cefaclor Fine Granules after powdered if necessary, equivalent to about 0.1 g (potency) of Cefaclor, shake vigorously with 60 mL of 0.1 mol/L phosphate buffer solution (pH 4.5) for 10 minutes, add the same buffer solution to make exactly 100 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg (potency) of Cefaclor RS, and dissolve in 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as standard solution. Proceed as directed in the Assay under Cefaclor.

Amount [mg (potency)] of cefaclor ($C_{15}H_{14}ClN_3O_4S$)
$= M_S \times Q_T/Q_S \times 2$

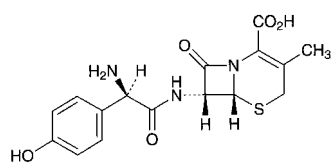
M_S: Amount [mg (potency)] of Cefaclor RS taken

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L phosphate buffer solution (pH 4.5) (1 in 700).

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Cefadroxil

セファドロキシル



$C_{16}H_{17}N_3O_5S$: 363.39
(6*R*,7*R*)-7-[(2*R*)-2-Amino-2-(4-hydroxyphenyl)acetylamino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
[*50370-12-2*]

Cefadroxil contains not less than 950 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefadroxil is expressed as mass (potency) of cefadroxil ($C_{16}H_{17}N_3O_5S$).

Description Cefadroxil occurs as a white to light yellow-white powder.

It is sparingly soluble in water, slightly soluble in methanol, and very slightly soluble in ethanol (95).

Identification **(1)** Determine the absorption spectrum of a solution of Cefadroxil (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefadroxil RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefadroxil as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Cefadroxil RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefadroxil in a mixture of heavy water for nuclear magnetic resonance spectroscopy and deuterated hydrochloric acid (3:1) (1 in 10), using sodium 3-(trimethylsilyl)propionate-d_4 for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy ⟨*2.21*⟩: it exhibits a single signal A at around δ 2.1 ppm, a double signal B at around δ 7.0 ppm, and a double signal C at around δ 7.5 ppm. The ratio of integrated intensity of each signal, A:B:C, is about 3:2:2.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{25}$: +164 – +182° (0.6 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 1.0 g of Cefadroxil in 200 mL of water: pH of the solution is between 4.0 and 6.0.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Cefadroxil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.1 g of Cefadroxil in 4 mL of a mixture of ethanol (99.5), water and diluted hydrochloric acid (1 in 5) (75:22:3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of ethanol (99.5), water and diluted hydrochloric acid (1 in 5) (75:22:3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of ethyl acetate, water, ethanol (99.5) and formic acid (14:5:5:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly ninhydrin-citric acid-acetic acid TS on the plate, and heat at 100°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water ⟨*2.48*⟩ Not less than 4.2% and not more than 6.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefadroxil and Cefadroxil RS, equivalent to about 50 mg (potency), dissolve each in water to make exactly 500 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of cefadroxil in each solution.

Amount [μg (potency)] of cefadroxil ($C_{16}H_{17}N_3O_5S$)
$= M_S \times A_T/A_S \times 1000$

M_S: amount [mg (potency)] of Cefadroxil RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 262 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of a solution of potassium dihydrogenphosphate (17 in 12,500) and methanol (17:3).

Flow rate: Adjust so that the retention time of cefadroxil is about 5 minutes.

System suitability—

System performance: Dissolve about 5 mg (potency) of Cefadroxil and about 10 mg (potency) of propylene glycol cefatrizine in 50 mL of water. When the procedure is run with 10 μL of this solution under the above operating conditions, cefadroxil and cefatrizine are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefadroxil is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cefadroxil Capsules

セファドロキシルカプセル

Cefadroxil Capsules contain not less than 95.0% and not more than 105.0% of the labeled potency of cefadroxil ($C_{16}H_{17}N_3O_5S$: 363.39).

Method of preparation Prepare as directed under Capsules, with Cefadroxil.

Identification Dissolve the contents of Cefadroxil Capsules, equivalent to 10 mg (potency) of Cefadroxil, in 500 mL of water, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 228 nm and 232 nm, and between 261 nm and 265 nm.

Water ⟨*2.48*⟩ Not more than 7.0% (0.15 g, volumetric titration, direct titration).

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Place 1 capsule of Cefadroxil Capsules in 300 mL of water, disperse with the aid of ultrasonic waves, shake for 30 minutes, and add water to make exactly 500 mL. Pipet 5 mL of this solution, and add water to make exactly V mL so that each mL contains about 0.1 mg (potency) of Cefadroxil. Filter the solution, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg (potency) of Cefadroxil RS, dissolve in water to make exactly 200 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefadroxil.

Amount [mg (potency)] of cefadroxil ($C_{16}H_{17}N_3O_5S$)
$= M_S \times A_T/A_S \times V/2$

M_S: Amount [mg (potency)] of Cefadroxil RS taken

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 0.05 mol/L acetic acid-sodium acetate buffer solution (pH 4.0) as the dissolution medium, the dissolution rate in 90 minutes of Cefadroxil Capsules is not less than 80%.

Start the test with 1 capsule of Cefadroxil Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 22 μg (potency) of Cefadroxil, and use this solution as the sample solution. Separately, weigh accurately about 22 mg (potency) of Cefadroxil RS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 263 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using water as the blank.

Dissolution rate (%) with respect to the labeled amount of cefadroxil ($C_{16}H_{17}N_3O_5S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount [mg (potency)] of Cefadroxil RS taken
C: Labeled amount [mg (potency)] of cefadroxil in 1 capsule

Assay Take out the contents of 20 Cefadroxil Capsules, and combine. Weigh accurately the mass of the combined contents, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg (potency) of Cefadroxil, add 300 mL of water, shake for 30 minutes, then add water to make exactly 500 mL, and filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Cefadroxil RS, equivalent to about 20 mg (potency), dissolve in water to make exactly 200 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefadroxil.

Amount [mg (potency)] of cefadroxil ($C_{16}H_{17}N_3O_5S$)
$= M_S \times A_T/A_S \times 5/2$

M_S: Amount [mg (potency)] of Cefadroxil RS taken

Containers and storage Containers—Tight containers.

Cefadroxil for Syrup

シロップ用セファドロキシル

Cefadroxil for Syrup is a preparation for syrup, which is suspended before use.

It contains not less than 95.0% and not more than 110.0% of the labeled potency of cefadroxil ($C_{16}H_{17}N_3O_5S$: 363.39).

Method of preparation Prepare as directed under Preparations for Syrups, with Cefadroxil.

Identification Dissolve an amount of Cefadroxil for Syrup, equivalent to 10 mg (potency) of Cefadroxil, in 500 mL of water, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 228 nm and 232 nm, and between 261 nm and 265 nm.

Water ⟨*2.48*⟩ Not more than 3.0% (0.5 g, volumetric titration, direct titration).

Uniformity of dosage units ⟨*6.02*⟩ The syrup in single-dose packages meets the requirement of the Mass variation test.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method (put the sample in the dissolution medium so that it disperses), using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Cefadroxil for Syrup is not less than 85%.

Start the test with accurately weighed amount of Cefadroxil for Syrup, equivalent to about 0.1 g (potency) of Cefadroxil, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 4 mL of the subsequent filtrate, add water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 22 mg (potency) of Cefadroxil RS, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 263 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of cefadroxil ($C_{16}H_{17}N_3O_5S$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 450$

M_S: Amount [mg (potency)] of Cefadroxil RS taken
M_T: Amount (g) of Cefadroxil for Syrup taken
C: Labeled amount [mg (potency)] of cefadroxil in 1 g

Assay Weigh accurately an amount of powdered Cefadroxil for Syrup, equivalent to about 50 mg (potency) of Cefadroxil, dissolve in water to make exactly 500 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefadroxil RS, equivalent to about 20 mg (potency), dissolve in water to make exactly 200 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefadroxil.

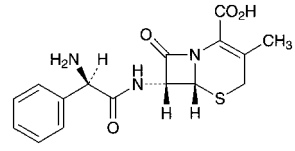
Amount [mg (potency)] of cefadroxil ($C_{16}H_{17}N_3O_5S$)
$= M_S \times A_T/A_S \times 5/2$

M_S: Amount [mg (potency)] of Cefadroxil RS taken

Containers and storage Containers—Tight containers.

Cefalexin

セファレキシン



$C_{16}H_{17}N_3O_4S$: 347.39
(6*R*,7*R*)-7-[(2*R*)-2-Amino-2-phenylacetylamino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
[*15686-71-2*]

Cefalexin contains not less than 950 μg (potency) and not more than 1030 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefalexin is expressed as mass (potency) of cefalexin ($C_{16}H_{17}N_3O_4S$).

Description Cefalexin occurs as a white to light yellowish white, crystals or crystalline powder.

It is sparingly soluble in water, slightly soluble in methanol, and practically insoluble in ethanol (95) and in *N*,*N*-dimethylformamide.

It is hygroscopic.

Identification **(1)** Determine the absorption spectrum of a solution of Cefalexin (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefalexin as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefalexin in heavy water for nuclear magnetic resonance spectroscopy (1 in 200) as directed under Nuclear Magnetic Resonance Spectroscopy ⟨*2.21*⟩, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ 1.8 ppm, and a single or a sharp multiple signal B

at around δ 7.5 ppm. The ratio of integrated intensity of these signals, A:B, is about 3:5.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +144 – +158° (0.125 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Cefalexin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Cefalexin by suspending in 10 mL of *N*,*N*-dimethylformamide, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve about 25 mg of Cefalexin in a solution of potassium dihydrogenphosphate (9 in 500) to make 5 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a solution of potassium dihydrogenphosphate (9 in 500) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the areas of each peak by the automatic integration method. If necessary, correct the change of the base-line due to the potassium dihydrogenphosphate solution by proceeding in the same manner with 20 μL of a solution of potassium dihydrogenphosphate (9 in 500): each peak area other than cefalexin from the sample solution is not larger than the peak area of cefalexin from the standard solution, and the total area of the peaks other than cefalexin from the sample solution which are larger than 1/50 times the peak area of cefalexin from the standard solution is not larger than 5 times of the peak area of cefalexin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Dissolve 1.0 g of sodium 1-pentanesulfonate in 1000 mL of water, add 15 mL of triethylamine, and adjust to pH 2.5 with phosphoric acid.

Mobile phase B: Dissolve 1.0 g of sodium 1-pentanesulfonate in 300 mL of water, add 15 mL of triethylamine, and adjust to pH 2.5 with phosphoric acid. To this solution add 350 mL of acetonitrile and 350 mL of methanol.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 1	100	0
1 – 34.5	100 → 0	0 → 100
34.5 – 35.5	0	100

Flow rate: 1.0 mL per minute.

Time span of measurement: About 2 times as long as the retention time of cefalexin, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, add a solution of potassium dihydrogenphosphate (9 in 500) to make exactly 100 mL. Confirm that the peak area of cefalexin obtained from 20 μL of this solution is equivalent to 1.8 to 2.2% of that obtained from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefalexin are not less than 150,000 and between 0.8 and 1.3, respectively.

System repeatability: When the test is repeated 3 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the retention time and the peak areas of cefalexin are not more than 2.0%, respectively.

Water ⟨*2.48*⟩ Not more than 8.0% (0.2 g, volumetric titration, back titration).

Assay Weigh accurately an amount of Cefalexin and Cefalexin RS, equivalent to about 25 mg (potency), dissolve each in 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 25 mL. Pipet 10 mL of these solutions, add exactly 5 mL of the internal standard solution, then add 0.1 mol/L phosphate buffer solution (pH 4.5) to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefalexin to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of cefalexin } (C_{16}H_{17}N_3O_4S) = M_S \times Q_T/Q_S \times 1000$$

M_S: amount [mg (potency)] of Cefalexin RS taken

Internal standard solution—A solution of *m*-hydroxyacetophenone in 0.1 mol/L phosphate buffer solution (pH 4.5) (1 in 1500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.8 g of potassium dihydrogenphosphate in 1000 mL of water, adjust to pH 3.0 with diluted phosphoric acid (3 in 500). To 800 mL of this solution add 200 mL of methanol.

Flow rate: Adjust so that the retention time of cefalexin is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefalexin and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefalexin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cefalexin Capsules

セファレキシンカプセル

Cefalexin Capsules contain not less than 93.0% and not more than 107.0% of the labeled potency of cefalexin ($C_{16}H_{17}N_3O_4S$: 347.39).

Method of preparation Prepare as directed under Capsules, with Cefalexin.

Identification Take out the contents of Cefalexin Capsules, to a quantity of the contents, equivalent to 70 mg (potency) of Cefalexin, add 25 mL of water, shake vigorously for 5 minutes, and filter. To 1 mL of the filtrate add water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 260 nm and 264 nm.

Water <*2.48*> Not more than 10.0% (0.2 g, volumetric titration, back titration).

Uniformity of dosage units <*6.02*> Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

Open 1 capsule of Cefalexin Capsules, add $3V/5$ mL of 0.1 mol/L phosphate buffer solution (pH 4.5), shake vigorously for 10 minutes, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly V mL so that each mL contains about 1.25 mg (potency) of Cefalexin. Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefalexin RS, equivalent to about 25 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefalexin to that of the internal standard.

Amount [mg (potency)] of cefalexin ($C_{16}H_{17}N_3O_4S$)
$= M_S \times Q_T/Q_S \times V/20$

M_S: Amount [mg (potency)] of Cefalexin RS taken

Internal standard solution—A solution of *m*-hydroxyacetophenone in 0.1 mol/L phosphate buffer solution (pH 4.5) (1 in 15,000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefalexin and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefalexin to that of the internal standard substance is not more than 1.0%.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rates in 30 minutes of 125-mg (potency) capsule and in 60 minutes of 250-mg (potency) capsule are not less than 75% and 80%, respectively.

Start the test with 1 capsule of Cefalexin Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 22 μg (potency) of Cefalexin, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefalexin RS, equivalent to about 22 mg (potency), and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 262 nm.

Dissolution rate (%) with respect to the labeled amount of cefalexin ($C_{16}H_{17}N_3O_4S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount [mg (potency)] of Cefalexin RS taken
C: Labeled amount [mg (potency)] of cefalexin ($C_{16}H_{17}N_3O_4S$) in 1 capsule

Assay Take out the contents of not less than 20 capsules of Cefalexin Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g (potency) of Cefalexin, add 60 mL of 0.1 mol/L phosphate buffer solution (pH 4.5), shake vigorously for 10 minutes, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 100 mL, and centrifuge. Pipet 2 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefalexin RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefalexin to that of the internal standard.

Amount [mg (potency)] of cefalexin ($C_{16}H_{17}N_3O_4S$)
$= M_S \times Q_T/Q_S \times 5$

M_S: Amount [mg (potency)] of Cefalexin RS taken

Internal standard solution—A solution of *m*-hydroxyacetophenone in 0.1 mol/L phosphate buffer solution (pH 4.5) (1 in 15,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.0 mm in inside diameter and 7.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.72 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 3.0 with diluted phosphoric acid (3 in 500). To 800 mL of this solution add 200 mL of methanol.

Flow rate: Adjust so that the retention time of cefalexin is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefalexin and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefalexin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cefalexin Combination Granules

セファレキシン複合顆粒

Cefalexin Combination Granules contain gastric-soluble granules and enteric-soluble granules in one package.

It contains not less than 90.0% and not more than 110.0% of cefalexin ($C_{16}H_{17}N_3O_4S$: 347.39) for the labeled total potency and the labeled potency of gastric-soluble granules, respectively.

Method of preparation Prepare as directed under Granules, with Cefalexin, and pack into single-dose packages.

Identification Powder Cefalexin Combination Granules, weigh a portion of the powder, equivalent to 30 mg (potency) of Cefalexin according to the labeled total potency, shake vigorously for 5 minutes with 100 mL of water, and centrifuge. To 2 mL of the supernatant liquid add water to make 20 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 260 nm and 264 nm.

Water <*2.48*> Not more than 5.0% (0.5 g, volumetric titration, direct titration).

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

(1) Total potency—To the total amount of the content of 1 package of Cefalexin Combination Granules add a small amount of 0.1 mol/L phosphate buffer solution (pH 4.5), grind, add 3 $V/5$ mL of 0.1 mol/L phosphate buffer solution (pH 4.5), shake vigorously for 10 minutes, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly V mL so that each mL contains about 2 mg (potency) of Cefalexin according to the labeled total potency, and centrifuge. Pipet 2 mL of the supernatant liquid, add exactly 20 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make 200 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay (1) Total potency.

$$\text{Amount [mg (potency)] of cefalexin } (C_{16}H_{17}N_3O_4S) = M_S \times Q_T/Q_S \times V/10$$

M_S: Amount [mg (potency)] of Cefalexin RS taken

Internal standard solution—A solution of *m*-hydroxyacetophenone in 0.1 mol/L phosphate buffer solution (pH 4.5) (1 in 15,000).

(2) Potency of gastric-soluble granules—To the total amount of the content of 1 package of Cefalexin Combination Granules, add 3 $V/5$ mL of 0.1 mol/L phosphate buffer solution (pH 4.5), shake gently for 5 minutes, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly V mL so that each mL contains about 0.6 mg (potency) of Cefalexin according to the labeled potency of gastric-soluble granules, and centrifuge. Pipet 7 mL of the supernatant liquid, add exactly 20 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make 200 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay (1) Total potency.

$$\text{Amount [mg (potency)] of cefalexin } (C_{16}H_{17}N_3O_4S) = M_S \times Q_T/Q_S \times V/35$$

M_S: Amount [mg (potency)] of Cefalexin RS taken

Internal standard solution—A solution of *m*-hydroxyacetophenone in 0.1 mol/L phosphate buffer solution (pH 4.5) (1 in 15,000).

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Cefalexin Combination Granules is between 25% and 35%.

Start the test with the total amount of the content of 1 package of Cefalexin Combination Granules, withdraw not less than about 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 22 μg (potency) of Cefalexin according to the labeled potency of gastric-soluble granules, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefalexin RS, equivalent to about 22 mg (potency), and dissolve in the dissolution medium to make exactly 50 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 262 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

$$\text{Dissolution rate (\%) of cefalexin } (C_{16}H_{17}N_3O_4S) \text{ with respect to the labeled potency} = M_S \times A_T/A_S \times V'/V \times 1/C \times 90$$

M_S: Amount [mg (potency)] of Cefalexin RS taken

C: Labeled total potency [mg (potency)] of Cefalexin in 1 package

When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 60 minutes of 200 mg (potency) preparation is not less than 80%, and the dissolution rate in 45 minutes of 500 mg (potency) preparation is not less than 75%.

Start the test with the total amount of the content of 1 package of Cefalexin Combination Granules, withdraw not less than about 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 22 μg (potency) of Cefalexin according to

the labeled total potency, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefalexin RS, equivalent to about 22 mg (potency), dissolve in the dissolution medium to make exactly 50 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 262 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) of cefalexin ($C_{16}H_{17}N_3O_4S$) with respect to the labeled potency
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount [mg (potency)] of Cefalexin RS taken
C: Labeled total potency [mg (potency)] of Cefalexin in 1 package

Assay (1) Total potency—Powder the total amount of the content obtained from not less than 20 packages of Cefalexin Combination Granules, weigh accurately a portion of the powder, equivalent to about 0.5 g (potency) of Cefalexin, shake vigorously for 10 minutes with 150 mL of 0.1 mol/L phosphate buffer solution (pH 4.5), add 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 250 mL, and centrifuge. Pipet 2 mL of this solution, add exactly 20 mL of the internal standard solution, and add 0.1 mol/L phosphate buffer solution (pH 4.5) to make 200 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefalexin RS, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and add 0.1 mol/L phosphate buffer solution (pH 4.5) to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefalexin to that of the internal standard.

Amount [mg (potency)] of cefalexin ($C_{16}H_{17}N_3O_4S$)
$= M_S \times Q_T/Q_S \times 25$

M_S: Amount [mg (potency)] of Cefalexin RS taken

Internal standard solution—A solution of *m*-hydroxyacetophenone in 0.1 mol/L phosphate buffer solution (pH 4.5) (1 in 15,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 mn).

Column: A stainless steel column 3.0 mm in inside diameter and 7.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.72 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to pH 3.0 with diluted phosphoric acid (3 in 500). To 800 mL of this solution add 200 mL of methanol.

Flow rate: Adjust so that the retention time of cefalexin is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefalexin and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefalexin to that of the internal standard is not more than 1.0%.

(2) Potency of gastric-soluble granules—Take out the content from not less than 20 packages of Cefalexin Combination Granules, weigh accurately a quantity, equivalent to about 0.3 g (potency) of Cefalexin according to the labeled potency of gastric-soluble granules, shake gently for 5 minutes with 200 mL of 0.1 mol/L phosphate buffer solution (pH 4.5), add 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 300 mL, and centrifuge. Pipet 2 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution, and add 0.1 mol/L phosphate buffer solution (pH 4.5) to make 100 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay (1) Total potency.

Amount [mg (potency)] of cefalexin ($C_{16}H_{17}N_3O_4S$)
$= M_S \times Q_T/Q_S \times 15$

M_S: Amount [mg (potency)] of Cefalexin RS taken

Internal standard solution—A solution of *m*-hydroxyacetophenone in 0.1 mol/L phosphate buffer solution (pH 4.5) (1 in 15,000).

Containers and storage Containers—Tight containers.

Cefalexin for Syrup

シロップ用セファレキシン

Cefalexin for Syrup is a preparation for syrup, which is dissolved or suspended before use.

It contains not less than 90.0% and not more than 110.0% of the labeled potency of cefalexin ($C_{16}H_{17}N_3O_4S$: 347.39).

Method of preparation Prepare as directed under Preparations for Syrups, with Cefalexin.

Identification Dissolve a quantity of Cefalexin for Syrup, equivalent to 3 mg (potency) of Cefalexin, in water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 260 nm and 264 nm.

Water <2.48> Not more than 5.0% (0.4 g, volumetric titration, back titration).

Uniformity of dosage units <6.02> Perform the test according to the following method: Cefalexin for Syrup in single-dose packages meets the requirement of the Content uniformity test.

Take out the total contents of 1 package of Cefalexin for Syrup, add $3V/5$ mL of 0.1 mol/L phosphate buffer solution (pH 4.5), shake vigorously for 10 minutes, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly V mL so that each mL contains about 1 mg (potency) of Cefalexin, and centrifuge. Pipet 2 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make 100 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount [mg (potency)] of cefalexin ($C_{16}H_{17}N_3O_4S$)
$= M_S \times Q_T/Q_S \times V/20$

M_S: Amount [mg (potency)] of Cefalexin RS taken

Internal standard solution—A solution of *m*-hydroxyacetophenone in 0.1 mol/L phosphate buffer solution (pH 4.5) (1 in 15,000).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Cefalexin for Syrup is not less than 80%.

Start the test with an accurately weighed amount of Cefalexin, equivalent to about 0.25 g (potency) of Cefalexin for Syrup, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add water to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefalexin RS, equivalent to about 22 mg (potency), and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and determine the absorbances, A_T and A_S, at 262 nm.

Dissolution rate (%) with respect to the labeled amount of cefalexin ($C_{16}H_{17}N_3O_4S$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 1125$

M_S: Amount [mg (potency)] of Cefalexin RS taken
M_T: Amount (g) of Cefalexin for Syrup taken
C: Labeled amount [mg (potency)] of cefalexin ($C_{16}H_{17}N_3O_4S$) in 1 g

Assay Powder Cefalexin for Syrup, if necessary, and weigh accurately a portion of the powder, equivalent to about 0.1 g (potency) of Cefalexin, add 60 mL of 0.1 mol/L phosphate buffer solution (pH 4.5), shake vigorously for 10 minutes, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 100 mL, and centrifuge. Pipet 2 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefalexin RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution (pH 4.5) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution (pH 4.5) to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefalexin to that of the internal standard.

Amount [mg (potency)] of cefalexin ($C_{16}H_{17}N_3O_4S$)
$= M_S \times Q_T/Q_S \times 5$

M_S: Amount [mg (potency)] of Cefalexin RS taken

Internal standard solution—A solution of *m*-hydroxyacetophenone in 0.1 mol/L phosphate buffer solution (pH 4.5) (1 in 15,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.0 mm in inside diameter and 7.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.72 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 3.0 with diluted phosphoric acid (3 in 500). To 800 mL of this solution add 200 mL of methanol.

Flow rate: Adjust so that the retention time of cefalexin is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefalexin and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

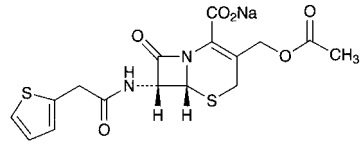
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefalexin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cefalotin Sodium

セファロチンナトリウム



$C_{16}H_{15}N_2NaO_6S_2$: 418.42
Monosodium (6*R*,7*R*)-3-acetoxymethyl-8-oxo-7-[2-(thiophen-2-yl)acetylamino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate
[*58-71-9*]

Cefalotin Sodium contains not less than 920 μg (potency) and not more than 980 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefalotin Sodium is expressed as mass (potency) of cefalotin ($C_{16}H_{16}N_2O_6S_2$: 396.44).

Description Cefalotin Sodium occurs as white to light yellowish white, crystals or crystalline powder.

It is freely soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

Identification **(1)** Determine the absorption spectrum of a solution of Cefalotin Sodium (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefalotin Sodium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefalotin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Cefalotin Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefalotin Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy ⟨*2.21*⟩, using sodium 3-trimethyl-

silylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ 2.1 ppm, a single or sharp multiple signal B at around δ 3.9 ppm, and a multiple signal C at around δ 7.0 ppm. The ratio of the integrated intensity of these signals, A:B:C, is about 3:2:2.

(4) Cefalotin Sodium responds to the Qualitative Tests ⟨*1.09*⟩ (1) for sodium salt.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{25}$: +124 – +134° (5 g, water, 100 mL, 100 mm).

pH ⟨*2.54*⟩ The pH of a solution obtained by dissolving 1.0 g of Cefalotin Sodium in 10 mL of water is between 4.5 and 7.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Cefalotin Sodium in 10 mL of water: the solution is clear. The absorbance of this solution at 450 nm, determined as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, is not more than 0.20.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Cefalotin Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Cefalotin Sodium according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Pipet 1 mL of the standard solution obtained in the Assay, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution obtained in the Assay and the standard solution prepared here as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peaks other than cefalotin from the sample solution is not larger than the peak area of cefalotin from the standard solution, and the total area of the peaks other than cefalotin from the sample solution is not larger than 3 times the peak area of cefalotin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 4 times as long as the retention time of cefalotin.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of cefalotin obtained from 10 μL of this solution is equivalent to 7 to 13% of that obtained from 10 μL of the standard solution.

System performance: Heat the standard solution in a water bath of 90°C for 10 minutes, and cool. Measure exactly 2.5 mL of this solution, and add the mobile phase to make exactly 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak of cefalotin and the peak, having the relative retention time of about 0.5 to cefalotin, is not less than 9, and the symmetry factor of the peak of cefalotin is not more than 1.8.

System repeatability: When the test is repeated 3 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefalotin is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 1.0% (0.5 g, volumetric titration, back titration).

Assay Weigh accurately an amount of Cefalotin Sodium and Cefalotin Sodium RS, equivalent to about 25 mg (potency), and dissolve each in the mobile phase to make exactly 25 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of cefalotin in each solution.

Amount [μg (potency)] of cefalotin ($C_{16}H_{16}N_2O_6S_2$) = $M_S \times A_T/A_S \times 1000$

M_S: Amount [mg (potency)] of Cefalotin Sodium RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 17 g of sodium acetate trihydrate in 790 mL of water, and add 0.6 mL of acetic acid (100). If necessary adjust the pH to 5.9 ± 0.1 with diluted sodium hydroxide TS (1 in 10) or acetic acid (100). To this solution add 150 mL of acetonitrile and 70 mL of ethanol (95).

Flow rate: Adjust so that the retention time of cefalotin is about 12 minutes.

System suitability—

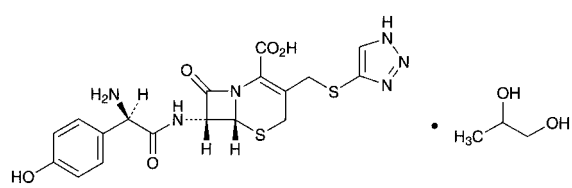
System performance: Heat the standard solution in a water bath of 90°C for 10 minutes, and cool. Measure exactly 2.5 mL of this solution, and add the mobile phase to make exactly 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak of cefalotin and the peak, having the relative retention time of about 0.5 to cefalotin is not less than 9, and the symmetry factor of the peak of cefalotin is not more than 1.8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefalotin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cefatrizine Propylene Glycolate

セファトリジンプロピレングリコール



$C_{18}H_{18}N_6O_5S_2.C_3H_8O_2$: 538.60

(6*R*,7*R*)-7-[(2*R*)-2-Amino-2-(4-hydroxyphenyl)acetylamino]-8-oxo-3-[2-(1*H*-1,2,3-triazol-4-yl)sulfanylmethyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monopropane-1,2-diolate (1/1)

[*51627-14-6*, Cefatrizine]

Cefatrizine Propylene Glycolate contains not less

than 816 μg (potency) and not more than 876 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefatrizine Propylene Glycolate is expressed as mass (potency) of cefatrizine ($C_{18}H_{18}N_6O_5S_2$: 462.50).

Description Cefatrizine Propylene Glycolate occurs as a white to yellowish white powder.

It is sparingly soluble in water, and practically insoluble in methanol and in ethanol (95).

Identification **(1)** Determine the absorption spectrum of a solution of Cefatrizine Propylene Glycolate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefatrizine Propylene Glycolate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefatrizine Propylene Glycolate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefatrizine Propylene Glycolate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefatrizine Propylene Glycolate in a mixture of heavy water for nuclear magnetic resonance spectroscopy and deuterated hydrochloric acid for nuclear magnetic resonance spectroscopy (3:1) (1 in 10), using sodium 3-(trimethylsilyl)propionate-d_4 for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>: it exhibits a double signal A at around δ 1.2 ppm, a double signal B at around δ 7.0 ppm, a double signal C at around δ 7.5 ppm and a single signal D at around δ 8.3 ppm. The ratio of integrated intensity of these signals, A:B:C:D, is about 3:2:2:1.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +52 – +58° (2.5 g calculated on the anhydrous bases, 1 mol/L hydrochloric acid TS, 50 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Cefatrizine Propylene Glycolate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Cefatrizine Propylene Glycolate according to Method 3, and perform the test (not more than 2 ppm). Use a solution of magnesium nitrate hexahydrate in ethanol (1 in 25).

(3) Related substances—Dissolve 25 mg of Cefatrizine Propylene Glycolate in 5 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly ninhydrin-citric acid-acetic acid TS on the plate, and heat at 100°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <*2.48*> Not more than 2.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefatrizine Propylene Glycolate and Cefatrizine Propylene Glycolate RS, equivalent to about 0.1 g (potency), dissolve each in water to make exactly 500 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of cefatrizine in each solution.

$$\text{Amount [}\mu\text{g (potency)] of cefatrizine } (C_{18}H_{18}N_6O_5S_2) = M_S \times A_T/A_S \times 1000$$

M_S: Amount [mg (potency)] of Cefatrizine Propylene Glycolate RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of a solution of potassium dihydrogenphosphate (17 in 12,500) and methanol (17:3).

Flow rate: Adjust so that the retention time of cefatrizine is about 11 minutes.

System suitability—

System performance: Dissolve about 10 mg (potency) of Cefatrizine Propylene Glycolate and about 5 mg (potency) of Cefadroxil in 50 mL of water. When the procedure is run with 10 μL of this solution under the above operating conditions, cefadroxil and cefatrizine are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of peak areas of cefatrizine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cefatrizine Propylene Glycolate for Syrup

シロップ用セファトリジンプロピレングリコール

Cefatrizine Propylene Glycolate for Syrup is a preparation for syrup, which is dissolved before use.

It contains not less than 90.0% and not more than 105.0% of the labeled potency of Cefatrizine ($C_{18}H_{18}N_6O_5S_2$: 462.50).

Method of preparation Prepare as directed under Preparations for Syrup, with Cefatrizine Propylene Glycolate.

Identification Powder Cefatrizine Propylene Glycolate for Syrup, weigh a portion of the powder, equivalent to 10 mg (potency) of Cefatrizine Propylene Glycolate, and dissolve in 10 mL of water. To 2 mL of this solution add water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 225 nm and 229 nm, and between 266 nm and 271 nm.

pH <*2.54*> Take an amount of Cefatrizine Propylene Glycolate for Syrup, equivalent to 0.4 g (potency) of Cefatrizine Propylene Glycolate, and suspend in 10 mL of water: the pH of this suspension is between 4.0 and 6.0.

Purity Related substances—Use the sample solution obtained in the Assay as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area in each solution by the automatic integration method: the area of each peak other than cefatrizine obtained from the sample solution is not larger than the peak area of cefatrizine obtained from the standard solution, and the total area of the peaks other than cefatrizine from the sample solution is not larger than 2 times the peak area of cefatrizine from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Cefatrizine Propylene Glycolate.

Time span of measurement: About 2.5 times as long as the retention time of cefatrizine, beginning after the solvent peak.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay under Cefatrizine Propylene Glycolate.

Test for required detectability: Pipet 2 mL of the standard solution, and add water to make exactly 10 mL. Confirm that the peak area of cefatrizine obtained from 10 μL of this solution is equivalent to 15 to 25% of that of cefatrizine obtained from 10 μL of the standard solution.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefatrizine is not more than 2.0%.

Uniformity of dosage units <6.02> Cefatrizine Propylene Glycolate for Syrup in single-dose packages meets the requirement of the Mass variation test.

Assay Powder Cefatrizine Propylene Glycolate for Syrup, weigh accurately a portion of the powder, equivalent to about 0.1 g (potency) of Cefatrizine Propylene Glycolate, dissolve in water to make exactly 500 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefatrizine Propylene Glycolate RS, equivalent to about 20 mg (potency), dissolve in water to make exactly 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefatrizine Propylene Glycolate.

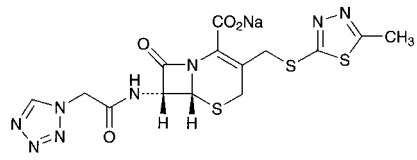
Amount [mg (potency)] of cefatrizine ($C_{18}H_{18}N_6O_5S_2$)
$= M_S \times A_T/A_S \times 5$

M_S: Amount [mg (potency)] of Cefatrizine Propylene Glycolate RS taken

Containers and storage Containers—Tight containers.

Cefazolin Sodium

セファゾリンナトリウム



$C_{14}H_{13}N_8NaO_4S_3$: 476.49
Monosodium (6*R*,7*R*)-3-(5-methyl-1,3,4-thiadiazol-2-ylsulfanylmethyl)-8-oxo-7-[2-(1*H*-tetrazol-1-yl)acetylamino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate
[*27164-46-1*]

Cefazolin Sodium contains not less than 900 μg (potency) and not more than 975 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefazolin Sodium is expressed as mass (potency) of cefazolin ($C_{14}H_{14}N_8O_4S_3$: 454.51).

Description Cefazolin Sodium occurs as a white to light yellow-white, crystals or crystalline powder.

It is freely soluble in water and in formamide, slightly soluble in methanol, and practically insoluble in ethanol (95).

Identification **(1)** Determine the absorption spectrum of a solution of Cefazolin Sodium (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefazolin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefazolin Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10), using sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.21>: it exhibits single signals, A and B, at around δ 2.7 ppm and at around δ 9.3 ppm, respectively. The ratio of integrated intensity of these signals, A:B, is about 3:1.

(4) Cefazolin Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: -19 – $-23°$ (2.5 g calculated as the anhydrous basis, water, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Cefazolin Sodium in 10 mL of water: pH of the solution is between 4.8 and 6.3.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Cefazolin Sodium in 10 mL of water: the solution is clear and colorless to pale yellow, and its absorbance at 400 nm determined as directed under Ultraviolet-visible Spectrophotometry <2.24> is not more than 0.35. The test should be performed within 10 minutes after preparing of the solution.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Cefazolin Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead

Solution (not more than 10 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 2.0 g of Cefazolin Sodium according to Method 3, and perform the test. When prepare the test solution, add 1.5 mL of hydrogen peroxide (30) after addition of 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), and then ignite (not more than 1 ppm).

(4) Related substances—Dissolve 0.10 g of Cefazolin Sodium in 20 mL of 0.1 mol/L phosphate buffer solution (pH 7.0) and use this solution as the sample solution. Prepare the sample solution before use. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of the peaks by the area percentage method: the amount of the peak, having the relative retention time of about 0.2 to cefazolin and the amount of the peak other than cefazolin and the peak mentioned above are not more than 1.5%, respectively. The total amount of the peaks other than cefazolin is not more than 2.5%. For the area of the peak, having the relative retention time of about 0.2 to the cefazolin, multiply the relative response factor, 1.43.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of cefazolin, beginning after the solvent peak.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Dissolve about 80 mg of Cefazolin RS in 0.1 mol/L phosphate buffer solution (pH 7.0) to make 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 20 mL. Confirm that the peak area of cefazolin obtained from 5 μL of this solution is equivalent to 3 to 7% of that obtained from 5 μL of the solution for system suitability test.

System repeatability: When the test is repeated 6 times with 5 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of cefazolin is not more than 1.0%.

Water <*2.48*> Not more than 2.5% (1.0 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

Assay Weigh accurately an amount of Cefazolin Sodium and Cefazolin RS, equivalent to about 20 mg (potency), dissolve each in the internal standard solution to make exactly 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefazolin to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of cefazolin } (C_{14}H_{14}N_8O_4S_3) = M_S \times Q_T/Q_S \times 1000$$

M_S: Amount [mg (potency)] of Cefazolin RS taken

Internal standard solution—A solution of *p*-acetanisidide in 0.1 mol/L phosphate buffer solution (pH 7.0) (11 in 20,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.27 g of disodium hydrogen phosphate dodecahydrate and 0.47 g of citric acid monohydrate in water to make 935 mL, and add 65 mL of acetonitrile.

Flow rate: Adjust so that the retention time of cefazolin is about 8 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, cefazolin and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefazolin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cefazolin Sodium for Injection

注射用セファゾリンナトリウム

Cefazolin Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled potency of cefazolin ($C_{14}H_{14}N_8O_4S_3$: 454.51).

Method of preparation Prepare as directed under Injections, with Cefazolin Sodium.

Description Cefazolin Sodium for Injection occurs as white to light yellowish white crystals or crystalline powder or masses.

Identification (1) Determine the absorption spectrum of a solution of Cefazolin Sodium for Injection (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 270 nm and 274 nm.

(2) Cefazolin Sodium for Injection responds to the Qualitative Tests <*1.09*> (1) for chloride.

Osmotic pressure ratio Being specified separately when the drug is granted approval based on the Law.

pH <*2.54*> The pH of a solution prepared by dissolving an amount of Cefazolin Sodium for Injection, equivalent to 1.0 g (potency) of Cefazolin Sodium, in 10 mL of water is 4.5 to 6.5.

Purity (1) Clarity and color of solution—Conduct this procedure within 10 minutes after the preparation of the solutions. A solution prepared by dissolving an amount of Cefazolin Sodium for Injection, equivalent to 1.0 g (potency) of Cefazolin Sodium, in 10 mL of water is clear, and the absorbance of this solution at 400 nm, determined as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, is not more than 0.35.

(2) Related substances—Dissolve an amount of Cefazolin Sodium for Injection, equivalent to 0.10 g (potency) of

Cefazolin Sodium, in 20 mL of 0.1 mol/L phosphate buffer solution (pH 7.0) and use this solution as the sample solution. Prepare the sample solution before use. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of each peak by the area percentage method: the amount of the peaks other than cefazolin is not more than 1.5%. Furthermore the total amount of the peaks other than cefazolin is not more than 2.5%. For the area of the peak, having the relative retention time of about 0.2 to cefazolin, multiply the relative response factor, 1.43.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Cefazolin Sodium.

Time span of measurement: About 3 times as long as the retention time of cefazolin, beginning after the solvent peak.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay under Cefazolin Sodium.

Test for required detectability: To 8 mL of the sample solution, add 0.1 mol/L phosphate buffer solution (pH 7.0) to make 50 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 20 mL. Confirm that the peak area of cefazolin obtained from 5 μL of this solution is equivalent to 3 to 7% of that obtained from 5 μL of the solution for system suitability test.

System repeatability: When the test is repeated 6 times with 5 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of cefazolin is not more than 1.0%.

Water <2.48> Not more than 3.0% (0.5 g, volumetric titration, direct titration). Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination.

Bacterial endotoxins <4.01> Less than 0.05 EU/mg (potency).

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Foreign insoluble matter <6.06> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 containers of Cefazolin Sodium for Injection. Weigh accurately an amount of the contents, equivalent to about 50 mg (potency) of Cefazolin Sodium, dissolve in the internal standard solution to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefazolin RS, equivalent to about 50 mg (potency), dissolve in the internal standard solution to make exactly 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefazolin Sodium.

Amount [mg (potency)] of cefazolin ($C_{14}H_{14}N_8O_4S_3$)
$= M_S \times Q_T/Q_S$

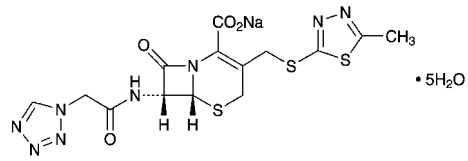
M_S: Amount [mg (potency)] of Cefazolin RS taken

Internal standard solution—A solution of *p*-acetanisidide in 0.1 mol/L phosphate buffer solution (pH 7.0) (11 in 20,000).

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Cefazolin Sodium Hydrate

セファゾリンナトリウム水和物



$C_{14}H_{13}N_8NaO_4S_3.5H_2O$: 566.57
Monosodium (6*R*,7*R*)-3-(5-methyl-1,3,4-thiadiazol-2-ylsulfanylmethyl)-8-oxo-7-[2-(1*H*-tetrazol-1-yl)acetylamino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate pentahydrate
[*115850-11-8*]

Cefazolin Sodium Hydrate contains not less than 920 μg (potency) and not more than 975 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefazolin Sodium Hydrate is expressed as mass (potency) of cefazolin ($C_{14}H_{14}N_8O_4S_3$: 454.51).

Description Cefazolin Sodium Hydrate occurs as white to pale yellowish white crystals.

It is freely soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification **(1)** Determine the absorption spectrum of a solution of Cefazolin Sodium Hydrate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefazolin Sodium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefazolin Sodium Hydrate in heavy water for nuclear magnetic resonance spectroscopy (1 in 10), using sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.21>: it exhibits single signals, A and B, at around δ 2.7 ppm and at around δ 9.3 ppm. The ratio of integrated intensity of each signal, A:B, is about 3:1.

(4) Cefazolin Sodium Hydrate responds to the Qualitative Tests <1.09> (1) for sodium salt.

Absorbance <2.24> $E^{1\%}_{1\,cm}$ (272 nm): 272 – 292 (80 mg calculated on the anhydrous basis, water, 5000 mL).

Optical rotation <2.49> $[\alpha]_D^{20}$: −20 – −25° (2.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Cefazolin Sodium Hydrate in 10 mL of water: the pH of the solution is between 4.8 and

6.3.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Cefazolin Sodium Hydrate in 10 mL of water: the solution is clear, and when determine the absorbance at 400 nm of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, it is not more than 0.15.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Cefazolin Sodium Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.10 of Cefazolin Sodium Hydrate in 20 mL of 0.1 mol/L phosphate buffer solution (pH 7.0) and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of the peak having the relative retention time of about 0.2 to cefazolin is not more than 1.0%, the amount of the peak other than cefazolin and the peak mentioned above is not more than 0.5%, and the total amount of the peaks other than cefazolin is not more than 2.0%. For the area of the peak, having the relative retention time of about 0.2 to cefazolin, multiply the relative response factor 1.43.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of cefazolin, beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add 0.1 mol/L phosphate buffer solution (pH 7.0) to make 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 10 mL. Confirm that the peak area of cefazolin obtained with 5 μL of this solution is equivalent to 7 to 13% of that obtained with 5 μL of the solution for system suitability test.

System performance: Dissolve 20 mg of Cefazolin Sodium Hydrate in 20 mL of a solution of *p*-acetanisidide in 0.1 mol/L phosphate buffer solution (pH 7.0) (11 in 20,000). When the procedure is run with 5 μL of this solution under the above operating conditions, cefazolin and *p*-acetanisidide are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 5 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of cefazolin is not more than 2.0%.

Water <*2.48*> Not less than 13.7% and not more than 16.0% (0.1 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

Bacterial endotoxins <*4.01*> Less than 0.10 EU/mg (potency).

Assay Weigh accurately an amount of Cefazolin Sodium Hydrate and Cefazolin RS, equivalent to about 20 mg (potency), dissolve in exactly 20 mL of the internal standard solution, and use these solutions as the sample solution and standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefazolin to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of cefazolin } (C_{14}H_{14}N_8O_4S_3) = M_S \times Q_T/Q_S \times 1000$$

M_S: Amount [mg (potency)] of Cefazolin RS taken

Internal standard solution—A solution of *p*-acetanisidide in 0.1 mol/L phosphate buffer solution (pH 7.0) (11 in 20,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.27 g of disodium hydrogen phosphate dodecahydrate and 0.47 g of citric acid monohydrate in water to make 935 mL. To this solution, add 65 mL of acetonitrile.

Flow rate: Adjust so that the retention time of cefazolin is about 8 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, cefazolin and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

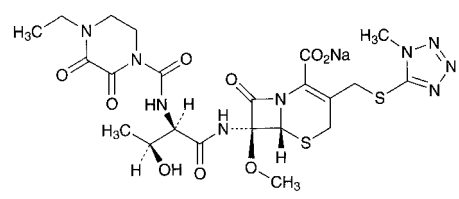
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefazolin is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant.

Cefbuperazone Sodium

セフブペラゾンナトリウム



$C_{22}H_{28}N_9NaO_9S_2$: 649.63

Monosodium (6*R*,7*S*)-7-{(2*R*,3*S*)-2-[(4-ethyl-2,3-dioxopiperazine-1-carbonyl)amino]-3-hydroxybutanoylamino}-7-methoxy-3-(1-methyl-1*H*-tetrazol-5-ylsulfanylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

[*76648-01-6*]

Cefbuperazone Sodium contains not less than 870 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefbuperazone Sodium is expressed as mass (potency) of cefbuperazone ($C_{22}H_{29}N_9O_9S_2$: 627.65).

Description Cefbuperazone Sodium occurs as white to light yellowish white, powder or masses.

It is very soluble in water, freely soluble in methanol and in pyridine, sparingly soluble in ethanol (95), and very

slightly soluble in acetonitrile.

Identification **(1)** Determine the absorption spectrum of a solution of Cefbuperazone Sodium (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 0.1 g of Cefbuperazone Sodium in 0.5 mL of deuterated pyridine for nuclear magnetic resonance spectroscopy and 1 drop of heavy water for nuclear magnetic resonance spectroscopy, and determine the ^1H spectrum of this solution as directed under Nuclear Magnetic Resonance Spectroscopy ⟨*2.21*⟩, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a triplet signal A at around δ 1.1 ppm, and two doublet signals, B and C, at around δ 1.6 ppm and at around δ 5.1 ppm, respectively. The ratio of the integrated intensity of each signal, A:B:C, is about 3:3:1.

(3) Cefbuperazone Sodium responds to the Qualitative Tests ⟨*1.09*⟩ (1) for sodium salt.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +48 – +56° (0.4 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 1.0 g of Cefbuperazone Sodium in 4 mL of water: the pH of the solution is between 4.0 and 6.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Cefbuperazone Sodium in 4 mL of water: the solution is clear and light yellow.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Cefbuperazone Sodium according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Cefbuperazone Sodium according to Method 4, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Cefbuperazone Sodium in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method. Calculate the percentages of each peak area of related substances from the sample solution against 50 times of the peak area of cefbuperazone from the standard solution; the amount of related substance I having the relative retention time of about 0.2 to cefbuperazone is not more than 2.0%, the amount of related substance II having the relative retention time of about 0.6 to cefbuperazone is not more than 4.5% and the amount of related substance III having the relative retention time of about 1.6 to cefbuperazone is not more than 1.0%, and the total amount of these related substances is not more than 6.0%. For the peak areas of the related substances I and III, multiply their relative response factors, 0.72 and 0.69, respectively.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of cefbuperazone.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of cefbuperazone obtained from 25 μL of this solution is equivalent to 7 to 13% of that obtained from 25 μL of the standard solution.

System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefbuperazone are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefbuperazone is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 1.0% (3 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefbuperazone Sodium and Cefbuperazone RS, equivalent to about 0.1 g (potency), and dissolve each in the mobile phase to make exactly 100 mL. Measure exactly 10 mL each of these solutions, add exactly 10 mL of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefbuperazone to that of the internal standard.

Amount [μg (potency)] of cefbuperazone ($C_{22}H_{29}N_9O_9S_2$) $= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Cefbuperazone RS taken

Internal standard solution—A solution of acetanilide in the mobile phase (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.0 g of tetra-*n*-propylammonium bromide in 1000 mL of a mixture of water, acetonitrile and acetic acid-sodium acetate buffer solution (pH 5.0) (83:13:4).

Flow rate: Adjust so that the retention time of cefbuperazone is about 16 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and cefbuperazone are eluted in this order with the resolution between these peaks being not less than 3.

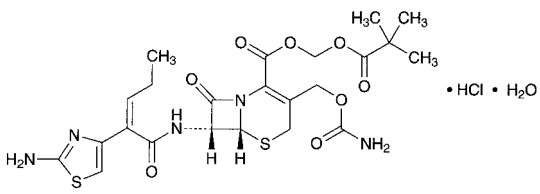
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefbuperazone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Storage—In a cold place.

Cefcapene Pivoxil Hydrochloride Hydrate

セフカペン　ピボキシル塩酸塩水和物



$C_{23}H_{29}N_5O_8S_2.HCl.H_2O$: 622.11

2,2-Dimethylpropanoyloxymethyl (6*R*,7*R*)-7-[(2*Z*)-2-(2-aminothiazol-4-yl)pent-2-enoylamino]-3-carbamoyloxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-caboxylate monohydrochloride monohydrate [*147816-24-8*]

Cefcapene Pivoxil Hydrochloride Hydrate contains not less than 722 μg (potency) and not more than 764 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefcapene Pivoxil Hydrochloride Hydrate is expressed as mass (potency) of cefcapene ($C_{17}H_{19}N_5O_6S_2$: 453.49).

Description Cefcapene Pivoxil Hydrochloride Hydrate occurs as a white to pale yellowish white, crystalline powder or mass. It has slightly a characteristic odor.

It is freely soluble in *N*,*N*-dimethylformamide and in methanol, soluble in ethanol (99.5), slightly soluble in water, and practically insoluble in diethyl ether.

Identification **(1)** Determine the absorption spectrum of a solution of Cefcapene Pivoxil Hydrochloride Hydrate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefcapene Pivoxil Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectra of Cefcapene Pivoxil Hydrochloride Hydrate and Cefcapene Pivoxil Hydrochloride RS as directed in the paste method under Infrared Spectrophotometry <*2.25*>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefcapene Pivoxil Hydrochloride Hydrate in deuterated methanol for nuclear magnetic resonance spectroscopy (1 in 50) as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a triplet signal A at around δ 6.3 ppm, and a single signal B at around δ 6.7 ppm, and the ratio of integrated intensity of each signal, A:B, is about 1:1.

(4) Dissolve 10 mg of Cefcapene Pivoxil Hydrochloride Hydrate in 2 mL of a mixture of water and methanol (1:1), and add 1 drop of silver nitrate TS: a white precipitate is formed.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +51 – +54° (0.1 g calculated on the anhydrous basis, methanol, 10 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Cefcapene Pivoxil Hydrochloride Hydrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substance I—Dissolve an amount of Cefcapene Pivoxil Hydrochloride Hydrate, equivalent to about 10 mg (potency), in 2 mL of methanol, add a mixture of water and methanol (1:1) to make 50 mL, and use this solution as the sample solution. Perform the test with 30 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method. If necessary, compensate the base-line by performing in the same manner as the test with 30 μL of a mixture of water and methanol (1:1). Measure the amount of the peak other than cefcapene pivoxil by the area percentage method: the amounts of the peaks, having the relative retention times of about 1.5 and about 1.7 to cefcapene pivoxil, are not more than 0.2%, respectively. The amount of the peaks other than the peaks mentioned above is not more than 0.1%, and the total of them is not more than 1.5%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase A: Dissolve 5.99 g of potassium dihydrogen phosphate in water to make 1100 mL. To this solution add a solution prepared by dissolving 1.89 g of tetra-*n*-pentylammonium bromide in methanol to make 1000 mL.

Mobile phase B: A mixture of methanol and water (22:3).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 20	98	2
20 – 40	98 → 50	2 → 50
40 – 50	50	50

Flow rate: 0.8 mL per minute.

Time span of measurement: About 2.5 times as long as the retention time of cefcapene pivoxil.

System suitability—

Test for required detectability: To exactly 1 mL of the sample solution add a mixture of water and methanol (1:1) to make 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mixture of water and methanol (1:1) to make exactly 10 mL. Confirm that the peak area of cefcapene pivoxil obtained from 30 μL of this solution is equivalent to 7 to 13% of that of cefcapene pivoxil obtained from 30 μL of the solution for system suitability test.

System performance: Dissolve 10 mg of Cefcapene Pivoxil Hydrochloride Hydrate and 10 mg of propyl parahydroxybenzoate in 25 mL of methanol, and add water to make 50 mL. To 5 mL of this solution add the mixture of water and methanol (1:1) to make 50 mL. When the procedure is run with 30 μL of this solution under the above operating conditions, cefcapene pivoxil and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 3 times with 30 μL of the solution for system suitability test under

the above operating conditions, the relative standard deviation of the peak area of cefcapene pivoxil is not more than 4.0%.

(3) Related substance II—Dissolve an amount of Cefcapene Pivoxil Hydrochloride Hydrate, equivalent to about 2 mg (potency), in *N*,*N*-dimethylformamide for liquid chromatography to make 20 mL, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks which appear earlier than cefcapene pivoxil is not more than 1.7% of the total area of the peaks other than the solvent.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 7.8 mm in inside diameter and 30 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography.

Column temperature: A constant temperature of about 25°C.

Mobile phase: A solution of lithium bromide in *N*,*N*-dimethylformamide for liquid chromatography (13 in 5000).

Flow rate: Adjust so that the retention time of cefcapene pivoxil is about 22 minutes.

Time span of measurement: About 1.8 times as long as the retention time of cefcapene pivoxil.

System suitability—

Test for required detectability: To exactly 1 mL of the sample solution add *N*,*N*-dimethylformamide for liquid chromatography to make 100 mL, and use this solution as the solution for system suitability test. Pipet 3 mL of the solution for system suitability test, and add *N*,*N*-dimethylformamide for liquid chromatography to make exactly 10 mL. Conform that the peak area of cefcapene pivoxil obtained from 20 μL of this solution is equivalent to 20 to 40% of that of cefcapene pivoxil obtained from 20 μL of the solution for system suitability test.

System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, the number of theoretical plates of the peak of cefcapene pivoxil is not less than 12,000.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of cefcapene pivoxil is not more than 4.0%.

Water <2.48> Not less than 2.8% and not more than 3.7% (0.5 g, volumetric titration, back titration).

Assay Weigh accurately an amount of Cefcapene Pivoxil Hydrochloride Hydrate and Cefcapene Pivoxil Hydrochloride RS, equivalent to about 20 mg (potency), and dissolve each in a mixture of water and methanol (1:1) to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL of the internal standard solution and the mixture of water and methanol (1:1) to them to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefcapene pivoxil to that of the internal standard.

Amount [μg (potency)] of cefcapene ($C_{17}H_{19}N_5O_6S_2$) $= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Cefcapene Pivoxil Hydrochloride RS taken

Internal standard solution—A solution of *p*-benzylphenol in a mixture of water and methanol (1:1) (7 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 3.0 mm in inside diameter and 7.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.56 g of sodium dihydrogenphosphate dihydrate and 1.22 g of sodium 1-decanesulfonate in water to make 1000 mL. To 700 mL of this solution add 300 mL of acetonitrile and 100 mL of methanol.

Flow rate: Adjust so that the retention time of cefcapene pivoxil is about 5 minutes.

System suitability—

System performance: Dissolve 0.2 g of Cefcapene Pivoxil Hydrochloride Hydrate in 10 mL of methanol, and warm in a water bath at 60°C for 20 minutes. After cooling, pipet 1 mL of this solution, and add exactly 10 mL of the internal standard solution and the mixture of water and methanol (1:1) to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, cefcapene pivoxil, *trans*-cefcapene pivoxil and the internal standard are eluted in this order, the relative retention time of trans-cefcapene pivoxil and the internal standard to that of cefcapene pivoxil are about 1.7 and about 2.0, respectively, and the resolution between the peaks of *trans*-cefcapene pivoxil and the internal standard is not less than 1.5.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefcapene pivoxil to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, at a temperature not exceeding 5°C.

Cefcapene Pivoxil Hydrochloride Fine Granules

セフカペン　ピボキシル塩酸塩細粒

Cefcapene Pivoxil Hydrochloride Fine Granules contain not less than 90.0% and not more than 110.0% of the labeled potency of cefcapene ($C_{17}H_{19}N_5O_6S_2$: 453.49).

Method of preparation Prepare as directed under Granules, with Cefcapene Pivoxil Hydrochloride Hydrate.

Identification Powder Cefcapene Pivoxil Hydrochloride Fine Granules. To a portion of the powder, equivalent to 10 mg (potency) of Cefcapene Pivoxil Hydrochloride Hydrate, add 40 mL of methanol, shake vigorously, and add methanol to make 50 mL. To 4 mL of this solution add methanol to make 50 mL, and filter through a membrane filter with a pore size of 0.45 μm. Determine the absorption spectrum of

the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 264 nm and 268 nm.

Purity (1) Related substances I—Powder Cefcapene Pivoxil Hydrochloride Fine Granules. To a portion of the powder, equivalent to 5 mg (potency) of Cefcapene Pivoxil Hydrochloride Hydrate, add 1 mL of methanol, and shake. Add 25 mL of a mixture of water and methanol (1:1), shake vigorously for 5 minutes, and filter through a membrane filter with a pore size of 0.45 μm. Discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Perform the test with 30 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method. If necessary, compensate the base-line by performing in the same manner as the test with 30 μL of a mixture of water and mothod (1:1). Calculate the amount of the peaks other than the peak of cefcapene pivoxil by the area percentage method: the amount of the substance, having the relative retention time of about 1.3 to cefcapene pivoxil, is not more than 0.4%, the amount of the trans-isomer of cefcapene pivoxil, having the relative retention time of about 1.5, is not more than 1.1%, the amount of the substance other than that mentioned above is not more than 0.3%, and the total amount of these substances is not more than 2.8%.

Operating conditions—

Proceed as directed in the operating conditions in the Purity (2) under Cefcapene Pivoxil Hydrochloride Hydrate.

System suitability—

Proceed as directed in the system suitability in the Purity (2) under Cefcapene Pivoxil Hydrochloride Hydrate.

(2) Related substances II—Powder Cefcapene Pivoxil Hydrochloride Fine Granules. To a portion of the powder, equivalent to 2 mg (potency) of Cefcapene Pivoxil Hydrochloride Hydrate, add 20 mL of *N*,*N*-dimethylformamide for liquid chromatography, shake vigorously for 10 minutes, and filter through a membrane filter with a pore size of 0.45 μm. Discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks eluted before that of cefcapene pivoxil is not more than 4.0% of the total area of all peaks other than the solvent peak.

Operating conditions—

Proceed as directed in the operating conitions in the Purity (3) under Cefcapene Pivoxil Hydrochloride Hydrate.

System suitability—

Proceed as directed in the system suitability in the Purity (3) under Cefcapene Pivoxil Hydrochloride Hydrate.

Water <2.48> Not more than 1.4% (0.5 g, volumetric titration, back titration). Perform the test without pulverizing the sample, and handling the sample under a relative humidity of less than 30%.

Uniformity of dosage units <6.02> The granules in single-dose packages meet the requirement of the Mass variation test.

Dissolution Being specified separately when the drug is granted approval based on the Law.

Assay Weigh accurately an amount of Cefcapene Pivoxil Hydrochloride Fine Granules, equivalent to about 0.2 g (potency) of and Cefcapene Pivoxil Hydrochloride Hydrate, add 100 mL of the mixture of water and methanol (1:1), shake vigorously for 10 minutes, add the mixture of water and methanol (1:1) to make exactly 200 mL, and centrifuge at 3000 rpm for 5 minutes. Filter the supernatant liquid through a membrane filter with a pore size of 0.45 μm, discard the first 1 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution and the mixture of water and methanol (1:1) to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg (potency) of Cefcapene Pivoxil Hydrochloride RS, and dissolve in the mixture of water and methanol (1:1) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the mixture of water and methanol (1:1) to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefcapene Pivoxil Hydrochloride Hydrate.

Amount [mg (potency)] of cefcapene ($C_{17}H_{19}N_5O_6S_2$) $= M_S \times Q_T/Q_S \times 10$

M_S: Amount [mg (potency)] of Cefcapene Pivoxil Hydrochloride RS taken

Internal standard solution—A solution of *p*-benzylphenol in the mixture of water and methanol (1:1) (7 in 4000).

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Cefcapene Pivoxil Hydrochloride Tablets

セフカペン　ピボキシル塩酸塩錠

Cefcapene Pivoxil Hydrochloride Tablets contain not less than 90.0% and not more than 105.0% of the labeled potency of cefcapene ($C_{17}H_{19}N_5O_6S_2$: 453.49).

Method of preparation Prepare as directed under Tablets, with Cefcapene Pivoxil Hydrochloride Hydrate.

Identification To an amount of powdered Cefcapene Pivoxil Hydrochloride Tablets, equivalent to about 10 mg (potency) of Cefcapene Pivoxil Hydrochloride Hydrate, add 40 mL of methanol, shake vigorously, and add methanol to make 50 mL. To 4 mL of this solution add methanol to make 50 mL, filter through a membrane filter with pore size of 0.45 μm, and use the filtrate as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 263 nm and 267 nm.

Purity (1) Related substances I—To an amount of powdered Cefcapene Pivoxil Hydrochloride Tables, equivalent to about 5 mg (potency) of Cefcapene Pivoxil Hydrochloride Hydrate, add 1 mL of methanol, and shake. Add 25 mL of a mixture of water and methanol (1:1), shake vigorously for 5 minutes, and filter through a membrane filter with pore size of 0.45 μm. Discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Perform the test with 30 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. If necessary, proceed with 30 μL of the mixture of water and methanol (1:1) in the same manner as the sample solution to compensate the base line. Calculate the amounts of the peaks other than cefcapene pivoxil by the

area percentage method: the amount of the peak, having the relative retention time of about 1.3 to cefcapene pivoxil, is not more than 0.4%, the amount of the peak of cefcapene pivoxil trans-isomer, having the relative retention time of about 1.5, is not more than 0.5%, the amount of the peaks other than the peaks mentioned above are not more than 0.3%, respectively, and the total amount of these peaks is not more than 2.0%.

Operating conditions—

Proceed as directed in the operating conditions in the Purity (2) under Cefcapene Pivoxil Hydrochloride Hydrate.

System suitability—

Proceed as directed in the system suitability in the Purity (2) under Cefcapene Pivoxil Hydrochloride Hydrate.

(2) Related substances II—To an amount of powdered Cefcapene Pivoxil Hydrochloride Tablets, equivalent to 2 mg (potency) of Cefcapene Pivoxil Hydrochloride Hydrate, add 20 mL of *N*,*N*-dimethylformamide for liquid chromatography, shake vigorously for 10 minutes, and filter through a membrane filter with pore size of 0.45 μm. Discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks which are eluted before cefcapene pivoxil is not more than 3.3% of the total area of the peaks other than the solvent peak.

Operating conditions—

Proceed as directed in the operating conditions in the Purity (3) under Cefcapene Pivoxil Hydrochloride Hydrate.

System suitability—

Proceed as directed in the system suitability in the Purity (3) under Cefcapene Pivoxil Hydrochloride Hydrate.

Water <*2.48*> Not more than 3.9% (0.5 g, volumetric titration, back titration). Powdering of the sample tablets and handling of the powder are performed under the relative humidity of not exceeding 30%.

Uniformity of dosage units <*6.02*> Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Cefcapene Pivoxil Hydrochloride Tablets add 5 mL of water, and shake vigorously for 5 minutes to disintegrate. Add 20 mL of methanol, shake vigorously for 5 minutes, add a mixture of methanol and water (4:1) to make exactly 50 mL, and centrifuge at 3000 rpm for 5 minutes. Filter the supernatant liquid through a membrane filter with pore size of 0.45 μm, and discard the first 1 mL of the filtrate. Pipet *V* mL of the subsequent filtrate, equivalent to about 6 mg (potency) of Cefcapene Pivoxil Hydrochloride Hydrate, add exactly 15 mL of the internal standard solution, add a mixture of water and methanol (1:1) to make 75 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount [mg (potency)] of cefcapene ($C_{17}H_{19}N_5O_6S_2$)
$= M_S \times Q_T/Q_S \times 15/V$

M_S: Amount [mg (potency)] of Cefcapene Pivoxil Hydrochloride RS taken

Internal standard solution—A solution of *p*-benzylphenol in a mixture of water and methanol (1:1) (7 in 4000).

Dissolution Being specified separately when the drug is granted approval based on the Law.

Assay To an amount of Cefcapene Pivoxil Hydrochloride Tablets, equivalent to about 0.6 g (potency) of Cefcapene Pivoxil Hydrochloride Hydrate, add 20 mL of water, and shake for 5 minutes to disintegrate. Add 80 mL of methanol, shake vigorously for 5 minutes, add a mixture of methanol and water (4:1) to make exactly 200 mL, and centrifuge at 3000 rpm for 5 minutes. Filter the supernatant liquid through a membrane filter with pore size of 0.45 μm, and discard the first 1 mL of the filtrate. Pipet 2 mL of the subsequent filtrate, add exactly 15 mL of the internal standard solution, add the mixture of water and methanol (1:1) to make 75 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefcapene Pivoxil Hydrochloride RS, equivalent to about 20 mg (potency), and dissolve in the mixture of water and methanol (1:1) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add the mixture of water and methanol (1:1) to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefcapene Pivoxil Hydrochloride Hydrate.

Amount [mg (potency)] of cefcapene ($C_{17}H_{19}N_5O_6S_2$)
$= M_S \times Q_T/Q_S \times 30$

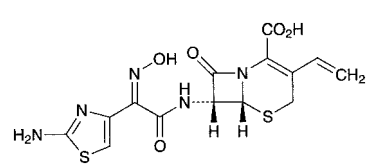
M_S: Amount [mg (potency)] of Cefcapene Pivoxil Hydrochloride RS taken

Internal standard solution—A solution of *p*-benzylphenol in the mixture of water and methanol (1:1) (7 in 4000).

Containers and storage Containers—Tight containers.

Cefdinir

セフジニル



$C_{14}H_{13}N_5O_5S_2$: 395.41
(6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetylamino]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
[*91832-40-5*]

Cefdinir contains not less than 930 μg (potency) and not more than 1020 μg (potency) per mg. The potency of Cefdinir is expressed as mass (potency) of cefdinir ($C_{14}H_{13}N_5O_5S_2$).

Description Cefdinir occurs as a white to light yellow crystalline powder.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in 0.1 mol/L phosphate buffer solution (pH 7.0).

Identification (1) Determine the absorption spectra of solutions of Cefdinir and Cefdinir RS in 0.1 mol/L phosphate buffer solution (pH 7.0) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectra of Cefdinir and Cefdinir RS as directed in the paste method under Infrared Spectrophotometry <*2.25*>, and compare these spectra: both spectra exhibit similar intensities of absorption at

the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefdinir in a mixture of deuterated dimethyl sulfoxide for nuclear magnetic resonance spectroscopy and heavy water for nuclear magnetic resonance spectroscopy (4:1) (1 in 10), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy ⟨*2.21*⟩: it exhibits multiple signals, A at around δ 5.0 – 6.1 ppm and B at around δ 6.4 – 7.5 ppm. The ratio of integrated intensity of each signal, A:B is about 2:1.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: -58 – $-66°$ (0.25 g, 0.1 mol/L phosphate buffer solution (pH 7.0), 25 mL, 100 mm).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Cefdinir according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve about 0.1 g of Cefdinir in 10 mL of 0.1 mol/L phosphate buffer solution (pH 7.0). To 3 mL of this solution add tetramethylammonium hydroxide TS (pH 5.5) to make 20 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amounts of their peaks by the area percentage method: the amount of the peaks, having the relative retention time of about 0.7, about 1.2 and about 1.5 to cefdinir, are not more than 0.7%, not more than 0.3% and not more than 0.8%, respectively, the total amount of the peaks, having the relative retention time of about 0.85, about 0.93, about 1.11 and about 1.14 to cefdinir, is not more than 0.4%, and the amount of the peak other than cefdinir and the peaks mentioned above is not more than 0.2%. And the total amount of the peaks other than cefdinir is not more than 3.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: To 1000 mL of tetramethylammonium hydroxide TS (pH 5.5) add 0.4 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS.

Mobile phase B: To 500 mL of tetramethylammonium hydroxide TS (pH 5.5) add 300 mL of acetonitrile for liquid chromatography and 200 mL of methanol, and add 0.4 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 2	95	5
2 – 22	95 → 75	5 → 25
22 – 32	75 → 50	25 → 50
32 – 37	50	50

Flow rate: 1.0 mL per minute (the retention time of cefdinir is about 22 minutes).

Time span of measurement: For 37 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add tetramethylammonium hydroxide TS (pH 5.5) to make 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, add tetramethylammonium hydroxide TS (pH 5.5) to make exactly 10 mL. Confirm that the peak area of cefdinir obtained from 10 μL of this solution is equivalent to 7 to 13% of that obtained from 10 μL of the solution for system suitability test.

System performance: Dissolve 30 mg of Cefdinir RS and 2 mg of cefdinir lactam ring-cleavage lactones in 3 mL of 0.1 mol/L phosphate buffer solution (pH 7.0), add tetramethylammonium hydroxide TS (pH 5.5) to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, peak 1 and peak 2 of cefdinir lactam ring-cleavage lactones separated into 4 peaks, cefdinir, peak 3 and peak 4 of remaining cefdinir lactam ring-cleavage lactones are eluted in this order. Relative retention time of peak 3 of cefdinir lactam ring-cleavage lactone to cefdinir is about 1.11. The number of theoretical plates and the symmetry factor of the peak of cefdinir are not less than 7000 and not more than 3.0, respectively.

System repeatability: When the test is repeated 3 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of cefdinir is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 2.0% (1 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

Assay Weigh accurately an amount of Cefdinir and Cefdinir RS equivalent to about 20 mg (potency), dissolve each in 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 5 μL of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of cefdinir in each solution.

$$\text{Amount [}\mu\text{g (potency)] of cefdinir } (C_{14}H_{13}N_5O_5S_2) = M_S \times A_T/A_S \times 1000$$

M_S: Amount [mg (potency)] of Cefdinir RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 1000 mL of tetramethylammonium hydroxide TS (pH 5.5) add 0.4 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS. To 900 mL of this solution add 60 mL of acetonitrile for liquid chromatography and 40 mL of methanol.

Flow rate: Adjust so that the retention time of cefdinir is about 8 minutes.

System suitability—

System performance: Dissolve 2 mg of Cefdinir RS and 5 mg of cefdinir lactam ring-cleavage lactones in 10 mL of 0.1 mol/L phosphate buffer solution, pH 7.0. When the procedure is run with 5 μL of this solution under the above operat-

ing conditions, peak 1 and peak 2 of cefdinir lactam ring-cleavage lactones separated into 4 peaks, cefdinir, peak 3 and peak 4 of remaining cefdinir lactam ring-cleavage lactones are eluted in this order. The resolution between the peak 2 of cefdinir lactam ring-cleavage lactone and that of cefdinir is not less than 1.2. The number of theoretical plates and the symmetry factor of the peak of cefdinir are not less than 2000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefdinir is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Cefdinir Capsules

セフジニルカプセル

Cefdinir Capsules contain not less than 90.0% and not more than 110.0% of the labeled potency of cefdinir ($C_{14}H_{13}N_5O_5S_2$: 395.41).

Method of preparation Prepare as directed under Capsules, with Cefdinir.

Identification To an amount of the contents of Cefdinir Capsules, equivalent to 10 mg (potency) of Cefdinir, add 100 mL of 0.1 mol/L phosphate buffer solution (pH 7.0), exposure to ultrasonic waves for 1 minute, and filter. To 2 mL of the filtrate add 0.1 mol/L phosphate buffer solution (pH 7.0) to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 221 nm and 225 nm and between 285 nm and 289 nm.

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate of a 50-mg capsule in 30 minutes is not less than 80%, and that of a 100-mg capsule in 45 minutes is not less than 75%.

Start the test with 1 capsule of Cefdinir Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 56 μg (potency) of Cefdinir, and use this solution as the sample solution. Separately, weigh accurately about 28 mg (potency) of Cefdinir RS, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 4 mL of this solution, add the dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01>, and determine the peak areas, A_T and A_S, of cefdinir in each solution.

Dissolution rate (%) with respect to the labeled amount of cefdinir ($C_{14}H_{13}N_5O_5S_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 180$

M_S: Amount [mg (potency)] of Cefdinir RS taken
C: Labeled amount [mg (potency)] of cefdinir ($C_{14}H_{13}N_5O_5S_2$) in 1 capsule

Operating conditions—
Proceed as directed in the operating conditions in the Assay under Cefdinir.
System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefdinir are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefdinir is not more than 1.0%.

Assay Weigh accurately not less than 5 Cefdinir Capsules, take out the contents, and powder. Wash the empty capsules with a little amount of diethyl ether, if necessary, allow to stand at a room temperature to vaporize the adhering diethyl ether, and weigh accurately the mass of the capsules to calculate the mass of the contents. Weigh accurately an amount of the contents, equivalent to about 0.1 g (potency) of Cefdinir, add 70 mL of 0.1 mol/L phosphate buffer solution (pH 7.0), shake for 30 minutes, and add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 100 mL. Centrifuge this solution at 3000 revolutions per minute for 10 minutes, pipet 4 mL of the supernatant liquid, add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefdinir RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 100 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefdinir.

Amount [mg (potency)] of cefdinir ($C_{14}H_{13}N_5O_5S_2$)
$= M_S \times A_T/A_S \times 5$

M_S: Amount [mg (potency)] of Cefdinir RS taken

Containers and storage Containers—Tight containers.

Cefdinir Fine Granules

セフジニル細粒

Cefdinir Fine Granules contain not less than 93.0% and not more than 107.0% of the labeled potency of cefdinir ($C_{14}H_{13}N_5O_5S_2$: 395.41).

Method of preparation Prepare as directed under Granules, with Cefdinir.

Identification To an amount of Cefdinir Fine Granules, equivalent to 10 mg (potency) of Cefdinir, add 100 mL of 0.1 mol/L phosphate buffer solution (pH 7.0), exposure to ultrasonic waves for 1 minute, and filter. To 2 mL of the filtrate add 0.1 mol/L phosphate buffer solution (pH 7.0) to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 221 nm and 225 nm and between 285 nm and 289 nm.

Uniformity of dosage units <6.02> The granules in single-dose packages meet the requirement of the Mass variation test.

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900

mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Cefdinir Fine Granules is not less than 75%.

Start the test with an accurate amount of Cefdinir Fine Granules, equivalent to about 0.1 g (potency) of Cefdinir, withdraw not less than about 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 28 mg (potency) of Cefdinir RS, and disslove in the dissolution medium to make exactly 50 mL. Pipet 4 mL of this solution, add the dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*>, and determine the peak areas, A_T and A_S, of cefdinir in each solution.

Dissolution rate (%) with respect to the labeled amount of cefdinir ($C_{14}H_{13}N_5O_5S_2$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 360$

M_S: Amount [mg (potency)] of Cefdinir RS taken
M_T: Amount (g) of Cefdinir Fine Granules taken
C: Labeled amount [mg (potency)] of cefdinir ($C_{14}H_{13}N_5O_5S_2$) in 1 g

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Cefdinir.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefdinir are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefdinir is not more than 1.0%.

Assay Powder, if necessary, and weigh accurately an amount of Cefdinir Fine Granules, equivalent to about 0.1 g (potency) of Cefdinir, add 70 mL of 0.1 mol/L phosphate buffer solution (pH 7.0), shake for 30 minutes, and add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 100 mL. Centrifuge at 3000 revolutions per minute for 10 minutes, pipet 4 mL of the supernatant liquid, add 0.1 mol/L phosphate buffer solution (pH 7.0) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefdinir RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 100 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefdinir.

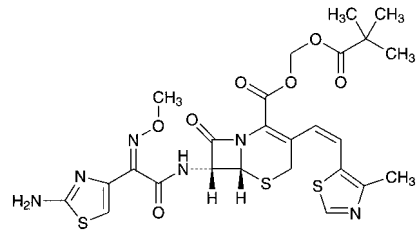
Amount [mg (potency)] of cefdinir ($C_{14}H_{13}N_5O_5S_2$)
$= M_S \times A_T/A_S \times 5$

M_S: Amount [mg (potency)] of Cefdinir RS taken

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Cefditoren Pivoxil

セフジトレン　ピボキシル



$C_{25}H_{28}N_6O_7S_3$: 620.72
2,2-Dimethylpropanoyloxymethyl (6*R*,7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetylamino]-3-[(1*Z*)-2-(4-methylthiazol-5-yl)ethenyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate
[*117467-28-4*]

Cefditoren Pivoxil contains not less than 770 μg (potency) and not more than 820 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefditoren Pivoxil is expressed as mass (potency) of cefditoren ($C_{19}H_{18}N_6O_5S_3$: 506.58).

Description Cefditoren Pivoxil occurs as a light yellowish white to light yellow crystalline powder.

It is sparingly soluble in methanol, slightly soluble in acetonitrile and in ethanol (95), very slightly sobuble in diethyleter and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

Identification **(1)** Dissolve 5 mg of Cefditoren Pivoxil in 3 mL of hydroxylammonium chloride-ethanol TS, allow to stand for 5 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS and shake: a red-brown color develops.

(2) Dissolve 1 mg of Cefditoren Pivoxil in 1 mL of dilute hydrochloric acid and 4 mL of water, add 3 drops of sodium nitrite TS under ice-cooling, shake, and allow to stand for 2 minutes. Then add 1 mL of ammonium amidosulfate TS, shake well, and allow to stand for 1 minute, and add 1 mL of *N*,*N*-diethyl-*N*′-1-naphthylethylenediamine oxalate TS: a purple color develops.

(3) Determine the absorption spectrum of a solution of Cefditoren Pivoxil in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefditoren Pivoxil RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the ^{1}H spectrum of a solution of Cefditoren Pivoxil in deuterated chloroform for nuclear magnetic resonance spectroscopy (1 in 50), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>: it exhibits single signals A, B and C, at around δ 1.1 ppm, at around δ 2.4 ppm and at around δ 4.0 ppm, double signals D and E, at around δ 6.4 ppm and at around δ 6.7 ppm, and a single signal F at around δ 8.6 ppm. The ratio of integrated intensity of each signal A:B:C:D:E:F, is about 9:3:3:1:1:1.

Absorbance <*2.24*> $E^{1\%}_{1\,cm}$ (231 nm): 340 – 360 (50 mg, methanol, 2500 mL).

Optical rotation <*2.49*> $[\alpha]^{20}_D$: −45 – −52° (50 mg, methanol, 10 mL, 100 mm).

Purity **(1)** Heavy metals <2.07>—Proceed with 2.0 g of Cefditoren Pivoxil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Being specified separately when the drug is granted approval based on the Law.

Water <2.48> Not more than 1.5% (0.5 g, volumetric titration, direct titration).

Residue on ignition Being specified separately when the drug is granted approval based on the Law.

Assay Conduct this procedure using light-resistant vessels. Weigh accurately an amount of Cefditoren Pivoxil and Cefditoren Pivoxil RS, equivalent to about 40 mg (potency), dissolve in 40 mL of acetonitrile, add exactly 10 mL each of the internal standard solution, and add acetonitrile to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefditoren pivoxil to that of the internal standard.

Amount [μg (potency)] of cefditoren ($C_{19}H_{18}N_6O_5S_3$) $= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Cefditoren Pivoxil RS taken

Internal standard solution—A solution of propyl *p*-hydroxybenzoate in acetonitrile (1 in 200).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.58 g of ammonium formate in 900 mL of water, adjust to pH 6.0 with diluted formic acid (1 in 250), and add water to make 1000 mL. To 450 mL of this solution add 275 mL of acetonitrile and 275 mL of methanol.

Flow rate: Adjust so that the retention time of cefditoren pivoxil is about 15 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and cefditoren pivoxil are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefditoren pivoxil to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cefditoren Pivoxil Fine Granules

セフジトレン ピボキシル細粒

Cefditoren Pivoxil Fine Granules contain not less than 90.0% and not more than 110.0% of the labeled potency of cefditoren ($C_{19}H_{18}N_6O_5S_3$: 506.58).

Method of preparation Prepare as directed under Granules, with Cefditoren Pivoxil.

Identification To an amount of powdered Cefditoren Pivoxil Fine Granules, equivalent to 0.1 g (potency) of Cefditoren Pivoxil, add 10 mL of acetonitrile, shake vigorously, and filter. To 1 mL of the filtrate add acetonitrile to make 50 mL. To 1 mL of this solution add acetonitrile to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 230 nm and 234 nm.

Purity Related substances—Being specified separately when the drug is granted approval based on the Law.

Loss on drying <2.41> Not more than 4.5% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Uniformity of dosage units <6.02> The granules in single-dose packages meet the requirement of the Mass variation test.

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 15 minutes of Cefditoren Pivoxil Fine Granules is not less than 80%.

Start the test with an accurately weighed amount of Cefditoren Pivoxil Fine Granules, equivalent to about 0.1 g (potency) of Cefditoren Pivoxil, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefditoren Pivoxil RS, equivalent to about 22 mg (potency), dissolve in 20 mL of diluted acetonitrile (3 in 4), and add the dissolution medium to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 272 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the control.

Dissolution rate (%) with respect to the labeled amount of cefditoren pivoxil ($C_{25}H_{28}N_6O_7S_3$) $= M_S/M_T \times A_T/A_S \times 1/C \times 450$

M_S: Amount [mg(potency)] of Cefditoren Pivoxil RS taken

M_T: Amount (g) of Cefditoren Pivoxil Fine Granules taken

C: Labeled amount [mg(potency)] of cefditoren pivoxil ($C_{25}H_{28}N_6O_7S_3$) in 1 g

Assay Conduct this procedure using light-resistant vessels. Weigh accurately an amount of powdered Cefditoren Pivoxil Fine Granules, equivalent to about 40 mg (potency) of Cefditoren Pivoxil, add 70 mL of diluted acetonitrile (3 in 4), and shake vigorously. To this solution add exactly 10 mL of the internal standard solution, then add acetonitrile to make

100 mL, filter, and use the filtrate as the sample solution. Separately, weigh accurately an amount of Cefditoren Pivoxil RS, equivalent to about 20 mg (potency), dissolve in 20 mL of acetonitrile, add exactly 5 mL of the internal standard solution, then add acetonitrile to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefditoren Pivoxil.

Amount [mg (potency)] of cefditoren ($C_{19}H_{18}N_6O_5S_3$)
$= M_S \times Q_T/Q_S \times 2$

M_S: Amount [mg (potency)] of Cefditoren Pivoxil RS taken

Internal standard solution—A solution of propyl parahydroxybenzoate in acetonitrile (1 in 200).

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Cefditoren Pivoxil Tablets

セフジトレン　ピボキシル錠

Cefditoren Pivoxil Tablets contain not less than 90.0% and not more than 110.0% of the labeled potency of cefditoren ($C_{19}H_{18}N_6O_5S_3$: 506.58).

Method of preparation Prepare as directed under Tablets, with Cefditoren Pivoxil.

Identification To an amount of powdered Cefditoren Pivoxil Tablets, equivalent to 35 mg (potency) of Cefditoren Pivoxil, add 100 mL of methanol, shake, and filter. To 5 mL of the filtrate add methanol to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 229 nm and 233 nm.

Purity Related substances—Being specified separately when the drug is granted approval based on the Law.

Loss on drying <*2.41*> Not more than 4.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Uniformity of dosage units <*6.02*> Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

Conduct this procedure using light-resistant vessels. To 1 tablet of Cefditoren Pivoxil Tablets add 12.5 mL of the 1st fluid for disintegration test, shake vigorously, add about 25 mL of acetonitrile, shake again, and add acetonitrile to make exactly 50 mL. Pipet V mL of this solution, equivalent to about 20 mg (potency) of Cefditoren Pivoxil, add exactly 5 mL of the internal standard solution, then add diluted acetonitrile (3 in 4) to make 50 mL, filter, and use the filtrate as the sample solution. Separately, weigh accurately an amount of Cefditoren Pivoxil RS, equivalent to about 20 mg (potency), dissolve in 20 mL of acetonitrile, add exactly 5 mL of the internal standard solution, then add acetonitrile to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefditoren Pivoxil.

Amount [mg (potency)] of cefditoren ($C_{19}H_{18}N_6O_5S_3$)
$= M_S \times Q_T/Q_S \times 50/V$

M_S: Amount [mg (potency)] of Cefditoren Pivoxil RS taken

Internal standard solution—A solution of propyl parahydroxybezoate in acetonitrile (1 in 200).

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 20 minutes of Cefditoren Pivoxil Tablets is not less than 85%.

Start the test with 1 tablet of Cefditoren Pivoxil Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 11 μg (potency) of Cefditoren Pivoxil, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefditoren Pivoxil RS, equivalent to about 22 mg (potency), dissolve in 20 mL of diluted acetonitrile (3 in 4), then add the dissolution medium to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 272 nm of the sample solution and standard solution as directed under Ultraviolelt-visible Spectrophotometry <*2.24*> using water as the control.

Dissolution rate (%) with respect to the labeled amount of cefditoren pivoxil ($C_{25}H_{28}N_6O_7S_3$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$

M_S: Amount [mg (potency)] of Cefditoren Pivoxil RS taken

C: Labeled amount [mg (potency)] of cefditoren pivoxil ($C_{25}H_{28}N_6O_7S_3$) in 1 tablet

Assay Conduct this procedure using light-resistant vessels. To an amount of Cefditoren Pivoxil Tablets, equivalent to 0.5 g (potency) of Cefditoren Pivoxil, add 63 mL of the 1st fluid for disintegration test, shake vigorously, add about 125 mL of acetonitrile, shake again, and add acetonitrile to make exactly 250 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, then add diluted acetonitrile (3 in 4) to make 50 mL, filter, and use the filtrate as the sample solution. Separately, weigh accurately an amount of Cefditoren Pivoxil RS, equivalent to about 20 mg (potency), dissolve in 20 mL of acetonitrile, add exactly 5 mL of the internal standard solution, then add acetonitrile to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefditoren Pivoxil.

Amount [mg (potency)] of cefditoren ($C_{19}H_{18}N_6O_5S_3$)
$= M_S \times Q_T/Q_S \times 25$

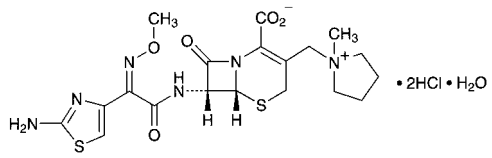
M_S: Amount [mg (potency)] of Cefditoren Pivoxil RS taken

Internal standard solution—A solution of propyl parahydroxybenzoate in acetonitrile (1 in 200).

Containers and storage Containers—Tight containers.

Cefepime Dihydrochloride Hydrate

セフェピム塩酸塩水和物



$C_{19}H_{24}N_6O_5S_2.2HCl.H_2O$: 571.50

(6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetylamino]-3-(1-methylpyrrolidinium-1-ylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate dihydrochloride monohydrate

[*123171-59-5*]

Cefepime Dihydrochloride Hydrate contains not less than 835 μg (potency) and not more than 886 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefepime Dihydrochloride Hydrate is expressed as mass (potency) of cefepime ($C_{19}H_{24}N_6O_5S_2$: 480.56).

Description Cefepime Dihydrochloride Hydrate occurs as a white to yellowish white, crystals or crystalline powder.

It is freely soluble in water and in methanol, and slightly soluble in ethanol (95), and practically in soluble in diethyl ether.

Identification **(1)** Dissolve 0.02 g of Cefepime Dihydrochloride Hydrate in 2 mL of water, add 1 mL of a solution of hydroxylammonium chloride (1 in 10) and 2 mL of sodium hydroxide TS, allow to stand for 5 minutes, then add 3 mL of 1 mol/L hydrochloric acid TS and 3 drops of iron (III) chloride TS: a red-brown color develops.

(2) Determine the absorption spectra of solutions (1 in 20,000) of Cefepime Dihydrochloride Hydrate and Cefepime Dihydrochloride RS as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectra of Cefepime Dihydrochloride Hydrate and Cefepime Dihydrochloride RS as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Determine the ^1H spectrum of a solution of Cefepime Dihydrochloride Hydrate in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>, using sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A and B, at around δ 3.1 ppm and at around δ 7.2 ppm, respectively, and the ratio of integrated intensity of each signal, A:B, is about 3:1.

(5) Dissolve 15 mg of Cefepime Dihydrochloride Hydrate in 5 mL of water, and add 2 drops of silver nitrate TS: a white turbidity is produced.

Absorbance <*2.24*> $E^{1\%}_{1\,cm}$ (259 nm): 310 – 340 (50 mg calculated on the anhydrous basis, water, 1000 mL).

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +39 – +47° (60 mg calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH <*2.54*> Dissolve 0.1 g of Cefepime Dihydrochloride Hydrate in 10 mL of water: the pH of this solution is between 1.6 and 2.1.

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Cefepime Dihydrochloride Hydrate in 5 mL of a solution of L-arginine (3 in 50): the solution is clear and has no more color than Matching Fluid H.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Cefepime Dihydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) *N*-Methylpyrrolidine—Weigh accurately an amount of Cefepime Dihydrochloride Hydrate equivalent to about 80 mg (potency), dissolve in diluted nitric acid (2 in 3125) to make exactly 10 mL, and use this solution as the sample solution. Separately, put 30 mL of water in a 100-mL volumetric flask, weigh accurately the mass of flask, then add about 0.125 g of *N*-methylpyrrolidine, weigh accurately the mass of the flask again, and add water to make exactly 100 mL. Pipet 4 mL of this solution, add diluted nitric acid (2 in 3125) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of *N*-methylpyrrolidine by the automatic integration method. Calculate the amount of *N*-methylpyrrolidine per 1 mg (potency) of Cefepime Dihydrochloride Hydrate by the following equation: not more than 0.5%. The sample solution must be tested within 20 minutes after preparation.

$$\text{Amount (\%) of } N\text{-methylpyrrolidine} = (M_S \times f)/M_T \times A_T/A_S \times 1/250$$

M_S: Amount (mg) of *N*-methylpyrrolidine taken

M_T: Amount [mg (potency)] of Cefepime Dihydrochloride Hydrate taken

f: Purity (%) of *N*-methylpyrrolidine

Operating conditions—

Detector: An electric conductivity detector.

Column: A plastic tube 4.6 mm in inside diameter and 5 cm in length, packed with hydrophilic silica gel for liquid chromatography carrying sulfonic acid groups having the exchange capacity of about 0.3 meq per g (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: To 990 mL of diluted nitric acid (2 in 3125) add 10 mL of acetonitrile.

Flow rate: 1.0 mL per minute.

System suitability—

System performance: To 20 mL of a solution of sodium chloride (3 in 1000) add 0.125 g of *N*-methylpyrrolidine, and add water to make 100 mL. To 4 mL of this solution add diluted nitric acid (2 in 3125) to make 100 mL. When the procedure is run with 100 μL of this solution under the above operating conditions, sodium and *N*-methylpyrrolidine are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 5 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of *N*-methylpyrrolidine is not more than 4.0%.

(4) Related substances—Dissolve about 0.1 g of Cefepime Dihydrochloride Hydrate in the mobile phase A to make 50 mL, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the fol-

lowing conditions, and determine the area of each peak by the automatic integration method. Calculate the total amount of the peaks other than cefepime by the area percentage method: not more than 0.5%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Dissolve 0.57 g of ammonium dihydrogenphosphate in 1000 mL of water.

Mobile phase B: Acetonitrile.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of the sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 25	100 → 75	0 → 25

Flow rate: Adjust so that the retention time of cefepime is about 9.5 minutes.

Time span of measurement: About 2.5 times as long as the retention time of cefepime.

System suitability—

Test for required detectability: To 1 mL of the sample solution add the mobile phase A to make 10 mL, and use this solution as the solution for system suitability test. To 1 mL of the solution for system suitability test add the mobile phase A to make 10 mL, and use this solution as the solution for test for required detectability. Pipet 1 mL of the solution for test for required detectability, add the mobile phase A to make exactly 10 mL. Conform that the peak area of cefepime obtained from 5 μL of this solution is equivalent to 7 to 13% of that of cefepime obtained from 5 μL of the solution for test for required detectability.

System performance: When the procedure is run with 5 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates of the peak of cefepime is not less than 6000.

System repeatability: When the test is repeated 3 times with 5 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of cefepime is not more than 2.0%.

Water ⟨*2.48*⟩ Not less than 3.0% and not more than 4.5% (Weigh accurately about 50 mg of Cefepime Dihydrochloride Hydrate, dissolve in exactly 2 mL of methanol for water determination and perform the test with exactly 0.5 mL of this solution; coulometric titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.04 EU/mg (potency).

Assay Weigh accurately an amount of Cefepime Dihydrochloride Hydrate and Cefepime Dihydrochloride RS, equivalent to about 60 mg (potency), dissolve in the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of cefepime in each solution.

Amount [μg (potency)] of cefepime ($C_{19}H_{24}N_6O_5S_2$) = $M_S \times A_T/A_S \times 1000$

M_S: Amount [mg (potency)] of Cefepime Dihydrochloride RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Adjust a solution of sodium 1-pentanesulfonate (261 in 100,000) to pH 3.4 with acetic acid (100), then adjust this solution to pH 4.0 with a solution of potassium hydroxide (13 in 20). To 950 mL of this solution add 50 mL of acetonitrile.

Flow rate: Adjust so that the retention time of cefepime is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of cefepime is not less than 1500.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefepime is not more than 2.0%.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant.

Cefepime Dihydrochloride for Injection

注射用セフェピム塩酸塩

Cefepime Dihydrochloride for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 95.0% and not more than 110.0% of the labeled potency of cefepime ($C_{19}H_{24}N_6O_5S_2$: 480.56).

Method of preparation Prepare as directed under Injections, with Cefepime Dihydrochloride Hydrate.

Description Cefepime Dihydrochloride for Injection occurs as a white to pale yellow powder.

Identification **(1)** Dissolve 40 mg of Cefepime Dihydrochloride in 2 mL of water, add 1 mL of a solution of hydroxylammonium chloride (1 in 10) and 2 mL of sodium hydroxide TS, allow to stand for 5 minutes, then add 3 mL of 1 mol/L hydrochloric acid TS and 3 drops of iron (III) chloride TS: a red-brown color develops.

(2) Determine the absorption spectrum of a solution of Cefepime Dihydrochloride for Injection (1 in 12,500) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 233 nm and 237 nm and between 255 nm and 259 nm.

pH ⟨*2.54*⟩ The pH of a solution obtained by dissolving an amount of Cefepime Dihydrochloride for Injection, equivalent to 0.5 g (potency) of Cefepime Dihydrochloride Hydrate, in 5 mL of water is between 4.0 and 6.0.

Purity **(1)** Clarity and color of solution—Dissolve an

amount of Cefepime Dihydrochloride for Injection, equivalent to 0.5 g (potency) of Cefepime Dihydrochloride Hydrate, in 5 mL of water: the solution is clear and colorless or light yellow. The color is not darker than Matching Fluid I.

(2) *N*-Methylpyrrolidine—Weigh accurately an amount of Cefepime Dihydrochloride for Injection, equivalent to about 0.2 g (potency) of Cefepime Dihydrochloride Hydrate, dissolve in diluted nitric acid (2 in 625) to make exactly 20 mL, and use this solution as the sample solution. Separately, transfer 30 mL of water into a 100-mL volumetric flask, weigh accurately the mass of the flask, add about 0.125 g of *N*-methylpyrrolidine, then weigh accurately the mass, and add water to make exactly 100 mL. Pipet 4 mL of this solution, add diluted nitric acid (2 in 3125) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas of *N*-methylpyrrolidine, A_T and A_S, by the automatic integration method within 20 minutes after the sample solution is prepared. Calculate the amount of *N*-methylpyrrolidine per mg (potency) of Cefepime Dihydrochloride for Injection by the following formula: not more than 1.0%.

$$\text{Amount (\%) of } N\text{-methylpyrrolidine} = (M_S \times f)/M_T \times A_T/A_S \times 1/125$$

M_S: Amount (mg) of *N*-methylpyrrolidine taken
M_T: Amount [mg (potency)] of Cefepime Dihydrochloride for Injection taken
f: Purity (%) of *N*-methylpyrrolidine

Operating conditions—

Proceed as directed in the operating conditions in the Purity (3) under Cefepime Dihydrochloride Hydrate.

System suitability—

Proceed as directed in the system suitability in the Purity (3) under Cefepime Dihydrochloride Hydrate.

Water <*2.48*> Not more than 4.0% (Weigh accurately about 50 mg of Cefepime Dihydrochloride for Injection, dissolve in exactly 2 mL of methanol for water determination, and perform the test with exactly 0.5 mL of this solution: coulometric titration).

Bacterial endotoxins <*4.01*> Less than 0.06 EU/mg (potency).

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test.

Foreign insoluble matter <*6.06*> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 Cefepime Dihydrochloride for Injection. Weigh accurately an amount of the content, equivalent to about 60 mg (potency) of Cefepime Dihydrochloride Hydrate, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefepime Dihydrochloride RS, equivalent to about 60 mg (potency), dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefepime Dihydrochloride Hydrate.

$$\text{Amount [}\mu\text{g (potency)] of cefepime } (C_{19}H_{24}N_6O_5S_2) = M_S \times A_T/A_S \times 1000$$

M_S: Amount [mg (potency)] of Cefepime Dihydrochloride RS taken

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

Cefixime Hydrate

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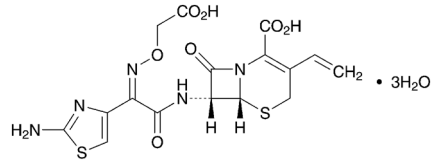


$C_{16}H_{15}N_5O_7S_2.3H_2O$: 507.50

(6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(carboxymethoxyimino)acetylamino]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid trihydrate

[*125110-14-7*]

Cefixime Hydrate contains not less than 930 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefixime Hydrate is expressed as mass (potency) of cefixime ($C_{16}H_{15}N_5O_7S_2$: 453.45).

Description Cefixime Hydrate occurs as a white to light yellow crystalline powder.

It is freely soluble in methanol and in dimethylsulfoxide, sparingly soluble in ethanol (99.5), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Cefixime Hydrate in 0.1 mol/L phosphate buffer solution (pH 7.0) (1 in 62,500) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefixime RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefixime Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefixime RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.05 g of Cefixime Hydrate in 0.5 mL of a mixture of deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy and heavy water for nuclear magnetic resonance spectroscopy (4:1). Determine the ^1H spectrum of this solution, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>: it exhibits a single signal A at around δ 4.7 ppm, and a multiple signal B between δ 6.5 ppm and δ 7.4 ppm. The ratio of integrated intensity of these signals, A:B, is about 1:1.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -75 – $-88°$ (0.45 g calculated on the anhydrous bases, a solution of sodium hydrogen carbonate (1 in 50), 50 mL, 100 mm).

Purity Dissolve 0.1 g of Cefixime Hydrate in 100 mL of 0.1 mol/L phosphate buffer solution (pH 7.0), and use this solu-

tion as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, determine the areas of the peaks by the automatic integration method, and calculate the amounts of these peak areas by the area percentage method: the amount of each peak other than cefixime is not more than 1.0%, and the total amount of the peaks other than cefixime is not more than 2.5%.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of cefixime beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the sample solution, and add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 100 mL. Confirm that the peak height of cefixime obtained from 10 μL of this solution is equivalent to 20 to 60 mm.

System performance: Dissolve about 2 mg of Cefixime RS in 200 mL of 0.1 mol/L phosphate buffer solution (pH 7.0) and use this solution as the solution for system suitability test. When the procedure is run with 10 μL of the solution according to the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefixime are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of cefixime is not more than 2.0%.

Water <2.48> Not less than 9.0 and not more than 12.0% (0.1 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Cefixime Hydrate and Cefixime RS, equivalent to about 0.1 g (potency), and dissolve in 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 100 mL each. Pipet 10 mL each of these solutions, add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 50 mL each, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of cefixime in each solution.

$$\text{Amount [}\mu\text{g (potency)] of } C_{16}H_{15}N_5O_7S_2 = M_S \times A_T/A_S \times 10000$$

M_S: Amount [mg (potency)] of Cefixime RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 125 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 25 mL of a solution of tetrabutylammonium hydroxide TS (10 in 13) add water to make 1000 mL, adjust to pH 6.5 with diluted phosphoric acid (1 in 10). To 300 mL of this solution add 100 mL of acetonitrile.

Flow rate: Adjust so that the retention time of cefixime is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefixime are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of peak areas of cefixime is not more than 2.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cefixime Capsules

セフィキシムカプセル

Cefixime Capsules contain not less than 90.0% and not more than 105.0% of the labeled potency of cefixime ($C_{16}H_{15}N_5O_7S_2$: 453.45).

Method of preparation Prepare as directed under Capsules, with Cefixime Hydrate.

Identification Take out the contents of Cefixime Capsules, to a quantity of the contents of Cefixime Capsules, equivalent to 70 mg (potency) of Cefixime Hydrate, add 100 mL of 0.1 mol/L phosphate buffer solution (pH 7.0), shake for 30 minutes, and filter. To 1 mL of the filtrate add 0.1 mol/L phosphate buffer solution (pH 7.0) to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 286 nm and 290 nm.

Purity Related substances—Take out the contents of Cefixime Capsules, to a quantity of the contents of Cefixime Capsules, equivalent to 0.1 g (potency) of Cefixime Hydrate, add 100 mL of 0.1 mol/L phosphate buffer solution (pH 7.0), shake for 30 minutes, filter, and use the filtrate as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area from the sample solution by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of each peak other than cefixime is not more than 1.0%, and the total amount of the peaks other than cefixime is not more than 2.5%.

Operating conditions—

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Cefixime Hydrate.

Time span for measurement: Proceed as directed in the operating conditions in the Purity under Cefixime Hydrate.

System suitability—

Test for required detectability: Pipet 1 mL of the sample solution, and add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 10 mL. Confirm that the peak area of cefixime obtained from 10 μL of this solution is equivalent to 7 to 13% of that obtained from 10 μL of the solution for system suitability test.

System performance: When the procedure is run with 10 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and

the symmetry factor of the peak of cefixime are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of cefixime is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 12.0% (0.1 g of the contents, volumetric titration, direct titration).

Uniformity of dosage units ⟨*6.02*⟩ Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

Take out the contents of 1 capsule of Cefixime Capsules, and to the contents and the capsule shells add $7V/10$ mL of 0.1 mol/L phosphate buffer solution (pH 7.0), shake for 30 minutes, and add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly V mL so that each mL contains about 1 mg (potency) of Cefixime Hydrate. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefixime RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefixime Hydrate.

Amount [mg (potency)] of cefixime ($C_{16}H_{15}N_5O_7S_2$)
$= M_S \times A_T/A_S \times V/20$

M_S: Amount [mg (potency)] of Cefixime RS taken

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of disodium hydrogen phosphate-citric acid buffer solution (pH 7.5) as the dissolution medium, the dissolution rates in 60 minutes of 50-mg (potency) capsule and in 90 minutes of 100-mg (potency) capsule are not less than 80%, respectively.

Start the test with 1 capsule of Cefixime Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 56 μg (potency) of Cefixime Hydrate, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefixime RS, equivalent to about 28 mg (potency), and dissolve in the dissolution medium to make exactly 100 mL. Pipet 4 mL of this solution, add the dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of cefixime in each solution.

Dissolution rate (%) with respect to the labeled amount of cefixime ($C_{16}H_{15}N_5O_7S_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 180$

M_S: Amount [mg (potency)] of Cefixime RS taken
C: Labeled amount [mg (potency)] of Cefixime Hydrate in 1 capsule

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Cefixime Hydrate.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefixime are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefixime is not more than 2.0%.

Assay Take out the contents of not less than 20 Cefixime Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g (potency) of Cefixime Hydrate, add 70 mL of 0.1 mol/L phosphate buffer solution (pH 7.0) and shake for 30 minutes, add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 100 mL. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefixime RS, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefixime Hydrate.

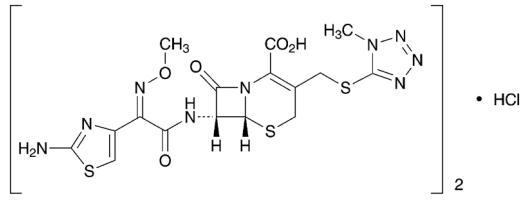
Amount [mg (potency)] of cefixime ($C_{16}H_{15}N_5O_7S_2$)
$= M_S \times A_T/A_S \times 5$

M_S: Amount [mg (potency)] of Cefixime RS taken

Containers and storage Containers—Tight containers.

Cefmenoxime Hydrochloride

セフメノキシム塩酸塩



$(C_{16}H_{17}N_9O_5S_3.)_2.HCl$: 1059.58
(6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetylamino]-3-(1-methyl-1*H*-tetrazol-5-ylsulfanylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid hemihydrochloride
[*75738-58-8*]

Cefmenoxime Hydrochloride contains not less than 890 μg (potency) and not more than 975 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefmenoxime Hydrochloride is expressed as mass (potency) of cefmenoxime ($C_{16}H_{17}N_9O_5S_3$: 511.56).

Description Cefmenoxime Hydrochloride occurs as white to light orange-yellow, crystals or crystalline powder.

It is freely soluble in formamide and in dimethylsulfoxide, slightly soluble in methanol, very slightly soluble in water, and practically insoluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Cefmenoxime Hydrochloride in 0.1 mol/L phosphate buffer solution (pH 6.8) (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefmenoxime Hydrochloride RS

prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefmenoxime Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Cefmenoxime Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefmenoxime Hydrochloride in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy ⟨*2.21*⟩, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits two single signals, A and B, at around δ 3.9 ppm, and a single signal C at around δ 6.8 ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 3:3:1.

(4) Dissolve 10 mg of Cefmenoxime Hydrochloride in 1 mL of diluted sodium carbonate TS (1 in 20), add 5 mL of acetic acid (100) and 2 drops of silver nitrate TS: a white precipitate is formed.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: -27 – $-35°$ (1 g, 0.1 mol/L phosphate buffer solution (pH 6.8), 100 mL, 100 mm).

pH ⟨*2.54*⟩ The pH of a solution obtained by dissolving 0.10 g of Cefmenoxime Hydrochloride in 150 mL of water is between 2.8 and 3.3.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 1.0 g of Cefmenoxime Hydrochloride in 10 mL of diluted sodium carbonate TS (1 in 4) is clear and colorless to light yellow.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Cefmenoxime Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Cefmenoxime Hydrochloride according to Method 4 and adding 10 mL of dilute hydrochloric acid to the residue after cooling, and perform the test (not more than 2 ppm).

(4) Related substances—Weigh accurately about 0.1 g of Cefmenoxime Hydrochloride, dissolve in 20 mL of 0.1 mol/L phosphate buffer solution (pH 6.8) and add the mobile phase to make exactly 100 mL. Pipet 4 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of 1-methyl-1*H*-tetrazol-5-thiol, and dissolve in the mobile phase to make exactly 100 mL. Pipet 4 mL of this solution, add the mobile phase to make exactly 250 mL, and use this solution as the standard solution (1). Weigh accurately about 0.1 g of Cefmenoxime Hydrochloride RS, dissolve in 20 mL of 0.1 mol/L phosphate buffer solution (pH 6.8) and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 250 mL, and use this solution as the standard solution (2). Perform the test immediately after preparation of these solutions with exactly 10 μL each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of these solutions by the automatic integration method, and calculate the amounts of 1-methyl-1*H*-tetrazol-5-thiol and the total related substance by the following formula: the amount of 1-methyl-1*H*-tetrazol-5-thiol is not more than 1.0%, and the total related substance is not more than 3.0%.

Amount (%) of 1-methyl-1*H*-tetrazol-5-thiol
$= M_{Sa}/M_T \times A_{Ta}/A_{Sa} \times 20$

Amount (%) of total related substances
$= M_{Sa}/M_T \times A_{Ta}/A_{Sa} \times 20 + M_{Sb}/M_T \times S_T/A_{Sb} \times 5$

M_{Sa}: Amount (g) of 1-methyl-1*H*-tetrazol-5-thiol taken
M_{Sb}: Amount (g) of Cefmenoxime Hydrochloride RS taken
M_T: Amount (g) of Cefmenoxime Hydrochloride taken
A_{Sa}: Peak area of 1-methyl-1*H*-tetrazol-5-thiol from the standard solution (1)
A_{Sb}: Peak area of cefmenoxime from the standard solution (2)
A_{Ta}: Peak area of 1-methyl-1*H*-tetrazol-5-thiol from the sample solution
S_T: Total area of the peaks other than 1-methyl-1*H*-tetrazol-5-thiol and other than cefmenoxime from the sample solution

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of cefmenoxime.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Measure exactly 5 mL of the standard solution (1), add the mobile phase to make exactly 100 mL. Confirm that the peak area of 1-methyl-1*H*-tetrazol-5-thiol obtained from 10 μL of this solution is equivalent to 4.5 to 5.5% of that obtained from 10 μL of the standard solution (1). Then, measure exactly 2 mL of the standard solution (2), add the mobile phase to make exactly 100 mL. Confirm that the peak area of cefmenoxime obtained from 10 μL of this solution is equivalent to 1.5 to 2.5% of that obtained from 10 μL of the standard solution (2).

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak area of 1-methyl-1*H*-tetrazol-5-thiol is not more than 1.0%.

Water ⟨*2.48*⟩ Not more than 1.5% (1 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1)).

Assay Weigh accurately an amount of Cefmenoxime Hydrochloride and Cefmenoxime Hydrochloride RS, equivalent to about 50 mg (potency), dissolve each in 10 mL of 0.1 mol/L phosphate buffer solution (pH 6.8) and add the mobile phase to make exactly 50 mL. Pipet 4 mL each of these solutions, add exactly 20 mL of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefmenoxime to that of the internal standard.

Amount [μg (potency)] of cefmenoxime ($C_{16}H_{17}N_9O_5S_3$)
$= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Cefmenoxime Hydrochloride RS taken

Internal standard solution—A solution of phthalimide in methanol (3 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (50:10:1).

Flow rate: Adjust so that the retention time of cefmenoxime is about 8 minutes.

System suitability—

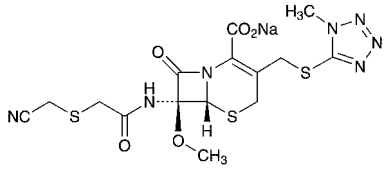
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefmenoxime and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak areas of cefmenoxime to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Cefmetazole Sodium

セフメタゾールナトリウム



$C_{15}H_{16}N_7NaO_5S_3$: 493.52

Monosodium (6*R*,7*R*)-7-{[(cyanomethylsulfanyl)acetyl]amino}-7-methoxy-3-(1-methyl-1*H*-tetrazol-5-ylsulfanylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

[*56796-20-4*]

Cefmetazole Sodium contains not less than 860 μg (potency) and not more than 965 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefmetazole Sodium is expressed as mass (potency) of cefmetazole ($C_{15}H_{17}N_7O_5S_3$: 471.53).

Description Cefmetazole Sodium occurs as a white to light yellowish white, powder or mass.

It is very soluble in water, freely soluble in methanol, slightly soluble in ethanol (95), and very slightly soluble in tetrahydrofuran.

It is hygroscopic.

Identification **(1)** Determine the absorption spectrum of a solution of Cefmetazole Sodium (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefmetazole Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefmetazole Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A, B and C, at around δ 3.6 ppm, at around δ 4.1 ppm and at around δ 5.2 ppm, respectively. The ratio of integrated intensity of each signal, A:B:C, is about 3:3:1.

(4) Cefmetazole Sodium responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +73 – +85° (0.25 g, water, 25 mL, 100 mm).

pH <*2.54*> Dissolve 1.0 g of Cefmetazole Sodium in 10 mL of water: the pH of the solution is between 4.2 and 6.2.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Cefmetazole Sodium in 10 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To a mixture of exactly 0.5 mL of Cobalt (II) Chloride CS and exactly 5 mL of Iron (III) Chloride CS add water to make exactly 50 mL. To exactly 15 mL of this solution add water to make exactly 20 mL.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Cefmetazole Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Cefmetazole Sodium according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.50 g of Cefmetazole Sodium in 10 mL of water, and use this solution as the sample solution. Pipet 4 mL, 2 mL, 1 mL, 0.5 mL and 0.25 mL of the sample solution, add water to them to make exactly 100 mL, and use these solutions as the standard solutions (1), (2), (3), (4) and (5), respectively. Separately, dissolve 0.10 g of 1-methyl-1*H*-tetrazole-5-thiol in water to make exactly 100 mL, and use this solution as the standard solution (6). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 1 μL each of the sample solution and standard solutions (1) to (6) on a plate of silica gel for thin-layer chromatography. Develop with a mixture of 1-butanol, water and acetic acid (100) (4:1:1) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the spot obtained from the sample solution corresponding to the spot obtained from the standard solution (6) is not more intense than the spot obtained from the standard solution (6), and the spots other than this spot and other than the principal spot are not more intense than the spot from the standard solution (1). Furthermore, the total amount of the spots other than the principal spot from the sample solution, calculated by the comparison with the spots from the standard solutions (1), (2), (3), (4) and (5), is not more than 8.0%.

Water <*2.48*> Not more than 1.0% (1 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefmetazole Sodium and Cefmetazole RS, equivalent to about 50 mg (potency), and dissolve each in the mobile phase to make exactly 25 mL. Pipet 1 mL each of these solutions, add exactly 10 mL of the internal standard solution, and use these solutions

as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefmetazole to that of the internal standard.

Amount [μg (potency)] of cefmetazole ($C_{15}H_{17}N_7O_5S_3$)
$= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Cefmetazole RS taken

Internal standard solution—A solution of methyl parahydroxybenzoate in the mobile phase (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.75 g of ammonium dihydrogenphosphate in 700 mL of water, add 280 mL of methanol, 20 mL of tetrahydrofuran and 3.2 mL of 40% tetrabutylammonium hydroxide TS, and adjust to pH 4.5 with phosphoric acid.

Flow rate: Adjust so that the retention time of cefmetazole is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefmetazole and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefmetazole to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Cefmetazole Sodium for Injection

注射用セフメタゾールナトリウム

Cefmetazole Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled potency of cefmetazole ($C_{15}H_{17}N_7O_5S_3$: 471.53).

Method of preparation Prepare as directed under Injections, with Cefmetazole Sodium.

Description Cefmetazole Sodium for Injection is a white to light yellow powder or masses.

It is hygroscopic.

Identification (1) Determine the absorption spectrum of a solution of Cefmetazole Sodium for Injection (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefmetazole Sodium for Injection as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <*2.54*> Take an amount of Cefmetazole Sodium for Injection equivalent to 1.0 g (potency) of Cefmetazole Sodium, and dissolve in 10 mL of water: the pH of the solution is 4.2 to 6.2.

Purity (1) Clarity and color of solution—Dissolve an amount of Cefmetazole Sodium for Injection, equivalent to 1.0 g (potency) of Cefmetazole Sodium, in 10 mL of water: the solution is clear and the color is not darker than the following control solution.

Control solution: Pipet 5 mL of Iron (III) Chloride CS and 0.5 mL of Cobalt (II) Chloride CS, and add water to make exactly 50 mL. Pipet 15 mL of this solution, and add water to make exactly 20 mL.

(2) Related substances—Proceed as directed in the Purity (4) under Cefmetazole Sodium.

Bacterial endotoxins <*4.01*> Less than 0.06 EU/mg (potency).

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test.

Foreign particulate matter <*6.06*> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Take 10 containers of Cefmetazole Sodium for Injection, dissolve the contents of each in the mobile phase, rinse each of the containers with the mobile phase, combine the rinse with the respective previous solution, and add the mobile phase to make exactly 500 mL. Take exactly a volume of this solution equivalent to about 0.2 g (potency) of Cefmetazole Sodium, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefmetazole RS, equivalent to about 50 mg (potency), and dissolve in the mobile phase to make exactly 25 mL. Pipet 1 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefmetazole Sodium.

Amount [mg (potency)] of cefmetazole ($C_{15}H_{17}N_7O_5S_3$)
$= M_S \times Q_T/Q_S \times 4$

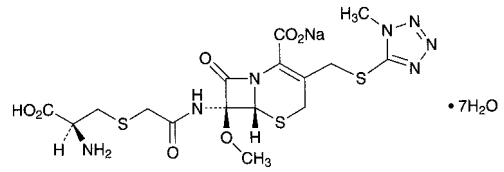
M_S: Amount [mg (potency)] of Cefmetazole RS taken

Internal standard solution—A solution of methyl parahydroxybenzoate in the mobile phase (1 in 10,000).

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Cefminox Sodium Hydrate

セフミノクスナトリウム水和物



$C_{16}H_{20}N_7NaO_7S_3.7H_2O$: 667.66
Monosodium (6*R*,7*S*)-7-{2-[(2*S*)-2-amino-2-carboxyethylsulfanyl]acetylamino}-7-methoxy-3-(1-methyl-1*H*-tetrazol-5-ylsulfanylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate heptahydrate
[*75498-96-3*]

Cefminox Sodium Hydrate contains not less than 900 μg (potency) and not more than 970 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefminox Sodium Hydrate is expressed as mass (potency) of cefminox ($C_{16}H_{21}N_7O_7S_3$: 519.58).

Description Cefminox Sodium Hydrate occurs as a white to light yellow crystalline powder.

It is freely soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (99.5).

Identification **(1)** Determine the absorption spectrum of a solution of Cefminox Sodium Hydrate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefminox Sodium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefminox Sodium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefminox Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefminox Sodium Hydrate in heavy water for nuclear magnetic resonance spectroscopy (1 in 30) as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a multiple signal, A, at around δ 3.2 ppm, a single signal, B, at around δ 3.5 ppm, a single signal, C, at around δ 4.0 ppm, and a single signal, D, at around δ 5.1 ppm. The ratio of integrated intensity of each signal, A:B:C:D, is about 2:3:3:1.

(4) Cefminox Sodium Hydrate responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +62 – +72° (50 mg, water, 10 mL, 100 mm).

pH <*2.54*> Dissolve 0.70 g of Cefminox Sodium Hydrate in 10 mL of water: the pH of the solution is between 4.5 and 6.0.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Cefminox Sodium Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 2.0 g of Cefminox Sodium Hydrate according to Method 3, and perform the test (not more than 1 ppm).

Water <*2.48*> Not less than 18.0% and not more than 20.0% (0.1 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism—*Escherichia coli* NIHJ

(ii) Culture medium—Use the medium iii in 3) under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 6.5 to 6.6 after sterilization.

(iii) Standard solution—Weigh accurately an amount of Cefminox Sodium RS, equivalent to about 40 mg (potency), dissolve in 0.05 mol/L phosphate buffer solution (pH 7.0) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.05 mol/L phosphate buffer solution (pH 7.0) to make solutions so that each mL contains 40 μg (potency) and 20 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

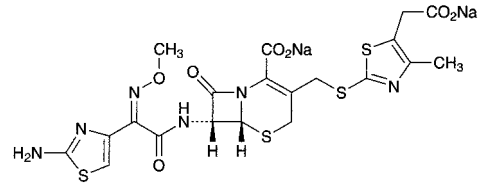
(iv) Sample solution—Weigh accurately an amount of Cefminox Sodium Hydrate equivalent to about 40 mg (potency), dissolve in 0.05 mol/L phosphate buffer solution (pH 7.0) to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.05 mol/L phosphate buffer solution (pH 7.0) to make solutions so that each mL contains 40 μg (potency) and 20 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

(v) Procedure—Incubate between 32°C and 35°C.

Containers and storage Containers—Hermetic containers.

Cefodizime Sodium

セフォジジムナトリウム



$C_{20}H_{18}N_6Na_2O_7S_4$: 628.63
Disodium (6*R*,7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetylamino]-3-[(5-carboxylatomethyl-4-methylthiazol-2-yl)sulfanylmethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate
[*86329-79-5*]

Cefodizime Sodium contains not less than 890 μg (potency) per mg, calculated on the anhydrous and ethanol-free basis. The potency of Cefodizime Sodium is expressed as mass (potency) of cefodizime ($C_{20}H_{20}N_6O_7S_4$: 584.67).

Description Cefodizime Sodium occurs as a white to light yellowish white crystalline powder.

It is very soluble in water, and practically insoluble in acetonitrile and in ethanol (99.5).

Identification **(1)** Determine the absorption spectrum of a

solution of Cefodizime Sodium (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefodizime Sodium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefodizime Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Cefodizime Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefodizime Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy ⟨*2.21*⟩, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A, B and C, at around δ 2.3 ppm, at around δ 4.0 ppm, and at around δ 7.0 ppm. The ratio of the integrated intensity of these signals, A:B:C, is about 3:3:1.

(4) Cefodizime Sodium responds to the Qualitative Tests ⟨*1.09*⟩ (1) for sodium salt.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: -56 – $-62°$ (0.2 g calculated on the anhydrous and ethanol-free basis, water, 20 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 1.0 g of Cefodizime Sodium in 10 mL of water: the pH of the solution is between 5.5 and 7.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Cefodizime Sodium in 10 mL of water: the solution is clear and pale yellow to light yellow.

(2) Heavy metals ⟨*1.07*⟩—Weigh 1.0 g of Cefodizime Sodium in a crucible, cover loosely, and carbonize by gentle heating. After cooling, add 2 mL of sulfuric acid, heat gradually until the white fumes are no longer evolved, and ignite between 500°C and 600°C. Proceed according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Cefodizime Sodium according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 30 mg of Cefodizime Sodium in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peaks other than cefodizime from the sample solution is not larger than the peak area of cefodizime from the standard solution, and the total area of the peaks other than cefodizime from the sample solution is not larger than 3 times the peak area of cefodizime from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 4 times as long as the retention time of cefodizime, beginning after the solvent peak.

System suitability—

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of cefodizime obtained from 5 μL of this solution is equivalent to 7 to 13% of that obtained from 5 μL of the standard solution.

(5) Ethanol—Weigh accurately about 1 g of Cefodizime Sodium, and dissolve in water to make exactly 10 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 2 g of ethanol for gas chromatography, and add water to make exactly 1000 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ethanol to that of the internal standard: the amount of ethanol is not more than 2.0%.

$$\text{Amount (\%) of ethanol} = M_S/M_T \times Q_T/Q_S$$

M_S: Amount (g) of ethanol for gas chromatography taken
M_T: Amount (g) of Cefodizime Sodium taken

Internal standard solution—A solution of 1-propanol (1 in 400).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3.2 mm in inside diameter and 3 m in length, packed with tetrafluoroethylene polymer for gas chromatography (180 – 250 μm in particle diameter) coated in 15% with polyethylene glycol 20 M.

Column temperature: A constant temperature of about 100°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the retention time of ethanol is about 3 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ethanol and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ethanol to that of the internal standard is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 4.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefodizime Sodium and Cefodizime Sodium RS, equivalent to about 50 mg (potency), add exactly 10 mL of the internal standard solution to dissolve, add water to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefodizime to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of cefodizime } (C_{20}H_{20}N_6O_7S_4) = M_S \times Q_T/Q_S \times 1000$$

M_S: Amount [mg (potency)] of Cefodizime Sodium RS taken

Internal standard solution—A solution of anhydrous caffeine (3 in 400).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 0.80 g of potassium dihydrogen phosphate and 0.20 g of anhydrous disodium hydrogen phosphate in a suitable amount of water, and add 80 mL of acetonitrile and water to make 1000 mL.

Flow rate: Adjust so that the retention time of cefodizime is about 5 minutes.

System suitability—

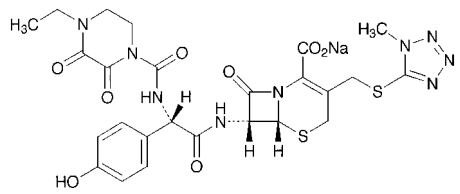
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefodizime and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefodizime to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Tight containers.

Cefoperazone Sodium

セフォペラゾンナトリウム



$C_{25}H_{26}N_9NaO_8S_2$: 667.65

Monosodium (6*R*,7*R*)-7-{(2*R*)-2-[(4-ethyl-2,3-dioxopiperazine-1-carbonyl)amino]-2-(4-hydroxyphenyl)acetylamino}-3-(1-methyl-1*H*-tetrazol-5-ylsulfanylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

[*62893-20-3*]

Cefoperazone Sodium contains not less than 871 μg (potency) and not more than 986 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefoperazone Sodium is expressed as mass (potency) of cefoperazone ($C_{25}H_{27}N_9O_8S_2$: 645.67).

Description Cefoperazone Sodium occurs as a white to yellowish white crystalline powder.

It is very soluble in water, soluble in methanol, and slightly soluble in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Cefoperazone Sodium (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the ^1H spectrum of a solution of Cefoperazone Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a triplet signal A at around δ 1.2 ppm, and a pair of double signals, B and C, at around δ 6.8 ppm and at around δ 7.3 ppm. The ratio of integrated intensity of these signals, A:B:C, is about 3:2:2.

(3) Cefoperazone Sodium responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −15 – −25° (1 g, water, 100 mL, 100 mm).

pH <*2.54*> Dissolve 1.0 g of Cefoperazone Sodium in 4 mL of water: the pH of the solution is between 4.5 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Cefoperazone Sodium in 10 mL of water: the solution is clear, and its absorbance at 400 nm, determined as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, is not more than 0.18.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Cefoperazone Sodium according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Cefoperazone Sodium according to Method 4, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.1 g of Cefoperazone Sodium in 100 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the areas of each peak by the automatic integration method. Calculate the percentages of each peak area from the sample solution to 50 times of the peak area of cefoperazone from the standard solution: the related substance I with the retention time of about 8 minutes is not more than 5.0%, the related substance II with that of about 17 minutes is not more than 1.5%, and the total of all related substances is not more than 7.0%. For the peak areas of the related substances I and II, multiply their relative response factors, 0.90 and 0.75, respectively.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of cefoperazone, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of cefoperazone obtained from 25 μL of this solution is equivalent to 3.5 to 6.5% of that obtained from 25 μL of the standard solution.

System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefoperazone are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times

with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefoperazone is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 1.0% (3 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefoperazone Sodium equivalent to about 0.1 g (potency), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefoperazone RS equivalent to about 20 mg (potency), dissolve in 1 mL of 0.1 mol/L phosphate buffer solution (pH 7.0) and add water to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefoperazone to that of the internal standard.

Amount [μg (potency)] of cefoperazone ($C_{25}H_{27}N_9O_8S_2$) $= M_S \times Q_T/Q_S \times 5000$

M_S: Amount [mg (potency)] of Cefoperazone RS taken

Internal standard solution—A solution of acetanilide in a mixture of water and acetonitrile (43:7) (3 in 8000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: To 57 mL of acetic acid (100) add 139 mL of triethylamine and water to make 1000 mL. To 20 mL of this solution add 835 mL of water, 140 mL of acetonitrile and 5 mL of dilute acetic acid.

Flow rate: Adjust so that the retention time of cefoperazone is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and cefoperazone are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefoperazone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Storage—In a cold place.

Cefoperazone Sodium and Sulbactam Sodium for Injection

注射用セフォペラゾンナトリウム·スルバクタムナトリウム

Cefoperazone Sodium and Sulbactam Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled potency of cefoperazone ($C_{25}H_{27}N_9O_8S_2$: 645.67), and not less than 95.0% and not more than 110.0% of the labeled potency of sulbactam ($C_8H_{11}NO_5S$: 233.24).

Method of Preparation Prepare as directed under Injections, with Cefoperazone Sodium and Sulbactam Sodium.

Description Cefoperazone Sodium and Sulbactam Sodium for Injection occurs as white to pale yellowish white, masses or powder.

Identification **(1)** The retention times of cefoperazone in the chromatogram obtained from the sample solution and the standard solution in the Assay are the same, and the peak area of cefoperazone obtained from the sample solution in the Assay is 0.8 to 1.1 times the peak area of cefoperazone obtained by the test performed with 10 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions.

Operating conditions—

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

(2) The retention times of sulbactam in the chromatogram obtained from the sample solution and the standard solution in the Assay are the same, and the peak area of sulbactam obtained from the sample solution in the Assay is 1.4 to 1.9 times the peak area of sulbactam obtained by the test performed with 10 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions.

Operating conditions—

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

pH ⟨*2.54*⟩ The pH of a solution prepared by dissolving an amount of Cefoperazone Sodium and Sulbactam Sodium for Injection, equivalent to 1.0 g (potency) of Cefoperazone Sodium, in 20 mL of water is 4.5 to 6.5.

Purity **(1)** Clarity and color of solution—A solution of an amount of Cefoperazone Sodium and Sulbactam Sodium for Injection, equivalent to 0.5 g (potency) of Cefoperazone Sodium, in 10 mL of water is clear. Perform the test with this

solution as directed under Ultraviolet Spectrophotometry ⟨2.24⟩: the absorbance at 425 nm is not more than 0.10.

(2) Related substances—Weigh accurately an amount of Cefoperazone Sodium and Sulbactam Sodium for Injection, equivalent to 0.1 g (potency) of Cefoperazone Sodium, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution (1). Weigh accurately about 40 mg of sulbactam sodium for sulbactam penicillamine, dissolve in 2 mL of water, add 0.5 mL of sodium hydroxide TS, allow to stand at room temperature for 10 minutes, then add 0.5 mL of 1 mol/L hydrochloric acid TS, and add the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution (2). Perform the test with exactly 10 μL each of the sample solution and the standard solutions (1) and (2) as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having a relative retention time of about 0.3 (related substance I) to cefoperazone, obtained from the sample solution is not larger than 1.75 times the peak area of cefoperazone obtained from the standard solution (1), the area of the peak, having a relative retention time of about 0.4 (related substance III) and about 1.3 (related substance II) to cefoperazone, obtained from the sample solution is not larger than 1/2 times the peak area of cefoperazone obtained from the standard solution (1). When determine the peak areas, A_T and A_S, of sulbactam penicillamine with the sample solution and the standard solution (2), and calculate the amount of sulbactam penicillamine by the following equation, it is not more than 1.0%. For the area of the peak of related substance III, multiply the relative response factor 0.4.

$$\text{Amount of sulbactam penicillamine (\%)} = M_S/M_T \times A_T/A_S \times 5$$

M_S: Amount (mg) of sulbactam sodium for sulbactam penicillamine taken
M_T: Amount (mg) of Cefoperazone Sodium and Sulbactam Sodium for Injection taken

Operating conditions—

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

System suitability—

System performance: To 1 mL of the standard solution (1) add 1 mL of the standard solution (2). When the procedure is run with 10 μL of this solution under the above operating conditions, sulbactam penicillamine, sulbactam and cefoperazone are eluted in this order with the resolutions between the peaks, sulbactam penicillamine and sulbactam, and sulbactam and cefoperazone, being not less than 4 and not less than 5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution (2) under the above operating conditions, the relative standard deviation of the peak area of sulbactam penicillamine is not more than 2.0%.

Water ⟨2.48⟩ Not more than 1.0% (1 g, volumetric titration, direct titration).

Bacterial endotoxins ⟨4.01⟩ Less than 0.060 EU/mg (potency).

Uniformity of dosage units ⟨6.02⟩ It meets the requirement of the Mass variation test (*T*: 105.0%).

Foreign insoluble matter ⟨6.06⟩ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter ⟨6.07⟩ It meets the requirement.

Sterility ⟨4.06⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the content of not less than 5 Cefoperazone Sodium and Sulbactam Sodium for Injection. Weigh accurately a portion of the content, equivalent to about 50 mg (potency) of Cefoperazone Sodium, dissolve in suitable amount of the mobile phase, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg (potency) each of Sulbactam RS and Cefoperazone RS, dissolve in suitable amount of the mobile phase, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions, and calculate the ratios, Q_{Ta} and Q_{Tb} of the peak areas of sulbactam and cefoperazone to that of the internal standard obtained from the sample solution, and the ratios, Q_{Sa} and Q_{Sb} of the peak areas of sulbactam and cefoperazone to that of the internal standard obtained from the standard solution.

$$\text{Amount [mg (potency)] of sulbactam } (C_8H_{11}NO_5S) = M_{S1} \times Q_{Ta}/Q_{Sa}$$

$$\text{Amount [mg (potency)] of cefoperazone } (C_{25}H_{27}N_9O_8S_2) = M_{S2} \times Q_{Tb}/Q_{Sb}$$

M_{S1}: Amount [mg (potency)] of Sulbactam RS taken
M_{S2}: Amount [mg (potency)] of Cefoperazone RS taken

Internal standard solution—A solution of ethyl parahydroxybenzoate (7 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of 0.005 mol/L tetrabutylammonium hydroxide TS and acetonitrile for liquid chromatography (3:1).

Flow rate: Adjust so that the retention time of sulbactam is about 7 minutes.

System suitability—

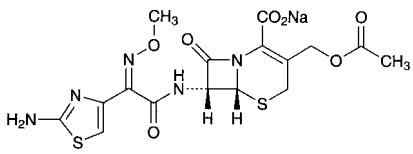
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, sulbactam, the internal standard, and cefoperazone are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak of sulbactam is not more than 1.0%.

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Cefotaxime Sodium

セフォタキシムナトリウム



$C_{16}H_{16}N_5NaO_7S_2$: 477.45
Monosodium (6*R*,7*R*)-3-acetoxymethyl-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetylamino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate
[*64485-93-4*]

Cefotaxime Sodium contains not less than 916 μg (potency) and not more than 978 μg (potency) per mg, calculated on the dried basis. The potency of Cefotaxime Sodium is expressed as mass (potency) of cefotaxime ($C_{16}H_{17}N_5O_7S_2$: 455.47).

Description Cefotaxime Sodium occurs as white to light yellowish white crystalline powder.

It is freely soluble in water, sparingly soluble in methanol, and very slightly soluble in ethanol (95).

Identification **(1)** Dissolve 2 mg of Cefotaxime Sodium in 0.01 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefotaxime Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefotaxime Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 125) as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits three single signals, A, B and C, at around δ 2.1 ppm, at around δ 4.0 ppm and at around δ 7.0 ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 3:3:1.

(4) Cefotaxime Sodium responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +58 – +64° (0.25 g calculated on the dried basis, water, 25 mL, 100 mm).

pH <*2.54*> The pH of a solution obtained by dissolving 1.0 g of Cefotaxime Sodium in 10 mL of water is between 4.5 and 6.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Cefotaxime Sodium in 10 mL of water: the solution is clear, and its absorbance at 430 nm, determined as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, is not more than 0.40.

(2) Sulfate <*1.14*>—Dissolve 2.0 g of Cefotaxime Sodium in 40 mL of water, add 2 mL of dilute hydrochloric acid and water to make 50 mL, shake well, and filter. Discard first 10 mL of the filtrate, and to the subsequent 25 mL of the filtrate add water to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution as follows: To 1.0 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Cefotaxime Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Cefotaxime Sodium according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Perform the test with 10 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area obtained from the chromatogram by the automatic integration method, and calculated the amounts of them by the area percentage method: the amount of the peak other than cefotaxime is not more than 1.0% and the total amount of these peaks is not more than 3.0%.

Operating conditions—

Detector, column, column temperature, mobile phase A, mobile phase B, flowing of mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3.5 times as long as the retention time of cefotaxime, beginning after the solvent peak.

System suitability—

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase A to make exactly 100 mL. Pipet 2 mL of this solution, and add the mobile phase A to make exactly 20 mL. Confirm that the peak area of cefotaxime obtained from 10 μL of this solution is equivalent to 0.15 to 0.25% of that obtained from 10 μL of the standard solution.

Loss on drying <*2.41*> Not more than 3.0% (1 g, 105°C, 3 hours).

Assay Weigh accurately an amount of Cefotaxime Sodium and Cefotaxime RS, equivalent to about 40 mg (potency), dissolve each in the mobile phase A to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of cefotaxime in each solution.

$$\text{Amount [}\mu\text{g (potency)] of cefotaxime } (C_{16}H_{17}N_5O_7S_2) = M_S \times A_T/A_S \times 1000$$

M_S: Amount [mg (potency)] of Cefotaxime RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase A: To 0.05 mol/L disodium hydrogen phosphate TS add phosphoric acid to adjust the pH to 6.25. To 860 mL of this solution add 140 mL of methanol.

Mobile phase B: To 0.05 mol/L disodium hydrogen phosphate TS add phosphoric acid to adjust the pH to 6.25. To

600 mL of this solution add 400 mL of methanol.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 7	100	0
7 – 9	100 → 80	0 → 20
9 – 16	80	20
16 – 45	80 → 0	20 → 100
45 – 50	0	100

Flow rate: 1.3 mL per minute (the retention time of cefotaxime is about 14 minutes).

System suitability—

System performance: To 1 mL of the standard solution add 7.0 mL of water and 2.0 mL of methanol, mix, then add 25 mg of sodium carbonate decahydrate, and shake. After allowing to stand for 10 minutes, add 3 drops of acetic acid (100) and 1 mL of the standard solution, and mix. When the procedure is run with 10 μL of this solution under the above operating conditions, desacetyl cefotaxime with the relative retention time being about 0.3 to cefotaxime and cefotaxime are eluted in this order with the resolution between these peaks being not less than 20, and the symmetry factor of the peak of cefotaxime is not more than 2.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefotaxime is not more than 2.0%.

Containers and storage Containers—Tight containers.

Cefotetan

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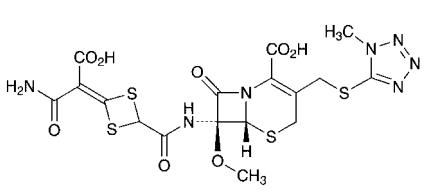

$C_{17}H_{17}N_7O_8S_4$: 575.62

(6*R*,7*R*)-7-{[4-(Carbamoylcarboxymethylidene)-1,3-dithietane-2-carbonyl]amino}-7-methoxy-3-(1-methyl-1*H*-tetrazol-5-ylsulfanylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

[*69712-56-7*]

Cefotetan contains not less than 960 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefotetan is expressed as mass (potency) of cefotetan ($C_{17}H_{17}N_7O_8S_4$).

Description Cefotetan occurs as white to light yellowish white powder.

It is sparingly soluble in methanol, and slightly soluble in water and in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Cefotetan in phosphate buffer solution for antibiotics, pH 6.5 (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefotetan RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefotetan as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefotetan RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 50 mg of Cefotetan in 0.5 mL of a solution of sodium hydrogen carbonate in heavy water for nuclear magnetic resonance spectroscopy (1 in 25). Determine the ^{1}H spectrum of this solution as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A, B, C and D, at around δ 3.6 ppm, at around δ 4.0 ppm, at around δ 5.1 ppm and at around δ 5.2 ppm, respectively. The ratio of the integrated intensity of each signal, A:B:C:D, is about 3:3:1:1.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +112 – +124° (0.5 g calculated on the anhydrous basis, a solution of sodium hydrogen carbonate (1 in 200), 50 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Cefotetan in 10 mL of a solution of sodium hydrogen carbonate (1 in 30): the solution is clear, and colorless or light yellow.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Cefotetan according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Weigh accurately about 0.1 g of Cefotetan, dissolve in a suitable amount of methanol, add exactly 2 mL of the internal standard solution and methanol to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 3 mg of 1-methyl-1*H*-tetrazole-5-thiol for liquid chromatography, previously dried in a desiccator (in vacuum, silica gel) for 2 hours, and about 2 mg of Cefotetan RS, calculated on the anhydrous basis, dissolve in methanol to make exactly 20 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution and methanol to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μL of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_{Ta}, Q_{Tb}, Q_{Tc}, Q_{Td}, Q_{Te} and Q_{Tf}, of the peak areas of 1-methyl-1*H*-tetrazole-5-thiol, cefotetan lactone having the relative retention time of about 0.5 to cefotetan, Δ_2-cefotetan having the relative retention time of about 1.2, isothiazole substance having the relative retention time of about 1.3, each of other related substances and the total of other related substances, to the peak area of the internal standard, respectively, obtained from the sample solution, and the ratios, Q_{Sa} and Q_{Sb}, of the peak areas of 1-methyl-1*H*-tetrazole-5-thiol and cefotetan, to the peak area of the internal standard, respectively, obtained from the standard solution. Calculate the amount of 1-methyl-1*H*-tetrazole-5-thiol, cefotetan lactone, Δ_2-cefotetan, isothiazole substance, each of other related substances and the total of other related substances from the following equations: the amount of 1-methyl-1*H*-tetrazole-5-thiol is not more than 0.3%, cefotetan lactone is not more than 0.3%, Δ_2-cefotetan is not more than 0.5%, isothiazole substance is not more than 0.5%, each of other related substances is not more than 0.2% and the total of other related substances is not more

than 0.4%.

1-Methyl-1*H*-tetrazole-5-thiol (%)
$= M_{Sa}/M_T \times Q_{Ta}/Q_{Sa} \times 1/100$

Cefotetan lactone (%)
$= M_{Sb}/M_T \times Q_{Tb}/Q_{Sb} \times 1/100$

Δ_2-Cefotetan (%)
$= M_{Sb}/M_T \times Q_{Tc}/Q_{Sb} \times 1/100$

Isothiazole substance (%)
$= M_{Sb}/M_T \times Q_{Td}/Q_{Sb} \times 1/100$

Each of other related substances (%)
$= M_{Sb}/M_T \times Q_{Te}/Q_{Sb} \times 1/100$

Total of other related substances (%)
$= M_{Sb}/M_T \times Q_{Tf}/Q_{Sb} \times 1/100$

M_{Sa}: Amount (mg) of 1-methyl-1*H*-tetrazole-5-thiol taken
M_{Sb}: Amount (mg) of Cefotetan RS, calculated on the anhydrous basis taken
M_T: Amount (g) of Cefotetan taken

Internal standard solution—A solution of anhydrous caffeine in methanol (3 in 10,000).
Operating conditions—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.
Time span of measurement: About 3.5 times as long as the retention time of cefotetan.
System suitability—
System performance: Proceed as directed in the system suitability in the Assay.
Test for required detectability: Measure exactly 15 mL of the standard solution, and add methanol to make exactly 100 mL. Confirm that the peak area of cefotetan obtained from 5 μL of this solution is equivalent to 12 to 18% of that obtained from 5 μL of the standard solution.
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefotetan to that of the internal standard is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 2.5% (1 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Isomer ratio Dissolve 10 mg of Cefotetan in 20 mL of methanol, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the adjacent two peaks appeared at around the retention time of 40 minutes, one having shorter retention time is *l*-substance and another having longer retention time is *d*-substance, by the area percentage method: the amount of *l*-substance is not less than 35% and not more than 45%.
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of 0.1 mol/L phosphate buffer solution (pH 7.0), water and a solution of tetrabutylammonium hydrogensulfate in acetonitrile (1 in 150) (9:9:2).
Flow rate: Adjust so that the retention time of *l*-substance is about 40 minutes.
System suitability—
System performance: When the procedure is run with 5 μL of the sample solution under the above operating conditions, *l*-substance and *d*-substance are eluted in this order with the resolution between these peaks being not less than 1.5.
System repeatability: To exactly 1 mL of the sample solution add methanol to make exactly 10 mL. When the test is repeated 6 times with 5 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of *l*-substance is not more than 5.0%.

Assay Weigh accurately an amount of Cefotetan and Cefotetan RS, equivalent to about 50 mg (potency), and dissolve each in phosphate buffer solution for antibiotics, pH 6.5 to make exactly 50 mL. Pipet 15 mL each of these solutions, add exactly 10 mL of the internal standard solution and phosphate buffer solution for antibiotics, pH 6.5 to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefotetan to that of the internal standard.

Amount [μg (potency)] of cefotetan ($C_{17}H_{17}N_7O_8S_4$)
$= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Cefotetan RS taken

Internal standard solution—A solution of anhydrous caffeine (1 in 1000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 11.53 g of phosphoric acid in 1000 mL of water. To 850 mL of this solution add 50 mL of acetonitrile, 50 mL of acetic acid (100) and 50 mL of methanol.
Flow rate: Adjust so that the retention time of cefotetan is about 17 minutes.
System suitability—
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard and cefotetan are eluted in this order with the resolution between these peaks being not less than 8.
System repeatability: When the test is repeated 5 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefotetan to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant, and at a temperature not exceeding 5°C.

Cefotiam Hexetil Hydrochloride

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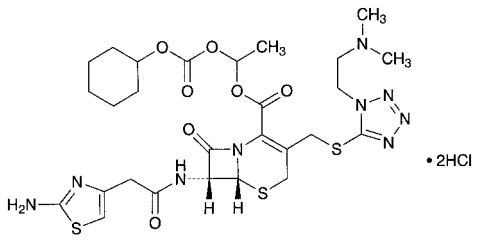


$C_{27}H_{37}N_9O_7S_3.2HCl$：768.76

(1*RS*)-1-Cyclohexyloxycarbonyloxyethyl (6*R*,7*R*)-7-[2-(2-aminothiazol-4-yl)acetylamino]-3-[1-(2-dimethylaminoethyl)-1*H*-tetrazol-5-ylsulfanylmethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate dihydrochloride

[*95789-30-3*]

Cefotiam Hexetil Hydrochloride contains not less than 615 μg (potency) and not more than 690 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefotiam Hexetil Hydrochloride is expressed as mass (potency) of cefotiam ($C_{18}H_{23}N_9O_4S_3$: 525.63).

Description Cefotiam Hexetil Hydrochloride occurs as a white to light yellow powder.

It is very soluble in water, in methanol and in ethanol (95), freely soluble in dimethylsulfoxide, and slightly soluble in acetonitrile.

It dissolves in 0.1 mol/L hydrochloric acid TS.

It is hygroscopic.

Identification **(1)** Determine the absorption spectrum of a solution of Cefotiam Hexetil Hydrochloride in 0.1 mol/L hydrochloric acid TS (3 in 125,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefotiam Hexetil Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the ^1H spectrum of a solution of Cefotiam Hexetil Hydrochloride in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 20) as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits two single signals, A and B, at around δ 2.8 ppm and at around δ 6.6 ppm, and a multiple signal, C, at around δ 6.9 ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 6:1:1.

(3) To a solution of Cefotiam Hexetil Hydrochloride (1 in 200) add 2 mL of dilute nitric acid and 1 mL of silver nitrate TS, and mix: a white precipitate is formed.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +52 – +60° (0.1 g calculated on the anhydrous basis, 0.1 mol/L hydrochloric acid TS, 10 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Cefotiam Hexetil Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 2.0 g of Cefotiam Hexetil Hydrochloride according to Method 3, and perform the test, using a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 5) (not more than 1 ppm).

(3) Related substance 1—Weigh accurately about 50 mg of Cefotiam Hexetil Hydrochloride, and dissolve in a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly 50 mL. Pipet 10 mL of this solution, add a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Cefotiam Hexetil Hydrochloride RS, and dissolve in a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly 50 mL. Pipet 1 mL of this solution, add a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the related substances by the following equation: the amount of the related substance having the relative retention time of about 1.2 to one of the peaks of cefotiam hexetil, which has the larger retention time, is not more than 2.0%, and each amount of the other related substances is not more than 0.5%. For the peak area, having the relative retention time of about 1.2 to one of the peaks of cefotiam hexetil, which has the larger retention time, multiply the relative response factor, 0.78.

$$\text{Amount (\%) of related substance} = M_S/M_T \times A_T/A_S \times 5$$

M_S: Amount (g) of Cefotiam Hexetil Hydrochloride RS taken

M_T: Amount (g) of Cefotiam Hexetil Hydrochloride taken

A_S: Total of two peak areas of cefotiam hexetil from the standard solution

A_T: Each peak area of related substance from the sample solution

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of diluted 0.2 mol/L potassium dihydrogen phosphate TS (1 in 2), acetonitrile and acetic acid (100) (72:28:1).

Mobile phase B: A mixture of acetonitrile, diluted 0.2 mol/L potassium dihydrogen phosphate TS (1 in 2) and acetic acid (100) (60:40:1).

Flowing of mobile phase: Adjust so that the mixing rate of the mobile phase A and the mobile phase B is changed linearly from 1:0 to 0:1 for 30 minutes.

Flow rate: 0.7 mL per minute.

Time span of measurement: As long as about 3 times of the retention time of one of the cefotiam hexetil peaks, which appears first, beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly 50 mL. Confirm that each area of the two peaks of cefotiam hexetil obtained from 10 μL of this solution is equivalent to 1.6 to 2.4% of that obtained from 10 μL of the standard so-

lution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the resolution between the two peaks of cefotiam hexetil is not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the total of the two peak areas of cefotiam hexetil is not more than 2.0%.

(4) Related substance 2—Weigh accurately about 20 mg of Cefotiam Hexetil Hydrochloride, dissolve in 2 mL of methanol, add a mixture of a solution of diammonium hydrogen phosphate (79 in 20,000) and acetic acid (100) (200:3) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Cefotiam Hydrochloride RS, and dissolve in the mobile phase to make exactly 50 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the related substances by the following equation: the amounts of the related substances having the relative retention time of about 0.1 and about 0.9 to cefotiam are not more than 1.0%, respectively, and each amount of the related substances other than the related substances having the relative retention time of about 0.1 and about 0.9 to cefotiam is not more than 0.5%. For the peak area, having the relative retention time of about 0.9 to cefotiam, multiply the relative response factor, 0.76.

$$\text{Amount (\%) of related substance} = M_S/M_T \times A_T/A_S \times 4$$

M_S: Amount (g) of Cefotiam Hydrochloride RS taken
M_T: Amount (g) of Cefotiam Hexetil Hydrochloride taken
A_S: Peak area of cefotiam from the standard solution
A_T: Each peak area from the sample solution

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of diammonium hydrogen phosphate (79 in 20,000), methanol and acetic acid (100) (200:10:3).

Flow rate: Adjust so that the retention time of cefotiam is about 15 minutes.

Time span of measurement: As long as about 2 times of the retention time of cefotiam, beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of cefotiam obtained from 10 μL of this solution is equivalent to 1.6 to 2.4% of that obtained from 10 μL of the standard solution.

System performance: To 1 mL of a solution of acetaminophen in the mobile phase (1 in 50,000) add 3 mL of the standard solution, and mix well. When the procedure is run with 10 μL of this solution under the above operating conditions, acetaminophen and cefotiam are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefotiam is not more than 2.0%.

(5) Total amount of related substances—The total of the amount of related substances obtained in the Related substance 1 and the Related substance 2 is not more than 6.5%.

Water <2.48> Not more than 3.5% (0.1 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Isomer ratio Proceed the test with 20 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography <2.01> according to the conditions directed in the Assay, and determine the areas of the two peaks, A_a for the faster peak and A_b for the later peak, closely appeared each other at the retention time of around 10 minutes: $A_a/(A_a + A_b)$ is not less than 0.45 and not more than 0.55.

Assay Weigh accurately an amount of Cefotiam Hexetil Hydrochloride and Cefotiam Hexetil Hydrochloride RS, equivalent to about 30 mg (potency), and dissolve each in a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly 50 mL. Measure exactly 5 mL each of these solutions, add exactly 5 mL of the internal standard solution and a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefotiam hexetil to that of the internal standard. For this calculation, the total of the areas of the two peaks appeared closely each other at the retention time of around 10 minutes is used as the peak area of cefotiam hexetil.

$$\text{Amount [μg (potency)] of cefotiam } (C_{18}H_{23}N_9O_4S_3) = M_S \times Q_T/Q_S \times 1000$$

M_S: Amount [mg (potency)] of Cefotiam Hexetil Hydrochloride RS taken

Internal standard solution—A solution of benzoic acid in a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) (7 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of diluted 0.2 mol/L potassium dihydrogen phosphate TS (1 in 2), acetonitrile and acetic acid (100) (72:28:1).

Flow rate: Adjust so that the retention time of the faster peak of cefotiam hexetil is about 9 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and cefotiam hexetil are eluted in this order with the resolution between the two peaks of cefotiam hexetil being not less than 2.0.

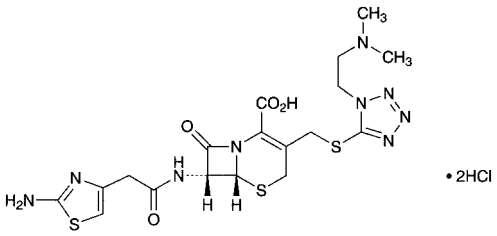
System repeatability: When the test is repeated 6 times

with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefotiam hexetil to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cefotiam Hydrochloride

セフォチアム塩酸塩



$C_{18}H_{23}N_9O_4S_3.2HCl$: 598.55
(6*R*,7*R*)-7-[2-(2-Aminothiazol-4-yl)acetylamino]-3-[1-(2-dimethylaminoethyl)-1*H*-tetrazol-5-ylsulfanylmethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid dihydrochloride
[*66309-69-1*]

Cefotiam Hydrochloride contains not less than 810 μg (potency) and not more than 890 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefotiam Hydrochloride is expressed as mass (potency) of cefotiam ($C_{18}H_{23}N_9O_4S_3$: 525.63).

Description Cefotiam Hydrochloride occurs as white to light yellow, crystals or crystalline powder.

It is freely soluble in water, in methanol and in formamide, slightly soluble in ethanol (95), and practically insoluble in acetonitrite.

Identification (1) Determine the absorption spectrum of a solution of Cefotiam Hydrochloride (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefotiam Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefotiam Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefotiam Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefotiam Hydrochloride in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A and B, at around δ 3.1 ppm and at around δ 6.7 ppm, respectively. The ratio of integrated intensity of each signal, A:B, is about 6:1.

(4) Dissolve 0.1 g of Cefotiam Hydrochloride in 5 mL of dilute nitric acid, and immediately add 1 mL of silver nitrate TS: a white precipitate is formed.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +60 – +72° (1 g calculated on the anhydrous bases, water, 100 mL, 100 mm).

pH <*2.54*> Dissolve 1.0 g of Cefotiam Hydrochloride in 10 mL of water: the pH of the solution is between 1.2 and 1.7.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Cefotiam Hydrochloride in 10 mL of water: the solution is clear, and colorless to yellow.

(2) Heavy metals <*1.07*>—To 1.0 g of Cefotiam Hydrochloride add 1 mL of sulfuric acid, and heat gently to carbonize. After cooling, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), fire the ethanol to burn, then heat gradually to incinerate. If a carbonized residue still retains, moisten the residue with a little amount of sulfuric acid, and ignite again to incinerate. After cooling, add 2 mL of hydrochloric acid to the residue, heat on a water bath to dissolve, then heat to dryness. Add 10 mL of water, and heat to dissolve. After cooling, add ammonia TS dropwise to adjust to pH 3 – 4, if necessary, filter, wash the residue on the filter with 10 mL of water, transfer the filtrate and washings into a Nessler tube, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution in the same manner as for preparation of the test solution (not more than 20 ppm).

(3) Arsenic <*1.11*>—Incinerate 1.0 g of Cefotiam Hydrochloride according to Method 4. After cooling, add 10 mL of dilute hydrochloric acid to the residue, heat to dissolve on the water bath, and use this solution as the test solution. Perform the test (not more than 2 ppm).

Water <*2.48*> Not more than 7.0% (0.25 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

Assay Weigh accurately an amount of Cefotiam Hydrochloride and Cefotiam Hydrochloride RS, equivalent to about 0.1 g (potency), and dissolve each in the mobile phase to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of cefotiam in each solution.

Amount [μg (potency)] of cefotiam ($C_{18}H_{23}N_9O_4S_3$) $= M_S \times A_T/A_S \times 1000$

M_S: Amount [mg (potency)] of Cefotiam Hydrochloride RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 125 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 800 mL of 0.05 mol/L disodium hydrogenphosphate TS add 0.05 mol/L potassium dihydrogenphosphate TS to adjust the pH to 7.7. To 440 mL of this solution add 60 mL of acetonitrile.

Flow rate: Adjust so that the retention time of cefotiam is about 14 minutes.

System suitability—

System performance: Dissolve 0.04 g of orcine in 10 mL of the standard solution. When the procedure is run with 10 μL

of the standard solution under the above operating conditions, orcine and cefotiam are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefotiam is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Cefotiam Hydrochloride for Injection

注射用セフォチアム塩酸塩

Cefotiam Hydrochloride for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled potency of cefotiam ($C_{18}H_{23}N_9O_4S_3$: 525.63).

Method of Preparation Prepare as directed under Injection, with Cefotiam Hydrochloride.

Description Cefotiam Hydrochloride for Injection occurs as a white to light yellow powder.

Identification (1) Determine the absorption spectrum of a solution of Cefotiam Hydrochloride for Injection (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 257 nm and 261 nm.

(2) Determine the ^{1}H spectrum of a solution of Cefotiam Hydrochloride for Injection in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A between δ 2.7 ppm and δ 3.0 ppm, and a single signal B at around δ 6.5 ppm. The ratio of the integrated intensity of each signal, A:B, is about 6:1.

pH <*2.54*> The pH of a solution prepared by dissolving an amount of Cefotiam Hydrochloride for Injection, equivalent to 0.5 g (potency), in 5 mL of water is between 5.7 and 7.2.

Purity Clarity and color of solution—Dissolve an amount of Cefotiam Hydrochloride for Injection, equivalent to 1.0 g (potency) of Cefotiam Hydrochloride, in 10 mL of water: the solution is clear, and the absorbance of this solution, determined at 450 nm 10 minutes after dissolving as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, is not more than 0.20.

Loss on drying <*2.41*> Not more than 6.0% (0.5 g, in vacuum, 60°C, 3 hours).

Bacterial endotoxins <*4.01*> Less than 0.125 EU/mg (potency).

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test.

Foreign insoluble matter <*6.06*> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the contents of not less than 10 Cefotiam Hydrochloride for Injection. Weigh accurately an amount of the content, equivalent to about 50 mg (potency) of Cefotiam Hydrochloride, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg (potency) of Cefotiam Hydrochloride RS, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefotiam Hydrochloride.

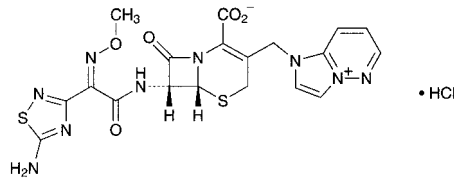
Amount [μg (potency)] of cefotiam ($C_{18}H_{23}N_9O_4S_3$) = $M_S \times A_T/A_S \times 1000$

M_S: Amount [mg (potency)] of Cefotiam Hydrochloride RS taken

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Cefozopran Hydrochloride

セフォゾプラン塩酸塩



$C_{19}H_{17}N_9O_5S_2$.HCl: 551.99

(6*R*,7*R*)-7-[(*Z*)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(methoxyimino)acetylamino]-3-(1*H*-imidazo[1,2-*b*]pyridazin-4-ium-1-ylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate monohydrochloride

[*113359-04-9*, Cefozopran]

Cefozopran Hydrochloride contains not less than 860 μg (potency) and not more than 960 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefozopran Hydrochloride is expressed as mass (potency) of cefozopran ($C_{19}H_{17}N_9O_5S_2$: 515.53).

Description Cefozopran Hydrochloride occurs as white to pale yellow, crystals or crystalline powder.

It is freely soluble in dimethylsulfoxide and in formamide, slightly soluble in water, in methanol and in ethanol (95), and practically insoluble in acetonitrile and diethyleter.

Identification (1) Dissolve 0.02 g of Cefozopran Hydrochloride in 10 mL of water, add 1 mL of a solution of hydroxylammonium chloride (1 in 10) and 2 mL of sodium hydroxide TS, allow to stand for 5 minutes, then add 3 mL of 1 mol/L hydrochloric acid TS and 3 drops of iron (III) chloride TS, and mix: a red-purple color develops.

(2) Determine the absorption spectra of solutions of Cefozopran Hydrochloride and Cefozopran Hydrochloride RS in a mixture of sodium chloride TS and methanol (3:2) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the ^{1}H spectrum of a solution of Cefozopran Hydrochloride in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 20) as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>, using tetramethylsilane for nuclear magnetic reso-

nance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ 3.9 ppm, a double signal B at around δ 5.2 ppm, and a quartet signal C at around δ 8.0 ppm, and the ratio of integrated intensity of each signal, A:B:C, is about 3:1:1.

(4) Dissolve 0.01 g of Cefozopran Hydrochloride in 1 mL of water and 2 mL of acetic acid (100), add 2 drops of silver nitrate TS, and mix: a white turbidity is formed.

Absorbance ⟨*2.24*⟩ $E^{1\%}_{1\,cm}$ (238 nm): 455 – 485 (50 mg calculated on the anhydrous basis, a mixture of sodium chloride TS and methanol (3:2), 5000 mL).

Optical rotation ⟨*2.49*⟩ $[\alpha]^{20}_{D}$: −73 – −78° (0.1 g calculated on the anhydrous basis, a mixture of sodium chloride TS and methanol (3:2), 10 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Being specified separately when the drug is granted approval based on the Law.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Cefozopran Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic—Being specified separately when the drug is granted approval based on the Law.

(4) Related substances—Being specified separately when the drug is granted approval based on the Law.

Water ⟨*2.48*⟩ Not more than 2.5% (0.5 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

Residue on ignition Being specified separately when the drug is granted approval based on the Law.

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.05 EU/mg (potency).

Assay Weigh accurately an amount of Cefozopran Hydrochloride and Cefozopran Hydrochloride RS, equivalent to about 50 mg (potency), and dissolve each in the mobile phase to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL of the internal standard solution and the mobile phase to make 25 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefozopran to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of cefozopran } (C_{19}H_{17}N_9O_5S_2) = M_S \times Q_T/Q_S \times 1000$$

M_S: Amount [mg (potency)] of Cefozopran Hydrochloride RS taken

Internal standard solution—A solution of 2,4-dihydroxybenzoic acid in the mobile phase (1 in 1250).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Mix 0.366 g of diethylamine with water to make 1000 mL, and add 60 mL of acetonitrile and 5 mL of acetic acid (100).

Flow rate: Adjust so that the retention time of cefozopran is about 9 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefozopran and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefozopran to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant.

Cefozopran Hydrochloride for Injection

注射用セフォゾプラン塩酸塩

Cefozopran Hydrochloride for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 115.0% of the labeled potency of cefozopran ($C_{19}H_{17}N_9O_5S_2$: 515.53).

Method of Preparation Prepare as directed under the Injections, with Cefozopran Hydrochloride.

Description Cefozopran Hydrochloride for Injection occurs as a white to light yellow, powder or masses.

Identification **(1)** Determine the absorption spectrum of a solution of Cefozopran Hydrochloride for Injection (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 236 nm and 241 nm.

(2) To 50 mg of Cefozopran Hydrochloride for Injection add 0.8 mL of deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy, and filter after shaking, and determine the ^{1}H spectrum of the filtrate as directed under Nuclear Magnetic Resonance Spectroscopy ⟨*2.21*⟩, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ 3.9 ppm, a double signal B at around δ 5.0 ppm, and a quartet signal C at around δ 8.0 ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 3:1:1.

pH ⟨*2.54*⟩ Dissolve an amount of Cefozopran Hydrochloride for Injection, equivalent to 0.5 g (potency) of Cefozopran Hydrochloride, in 5 mL of water: the pH of this solution is between 7.5 and 9.0.

Purity **(1)** Clarity and color of solution—Dissolve an amount of Cefozopran Hydrochloride for Injection, equivalent to 1 g (potency) of Cefozopran Hydrochloride, in 10 mL of water: the solution is clear and has no more color than Matching Fluid N.

(2) Related substances—Being specified separately when the drug is granted approval based on the Law.

Water ⟨*2.48*⟩ Not more than 2.5% (0.5 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.05 EU/mg (potency).

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Foreign insoluble matter ⟨*6.06*⟩ It meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 Cefozopran Hydrochloride for Injection. Weigh accurately an amount of the contents, equivalent to about 0.5 g (potency) of Cefozopran Hydrochloride, and add water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, add the mobile phase to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefozopran Hydrochloride RS, equivalent to about 50 mg (potency), and dissolve in the mobile phase to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add the mobile phase to make 25 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefozopran Hydrochloride.

Amount [mg (potency)] of cefozopran ($C_{19}H_{17}N_9O_5S_2$)
$= M_S \times Q_T/Q_S \times 10$

M_S: Amount [mg (potency)] of Cefozopran Hydrochloride RS taken

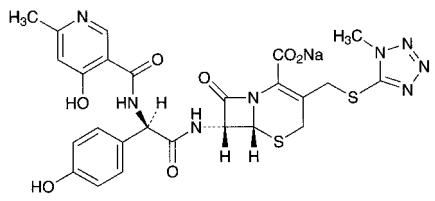
Internal standard solution—A solution 2,4-dihydroxybenzoic acid in the mobile phase (1 in 1250).

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Storage—Light-resistant.

Cefpiramide Sodium

セフピラミドナトリウム



$C_{25}H_{23}N_8NaO_7S_2$: 634.62

Monosodium (6*R*,7*R*)-7-{(2*R*)-2-[(4-hydroxy-6-methylpyridine-3-carbonyl)amino]-2-(4-hydroxyphenyl)acetylamino}-3-(1-methyl-1*H*-tetrazol-5-ylsulfanylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

[*74849-93-7*]

Cefpiramide Sodium contains not less than 900 μg (potency) and not more than 990 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefpiramide Sodium is expressed as mass (potency) of cefpiramide ($C_{25}H_{24}N_8O_7S_2$: 612.64).

Description Cefpiramide Sodium occurs as white to yellowish white powder.

It is very soluble in dimethylsulfoxide, freely soluble in water, sparingly soluble in methanol, and slightly soluble in ethanol (95).

Identification **(1)** Determine the absorption spectrum of a solution of Cefpiramide Sodium in 0.05 mol/L phosphate buffer solution (pH 7.0) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the ^1H spectrum of a solution of Cefpiramide Sodium in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy ⟨*2.21*⟩, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A, B and C, at around δ 2.3 ppm, at around δ 3.9 ppm and at around δ 8.2 ppm, respectively. The ratio of the integrated intensity of each signal, A:B:C, is about 3:3:1.

(3) Cefpiramide Sodium responds to the Qualitative Tests ⟨*1.09*⟩ (1) for sodium salt.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: -33 – $-40°$ (0.2 g calculated on the anhydrous basis, 0.05 mol/L phosphate buffer solution (pH 7.0), 10 mL, 100 mm).

pH ⟨*2.54*⟩ The pH of a solution obtained by dissolving 2.0 g of Cefpiramide Sodium in 20 mL of water is between 5.5 and 8.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Cefpiramide Sodium in 10 mL of 0.05 mol/L phosphate buffer solution (pH 7.0): the solution is clear, and colorless or light yellow.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Cefpiramide Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Weigh accurately about 25 mg of Cefpiramide Sodium, dissolve in 0.03 mol/L phosphate buffer solution (pH 7.5) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of 1-methyl-1*H*-tetrazole-5-thiol for liquid chromatography, previously dried in a desiccator (in vacuum, silica gel) for 2 hours, and an amount of Cefpiramide RS, equivalent to about 75 mg (potency), dissolve them in 0.03 mol/L phosphate buffer solution (pH 7.5) to make exactly 100 mL. Pipet 2 mL of this solution, add 0.03 mol/L phosphate buffer solution (pH 7.5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of 1-methyl-1*H*-tetrazole-5-thiol, each of the other related substances and the total of the other related substances by the following equations: the amount of 1-methyl-1*H*-tetrazole-5-thiol, each of the other related substances and the total of the other related substances are not more than 1.0%, not more than 1.5% and not more than 4.0%, respectively.

Amount (%) of 1-methyl-1*H*-tetrazole-5-thiol ($C_2H_4N_4S$)
$= M_{Sa}/M_T \times A_{Ta}/A_{Sa}$

Amount (%) of each of other related substances
$= M_{Sb}/M_T \times A_{Tc}/A_{Sb}$

M_{Sa}: Amount (mg) of 1-methyl-1*H*-tetrazole-5-thiol taken
M_{Sb}: Amount [mg (potency)] of Cefpiramide RS taken

M_T: Amount (mg) of Cefpiramide Sodium taken
A_{Sa}: Peak area of 1-methyl-1*H*-tetrazole-5-thiol from the standard solution
A_{Sb}: Peak area of cefpiramide from the standard solution
A_{Ta}: Peak area of 1-methyl-1*H*-tetrazole-5-thiol from the sample solution
A_{Tc}: Area of each peak other than 1-methyl-1*H*-tetrazole-5-thiol and cefpiramide from the sample solution

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octylsilanized silica gel for liquid chromatography (10 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of 0.03 mol/L phosphate buffer solution (pH 7.5) and methanol (3:1).
Flow rate: Adjust so that the retention time of cefpiramide is about 11 minutes.
Time span of measurement: About 2 times as long as the retention time of cefpiramide.
System suitability—
Test for required detectability: Measure exactly 5 mL of the standard solution, and add 0.03 mol/L phosphate buffer solution (pH 7.5) to make exactly 50 mL. Confirm that the peak area of 1-methyl-1*H*-tetrazole-5-thiol obtained from 5 μL of this solution is equivalent to 8 to 12% of that obtained from 5 μL of the standard solution.
System performance: Dissolve 25 mg of Cefpiramide RS and 7 mg of cinnamic acid in the mobile phase to make 50 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, cinnamic acid and cefpiramide are eluted in this order with the resolution between these peaks being not less than 3.
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of 1-methyl-1*H*-tetrazole-5-thiol is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 7.0% (0.35 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefpiramide Sodium and Cefpiramide RS, equivalent to about 50 mg (potency), add exactly 5 mL of the internal standard solution to dissolve, then add the mobile phase to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefpiramide to that of the internal standard.

Amount [μg (potency)] of cefpiramide ($C_{25}H_{24}N_8O_7S_2$) $= M_S \times Q_T/Q_S \times 1000$

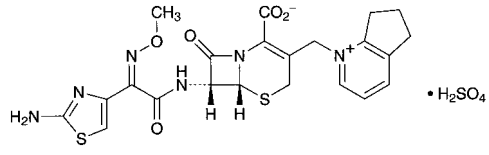
M_S: Amount [mg (potency)] of Cefpiramide RS taken

Internal standard solution—A solution of 4-dimethylaminoantipyrine (1 in 100).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octylsilanized silica gel for liquid chromatography (10 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: A mixture of 0.01 mol/L phosphate buffer solution (pH 6.8), acetonitrile, methanol and tetrahydrofuran (22:1:1:1).
Flow rate: Adjust so that the retention time of cefpiramide is about 7 minutes.
System suitability—
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, cefpiramide and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefpiramide to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant, and at a temperature not exceeding 5°C.

Cefpirome Sulfate

セフピロム硫酸塩



$C_{22}H_{22}N_6O_5S_2.H_2SO_4$: 612.66
(6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetylamino]-3-(6,7-dihydro-5*H*-cyclopenta[*b*]pyridinium-1-ylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate monosulfate
[*98753-19-6*]

Cefpirome Sulfate contains not less than 760 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefpirome Sulfate is expressed as mass (potency) of cefpirome ($C_{22}H_{22}N_6O_5S_2$: 514.58).

Description Cefpirome Sulfate occurs as a white to pale yellowish white crystalline powder, and has a slight, characteristic odor.
It is soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.
It is hygroscopic.

Identification **(1)** Dissolve 10 mg of Cefpirome Sulfate in 2 mL of water, add 3 mL of hydroxylammonium hydrochloride-ethanol TS, allow to stand for 5 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS, and shake: a red-brown color develops.
(2) Dissolve 1 mg of Cefpirome Sulfate in 4 mL of water, add 1 mL of dilute hydrochloric acid while cooling in ice, add 1 mL of a freshly prepared solution of sodium nitrite (1 in 100), and allow to stand for 2 minutes. Add 1 mL of ammonium amidosulfuric acid TS while cooling in ice bath, allow to stand for 1 minute, and add 1 mL of a solution of *N*-1-naphthylethylene dihydrochloride (1 in 1000): a purple color develops.
(3) Take 5 mg of Cefpirome Sulfate, dissolve in 1 mL of ethanol (95) and 1 mL of water, add 100 mg of 1-chloro-2,4-dinitrobenzene, and heat on a water bath for 5 minutes. After cooling, add 2 or 3 drops of a solution of sodium hydroxide (1 in 10) and 3 mL of ethanol (95): a red-brown

color develops.

(4) Determine the absorption spectra of solutions of Cefpirome Sulfate and Cefpirome Sulfate RS in 0.01 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(5) Determine the ^1H spectrum of a solution of Cefpirome Sulfate in heavy water for nuclear magnetic resonance spectroscopy (1 in 25) as directed under Nuclear Magnetic Resonance Spectroscopy ⟨*2.21*⟩, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ 4.1 ppm, a double signal B at around δ 5.9 ppm, a single signal C at around δ 7.1 ppm, and a multiple signal D at around δ 7.8 ppm. The ratio of integrated intensity of each signal, A:B:C:D, is about 3:1:1:1.

(6) A solution of Cefpirome Sulfate (1 in 250) responds to the Qualitative Tests ⟨*1.09*⟩ (1) for sulfate salt.

Absorbance ⟨*2.24*⟩ $E^{1\%}_{1\,cm}$ (270 nm): 405 – 435 (50 mg calculated on the anhydrous basis, 0.01 mol/L hydrochloric acid TS, 2500 mL).

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −27 – −33° (50 mg calculated on the anhydrous basis, a solution prepared by addition of water to 25 mL of acetonitrile to make 50 mL, 20 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 0.1 g of Cefpirome Sulfate in 10 mL of water: the pH of the solution is between 1.6 and 2.6.

Purity **(1)** Clarity and color of solution—Being specified separately when the drug is granted approval based on the Law.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Cefpirome Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic—Being specified separately when the drug is granted approval based on the Law.

(4) Related substances—Being specified separately when the drug is granted approval based on the Law.

Water ⟨*2.48*⟩ Not more than 2.5% (0.5 g, volumetric titration, direct titration).

Residue on ignition Being specified separately when the drug is granted approval based on the Law.

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.10 EU/mg (potency).

Assay Weigh accurately an amount of Cefpirome Sulfate and Cefpirome Sulfate RS, equivalent to about 50 mg (potency), dissolve each in water to make exactly 100 mL. Pipet 5 mL of these solutions, add each in water to make exactly 20 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 μL of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of cefpirome in each solution.

Amount [μg (potency)] of cefpirome ($C_{22}H_{22}N_6O_5S_2$) = $M_S \times A_T/A_S \times 1000$

M_S: Amount [mg (potency)] of Cefpirome Sulfate RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 3.45 g of ammonium dihydrogenphosphate in 1000 mL of water, and adjust the pH to 3.3 with phosphoric acid. To 800 mL of this solution add 100 mL of acetonitrile.

Flow rate: Adjust so that the retention time of cefpirome is about 7.5 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of cefpirome is not less than 3600.

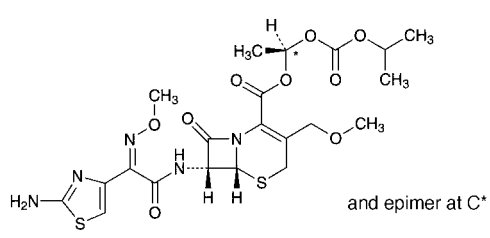
System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefpirome is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Storage—At a temperature between 2°C and 8°C.

Cefpodoxime Proxetil

セフポドキシム　プロキセチル



$C_{21}H_{27}N_5O_9S_2$: 557.60

(1*RS*)-1-[(1-Methylethoxy)carbonyloxy]ethyl (6*R*,7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetylamino]-3-methoxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

[*87239-81-4*]

Cefpodoxime Proxetil contains not less than 706 μg (potency) and not more than 774 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefpodoxime Proxetil is expressed as mass (potency) of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$: 427.46).

Description Cefpodoxime Proxetil occurs as a white to light brownish white powder.

It is very soluble in acetonitrile, in methanol and in chloroform, freely soluble in ethanol (99.5), and very slightly soluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Cefpodoxime Proxetil in acetonitrile (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefpodoxime Proxetil RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cef-

podoxime Proxetil as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefpodoxime Proxetil RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefpodoxime Proxetil in deuterochloroform for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21>, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits double signals, A and B, at around δ 1.3 ppm and at around δ 1.6 ppm, and single signals, C and D, at around δ 3.3 ppm and at around δ 4.0 ppm. The ratio of the integrated intensity of these signals, A:B:C:D, is about 2:1:1:1.

Optical rotation <2.49> $[\alpha]_D^{20}$: +24.0 – +31.4° (0.1 g calculated on the anhydrous basis, acetonitrile, 20 mL, 100 mm).

Purity **(1)** Heavy metals <1.07>—Proceed with 1.0 g of Cefpodoxime Proxetil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Cefpodoxime Proxetil in 50 mL of a mixture of water, acetonitrile and acetic acid (100) (99:99:2), and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the amount of the peak, having the relative retention time of about 0.8 to the isomer B of cefpodoxime proxetil, is not more than 2.0%, the amount of the peak other than cefpodoxime proxetil is not more than 1.0%, and the total amount of the peaks other than cefpodoxime proxetil is not more than 6.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 22°C.

Mobile phase A: A mixture of water, methanol and a solution of formic acid (1 in 50) (11:8:1).

Mobile phase B: A mixture of methanol and a solution of formic acid (1 in 50) (19:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 65	95	5
65 – 145	95 → 15	5 → 85
145 – 155	15	85

Flow rate: 0.7 mL per minute (the retention time of the isomer B of cefpodoxime proxetil is about 60 minutes).

Time span of measurement: For 155 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: To 5 mL of the sample solution add a mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make 200 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add the mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make exactly 100 mL. Confirm that the peak areas of the isomer A and the isomer B of cefpodoxime proxetil obtained from 20 μL of this solution are equivalent to 1.4 to 2.6% of them obtained from 20 μL of the solution for system suitability test, respectively.

System performance: When the procedure is run with 20 μL of the solution for system suitability test under the above operating conditions, the isomer A and the isomer B of cefpodoxime proxetil are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 5 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of the isomer A and the isomer B of cefpodoxime proxetil is not more than 2.0%.

Water <2.48> Not more than 2.5% (0.5 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Isomer ratio Perform the test with 5 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas of the two isomers of cefpodoxime proxetil, A_a, for the isomer having the smaller retention time, and A_b, for the isomer having the larger retention time, by the automatic integration method: $A_b/(A_a + A_b)$ is between 0.50 and 0.60.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution obtained in the Assay under the above operating conditions, the internal standard, the isomer A and the isomer B of cefpodoxime proxetil are eluted in this order with the resolution between the peaks of the isomers being not less than 4.

System repeatability: When the test is repeated 5 times with 5 μL of the standard solution obtained in the Assay under the above operating conditions, the relative standard deviation of the ratio of the peak area of the isomer B of cefpodoxime proxetil to that of the internal standard is not more than 1.0%.

Assay Weigh accurately an amount of Cefpodoxime Proxetil and Cefpodoxime Proxetil RS, equivalent to about 60 mg (potency), dissolve in 80 mL of acetonitrile, add exactly 4 mL of the internal standard solution, add acetonitrile to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_{T1}, Q_{S1}, Q_{T2} and Q_{S2}, of the areas of the two peaks of the isomers of cefpodoxime proxetil to the peak area of the internal standard.

Amount [μg (potency)] of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$) $= M_S \times (Q_{T1} + Q_{T2})/(Q_{S1} + Q_{S2}) \times 1000$

M_S: Amount [mg (potency)] of Cefpodoxime Proxetil RS taken

Internal standard solution—Dissolve 0.3 g of ethyl parahydroxybenzoate in a solution of citric acid in acetonitrile (1 in 2000) to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and methanol (11:9).

Flow rate: Adjust so that the retention time of the internal standard is about 11 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard, the isomer A and the isomer B are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of the isomer B of cefpodoxime proxetil to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cefpodoxime Proxetil for Syrup

シロップ用セフポドキシム　プロキセチル

Cefpodoxime Proxetil for Syrup is a preparation for syrups which is suspended before use.

It contains not less than 93.0% and not more than 107.0% of the labeled potency of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$: 427.46).

Method of preparation Prepare as directed under Syrups, with Cefpodoxime Proxetil.

Identification To an amount of Cefpodoxime Proxetil for Syrup, equivalent to 15 mg (potency) of Cefpodoxime Proxetil, add 10 mL of 0.1 mol/L phosphate buffer solution (pH 8.0), treat with ultrasonic waves for 5 minutes while occasional shaking. Then, add 20 mL of ethyl acetate, shake for 5 minutes, and centrifuge. Take 3 mL of the supernatant liquid, evaporate the ethyl acetate by warming at 40°C under reduced pressure. Dissolve the residue in acetonitrile to make 200 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 232 nm and 236 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: Cefpodoxime Proxetil for Syrup in single-dose packages meet the requirement of the Content uniformity test.

To the total content of 1 package of Cefpodoxime Proxetil for Syrup add exactly 30 mL of the internal standard solution, treat with ultrasonic waves for 10 minutes while occasional shaking, and centrifuge. Take 3 mL of the supernatant liquid, add a mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefpodoxime Proxetil RS, equivalent to about 50 mg (potency), dissolve in a suitable amount of a mixture of water, acetonitrile and acetic acid (100) (99:99:2), add exactly 15 mL of the internal standard solution, then add a mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefpodoxime Proxetil.

Amount [mg (potency)] of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$)
$= M_S \times (Q_{T1} + Q_{T2})/(Q_{S1} + Q_{S2}) \times 2$

M_S: Amount [mg (potency)] of Cefpodoxime Proxetil RS taken

Internal standard solution—Dissolve 0.2 g of ethyl parahydroxybenzoate in a mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make 300 mL.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rates in 15 minutes of Cefpodoxime Proxetil for Syrup is not less than 85%.

Start the test with an accurately weighed amount of Cefpodoxime Proxetil for Syrup, equivalent to about 50 mg (potency) of Cefpodoxime Proxetil, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add a solution of citric acid monohydrate in the mobile phase (1 in 2000) to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefpodoxime Proxetil RS, equivalent to about 22 mg (potency), dissolve in a solution of citric acid monohydrate in the mobile phase (1 in 2000) to make exactly 100 mL. Pipet 5 mL of this solution, add a solution of citric acid monohydrate in the mobile phase (1 in 2000) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the areas, A_{Ta} and A_{Sa}, of the one peak which appears at the retention time of about 24 minutes among the two peaks obtainable from cefpodoxime proxetil, and the areas, A_{Tb} and A_{Sb}, of the peak which appears at the retention time of about 30 minutes, in each solution.

Dissolution rate (%) with respect to the labeled amount of cefpodoxime proxetil ($C_{21}H_{27}N_5O_9S_2$)
$= M_S/M_T \times (A_{Ta} + A_{Tb})/(A_{Sa} + A_{Sb}) \times 1/C \times 225$

M_S: Amount [mg (potency)] of Cefpodoxime Proxetil RS taken

M_T: Amount (g) of Cefpodoxime Proxetil for Syrup taken

C: Labeled amount [mg (potency)] of cefpodoxime proxetil ($C_{21}H_{27}N_5O_9S_2$) in 1 g

Operating conditions—

Detector, column, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay under Cefpodoxime Proxetil.

Flow rate: Adjust so that the retention time of the peak, which elutes faster among the two peaks obtained from cefpodoxime proxetil, is about 24 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the resolution between the two peaks obtained from cefpodoxime proxetil is not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the sum of the areas of the two peaks obtained from cefpodoxime proxetil is not more than 2.0%.

Assay Weigh accurately an amount of powdered Cefpodoxime Proxetil for Syrup, equivalent to about 0.1 g (potency) of Cefpodoxime Proxetil, add exactly 30 mL of the internal standard solution, treat with ultrasonic waves for 10 minutes while occasional shaking, and centrifuge. Take 3 mL of the supernatant liquid, add a mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefpodoxime Proxetil RS, equivalent to about 50 mg (potency), dissolve in a suitable amount of a mixture of water, acetonitrile and acetic acid (100) (99:99:2), add exactly 15 mL of the internal standard solution, then add a mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefpodoxime Proxetil.

Amount [mg (potency)] of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$) $= M_S \times (Q_{T1} + Q_{T2})/(Q_{S1} + Q_{S2}) \times 2$

M_S: Amount [mg (potency)] of Cefpodoxime Proxetil RS taken

Internal standard solution—Dissolve 0.2 g of ethyl parahydroxybenzoate in a mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make 300 mL.

Containers and storage Containers—Tight containers.

Cefpodoxime Proxetil Tablets

セフポドキシム　プロキセチル錠

Cefpodoxime Proxetil Tablets contain not less than 93.0% and not more than 107.0% of the labeled potency of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$: 427.46).

Method of preparation Prepare as directed under Tablets, with Cefpodoxime Proxetil.

Identification Powder Cefpodoxime Proxetil Tablets. To a portion of the powder, equivalent to 65 mg (potency) of Cefpodoxime Proxetil, add 25 mL of acetonitrile, shake thoroughly, and centrifuge. To 2 mL of the supernatant liquid add acetonitrile to make 50 mL. To 5 mL of this solution add acetonitrile to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 232 nm and 236 nm.

Uniformity of dosage units <*6.02*> Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Cefpodoxime Proxetil Tablets, add exactly 20 mL of a mixture of water, acetonitrile and acetic acid (100) (99:99:2), agitate with the aid of ultrasonic waves for 10 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, equivalent to 30 mg (potency) of Cefpodoxime Proxetil, add exactly 6 mL of the internal standard solution, then add a mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefpodoxime Proxetil RS, equivalent to about 60 mg (potency), dissolve in 60 mL of a mixture of water, acetonitrile and acetic acid (100) (99:99:2), add exactly 12 mL of the internal standard solution, then add a mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefpodoxime Proxetil.

Amount [mg (potency)] of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$) $= M_S \times (Q_{T1} + Q_{T2})/(Q_{S1} + Q_{S2}) \times 10/V$

M_S: Amount [mg (potency)] of Cefpodoxime Proxetil RS taken

Internal standard solution—Dissolve 0.1 g of ethyl parahydroxybenzoate in a mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make 100 mL.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Cefpodoxime Proxetil Tablets is not less than 70%.

Start the test with 1 tablet of Cefpodoxime Proxetil Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add a solution of citric acid monohydrate in the mobile phase (1 in 2000) to make exactly V' mL so that each mL contains about 11 μg (potency) of Cefpodoxime Proxetil, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefpodoxime Proxetil RS, equivalent to about 22 mg (potency), and dissolve in a solution of citric acid monohydrate in the mobile phase (1 in 2000) to make exactly 100 mL. Pipet 5 mL of this solution, add a solution of citric acid monohydrate in the mobile phase (1 in 2000) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the areas of separated two peaks, one has the retention time of about 24 minutes, A_{Ta} and A_{Sa}, and another one has the retention time of about 30 minutes, A_{Tb} and A_{Sb}, in each solution.

Dissolution rate (%) with respect to the labeled amount of cefpodoxime proxetil ($C_{21}H_{27}N_5O_9S_2$) $= M_S \times (A_{Ta} + A_{Tb})/(A_{Sa} + A_{Sb}) \times V'/V \times 1/C \times 45$

M_S: Amount [mg (potency)] of Cefpodoxime Proxetil RS taken

C: Labeled amount [mg (potency)] of cefpodoxime proxetil ($C_{21}H_{27}N_5O_9S_2$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and methanol (11:9).

Flow rate: Adjust so that the retention time of one of the two peaks that elutes firster is about 24 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the resolution between the two peaks of cefpodoxime proxetil is not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the total area of the two peaks of cefpodoxime proxetil is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Cefpodoxime Proxetil Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.3 g (potency) of Cefpodoxime Proxetil, add 80 mL of a mixture of water, acetonitrile and acetic acid (100) (99:99:2), agitate for 10 minutes with the aid of ultrasonic waves, and add a mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make exactly 100 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add exactly 6 mL of the internal standard solution, then, add a mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefpodoxime Proxetil RS, equivalent to about 60 mg (potency), dissolve in 60 mL of a mixture of water, acetonitrile and acetic acid (100) (99:99:2), add exactly 12 mL of the internal standard solution, then add a mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefpodoxime Proxetil.

Amount [mg (potency)] of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$) = $M_S \times (Q_{T1} + Q_{T2})/(Q_{S1} + Q_{S2}) \times 5$

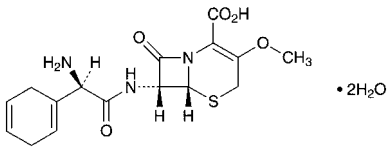
M_S: Amount [mg (potency)] of Cefpodoxime Proxetil RS taken

Internal standard solution—Dissolve 0.1 g of ethyl parahydroxybenzoate in a mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make 100 mL.

Containers and storage Containers—Tight containers.

Cefroxadine Hydrate

セフロキサジン水和物



$C_{16}H_{19}N_3O_5S.2H_2O$: 401.43

(6*R*,7*R*)-7-[(2*R*)-2-Amino-2-cyclohexa-1,4-dienylacetylamino]-3-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid dihydrate [*51762-05-1*, anhydride]

Cefroxadine Hydrate contains not less than 930 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefroxadine Hydrate is expressed as mass (potency) of cefroxadine ($C_{16}H_{19}N_3O_5S$: 365.40).

Description Cefroxadine Hydrate occurs as pale yellowish white to light yellow, crystalline particles or powder.

It is very soluble in formic acid, slightly soluble in water and in methanol, and very slightly soluble in acetonitrile and in ethanol (95).

It dissolves in 0.001 mol/L hydrochloric acid TS and in dilute acetic acid.

Identification **(1)** Determine the absorption spectrum of a solution of Cefroxadine Hydrate in 0.001 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefroxadine RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefroxadine Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefroxadine RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefroxadine Hydrate in deuterated formic acid for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits three sharp single signals, A, B and C, at around δ 2.8 ppm, at around δ 4.1 ppm and at around δ 6.3 ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 4:3:1.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +95 – +108° (0.1 g calculated on the anhydrous basis, diluted acetic acid (100) (3 in 25), 100 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Weigh 1.0 g of Cefroxadine Hydrate in a porcelain crucible, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), mix, burn the ethanol, and carbonize by gently heating. After cooling, add 2 mL of nitric acid, heat carefully, and incinerate by ignition at 500 – 600°C. If a carbonized substance still remains, moisten it with a small amount of nitric acid, and incinerate again by ignition. After cooling, add 6 mL of hydrochloric acid, and evaporate on a water bath to dryness. Moisten the residue with 3 drops of hydrochloric acid, and add 10 mL of hot water to dissolve the residue by heating on a water bath. After cooling, adjust the pH between 3 and 4 with ammonia TS, add 2 mL of dilute acetic acid, filter if necessary, transfer to a Nessler tube, wash the crucible with 10 mL of water, and add the washing and water to the tube to make 50 mL. Perform the test with this solution. Prepare the control solution as follows: Put 2.0 mL of Standard Lead Solution and 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) in a porcelain crucible, and proceed as directed for the preparation of the test solution (not more than 20 ppm).

(2) Related substances—Dissolve 10 mg of Cefroxadine Hydrate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the areas of the peaks, having the relative retention times of about 0.07, about 0.6 and about 0.8 to cefroxadine obtained from the sample solution are not larger than 2 times, 4 times and 1 time the peak area of cefroxadine obtained from the standard solution, respectively, and any peak area other than cefroxadine and other than the peaks mentioned above is not larger than 1/2 times the peak area of cefroxadine from the standard solution, and the total area of the peaks other than cefroxadine is not larger than 6 times the peak area of cefroxadine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica

gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.4 g of sodium perchlorate in 1000 mL of a mixture of water and acetonitrile (489:11).

Flow rate: Adjust so that the retention time of cefroxadine is about 20 minutes.

Time span of measurement: About 2 times as long as the retention time of cefroxadine.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of cefroxadine obtained with 40 μL of this solution is equivalent to 7 to 13% of that obtained with 40 μL of the standard solution.

System performance: Dissolve 3 mg of Cefroxadine Hydrate and 15 mg of orcin in 100 mL of the mobile phase. When the procedure is run with 40 μL of this solution under the above operating conditions, orcin and cefroxadine are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefroxadine is not more than 2.0%.

Water ⟨*2.48*⟩ Not less than 8.5% and not more than 12.0% (0.1 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefroxadine Hydrate and Cefroxadine RS, equivalent to about 50 mg (potency), dissolve each in a suitable amount of a mixture of dilute acetic acid and phosphoric acid (500:1), add exactly 5 mL of the internal standard solution and a mixture of dilute acetic acid and phosphoric acid (500:1) to make 200 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefroxadine to that of the internal standard.

Amount [μg (potency)] of cefroxadine ($C_{16}H_{19}N_3O_5S$)
$= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Cefroxadine RS taken

Internal standard solution—Dissolve 1.6 g of vanillin in 5 mL of methanol, and add a mixture of dilute acetic acid and phosphoric acid (500:1) to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of ammonium sulfate (1 in 50) and acetonitrile (97:3).

Flow rate: Adjust so that the retention time of cefroxadine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefroxadine and the internal standard are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak areas of cefroxadine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cefroxadine for Syrup

シロップ用セフロキサジン

Cefroxadine for Syrup is a preparation for syrup, which is suspended before use.

It contains not less than 93.0% and not more than 107.0% of the labeled potency of cefroxadine ($C_{16}H_{19}N_3O_5S$: 365.40).

Method of preparation Prepare as directed under Preparations for Syrups, with Cefroxadine Hydrate.

Identification Powder Cefroxadine for Syrup, if necessary. To a portion of the powder, equivalent to 2 mg (potency) of Cefroxadine Hydrate, add 100 mL of 0.001 mol/L hydrochloric acid TS, shake well, and filter. Determine the absorption spectrum of this filtrate as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 267 nm and 271 nm.

Water ⟨*2.48*⟩ Not more than 4.5% (0.1 g, volumetric titration, direct titration).

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: Cefroxadine for Syrup in single-dose packages meet the requirement of the Content uniformity test.

Take out the total contents of 1 package of Cefroxadine for Syrup, add $4V/5$ mL of a mixture of dilute acetic acid and phosphoric acid (500:1), shake well for 15 minutes, add exactly 5 mL of the internal standard solution per 50 mg (potency) of Cefroxadine Hydrate, and add a mixture of dilute acetic acid and phosphoric acid (500:1) to make V mL so that each mL contains about 0.25 mg (potency) of Cefroxadine Hydrate. Filter this solution through a membrane filter with pore size of not exceeding 0.45 μm, and use the filtrate as the sample solution. Separately, weigh accurately an amount of Cefroxadine RS, equivalent to about 50 mg (potency), dissolve in a mixture of dilute acetic acid and phosphoric acid (500:1), add exactly 5 mL of the internal standard solution, add a mixture of dilute acetic acid and phosphoric acid (500:1) to make 200 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefroxadine Hydrate.

Amount [mg (potency)] of cefroxadine ($C_{16}H_{19}N_3O_5S$)
$= M_S \times Q_T/Q_S \times V/200$

M_S: Amount [mg (potency)] of Cefroxadine RS taken

Internal standard solution—Dissolve 1.6 g of vanillin in 5 mL of methanol, and add a mixture of dilute acetic acid and phosphoric acid (500:1) to make 100 mL.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Cefroxadine for Syrup is not less than 85%.

Start the test with an accurately weighed amount of Cefroxadine for Syrup, equivalent to about 0.1 g (potency) of Cefroxadine Hydrate, withdraw not less than 10 mL of the medium at the specified minute after starting the test,

and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 5 mL of the filtrate, pipet 4 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefroxadine RS, equivalent to about 22 mg (potency), and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 5 mL of this solution, add 10 mL of water, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 267 nm.

Dissolution rate (%) with respect to the labeled amount of cefroxadine ($C_{16}H_{19}N_3O_5S$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 450$

M_S: Amount [mg (potency)] of Cefroxadine RS taken
M_T: Amount (g) of Cefroxadine for Syrup taken
C: Labeled amount [mg (potency)] of cefroxadine ($C_{16}H_{19}N_3O_5S$) in 1 g

Assay Powder Cefroxadine for Syrup, if necessary, weigh accurately a portion of the powder, equivalent to about 50 mg (potency) of Cefroxadine Hydrate, add 160 mL of a mixture of dilute acetic acid and phosphoric acid (500:1), shake well for 15 minutes, add exactly 5 mL of the internal standard solution, and add a mixture of dilute acetic acid and phosphoric acid (500:1) to make 200 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Separately, weigh accurately an amount of Cefroxadine RS, equivalent to about 50 mg (potency), dissolve in a mixture of dilute acetic acid and phosphoric acid (500:1), add exactly 5 mL of the internal standard solution, add a mixture of dilute acetic acid and phosphoric acid (500:1) to make 200 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefroxadine Hydrate.

Amount [mg (potency)] of cefroxadine ($C_{16}H_{19}N_3O_5S$)
$= M_S \times Q_T/Q_S$

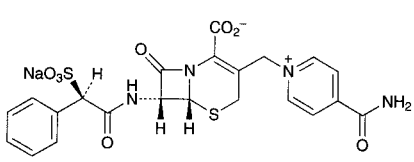
M_S: Amount [mg (potency)] of Cefroxadine RS taken

Internal standard solution—Dissolve 1.6 g of vanillin in 5 mL of methanol, and add a mixture of dilute acetic acid and phosphoric acid (500:1) to make 100 mL.

Containers and storage Containers—Tight containers.

Cefsulodin Sodium

セフスロジンナトリウム



$C_{22}H_{19}N_4NaO_8S_2$: 554.53
Monosodium (6*R*,7*R*)-3-(4-carbamoylpyridinium-1-ylmethyl)-8-oxo-7-[(2*R*)-2-phenyl-2-sulfonatoacetylamino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate
[*52152-93-9*]

Cefsulodin Sodium contains not less than 900 μg (potency) and not more than 970 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefsulodin Sodium is expressed as mass (potency) of cefsulodin ($C_{22}H_{20}N_4O_8S_2$: 532.55).

Description Cefsulodin Sodium occurs as white to light yellow, crystals or crystalline powder.

It is freely soluble in water and in formamide, slightly soluble in methanol, and very slightly soluble in ethanol (95).

It is hygroscopic.

Identification **(1)** Determine the absorption spectrum of a solution of Cefsulodin Sodium (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefsulodin Sodium RS prepared in the same manner as sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefsulodin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefsulodin Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefsulodin Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a multiple signal A between δ 7.3 ppm and δ 7.7 ppm, and double signals, B and C, at around δ 8.4 ppm and at around δ 9.1 ppm, respectively. The ratio of integrated intensity of these signals, A:B:C, is about 5:2:2.

(4) Cefsulodin Sodium responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +16.5 – +20.0° (0.1 g calculated on the anhydrous basis, water, 10 mL, 100 mm).

pH <*2.54*> Dissolve 1.0 g of Cefsulodin Sodium in 10 mL of water: the pH of the solution is not less than 3.3 and not more than 4.8.

Purity **(1)** Clarity of solution—Dissolve 1.0 g of Cefsulodin Sodium in 10 mL of water: the solution is clear.

(2) Heavy metals <*1.07*>—To 1.0 g of Cefsulodin Sodium add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 5), mix, fire the ethanol to burn, then heat gradually to carbonize. After cooling, add 2 mL of nitric acid, heat carefully, then heat at 500 – 600°C to incinerate. If a carbonized residue still retains, add a little amount of nitric acid, and heat again to incinerate. After cooling, add 6 mL of hydrochloric acid to the residue, heat to dryness on a water bath, then moisten the residue with 3 drops of hydrochloric acid, add 10 mL of hot water, and heat on a water bath to dissolve. Add ammonia TS dropwise to adjust to pH 3 – 4, and add 2 mL of dilute acetic acid. If necessary, filter, wash the crucible and residue on the filter with 10 mL of water, transfer the filtrate and washings into a Nessler tube, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution as follows: To 2.0 mL of Standard Lead Solution add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 5), fire the ethanol to burn. After cooling, add 2 mL of nitric acid, heat carefully, then heat at 500 – 600°C. After cooling, add 6 mL of hydrochloric acid, then proceed in the same manner as for the preparation of the test solution (not more than 20 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Cefsulodin Sodium according to Method 3, using a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in

5) and 15 mL of dilute hydrochloric acid instead of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50) and 3 mL of hydrochloric acid, and perform the test (not more than 2 ppm).

(4) Related substances—Weigh accurately 0.10 g of Cefsulodin Sodium, dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of isonicotinic acid amide and about 20 mg of Cefsulodin Sodium RS (separately determine the water ⟨*2.48*⟩ in the same manner as Cefsulodin Sodium), and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the areas of each peak by the automatic integration method. Calculate the amount of the related substances by the following formula: the amount of isonicotinic acid amide is not more than 1.0%, and the total amount of other related substances is not more than 1.2%.

Amount (%) of isonicotinic acid amide
$= A/B_I \times M_I/M_T \times 5$

Total amount (%) of the other related substances
$= B/B_S \times M_S/M_T \times 5$

A: Peak area of isonicotinic acid amide from the sample solution
B: Total peak area other than cefsulodin and other than isonicotinic acid amide from the sample solution
B_I: Peak area of isonicotinic acid amide from the standard solution
B_S: Peak area of cefsulodin from the standard solution
M_T: Amount (g) of Cefsulodim Sodium taken
M_S: Amount (g) of Cefsulodin Sodium RS taken
M_I: Amount (g) of isonicotinic acid amide taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of a solution of ammonium sulfate (1 in 100) and acetonitrile (97:3).

Mobile phase B: A mixture of a solution of ammonium sulfate (1 in 100) and acetonitrile (23:2).

Flowing of mobile phase: Change the mobile phase A to B at 14 minutes after the injection of sample.

Flow rate: Adjust so that the retention time of cefsulodin is about 9 minutes.

Time span of measurement: About 4 times as long as the retention time of cefsulodin.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, add water to make exactly 10 mL. Confirm that the peak areas of isonicotinic acid amide and cefsulodin obtained from 10 μL of this solution are equivalent to 7 to 13% of those of isonicotinic acid amide and cefsulodin obtained from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, isonicotinic acid amide and cefsulodin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefsulodin is not more than 1.0%.

Water ⟨*2.48*⟩ Not more than 5.0% (1 g, volumetric titration, direct titration, avoiding moisture absorption of the sample, using a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

Assay Weigh accurately an amount of Cefsulodin Sodium and Cefsulodin Sodium RS, equivalent to about 0.1 g (potency), dissolve each in water to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of cefsulodin in each solution.

Amount [μg (potency)] of cefsulodin ($C_{22}H_{20}N_4O_8S_2$)
$= M_S \times A_T/A_S \times 1000$

M_S: Amount [mg (potency)] of Cefsulodin Sodium RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of ammonium sulfate (1 in 100) and acetonitrile (97:3).

Flow rate: Adjust so that the retention time of cefsulodin is about 9 minutes.

System suitability—

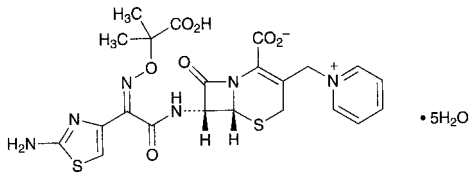
System performance: Dissolve 40 mg of isonicotinic acid amide in 25 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, isonicotinic acid amide and cefsulodin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefsulodin is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Ceftazidime Hydrate

セフタジジム水和物



$C_{22}H_{22}N_6O_7S_2.5H_2O$: 636.65

(6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxyimino)acetylamino]-3-(pyridinium-1-ylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate pentahydrate

[*78439-06-2*]

Ceftazidime Hydrate contains not less than 950 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Ceftazidime Hydrate is expressed as mass (potency) of ceftazidime ($C_{22}H_{22}N_6O_7S_2$: 546.58).

Description Ceftazidime Hydrate occurs as a white to light yellowish white crystalline powder.

It is slightly soluble in water, and very slightly soluble in acetonitrile and in ethanol (95).

Identification **(1)** Determine the absorption spectrum of a solution of Ceftazidime Hydrate in phosphate buffer solution (pH 6.0) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ceftazidime RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ceftazidime Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Ceftazidime RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 0.05 g of Ceftazidime Hydrate add 5 mg of dried sodium carbonate, and add 0.5 mL of heavy water for nuclear magnetic resonance spectroscopy to dissolve. Determine the ^1H spectrum of this solution as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>, using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A and B, at around δ 1.5 ppm and at around δ 6.9 ppm, and a multiple signal C between δ 7.9 ppm and δ 9.2 ppm. The ratio of integrated intensity of each signal, A:B:C, is about 6:1:5.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -28 – $-34°$ (0.5 g calculated on the anhydrous basis, phosphate buffer solution (pH 6.0), 100 mL, 100 mm).

pH <*2.54*> Dissolve 0.5 g of Ceftazidime Hydrate in 100 mL of water: the pH of the solution is between 3.0 and 4.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Ceftazidime Hydrate in 10 mL of a solution obtained by dissolving 5 g of anhydrous disodium hydrogen phosphate and 1 g of potassium dihydrogen phosphate in water to make 100 mL: the solution is clear, and its absorbance at 420 nm, determined as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, is not more than 0.20.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Ceftazidime Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances (i) Trityl-*t*-butyl substance and *t*-butyl substance—Dissolve 0.10 g of Ceftazidime Hydrate in 2 mL of diluted disodium hydrogen phosphate TS (1 in 3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted disodium hydrogenphosphate TS (1 in 3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop with a mixture of acetic acid (100), *n*-butyl acetate, acetate buffer solution (pH 4.5) and 1-butanol (16:16:13:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots which appear upper in position than the principal spot obtained from the sample solution are not more intense than the spot obtained from the standard solution.

(ii) Other related substances—Dissolve 20 mg of Ceftazidime Hydrate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than ceftazidime obtained from the sample solution is not larger than that of ceftazidime obtained from the standard solution, and the total of peak areas other than ceftazidime from the sample solution is not larger than 5 times the peak area of ceftazidime from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.0 g of ammonium dihydrogenphosphate in 750 mL of water, adjust to pH 3.5 with phosphoric acid, and add water to make 870 mL. To this solution add 130 mL of acetonitrile.

Flow rate: Adjust so that the retention time of ceftazidime is about 4 minutes.

Time span of measurement: About 3 times as long as the retention time of ceftazidime, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, add the mobile phase to make exactly 5 mL, and confirm that the peak area of ceftazidime obtained with 5 μL of this solution is equivalent to 15 to 25% of that obtained with 5 μL of the standard solution.

System performance: Dissolve about 10 mg each of Ceftazidime Hydrate and acetanilide in 20 mL of the mobile phase. When the procedure is run with 5 μL of this solution under the above operating conditions, ceftazidime and acetanilide are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times

with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ceftazidime is not more than 2.0%.

(4) Free pyridine—Weigh accurately about 50 mg of Ceftazidime Hydrate, dissolve in the mobile phase to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of pyridine, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak heights, H_T and H_S, of pyridine in each solution: the amount of free pyridine is not more than 0.3%.

$$\text{Amount (mg) of free pyridine} = M_S \times H_T/H_S \times 1/1000$$

M_S: Amount (mg) of pyridine taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.88 g of ammonium dihydrogenphosphate in 500 mL of water, add 300 mL of acetonitrile and water to make 1000 mL, and adjust to pH 7.0 with ammonia solution (28).

Flow rate: Adjust so that the retention time of pyridine is about 4 minutes.

System suitability—

System performance: Dissolve 5 mg of Ceftazidime Hydrate in 100 mL of a solution of pyridine in the mobile phase (1 in 20,000). When the procedure is run with 10 μL of this solution under the above operating conditions, ceftazidime and pyridine are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak height of pyridine is not more than 5.0%.

Water <2.48> 13.0 – 15.0% (0.1 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Ceftazidime Hydrate, equivalent to about 0.1 g (potency), and dissolve in 0.05 mol/L phosphate buffer solution (pH 7.0) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, then add 0.05 mol/L phosphate buffer solution (pH 7.0) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Ceftazidime RS, equivalent to about 20 mg (potency), dissolve in 0.05 mol/L phosphate buffer solution (pH 7.0) to make exactly 20 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, then add 0.05 mol/L phosphate buffer solution (pH 7.0) to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ceftazidime to that of the internal standard.

$$\text{Amount [μg (potency)] of ceftazidime } (C_{22}H_{22}N_6O_7S_2) = M_S \times Q_T/Q_S \times 5000$$

M_S: Amount [mg (potency)] of Ceftazidime RS taken

Internal standard solution—A solution of dimedon in 0.05 mol/L phosphate buffer solution (pH 7.0) (11 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with hexasilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 4.26 g of anhydrous disodium hydrogen phosphate and 2.72 g of potassium dihydrogen phosphate in 980 mL of water, and add 20 mL of acetonitrile.

Flow rate: Adjust so that the retention time of ceftazidime is about 4 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard and ceftazidime are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ceftazidime to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Ceftazidime for Injection

注射用セフタジジム

Ceftazidime for Injection is a preparation for injection which is dissolved before use.

It contains not less than 93.0% and not more than 107.0% of the labeled potency of ceftazidime ($C_{22}H_{22}N_6O_7S_2$: 546.58).

Method of preparation Prepare as directed under Injections, with Ceftazidime Hydrate.

Description Ceftazidime for Injection is a white to pale yellowish white powder.

Identification Determine the absorption spectrum of a solution of Ceftazidime for Injection (1 in 100,000) in phosphate buffer solution (pH 6.0) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 255 nm and 259 nm.

pH <2.54> Dissolve an amount of Ceftazidime for Injection, equivalent to 1.0 g (potency) of Ceftazidime Hydrate, in 10 mL of water: the pH of this solution is 5.8 to 7.8.

Purity Clarity and color of solution—Dissolve 5 g of disodium hydrogen phosphate and 1 g of potassium dihydrogen phosphate in water to make 100 mL. In 10 mL of this solution dissolve an amount of Ceftazidime for Injection, equivalent to 1.0 g (potency) of Ceftazidime Hydrate: the solution is clear. Also, determine the absorption spectra of this solution as directed under Ultraviolet-visible Spectropho-

tometry ⟨*2.24*⟩: the absorbance at 420 nm is not more than 0.3.

Loss on drying ⟨*2.41*⟩ Not more than 14.0% (0.1 g, in vacuum not exceeding 0.67 kPa, 60°C, 3 hours).

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.067 EU/mg (potency).

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filter method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 containers of Ceftazidime for Injection. Weigh accurately an amount of Ceftazidime Hydrate, equivalent to about 0.25 g (potency), and dissolve in 0.05 mol/L phosphate buffer solution (pH 7.0) to make exactly 250 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, add more 0.05 mol/L phosphate buffer solution (pH 7.0) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Ceftazidime RS, equivalent to about 25 mg (potency), and dissolve in 0.05 mol/L phosphate buffer solution (pH 7.0) to make exactly 25 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, then add 0.05 mol/L phosphate buffer solution (pH 7.0) to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Ceftazidime Hydrate.

Amount [mg (potency)] of ceftazidime ($C_{22}H_{22}N_6O_7S_2$) $= M_S \times Q_T/Q_S \times 10$

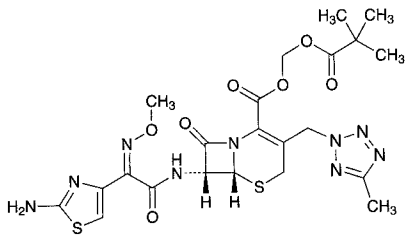
M_S: Amount [mg(potency)] of Ceftazidime RS taken

Internal standard solution—A solution of dimedon in 0.05 mol/L phosphate buffer solution (pH 7.0) (11 in 10,000).

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant.

Cefteram Pivoxil

セフテラム　ピボキシル



$C_{22}H_{27}N_9O_7S_2$: 593.64
2,2-Dimethylpropanoyloxymethyl (6*R*,7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetylamino]-3-(5-methyl-2*H*-tetrazol-2-ylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate
[*82547-58-8*, Cefteram]

Cefteram Pivoxil contains not less than 743 μg (potency) and not more than 824 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefteram Pivoxil is expressed as mass (potency) of cefteram ($C_{16}H_{17}N_9O_5S_2$: 479.49).

Description Cefteram Pivoxil occurs as a white to pale yellowish white powder.

It is very soluble in acetonitrile, freely soluble in methanol, in ethanol (95) and in chloroform, and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Cefteram Pivoxil in 0.05 mol/L hydrochloric acid-methanol TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefteram Pivoxil as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefteram Pivoxil in deuterated chloroform for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy ⟨*2.21*⟩, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals A, B and C, at around δ 1.2 ppm, at around δ 2.5 ppm and at around δ 4.0 ppm, respectively. The ratio of the integrated intensity of these signals, A:B:C, is about 3:1:1.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +35 – +43° (0.4 g calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Cefteram Pivoxil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Cefteram Pivoxil in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the each area of the peaks, having the relative retention time of about 0.2 and about 0.9 to cefteram pivoxil, obtained from the sample solution is not larger than 1/2 times and 1.25 times the peak area of cefteram pivoxil obtained from the standard solution, respectively, the area of the peak other than cefteram pivoxil and the peaks mentioned above is not larger than 1/4 times the peak area of cefteram pivoxil from the standard solution, and the total area of the peaks other than cefteram pivoxil is not larger than 2.75 times the peak area of cefteram pivoxil from the standard solution. For the area of the peak, having the relative retention time of about 0.1 to cefteram pivoxil, multiply the relative response factor, 0.74.
Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of cefteram pivoxil.
System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 10

mL. Confirm that the peak area of cefteram pivoxil obtained from 10 μL of this solution is equivalent to 7 to 13% of that obtained from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefteram pivoxil are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefteram pivoxil is not more than 3.0%.

Water ⟨*2.48*⟩ Not more than 3.0% (0.3 g, coulometric titration).

Assay Weigh accurately an amount of Cefteram Pivoxil and Cefteram Pivoxil Mesitylene Sulfonate RS, equivalent to about 40 mg (potency), dissolve each in 20 mL of diluted acetonitrile (1 in 2), add exactly 5 mL of the internal standard solution and diluted acetonitrile (1 in 2) to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefteram pivoxil to that of the internal standard.

Amount [μg (potency)] of cefteram ($C_{16}H_{17}N_9O_5S_2$) $= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Cefteram Pivoxil Mesitylene Sulfonate RS taken

Internal standard solution—A solution of methyl parahydroxybenzoate in diluted acetonitrile (1 in 2) (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 100 mL of acetic acid-sodium acetate buffer solution (pH 5.0) add 375 mL of acetonitrile and water to make 1000 mL.

Flow rate: Adjust so that the retention time of cefteram pivoxil is about 14 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and cefteram pivoxil are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefteram pivoxil to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—In a cold place.

Cefteram Pivoxil Fine Granules

セフテラム　ピボキシル細粒

Cefteram Pivoxil Fine Granules contain not less than 90.0% and not more than 110.0% of the labeled potency of cefteram ($C_{16}H_{17}N_9O_5S_2$: 479.49).

Method of preparation Prepare as directed under Granules, with Cefteram Pivoxil.

Identification Powder Cefteram Pivoxil Fine Granules. To a portion of the powder, equivalent to 0.1 g (potency) of Cefteram Pivoxil, add 20 mL of methanol, shake well, and filter. To 1 mL of the filtrate add 0.05 mol/L hydrochloric acid-methanol TS to make 500 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 262 nm and 266 nm.

Purity Related substances—Powder Cefteram Pivoxil Fine Granules, if necessary. To a portion, equivalent to 0.1 g (potency) of Cefteram Pivoxil, add diluted acetonitrile (1 in 2) to make 100 mL, disperse the particle with the aid of ultrasonic waves, then filter, and use the filtrate as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.9 to cefteram pivoxil obtained from the sample solution, is not larger than 1.75 times the peak area of cefteram pivoxil obtained from the standard solution, the area of the peak, having the relative retention time of about 0.1 from the sample solution, is not larger than 17/25 times the peak area of cefteram pivoxil from the standard solution, and the total area of the peaks other than cefteram pivoxil from the sample solution is not larger than 3.7 times the peak area of cefteram pivoxil from the standard solution. For the area of the peak, having the relative retention time of about 0.1 to cefteram pivoxil, multiply the relative response factor, 0.74.

Operating conditions—

Proceed as directed in the operating conditions in tne Purity (2) under Cefteram Pivoxil.

System suitability—

Proceed as directed in the system suitability in the Purity (2) under Cefteram Pivoxil.

Water ⟨*2.48*⟩ Not more than 0.3% (0.1 g (potency), coulometric titration).

Uniformity of dosage units ⟨*6.02*⟩ The Granules in single-dose packages meet the requirement of the Mass variation test.

Dissolution Being specified separately when the drug is granted approval based on the Law.

Assay Powder Cefteram Pivoxil Fine Granules, if necessary. Weigh accurately an amount of the powder, equivalent to about 0.3 g (potency) of Ceteram Pivoxil, add exactly 30 mL of the internal standard solution and diluted acetonitrile (1 in 2) to make 300 mL. Disperse the particle with the aid of ultrasonic waves, then filter, and use the filtrate as the sample solution. Separately, weigh accurately an amount of Cefteram Pivoxil Mesitylene Sulfonate RS, equivalent to

about 50 mg (potency), dissolve in 20 mL of diluted acetonitrile (1 in 2), add exactly 5 mL of the internal standard solution and diluted acetonitrile (1 in 2) to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefteram pivoxil to that of the internal standard.

Amount [mg (potency)] of cefteram ($C_{16}H_{17}N_9O_5S_2$)
$= M_S \times Q_T/Q_S \times 6$

M_S: Amount [mg (potency)] of Cefteram Pivoxil Mesitylene Sulfonate RS taken

Internal standard solution—A solution of methyl parahydroxybenzoate in diluted acetonitrile (1:2) (1 in 1000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Cefteram Pivoxil.

System suitability—

Proceed as directed in the system suitability in the Assay under Cefteram Pivoxil.

Containers and storage Containers—Tight containers.

Cefteram Pivoxil Tablets

セフテラム　ピボキシル錠

Cefteram Pivoxil Tablets contain not less than 90.0% and not more than 110.0% of the labeled potency of cefteram ($C_{16}H_{17}N_9O_5S_2$: 479.49).

Method of preparation Prepare as directed under Tablets, with Cefteram Pivoxil.

Identification To a quantity of powdered Cefteram Pivoxil Tablets, equivalent to 0.1 g (potency) of Cefteram Pivoxil, add 20 mL of methanol, shake well, and filter. To 1 mL of the filtrate add 0.05 mol/L hydrochloric acid-methanol TS to make 500 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 262 nm and 266 nm.

Purity Related substances—To a quantity of powdered Cefteram Pivoxil Tablets, equivalent to 0.1 g (potency) of Cefteram Pivoxil, add diluted acetonitrile (1 in 2) to make 100 mL. Disperse this solution with ultrasonic waves, filter, and use the filtrate as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak, having the relative retention time of about 0.9 to cefteram pivoxil, obtained from the sample solution is not larger than 1.75 times the peak area of cefteram pivoxil obtained from the standard solution, and the area of the peak, having the relative retention time of about 0.1 from the sample solution is not larger than 17/25 times the peak area of cefteram pivoxil from the standard solution. Furthermore, the total area of the peaks other than cefteram pivoxil from the sample solution is not larger than 3.7 times the peak area of cefteram pivoxil from the standard solution. For the area of the peak, having the relative retention time of about 0.1 to cefteram pivoxil, multiply the relative response factor, 0.74.

Operating conditions—

Proceed as directed in the operating conditions in the Purity (2) under Cefteram Pivoxil.

System suitability—

Proceed as directed in the system suitability in the Purity (2) under Cefteram Pivoxil.

Water <2.48> Not more than 4.0% (a quantity equivalent to 0.2 g (potency) of powdered Cefteram Pivoxil Tablets, volumetric titration, direct titration).

Uniformity of dosage units <6.02> Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Cefteram Pivoxil Tablets add exactly 5 mL of the internal standard solution per 50 mg (potency) of Cefteram Pivoxil, and add diluted acetonitrile (1 in 2) to make V mL so that each mL contains about 1 mg (potency) of Cefteram Pivoxil. Disperse this solution with ultrasonic waves, filter through a membrane filter with pore size of not exceeding 0.45 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Cefteram Pivoxil Mesitylene Sulfonate RS, equivalent to about 50 mg (potency), dissolve in 20 mL of diluted acetonitrile (1 in 2), add exactly 5 mL of the internal standard solution, add diluted acetonitrile (1 in 2) to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefteram Pivoxil.

Amount [mg (potency)] of cefteram ($C_{16}H_{17}N_9O_5S_2$)
$= M_S \times Q_T/Q_S \times V/50$

M_S: Amount [mg (potency)] of Cefteram Pivoxil Mesitylene Sulfonate RS taken

Internal standard solution—A solution of methyl parahydroxybenzoate in diluted acetonitrile (1 in 2) (1 in 1000).

Dissolution <6.10> When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Cefteram Pivoxil Tablets is not less than 75%.

Start the test with 1 tablet of Cefteram Pivoxil Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 22 μg (potency) of Cefteram Pivoxil, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefteram Pivoxil Mesitylene Sulfonate RS, equivalent to about 22 mg (potency), and dissolve in 20 mL of methanol, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank, and determine the absorbances, A_T and A_S, at 300 nm.

Dissolution rate (%) with respect to the labeled amount of cefteram ($C_{16}H_{17}N_9O_5S_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount [mg (potency)] of Cefteram Pivoxil Mesitylene Sulfonate RS taken

C: Labeled amount [mg (potency)] of cefteram

($C_{16}H_{17}N_9O_5S_2$) in 1 tablet

Assay To a number of tablet of Cefteram Pivoxil Tablets, equivalent to about 1.0 g (potency) of Cefteram Pivoxil, add 120 mL of diluted acetonitrile (1 in 2), disperse with ultrasonic waves, and add diluted acetonitrile (1 in 2) to make exactly 200 mL. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add diluted acetonitrile (1 in 2) to make 50 mL, filter through a membrane filter with pore size not exceeding 0.45 μm, discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately an amount of Cefteram Pivoxil Mesitylene Sulfonate RS, equivalent to about 50 mg (potency), dissolve in 20 mL of diluted acetonitrile (1 in 2), add exactly 5 mL of the internal standard solution, add diluted acetonitrile (1 in 2) to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefteram Pivoxil.

Amount [mg (potency)] of cefteram ($C_{16}H_{17}N_9O_5S_2$)
$= M_S \times Q_T/Q_S \times 20$

M_S: Amount [mg (potency)] of Cefteram Pivoxil Mesitylene Sulfonate RS taken

Internal standard solution—A solution of methyl parahydroxybenzoate in diluted acetonitrile (1 in 2) (1 in 1000).

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Ceftibuten Hydrate

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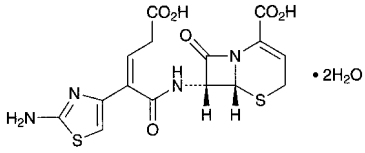

$C_{15}H_{14}N_4O_6S_2.2H_2O$: 446.46
(6*R*,7*R*)-7-[(2*Z*)-2-(2-Aminothiazol-4-yl)-4-carboxybut-2-enoylamino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid dihydrate
[*118081-34-8*]

Ceftibuten Hydrate contains not less than 900 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Ceftibuten Hydrate is expressed as mass (potency) of ceftibuten ($C_{15}H_{14}N_4O_6S_2$: 410.42).

Description Ceftibuten Hydrate occurs as a white to pale yellowish white crystalline powder.

It is freely soluble in *N*,*N*-dimethylformamide and in dimethyl sulfoxide, and practically insoluble in water, in ethanol (95) and in diethyl ether.

Identification (1) Determine the absorption spectrum of a solution of Ceftibuten Hydrate in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ceftibuten Hydrate as directed in the paste method under the Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^{1}H spectrum of a solution of Ceftibuten Hydrate in deuterated dimethyl sulfoxide for nuclear magnetic resonance spectroscopy (1 in 30), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>: it exhibits double signals A and B, at around δ 3.2 ppm and at around δ 5.1 ppm, a quartet signal C, at around δ 5.8 ppm, and a single signal D, at around δ 6.3 ppm. The ratio of integrated intensity of each signal except the signal at around δ 3.2 ppm, B:C:D is about 1:1:1.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +135 – +155° (0.3 g calculated on the anhydrous basis, 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0, 50 mL, 100 mm).

Purity (1) Heavy metals <*1.07*>—Proceed with 2.0 g of Ceftibuten Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—(i) Keep the sample solution and the standard solution at not exceeding 5°C and use within 2 hours after preparation. Dissolve 25 mg of Ceftibuten Hydrate in 20 mL of 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0. To 4 mL of this solution add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make 20 mL, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than ceftibuten obtained from the sample solution is not larger than 1/5 times the peak area of ceftibuten obtained from the standard solution, and the total area of the peaks other than ceftibuten from the sample solution is not larger than the peak area of ceftibuten from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 1.7 times as long as the retention time of ceftibuten, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) to make exactly 20 mL. Confirm that the peak area of ceftibuten obtained from 5 μL of this solution is equivalent to 7 to 13% of that of ceftibuten obtained from 5 μL of the standard solution.

System performance: Dissolve 5 mg of Ceftibuten Hydrate in 20 mL of 0.1 mol/L hydrochloric acid TS, and allow to stand at 40°C for 1 hour. To 4 mL of this solution add 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) to make 25 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, trans-isomer of ceftibuten and ceftibuten are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 5 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ceftibuten is not more than 2.0%.

(ii) Keep the sample solution at not exceeding 5°C, and use within 24 hours after preparation. To 5 mg of Ceftibuten Hydrate add 20 mL of the mobile phase, agitate with the aid of ultrasonic waves, if necessary, then shake to dissolve, filter through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate their amounts by the area percentage method: the total amount of the peaks that are eluted faster than ceftibuten is not more than 5.0%. For the areas of these peaks, multiply the relative response factor, 1.63, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 263 nm).

Column: A stainless steel column 7.5 mm in inside diameter and 60 cm in length, packed with glycol etherified silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.05 g of disodium hydrogen phosphate dodecahydrate and 0.58 g of potassium dihydrogen phosphate in water to make 1000 mL.

Flow rate: Adjust so that the retention time of ceftibuten is about 20 minutes.

Time span of measurement: About 1.6 times as long as the retention time of ceftibuten.

System suitability—

Test for required detectability: To 1 mL of the sample solution add the mobile phase to make 20 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of ceftibuten obtained from 10 μL of this solution is equivalent to 7 to 13% of that of ceftibuten obtained from 10 μL of the solution for system suitability test.

System performance: When the procedure is run with 10 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ceftibuten are not less than 10,000 and 0.8 – 1.2, respectively.

System repeatability: When the test is repeated 5 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of ceftibuten is not more than 1.7%.

Water <2.48> Not less than 8.0% and not more than 13.0% (0.2 g, volumetric titration, direct titration. Use a mixture of pyridine for water determination and ethylene glycol for water determination (5:1) instead of methanol for water determination).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Keep the sample solution and the standard solution at not exceeding 5°C and use within 2 hours after preparation. Weigh accurately an amount of Ceftibuten Hydrate and Ceftibuten Hydrochloride RS, equivalent to about 10 mg (potency), dissolve each in about 36 mL of 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0), add exactly 4 mL each of the internal standard solution, shake, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ceftibuten to that of the internal standard.

Amount [μg (potency)] of ceftibuten ($C_{15}H_{14}N_4O_6S_2$) = $M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Ceftibuten Hydrochloride RS taken

Internal standard solution—A solution of methyl parahydroxybenzoate in acetonitrile (3 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 263 nm).

Column: A stainless steel column 4 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.005 mol/L *n*-decyl trimethylammonium bromide TS and acetonitrile (4:1).

Flow rate: Adjust so that the retention time of ceftibuten is about 10 minutes.

System suitability—

System performance: Dissolve 5 mg of Ceftibuten Hydrate in 20 mL of 0.1 mol/L hydrochloric acid TS, and allow to stand at 40°C for 1 hour. To 4 mL of this solution add 0.1 mol/L phosphate buffer solution for antibiotics (pH 8.0) to make 25 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, trans-isomer of ceftibuten and ceftibuten are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ceftibuten to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and not exceeding 5°C.

Ceftizoxime Sodium

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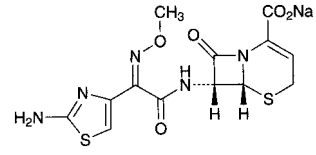

$C_{13}H_{12}N_5NaO_5S_2$: 405.38

Monosodium (6*R*,7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetylamino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

[*68401-82-1*]

Ceftizoxime Sodium contains not less than 925 μg (potency) and not more than 965 μg (potency) per mg, calculated on the anhydrous basis. The potency of Ceftizoxime Sodium is expressed as mass (potency) of ceftizoxime ($C_{13}H_{13}N_5O_5S_2$: 383.40).

Description Ceftizoxime Sodium occurs as a white to light yellow, crystals or crystalline powder.

It is very soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Ceftizoxime Sodium (1 in 63,000) as directed

under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ceftizoxime Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Ceftizoxime Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21>, using sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ 4.0 ppm, a multiple signal B around δ 6.3 ppm, and a single signal C at around δ 7.0 ppm. The ratio of integrated intensity of each signal, A:B:C, is about 3:1:1.

(4) Ceftizoxime Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +125 – +145° (0.25 g calculated on the anhydrous bases, water, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Ceftizoxime Sodium in 10 mL of water: the pH of the solution is between 6.0 and 8.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Ceftizoxime Sodium in 10 mL of water: the solution is clear, and colorless to light yellow.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Ceftizoxime Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 2.0 g of Ceftizoxime Sodium according to Method 3, and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 0.11 g of Ceftizoxime Sodium in 100 mL of 0.1 mol/L phosphate buffer solution (pH 7.0) and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the areas of each peak by the automatic integration method: each peak area other than ceftizoxime is not more than 0.5% of the peak area of ceftizoxime, and the total area of peaks other than ceftizoxime is not more than 1.0% of that of ceftizoxime.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: Dissolve 2.31 g of disodium hydrogenphosphate dodecahydrate and 1.42 g of citric acid monohydrate in 1000 mL of water, adjust to pH 3.6 with diluted phosphoric acid (1 in 10) or dilute sodium hydroxide TS. To 200 mL of this solution add 10 mL of acetonitrile.

Flow rate: Adjust so that the retention time of ceftizoxime is about 12 minutes.

Time span of measurement: About 5 times as long as the retention time of ceftizoxime, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the sample solution, add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 100 mL, and use this solution as the solution for test for required detectability. Pipet 1 mL of the solution, add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 10 mL, and confirm that the peak area of ceftizoxime obtained from 5 μL of this solution is equivalent to 7 to 13% of that of ceftizoxime obtained from 5 μL of the solution for test for required detectability.

System performance: Dissolve about 10 mg of Ceftizoxime RS in 100 mL of 0.1 mol/L phosphate buffer solution (pH 7.0) and use this solution as the solution for system suitability test. When the procedure is run with 5 μL of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ceftizoxime are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of ceftizoxime is not more than 2.0%.

Water <2.48> Not more than 8.5% (0.4 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Ceftizoxime Sodium and Ceftizoxime RS, equivalent to about 0.1 g (potency), and dissolve each in 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 20 mL. Pipet 2 mL each of these solutions, add exactly 10 mL of the internal standard solution, then add 0.1 mol/L phosphate buffer solution (pH 7.0) to make 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ceftizoxime to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of ceftizoxime } (C_{13}H_{13}N_5O_5S_2) = M_S \times Q_T/Q_S \times 1000$$

M_S: Amount [mg (potency)] of Ceftizoxime RS taken

Internal standard solution—A solution of 3-hydroxybenzoic acid in 0.1 mol/L phosphate buffer solution (pH 7.0) (3 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 2.31 g of disodium hydrogenphosphate dodecahydrate and 1.42 g of citric acid monohydrate in 1000 mL of water, and adjust to pH 3.6 with diluted phosphoric acid (1 in 10) or dilute sodium hydroxide TS. To 450 mL of this solution add 50 mL of acetonitrile.

Flow rate: Adjust so that the retention time of ceftizoxime is about 4 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, ceftizoxime and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.0 and the symmetry factor of each peak is not more than 2.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ceftizoxime to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Ceftriaxone Sodium Hydrate

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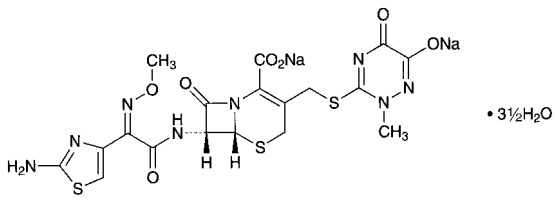


$C_{18}H_{16}N_8Na_2O_7S_3.3\frac{1}{2}H_2O$: 661.60
Disodium (6*R*,7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetylamino]-3-(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-ylsulfanylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate hemiheptahydrate
[*104376-79-6*]

Ceftriaxone Sodium Hydrate contains not less than 905 μg (potency) and not more than 935 μg (potency) per mg, calculated on the anhydrous basis. The potency of Ceftriaxone Sodium Hydrate is expressed as mass (potency) of ceftriaxone ($C_{18}H_{18}N_8O_7S_3$: 554.58).

Description Ceftriaxone Sodium Hydrate occurs as a white to yellowish white crystalline powder.

It is freely soluble in water and in dimethylsulfoxide, sparingly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in acetonitrile.

Identification **(1)** Determine the absorption spectrum of a solution of Ceftriaxone Sodium Hydrate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ceftriaxone Sodium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the ^1H spectrum of a solution of Ceftriaxone Sodium Hydrate in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A, B, C and D, at around δ 3.5 ppm, at around δ 3.8 ppm, at around δ 6.7 ppm and at around δ 7.2 ppm, respectively. The ratio of integrated intensity of each signal, A: B: C: D, is about 3:3:1:2. When the signal at around δ 3.5 ppm overlaps with the signal of water, perform the measurement in the probe kept at about 50°C.

(3) Ceftriaxone Sodium Hydrate responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -153 – $-170°$ (50 mg calculated on the anhydrous basis, water, 2.5 mL, 20 mm).

pH <*2.54*> Dissolve 0.6 g of Ceftriaxone Sodium Hydrate in 5 mL of water: the pH of the solution is between 6.0 and 8.0.

Purity **(1)** Clarity and color of solution—Dissolve 0.6 g of Ceftriaxone Sodium Hydrate in 5 mL of water: the solution is clear and light yellow.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Ceftriaxone Sodium Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Ceftriaxone Sodium Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances 1—Dissolve 20 mg of Ceftriaxone Sodium Hydrate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and acetonitrile for liquid chromatography (11:9) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of the impurity 1 having the relative retention time of about 0.5 and the impurity 2 having the relative retention time of about 1.3 to ceftriaxone from the sample solution are not larger than the peak area of ceftriaxone from the standard solution. For the areas of the peaks, the impurity 1 and the impurity 2, multiply their relative response factors 0.9 and 1.2, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.796 g of anhydrous disodium hydrogen phosphate and 3.522 g of potassium dihydrogen phosphate in water to make exactly 1000 mL, and use this solution as the solution A. Separately, dissolve 20.256 g of citric acid monohydrate and 7.840 g of sodium hydroxide in water to make exactly 1000 mL, and use this solution as the solution B. Dissolve 4.00 g of tetra-*n*-heptylammonium bromide in 450 mL of acetonitrile for liquid chromatography, add 55 mL of the solution A, 5 mL of the solution B and 490 mL of water.

Flow rate: Adjust so that the retention time of ceftriaxone is about 7 minutes.

Time span of measurement: About 2 times as long as the retention time of ceftriaxone.

System suitability—

Test for required detectability: To 5 mL of the sample solution, add a mixture of water and acetonitrile for liquid chromatography (11:9) to make 200 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add a mixture of water and acetonitrile for liquid chromatography (11:9) to make exactly 100 mL. Confirm that the peak area of ceftriaxone obtained from 10 μL of this solution is equivalent to 0.9 to 1.1% of that obtained from 10 μL of the solution for system suitability test.

System performance: Dissolve 10 mg of Ceftriaxone Sodium Hydrate in a mixture of water and acetonitrile for liquid chromatography (11:9) to make 5 mL, add 5 mL of a solution of diethyl terephthalate in a mixture of water and acetonitrile for liquid chromatography (11:9) (9 in 5000), and add a mixture of water and acetonitrile for liquid chromatography (11:9) to make 200 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ceftriaxone and diethyl terephthalate are eluted in this order, with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of ceftriaxone is not more than 1.0%.

(5) Related substances 2—Dissolve 10 mg of Ceftriaxone Sodium Hydrate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetonitrile for liquid chromatography and water (23:11) to make exactly 100 mL, and use this solution as standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the each peak area of the impurities which appear after the peak of ceftriaxone from the sample solution is not larger than the peak area of ceftriaxone from the standard solution, and the total peak area of these impurities is not larger than 2.5 times of the peak area from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.796 g of anhydrous disodium hydrogen phosphate and 3.522 g of potassium dihydrogen phosphate in water to make exactly 1000 mL, and use this solution as the solution A. Separately, dissolve 20.256 g of citric acid monohydrate and 7.840 g of sodium hydroxide in water to make exactly 1000 mL, and use this solution as the solution B. Dissolve 4.00 g of tetra-*n*-heptylammonium bromide in 450 mL of acetonitrile for liquid chromatography, and add 55 mL of the solution A, 5 mL of the solution B, 490 mL of water and 700 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust so that the retention time of ceftriaxone is about 3 minutes.

Time span of measurement: About 10 times as long as the retention time of ceftriaxone.

System suitability—

Test for required detectability: Measure 5 mL of the sample solution, add a mixture of acetonitrile for liquid chromatography and water (23:11) to make 100 mL, and use this solution as the solution for system suitability test. Measure exactly 1 mL of the solution for system suitability test, and add a mixture of acetonitrile for liquid chromatography and water (23:11) to make exactly 100 mL. Confirm that the peak area of ceftriaxone obtained from 10 μL of this solution is equivalent to 0.9 to 1.1% of that obtained from 10 μL of the solution for system suitability test.

System performance: Dissolve 10 mg of Ceftriaxone Sodium Hydrate in a mixture of acetonitrile for liquid chromatography and water (23:11) to make 5 mL, add 5 mL of a solution of diethyl terephthalate in a mixture of water and acetonitrile for liquid chromatography (11:9) (9 in 5000), and add a mixture of acetonitrile for liquid chromatography and water (23:11) to make 200 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ceftriaxone and diethyl terephthalate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of ceftriaxone is not more than 1.0%.

Water <2.48> Not less than 8.0% and not more than 11.0% (0.15 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Ceftriaxone Sodium Hydrate and Ceftriaxone Sodium RS, equivalent to about 0.1 g (potency), dissolve each in a mixture of water and acetonitrile for liquid chromatography (11:9) to make exactly 50 mL. Pipet 5 mL of each solution, add exactly 5 mL of the internal standard solution and a mixture of water and acetonitrile for liquid chromatography (11:9) to make 200 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ceftriaxone to that of the internal standard.

Amount [μg (potency)] of ceftriaxone ($C_{18}H_{18}N_8O_7S_3$) $= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Ceftriaxone Sodium RS taken

Internal standard solution—A solution of diethyl terephthalate in a mixture of water and acetonitrile for liquid chromatography (11:9) (9 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.796 g of anhydrous disodium hydrogen phosphate and 3.522 g of potassium dihydrogen phosphate in water to make exactly 1000 mL, and use this solution as solution A. Dissolve 20.256 g of citric acid monohydrate and 7.840 g of sodium hydroxide in water to make exactly 1000 mL, and use this solution as solution B. Dissolve 4.00 g of tetra-*n*-heptylammonium bromide in 450 mL of acetonitrile for liquid chromatography, and add 490 mL of water, 55 mL of solution A, and 5 mL of solution B.

Flow rate: Adjust so that the retention time of ceftriaxone is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ceftriaxone and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

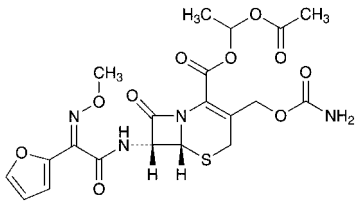
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ceftriaxone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cefuroxime Axetil

セフロキシム　アキセチル



$C_{20}H_{22}N_4O_{10}S$: 510.47

(1*RS*)-1-Acetoxyethyl (6*R*,7*R*)-3-carbamoyloxymethyl-7-[(*Z*)-2-furan-2-yl-2-(methoxyimino)acetylamino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

[*64544-07-6*]

Cefuroxime Axetil contains not less than 800 μg (potency) and not more than 850 μg (potency) per mg, calculated on the anhydrous and acetone-free basis. The potency of Cefuroxime Axetil is expressed as mass (potency) of cefuroxime ($C_{16}H_{16}N_4O_8S$: 424.39).

Description Cefuroxime Axetil occurs as white to yellowish white, non-crystalline powder.

It is freely soluble in dimethylsulfoxide, soluble in methanol, sparingly soluble in ethanol (95), and very slightly soluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Cefuroxime Axetil in methanol (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefuroxime Axetil RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefuroxime Axetil as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefuroxime Axetil RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the ^1H spectrum of a solution of Cefuroxime Axetil in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 20) as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a double signal or a pair of double signals A at around δ 1.5 ppm, a pair of single signals B at around δ 2.1 ppm, and a single signal C at around δ 3.9 ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 1:1:1.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +41 – +47° (0.5 g, methanol, 50 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Cefuroxime Axetil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 25 mg of Cefuroxime Axetil in 4 mL of methanol, add a solution of ammonium dihydrogenphosphate (23 in 1000) to make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add 40 mL of methanol and a solution of ammonium dihydrogenphosphate (23 in 1000) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than cefuroxime axetil obtained from the sample solution is not larger than 1.5 times the total area of the two peaks of cefuroxime axetil obtained from the standard solution, and the total area of the peaks other than cefuroxime axetil from the sample solution is not larger than 4 times the total area of the two peaks of cefuroxime axetil from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of the peak having the larger retention time of the two peaks of cefuroxime axetil, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add 4 mL of methanol and a solution of ammonium dihydrogenphosphate (23 in 1000) to make exactly 10 mL. Confirm that the total area of the two peaks of cefuroxime axetil obtained with 2 μL of this solution is equivalent to 7 to 13% of that obtained with 2 μL of the standard solution.

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the resolution between the two peaks of cefuroxime axetil is not less than 1.5.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the total area of the two peaks of cefuroxime axetil is not more than 2.0%.

(3) Acetone—Weigh accurately about 1 g of Cefuroxime Axetil, add exactly 0.2 mL of the internal standard solution and dimethylsulfoxide to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.5 g of acetone, and add dimethylsulfoxide to make exactly 100 mL. Pipet 0.2 mL of this solution, add exactly 0.2 mL of the internal standard solution and dimethylsulfoxide to make 10 mL, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of acetone to that of the internal standard: the amount of acetone is not more than 1.3%.

$$\text{Amount (\%) of acetone} = M_S/M_T \times Q_T/Q_S \times 1/5$$

M_S: Amount (g) of acetone taken

M_T: Amount (g) of Cefuroxim Axetil taken

Internal standard solution—A solution of 1-propanol in dimethylsulfoxide (1 in 200).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography coated with a mixture of polyethylene glycol 600 for gas chromatography and polyethylene glycol 1500 for gas chromatography (1:1) in the ratio of 20% (125 – 150 μm in particle diameter).

Column temperature: A constant temperature of about 90°C.

Temperature of injection port: A constant temperature of about 115°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the retention time of the internal standard is about 4 minutes.

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, acetone and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of acetone to that of the internal standard is not more than 5.0%.

Water ⟨*2.48*⟩ Not more than 2.0% (0.4 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (0.5 g).

Isomer ratio Perform the test with 10 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the area, A_a, of the peak having the smaller retention time and the area, A_b, of the peak having the bigger retention time of the two peaks of cefuroxime axetil: $A_b/(A_a + A_b)$ is between 0.48 and 0.55.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Assay Weigh accurately an amount of Cefuroxime Axetil and Cefuroxime Axetil RS, equivalent to about 50 mg (potency), and dissolve each in methanol to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 5 mL of the internal standard solution, 5 mL of methanol and a solution of ammonium dihydrogen phosphate (23 in 1000) to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the sum area of the two peaks of cefuroxime axetil to the peak area of the internal standard.

Amount [μg (potency)] of cefuroxime ($C_{16}H_{16}N_4O_8S$) $= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Cefuroxime Axetil RS taken

Internal standard solution—A solution of acetanilide in methanol (27 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 278 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 20 cm in length, packed with trimethylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of ammonium dihydrogen phosphate (23 in 1000) and methanol (5:3).

Flow rate: Adjust so that the retention time of the peak having the smaller retention time of the two peaks of cefuroxime axetil is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and cefuroxime axetil are eluted in this order with the resolution between the two peaks of cefuroxime axetil being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the sum area of the two peaks of cefuroxime axetil to the peak area of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cellacefate

Cellulose Acetate Phthalate

セラセフェート

[*9004-38-0*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Cellacefate is a reaction product of phthalic anhydride and partially acetylated cellulose.

It contains not less than 21.5% and not more than 26.0% of acetyl group (-$COCH_3$: 43.04), and not less than 30.0% and not more than 36.0% of carboxybenzoyl group (-COC_6H_4COOH: 149.12), calculated on the anhydrous and free acid-free basis.

◆**Description** Cellacefate occurs as a white powder or grain.

It is freely soluble in acetone, and practically insoluble in water and in ethanol (99.5).◆

Identification Determine the infrared absorption spectrum of Cellacefate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Cellacefate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Viscosity ⟨*2.53*⟩ Weigh accurately a quantity of Cellacefate, equivalent to 15 g calculated on the anhydrous basis, dissolve in 85 g of a mixture of acetone and water (249: 1 in mass), and use this solution as the sample solution. Perform the test with the sample solution at 25 ± 0.2°C as directed in Method 1 to obtain the kinematic viscosity v. Separately, determine the density, ρ, of the sample solution as directed under Determination of Specific Gravity and Density ⟨*2.56*⟩, and calculate the viscosity of the sample solution, η, as $\eta = \rho v$: not less than 45 mPa·s and not more than 90 mPa·s.

Purity **(1)** ◆Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Cellacefate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).◆

(2) Free acids—Weigh accurately about 3 g of Cellacefate, put in a glass-stoppered conical flask, add 100 mL of diluted methanol (1 in 2), stopper tightly, and filter after shaking for 2 hours. Wash both the flask and residue with

two 10-mL portions each of diluted methanol (1 in 2), combine the washes to the filtrate, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 2-3 drops of phenolphthalein TS). Perform the blank determination with 120 mL of diluted methanol (1 in 2), and make any necessary correction.

$$\text{Amount (\%) of free acids} = 0.8306A/M$$

A: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed
M: Amount (g) of Cellacefate taken, calculated on the anhydrous basis

The amount of free acids is not more than 3.0%, calculated as phthalic acid ($C_8H_6O_4$: 166.13).

Water <2.48> Not more than 5.0% (0.5 g, volumetric titration, direct titration, using a mixture of ethanol (99.5) and dichloromethane (3:2) instead of methanol for water determination).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay (1) Carboxybenzoyl group—Weigh accurately about 1 g of Cellacefate, dissolve in 50 mL of a mixture of ethanol (95) and acetone (3:2), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 2 – 3 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

$$\text{Content (\%) of carboxybenzoyl group } (C_8H_5O_3) = \frac{\dfrac{1.491 \times A}{M} - (1.795 \times B)}{100 - B} \times 100$$

A: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed
B: Amount (%) of free acids obtained in the Purity (2)
M: Amount (g) of Cellacefate taken, calculated on the anhydrous basis

(2) Acetyl group—Weigh accurately about 0.1 g of Cellacefate, put in a glass-stoppered conical flask, add exactly 25 mL of 0.1 mol/L sodium hydroxide VS, and boil for 30 minutes under a reflux condenser. After cooling, add 2 – 3 drops of phenolphthalein TS, and titrate <2.50> the excess of sodium hydroxide with 0.1 mol/L hydrochloric acid VS. Perform a blank determination.

$$\text{Content (\%) of free acids and bound acetyl group } (C_2H_3O) = 0.4305A/M$$

A: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed, corrected by the blank determination
M: Amount (g) of Cellacefate taken, calculated on the anhydrous basis

$$\text{Content (\%) of acetyl group } (C_2H_3O) = 100 \times (P - 0.5182B)/(100 - B) - 0.5772C$$

B: Amount (%) of free acids obtained in the Purity (2)
C: Content (%) of carboxybenzoyl group
P: Content (%) of free acids and bound acetyl group (C_2H_3O)

◆**Containers and storage** Containers—Tight containers.◆

Microcrystalline Cellulose

結晶セルロース

[*9004-34-6*, cellulose]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Microcrystalline Cellulose is purified, partially depolymerized α-cellulose, obtained as a pulp from fibrous plant material, with mineral acids.

◆The label indicates the mean degree of polymerization, loss on drying, and bulk density values with a range.◆

◆**Description** Microcrystalline Cellulose occurs as a white crystalline powder having fluidity.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It swells with sodium hydroxide TS on heating.◆

Identification (1) Dissolve 20 g of zinc chloride and 6.5 g of potassium iodide in 10.5 mL of water, add 0.5 g of iodine, and shake for 15 minutes. Place about 10 mg of Microcrystalline Cellulose on a watch glass, and disperse in 2 mL of this solution: the substance develops a blue-violet color.

◆**(2)** Sieve 20 g of Microcrystalline Cellulose for 5 minutes on an air-jet sieve equipped with a screen (No.391, 200 mm in inside diameter) having 38-μm openings. If more than 5% is retained on the screen, mix 30 g of Microcrystalline Cellulose with 270 mL of water; otherwise, mix 45 g with 255 mL of water. Perform the mixing for 5 minutes in a high-speed (18,000 revolutions per minute or more) power blender. Transfer 100 mL of the dispersion to a 100-mL graduated cylinder, and allow to stand for 3 hours: a white, opaque, bubble-free dispersion, which does not form a supernatant liquid at the surface, is obtained.◆

(3) Transfer 1.3 g of Microcrystalline Cellulose, accurately weighed, to a 125-mL conical flask, and add exactly 25 mL each of water and 1 mol/L cupriethylenediamine TS. Immediately purge the solution with nitrogen, insert the stopper, and shake on a suitable mechanical shaker to dissolve. Perform the test with a suitable amount of this solution, taken exactly, according to Method 1 under Viscosity Determination <2.53> using a capillary viscometer having the viscosity constant (K) of approximately 0.03, at 25 ± 0.1°C, and determine the kinematic viscosity, ν. Separately, perform the test with a mixture of exactly 25 mL each of water and 1 mol/L cupriethylenediamine TS in the same manner as above, using a capillary viscometer having K of approximately 0.01, and determine the kinematic viscosity, ν_o.

Calculate the relative viscosity, η_{rel}, of Microcrystalline Cellulose by the formula:

$$\eta_{rel} = \nu/\nu_o$$

Obtain the product, $[\eta]C$, of intrinsic viscosity $[\eta]$(mL/g) and concentration C (g/100 mL) from the value η_{rel} of the Table. When calculate the degree of polymerization, P, by the following formula, P is not more than 350 ◆and within the labeled range.◆

$$P = (95)[\eta]C/M_T$$

M_T: Amount (g) of the sample taken, calculated on the dried basis

pH ⟨*2.54*⟩ Shake 5.0 g of Microcrystalline Cellulose with 40 mL of water for 20 minutes, and centrifuge: the pH of the supernatant liquid is between 5.0 and 7.5.

Purity ◆**(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Microcrystalline Cellulose according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).◆

(2) Water-soluble substances—Shake 5.0 g of Microcrystalline Cellulose with 80 mL of water for 10 minutes, filter with the aid of vacuum through a filter paper for quantitative analysis (5C) into a vacuum flask. Evaporate the clear filtrate in a tared evaporating dish to dryness without charring, dry at 105°C for 1 hour, cool in a desiccator, and weigh: the difference between the mass of the residue and the mass obtained from a blank determination does not exceed 12.5 mg.

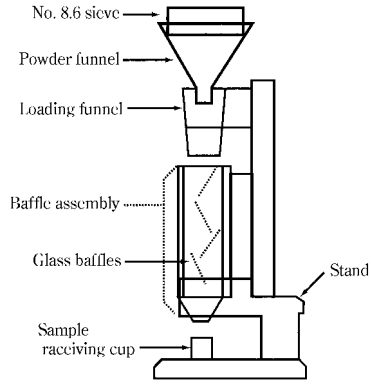
(3) Diethyl ether-soluble substances—Place 10.0 g of Microcrystalline Cellulose in a column having an internal diameter of about 20 mm, and pass 50 mL of peroxide-free diethyl ether through the column. Evaporate the eluate to dryness in a previously dried and tared evaporation dish. Dry the residue at 105°C for 30 minutes, allow to cool in a desiccator, and weigh: the difference between the mass of the residue and the mass obtained from a blank determination does not exceed 5.0 mg.

Conductivity ⟨*2.51*⟩ Perform the test as directed in the Conductivity Measurement with the supernatant liquid obtained in the pH as the sample solution, and determine the conductivity ◆at 25 ± 0.1°C.◆ Determine in the same way the conductivity of water used for the preparation of the sample solution: the difference between these conductivities is not more than 75 $\mu S \cdot cm^{-1}$.

Loss on drying ⟨*2.41*⟩ Not more than 7.0% ◆and within a range as specified on the label◆ (1 g, 105°C. 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% ◆(2 g).◆

Bulk density (i) Apparatus—Use a volumeter shown in the figure. Put a No.8.6 sieve (2000 μm) on the top of the volumeter. A funnel is mounted over a baffle box, having four glass baffle plates inside which the sample powder slides



as it passes. At the bottom of the baffle box is a funnel that collect the powder, and allows it to pour into a sample receiving cup mounted directly below it.

(ii) Procedure—Weigh accurately the mass of a brass or stainless steel cup, which has a capacity of 25.0 ± 0.05 mL and an inside diameter of 30.0 ± 2.0 mm, and put the cup directly below the funnel of the volumeter. Slowly pour Microcrystalline Cellulose 5.1 cm height from the upper part of the powder funnel through the sieve, at a rate suitable to prevent clogging, until the cup overflows. If the clogging occurs, take out the sieve. Level the excess powder with the aid of a slide glass, weigh the filled cup, and weigh accurately the content of the cup, and then calculate the bulk density by the following expression: the bulk density is within the labeled specification.

$$\text{Bulk density } (g/cm^3) = A/25$$

A: Measured mass (g) of the content of the cup

◆**Microbial limit** ⟨*4.05*⟩ The acceptance criteria of TAMC and TYMC are 10^3 CFU/g and 10^2 CFU/g, respectively. *Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are not observed.◆

◆**Containers and storage** Containers—Tight containers.◆

Table for Conversion of Relative Viscosity (η_{rel}) into the Product of Limiting Viscosity and Concentration ($[\eta]C$)

	$[\eta]C$									
η_{rel}	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
1.1	0.098	0.106	0.115	0.125	0.134	0.143	0.152	0.161	0.170	0.180
1.2	0.189	0.198	0.207	0.216	0.225	0.233	0.242	0.250	0.259	0.268
1.3	0.276	0.285	0.293	0.302	0.310	0.318	0.326	0.334	0.342	0.350
1.4	0.358	0.367	0.375	0.383	0.391	0.399	0.407	0.414	0.422	0.430
1.5	0.437	0.445	0.453	0.460	0.468	0.476	0.484	0.491	0.499	0.507
1.6	0.515	0.522	0.529	0.536	0.544	0.551	0.558	0.566	0.573	0.580
1.7	0.587	0.595	0.602	0.608	0.615	0.622	0.629	0.636	0.642	0.649
1.8	0.656	0.663	0.670	0.677	0.683	0.690	0.697	0.704	0.710	0.717
1.9	0.723	0.730	0.736	0.743	0.749	0.756	0.762	0.769	0.775	0.782
2.0	0.788	0.795	0.802	0.809	0.815	0.821	0.827	0.833	0.840	0.846
2.1	0.852	0.858	0.864	0.870	0.876	0.882	0.888	0.894	0.900	0.906
2.2	0.912	0.918	0.924	0.929	0.935	0.941	0.948	0.953	0.959	0.965
2.3	0.971	0.976	0.983	0.988	0.994	1.000	1.006	1.011	1.017	1.022
2.4	1.028	1.033	1.039	1.044	1.050	1.056	1.061	1.067	1.072	1.078
2.5	1.083	1.089	1.094	1.100	1.105	1.111	1.116	1.121	1.126	1.131
2.6	1.137	1.142	1.147	1.153	1.158	1.163	1.169	1.174	1.179	1.184
2.7	1.190	1.195	1.200	1.205	1.210	1.215	1.220	1.225	1.230	1.235
2.8	1.240	1.245	1.250	1.255	1.260	1.265	1.270	1.275	1.280	1.285
2.9	1.290	1.295	1.300	1.305	1.310	1.314	1.319	1.324	1.329	1.333

	$[\eta]C$									
η_{rel}	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
3.0	1.338	1.343	1.348	1.352	1.357	1.362	1.367	1.371	1.376	1.381
3.1	1.386	1.390	1.395	1.400	1.405	1.409	1.414	1.418	1.423	1.427
3.2	1.432	1.436	1.441	1.446	1.450	1.455	1.459	1.464	1.468	1.473
3.3	1.477	1.482	1.486	1.491	1.496	1.500	1.504	1.508	1.513	1.517
3.4	1.521	1.525	1.529	1.533	1.537	1.542	1.546	1.550	1.554	1.558
3.5	1.562	1.566	1.570	1.575	1.579	1.583	1.587	1.591	1.595	1.600
3.6	1.604	1.608	1.612	1.617	1.621	1.625	1.629	1.633	1.637	1.642
3.7	1.646	1.650	1.654	1.658	1.662	1.666	1.671	1.675	1.679	1.683
3.8	1.687	1.691	1.695	1.700	1.704	1.708	1.712	1.715	1.719	1.723
3.9	1.727	1.731	1.735	1.739	1.742	1.746	1.750	1.754	1.758	1.762
4.0	1.765	1.769	1.773	1.777	1.781	1.785	1.789	1.792	1.796	1.800
4.1	1.804	1.808	1.811	1.815	1.819	1.822	1.826	1.830	1.833	1.837
4.2	1.841	1.845	1.848	1.852	1.856	1.859	1.863	1.867	1.870	1.874
4.3	1.878	1.882	1.885	1.889	1.893	1.896	1.900	1.904	1.907	1.911
4.4	1.914	1.918	1.921	1.925	1.929	1.932	1.936	1.939	1.943	1.946
4.5	1.950	1.954	1.957	1.961	1.964	1.968	1.971	1.975	1.979	1.982
4.6	1.986	1.989	1.993	1.996	2.000	2.003	2.007	2.010	2.013	2.017
4.7	2.020	2.023	2.027	2.030	2.033	2.037	2.040	2.043	2.047	2.050
4.8	2.053	2.057	2.060	2.063	2.067	2.070	2.073	2.077	2.080	2.083
4.9	2.087	2.090	2.093	2.097	2.100	2.103	2.107	2.110	2.113	2.116
5.0	2.119	2.122	2.125	2.129	2.132	2.135	2.139	2.142	2.145	2.148
5.1	2.151	2.154	2.158	2.160	2.164	2.167	2.170	2.173	2.176	2.180
5.2	2.183	2.186	2.190	2.192	2.195	2.197	2.200	2.203	2.206	2.209
5.3	2.212	2.215	2.218	2.221	2.224	2.227	2.230	2.233	2.236	2.240
5.4	2.243	2.246	2.249	2.252	2.255	2.258	2.261	2.264	2.267	2.270
5.5	2.273	2.276	2.279	2.282	2.285	2.288	2.291	2.294	2.297	2.300
5.6	2.303	2.306	2.309	2.312	2.315	2.318	2.320	2.324	2.326	2.329
5.7	2.332	2.335	2.338	2.341	2.344	2.347	2.350	2.353	2.355	2.358
5.8	2.361	2.364	2.367	2.370	2.373	2.376	2.379	2.382	2.384	2.387
5.9	2.390	2.393	2.396	2.400	2.403	2.405	2.408	2.411	2.414	2.417
6.0	2.419	2.422	2.425	2.428	2.431	2.433	2.436	2.439	2.442	2.444
6.1	2.447	2.450	2.453	2.456	2.458	2.461	2.464	2.467	2.470	2.472
6.2	2.475	2.478	2.481	2.483	2.486	2.489	2.492	2.494	2.497	2.500
6.3	2.503	2.505	2.508	2.511	2.513	2.516	2.518	2.521	2.524	2.526
6.4	2.529	2.532	2.534	2.537	2.540	2.542	2.545	2.547	2.550	2.553
6.5	2.555	2.558	2.561	2.563	2.566	2.568	2.571	2.574	2.576	2.579
6.6	2.581	2.584	2.587	2.590	2.592	2.595	2.597	2.600	2.603	2.605
6.7	2.608	2.610	2.613	2.615	2.618	2.620	2.623	2.625	2.627	2.630
6.8	2.633	2.635	2.637	2.640	2.643	2.645	2.648	2.650	2.653	2.655
6.9	2.658	2.660	2.663	2.665	2.668	2.670	2.673	2.675	2.678	2.680
7.0	2.683	2.685	2.687	2.690	2.693	2.695	2.698	2.700	2.702	2.705
7.1	2.707	2.710	2.712	2.714	2.717	2.719	2.721	2.724	2.726	2.729
7.2	2.731	2.733	2.736	2.738	2.740	2.743	2.745	2.748	2.750	2.752
7.3	2.755	2.757	2.760	2.762	2.764	2.767	2.769	2.771	2.774	2.776
7.4	2.779	2.781	2.783	2.786	2.788	2.790	2.793	2.795	2.798	2.800
7.5	2.802	2.805	2.807	2.809	2.812	2.814	2.816	2.819	2.821	2.823
7.6	2.826	2.828	2.830	2.833	2.835	2.837	2.840	2.842	2.844	2.847
7.7	2.849	2.851	2.854	2.856	2.858	2.860	2.863	2.865	2.868	2.870
7.8	2.873	2.875	2.877	2.879	2.881	2.884	2.887	2.889	2.891	2.893
7.9	2.895	2.898	2.900	2.902	2.905	2.907	2.909	2.911	2.913	2.915
8.0	2.918	2.920	2.922	2.924	2.926	2.928	2.931	2.933	2.935	2.937
8.1	2.939	2.942	2.944	2.946	2.948	2.950	2.952	2.955	2.957	2.959
8.2	2.961	2.963	2.966	2.968	2.970	2.972	2.974	2.976	2.979	2.981
8.3	2.983	2.985	2.987	2.990	2.992	2.994	2.996	2.998	3.000	3.002
8.4	3.004	3.006	3.008	3.010	3.012	3.015	3.017	3.019	3.021	3.023
8.5	3.025	3.027	3.029	3.031	3.033	3.035	3.037	3.040	3.042	3.044
8.6	3.046	3.048	3.050	3.052	3.054	3.056	3.058	3.060	3.062	3.064
8.7	3.067	3.069	3.071	3.073	3.075	3.077	3.079	3.081	3.083	3.085
8.8	3.087	3.089	3.092	3.094	3.096	3.098	3.100	3.102	3.104	3.106
8.9	3.108	3.110	3.112	3.114	3.116	3.118	3.120	3.122	3.124	3.126
9.0	3.128	3.130	3.132	3.134	3.136	3.138	3.140	3.142	3.144	3.146
9.1	3.148	3.150	3.152	3.154	3.156	3.158	3.160	3.162	3.164	3.166
9.2	3.168	3.170	3.172	3.174	3.176	3.178	3.180	3.182	3.184	3.186
9.3	3.188	3.190	3.192	3.194	3.196	3.198	3.200	3.202	3.204	3.206
9.4	3.208	3.210	3.212	3.214	3.215	3.217	3.219	3.221	3.223	3.225
9.5	3.227	3.229	3.231	3.233	3.235	3.237	3.239	3.241	3.242	3.244

	[η]C									
η_{rel}	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
9.6	3.246	3.248	3.250	3.252	3.254	3.256	3.258	3.260	3.262	3.264
9.7	3.266	3.268	3.269	3.271	3.273	3.275	3.277	3.279	3.281	3.283
9.8	3.285	3.287	3.289	3.291	3.293	3.295	3.297	3.298	3.300	3.302
9.9	3.304	3.305	3.307	3.309	3.311	3.313	3.316	3.318	3.320	3.321
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
10	3.32	3.34	3.36	3.37	3.39	3.41	3.43	3.45	3.46	3.48
11	3.50	3.52	3.53	3.55	3.56	3.58	3.60	3.61	3.63	3.64
12	3.66	3.68	3.69	3.71	3.72	3.74	3.76	3.77	3.79	3.80
13	3.80	3.83	3.85	3.86	3.88	3.89	3.90	3.92	3.93	3.95
14	3.96	3.97	3.99	4.00	4.02	4.03	4.04	4.06	4.07	4.09
15	4.10	4.11	4.13	4.14	4.15	4.17	4.18	4.19	4.20	4.22
16	4.23	4.24	4.25	4.27	4.28	4.29	4.30	4.31	4.33	4.34
17	4.35	4.36	4.37	4.38	4.39	4.41	4.42	4.43	4.44	4.45
18	4.46	4.47	4.48	4.49	4.50	4.52	4.53	4.54	4.55	4.56
19	4.57	4.58	4.59	4.60	4.61	4.62	4.63	4.64	4.65	4.66

Powdered Cellulose

粉末セルロース

[*9004-34-6*, Cellulose]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Powdered Cellulose is a purified, mechanically disintegrated alpha cellulose obtained as a pulp, ◆after partial hydrolysis as occasion demands◆, from fibrous plant materials.

The label indicates the mean degree of polymerization value with a range.

◆**Description** Powdered Cellulose occurs as a white powder.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.◆

Identification **(1)** Dissolve 20 g of zinc chloride and 6.5 g of potassium iodide in 10.5 mL of water, add 0.5 g of iodine, and shake for 15 minutes. Place about 10 mg of Powdered Cellulose on a watch glass, and disperse in 2 mL of this solution: the substance develops a blue-violet color.

◆**(2)** Mix 30 g of Powdered Cellulose with 270 mL of water in a high-speed (18,000 revolutions per minute or more) blender for 5 minutes, transfer 100 mL of the dispersion to a 100-mL graduated cylinder, and allow to stand for 1 hour: a supernatant liquid appears above the layer of the cellulose.◆

(3) Transfer 0.25 g of Powdered Cellulose, accurately weighed, to a 125-mL conical flask, add exactly 25 mL each of water and 1 mol/L cupriethylenediamine TS, and proceed as directed in the Identification (3) under Microcrystalline Cellulose. The mean degree of polymerization, P, is not less than 440 and is within the labeled specification.

pH ⟨*2.54*⟩ Mix 10 g of Powdered Cellulose with 90 mL of water, and allow to stand for 1 hour with occasional stirring: the pH of the supernatant liquid is between 5.0 and 7.5.

Purity ◆**(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Powdered Cellulose according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).◆

(2) Water-soluble substances—Shake 6.0 g of Powdered Cellulose with 90 mL of recently boiled and cooled water, and allow to stand for 10 minutes with occasional shaking. Filter, with the aid of vacuum through a filter paper, discard the first 10 mL of the filtrate, and pass the subsequent filtrate through the same filter, if necessary, to obtain a clear filtrate. Evaporate a 15.0-mL portion of the filtrate in a tared evaporating dish to dryness without charring, dry at 105°C for 1 hour, and weigh after allowing to cool in a desiccator: the difference between the mass of the residue and the mass obtained from a blank determination does not exceed 15.0 mg (1.5%).

(3) Diethyl ether-soluble substances—Place 10.0 g of Powdered Cellulose in a column having an internal diameter of about 20 mm, and pass 50 mL of peroxide-free diethyl ether through the column. Evaporate the eluate to dryness in a previously dried and tared evaporation dish. Dry the residue at 105°C for 30 minutes, and weigh after allowing to cool in a desiccator: the difference between the mass of the residue and the mass obtained from a blank determination does not exceed 15.0 mg (0.15%).

Loss on drying ⟨*2.41*⟩ Not more than 6.5% (1 g, 105°C, 3 hours).

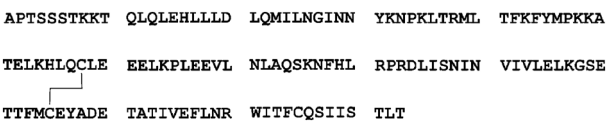
Residue on ignition ⟨*2.44*⟩ Not more than 0.3% (1 g calculated on the dried basis).

◆**Microbial limit** ⟨*4.05*⟩ The acceptance criteria of TAMC and TYMC are 10^3 CFU/g and 10^2 CFU/g, respectively. *Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are not observed.◆

◆**Containers and storage** Containers—Tight containers.◆

Celmoleukin (Genetical Recombination)

セルモロイキン(遺伝子組換え)



$C_{693}H_{1118}N_{178}O_{203}S_7$: 15415.82
[*94218-72-1*]

Celmoleukin (Genetical Recombination) is genetical recombinant human interleukin-2, and is a protein consisting of 133 amino acid residues. It is a solution. It has a T-lymphocyte activating effect.

It contains not less than 0.5 and not more than 1.5 mg of protein per mL, and 1 mg of this protein contains potency not less than 8.0×10^6 units.

Description Celmoleukin (Genetical Recombination) occurs as a clear and colorless liquid.

Identification **(1)** Add 100 μL of protein digestive enzyme TS to 100 μL of Celmoleukin (Genetical Recombination), shake, leave standing at 37°C for 18 to 24 hours, and then add 2 μL of 2-mercaptoethanol. Leave at 37°C for a further 30 minutes, and add 5 μL of trifluoroacetic acid solution (1 in 10). Use this solution as the sample solution. Separately, process with celmoleukin for liquid chromatography by using the same method. Use this solution as the standard solution. Perform the test with 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and compare the chromatograms obtained from the sample solution and standard solution: the similar peaks are observed at the same retention time.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (particle size: 5 μm).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A solution of trifluoroacetic acid (1 in 1000).

Mobile phase B: A solution of trifluoroacetic acid in a mixture of acetonitrile and water (17:3) (1 in 1000).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 5	100	0
5 – 45	100 → 60	0 → 40
45 – 75	60 → 0	40 → 100
75 – 85	0	100

Flow rate: Adjust so that the retention time of celmoleukin is about 70 minutes.

System suitability—

System performance: Add 2 μL of 2-mercaptoethanol to 100 μL of celmoleukin for liquid chromatography, leave at 37°C for 2 hours, and then run this solution under the above operating conditions. Celmoleukin and its reduced form are eluted in this order with the resolution between these peaks being not less than 1.5.

(2) Accurately measure an appropriate amount of Celmoleukin (Genetical Recombination), dilute by adding culture medium for celmoleukin, and prepare a sample solution containing 800 units per mL. Add 25 μL of the sample solution to 2 wells (A and B) of a flat-bottomed microtest plate for tissue culture, and then add 25 μL of reference anti-interleukin-2 antiserum solution diluted with culture medium for celmoleukin to well A and 25 μL of culture medium for celmoleukin to well B. Add 50 μL of culture medium for celmoleukin to another well (well C). After shaking the microtest plate, warm in air containing 5% carbon dioxide at 37°C for 30 minutes to 2 hours. Next, add to each well 50 μL of culture medium for celmoleukin containing the interleukin-2 dependent mouse natural killer cells NKC3 and culture at 37°C for 16 to 24 hours. Add 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide TS, culture at 37°C for 4 to 6 hours, and add sodium lauryl sulfate TS and leave for 24 to 48 hours. When the absorbance at 590 nm of the solution in each well is measured, the difference in absorbance between the solutions from wells A and C is not more than 3% of the difference in absorbance between the solutions from wells B and C.

Constituent amino acid When hydrolyze Celmoleukin (Genetical Recombination) according to Method 1 and Method 4 described in ''1. Hydrolysis of Protein and Peptide'', and perform the test according to Method 1 described in ''2. Methodologies of Amino Acid Analysis'' under Amino Acid Analysis of Proteins <*2.04*>, the molar ratios of the respective amino acids are as follows: glutamic acid (or glutamine) is 17 or 18, threonine is 11 to 13, aspartic acid (or asparagine) is 11 or 12, lysine is 11, isoleucine is 7 or 8, serine is 6 to 9, phenylalanine is 6, alanine is 5, proline is 5 or 6, arginine is 4, methionine is 4, cysteine is 3 or 4, valine is 3 or 4, tyrosine is 3, histidine is 3, glycine is 2, and tryptophan is 1.

Procedure

(i) Hydrolysis Based on the results of the Assay (1), place an amount of Celmoleukin (Genetical Recombination), equivalent to about 50 μg as the total protein in two hydrolysis tubes, and evaporate to dryness under vacuum. To one of the hydrolysis tubes add 100 μL of a mixture of diluted hydrochloric acid (59 in 125), mercapto acetic acid and phenol (100:10:1), and shake. Place this hydrolysis tube in a vial and humidify the inside of the vial with 200 μL of the mixture of diluted hydrochloric acid (59 in 125), mercapto acetic acid and phenol (100:10:1). Replace the vial interior with inert gas or reduce the pressure, and heat at about 115°C for 24 hours. After drying under vacuum, dissolve in 0.5 mL of 0.02 mol/L hydrochloric acid TS, and use this solution as the sample solution (1). To the other hydrolysis tube, add 100 μL of ice cold performic acid, oxidize for 1.5 hours on ice, add 50 μL of hydrobromic acid, and dry under vacuum. Add 200 μL of water, repeat the dry under vacuum procedure two more times, place the hydrolysis tube in a vial, and humidify the inside of the vial with 200 μL of diluted hydrochloric acid (59 in 125). Replace the vial interior with inert gas or reduce the pressure, and heat at about 115°C for 24 hours. After drying under vacuum, dissolve in 0.5 mL of 0.02 mol/L hydrochloric acid TS, and use this solution as the sample solution (2). Separately, weigh exactly 60 mg of L-aspartic acid, 100 mg of L-glutamic acid, 17 mg of L-alanine, 23 mg of L-methionine, 21 mg of L-tyrosine, 24 mg of

L-histidine hydrochloride monohydrate, 58 mg of L-threonine, 22 mg of L-proline, 14 mg of L-cystine, 45 mg of L-isoleucine, 37 mg of L-phenylalanine, 32 mg of L-arginine hydrochloride, 32 mg of L-serine, 6 mg of glycine, 18 mg of L-valine, 109 mg of L-leucine, 76 mg of L-lysine hydrochloride, and 8 mg of L-tryptophan, dissolve with 0.1 mol/L hydrochloric acid TS to make exactly 500 mL, and use this solution as the standard solution. Transfer 40 μL each of the standard solution to two hydrolysis tubes, evaporate to dryness under vacuum, and proceed in the same way for each respective sample solution to make the standard solutions (1) and (2).

(ii) Amino acid analysis Perform the test with exactly 250 μL each of the sample solutions (1) and (2) and standard solutions (1) and (2) as directed under Liquid Chromatography <2.01> according to the following conditions, and from the peak areas for each amino acid obtained from the sample solutions (1) and (2) and standard solutions (1) and (2) calculate the molar number of the amino acids contained in 1 mL of the sample solutions (1) and (2). Furthermore, calculate the number of amino acids assuming there are 22 leucine residues in one mole of celmoleukin.

Operating conditions—

Detector: A visible absorption photometer [wavelength: 440 nm (proline) and 570 nm (amino acids other than proline)].

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with strongly acidic ionexchange resin for liquid chromatography (Na type) (sulfonic acid group bound divinylbenzenepolystyrene) (5 μm in particle diameter).

Column temperature: Maintaining a constant temperature of about 48°C for 28 minutes after sample injection, then a constant temperature of about 62°C until 121 minutes after the injection.

Reaction temperature: A constant temperature of about 135°C.

Color developing time: About 1 minute.

Mobile phases A, B, C and D: Prepare according to the following table.

Mobile phase	A	B	C	D
Citric acid monohydrate	17.70 g	10.50 g	6.10 g	—
Trisodium citrate dihydrate	7.74 g	15.70 g	26.67 g	—
Sodium chloride	7.07 g	2.92 g	54.35 g	—
Sodium hydroxide	—	—	2.30 g	8.00 g
Methanol (99.5)	40 mL	—	—	—
Benzyl alcohol	—	10 mL	5 mL	—
Thiodiglycol	5 mL	5 mL	5 mL	—
Lauromacrogol solution (1 in 4)	4 mL	4 mL	4 mL	4 mL
Caprylic acid	0.1 mL	0.1 mL	0.1 mL	0.1 mL
Water	a sufficient quantity	a sufficient quantity	a sufficient quantity	a sufficient quantity
Total	1000 mL	1000 mL	1000 mL	1000 mL

Flowing of mobile phase: Control the gradient by mixing the mobile phases A, B, C and D as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)	Mobile phase C (vol%)	Mobile phase D (vol%)
0 – 35	100	0	0	0
35 – 60	0	100	0	0
60 – 111	0	0	100	0
111 – 121	0	0	0	100

Reaction reagent: Mix 407 g of lithium acetate dihydrate, 245 mL of acetic acid (100) and 801 mL of 1-methoxy-2-propanol, add water to make 2000 mL, stir for 10 minutes while passing a current of nitrogen, and assign as solution A. Separately, to 1957 mL of 1-methoxy-2-propanol add 77 g of ninhydrin and 0.134 g of sodium borohydride, stir for 30 minutes while passing a current of nitrogen, and assign as solution B. Mix solutions A and B before use.

Flow rate of mobile phase: Adjust so that the retention times of serine and leucine are about 30 minutes and about 73 minutes, respectively (about 0.21 mL per minute).

Flow rate of reaction reagent: About 0.25 mL per minute.

System suitability—

System performance: To 2 mL of the standard solution add 0.02 mol/L hydrochloric acid TS to make 25 mL. When the procedure is run with 250 μL of this solution under the above operating conditions, the resolution between the peaks of threonine and serine is not less than 1.2.

System repeatability: To 2 mL of the standard solution add 0.02 mol/L hydrochloric acid TS to make 25 mL. When the test is repeated 3 times with 250 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of aspartic acid, serine, arginine and proline is not more than 2.4%.

Molecular mass Based on the results of the Assay (1), add buffer for celmoleukin and dilute to prepare a sample solution so that there is about 0.5 mg of protein per mL. To vertical uncontinuous buffer SDS-polyacrylamide gel prepared from resolving gel for celmoleukin and stacking gel for celmoleukin add 20 μL of the sample solution or 20 μL of marker protein for celmoleukin molecular mass determination to each stacking gel well, and perform the electrophoresis. The molecular mass of the main electrophoretic band is between 12,500 and 13,800 when the band is stained by immersion in Coomassie staining TS.

pH <2.54> 4.5 – 5.5

Purity (1) Related substances—Perform the test with 10 μL each of Celmoleukin (Genetical Recombination) and 0.01 mol/L acetic acid buffer solution (pH 5.0) as directed under Liquid Chromatography <2.01> under the following conditions, and measure the area of each peak by an automatic integration method. When the amounts of related substances other than celmoleukin are calculated by the area percentage method, the total amount is not more than 5%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: Stainless steel tube with an inside diameter of 4 mm and a length of 30 cm packed with octadecylsilanized silica gel for liquid chromatography (particle size: 5 μm).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A solution of trifluoroacetic acid in a mixture of acetic acid and water (3:2) (1 in 1000).

Mobile phase B: A solution of trifluoroacetic acid in a mixture of acetic acid and water (13:7) (1 in 1000).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 60	70 → 10	30 → 90

Flow rate: Adjust so that the retention time of celmoleukin is about 50 minutes.

Time span of measurement: About 1.3 times as long as the retention time of celmoleukin, beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 0.5 mL of Celmoleukin (Genetical Recombination), and add 0.01 mol/L acetic acid buffer solution (pH 5.0) to make exactly 50 mL. Confirm that the peak area of celmoleukin obtained from 10 μL of this solution is equivalent to 0.9 to 1.1% of the peak area obtained from 10 μL of Celmoleukin (Genetical Recombination).

System performance: Add 2 μL of 2-mercaptoethanol to 100 μL of Celmoleukin (Genetical Recombination), leave at 37°C for 2 hours, and then run this solution under the above conditions. Celmoleukin and its reduced form are eluted in this order with the resolution between these peaks being not less than 3.0.

(2) Multimers—Dilute (at least 4 steps) the sample solution prepared in the Molecular mass with buffer solution for celmoleukin so that the protein content is within the range of 2 to 32 μg per mL to prepare a series of standard solutions. Pipet 20 μL each of the sample solution and the standard solutions into the stacking gel well, and perform vertical uncoupled buffer SDS-polyacrylamide gel electrophoresis followed by immersion in Coomassie staining TS. Each electrophoretic band is stained blue. Next, determine the peak area of the electrophoretic bands obtained from each standard solution using a densitometer and calculate the protein content using the calibration curve mentioned above. When determining the polymer proteins derived from celmoleukin, other than celmoleukin monomer, the amount is not more than 2% in relation to the total protein.

(3) Host cell proteins—Being specified separately when the drug is granted approval based on the Law.

(4) DNA—Being specified separately when the drug is granted approval based on the Law.

Bacterial endotoxins <*4.01*> Less than 100 EU/mL.

Ammonium acetate Measure exactly 0.1 mL of Celmoleukin (Genetical Recombination), add water to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of ammonium chloride, and add water to make exactly 100 mL. Measure exactly 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard stock solution. Measure exactly 3 mL of the standard stock solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. When determining the area of the ammonium ion peak A_T and A_S, Celmoleukin (Genetical Recombination) contains not less than 0.28 mg and not more than 0.49 mg of ammonium acetate per mL.

Amount (mg) of ammonium acetate (CH_3COONH_4) per mL
$= A_T/A_S \times M_S \times 0.003 \times 1.441$

M_S: Amount (mg) of ammonium chloride taken
0.003: Dilution correction factor
1.441: Molecular mass conversion coefficient for converting ammonium chloride to ammonium acetate

Operating conditions—

Detector: An electric conductivity detector.

Column: Resin column 5 mm in inside diameter and 25 cm in length, packed with weakly acidic ion exchange resin for liquid chromatography (particle size: 5.5 μm).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Diluted 0.1 mol/L methanesulfonic acid TS (3 in 10).

Flow rate: Adjust so that the retention time of ammonium is about 8 minutes.

System suitability—

System performance: Measure exactly 1 mL of Standard Sodium Stock Solution and 0.2 mL of Standard Potassium Stock Solution, and then add water to make exactly 100 mL. Measure exactly 5 mL of this solution and 3 mL of Standard Ammonium Solution, and then add water to make exactly 50 mL. When 25 μL of this solution is run under the above conditions, sodium, ammonium and potassium are eluted in this order with the resolution between the peaks of sodium and ammonium being not less than 3.0.

System repeatability: When the test is repeated 5 times with 25 μL of the standard solution under the above conditions, the relative standard deviation of the ammonium peak area is not more than 10%.

Assay **(1)** Total protein content—Measure accurately 1 mL of Celmoleukin (Genetical Recombination) and add water to make exactly 10 mL. Use this solution as the sample solution. Separately, weigh accurately about 50 mg of bovine serum albumin for assay in water to prepare standard dilution solutions of 50, 100, and 150 μg/mL. Measure exactly 1 mL of the sample solution and each standard dilution solution, add exactly 2.5 mL of alkaline copper TS for protein content determination, shake, and leave for 15 minutes. Next, add exactly 2.5 mL of water and 0.5 mL of dilute Folin's TS, and leave at 37°C for 30 minutes. Measure the absorbances of these solutions at 750 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using 1 mL of water processed in the same way as control. Using the calibration curve prepared from the absorbance of the standard dilution solution, calculate the protein content of Celmoleukin (Genetical Recombination).

(2) Specific activity—Measure exactly 0.1 mL of Celmoleukin (Genetical Recombination) and add exactly 0.9 mL of culture medium for celmoleukin to make the sample solution. Separately, take one Interleukin-2 RS and add exactly 1 mL of water to dissolve. This is the standard solution. Dilute exactly the sample and standard solutions in serially two-fold steps with culture medium for celmoleukin, and add equal volumes of interleukin-2 dependent mouse natural killer NKC3 cells to the serially diluted solutions. The control solution is a mixture of equal volumes of interleukin-2 dependent mouse natural killer NKC3 and culture medium for celmoleukin. Incubate these solutions at 37°C for 16 to 24 hours. Following this, add a volume of 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide TS that is 1/5 that of the volume of culture medium for celmoleukin, incubate at 37°C for 4 to 6 hours, add a volume of sodium lauryl sulfate TS equivalent to the volume of the culture medium for celmoleukin, and leave for 24 to 48 hours. After eluting the blue-colored pigment generated, perform the test on these solutions as directed under Ultravi-

olet-visible Spectrophotometry <2.24>, and measure the absorbance at 590 nm. Taking the absorbance obtained when 1000 to 2000 units of celmoleukin per mL are added as 100% and the absorbance of the control solution as 0%, determine the dilution factor (A) of the Interleukin-2 RS that shows an absorbance of 50% and dilution factor of Celmoleukin (Genetical Recombination) (B). Multiply the B/A value by the unit number of the Interleukin-2 RS to calculate the biological activity of 1 mL of Celmoleukin (Genetical Recombination). Calculate the ratio of biological activity in relation to protein content determined in the total protein content test.

Containers and storage Containers—Tight containers.
Storage—At −20°C or lower.

Cetanol

セタノール

Cetanol is a mixture of solid alcohols, and consists chiefly of cetanol ($C_{16}H_{34}O$: 242.44).

Description Cetanol occurs as unctuous, white flakes, granules, or masses. It has a faint, characteristic odor. It is tasteless.

It is very soluble in pyridine, freely soluble in ethanol (95), in ethanol (99.5) and in diethyl ether, very slightly soluble in acetic anhydride, and practically insoluble in water.

Melting point <1.13> 47 – 53°C Prepare the sample according to Method 2, then attach tightly a capillary tube to the bottom of the thermometer by means of a rubber band or by any suitable means, and make the bottom of the capillary tube equal in position to the lower end of the thermometer. Insert this thermometer into a test tube 17 mm in inside diameter and about 170 mm in height, fasten the thermometer with cork stopper so that the lower end of the thermometer is about 25 mm distant from the bottom of the test tube. Suspend the test tube in a beaker containing water, and heat the beaker with constant stirring until the temperature rises to 5°C below the expected melting point. Then regulate the rate of increase to 1°C per minute. The temperature at which the sample is transparent and no turbidity is produced is taken as the melting point.

Acid value <1.13> Not more than 1.0.

Ester value <1.13> Not more than 2.0.

Hydroxyl value <1.13> 210 – 232

Iodine value <1.13> Not more than 2.0.

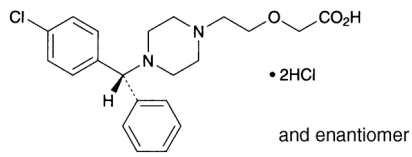
Purity (1) Clarity of solution—Dissolve 3.0 g of Cetanol in 25 mL of ethanol (99.5) by warming: the solution is clear.
(2) Alkalinity—To the solution obtained in (1) add 2 drops of phenolphthalein TS: no red color develops.

Residue on ignition <2.44> Not more than 0.05% (2 g).

Containers and storage Containers—Well-closed containers.

Cetirizine Hydrochloride

セチリジン塩酸塩



$C_{21}H_{25}ClN_2O_3.2HCl$: 461.81
2-(2-{4-[(*RS*)-(4-Chlorophenyl)(phenyl)methyl]piperazin-1-yl}ethoxy)acetic acid dihydrochloride
[*83881-52-1*]

Cetirizine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3.2HCl$).

Description Cetirizine Hydrochloride occurs as a white crystalline powder.

It is very soluble in water, and slightly soluble in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS.

A solution of Cetirizine Hydrochloride (1 in 10) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Cetirizine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.
(2) Determine the infrared absorption spectrum of Cetirizine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.
(3) A solution of Cetirizine Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Cetirizine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).
(2) Related substances—Dissolve 0.10 g of Cetirizine Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of each solution by the automatic integration method: the area of each peak other than cetirizine obtained from the sample solution is not larger than the peak area of cetirizine obtained from the standard solution. And the total area of the peaks other than cetirizine from the sample solution is not larger than 2.5 times the peak area of cetirizine from the standard solution.
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 230 nm).
Column: A stainless steel column 4.0 mm in inside diameter and 25 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetonitrile and diluted 0.5 mol/L sulfuric acid TS (2 in 25) (47:3).

Flow rate: Adjust so that the retention time of cetirizine is about 9 minutes.

Time span of measurement: About 3 times as long as the retention time of cetirizine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of cetirizine obtained from 10 μL of this solution is equivalent to 35 to 65% of that obtained from 10 μL of the standard solution.

System performance: Dissolve 20 mg of Cetirizine Hydrochloride in the mobile phase to make 100 mL. To 5 mL of this solution, add 3 mL of a solution of aminopyrine in the mobile phase (1 in 2500), and add the mobile phase to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, cetirizine and aminopyrine are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cetirizine is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Assay Weigh accurately about 0.1 g of Cetirizine Hydrochloride, previously dried, dissolve in 70 mL of a mixture of acetone and water (7:3), and titrate ⟨*2.50*⟩ to the second equivalence point with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 15.39 mg of $C_{21}H_{25}ClN_2O_3.2HCl$

Containers and storage Containers—Well-closed containers.

Cetirizine Hydrochloride Tablets

セチリジン塩酸塩錠

Cetirizine Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3.2HCl$: 461.81).

Method of preparation Prepare as directed under Tablets, with Cetirizine Hydrochloride.

Identification To a quantity of powdered Cetirizine Hydrochloride Tablets, equivalent to 10 mg of Cetirizine Hydrochloride, add about 70 mL of 0.1 mol/L hydrochloric acid TS, shake, add 0.1 mol/L hydrochloric acid TS to make 100 mL, and filter. To 4 mL of the filtrate add 0.1 mol/L hydrochloric acid TS to make 25 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 230 nm and 234 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take 1 tablet of Cetirizine Hydrochloride Tablets, add $4V/5$ mL of sodium 1-heptanesulfonate solution (1 in 5000) adjusted to pH 3.0 with 0.5 mol/L sulfuric acid TS, treat with ultrasonic waves for 20 minutes, add sodium 1-heptanesulfonate solution (1 in 5000) adjusted to pH 3.0 with 0.5 mol/L sulfuric acid TS to exactly V mL so that each mL contains about 0.2 mg of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3.2HCl$), and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 2 mL of the internal standard solution, add acetonitrile to make 10 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3.2HCl$)
$= M_S \times Q_T/Q_S \times V/100$

M_S: Amount (mg) of cetirizine hydrochloride for assay taken

Internal standard solution—A solution of propyl parahydroxybenzoate in the mobile phase (1 in 1000).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rates in 15 minutes of 5-mg tablet and in 30 minutes of 10-mg tablet are not less than 85% and not less than 80%, respectively.

Start the test with 1 tablet of Cetirizine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 5.6 μg of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3.2HCl$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of cetirizine hydrochloride for assay, previously dried in vacuum at 60°C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 230 nm of the sample solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3.2HCl$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of cetirizine hydrochloride for assay taken

C: Labeled amount (mg) of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3.2HCl$) in 1 tablet

Assay Weigh accurately not less than 20 Cetirizine Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3.2HCl$), add 40 mL of sodium 1-heptanesulfonate solution (1 in 5000) adjusted to pH 3.0 with 0.5 mol/L sulfuric acid TS, treat with ultrasonic waves for 20 minutes, add sodium 1-heptanesulfonate solution (1 in 5000) adjusted to pH 3.0 with 0.5 mol/L sulfuric acid TS, to make exactly 50 mL, and filter this solution through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 2 mL of the internal standard solution, add acetonitrile to make exactly 10 mL, and use this solution

as the sample solution. Separately, weigh accurately about 20 mg of cetirizine hydrochloride for assay, previously dried in vacuum at 60°C for 3 hours, and add sodium 1-heptanesulfonate solution (1 in 5000) adjusted to pH 3.0 with 0.5 mol/L sulfuric acid TS, to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 2 mL of the internal standard solution, add acetonitrile to make 10 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cetirizine to that of the internal standard.

Amount (mg) of cetirizine hydrochloride ($C_{21}H_{25}ClN_2O_3.2HCl$) $= M_S \times Q_T/Q_S \times 1/2$

M_S: Amount (mg) of cetirizine hydrochloride for assay taken

Internal standard solution—A solution of propyl parahydroxybenzoate in the mobile phase (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of sodium 1-heptansulfonate (1 in 2900) and acetonitrile (29:21), adjusted to pH 3.0 with 0.5 mol/L sulfuric acid TS.

Flow rate: Adjust so that the retention time of cetirizine is about 5 minutes.

System suitability—

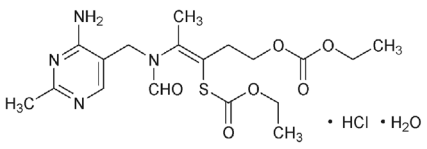
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, cetirizine and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cetirizine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Cetotiamine Hydrochloride Hydrate

セトチアミン塩酸塩水和物



$C_{18}H_{26}N_4O_6S.HCl.H_2O$: 480.96

(3*Z*)-4-{*N*-[(4-Amino-2-methylpyrimidin-5-yl)methyl]-*N*-formylamino}-3-(ethoxycarbonylsulfanyl)pent-3-enyl ethyl carbonate monohydrochloride monohydrate

[*616-96-6*, anhydride]

Cetotiamine Hydrochloride Hydrate contains not less than 98.0% and not more than 102.0% of cetotiamine hydrochloride ($C_{18}H_{26}N_4O_6S.HCl$: 462.95), calculated on the anhydrous basis.

Description Cetotiamine Hydrochloride Hydrate occurs as white, crystals or crystalline powder. It is odorless or has a faint characteristic odor.

It is freely soluble in water and in ethanol (99.5).

It dissolves in 0.01 mol/L hydrochloric acid TS.

Melting point: about 132°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Cetotiamine Hydrochloride Hydrate in 0.01 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cetotiamine Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cetotiamine Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cetotiamine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Cetotiamine Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 1.0 g of Cetotiamine Hydrochloride Hydrate in 10 mL of water is clear and has no more color than the following control solution.

Control solution: Mix exactly 1.5 mL of Cobalt (II) Chloride CS, exactly 36 mL of Iron (III) Chloride CS and exactly 12.5 mL of diluted dilute hydrochloric acid (1 in 10). Pipet 1 mL of this mixture, and add diluted dilute hydrochloric acid (1 in 10) to make exactly 100 mL.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Cetotiamine Hydrochloride Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 50 mg of Cetotiamine Hydrochloride Hydrate in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than cetotiamine from the sample solution is not larger than the peak area of cetotiamine from the standard solution, and the total area of the peaks other than cetotiamine from the sample solution is not larger than 2 times the peak area of cetotiamine from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of cetotiamine, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of cetotiamine obtained

with 10 μL of this solution is equivalent to 7 to 13% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cetotiamine are not less than 3000 and 0.7 – 1.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cetotiamine is not more than 2.0%.

Water ⟨*2.48*⟩ 3.0 – 5.0% (40 mg, coulometric titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Assay Weigh accurately about 30 mg each of Cetotiamine Hydrochloride Hydrate and Cetotiamine Hydrochloride RS (separately determine the water ⟨*2.48*⟩ in the same manner as Cetotiamine Hydrochloride Hydrate), add exactly 10 mL each of the internal standard solution, then add a mixture of water and methanol (1:1) to make 50 mL. To 2 mL each of these solutions add a mixture of water and methanol (1:1) to make 10 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cetotiamine to that of the internal standard.

Amount (mg) of cetotiamine hydrochloride ($C_{18}H_{26}N_4O_6S.HCl$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of Cetotiamine Hydrochloride RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of propyl parahydroxybenzoate in a mixture of water and methanol (1:1) (1 in 800).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.0 g of sodium 1-heptanesulfonate in diluted acetic acid (100) (1 in 100) to make 1000 mL. To 1 volume of this solution add 1 volume of methanol.

Flow rate: Adjust so that the retention time of cetotiamine is about 10 minutes.

System suitability—

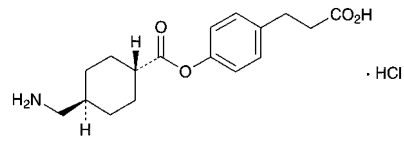
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, cetotiamine and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cetotiamine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cetraxate Hydrochloride

セトラキサート塩酸塩



$C_{17}H_{23}NO_4.HCl$: 341.83

3-{4-[*trans*-4-(Aminomethyl)cyclohexylcarbonyloxy]-phenyl}propanoic acid monohydrochloride

[*27724-96-5*]

Cetraxate Hydrochloride, when dried, contains not less than 98.5% of cetraxate hydrochloride ($C_{17}H_{23}NO_4.HCl$).

Description Cetraxate Hydrochloride occurs as white, crystals or crystalline powder.

It is soluble in methanol, sparingly soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 236°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Cetraxate Hydrochloride in methanol (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 0.5 g of Cetraxate Hydrochloride in 5 mL of a mixture of water and 2-propanol (1:1) by warming, cool to below 25°C. Filter, dry the formed crystals in vacuum for 4 hours, and further dry at 105°C for 1 hour. Determine the infrared absorption spectrum of the dried matter as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Cetraxate Hydrochloride (1 in 100) responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Cetraxate Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Cetraxate Hydrochloride according to Method 3, and perform the test with a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 5) (not more than 2 ppm).

(3) *cis* Isomer—Dissolve 0.10 g of Cetraxate Hydrochloride in 10 mL of water, and use this solution as the sample solution. To exactly 5 mL of the sample solution add water to make exactly 100 mL. To exactly 2 mL of this solution add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak, having the relative retention time 1.3 to 1.6 to cetraxate from the sample solution is not larger than the peak area of cetraxate from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 6 mm in inside diameter

and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Adjust the pH of a mixture of water, methanol and 0.5 mol/L ammonium acetate TS (15:10:4) to 6.0 with acetic acid (31).

Flow rate: Adjust so that the retention time of cetraxate is about 10 minutes.

System suitability—

System performance: Dissolve 0.02 g of Cetraxate Hydrochloride and 0.01 g of phenol in 100 mL of water. To 2 mL of this solution add water to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, cetraxate and phenol are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cetraxate is not more than 2.0%.

(4) 3-(*p*-Hydroxyphenyl)propionic acid—To 0.10 g of Cetraxate Hydrochloride add exactly 2 mL of the internal standard solution and methanol to make 10 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of 3-(*p*-hydroxyphenyl)propionic acid in methanol to make exactly 100 mL. To exactly 2 mL of this solution add exactly 2 mL of the internal standard solution and methanol to make 10 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of 3-(*p*-hydroxyphenyl)propionic acid to that of the internal standard: Q_T is not larger than Q_S.

Internal standard solution—A solution of caffeine in methanol (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Adjust the pH of a mixture of water, methanol and 0.5 mol/L ammonium acetate TS (15:5:2) to 5.5 with acetic acid (31).

Flow rate: Adjust so that the retention time of 3-(*p*-hydroxyphenyl)propionic acid is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, 3-(*p*-hydroxyphenyl)propionic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of 3-(*p*-hydroxyphenyl)propionic acid to that of the internal standard is not more than 1.0%.

(5) Related substances—Dissolve 0.10 g of Cetraxate Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and acetic acid (100) (20:4:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin TS on the plate, and heat at 90°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (0.5 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

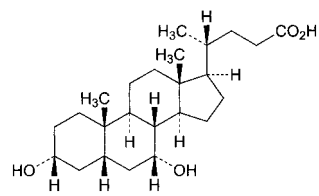
Assay Weigh accurately about 0.5 g of Cetraxate Hydrochloride, previously dried, dissolve in 100 mL of water, and adjust the pH of this solution to between 7.0 and 7.5 with dilute sodium hydroxide TS. To this solution add 10 mL of formaldehyde solution, stir for about 5 minutes, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS by taking over about 20 minutes (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 34.18 mg of $C_{17}H_{23}NO_4.HCl$

Containers and storage Containers—Tight containers.

Chenodeoxycholic Acid

ケノデオキシコール酸



$C_{24}H_{40}O_4$: 392.57

3α,7α-Dihydroxy-5β-cholan-24-oic acid

[*474-25-9*]

Chenodeoxycholic Acid, when dried, contains not less than 98.0% and not more than 101.0% of chenodeoxycholic acid ($C_{24}H_{40}O_4$).

Description Chenodeoxycholic Acid occurs as white, crystals, crystalline powder or powder.

It is freely soluble in methanol and in ethanol (99.5), soluble in acetone, and practically insoluble in water.

Identification Determine the infrared absorption spectrum of Chenodeoxycholic Acid, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +11.0 – +13.0° (after drying, 0.4 g, ethanol (99.5), 20 mL, 100 mm).

Melting point <*2.60*> 164 – 169°C

Purity (1) Chloride <*1.03*>—Dissolve 0.36 g of Chenodeoxycholic Acid in 30 mL of methanol, add 10 mL of dilute nitric acid and water to make 50 mL, and perform the test with this solution. Prepare the control solution as follows: To 1.0 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of methanol, 10 mL of dilute nitric acid and water to make 50 mL (not more than 0.1%).

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Chenodeoxycholic Acid according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Barium—To 2.0 g of Chenodeoxycholic Acid add 100 mL of water, and boil for 2 minutes. To this solution add 2 mL of hydrochloric acid, boil for 2 minutes, filter after cooling, and wash the filter with water until to get 100 mL of the filtrate. To 10 mL of the filtrate add 1 mL of dilute sulfuric acid: no turbidity appears.

(4) Related substances—Dissolve 0.20 g of Chenodeoxycholic Acid in a mixture of acetone and water (9:1) to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of lithocholic acid for thin-layer chromatography in the mixture of acetone and water (9:1) to make exactly 10 mL. Pipet 2 mL of this solution, add the mixture of acetone and water (9:1) to make exactly 100 mL, and use this solution as the standard solution (1). Separately, dissolve 10 mg of ursodeoxycholic acid in the mixture of acetone and water (9:1) to make exactly 100 mL, and use this solution as the standard solution (2). Separately, dissolve 10 mg of cholic acid for thin-layer chromatography in the mixture of acetone and water (9:1) to make exactly 100 mL, and use this solution as the standard solution (3). Pipet 1 mL of the sample solution, and add the mixture of acetone and water (9:1) to make exactly 20 mL. Pipet 0.5 mL, 1 mL, 2 mL, 3 mL and 5 mL of this solution, add the mixture of acetone and water (9:1) to each of them to make exactly 50 mL, and designate these solutions as standard solution A, standard solution B, standard solution C, standard solution D and standard solution E, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution, standard solutions (1), (2), (3) and standard solutions A, B, C, D and E on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 4-methyl-2-pentanone, toluene and formic acid (16:6:1) to a distance of about 15 cm, air-dry the plate, and further dry at 120°C for 30 minutes. Immediately, spray evenly a solution of phosphomolybdic acid *n*-hydrate in ethanol (95) (1 in 5) on the plate, and heat at 120°C for 2 to 3 minutes: the spot corresponding to the spot with the standard solution (1) is not more intense than the spot with the standard solution (1), the spot corresponding to the spot with the standard solution (2) is not more intense than the spot with the standard solution (2), and the spot corresponding to the spot with the standard solution (3) is not more intense than the spot with the standard solution (3). As compared to the spots with the standard solutions A, B, C, D and E, the spots other than the principal spot and the spots mentioned above with the sample solution are not more intense than the spot with the standard solution E, and the total amount of them is not more than 1.5%.

Loss on drying ⟨*2.41*⟩ Not more than 1.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

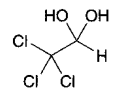
Assay Weigh accurately about 0.5 g of Chenodeoxycholic Acid, previously dried, dissolve in 40 mL of ethanol (95) and 20 mL of water, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 39.26 mg of $C_{24}H_{40}O_4$

Containers and storage Containers—Tight containers.

Chloral Hydrate

抱水クロラール



$C_2H_3Cl_3O_2$: 165.40
2,2,2-Trichloroethane-1,1-diol
[*302-17-0*]

Chloral Hydrate contains not less than 99.5% of chloral hydrate ($C_2H_3Cl_3O_2$).

Description Chloral Hydrate occurs as colorless crystals. It has a pungent odor and an acrid, slightly bitter taste.

It is very soluble in water, and freely soluble in ethanol (95) and in diethyl ether.

It slowly volatilizes in air.

Identification **(1)** Dissolve 0.2 g of Chloral Hydrate in 2 mL of water, and add 2 mL of sodium hydroxide TS: the turbidity is produced, and it separates into two clear layers by warming.

(2) Heat 0.2 g of Chloral Hydrate with 3 drops of aniline and 3 drops of sodium hydroxide TS: the disagreeable odor of phenylisocyanide (poisonous) is perceptible.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Chloral Hydrate in 2 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 0.20 g of Chloral Hydrate in 2 mL of water, and add 1 drop of methyl orange TS: a yellow color develops.

(3) Chloride ⟨*1.03*⟩—Perform the test with 1.0 g of Chloral Hydrate. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(4) Chloral alcoholate—Warm 1.0 g of Chloral Hydrate with 10 mL of sodium hydroxide TS, filter the upper layer, add iodine TS to the filtrate until a yellow color develops, and allow the solution to stand for 1 hour: no yellow precipitate is produced.

(5) Benzene—Warm the solution obtained in (1) with 3 mL of water: no odor of benzene is perceptible.

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

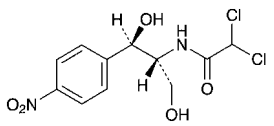
Assay Weigh accurately about 4 g of Chloral Hydrate in a glass-stoppered flask, add 10 mL of water and exactly 40 mL of 1 mol/L sodium hydroxide VS, and allow the mixture to stand for exactly 2 minutes. Titrate ⟨*2.50*⟩ the excess sodium hydroxide immediately with 0.5 mol/L sulfuric acid VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 1 mol/L sodium hydroxide VS
= 165.4 mg of $C_2H_3Cl_3O_2$

Containers and storage Containers—Tight containers.

Chloramphenicol

クロラムフェニコール



$C_{11}H_{12}Cl_2N_2O_5$: 323.13
2,2-Dichloro-*N*-[(1*R*,2*R*)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]acetamide
[*56-75-7*]

Chloramphenicol contains not less than 980 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the dried basis. The potency of Chloramphenicol is expressed as mass (potency) of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$).

Description Chloramphenicol occurs as white to yellowish white, crystals or crystalline powder.

It is freely soluble in methanol and in ethanol (99.5), and slightly soluble in water.

Identification **(1)** Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Chloramphenicol RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Chloramphenicol as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Chloramphenicol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +18.5 – +21.5° (1.25 g, ethanol (99.5), 25 mL, 100 mm).

Melting point <*2.60*> 150 – 155°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Chloramphenicol according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 25 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 2.0 g of Chloramphenicol according to Method 4, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 0.10 g of Chloramphenicol in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 10 mL of the standard solution (1), add methanol to make exactly 20 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of chloroform, methanol and acetic acid (100) (79:14:7) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and the spot on the original obtained from the sample solution are not more intense than the spot obtained from the standard solution (1), and the total amount of these spots from the sample solution is not more than 2.0%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Chloramphenicol and Chloramphenicol RS, equivalent to about 0.1 g (potency), dissolve each in 20 mL of methanol, and add water to make exactly 100 mL. Pipet 20 mL each of these solutions, and add water to make exactly 100 mL. Pipet 10 mL each of these solutions, add water to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Determine the absorbances, A_T and A_S, at 278 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

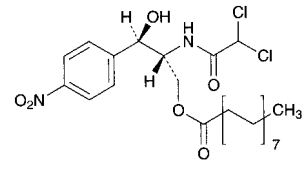
Amount [μg (potency)] of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$) $= M_S \times A_T/A_S \times 1000$

M_S: Amount [mg (potency)] of Chloramphenicol RS taken

Containers and storage Containers—Tight containers.

Chloramphenicol Palmitate

クロラムフェニコールパルミチン酸エステル



$C_{27}H_{42}Cl_2N_2O_6$: 561.54
(2*R*,3*R*)-2-(Dichloroacetyl)amino-3-hydroxy-3-(4-nitrophenyl)propan-1-yl palmitate
[*530-43-8*]

Chloramphenicol Palmitate contains not less than 558 μg (potency) and not more than 587 μg (potency) per mg, calculated on the dried basis. The potency of Chloramphenicol Palmitate is expressed as mass (potency) of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$: 323.13).

Description Chloramphenicol Palmitate occurs as a white to grayish white, crystalline powder.

It is freely soluble in acetone, sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification **(1)** Determine the absorption spectrum of a solution of Chloramphenicol Palmitate in ethanol (99.5) (1 in 33,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Chloramphenicol Palmitate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 5 mg each of Chloramphenicol Palmitate and Chloramphenicol Palmitate RS in 1 mL of acetone, and use these solutions as the sample solution and standard soution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone and cyclohexane (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm):

the principal spot obtained from the sample solution has the same *R*f value as the spot obtained from the standard solution.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{25}$: +21 – +25° (1 g calculated on the dried basis, ethanol (99.5), 20 mL, 100 mm).

Melting point ⟨*2.60*⟩ 91 – 96°C

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Chloramphenicol Palmitate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Chloramphenicol Palmitate according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Chloramphenicol Palmitate in 50 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. The test should be performed within 30 minutes after the sample solution and standard solution are prepared. Determine each peak area by the automatic integration method: the total area of the peaks other than the peak of chloramphenicol palmitate from the sample solution is not larger than 3.5 times the peak area of chloramphenicol palmitate from the standard solution. For the peak areas for chloramphenicol, having the relative retention time of about 0.5 to chloramphenicol palmitate, and for chloramphenicol dipalmitate, having the relative retention time of about 5.0 to chloramphenicol palmitate, multiply their relative response factors, 0.5 and 1.4, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column 6.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: Methanol.

Flow rate: Adjust so that the retention time of chloramphenicol palmitate is about 5 minutes.

Time span of measurement: About 6 times as long as the retention time of chloramphenicol palmitate.

System suitability—

Test for required detectability: Dissolve 50 mg of Chloramphenicol Palmitate in 50 mL of methanol. To 1 mL of this solution, add methanol to make 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitabillity test, and add methanol to make exactly 50 mL. Confirm that the peak area of chloramphenicol palmitate obtained from 20 μL of this solution is equivalent to 7 to 13% of that obtained from 20 μL of the solution for system suitability test.

System performance: When the procedure is run with 20 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates of the peak of chloramphenicol palmitate is not less than 5000.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of chloramphenicol palmitate is not more than 1.0%.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Weigh accurately an amount of Chloramphenicol Palmitate and Chloramphenicol Palmitate RS, equivalent to about 37 mg (potency), dissolve each in 40 mL of methanol and 1 mL of acetic acid (100), and add methanol to make exactly 50 mL. Pipet 10 mL each of these solutions, add the mobile phase to make exactly 25 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of chloramphenicol palmitate in each solution.

Amount [μg (potency)] of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$) $= M_S \times A_T/A_S \times 1000$

M_S: Amount [mg (potency)] of Chloramphenicol Palmitate RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of methanol, water and acetic acid (100) (172:27:1).

Flow rate: Adjust so that the retention time of chloramphenicol palmitate is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of chloramphenicol palmitate is not less than 2400.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of chloramphenicol palmitate is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Chloramphenicol Sodium Succinate

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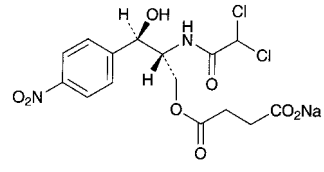


$C_{15}H_{15}Cl_2N_2NaO_8$: 445.18

Monosodium (2*R*,3*R*)-2-(dichloroacetyl)amino-3-hydroxy-3-(4-nitrophenyl)propan-1-yl succinate

[*982-57-0*]

Chloramphenicol Sodium Succinate contains not less than 711 μg (potency) and not more than 740 μg (potency) per mg, calculated on the anhydrous basis. The potency of Chloramphenicol Sodium Succinate is expressed as mass (potency) of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$: 323.13).

Description Chloramphenicol Sodium Succinate occurs as white to yellowish white, crystals or crystalline powder.

It is very soluble in water, and freely soluble in methanol and in ethanol (99.5).

It is hygroscopic.

Identification **(1)** Determine the absorption spectrum of a solution of Chloramphenicol Sodium Succinate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Chloramphenicol Sodium Succinate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Chloramphenicol Sodium Succinate responds to the Qualitative Tests ⟨*1.09*⟩ (1) for sodium salt.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{25}$: +5 – +8° (1.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH ⟨*2.54*⟩ The pH of a solution obtained by dissolving 1.4 g of Chloramphenicol Sodium Succinate in 5 mL of water is between 6.0 and 7.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Chloramphenicol Sodium Succinate in 10 mL of water: the solution is clear and colorless to yellowish.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Chloramphenicol Sodium Succinate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Chloramphenicol Sodium Succinate according to Method 1, and perform the test (not more than 2 ppm).

Water ⟨*2.48*⟩ Not more than 2.0% (1.0 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Chloramphenicol Sodium Succinate, equivalent to about 20 mg (potency), dissolve in water to make exactly 1000 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Chloramphenicol Succinate RS, equivalent to about 20 mg (potency), add about 50 mL of water to make a suspension, and add gradually about 7 mL of 0.01 mol/L sodium hydroxide TS while stirring to adjust the pH to 7.0. To this solution add water to make exactly 1000 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 276 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

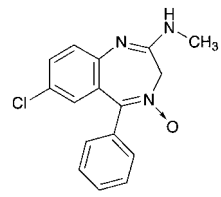
Amount [μg (potency)] of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$) = $M_S \times A_T/A_S \times 1000$

M_S: Amount [mg (potency)] of Chloramphenicol Succinate RS taken

Containers and storage Containers—Hermetic containers.

Chlordiazepoxide

クロルジアゼポキシド



$C_{16}H_{14}ClN_3O$: 299.75

7-Chloro-2-methylamino-5-phenyl-3*H*-1,4-benzodiazepin-4-oxide

[*58-25-3*]

Chlordiazepoxide, when dried, contains not less than 98.5% of chlordiazepoxide ($C_{16}H_{14}ClN_3O$).

Description Chlordiazepoxide occurs as white to light yellow, crystals or crystalline powder.

It is freely soluble in acetic acid (100), sparingly soluble in ethanol (95), very slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

It is gradually affected by light.

Melting point: about 240°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Chlordiazepoxide in 0.1 mol/L hydrochloric acid TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Chlordiazepoxide RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectra of Chlordiazepoxide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of dried Chlordiazepoxide RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Proceed with Chlordiazepoxide as directed under Flame Coloration Test ⟨*1.04*⟩ (2), and perform the test: a green color develops.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Chlordiazepoxide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.20 g of Chlordiazepoxide in exactly 10 mL of a mixture of methanol and ammonia TS (97:3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and ammonia TS (97:3) to make exactly 200 mL, and use this solution as the standard solution (1). Separately, dissolve 10 mg of 2-amino-5-chlorobenzophenone for thin-layer chromatography in methanol to make exactly 200 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 25 μL of the sample solution and 5 μL each of the standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and ethanol (99.5) (19:1) to a distance of

about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (1). Spray evenly a solution of sodium nitrite in 1 mol/L hydrochloric acid TS (1 in 100) on the plate, allow to stand for 1 minute, and spray evenly *N*-(1-naphthyl)-*N'*-diethylethylenediamine oxalate-acetone TS on the plate: the spots from the sample solution are not more intense than the spots from the standard solution (2).

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Chlordiazepoxide, previously dried, and dissolve in 50 mL of acetic acid (100). Titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS until the color of the supernatant liquid changes from purple through blue-purple to blue (indicator: 3 drops of crystal violet TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.98 mg of $C_{16}H_{14}ClN_3O$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Chlordiazepoxide Powder

クロルジアゼポキシド散

Chlordiazepoxide Powder contains not less than 93.0% and not more than 107.0% of the labeled amount of chlordiazepoxide ($C_{16}H_{14}ClN_3O$: 299.75).

Method of preparation Prepare as directed under Granules or Powders, with Chlordiazepoxide.

Identification **(1)** Weigh a portion of Chlordiazepoxide Powder, equivalent to 0.01 g of Chlordiazepoxide, add 100 mL of 0.1 mol/L hydrochloric acid TS, shake, and filter. To 5 mL of the filtrate add 0.1 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 244 nm and 248 nm and between 306 nm and 310 nm, and a minimum between 288 nm and 292 nm.

(2) Weigh a portion of Chlordiazepoxide Powder, equivalent to 0.02 g of Chlordiazepoxide, add 10 mL of methanol, shake for 5 minutes, then filter by suction through a glass filter (G4), evaporate the filtrate with the aid of a current of air to dryness, and dry the residue in vacuum at 60°C for 1 hour. Determine the infrared absorption spectrum of the residue as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩: it exhibits absorption at the wave numbers of about 1625 cm^{-1}, 1465 cm^{-1}, 1265 cm^{-1}, 850 cm^{-1} and 765 cm^{-1}.

Purity Conduct this procedure without exposure to light, using light-resistant vessels. To a portion of Chlordiazepoxide Powder, equivalent to 50 mg of Chlordiazepoxide, add exactly 5 mL of a mixture of methanol and ammonia TS (97:3), shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 50 mg of Chlordiazepoxide RS in a mixture of methanol and ammonia TS (97:3) to make exactly 50 mL, and use this solution as the standard solution (1). Dissolve 5.0 mg of 2-amino-5-chlorobenzophenone for thin-layer chromatography in methanol to make exactly 200 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 25 μL of the sample solution and 10 μL each of the standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Proceed as directed in the Purity (2) under Chlordiazepoxide.

Dissolution ⟨*6.10*⟩ When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 60 minutes of Chlordiazepoxide Powder is not less than 70%.

Start the test with an accurately weighed amount of Chlordiazepoxide Powder, equivalent to about 3.3 mg of chlordiazepoxide ($C_{16}H_{14}ClN_3O$), withdraw not less than 15 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 12 mg of Chlordiazepoxide RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 60°C) for 4 hours, dissolve in 20 mL of 0.1 mol/L hydrochloric acid TS, and add the dissolution medium to make exactly 200 mL. Pipet 3 mL of this solution, add the dissolution medium to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 260 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of chlordiazepoxide ($C_{16}H_{14}ClN_3O$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 27$

M_S: Amount (mg) of Chlordiazepoxide RS taken
M_T: Amount (g) of Chlordiazepoxide Powder taken
C: Labeled amount (mg) of chlordiazepoxide ($C_{16}H_{14}ClN_3O$) in 1 g

Assay Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately a quantity of Chlordiazepoxide Powder, equivalent to about 0.1 g of chlordiazepoxide ($C_{16}H_{14}ClN_3O$), transfer to a glass-stoppered flask, wet with exactly 10 mL of water, add exactly 90 mL of methanol, stopper, shake vigorously for 15 minutes, and centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Chlordiazepoxide RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 60°C) for 4 hours, and dissolve in exactly 10 mL of water and exactly 90 mL of methanol. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of chlordiazepoxide to that of the internal standard.

Amount (mg) of chlordiazepoxide ($C_{16}H_{14}ClN_3O$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of Chlordiazepoxide RS taken

Internal standard solution—A solution of isobutyl salicylate

in methanol (1 in 20).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and 0.02 mol/L ammonium dihydrogenphosphate TS (7:3).

Flow rate: Adjust so that the retention time of chlordiazepoxide is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, chlordiazepoxide and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of chlordiazepoxide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage-Light-resistant.

Chlordiazepoxide Tablets

クロルジアゼポキシド錠

Chlordiazepoxide Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of chlordiazepoxide ($C_{16}H_{14}ClN_3O$: 299.75).

Method of preparation Prepare as directed under Tablets, with Chlordiazepoxide.

Identification (1) Weigh a portion of powdered Chlordiazepoxide Tablets, equivalent to 0.01 g of Chlordiazepoxide, add 100 mL of 0.1 mol/L hydrochloric acid TS, shake, and filter. To 5 mL of the filtrate add 0.1 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 244 nm and 248 nm and between 306 nm and 310 nm, and a minimum between 288 nm and 292 nm.

(2) Weigh a portion of powdered Chlordiazepoxide Tablets, equivalent to 0.01 g of Chlordiazepoxide, add 10 mL of diethyl ether, shake vigorously, and centrifuge. Evaporate 5 mL of the supernatant liquid by warming on a water bath to dryness. Determine the infrared absorption spectrum of the residue as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1625 cm^{-1}, 1465 cm^{-1}, 1265 cm^{-1}, 850 cm^{-1} and 765 cm^{-1}.

Purity Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. To a portion of powdered Chlordiazepoxide Tablets, equivalent to 50 mg of Chlordiazepoxide, add exactly 5 mL of a mixture of methanol and ammonia TS (97:3), shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 50 mg of Chlordiazepoxide RS in a mixture of methanol and ammonia TS (97:3) to make exactly 50 mL, and use this solution as the standard solution (1). Dissolve 5.0 mg of 2-amino-5-chlorobenzophenone for thin-layer chromatography in methanol to make exactly 200 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 25 μL of the sample solution and 10 μL each of the standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Proceed as directed in the Purity (2) under Chlordiazepoxide.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Conduct this procedure without exposure to light, using light-resistant vessels. To 1 tablet of Chlordiazepoxide Tablets add 1 mL of water, shake to disintegrate the tablet, then add 20 mL of methanol, shake, add methanol to make exactly 25 mL, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 5 mL of the filtrate, take exactly V mL of the subsequent filtrate equivalent to about 2 mg of chlordiazepoxide ($C_{16}H_{14}ClN_3O$), add exactly 1 mL of the internal standard solution, then add methanol to make 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of chlordiazepoxide } (C_{16}H_{14}ClN_3O) = M_S \times Q_T/Q_S \times 5/V$$

M_S: Amount (mg) of Chlordiazepoxide RS taken

Internal standard solution—A solution of isobutyl salicylate in methanol (1 in 20).

Dissolution <6.10> When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 60 minutes of Chlordiazepoxide Tablets is not less than 70%.

Conduct this procedure without exposure to light, using light-resistant vessels. Start the test with 1 tablet of Chlordiazepoxide Tablets, withdraw not less than 30 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 3.7 μg of chlordiazepoxide ($C_{16}H_{14}ClN_3O$), and use this solution as the sample solution. Separately, weigh accurately about 12 mg of Chlordiazepoxide RS, previously dried under reduced pressure with phosphorus (V) oxide as a dessicant at 60°C for 4 hours, dissolve in 5 mL of 0.1 mol/L hydrochloric acid TS, and add the dissolution medium to make exactly 200 mL. Pipet 3 mL of this solution, add the dissolution medium to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 260 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\text{Dissolution rate (\%) with respect to the labeled amount of chlordiazepoxide } (C_{16}H_{14}ClN_3O) = M_S \times A_T/A_S \times V'/V \times 1/C \times 27$$

M_S: Amount (mg) of Chlordiazepoxide RS taken

C: Labeled amount (mg) of chlordiazepoxide ($C_{16}H_{14}ClN_3O$) in 1 tablet

Assay Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately a quantity of Chlordiazepoxide Tablets, equivalent to about 0.1 g of chlordiazepoxide ($C_{16}H_{14}ClN_3O$), add 10 mL of water, and shake well to disintegrate. Add 60 mL of methanol, shake well, add methanol to make exactly 100 mL, and centrifuge.

Pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Chlordiazepoxide RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 60°C) for 4 hours, dissolve in 1 mL of water and a suitable amount of methanol, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of chlordiazepoxide to that of the internal standard.

Amount (mg) of chlordiazepoxide ($C_{16}H_{14}ClN_3O$)
$= M_S \times Q_T/Q_S \times 10$

M_S: Amount (mg) of Chlordiazepoxide RS taken

Internal standard solution—A solution of isobutyl salicylate in methanol (1 in 20).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and 0.02 mol/L ammonium dihydrogenphosphate TS (7:3).

Flow rate: Adjust so that the retention time of chlordiazepoxide is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, chlordiazepoxide and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of chlordiazepoxide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Chlorhexidine Gluconate Solution

クロルヘキシジングルコン酸塩液

Chlorhexidine Gluconate Solution is a solution of digluconate of chlorhexidine.

It contains not less than 19.0 w/v% and not more than 21.0 w/v% of chlorhexidine gluconate ($C_{22}H_{30}Cl_2N_{10}.2C_6H_{12}O_7$: 897.76).

Description Chlorhexidine Gluconate Solution is a clear, colorless or pale yellow liquid. It is odorless, and has a bitter taste.

It is miscible with water and with acetic acid (100). 1 mL of Chlorhexidine Gluconate Solution is miscible with not more than 5 mL of ethanol (99.5) and with not more than 3 mL of acetone. By further addition of each of these solvents, a white turbidity is formed.

It is gradually colored by light.

Specific gravity d_{20}^{20}: 1.06 – 1.07

Identification **(1)** To 0.05 mL of Chlorhexidine Gluconate Solution add 5 mL of methanol, 1 mL of bromine TS and 1 mL of 8 mol/L sodium hydroxide TS: a deep red color is produced.

(2) To 0.5 mL of Chlorhexidine Gluconate Solution add 10 mL of water and 0.5 mL of copper (II) sulfate TS: a white precipitate is formed. Heat to boiling: the precipitate changes to light purple.

(3) To 10 mL of Chlorhexidine Gluconate Solution add 5 mL of water, cool on ice, and add 5 mL of sodium hydroxide TS dropwise with stirring: a white precipitate is formed. Collect the precipitate by filtration, wash with water, recrystallize from diluted ethanol (95) (7 in 10), and dry at 105°C for 30 minutes: the crystals thus obtained melt <2.60> between 130°C and 134°C.

(4) Neutralize the filtrate obtained in (3) with 5 mol/L hydrochloric acid TS. To 5 mL of this solution add 0.65 mL of acetic acid (100) and 1 mL of freshly distilled phenylhydrazine, and heat on a water bath for 30 minutes. After cooling, scratch the inner wall of the vessel with a glass rod to induce crystallization. Collect the crystals, dissolve in 10 mL of hot water, add a small amount of activated charcoal, and filter. Cool the filtrate, scratch the inner side of the vessel, collect the formed crystals, and dry: the crystals thus obtained melt <2.60> at about 195°C (with decomposition).

pH <2.54> To 5.0 mL of Chlorhexidine Gluconate Solution add water to make 100 mL: the pH of the solution is between 5.5 and 7.0.

Purity 4-Chloroaniline—To 2.0 mL of Chlorhexidine Gluconate Solution add water to make exactly 100 mL. Pipet 5 mL of the solution, and add 20 mL of water and 5 mL of 1 mol/L hydrochloric acid TS. Add 0.3 mL of sodium nitrite TS, shake, and allow to stand for 2 minutes. Add 4 mL of ammonium amidosulfate TS, and then allow to stand for 1 minute. Add 5 mL of *N*-(1-naphthyl)-*N'*-diethylethylenediamine oxalate-acetone TS, allow to stand for 10 minutes, add 1 mL of ethanol (95), and then add water to make 50 mL: the color of the solution is not more intense than the following control solution.

Control solution: Dissolve 20 mg of 4-chloroaniline in 10 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of the solution, and add water to make exactly 100 mL. Pipet 5 mL of the solution, add 20 mL of water and 5 mL of 1 mol/L hydrochloric acid TS, and proceed as directed for the preparation of the sample solution.

Residue on ignition <2.44> Not more than 0.1% (2 g, after evaporation).

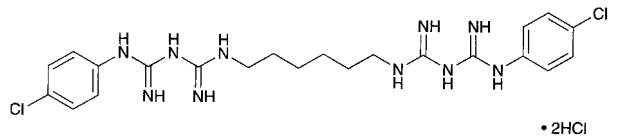
Assay Pipet 2 mL of Chlorhexidine Gluconate Solution, evaporate to dryness on a water bath, dissolve the residue in 60 mL of acetic acid for nonaqueous titration, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
$= 22.44$ mg of $C_{22}H_{30}Cl_2N_{10}.2C_6H_{12}O_7$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Chlorhexidine Hydrochloride

クロルヘキシジン塩酸塩



$C_{22}H_{30}Cl_2N_{10}.2HCl$: 578.37
1,1′-Hexamethylenebis[5-(4-chlorophenyl)biguanide] dihydrochloride
[*3697-42-5*]

Chlorhexidine Hydrochloride, when dried, contains not less than 98.0% of chlorhexidine hydrochloride ($C_{22}H_{30}Cl_2N_{10}.2HCl$).

Description Chlorhexidine Hydrochloride occurs as a white crystalline powder. It is odorless, and has a bitter taste.

It is soluble in formic acid, slightly soluble in methanol and in warm methanol, and practically insoluble in water, in ethanol (95) and in diethyl ether.

It is gradually colored by light.

Identification **(1)** Dissolve 0.01 g of Chlorhexidine Hydrochloride in 5 mL of methanol by warming, and add 1 mL of bromine TS and 1 mL of 8 mol/L sodium hydroxide TS: a deep red color is produced.

(2) Dissolve 0.3 g of Chlorhexidine Hydrochloride in 10 mL of 6 mol/L hydrochloric acid TS, cool in ice, and add 10 mL of 8 mol/L sodium hydroxide TS dropwise with stirring: a white precipitate is produced. Collect the precipitate, wash with water, recrystallize from diluted ethanol (95) (7 in 10), and dry at 105°C for 30 minutes: the crystals so obtained melt ⟨*2.60*⟩ between 130°C and 134°C.

(3) Dissolve 0.1 g of Chlorhexidine Hydrochloride in 50 mL of dilute nitric acid: the solution responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Chlorhexidine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic ⟨*1.11*⟩—To 1.0 g of Chlorhexidine Hydrochloride in a crucible add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), fire the ethanol (95) to burn, and heat gradually to incinerate. If a carbonized substance remains, moisten with a small amount of nitric acid, and ignite to incinerate. Cool, add 10 mL of dilute hydrochloric acid to the residue, dissolve by warming on a water bath, use this solution as the test solution, and perform the test (not more than 2 ppm).

(3) *p*-Chloroaniline—Dissolve 0.10 g of Chlorhexidine Hydrochloride in 2 mL of formic acid, and add 15 mL of 1 mol/L hydrochloric acid TS and 20 mL of water immediately. Add 0.3 mL of sodium nitrite TS, shake, and allow to stand for 2 minutes. Add 4 mL of ammonium amidosulfate TS, and then allow to stand for 1 minute. Add 5 mL of *N*-(1-naphthyl)-*N*′-diethylethylenediamine oxalate-acetone TS, allow to stand for 10 minutes, and add 1 mL of ethanol (95) and water to make 50 mL: the solution has no more color than the following control solution.

Control solution: Dissolve 20 mg of 4-chloroaniline in 10 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of the solution, and add water to make exactly 100 mL. To 2.0 mL of the solution add 2 mL of formic acid, 15 mL of 1 mol/L hydrochloric acid TS and 20 mL of water, and proceed in the same manner.

Loss on drying ⟨*2.41*⟩ Not more than 2.0% (1 g, 130°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Chlorhexidine Hydrochloride, previously dried, dissolve in 2.0 mL of formic acid, add 60 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 14.46 mg of $C_{22}H_{30}Cl_2N_{10}.2HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Chlorinated Lime

サラシ粉

Chlorinated Lime contains not less than 30.0% of available chlorine (Cl: 35.45).

Description Chlorinated Lime occurs as a white powder. It has a chlorine-like odor.

It dissolves partially in water. The solution changes red litmus paper to blue, then gradually decolorizes.

Identification **(1)** To Chlorinated Lime add dilute hydrochloric acid: a gas, which has the odor of chlorine, evolves, and the gas changes moistened starch-potassium iodide paper to blue.

(2) Shake 1 g of Chlorinated Lime with 10 mL of water, and filter: the filtrate responds to the Qualitative Tests ⟨*1.09*⟩ (2) and (3) for calcium salt.

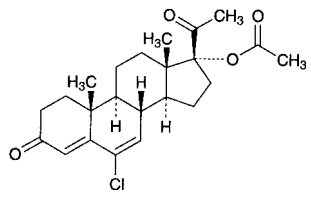
Assay Weigh accurately about 5 g of Chlorinated Lime, transfer to a mortar, and triturate thoroughly with 50 mL of water. Transfer to a 500-mL volumetric flask with the aid of water, and add water to make 500 mL. Mix well, immediately take exactly 50 mL of the mixture in an iodine flask, add 10 mL of potassium iodide TS and 10 mL of dilute hydrochloric acid, and titrate ⟨*2.50*⟩ the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 3.545 mg of Cl

Containers and storage Containers—Tight containers.
Storage—Light-resistant, and in a cold place.

Chlormadinone Acetate

クロルマジノン酢酸エステル



$C_{23}H_{29}ClO_4$: 404.93
6-Chloro-3,20-dioxopregna-4,6-dien-17-yl acetate
[*302-22-7*]

Chlormadinone Acetate, when dried, contains not less than 98.0% of chlormadinone acetate ($C_{23}H_{29}ClO_4$).

Description Chlormadinone Acetate occurs as white to light yellow, crystals or crystalline powder. It is odorless.

It is freely soluble in chloroform, soluble in acetonitrile, slightly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

Identification **(1)** Dissolve 2 mg of Chlormadinone Acetate in 1 mL of ethanol (95), and add 1 mL of 1,3-dinitrobenzene TS and 1 mL of a solution of potassium hydroxide (1 in 5): a red-purple color develops.

(2) To 0.05 g of Chlormadinone Acetate add 2 mL of potassium hydroxide-ethanol TS, and boil on a water bath for 5 minutes. After cooling, add 2 mL of diluted sulfuric acid (2 in 7), and boil gently for 1 minute: the odor of ethyl acetate is perceptible.

(3) Determine the infrared absorption spectrum of Chlormadinone Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Chlormadinone Acetate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Perform the test with Chlormadinone Acetate as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -10.0 – $-14.0°$ (after drying, 0.2 g, acetonitrile, 10 mL, 100 mm).

Melting point <*2.60*> 211 – 215°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Chlormadinone Acetate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Chlormadinone Acetate according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 20 mg of Chlormadinone Acetate in 10 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the total area of peaks other than the peak of chlormadinone acetate from the sample solution is not larger than the peak area of chlormadinone acetate from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 236 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of acetonitrile and water (13:7).

Flow rate: Adjust so that the retention time of chlormadinone acetate is about 10 minutes.

Time span of measurement: About 1.5 times as long as the retention time of chlormadinone acetate, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add acetonitorile to make exactly 50 mL. Confirm that the peak area of chlormadinone acetate obtained from 10 μL of this solution is equivalent to 7 to 13% of that of chlormadinone acetate obtained from 10 μL of the standard solution.

System performance: Dissolve 8 mg of Chlormadinone Acetate and 2 mg of butyl parahydroxybenzoate in 100 mL of acetonitrile. When the procedure is run with 10 μL of this solution under the above operating conditions, butyl parahydroxybenzoate and chlormadinone acetate are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of chlormadinone acetate is not more than 1.0%.

Loss on drying <*2.41*> Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 20 mg each of Chlormadinone Acetate and Chlormadinone Acetate RS, previously dried, and dissolve in ethanol (95) to make exactly 100 mL. Pipet 5 mL each of these solutions, to each add ethanol (95) to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 285 nm.

$$\text{Amount (mg) of chlormadinone acetate } (C_{23}H_{29}ClO_4) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Chlormadinone Acetate RS taken

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Chlorobutanol

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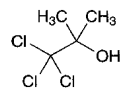

$C_4H_7Cl_3O$: 177.46
1,1,1-Trichloro-2-methylpropan-2-ol
[*57-15-8*]

Chlorobutanol contains not less than 98.0% of chlorobutanol ($C_4H_7Cl_3O$), calculated on the anhydrous basis.

Description Chlorobutanol occurs as colorless or white crystals. It has a camphoraceous odor.

It is very soluble in methanol, in ethanol (95) and in diethyl ether, and slightly soluble in water.

It slowly volatilizes in air.

Melting point: not lower than about 76°C.

Identification **(1)** To 5 mL of a solution of Chlorobutanol (1 in 200) add 1 mL of sodium hydroxide TS, then slowly add 3 mL of iodine TS: a yellow precipitate is produced and the odor of iodoform is perceptible.

(2) To 0.1 g of Chlorobutanol add 5 mL of sodium hydroxide TS, shake well the mixture, add 3 to 4 drops of aniline, and warm gently: the disagreeable odor of phenyl isocyanide (poisonous) is perceptible.

Purity **(1)** Acidity—Shake thoroughly 0.10 g of the powder of Chlorobutanol with 5 mL of water: the solution is neutral.

(2) Chloride <*1.03*>—Dissolve 0.5 g of Chlorobutanol in 25 mL of dilute ethanol, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS by adding 25 mL of dilute ethanol, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.071%).

Water <*2.48*> Not more than 6.0% (0.2 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Transfer about 0.1 g of Chlorobutanol, accurately weighed, to a 200-mL conical flask, and dissolve in 10 mL of ethanol (95). Add 10 mL of sodium hydroxide TS, boil under a reflux condenser for 10 minutes, cool, add 40 mL of dilute nitric acid and exactly 25 mL of 0.1 mol/L silver nitrate VS, and shake well. Add 3 mL of nitrobenzene, and shake vigorously until the precipitate is coagulated. Titrate <*2.50*> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L silver nitrate VS
= 5.915 mg of $C_4H_7Cl_3O$

Containers and storage Containers—Tight containers.

Chlorphenesin Carbamate

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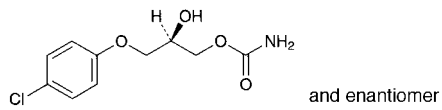

$C_{10}H_{12}ClNO_4$: 245.66
(2*RS*)-3-(4-Chlorophenoxy)-2-hydroxypropyl carbamate
[*886-74-8*]

Chlorphenesin Carbamate, when dried, contains not less than 98.0% and not more than 102.0% of chlorphenesin carbamate ($C_{10}H_{12}ClNO_4$).

Description Chlorphenesin Carbamate occurs as white, crystals or a crystalline powder.

It is freely soluble in methanol, in ethanol (95) and in pyridine, and slightly soluble in water.

A solution of Chlorphenesin Carbamate in ethanol (95) (1 in 20) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Chlorphenesin Carbamate in ethanol (95) (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Chlorphenesin Carbamate, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Chlorphenesin Carbamate as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Melting point <*2.60*> 88 – 91°C

Purity **(1)** Heavy metals <*1.07*>—Dissolve 2.0 g of Chlorphenesin Carbamate in 20 mL of ethanol (95), and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 20 mL of ethanol (95), 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Chlorphenesin Carbamate according to Method 3, and perform the test (not more than 2 ppm).

(3) Chlorphenesin-2-carbamate—Dissolve 0.10 g of Chlorphenesin Carbamate in 20 mL of a mixture of hexane for liquid chromatography and 2-propanol (7:3), and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak area, A_a, of chlorphenesin carbamate and the peak area, A_b, of chlorphenesin-2-carbamate by the automatic integration method: the ratio, $A_b/(A_a + A_b)$, is not more than 0.007.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about

40°C.

Mobile phase: A mixture of hexane for liquid chromatography, 2-propanol and acetic acid (100) (700:300:1).

Flow rate: Adjust so that the retention time of chlorphenesin carbamate is about 9 minutes.

System suitability—

Test for required detectability: To 1 mL of the sample solution, add a mixture of hexane for liquid chromatography and 2-propanol (7:3) to make 100 mL, and use this solution as the solution for system suitability test. To exactly 5 mL of the solution for system suitability test add the mixture of hexane for liquid chromatography and 2-propanol (7:3) to make exactly 10 mL. Confirm that the peak area of chlorphenesin carbamate obtained from 10 μL of this solution is equivalent to 40 to 60% of that of chlorphenesin carbamate obtained from 10 μL of the solution for system suitability test.

System performance: Dissolve 0.1 g of Chlorphenesin Carbamate in 50 mL of methanol. To 25 mL of this solution add 25 mL of dilute sodium hydroxide TS, and warm at 60°C for 20 minutes. To 20 mL of this solution add 5 mL of 1 mol/L hydrochloric acid TS, shake well with 20 mL of ethyl acetate, and allow to stand to separate the upper layer. When the procedure is run with 10 μL of this layer under the above operating conditions, chlorphenesin, chlorphenesin carbamate and chlorphenesin-2-carbamate are eluted in this order, with the relative retention times of chlorphenesin and chlorphenesin-2-carbamate to chlorphenesin carbamate being about 0.7 and about 1.2, respectively, and with the resolution between the peaks of chlorphenesin and chlorphenesin carbamate being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of chlorphenesin carbamate is not more than 2.0%.

(4) Related substances—Dissolve 0.10 g of Chlorphenesin Carbamate in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 20 mL. Pipet 2 mL of this solution, add ethanol (95) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 50 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (17:2:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 20 minutes: the spot other than the principal spot from the sample solution is not more than one, and it is not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.20% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Chlorphenesin Carbamate, previously dried, dissolve in 20 mL of pyridine, add exactly 50 mL of 0.1 mol/L potassium hydroxide-ethanol TS, and warm at 70°C for 40 minutes. After cooling, add 100 mL of ethanol (95), and titrate <*2.50*> the excess potassium hydroxide with 0.1 mol/L hydrochloric acid VS until the color of the solution changes from blue through blue-green to yellow (indicator: 1 mL of thymol blue TS). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L potassium hydroxide-ethanol TS
= 24.57 mg of $C_{10}H_{12}ClNO_4$

Containers and storage Containers—Tight containers.

Chlorphenesin Carbamate Tablets

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Chlorphenesin Carbamate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of chlorphenesin carbamate ($C_{10}H_{12}ClNO_4$: 245.66).

Method of preparation Prepare as directed under Tablets, with Chlorphenesin Carbamate.

Identification To a quantity of powdered Chlorphenesin Carbamate Tablets, equivalent to 0.15 g of Chlorphenesin Carbamate, add 60 mL of ethanol (95), treat with ultrasonic waves, and add ethanol (95) to make 100 mL. Centrifuge 20 mL of this solution, add ethanol (95) to 1 mL of the supernatant liquid to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 226 nm and 230 nm, between 279 nm and 283 nm, and between 286 nm and 290 nm.

Uniformity of dosage units <*6.02*> Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Chlorphenesin Carbamate Tablets add 10 mL of water to disintegrate the tablet, add 70 mL of a mixture of water and methanol (1:1), treat with ultrasonic waves for 15 minutes with occasional stirring, then add the mixture of water and methanol (1:1) to make exactly 100 mL. Centrifuge this solution, pipet V mL of the supernatant liquid equivalent to about 2.5 mg of chlorphenesin carbamate ($C_{10}H_{12}ClNO_4$), add the mixture of water and methanol (1:1) to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of chlorphenesin carbamate for assay, previously dried in a desiccator (in vacuum, silica gel) for 4 hours, and dissolve in the mixture of water and methanol (1:1) to make exactly 50 mL. Pipet 2 mL of this solution, add the mixture of water and methanol (1:1) to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances at 280 nm, A_T and A_S, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount (mg) of chlorphenesin carbamate ($C_{10}H_{12}ClNO_4$)
$= M_S \times A_T/A_S \times 1/V \times 5$

M_S: Amount (mg) of chlorphenesin carbamate for assay taken

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Chlorphenesin Carbamate Tablets is not less than 85%.

Start the test with 1 tablet of Chlorphenesin Carbamate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each

mL contains about 0.14 mg of chlorphenesin carbamate ($C_{10}H_{12}ClNO_4$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of chlorphenesin carbamate for assay, previously dried in a desiccator (in vacuum, silica gel) for 4 hours, dissolve in 1 mL of methanol, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 278 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of chlorphenesin carbamate ($C_{10}H_{12}ClNO_4$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 450$

M_S: Amount (mg) of chlorphenesin carbamate for assay taken
C: Labeled amount (mg) of chlorphenesin carbamate ($C_{10}H_{12}ClNO_4$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Chlorphenesin Carbamate Tablets, and powder them in an agate mortar. Weigh accurately a portion of the powder, equivalent to about 0.25 g of chlorphenesin carbamate ($C_{10}H_{12}ClNO_4$), add 30 mL of ethyl acetate, disperse using ultrasonic waves, then add ethyl acetate to make exactly 50 mL. Centrifuge 20 mL of this solution, pipet 2 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, add ethyl acetate to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of chlorphenesin carbamate for assay, previously dried in a desiccator (in vacuum, silica gel) for 4 hours, and dissolve in ethyl acetate to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 2 mL of the internal standard solution, then add ethyl acetate to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of chlorphenesin carbamate to that of the internal standard.

Amount (mg) of chlorphenesin carbamate ($C_{10}H_{12}ClNO_4$)
$= M_S \times Q_T/Q_S \times 5/2$

M_S: Amount (mg) of chlorphenesin carbamate for assay taken

Internal standard solution—A solution of ethenzamide in ethyl acetate (1 in 400).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 280 nm).
Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of hexane for liquid chromatography, 2-propanol and acetic acid (100) (700:300:1).
Flow rate: Adjust so that the retention time of chlorphenesin carbamate is about 9 minutes.
System suitability—
System performance: Proceed as directed in the system suitability in the Purity (3) under Chlorphenesin Carbamate.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of chlorphenesin carbamate to that of the internal standard is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Chlorpheniramine Maleate

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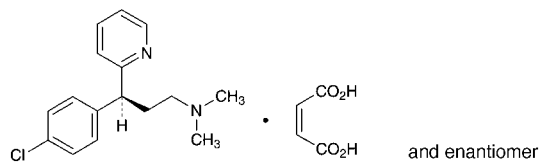

$C_{16}H_{19}ClN_2.C_4H_4O_4$: 390.86
(3*RS*)-3-(4-Chlorophenyl)-*N*,*N*-dimethyl-3-pyridin-2-ylpropylamine monomaleate
[*113-92-8*]

Chlorpheniramine Maleate, when dried, contains not less than 98.0% and not more than 101.0% of *dl*-chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$).

Description Chlorpheniramine Maleate occurs as white, fine crystals.
It is very soluble in acetic acid (100), freely soluble in water and in methanol, and soluble in ethanol (99.5).
It dissolves in dilute hydrochloric acid.
A solution of Chlorpheniramine Maleate (1 in 20) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Chlorpheniramine Maleate in 0.1 mol/L hydrochloric acid TS (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Chlorpheniramine Maleate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.
(2) Determine the infrared absorption spectrum of Chlorpheniramine Maleate, previously dried, as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Chlorpheniramine Maleate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.
(3) Dissolve 0.10 g of Chlorpheniramine Maleate in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 56 mg of maleic acid in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether, methanol, acetic acid (100) and water (70:20:7:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a spot among two of the spots obtained with the sample solution shows the same intense and *R*f value with the spot obtained with the standard solution.

pH <2.54> Dissolve 1.0 g of Chlorpheniramine Maleate in 100 mL of freshly boiled and cooled water: the pH of this solution is between 4.0 and 5.5.

Melting point <2.60> 130 – 135°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g

of Chlorpheniramine Maleate in 50 mL of water: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Chlorpheniramine Maleate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of Chlorpheniramine Maleate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 3 mL of the sample solution, and add the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than maleic acid and chlorpheniramine obtained with the sample solution is not larger than 2/3 times the peak area of chlorpheniramine obtained with the standard solution, and the total area of the peaks other than maleic acid and chlorpheniramine with the sample solution is not larger than the peak area of chlorpheniramine with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 8.57 g of ammonium dihydrogenphosphate and 1 mL of phosphoric acid in water to make 1000 mL. To 800 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust so that the retention time of chlorpheniramine is about 11 minutes.

Time span of measurement: About 4 times as long as the retention time of chlorpheniramine, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2.5 mL of the standard solution add the mobile phase to make exactly 25 mL. Confirm that the peak area of chlorpheniramine obtained with 20 μL of this solution is equivalent to 7 to 13% of that obtained with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of chlorpheniramine are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of chlorpheniramine is not more than 4.0%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Dissolve about 0.4 g of Chlorpheniramine Maleate, previously dried and accurately weighed, in 20 mL of acetic acid (100). Titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-green to green (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 19.54 mg of $C_{16}H_{19}ClN_2.C_4H_4O_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Chlorpheniramine Maleate Injection

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Chlorpheniramine Maleate Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of *dl*-chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$: 390.86).

Method of preparation Prepare as directed under Injections, with Chlorpheniramine Maleate.

Description Chlorpheniramine Maleate Injection is a clear, colorless liquid.
pH: 4.5 – 7.0

Identification Take a volume of Chlorpheniramine Maleate Injection, equivalent to 25 mg of Chlorpheniramine Maleate, add 5 mL of dilute sodium hydroxide TS, and extract with 20 mL of hexane. Wash the hexane layer with 10 mL of water, shake with 0.5 g of anhydrous sodium sulfate for several minutes, and filter. Evaporate the filtrate in a water bath at 50°C under a reduced pressure, and determine the infrared absorption spectrum of the residue as directed in the liquid film method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 2940 cm^{-1}, 2810 cm^{-1}, 2770 cm^{-1}, 1589 cm^{-1}, 1491 cm^{-1}, 1470 cm^{-1}, 1434 cm^{-1}, 1091 cm^{-1} and 1015 cm^{-1}.

Bacterial endotoxins <*4.01*> Less than 8.8 EU/mg.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Transfer an exactly measured volume of Chlorpheniramine Maleate Injection, equivalent to about 3 mg of chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$), to a 100-mL separator, add 20 mL of water and 2 mL of sodium hydroxide TS, and extract with two 50-mL portions of diethyl ether. Combine the diethyl ether extracts, wash with 20 mL of water, and then extract with 20-mL, 20-mL and 5-mL portions of 0.25 mol/L sulfuric acid TS successively. Combine all acid extracts, and add 0.25 mol/L sulfuric acid TS to make exactly 50 mL. Pipet 10 mL of this solution, add 0.25 mol/L sulfuric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Chlorpheniramine Maleate RS, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 200 mL. Pipet 20 mL of this solution, transfer to a 100-mL separator, add 2 mL of sodium hydroxide TS, and extract with two 50-mL portions of diethyl ether. Proceed in the same manner as for the preparation of the sample solution, and use the solution so obtained as the standard solution. Determine the absorbances A_T and A_S of the sample solution and standard solution at a wavelength of the maxi-

mum absorbance at about 265 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$)
$= M_S \times A_T/A_S \times 1/10$

M_S: Amount (mg) of Chlorpheniramine Maleate RS taken

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant.

Chlorpheniramine Maleate Powder

クロルフェニラミンマレイン酸塩散

Chlorpheniramine Maleate Powder contains not less than 93.0% and not more than 107.0% of the labeled amount of *dl*-chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$: 390.86).

Method of preparation Prepare as directed under Granules or Powders, with Chlorpheniramine Maleate.

Identification Weigh a portion of Chlorpheniramine Maleate Powder, equivalent to 50 mg of Chlorpheniramine Maleate, shake with 40 mL of 0.1 mol/L hydrochloric acid TS, and filter. Transfer the filtrate to a separator, and wash with 40 mL of hexane. Add 10 mL of sodium hydroxide TS, and extract with 20 mL of hexane. Wash the hexane layer with 5 mL of water. Centrifuge, if necessary, shake the hexane extract with 0.5 g of anhydrous sodium sulfate for several minutes, and filter. Evaporate the filtrate in a water bath at about 50°C under reduced pressure, and determine the infrared absorption spectrum of the residue as directed in the liquid film method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave number of about 2940 cm^{-1}, 2810 cm^{-1}, 2770 cm^{-1}, 1589 cm^{-1}, 1491 cm^{-1}, 1470 cm^{-1}, 1434 cm^{-1}, 1091 cm^{-1} and 1015 cm^{-1}.

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Chlorpheniramine Maleate Powder is not less than 85%.

Start the test with an accurately weighed amount of Chlorpheniramine Maleate Powder, equivalent to about 4 mg of chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$), withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 22 mg of Chlorpheniramine Maleate RS, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of chlorpheniramine in each solution.

Dissolution rate (%) with respect to the labeled amount of chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 18$

M_S: Amount (mg) of Chlorpheniramine Maleate RS taken
M_T: Amount (g) of Chlorpheniramine Maleate Powder taken
C: Labeled amount (mg) of chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$) in 1 g

Operating conditions—
Proceed as directed in the operating conditions in the Assay.
System suitability—
System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of chlorpheniramine are not less than 2000 and not more than 2.5, respectively.
System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of chlorpheniramine is not more than 2.0%.

Assay Weigh accurately an amount of Chlorpheniramine Maleate Powder, equivalent to about 4 mg of chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$), add 70 mL of the internal standard solution, shake for 15 minutes, then add the internal standard solution to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Chlorpheniramine Maleate RS, previously dried at 105°C for 3 hours, and add the internal standard to make exactly 100 mL. Pipet 20 mL of this solution, add the internal standard solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 30 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of chlorpheniramine to that of the internal standard.

Amount (mg) of chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$)
$= M_S \times Q_T/Q_S \times 1/5$

M_S: Amount (mg) of Chlorpheniramine Maleate RS taken

Internal standard solution—To 7 mL of a solultion of methyl parahydroxybenzoate in methanol (1 in 1000) add water to make 1000 mL.
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 265 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 1 g of sodium 1-heptane sulfonate in 900 mL of water, add 10 mL of acetic acid (100) and water to make 1000 mL. To 650 mL of this solution add 350 mL of acetonitrile.
Flow rate: Adjust so that the retention time of chlorpheniramine is about 8 minutes.
System suitability—
System performance: When the procedure is run with 30 μL of the standard solution under the above operating conditions, the internal standard and chlorpheniramine are eluted in this order with the resolution between these peaks being not less than 2.0.
System repeatability: When the test is repeated 6 times with 30 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of chlorpheniramine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Chlorpheniramine Maleate Tablets

クロルフェニラミンマレイン酸塩錠

Chlorpheniramine Maleate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of *dl*-chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$: 390.86).

Method of preparation Prepare as directed under Tablets, with Chlorpheniramine Maleate.

Identification Weigh a portion of powdered Chlorpheniramine Maleate Tablets, equivalent to 50 mg of Chlorpheniramine Maleate, shake with 40 mL of 0.1 mol/L hydrochloric acid TS, and filter. Transfer the filtrate to a separator, and wash with 40 mL of hexane. Add 10 mL of sodium hydroxide TS, and extract with 20 mL of hexane. Wash the hexane layer with 5 mL of water. Centrifuge, if necessary, shake the hexane extract with 0.5 g of anhydrous sodium sulfate for several minutes, and filter. Evaporate the filtrate in a water bath at about 50°C under a reduced pressure, and determine the infrared absorption spectrum of the residue as directed in the liquid film method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 2940 cm^{-1}, 2810 cm^{-1}, 2770 cm^{-1}, 1589 cm^{-1}, 1491 cm^{-1}, 1470 cm^{-1}, 1434 cm^{-1}, 1091 cm^{-1} and 1015 cm^{-1}.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Chlorpheniramine Maleate Tablets add 10 mL of water, shake to disintegrate the tablet, then add water to make exactly V mL of a solution containing about 80 μg of chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$) per mL, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Pipet 5 mL of the filtrate, add exactly 2.5 mL of the internal standard solution, add water to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Chlorpheniramine Maleate RS, previously dried at 105°C for 3 hours, and add water to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 25 mL of the internal standard solution, add water to make 100 mL, and use this solution as the standard solution. Perform the test with 30 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the conditions described in the Assay, and calculate the ratios, Q_T and Q_S, of the peak area of chlorpheniramine to that of the internal standard.

Amount (mg) of chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$)
$= M_S \times Q_T/Q_S \times V/250$

M_S: Amount (mg) of Chlorpheniramine Maleate RS taken

Internal standard solution—To 7 mL of a solution of methyl parahydroxybenzoate (1 in 250) add water to make 1000 mL.

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Chlorpheniramine Maleate Tablets is not less than 75%.

Start the test with 1 tablet of Chlorpheniramine Maleate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 4.4 μg of chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Chlorpheniramine Maleate RS, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01>, and determine the peak areas, A_T and A_S, of chlorpheniramine in each solution.

Dissolution rate (%) with respect to the labeled amount of chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of Chlorpheniramine Maleate RS taken
C: Labeled amount (mg) of chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1 g of sodium 1-heptane sulfonate in 900 mL of water, add 10 mL of acetic acid (100), and add water to make 1000 mL. To 650 mL of this solution add 350 mL of acetonitrile.

Flow rate: Adjust so that the retention time of chlorpheniramine is about 8 minutes.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of chlorpheniramine are not less than 2000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of chlorpheniramine is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Chlorpheniramine Maleate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 4 mg of chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$), add 70 mL of the internal standard solution, shake for 15 minutes, then add the internal standard solution to make exactly 100 mL, filter through a membrane filter with a pore size not exceeding 0.5 μm, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Chlorpheniramine Maleate RS, previously dried at 105°C for 3 hours, and add the internal standard to make exactly 100 mL. Pipet 20 mL of this solution, add the internal standard solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 30 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of chlorpheniramine to that of the internal standard.

Amount (mg) of chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$)
$= M_S \times Q_T/Q_S \times 1/5$

M_S: Amount (mg) of Chlorpheniramine Maleate RS taken

Internal standard solution—To 7 mL of a solution of methyl parahydroxybenzoate in methanol (1 in 1000) add water to make 1000 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1 g of sodium 1-heptane sulfonate in 900 mL of water, add 10 mL of acetic acid (100) and water to make 1000 mL. To 650 mL of this solution add 350 mL of acetonitrile.

Flow rate: Adjust so that the retention time of chlorpheniramine is about 8 minutes.

System suitability—

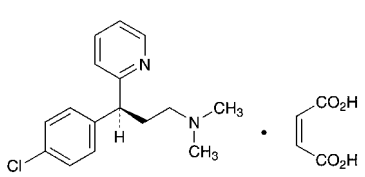
System performance: When the procedure is run with 30 μL of the standard solution under the above operating conditions, the internal standard and chlorpheniramine are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 30 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of chlorpheniramine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

d-Chlorpheniramine Maleate

d-クロルフェニラミンマレイン酸塩



$C_{16}H_{19}ClN_2.C_4H_4O_4$: 390.86
(3*S*)-3-(4-Chlorophenyl)-*N*,*N*-dimethyl-3-pyridin-2-ylpropylamine monomaleate
[*2438-32-6*]

d-Chlorpheniramine Maleate, when dried, contains not less than 99.0% and not more than 101.0% of *d*-chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$).

Description *d*-Chlorpheniramine Maleate occurs as a white, crystalline powder.

It is very soluble in water, in methanol and in acetic acid (100), and freely soluble in *N*,*N*-dimethylformamide and in ethanol (99.5).

It dissolves in dilute hydrochloric acid.

Identification (1) Determine the absorption spectrum of a solution of *d*-Chlorpheniramine Maleate in 0.1 mol/L hydrochloric acid TS (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of *d*-Chlorpheniramine Maleate, previously dried, as directed in the paste method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.10 g of *d*-Chlorpheniramine Maleate in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 56 mg of maleic acid in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether, methanol, acetic acid (100) and water (70:20:7:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a spot among two of the spots obtained with the sample solution shows the same intense to the spot obtained with the standard solution, and its *R*f value is about 0.4.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +39.5 – +43.0° (after drying, 0.5 g, *N*,*N*-dimethylformamide, 10 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 1.0 g of *d*-Chlorpheniramine Maleate in 100 mL of freshly boiled and cooled water: the pH of this solution is between 4.0 and 5.0.

Melting point ⟨*2.60*⟩ 111 – 115°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of *d*-Chlorpheniramine Maleate in 50 mL of water: the solution is clear and colorless.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of *d*-Chlorpheniramine Maleate according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of *d*-Chlorpheniramine Maleate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than maleic acid and *d*-chlorpheniramine obtained with the sample solution is not larger than 2/3 times the peak area of *d*-chlorpheniramine obtained with the standard solution, and the total area of these peaks is not larger than the peak area of *d*-chlorpheniramine with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 8.57 g of ammonium dihydrogen phosphate and 1 mL of phosphoric acid in water to make 1000 mL. To 800 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust so that the retention time of *d*-chlorpheniramine is about 11 minutes.

Time span of measurement: About 4 times as long as the

retention time of *d*-chlorpheniramine, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2.5 mL of the standard solution add the mobile phase to make exactly 25 mL. Confirm that the peak area of *d*-chlorpheniramine obtained with 20 μL of this solution is equivalent to 7 to 13% of that obtained with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of *d*-chlorpheniramine are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of *d*-chlorpheniramine is not more than 4.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 65°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

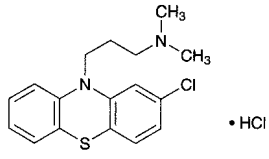
Assay Weigh accurately about 0.3 g of *d*-Chlorpheniramine Maleate, previously dried, and dissolve in 20 mL of acetic acid (100). Titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-green to green (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 19.54 mg of $C_{16}H_{19}ClN_2.C_4H_4O_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Chlorpromazine Hydrochloride

クロルプロマジン塩酸塩



$C_{17}H_{19}ClN_2S.HCl$: 355.33
3-(2-Chloro-10*H*-phenothiazin-10-yl)-*N*,*N*-dimethylpropylamine monohydrochloride
[*69-09-0*]

Chlorpromazine Hydrochloride, when dried, contains not less than 99.0% of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S.HCl$).

Description Chlorpromazine Hydrochloride occurs as a white to pale yellow, crystalline powder. It is odorless, or has a faint, characteristic odor.

It is very soluble in water, freely soluble in ethanol (95) and in acetic acid (100), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

It is gradually colored by light.

Identification **(1)** To 5 mL of a solution of Chlorpromazine Hydrochloride (1 in 1000) add 1 drop of iron (III) chloride TS: a red color develops.

(2) Dissolve 0.1 g of Chlorpromazine Hydrochloride in 20 mL of water and 3 drops of dilute hydrochloric acid, add 10 mL of 2,4,6-trinitrophenol TS, and allow to stand for 5 hours. Collect the resulting precipitate, wash with water, recrystallize from a small portion of acetone, and dry at 105°C for 1 hour: the crystals so obtained melt ⟨*2.60*⟩ between 175°C and 179°C.

(3) Dissolve 0.5 g of Chlorpromazine Hydrochloride in 5 mL of water, add 2 mL of ammonia TS, and heat on a water bath for 5 minutes. Cool, filter, and render the filtrate acidic with dilute nitric acid: the solution responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

Melting point ⟨*2.60*⟩ 194 – 198°C

pH ⟨*2.54*⟩ Dissolve 1.0 g of Chlorpromazine Hydrochloride in 20 mL of freshly boiled and cooled water, and measure within 10 minutes: the pH of this solution is between 4.0 and 5.0.

Purity **(1)** Clarity and color of solution—A solution of 1.0 g of Chlorpromazine Hydrochloride in 20 mL of water, when observed within 10 minutes, is clear and colorless to pale yellow.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Chlorpromazine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.7 g of Chlorpromazine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 35.53 mg of $C_{17}H_{19}ClN_2S.HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Chlorpromazine Hydrochloride Injection

クロルプロマジン塩酸塩注射液

Chlorpromazine Hydrochloride Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S.HCl$: 355.33).

Method of preparation Prepare as directed under Injections, with Chlorpromazine Hydrochloride.

Description Chlorpromazine Hydrochloride Injection is a clear, colorless or pale yellow liquid.

pH: 4.0 – 6.5

Identification **(1)** Proceed with a volume of Chlorpromazine Hydrochloride Injection, equivalent to 5 mg of Chlorpromazine Hydrochloride, as directed in the Identification (1) under Chlorpromazine Hydrochloride.

(2) Proceed with a volume of Chlorpromazine Hydrochloride Injection, equivalent to 0.1 g of Chlorpromazine Hydrochloride, as directed in the Identification (2) under

Chlorpromazine Hydrochloride.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Transfer an exactly measured volume of Chlorpromazine Hydrochloride Injection, equivalent to about 0.15 g of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S.HCl$) to a separator, add 30 mL of water and 10 mL of a solution of sodium hydroxide (1 in 5), and extract with two 30-mL portions and three 20-mL portions of diethyl ether. Wash the combined diethyl ether extracts with successive 10-mL portions of water until the last washing shows no red color upon the addition of phenolphthalein TS. Concentrate the diethyl ether extracts on a water bath to 20 mL, add 5 g of anhydrous sodium sulfate, allow to stand for 20 minutes, and filter through a pledget of absorbent cotton. Wash with diethyl ether, combine the washings with the filtrate, and evaporate the diethyl ether on a water bath. Dissolve the residue in 50 mL of acetone and 5 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.05 mol/L perchloric acid VS until the color of the solution changes from red-purple to blue-purple (indicator: 3 drops of bromocresol green-methylrosaniline chloride TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 17.77 mg of $C_{17}H_{19}ClN_2S.HCl$

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Chlorpromazine Hydrochloride Tablets

クロルプロマジン塩酸塩錠

Chlorpromazine Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S.HCl$: 355.33).

Method of preparation Prepare as directed under Tablets, with Chlorpromazine Hydrochloride.

Identification **(1)** Shake a quantity of powdered Chlorpromazine Hydrochloride Tablets, equivalent to 0.2 g of Chlorpromazine Hydrochloride, with 40 mL of 0.1 mol/L hydrochloric acid TS, and filter. To 1 mL of the filtrate add 4 mL of water and 1 drop of iron (III) chloride TS: a red color develops.

(2) To 20 mL of the filtrate obtained in (1) add 10 mL of 2,4,6-trinitrophenol TS dropwise, and proceed as directed in the Identification (2) under Chlorpromazine Hydrochloride.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Conduct this procedures using light-resistant vessels. To 1 tablet of Chlorpromazine Hydrochloride Tablets add an amount of a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) so that each mL contains about 0.83 mg of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S.HCl$), treat with the ultrasonic waves for 5 minutes, then shake vigorously for 20 minutes, and add the mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make exactly V mL so that each mL contains about 0.5 mg of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S.HCl$). Filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, pipet 2.5 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution, then add the mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make 25 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S.HCl$)
$= M_S \times Q_T/Q_S \times V/50$

M_S: Amount (mg) of chlorpromazine hydrochloride for assay taken

Internal standard solution—A solution of ethyl parahydroxybenzoate in the mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) (1 in 4500).

Dissolution ⟨*6.10*⟩ When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 60 minutes of Chlorpromazine Hydrochloride Tablets is not less than 75%.

Start the test with 1 tablet of Chlorpromazine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 5.6 μg of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S.HCl$), and use this solution as the sample solution. Separately, weigh accurately about 90 mg of chlorpromazine hydrochloride for assay, previously dried at 105°C for 2 hours, dissolve in the dissolution medium to make exactly 200 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, further pipet 5 mL of this solution, add the dissolution medium to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 254 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to labeled amount of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S.HCl$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45/8$

M_S: Amount (mg) of chlorpromazine hydrochloride for assay taken

C: Labeled amount (mg) of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S.HCl$) in 1 tablet

Assay Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately, and powder not less than 20 Chlorpromazine Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S.HCl$), add 60 mL of a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1), treat with ultrasonic waves for 5 minutes, then shake vigorously for 20 minutes, and add the mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make exactly 100 mL. Filter the solution through a membrane filter with a pore size

not exceeding 0.45 μm, and discard the first 3 mL of the filtrate. To exactly 2.5 mL of the subsequent filtrate add exactly 5 mL of the internal standard solution, then add the mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of chlorpromazine hydrochloride for assay, previously dried at 105°C for 2 hours, and dissolve in the mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and the mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make 25 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of chlorpromazine to that of the internal standard.

Amount (mg) of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S.HCl$) = $M_S \times Q_T/Q_S \times 2$

M_S: Amount (mg) of chlorpromazine hydrochloride for assay taken

Internal standard solution—A solution of ethyl parahydroxybenzoate in a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) (1 in 4500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 256 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2) and acetonitrile (27:13).

Flow rate: Adjust so that the retention time of chlorpromazine is about 15 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and chlorpromazine are eluted in this order with the resolution between these peaks being not less than 10.

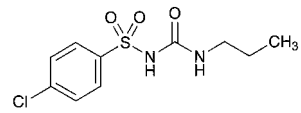
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of chlorpromazine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Chlorpropamide

クロルプロパミド



$C_{10}H_{13}ClN_2O_3S$: 276.74

4-Chloro-*N*-(propylcarbamoyl)benzenesulfonamide

[*94-20-2*]

Chlorpropamide, when dried, contains not less than 98.0% of chlorpropamide ($C_{10}H_{13}ClN_2O_3S$).

Description Chlorpropamide occurs as white, crystals or crystalline powder.

It is freely soluble in methanol and in acetone, soluble in ethanol (95), and slightly soluble in diethyl ether, and practically insoluble in water.

Identification **(1)** Dissolve 0.08 g of Chlorpropamide in 50 mL of methanol. To 1 mL of the solution add 0.01 mol/L hydrochloric acid TS to make 200 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Chlorpropamide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Chlorpropamide as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Melting point <*2.60*> 127 – 131°C

Purity **(1)** Acidity—To 3.0 g Chlorpropamide add 150 mL of water, and warm at 70°C for 5 minutes. Allow to stand in ice water for 1 hour, and filter. To 25 mL of the filtrate add 2 drops of methyl red TS and 0.30 mL of 0.1 mol/L sodium hydroxide VS: a yellow color develops.

(2) Chloride <*1.03*>—To 40 mL of the filtrate obtained in (1) add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(3) Sulfate <*1.14*>—To 40 mL of the filtrate obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.021%).

(4) Heavy metals <*1.07*>—Proceed with 2.0 g of Chlorpropamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Related substances—Dissolve 0.6 g of Chlorpropamide in acetone to make exactly 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 300 mL, and use this solution as the standard solution (1). Separately, dissolve 60 mg of 4-chlorobenzene sulfonamide in acetone to make exactly 300 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under

Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, 3-methyl-1-butanol, methanol and ammonia solution (28) (15:10:5:1) to a distance of about 10 cm, and air-dry the plate. After drying the plate at 100°C for 1 hour, spray evenly sodium hypochlorite TS on the plate, and air-dry for 15 minutes. Then spray evenly potassium iodide-starch TS on the plate: the spot from the sample solution equivalent to the spot from the standard solution (2) is not more intense than the spot from the standard solution (2), and the spots other than the spot mentioned above and other than the principal spot is not more intense than the spot from the standard solution (1).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.5 g of Chlorpropamide, previously dried, dissolve in 30 mL of neutralized ethanol, and add 20 mL of water. Titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 27.67 mg of $C_{10}H_{13}ClN_2O_3S$

Containers and storage Containers—Well-closed containers.

Chlorpropamide Tablets

クロルプロパミド錠

Chlorpropamide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of chlorpropamide ($C_{10}H_{13}ClN_2O_3S$: 276.74).

Method of preparation Prepare as directed under Tablets, with Chlorpropamide.

Identification Take a quantity of powdered Chlorpropamide Tablets, equivalent to 0.08 g of Chlorpropamide, add 50 mL of methanol, shake, and filter. To 1 mL of the filtrate add 0.01 mol/L hydrochloric acid TS to make 200 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 231 nm and 235 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Chlorpropamide Tablets add 75 mL of the mobile phase, treat with the ultrasonic waves for 20 minutes with occasional strong shaking, then add the mobile phase to make exactly V mL so that each mL contains about 2.5 mg of Chlorpropamide. Centrifuge the solution, pipet 2 mL of the supernatant liquid, add the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of chlorpropamide ($C_{10}H_{13}ClN_2O_3S$)
$= M_S \times A_T/A_S \times V/20$

M_S: Amount (mg) of chlorpropamide for assay taken

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of Chlorpropamide Tablets is not less than 70%.

Start the test with 1 tablet of Chlorpropamide Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 10 μg of chlorpropamide ($C_{10}H_{13}ClN_2O_3S$), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of chlorpropamide for assay, previously dried at 105°C for 3 hours, dissolve in 10 mL of methanol, and add water to make exactly 50 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 232 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of chlorpropamide ($C_{10}H_{13}ClN_2O_3S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of chlorpropamide for assay taken
C: Labeled amount (mg) of chlorpropamide ($C_{10}H_{13}ClN_2O_3S$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Chlorpropamide Tablets. Weigh accurately a quantity of the powder, equivalent to about 50 mg of chlorpropamide ($C_{10}H_{13}ClN_2O_3S$), add 75 mL of the mobile phase, shake for 10 minutes, and add the mobile phase to make exactly 100 mL. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of chlorpropamide for assay, previously dried at 105°C for 3 hours, dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_T and A_S, of chlorpropamide in each solution.

Amount (mg) of chlorpropamide ($C_{10}H_{13}ClN_2O_3S$)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of chlorpropamide for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of diluted acetic acid (100) (1 in 100) and acetonitrile (1:1).

Flow rate: Adjust so that the retention time of chlorpropamide is about 5 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of chlorpropamide are not less than 1500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operat-

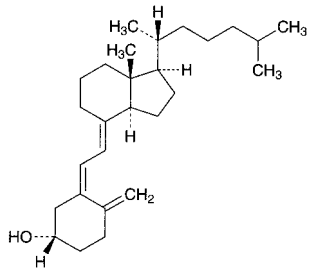
ing conditions, the relative standard deviation of the peak area of chlorpropamide is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Cholecalciferol

Vitamin D_3

コレカルシフェロール



$C_{27}H_{44}O$: 384.64
(3*S*,5*Z*,7*E*)-9,10-Secocholesta-5,7,10(19)-trien-3-ol
[*67-97-0*]

Cholecalciferol contains not less than 97.0% and not more than 103.0% of cholecalciferol ($C_{27}H_{44}O$).

Description Cholecalciferol occurs as white crystals. It is odorless.

It is freely soluble in ethanol (95), in chloroform, in diethyl ether and in isooctane, and practically insoluble in water.

It is affected by air and by light.

Melting point: 84 – 88°C Transfer Cholecalciferol to a capillary tube, and dry for 3 hours in a desiccator (in vacuum at a pressure not exceeding 2.67 kPa). Immediately fireseal the capillary tube, put it in a bath fluid, previously heated to a temperature about 10°C below the expected melting point, and heat at a rate of rise of about 3°C per minute, and read the melting point.

Identification **(1)** Dissolve 0.5 mg of Cholecalciferol in 5 mL of chloroform, add 0.3 mL of acetic anhydride and 0.1 mL of sulfuric acid, and shake: a red color is produced, and rapidly changes through purple and blue to green.

(2) Determine the infrared absorption spectrum of Cholecalciferol as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Cholecalciferol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance <*2.24*> $E^{1\%}_{1\,cm}$ (265 nm): 450 – 490 (10 mg, ethanol (95), 1000 mL).

Optical rotation <*2.49*> $[\alpha]_D^{20}$ +103 – +112° (50 mg, ethanol (95), 10 mL, 100 mm). Prepare the solution without delay, using Cholecalciferol from a container opened not longer than 30 minutes, previously, and determine the rotation within 30 minutes after the solution has been prepared.

Purity 7-Dehydrocholesterol—Dissolve 10 mg of Cholecalciferol in 2.0 mL of diluted ethanol (95) (9 in 10), add a solution prepared by dissolving 20 mg of digitonin in 2.0 mL of diluted ethanol (95) (9 in 10), and allow the mixture to stand for 18 hours: no precipitate is formed.

Assay Proceed with the operation avoiding contact with air or other oxidizing agents and using light-resistant containers. Dissolve separately about 30 mg each of Cholecalciferol and Cholecalciferol RS, accurately weighed, in isooctane to make exactly 50 mL. Pipet 10 mL each of these solutions, add 3 mL each of the internal standard solution, then add the mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cholecalciferol to that of the internal standard.

Amount (mg) of cholecalciferol ($C_{27}H_{44}O$ = $M_S \times Q_T/Q_S$)

M_S: Amount (mg) of Cholecalciferol RS taken

Internal standard solution—A solution of dimethyl phthalate in isooctane (1 in 100).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and 10 to 30 cm in length, packed with silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: Ordinary temperature.

Mobile phase: A mixture of hexane and *n*-amylalcohol (997:3).

Flow rate: Adjust so that the retention time of cholecalciferol is about 25 minutes.

Selection of column: Dissolve 15 mg of Cholecalciferol RS in 25 mL of isooctane. Transfer this solution to a flask, heat under a reflux condenser in an oil bath for 2 hours, and cool to room temperature rapidly. Transfer this solution to a quartz test tube, and irradiate under a short-wave lamp (main wavelength: 254 nm) and a long-wave lamp (main wavelength: 365 nm) for 3 hours. To this solution add the mobile phase to make 50 mL. Proceed with 10 μL of this solution under the above operating conditions. Use a column with the relative retention time of previtamin D_3, trans-vitamin D_3 and tachysterol D_3 to cholecalciferol being about 0.5, about 0.6 and about 1.1, respectively, and with resolution between previtamin D_3 and trans-vitamin D_3, and that between cholecalciferol and tachysterol D_3 being not less than 1.0.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant, under nitrogen atmosphere, and in a cold place.

Cholera Vaccine

コレラワクチン

Cholera Vaccine is a liquid for injection containing inactivated *Vibrio cholerae* of the Ogawa and Inaba strains.

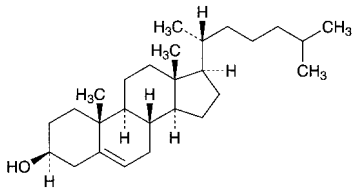
Monotypic products may be manufactured, if necessary.

It conforms to the requirements of Cholera Vaccine in the Minimum Requirements for Biological Products.

Description Cholera Vaccine is a white-turbid liquid.

Cholesterol

コレステロール



$C_{27}H_{46}O$: 386.65
Cholest-5-en-3β-ol
[*57-88-5*]

Description Cholesterol occurs as white to pale yellow crystals or granules. It is odorless, or has a slight odor. It is tasteless.

It is freely soluble in chloroform and in diethyl ether, soluble in 1,4-dioxane, sparingly soluble in ethanol (99.5), and practically insoluble in water.

It gradually changes to a yellow to light yellow-brown color by light.

Identification **(1)** Dissolve 0.01 g of Cholesterol in 1 mL of chloroform, add 1 mL of sulfuric acid, and shake: a red color develops in the chloroform layer, and the sulfuric acid layer shows a green fluorescence.

(2) Dissolve 5 mg of Cholesterol in 2 mL of chloroform, add 1 mL of acetic anhydride and 1 drop of sulfuric acid, and shake: a red color is produced, and it changes to green through blue.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{25}$: -34 – $-38°$ (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

Melting point ⟨*2.60*⟩ 147 – 150°C

Purity **(1)** Clarity of solution—Place 0.5 g of Cholesterol in a glass-stoppered flask, dissolve in 50 mL of warm ethanol (95), and allow to stand at room temperature for 2 hours: no turbidity or deposit is produced.

(2) Acidity—Place 1.0 g of Cholesterol in a flask, dissolve in 10 mL of diethyl ether, add 10.0 mL of 0.1 mol/L sodium hydroxide VS, and shake for 1 minute. Expel the diethyl ether, and boil for 5 minutes. Cool, add 10 mL of water, and titrate ⟨*2.50*⟩ with 0.05 mol/L sulfuric acid VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination in the same manner.

The volume of 0.1 mol/L sodium hydroxide VS consumed is not more than 0.30 mL.

Loss on drying ⟨*2.41*⟩ Not more than 0.30% (1 g, in vacuum, 60°C, 4 hours).

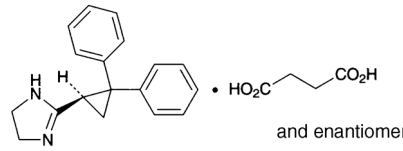
Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cibenzoline Succinate

シベンゾリンコハク酸塩



$C_{18}H_{18}N_2.C_4H_6O_4$: 380.44
2-[(1*RS*)-2,2-Diphenylcyclopropan-1-yl]-4,5-dihydro-1*H*-imidazole monosuccinate
[*100678-32-8*]

Cibenzoline Succinate, when dried, contains not less than 98.5% and not more than 101.0% of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$).

Description Cibenzoline Succinate occurs as a white crystalline powder.

It is freely soluble in methanol and in acetic acid (100), and sparingly soluble in water and in ethanol (99.5).

A solution of Cibenzoline Succinate in methanol (1 in 10) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Cibenzoline Succinate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cibenzoline Succinate as directed in the paste method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Shake 0.4 g of Cibenzoline Succinate with 2.5 mL of sodium hydroxide TS and 5 mL of ethyl acetate, allow to stand, and to 1 mL of the water layer so obtained add 0.5 mL of 1 mol/L hydrochloric acid TS and 0.5 mL of iron (III) chloride TS: a blown precipitate is formed.

Melting point ⟨*2.60*⟩ 163 – 167°C

pH ⟨*2.54*⟩ Dissolve 0.20 g of Cibenzoline Succinate in 10 mL of water: the pH of this solution is between 4.0 and 6.0.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 0.20 g of Cibenzoline Succinate in 10 mL of water is clear and colorless.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Cibenzoline Succinate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Cibenzoline Succinate according to Method 3, using a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 25), and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Cibenzoline Succinate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 5 mL and 2 mL of this solution, add methanol to make them exactly 10 mL, and use these solutions as the standard solution (1) and the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solutions (1)

and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (20:3:2) to a distance of about 10 cm, air-dry the plate, and dry more at 80°C for 30 minutes. After cooling, examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained with the sample solution is not more intense than the spot obtained with the standard solution (1). Allow the plate to stand for 30 minutes in iodine vapor: the spot other than the principal spot obtained with the sample solution is not more intense than the spot obtained with the standard solution (1), and the spot, which is more intense than the spot with the standard solution (2), is not more than two.

Loss on drying ⟨*2.41*⟩ Not more than 0.3% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Cibenzoline Succinate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS until the color of the solution changes from violet to blue-green through blue (indicator: 2 drops of crystal violet TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 38.04 mg of $C_{18}H_{18}N_2.C_4H_6O_4$

Containers and storage Containers—Tight containers.

Cibenzoline Succinate Tablets

シベンゾリンコハク酸塩錠

Cibenzoline Succinate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$: 380.44).

Method of preparation Prepare as directed under Tablets, with Cibenzoline Succinate.

Identification To a quantity of powdered Cibenzoline Succinate Tablets, equivalent to 50 mg of Cibenzoline Succinate, add 100 mL of water, shake for 10 minutes, and centrifuge. To 2 mL of the supernatant liquid add water to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 221 nm and 225 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Cibenzoline Succinate Tablets add a suitable amount of water so that each mL contains about 10 mg of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$), and allow standing for 10 minutes while occasional shaking. To this solution add methanol so that each mL contains about 2 mg of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$), add exactly 1 mL of the internal standard solution per 10 mg of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$), then add methanol so that each mL contains about 1 mg of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$). Centrifuge the solution, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$)
$= M_S \times Q_T/Q_S \times C/100$

M_S: Amount (mg) of cibenzoline succinate for assay taken
C: Labeled amount (mg) of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$) in 1 tablet

Internal standard solution—Dissolve 0.1 g of 2-ethylhexyl parahydroxybenzoate in methanol to make 100 mL.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Cibenzoline Succinate Tablets is not less than 80%.

Start the test with 1 tablet of Cibenzoline Succinate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 11 μg of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of cibenzoline succinate for assay, previously dried at 105°C for 2 hours, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 222 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of cibenzoline succinate for assay taken
C: Labeled amount (mg) of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$) in 1 tablet

Assay Weigh accurately not less than 20 Cibenzoline Succinate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$), add 10 mL of water, shake, and add 40 mL of methanol and exactly 10 mL of the internal standard solution. Shake for 20 minutes, add methanol to make 100 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 0.1 g of cibenzoline succinate for assay, previously dried at 105°C for 2 hours, add 10 mL of water and 40 mL of methanol to dissolve, then add exactly 10 mL of the internal standard solution and methanol to make 100 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cibenzoline to that of the internal standard.

Amount (mg) of cibenzoline succinate ($C_{18}H_{18}N_2.C_4H_6O_4$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of cibenzoline succinate for assay taken

Internal standard solution—Dissolve 0.1 g of 2-ethylhexyl parahydroxybenzoate in methanol to make 100 mL.
Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.67 g of sodium di-2-ethylhexyl sulfosuccinate in 2000 mL of a mixture of water, acetonitrile and diluted phosphoric acid (1 in 10) (1000:1000:1).

Flow rate: Adjust so that the retention time of cibenzoline is about 3 minutes.

System suitability—

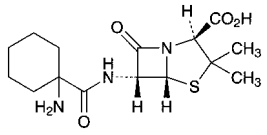
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, cibenzoline and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cibenzoline to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Ciclacillin

シクラシリン



$C_{15}H_{23}N_3O_4S$: 341.43

(2*S*,5*R*,6*R*)-6-[(1-Aminocyclohexanecarbonyl)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid

[*3485-14-1*]

Ciclacillin contains not less than 920 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the anhydrous basis. The potency of Ciclacillin is expressed as mass (potency) of ciclacillin ($C_{15}H_{23}N_3O_4S$).

Description Ciclacillin occurs as white to light yellowish white crystalline powder.

It is sparingly soluble in water, slightly soluble in methanol, and practically insoluble in acetonitrile and in ethanol (99.5).

Identification Determine the infrared absorption spectrum of Ciclacillin as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Ciclacillin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +300 – +315° (2 g, water, 100 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Ciclacillin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Ciclacillin according to Method 3, and perform the test (not more than 2 ppm).

Water <*2.48*> Not more than 2.0% (1 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Ciclacillin and Ciclacillin RS, equivalent to about 50 mg (potency), dissolve each in a suitable amount of the mobile phase, add exactly 5 mL of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ciclacillin to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of ciclacillin } (C_{15}H_{23}N_3O_4S) = M_S \times Q_T/Q_S \times 1000$$

M_S: Amount [mg (potency)] of Ciclacillin RS taken

Internal standard solution—A solution of orcin in the mobile phase (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 0.771 g of ammonium acetate in about 900 mL of water, adjust the pH to 4.0 with acetic acid (100), and add water to make 1000 mL. To 850 mL of this solution add 150 mL of acetonitrile.

Flow rate: Adjust so that the retention time of ciclacillin is about 4 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ciclacillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

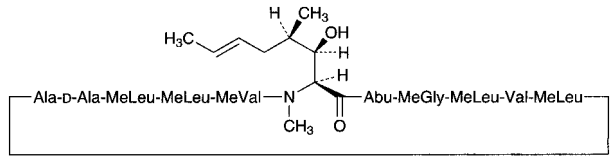
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak areas of ciclacillin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Ciclosporin

Ciclosporin A

シクロスポリン



Abu = (2*S*)-2-Aminobutyric acid
MeGly = *N*-Methylglycine
MeLeu = *N*-Methylleucine
MeVal = *N*-Methylvaline

$C_{62}H_{111}N_{11}O_{12}$: 1202.61
cyclo{-[(2*S*,3*R*,4*R*,6*E*)-3-Hydroxy-4-methyl-2-methylaminooct-6-enoyl]-L-2-aminobutanoyl-*N*-methylglycyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl-}
[*59865-13-3*]

Ciclosporin contains not less than 98.5% and not more than 101.5% of ciclosporin ($C_{62}H_{111}N_{11}O_{12}$), calculated on the dried basis.

Description Ciclosporin occurs as a white powder.

It is very soluble in acetonitrile, in methanol and in ethanol (95), freely soluble in diethyl ether, and practically insoluble in water.

Identification Determine the infrared absorption spectrum of Ciclosporin as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Ciclosporin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -185 – $-193°$ (0.1 g calculated on the dried basis, methanol, 20 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Ciclosporin in 10 mL of ethanol (95): the solution is clear, and has no more color than the following control solutions (1), (2) or (3).

Control solution (1): To exactly 3.0 mL of Iron (III) Chloride CS and exactly 0.8 mL of Cobalt (II) Chloride CS add diluted hydrochloric acid (1 in 40) to make exactly 100 mL.

Control solution (2): To exactly 3.0 mL of Iron (III) Chloride CS, exactly 1.3 mL of Cobalt (II) Chloride CS and exactly 0.5 mL of Copper (II) Sulfate CS add diluted hydrochloric acid (1 in 40) to make exactly 100 mL.

Control solution (3): To exactly 0.5 mL of Iron (III) Chloride CS and exactly 1.0 mL of Cobalt (II) Chloride CS add diluted hydrochloric acid (1 in 40) to make exactly 100 mL.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Ciclosporin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Use the sample solution obtained in the Assay as the sample solution. Pipet 2 mL of the sample solution, add a mixture of water and acetonitrile (1:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard soluton as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak other than the ciclosporin from the sample solution is not larger than 7/10 times the peak area of ciclosporin from the standard solution, and the total area of all peaks other than the ciclosporin from the sample solution is not larger than 1.5 times the peak area of ciclosporin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of ciclosporin, beginning after the solvent peak.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: To exactly 2 mL of the standard solution add a mixture of water and acetonitrile (1:1) to make exactly 20 mL. Confirm that the peak area of ciclosporin obtained from 20 μL of this solution is equivalent to 7 to 13% of that of ciclosporin obtained from 20 μL of the standard solution.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ciclosporin is not more than 3.0%.

Loss on drying <*2.41*> Not more than 2.0% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 30 mg each of Ciclosporin and Ciclosporin RS (separately determine the loss on drying <*2.41*> under the same conditions as Ciclosporin), and dissolve each in a mixture of water and acetonitrile (1:1) to make exactly 25 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of ciclosporin in each solution.

$$\text{Amount (mg) of ciclosporin } (C_{62}H_{111}N_{11}O_{12}) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Ciclosporin RS taken, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter). Connect the sample injection port and the column with a stainless steel tube 0.3 mm in inside diameter and 1 m in length.

Column temperature: A constant temperature of about 80°C (including the sample injection port and the connecting tube).

Mobile phase: A mixture of water, acetonitrile, tert-butyl methyl ether and phosphoric acid (520:430:50:1).

Flow rate: Adjust so that the retention time of ciclosporin is about 27 minutes.

System suitability—

System performance: Dissolve 3 mg of Ciclosporin U in 2.5 mL of a mixture of water and acetonitrile (1:1), and add

2.5 mL of the standard solution. When the procedure is run with 20 μL of this solution under the above operating conditions, ciclosporin U and ciclosporin are eluted in this order with the resolution between these peaks being not less than 1.2.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ciclosporin is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Cilastatin Sodium

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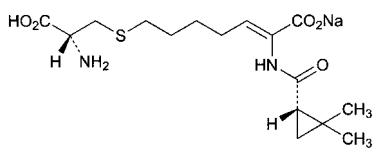

$C_{16}H_{25}N_2NaO_5S$: 380.43
Monosodium (2*Z*)-7-{[(2*R*)-2-amino-2-carboxyethyl]sulfanyl}-2-({[(1*S*)-2,2-dimethylcyclopropyl]carbonyl}amino)hept-2-enoate
[*81129-83-1*]

Cilastatin Sodium contains not less than 98.0% and not more than 101.0% of cilastatin sodium ($C_{16}H_{25}N_2NaO_5S$), calculated on the anhydrous and residual solvent-free basis.

Description Cilastatin Sodium occurs as a white to pale yellowish white powder.

It is very soluble in water, freely soluble in methanol, and slightly soluble in ethanol (99.5).

It is hygroscopic.

Identification **(1)** Determine the infrared absorption spectrum of Cilastatin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Cilastatin Sodium (1 in 10) responds to the Qualitative Tests <*1.09*> for sodium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +41.5 – +44.5° (0.1 g calculated on the anhydrous and residual solvent-free basis, a solution of hydrochloric acid in methanol (9 in 1000), 10 mL, 100 mL).

pH <*2.54*> The pH of a solution prepared by dissolving 1.0 g of Cilastatin Sodium in 100 mL of water is between 6.5 and 7.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Cilastatin Sodium in 100 mL of water: the solution is clear and the solution has no more color than the following control solution.

Control solution: To a mixture of 2.4 mL of Iron (III) Chloride CS and 0.6 mL of Cobalt (II) Chloride CS add water to make 10 mL, pipet 5 mL of this solution, and add water to make exactly 100 mL.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Cilastatin Sodium according to Method 2, and perform the test. After carbonization, add 0.5 mL of sulfuric acid instead of nitric acid. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <*1.11*>—To 2.0 g of Cilastatin Sodium add 5 mL of nitric acid and 1 mL of sulfuric acid, and heat carefully until white fumes are evolved. After cooling, heat with two 2-mL portions of nitric acid, then heat with several 2-mL portions of hydrogen peroxide (30) until a colorless or pale yellow solution is obtained. After cooling, heat again until white fumes are evolved. After cooling, add water to make 5 mL, and perform the test with this solution as the test solution: it shows no more color than the following color standard.

Color standard: Prepare a solution according to the above procedure without using Cilastatin Sodium, add exactly 2 mL of Standard Arsenic Solution, and perform the test in the same manner as the test solution (not more than 1 ppm).

(4) Related substances—Dissolve about 40 mg of Cilastatin Sodium in 25 mL of water, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than cilastatin from the sample solution is not larger than 1/6 times the peak area of cilastatin from the standard solution, and the total area of the peaks other than the peak of cilastatin from the sample solution is not larger than the peak area of cilastatin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.5 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase A: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (7:3).

Mobile phase B: Diluted phosphoric acid (1 in 1000).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 30	15 → 100	85 → 0
30 – 40	100	0

Flow rate: 2.0 mL per minute.

Time span of measurement: For 40 minutes.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add water to make exactly 30 mL. Confirm that the peak area of cilastatin obtained with 20 μL of this solution is equivalent to 2.3 to 4.5% of that obtained with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the retention time of cilastatin is about 20 minutes, and the number of theoretical plates and the symmetry factor of the peak of cilastatin are not less than 10,000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 3 times with 20 μL of the standard solution under the above operat-

ing conditions, the relative standard deviation of the peak area of cilastatin is not more than 2.0%.

(5) Residual solvents ⟨*2.46*⟩—Weigh accurately about 0.2 g of Cilastatin Sodium, add exactly 2 mL of the internal standard solution, dissolve in water to make 10 mL, and use this solution as the sample solution. Separately, measure exactly 2 mL of acetone, 0.5 mL of methanol and 0.5 mL of mesityl oxide, and add water to make exactly 1000 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, add water to make 10 mL, and use this solution as the standard solution. Perform the test with 2 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions. Calculate the ratios of the peak areas of acetone, methanol and mesityl oxide and to the peak area of the internal standard, Q_{Ta} and Q_{Sa}, Q_{Tb} and Q_{Sb}, Q_{Tc} and Q_{Sc}, and calculate the amounts of acetone, methanol and mesityl oxide by the following equation: they are not more than 1.0%, not more 0.5% and not more than 0.4%, respectively.

Amount (%) of acetone (CH_3COCH_3)
$= 1/M_T \times Q_{Ta}/Q_{Sa} \times 400 \times 0.79$

Amount (%) of methanol (CH_3OH)
$= 1/M_T \times Q_{Tb}/Q_{Sb} \times 100 \times 0.79$

Amount (%) of mesityl oxide ($CH_3COCH = C(CH_3)_2$)
$= 1/M_T \times Q_{Tc}/Q_{Sc} \times 100 \times 0.86$

M_T: Amount (mg) of Cilastatin Sodium taken
0.79: Density (g/mL) of acetone and methanol
0.86: Density (g/mL) of mesityl oxide

Internal standard solution—To 0.5 mL of 1-propanol add water to make 1000 mL.
Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A glass column 3.2 mm in inside diameter and 2.1 m in length, packed with tetrafluoroethylene polymer for gas chromatography (250 – 420 μm) coated with polyethylene glycol 20 M for gas chromatography at the ratio of 10%.
Column temperature: A constant temperature of about 70°C.
Carrier gas: Helium.
Flow rate: Adjust so that the retention time of the internal standard is about 5 minutes.
Time span of measurement: About 3 times as long as the retention time of the internal standard.
System suitability—
System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, acetone, methanol, 1-propanol and mesityl oxide are eluted in this order, and these peaks completely separate each other.
System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviations of the ratio of the peak area of acetone, methanol and mesityl oxide to that of the internal standard are not more than 4.0%, respectively.

Water ⟨*2.48*⟩ Not more than 2.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.3 g of Cilastatin Sodium, dissolve in 30 mL of methanol, add 5 mL of water, and adjust to pH 3.0 with 0.1 mol/L hydrochloric acid TS. Titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS from the first equivalence point to the third equivalence point (potentiometric titration).

Each mL of 0.1 mol/L sodium hydroxide VS
= 19.02 mg of $C_{16}H_{25}N_2NaO_5S$

Containers and storage Containers—Tight containers.
Storage—In a cold place.

Cilazapril Hydrate

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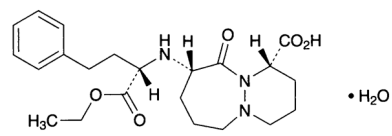


$C_{22}H_{31}N_3O_5.H_2O$: 435.51
(1*S*,9*S*)-9-[(1*S*)-(1-Ethoxycarbonyl-3-phenylpropyl)amino]-10-oxooctahydro-6*H*-pyridazino[1,2-*a*][1,2]diazepine-1-carboxylic acid monohydrate
[*92077-78-6*]

Cilazapril Hydrate contains not less than 98.5% and not more than 101.0% of cilazapril ($C_{22}H_{31}N_3O_5$: 417.50), calculated on the anhydrous basis.

Description Cilazapril Hydrate occurs as white to yellowish white, crystals or crystalline powder.
It is very soluble in methanol, freely soluble in ethanol (99.5) and in acetic acid (100), and slightly soluble in water.
It gradually turns yellow on exposure to light.
Melting point: about 101°C (with decomposition).

Identification **(1)** To 4 mL of a solution of Cilazapril Hydrate (1 in 1000) add 2 mL of Dragendorff's TS: an orange precipitate is produced.
(2) Determine the infrared absorption spectrum of Cilazapril Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −53 – −58° (0.2 g calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

Purity **(1)** Chloride ⟨*1.03*⟩—Perform the test using 1.0 g of Cilazapril Hydrate. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.009%).
(2) Sulfate ⟨*1.14*⟩—Dissolve 1.0 g of Cilazapril Hydrate in 40 mL of water and 1.5 mL of dilute hydrochloric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019%).
(3) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Cilazapril Hydrate according to Method 4, and perform the test. However, use 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 8). Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).
(4) Related substances—Dissolve 0.10 g of Cilazapril Hydrate in 20 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 3 mL of the standard solu-

tion (1), add methanol to make exactly 10 mL, and use this solution as the standard solution (2). Separately, pipet 2 mL of the standard solution (1), add methanol to make exactly 10 mL, and use this solution as the standard solution (3). Perform the test with these solutions as directed under Thin-layer Chromatography ⟨2.03⟩. Spot 20 μL each of the sample solution and three standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol, acetic acid (100), hexane, and water (62:15:10:10:3) to a distance of about 15 cm, and air-dry the plate. Leave the plate in iodine vapor for 2 hours, and examine the plate under ultraviolet light (main wavelength: 254 nm): of the spots other than the principal spot with an *R*f value close to 0.40 obtained from the sample solution, the spot in the vicinity of *R*f value 0.17 is not more intense than the spot obtained from the standard solution (1), and the spot in the vicinity of *R*f value 0.44 is not more intense than the spot from the standard solution (2). The number of all other spot does not exceed 3, and of these spots, no more than one is more intense than the spot from the standard solution (3) and none are more intense than the spot from the standard solution (2).

Water ⟨2.48⟩ 3.5 – 5.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition ⟨2.44⟩ Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.2 g of Cilazapril Hydrate, dissolve in 50 mL of acetic acid (100), and titrate ⟨2.50⟩ with 0.02 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner and make any necessary correction.

Each mL of 0.02 mol/L perchloric acid VS
= 8.350 mg of $C_{22}H_{31}N_3O_5$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Cilazapril Tablets

シラザプリル錠

Cilazapril Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of cilazapril ($C_{22}H_{31}N_3O_5$: 417.50).

Method of preparation Prepare as directed under Tablets, with Cilazapril Hydrate.

Identification To a quantity of powdered Cilazapril Tablets, equivalent to 2 mg of cilazapril ($C_{22}H_{31}N_3O_5$), add 2 mL of a mixture of acetonitrile and ethyl acetate (3:1), shake, treat with ultrasonic waves for 30 seconds, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 5 mg of cilazapril in 5 mL of the mixture of acetonitrile and ethyl acetate (3:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨2.03⟩. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol, acetic acid (100), hexane and water (62:15:10:10:3) to a distance of about 15 cm, and air-dry the plate. Place the plate in iodine vapor for 2 hours, and immediately examine under ultraviolet light (main wavelength: 254 nm): the spots obtained from the sample and standard solutions are dark brown and they show the same *R*f value.

Uniformity of dosage units ⟨6.02⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Cilazapril Tablets add 5 mL of a mixture of water and acetonitrile (7:3), shake well until disintegration, add the mixture of water and acetonitrile (7:3) to make exactly *V* mL so that each mL contains about 25 μg of cilazapril ($C_{22}H_{31}N_3O_5$), and centrifuge. Pipet 4 mL of the supernatant liquid, add exactly 1 mL of the internal standard solution, add the mixture of water and acetonitrile (7:3) to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 26 mg of cilazapril for assay (separately determine the water ⟨2.48⟩ in the same manner as Cilazapril Hydrate), and dissolve in the mixture of water and acetonitrile (7:3) to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, add the mixture of water and acetonitrile (7:3) to make 100 mL, and use this solution as the standard solution. Perform the test with 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cilazapril to that of the internal standard.

Amount (mg) of cilazapril ($C_{22}H_{31}N_3O_5$)
$= M_S \times Q_T/Q_S \times V/1000$

M_S: Amount (mg) of cilazapril for assay taken, calculated on the anhydrous basis

Internal standard solution—A solution of dimethyl phthalate in a mixture of water and acetonitrile (7:3) (1 in 12,500).
Operating conditions—

Proceed as directed in the operating conditions in the Assay.
System suitability—

System performance: When the procedure is run with 100 μL of the standard solution under the above conditions, cilazapril and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above conditions, the relative standard deviation of the ratio of the peak area of cilazapril to that of the internal standard is not more than 2.0%.

Dissolution ⟨6.10⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Cilazapril Tablets is not less than 85%.

Start the test with 1 tablet of Cilazapril Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet *V* mL of the subsequent filtrate, and add water to make exactly *V′* mL so that each mL contains about 0.28 μg of cilazapril ($C_{22}H_{31}N_3O_5$). Pipet 10 mL of the solution, add exactly 5 mL of acetonitrile, and use this solution as the sample solution. Separately, weigh accurately about 29 mg of cilazapril for assay (separately determine the water ⟨2.48⟩ in the same manner as Cilazapril Hydrate), and dissolve in water to make exactly 100 mL. Pipet 5 mL of the solution, add water to make exactly 100 mL. Then, pipet 2 mL of this solution, add water to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of acetonitrile, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution

and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of cilazapril in each solution.

Dissolution rate (%) with respect to the labeled amount of cilazapril ($C_{22}H_{31}N_3O_5$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/10$

M_S: Amount (mg) of cilazapril for assay taken, calculated on the anhydrous basis
C: Labeled amount (mg) of cilazapril ($C_{22}H_{31}N_3O_5$) in 1 tablet

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: To a solution consisting of 180 mL of tetrahydrofuran for liquid chromatography, 120 mL of acetonitrile for liquid chromatography and 3 mL of triethylamine add water to make 1000 mL, and adjust the pH to 2.5 with phosphoric acid.
Flow rate: Adjust so that the retention time of cilazapril is about 10 minutes.
System suitability—
System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cilazapril are not less than 3000 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cilazapril is not more than 2.0%.

Assay Weigh acurately the mass of not less than 20 Cilazapril Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 1 mg of cilazapril ($C_{22}H_{31}N_3O_5$), add 30 mL of a mixture of water and acetonitrile (7:3), and treat with ultrasonic waves for 5 minutes. Next, add exactly 5 mL of the internal standard solution, add the mixture of water and acetonitrile (7:3) to make 50 mL, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.5 μm, and use the filtrate as the sample solution. Separately, weigh accurately about 26 mg of cilazapril for assay (separately determine the water <2.48> in the same manner as Cilazapril Hydrate), and dissolve in the mixture of water and acetonitrile (7:3) to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution, add the mixture of water and acetonitrile (7:3) to make 50 mL, and use this solution as the standard solution. Perform the test with 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cilazapril to that of the internal standard.

Amount (mg) of cilazapril ($C_{22}H_{31}N_3O_5$)
$= M_S \times Q_T/Q_S \times 1/25$

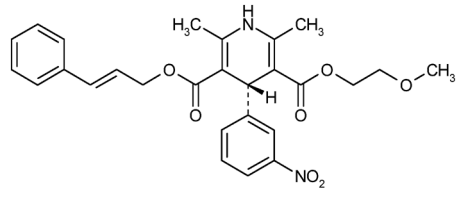
M_S: Amount (mg) of cilazapril for assay taken, calculated on the anhydrous basis

Internal standard solution—A solution of dimethyl phthalate in a mixture of water and acetonitrile (7:3) (1 in 12,500).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 23°C.
Mobile phase: To a solution consisting of 180 mL of tetrahydrofuran for liquid chromatography, 120 mL of acetonitrile for liquid chromatography and 3 mL of triethylamine add water to make 1000 mL, and adjust the pH to 2.5 with phosphoric acid.
Flow rate: Adjust so that the retention time of cilazapril is about 10 minutes.
System suitability—
System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, cilazapril and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.
System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cilazapril to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cilnidipine

シルニジピン



and enantiomer

$C_{27}H_{28}N_2O_7$: 492.52
3-(2-Methoxyethyl) 5-[(2*E*)-3-phenylprop-2-en-1-yl] (4*RS*)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate
[*132203-70-4*]

Cilnidipine, when dried, contains not less than 98.0% and not more than 102.0% of cilnidipine ($C_{27}H_{28}N_2O_7$).

Description Cilnidipine occurs as a faint yellow crystalline powder.
It is freely soluble in acetonitrile, sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water.
A solution of Cilnidipine in acetonitrile (1 in 100) shows no optical rotation.
It is gradually colored to reddish yellow and decomposed by light.

Identification **(1)** Determine the absorption spectrum of a solution of Cilnidipine in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cilnidipine RS prepared in the same manner as the sample solution: both spectra exhibit

similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of previously dried Cilnidipine as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨2.25⟩, and compare the spectrum with the Reference Spectrum or the spectrum of dried Cilnidipine RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨2.60⟩ 107 – 112°C

Purity **(1)** Heavy metals ⟨1.07⟩—Proceed with 2.0 g of Cilnidipine according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Conduct this procedure using light-resistant vessels. Dissolve 50 mg of Cilnidipine in 20 mL of acetonitrile, add the mobile phase to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.5 to cilnidipine, obtained from the sample solution is not larger than 2/5 times the peak area of cilnidipine obtained from the standard solution, the area of the peaks other than cilnidipine and the above mentioned peak from the sample solution is not larger than 1/5 times the peak area of cilnidipine from the standard solution, and the total area of the peaks other than cilnidipine from the sample solution is not larger than the peak area of cilnidipine from the standard solution. For the area of the peak, having the relative retention time of about 1.15, about 1.6, and about 1.7 to cilnidipine, multiply the relative response factor, 1.5, 1.4, and 1.6, respectively.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of cilnidipine, beginning after the solvent peak.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Pipet 5 mL of the standard solution, add the mobile phase to make exactly 50 mL. Confirm that the peak area of cilnidipine obtained from 10 μL of this solution is equivalent to 7 to 13% of that obtained from 10 μL of the standard solution.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cilnidipine is not more than 2.0%.

Loss on drying ⟨2.41⟩ Not more than 0.5% (1.0 g, in vacuum, 60°C, 3 hours).

Residue on ignition ⟨2.44⟩ Not more than 0.1% (1 g).

Assay Conduct this procedure using light-resistant vessels. Weigh accurately about 50 mg each of Cilnidipine and Cilnidipine RS, both previously dried, dissolve in 20 mL of acetonitrile, and add the mobile phase to make exactly 100 mL, respectively. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, add the mobile phase to make 25 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cilnidipine to that of the internal standard.

Amount (mg) of cilnidipine ($C_{27}H_{28}N_2O_7$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of Cilnidipine RS taken

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with perfluorohexylpropylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.36 g of sodium acetate trihydrate in water to make 1000 mL, and adjust to pH 5.5 with diluted acetic acid (100) (1 in 100). To 400 mL of this solution add 600 mL of methanol.

Flow rate: Adjust so that the retention time of cilnidipine is about 20 minutes.

System suitability—

System performance: After exposing Cilnidipine to a fluorescent light (15,000 lx·h), take 10 mg, dissolve in 4 mL of acetonitrile, and add the mobile phase to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak of cilnidipine and the peak having the relative retention time of about 1.07 to cilnidipine is not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cilnidipine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Cilnidipine Tablets

シルニジピン錠

Cilnidipine Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of cilnidipine ($C_{27}H_{28}N_2O_7$: 492.52).

Method of preparation Prepare as directed under Tablets, with Cilnidipine.

Identification Powder Cilnidipine Tablets. To a portion of the powder, equivalent to 20 mg of Cilnidipine, add 20 mL of methanol, shake well, and centrifuge. To 1 mL of the supernatant liquid add methanol to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨2.24⟩: it exhibits maxima between 238 nm and 242 nm and between 350 nm and 360 nm.

Purity Related substances—Conduct this procedure using light-resistant vessels. Powder Cilnidipine Tablets. To a portion of the powder, equivalent to 25 mg of Cilnidipine, add 40 mL of the mobile phase, shake well, and add the mobile

phase to make 50 mL. Centrifuge, and use the supernatant liquid as the sample solution. Pipet 3 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 1.09 to cilnidipine, obtained from the sample solution is not larger than 1/3 times the peak area of cilnidipine obtained from the standard solution, the area of the peaks other than cilnidipine and the peak mentioned above from the sample solution is not larger than 2/15 times the peak area of cilnidipine from the standard solution, and the total area of the peaks other than cilnidipine from the sample solution is not larger than the peak area of cilnidipine from the standard solution. For the area of the peak, having the relative retention time of about 1.09 to cilnidipine, multiply the relative response factor, 1.4.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of cilnidipine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, add the mobile phase to make exactly 150 mL. Confirm that the peak area of cilnidipine obtained with 20 μL of this solution is equivalent to 2.4 to 4.3% of that obtained with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cilnidipine are not less than 15,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cilnidipine is not more than 2.0%.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Conduct this procedure using light-resistant vessels. To 1 tablet of Cilnidipine Tablets add V/10 mL of water, and shake to completely disintegrate the tablet. Add acetonitrile to make exactly V mL so that each mL contains about 0.2 mg of cilnidipine ($C_{27}H_{28}N_2O_7$), and centrifuge. Pipet 4 mL of the supernatant liquid, add a mixture of acetonitrile and water (9:1) to make exactly 20 mL, filter, if necessary, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Cilnidipine RS, previously dried in vacuum at 60°C for 3 hours, dissolve in a mixture of acetonitrile and water (9:1) to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of acetonitrile and water (9:1) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 355 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*> using a mixture of acetonitrile and water (9:1) as the control.

$$\text{Amount (mg) of cilnidipine } (C_{27}H_{28}N_2O_7) = M_S \times A_T/A_S \times V/100$$

M_S: Amount (mg) of Cilnidipine RS taken

Dissolution <*6.10*> When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of a solution of polysorbate 80 (dissolving 1 g of polysorbate 80 in 1000 mL of 2nd fluid for dissolution test) as the dissolution medium, the dissolution rate in 90 minutes of Cilnidipine Tablets is not less than 70%.

Start the test with 1 tablet of Cilnidipine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 5.6 μg of cilnidipine ($C_{27}H_{28}N_2O_7$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Cilnidipine RS, previously dried in vacuum at 60°C for 3 hours, dissolve in acetonitrile to make exactly 100 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of cilnidipine in each solution.

$$\text{Dissolution rate (\%) with respect to the labeled amount of cilnidipine } (C_{27}H_{28}N_2O_7) = M_S \times A_T/A_S \times V'/V \times 1/C \times 18$$

M_S: Amount (mg) of Cilnidipine RS taken

C: Labeled amount (mg) of cilnidipine ($C_{27}H_{28}N_2O_7$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wave length: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.58 g of disodium hydrogen phosphate dodecahydrate in 1000 mL of water, and adjust to pH 6.0 with phosphoric acid. To 400 mL of this solution add 600 mL of acetonitrile.

Flow rate: Adjust so that the retention time of cilnidipine is about 8 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cilnidipine are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cilnidipine is not more than 2.0%.

Assay Conduct this procedure using light-resistant vessels. Weigh accurately the mass of not less than 20 Cilnidipine Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 25 mg of cilnidipine ($C_{27}H_{28}N_2O_7$), add 40 mL of the mobile phase, shake well, and add the mobile phase to make exactly 50 mL. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add exactly 2.5 mL of the internal standard solution, add the mobile phase to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Cilnidipine RS, previously dried in vacuum at 60°C for 3 hours, dissolve in the mobile phase to make exactly 50 mL.

Pipet 5 mL of this solution, add exactly 2.5 mL of the internal standard solution, add the mobile phase to make 25 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cilnidipine to that of the internal standard.

Amount (mg) of cilnidipine ($C_{27}H_{28}N_2O_7$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of Cilnidipine RS taken

Internal standard solution—A solution of 4,4'-difluorobenzophenone in the mobile phase (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 6 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.58 g of disodium hydrogen phosphate dodecahydrate in 1000 mL of water, and adjust to pH 6.0 with phosphoric acid. To 400 mL of this solution add 600 mL of acetonitrile.

Flow rate: Adjust so that the retention time of cilnidipine is about 23 minutes.

System suitability—

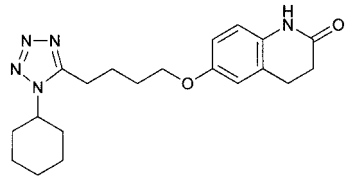
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and cilnidipine are eluted in this order with the resolution between these peaks being not less than 15.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cilnidipine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Cilostazol

シロスタゾール



$C_{20}H_{27}N_5O_2$: 369.46

6-[4-(1-Cyclohexyl-1*H*-tetrazol-5-yl)butyloxy]-3,4-dihydroquinolin-2(1*H*)-one

[*73963-72-1*]

Cilostazol, when dried, contains not less than 98.5% and not more than 101.5% of cilostazol ($C_{20}H_{27}N_5O_2$).

Description Cilostazol occurs as white to pale yellowish white, crystals or crystalline powder.

It is slightly soluble in methanol, in ethanol (99.5) and in acetonitrile, and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Cilostazol in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cilostazol RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cilostazol as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cilostazol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 158 – 162°C

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Cilostazol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 25 mg of Cilostazol in 25 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetonitrile to make exactly 100 mL. Pipet 10 mL of this solution, add acetonitrile to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than cilostazol obtained with the sample solution is not larger than 7/10 times the peak area of cilostazol obtained with the standard solution, and the total area of the peaks other than the peak of cilostazol with the sample solution is not larger than 1.2 times the peak area of cilostazol with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of hexane, ethyl acetate and methanol (10:9:1).

Flow rate: Adjust so that the retention time of cilostazol is about 7 minutes.

Time span of measurement: About 3 times as long as the retention time of cilostazol, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add acetonitrile to make exactly 10 mL. Confirm that the peak area of cilostazol obtained with 10 μL of this solution is equivalent to 7 to 13% of that obtained with 10 μL of the standard solution.

System performance: To 1 mL of the sample solution, add 1 mL of a solution prepared by dissolving 5 mg of 3,4-dihydro-6-hydroxy-2(1*H*)-quinolinone in 10 mL of acetonitrile and acetonitrile to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, 3,4-dihydro-6-hydroxy-2(1*H*)-quinolinone and cilostazol are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cilostazol is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.1% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Cilostazol and Cilostazol RS, previously dried, dissolve each in a suitable amount of methanol, add exactly 5 mL of the internal standard solution and methanol to make 50 mL. To 1 mL each of these solutions add methanol to make 10 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cilostazol to that of the internal standard.

Amount (mg) of cilostazol ($C_{20}H_{27}N_5O_2$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of Cilostazol RS taken

Internal standard solution—A solution of benzophenone in methanol (1 in 250).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, acetonitrile and methanol (10:7:3).

Flow rate: Adjust so that the retention time of cilostazol is about 9 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cilostazol and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cilostazol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Cilostazol Tablets

シロスタゾール錠

Cilostazol Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of cilostazol ($C_{20}H_{27}N_5O_2$: 369.46).

Method of preparation Prepare as directed under Tablets, with Cilostazol.

Identification Mix well an amount of powdered Cilostazol Tablets, equivalent to 50 mg of Cilostazol, with 10 mL of acetone, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 25 mg of Cilostazol RS in 5 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 6 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, acetonitrile, methanol and formic acid (75:25:5:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: the principal spot with the sample solution and the spot with the standard solution are orange in color and have the same *Rf* value.

Uniformity of dosage units ⟨*6.02*⟩ Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Cilostazol Tablets add 2 mL of water to disintegrate the tablet, add the internal standard solution exactly 5 mL for a 50-mg tablet and exactly 10 mL for a 100-mg tablet, and add methanol to make 50 mL. Shake for 10 minutes for the 50-mg tablet and for 20 minutes for the 100-mg tablet. To 1 mL of the solution add methanol to make 10 mL for the 50-mg tablet and 20 mL for the 100-mg tablet, filter through a membrane filter with a pore size not exceeding 0.5 μm, and use the filtrate as the sample solution. Proceed as directed in the Assay.

Amount (mg) of cilostazol ($C_{20}H_{27}N_5O_2$)
= $M_S \times Q_T/Q_S \times C/50$

M_S: Amount (mg) of Cilostazol RS taken
C: Labeled amount (mg) of cilostazol ($C_{20}H_{27}N_5O_2$) in 1 tablet

Internal standard solution—A solution of benzophenone in methanol (1 in 250).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of a solution of sodium lauryl sulfate (3 in 1000) as the dissolution medium, the dissolution rates of a 50-mg tablet in 45 minutes and a 100-mg tablet in 60 minutes are not less than 75% and not less than 70%, respectively.

Start the test with 1 tablet of Cilostazol Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 5.6 μg of cilostazol ($C_{20}H_{27}N_5O_2$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Cilostazol RS, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 100 mL. Pipet 4 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 257 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩ using the dissolution medium as the control.

Dissolution rate (%) with respect to the labeled amount of cilostazol ($C_{20}H_{27}N_5O_2$)
= $M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of Cilostazol RS taken
C: Labeled amount (mg) of cilostazol ($C_{20}H_{27}N_5O_2$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Cilostazol Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of cilostazol ($C_{20}H_{27}N_5O_2$), add exactly 5 mL of the internal standard solution and methanol to make 50 mL, and shake well for 10 minutes. To 1 mL of this solution add methanol to make 10 mL, filter through a membrane filter with a pore size not exceeding 0.5 μm, and use the filtrate as the sample solution.

Separately, weigh accurately about 50 mg of Cilostazol RS, previously dried at 105°C for 2 hours, dissolve in a suitable amount of methanol, and add exactly 5 mL of the internal standard solution, and add methanol to make 50 mL. To 1 mL of this solution add methanol to make 10 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cilostazol to that of the internal standard.

Amount (mg) of cilostazol ($C_{20}H_{27}N_5O_2$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of Cilostazol RS taken

Internal standard solution—A solution of benzophenone in methanol (1 in 250).

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Cilostazol.

System suitability—

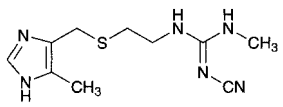
System performance: Proceed as directed in the system suitability in the Assay under Cilostazol.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cilostazol to that of the internal standard is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Cimetidine

シメチジン



$C_{10}H_{16}N_6S$: 252.34

2-Cyano-1-methyl-3-{2-[(5-methyl-1*H*-imidazol-4-yl)methylsulfanyl]ethyl}guanidine

[*51481-61-9*]

Cimetidine, when dried, contains not less than 99.0% of cimetidine ($C_{10}H_{16}N_6S$).

Description Cimetidine occurs as a white crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in methanol and in acetic acid (100), sparingly soluble in ethanol (95), slightly soluble in water, and practically insoluble in diethyl ether.

It dissolves in dilute hydrochloric acid.

It is gradually colored by light.

Identification (1) To 0.1 mL of a solution of Cimetidine in ethanol (95) (1 in 100) add 5 mL of citric acid-acetic anhydride TS, and heat in a water bath for 15 minutes: a red-purple color develops.

(2) Determine the infrared absorption spectrum of Cimetidine, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <2.54> Dissolve 0.5 g of Cimetidine in 50 mL of freshly boiled and cooled water, shake for 5 minutes and filter: the pH of the filtrate is between 9.0 and 10.5.

Melting point <2.60> 140 – 144°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Cimetidine in 10 mL of methanol: the solution is clear and colorless to pale yellow in color.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Cimetidine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Dissolve 1.0 g of Cimetidine in 5 mL of dilute hydrochloric acid, and perform the test with this solution (not more than 2 ppm).

(4) Related substances—Dissolve 0.5 g of Cimetidine in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL. Pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 4 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (21:2:2) to a distance of about 15 cm, air-dry the plate, and then dry at 80°C for 30 minutes. Allow the plate to stand in iodine vapor for 45 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.24 g of Cimetidine, previously dried, dissolve in 75 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

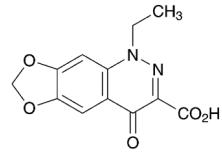
Each mL of 0.1 mol/L perchloric acid VS
= 25.23 mg of $C_{10}H_{16}N_6S$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Cinoxacin

シノキサシン



$C_{12}H_{10}N_2O_5$: 262.22

5-Ethyl-8-oxo-5,8-dihydro[1,3]dioxolo[4,5-*g*]cinnoline-7-carboxylic acid

[*28657-80-9*]

Cinoxacin, when dried, contains not less than 98.0% and not more than 101.0% of cinoxacin ($C_{12}H_{10}N_2O_5$).

Description Cinoxacin occurs as a white to pale yellow crystalline powder. It is odorless or has a slight, characteris-

tic odor. It has a bitter taste.

It is slightly soluble in *N*,*N*-dimethylformamide and in acetone, very slightly soluble in ethanol (99.5), and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Melting point: about 265°C (with decomposition).

Identification **(1)** Dissolve 30 mg of Cinoxacin in 10 mL of dilute sodium hydroxide TS, and add water to make 100 mL. To 1 mL of this solution add 0.1 mol/L hydrochloric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cinoxacin as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Sulfate <*1.14*>—Dissolve 0.20 g of Cinoxacin in 10 mL of dilute sodium hydroxide TS, add 20 mL of 0.1 mol/L hydrochloric acid TS, shake, filter, and add water to the filtrate to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.005 mol/L sulfuric acid VS by adding 10 mL of dilute sodium hydroxide TS, 20 mL of 0.1 mol/L hydrochloric acid TS, and water to make 50 mL (not more than 0.048%).

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Cinoxacin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 10 mg of Cinoxacin in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetonitrile, water and ammonia solution (28) (14:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot obtained from the sample solution are not more intense than the spot obtained from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 1 hour).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.4 g of Cinoxacin, previously dried, add 60 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and dissolve by warming. After cooling, titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 26.22 mg of $C_{12}H_{10}N_2O_5$

Containers and storage Containers—Tight containers.

Cinoxacin Capsules

シノキサシンカプセル

Cinoxacin Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of cinoxacin ($C_{12}H_{10}N_2O_5$: 262.22).

Method of preparation Prepare as directed under Capsules, with Cinoxacin.

Identification To a quantity of the contents of Cinoxacin Capsules, equivalent to 10 mg of Cinoxacin, add 20 mL of acetone, shake well, and centrifuge. To 3 mL of the supernatant liquid add acetone to make 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of cinoxacin for assay in 20 mL of acetone. To 3 mL of this solution add acetone to make 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetonitrile, water and ammonia solution (28) (14:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution and the spot obtained from the standard solution show a blue-purple color and the same *R*f value.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 capsule of Cinoxacin Capsules add 40 mL of dilute sodium hydroxide TS, and dissolve the capsule in lukewarm water with occasional shaking. After cooling, add water and shake well, add water to make exactly *V* mL so that each mL contains about 1 mg of cinoxacin ($C_{12}H_{10}N_2O_5$), and filter. Discard the first 20 mL of the filtrate, pipet 1 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.2 g of cinoxacin for assay, previously dried at 105°C for 1 hour, dissolve in 40 mL of dilute sodium hydroxide TS, and add water to make exactly 200 mL. Pipet 1 mL of this solution, add 0.1 mol/L of hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and determine the absorbances, A_T and A_S, at 354 nm.

Amount (mg) of cinoxacin ($C_{12}H_{10}N_2O_5$)
$= M_S \times A_T/A_S \times V/200$

M_S: Amount (mg) of cinoxacin for assay taken

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of 2nd solution for dissolution test as the dissolution medium, the dissolution rate in 90 minutes of Cinoxacin Capsules is not less than 70%.

Start the test with 1 capsule of Cinoxacin Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet *V* mL of the subsequent filtrate, add the dissolution medium to make exactly *V'* mL so that each mL contains about 11 μg of cinoxacin

($C_{12}H_{10}N_2O_5$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of cinoxacin for assay, previously dried at 105°C for 1 hour, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_T and A_S, at 351 nm.

Dissolution rate (%) with respect to the labeled amount of cinoxacin ($C_{12}H_{10}N_2O_5$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$

M_S: Amount (mg) of cinoxacin for assay taken
C: Labeled amount (mg) of cinoxacin ($C_{12}H_{10}N_2O_5$) in 1 capsule

Assay Weigh accurately the mass of not less than 20 Cinoxacin Capsules, take out the contents, and powder. Wash the capsule shells with a small amount of diethyl ether, allow to stand at room temperature to vaporize the diethyl ether, weigh accurately the mass of the capsule shells, and calculate the mass of the contents. Weigh accurately a portion of the powder, equivalent to about 50 mg of cinoxacin ($C_{12}H_{10}N_2O_5$), add 10 mL of dilute sodium hydroxide TS, shake, add water to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet 1 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of cinoxacin for assay, previously dried at 105°C for 1 hour, dissolve in 10 mL of dilute sodium hydroxide TS, and add water to make exactly 100 mL. Pipet 1 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_T and A_S, at 354 nm.

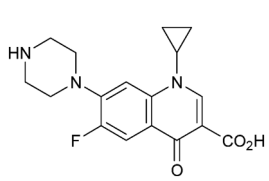
Amount (mg) of cinoxacin ($C_{12}H_{10}N_2O_5$)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of cinoxacin for assay taken

Containers and storage Containers—Well-closed containers.

Ciprofloxacin

シプロフロキサシン



$C_{17}H_{18}FN_3O_3$: 331.34
1-Cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid
[*85721-33-1*]

Ciprofloxacin, when dried, contains not less than 98.5% and not more than 101.0% of ciprofloxacin ($C_{17}H_{18}FN_3O_3$).

Description Ciprofloxacin occurs as a white to light yellowish white, crystalline powder.

It is practically insoluble in water and in ethanol (99.5).

It dissolves in ammonia TS.

It is gradually colored to yellow tint by light.

Melting point: about 270°C (with decomposition).

Identification **(1)** Determine the infrared absorption spectrum of Ciprofloxacin, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of the Ciprofloxacin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Conduct this procedure using light-resistant vessels. Dissolve 50 mg each of Ciprofloxacin and Ciprofloxacin RS in 5 mL of ammonia TS, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. After allowing to stand this plate in the vapor of ammonia for 15 minutes, develop the plate with a mixture of methanol, dichloromethane, ammonia solution (28) and acetonitrile (4:4:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution and the spot obtained from the standard solution show the same *R*f value.

Purity **(1)** Chloride <1.03>—To 1.5 g of Ciprofloxacin add 75 mL of water, and boil for 5 minutes. After cooling, add water to make 75 mL, and filter. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.30 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute sulfuric acid and water to make 50 mL (not more than 0.021%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Ciprofloxacin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Fluoroquinolonic acid—Conduct this procedure using light-resistant vessels. Dissolve 50 mg of Ciprofloxacin in ammonia TS to make exactly 5 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of fluoroquinolonic acid for thin-layer chromatography in 0.1 mL of ammonia TS and water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. After allowing to stand this plate in the vapor of ammonia for 15 minutes, develop the plate with a mixture of methanol, dichloromethane, ammonia solution (28) and acetonitrile (4:4:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot obtained from the sample solution, corresponding to the spot obtained from the standard solution, is not more intense than that obtained from the standard solution.

(4) Related substances—Conduct this procedure using light-resistant vessels. To 25 mg of Ciprofloxacin add 2 mL of a mixture of water and phosphoric acid (13:1), then add the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 20 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 50 mL, and use

this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than ciprofloxacin obtained from the sample solution is not larger than the peak area of ciprofloxacin obtained from the standard solution, and the total area of the peaks other than ciprofloxacin from the sample solution is not larger than 2.5 times the peak area of ciprofloxacin from the standard solution. For the area of peak, having the relative retention time of about 0.4, about 0.5, and about 1.2 to ciprofloxacin, multiply the relative response factor, 6.7, 1.3, and 1.4, respectively.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.3 times as long as the retention time of ciprofloxacin, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of ciprofloxacin obtained with 50 μL of this solution is equivalent to 20 to 30% of that obtained with 50 μL of the standard solution.

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ciprofloxacin are not less than 3500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ciprofloxacin is not more than 2.0%.

Loss on drying <2.41> Not more than 1.0% (2 g, in vacuum, 120°C, 6 hours).

Residue on ignition <2.44> Not more than 0.1% (2 g).

Assay Conduct this procedure using light-resistant vessels. Weigh accurately about 25 mg each of Ciprofloxacin and Ciprofloxacin RS, both dried previously, add 2 mL of a mixture of water and phosphoric acid (13:1), add the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of ciprofloxacin in each solution.

$$\text{Amount (mg) of ciprofloxacin } (C_{17}H_{18}FN_3O_3) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Ciprofloxacin RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 278 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 2.88 g of phosphoric acid add water to make 1000 mL, and adjust to pH 3.0 with triethylamine. To 870 mL of this solution add 130 mL of acetonitrile.

Flow rate: Adjust so that the retention time of ciprofloxacin is about 7 minutes.

System suitability—

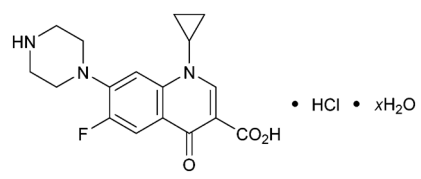
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ciprofloxacin are not less than 3500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ciprofloxacin is not more than 1.0%.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Ciprofloxacin Hydrochloride Hydrate

シプロフロキサシン塩酸塩水和物



$C_{17}H_{18}FN_3O_3.HCl.xH_2O$

1-Cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid monohydrochloride hydrate [*86393-32-0*, monohydrochloride monohydrate]

Ciprofloxacin Hydrochloride Hydrate contains not less than 98.0% and not more than 102.0% of ciprofloxacin hydrochloride ($C_{17}H_{18}FN_3O_3.HCl$: 367.80), calculated on the anhydrous basis.

Description Ciprofloxacin Hydrochloride Hydrate occurs as a white to pale yellow crystalline powder.

It is sparingly soluble in water, slightly soluble in methanol, and very slightly soluble in ethanol (99.5).

It is gradually colored to a slightly brownish light yellow by light.

Identification **(1)** Determine the infrared absorption spectrum of Ciprofloxacin Hydrochloride Hydrate, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Conduct this procedure using light-resistant vessels. Dissolve 50 mg of Ciprofloxacin Hydrochloride Hydrate in 5 mL of water, and use this solution as the sample solution. Separately, dissolve 45 mg of Ciprofloxacin RS in 5 mL of ammonia TS, and use this solution as the standard solution. Perform the test with these solutions, as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. After allowing to stand the plate in the vapor of ammonia for 15 minutes, develop the plate with a mixture of methanol, dichloromethane, ammonia solution (28) and acetonitrile (4:4:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution and the spot obtained from the standard solution show the same *R*f value.

(3) A solution of Ciprofloxacin Hydrochloride Hydrate (1 in 500) responds to the Qualitative Tests <*1.09*> for chloride.

Purity **(1)** Sulfate <*1.14*>—Perform the test with 0.5 g of Ciprofloxacin Hydrochloride Hydrate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Ciprofloxacin Hydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 1.0 ml of Standard Lead Solution (not more than 10 ppm).

(3) Fluoroquinolonic acid—Conduct this procedure using light-resistant vessels. Dissolve 50 mg of Ciprofloxacin Hydrochloride Hydrate in water to make exactly 5 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of fluoroquinolonic acid for thin-layer chromatography in 0.1 mL of ammonia TS and water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. After allowing to stand the plate in the vapor of ammonia for 15 minutes, develop the plate with a mixture of methanol, dichloromethane, ammonia solution (28) and acetonitrile (4:4:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot obtained from the sample solution, corresponding to the spot obtained from the standard solution, is not more intense than that from the standard solution.

(4) Related substances—Conduct this procedure using light-resistant vessels. Dissolve 25 mg of Ciprofloxacin Hydrochloride Hydrate in 50 mL of mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 20 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peaks other than ciprofloxacin obtained from the sample solution is not larger than the peak area of ciprofloxacin obtained from the standard solution, and the total area of the peaks other than ciprofloxacin from the sample solution is not larger than 2.5 times the peak area of ciprofloxacin from the standard solution. For the area of the peaks, having the relative retention times of about 0.4, about 0.5, and about 1.2 to ciprofloxacin, multiply the relative response factors, 6.7, 1.3, and 1.4, respectively.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of ciprofloxacin, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of ciprofloxacin obtained with 50 μL of this solution is equivalent to 20 to 30% of that obtained with 50 μL of the standard solution.

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ciprofloxacin are not less than 3500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ciprofloxacin is not more than 2.0%.

Water <*2.48*> 4.7 – 6.7% (0.2 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Conduct this procedure using light-resistant vessels. Weigh accurately about 25 mg of Ciprofloxacin Hydrochloride Hydrate, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 22.5 mg of Ciprofloxacin RS, previously dried at 120°C in vacuum for 6 hours, add 2 mL of a mixture of water and phosphoric acid (13:1), then add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following operating conditions, and determine the peak areas, A_T and A_S, of ciprofloxacin in each solution.

$$\text{Amount (mg) of ciprofloxacin hydrochloride } (C_{17}H_{18}FN_3O_3.HCl) = M_S \times A_T/A_S \times 1.110$$

M_S: Amount (mg) of Ciprofloxacin RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 278 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 2.88 g of phosphoric acid add water to make 1000 mL, and adjust to pH 3.0 with triethylamine. To 870 mL of this solution add 130 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust so that the retention time of ciprofloxacin is about 7 minutes.

System suitability—

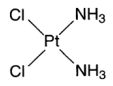
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ciprofloxacin are not less than 3500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ciprofloxacin is not more than 1.0%.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Cisplatin

シスプラチン



$Cl_2H_6N_2Pt$: 300.05
(*SP*-4-2)-Diamminedichloroplatinum
[*15663-27-1*]

Cisplatin, when dried, contains not less than 98.0% and not more than 102.0% of cisplatin ($Cl_2H_6N_2Pt$).

Description Cisplatin occurs as a yellow crystalline powder.

It is sparingly soluble in *N*,*N*-dimethylformamide, slightly soluble in water, and practically insoluble in ethanol (99.5).

Identification **(1)** To 5 mL of a solution of Cisplatin (1 in 2000) add 2 to 3 drops of a solution of tin (II) chloride dihydrate (1 in 100): a brown precipitate is formed.

(2) Determine the absorption spectrum of a solution of Cisplatin in a solution of sodium chloride in 0.01 mol/L hydrochloric acid TS (9 in 1000) (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cisplatin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Cisplatin as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Cisplatin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Cisplatin (1 in 2000) responds to the Qualitative Tests <*1.09*> (1) for chloride.

Purity Ammonium amminetrichloroplatinate—Conduct this procedure using light-resistant vessels. Dissolve 50 mg of Cisplatin in a solution of sodium chloride (9 in 1000) to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of ammonium amminetrichloroplatinate for liquid chromatography, previously dried at 80°C for 3 hours, in the solution of sodium chloride (9 in 1000) to make exactly 200 mL. Pipet 2 mL of this solution, add the solution of sodium chloride (9 in 1000) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak area of ammonium amminetrichloroplatinate by the automatic integration method: the peak area from the sample solution is not larger than that from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 209 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with silica gel for liquid chromatography having quaternary ammonium groups (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A solution of ammonium sulfate (1 in 800).

Flow rate: Adjust so that the retention time of ammonium amminetrichloroplatinate is about 8 minutes.

System suitability—

System performance: When the procedure is run with 40 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ammonium amminetrichloroplatinate are not less than 1500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ammonium amminetrichloroplatinate is not more than 3.0%.

Loss on drying <*2.41*> Not more than 0.1% (1 g, 105°C, 4 hours).

Assay Conduct this procedure using light-resistant vessels. Weigh accurately about 25 mg each of Cisplatin and Cisplatin RS, previously dried, dissolve in *N*,*N*-dimethylformamide to make exactly 25 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of cisplatin in each solution.

$$\text{Amount (mg) of cisplatin } (Cl_2H_6N_2Pt) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Cisplatin RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 310 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with aminopropylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of ethyl acetate, methanol, water and *N*,*N*-dimethylformamide (25:16:5:5).

Flow rate: Adjust so that the retention time of cisplatin is about 4 minutes.

System suitability—

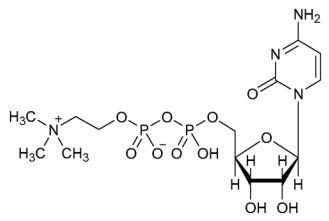
System performance: When the procedure is run with 40 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cisplatin are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cisplatin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Citicoline

シチコリン



$C_{14}H_{26}N_4O_{11}P_2$: 488.32
P'-[2-(Trimethylammonio)ethyl] cytidine 5'-(monohydrogen diphosphate)
[*987-78-0*]

Citicoline contains not less than 98.0% and not more than 102.0% of citicoline ($C_{14}H_{26}N_4O_{11}P_2$), calculated on the dried basis.

Description Citicoline occurs as a white crystalline powder.

It is very soluble in water, and practically insoluble in ethanol (99.5).

It dissolves in 0.01 mol/L hydrochloric acid TS.

Identification **(1)** Determine the absorption spectrum of a solution of Citicoline in 0.01 mol/L hydrochloric acid TS (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Citicoline RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Citicoline as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Citicoline RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <*2.54*> The pH of a solution obtained by dissolving 1.0 g of Citicoline in 100 mL of water is between 2.5 and 3.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Citicoline in 8 mL of water: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Citicoline according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Citicoline according to Method 4, and perform the test (not more than 2 ppm).

(4) Free phosphoric acid—Weigh accurately about 0.1 g of Citicoline, dissolve in water to make exactly 10 mL, and use this solution as the sample solution. Separately, pipet 4 mL of Standard Phosphoric Acid Solution, add water to make exactly 10 mL, and use this solution as the standard solution. To each of the sample solution and the standard solution, add exactly 1 mL of hexaammonium heptamolybdate-sulfuric acid TS and exactly 0.5 mL of 1-amino-2-naphthol-4-sulfonic acid TS, and after shaking, allow to stand for 30 minutes at 20 ± 1°C. To exactly 2 mL each of these solutions add water to make exactly 10 mL, and determine the absorbances, A_T and A_S, of the solutions obtained from the sample solution and the standard solution at 730 nm as directed under Ultraviolet-visible Spectrometry <*2.24*>, using the solution, obtained by proceeding with 10 mL of water in the same manner as the sample solution, as the blank. The amount of free phosphoric acid is not more than 0.1%.

$$\text{Amount (\%) of free phosphoric acid } (H_3PO_4) = 1/M \times A_T/A_S \times 10.32$$

M: Amount (mg) of Citicoline taken, calculated on the dried basis

(5) Related substances—Dissolve 0.10 g of Citicoline in water to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peaks other than citicoline obtained from the sample solution is not larger than 3/5 times the peak area of citicolins obtained from the standard solution, and the total area of the peaks other than citicoline from the sample solution is not larger than the peak area of citicoline from the standard solution. For the area of the peaks, having the relative retention times of about 0.62, about 0.64 and about 1.3 to citicoline, multiply the relative response factors, 1.2, 0.7 and 0.5, respectively.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of citicoline.

System suitability—

Test for required detectability: Pipet 4 mL of the standard solution, and add water to make exactly 50 mL. Confirm that the peak area of citicoline obtained with 10 μL of this solution is equivalent to 5.6 to 10.4% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of citicoline are not less than 2000 and 0.9 to 1.6, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of citicoline is not more than 2.0%.

Loss on drying <*2.41*> Not more than 5.0% (1 g, in vacuum, phosphorus (V) oxide, 100°C, 4 hours).

Assay Weigh accurately about 0.1 g of Citicoline, and dissolve in water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Citicoline RS (separately determine the loss on drying <*2.41*> under the same conditions as Citicoline), and dissolve in water to make exactly 25 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of citicoline in each solution.

$$\text{Amount (mg) of citicoline } (C_{14}H_{26}N_4O_{11}P_2) = M_S \times A_T/A_S \times 4$$

M_S: Amount (mg) of Citicoline RS taken, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: Combine 2 stainless steel columns (4 mm in inside diameter and 25 cm in length) packed with strongly basic ion exchange resin for liquid chromatography (10 μm in particle diameter) in series.

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 8.17 g of potassium dihydrogen phosphate in water to make 1000 mL. Adjust the pH of this solution to 3.5 with phosphoric acid.

Flow rate: Adjust so that the retention time of citicoline is about 26 minutes.

System suitability—

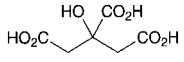
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of citicoline are not less than 2000 and 0.9 to 1.6, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of citicoline is not more than 1.0%.

Containers and storage Containers—Tight containers.

Anhydrous Citric Acid

無水クエン酸



$C_6H_8O_7$: 192.12
2-Hydroxypropane-1,2,3-tricarboxylic acid
[*77-92-9*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbol (◆ ◆).

Anhydrous Citric Acid contains not less than 99.5% and not more than 100.5% of anhydrous citric acid ($C_6H_8O_7$), calculated on the anhydrous basis.

◆**Description** Anhydrous Citric Acid occurs as colorless crystals, white granules or crystalline powder.

It is very soluble in water, and freely soluble in ethanol (99.5).◆

Identification Determine the infrared absorption spectrum of Anhydrous Citric Acid, previously dried at 105°C for 2 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Clarity and color of solution—Dissolve 2.0 g of Anhydrous Citric Acid in water to make 10 mL: the solution is clear and colorless or has no more color than the following control solutions (1), (2) or (3).

Control solution (1): To 1.5 mL of Cobalt (II) Chloride CS and 6.0 mL of Iron (III) Chloride CS add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

Control solution (2): To 2.5 mL of Cobalt (II) Chloride CS, 6.0 mL of Iron (III) Chloride CS and 1.0 mL of Copper (II) Sulfate CS add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

Control solution (3): To 0.15 mL of Cobalt (II) Chloride CS, 7.2 mL of Iron (III) Chloride CS and 0.15 mL of Copper (II) Sulfate CS add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

(2) Sulfates—Dissolve 2.0 g of Anhydrous Citric Acid in water to make 30 mL, and use this solution as the sample solution. Separately, dissolve 0.181 g of potassium sulfate in diluted ethanol (3 in 10) to make exactly 500 mL. Pipet 5 mL of this solution, and add diluted ethanol (3 in 10) to make exactly 100 mL. To 4.5 mL of this solution add 3 mL of a solution of barium chloride dihydrate (1 in 4), shake, and allow to stand for 1 minute. To 2.5 mL of this solution add 15 mL of the sample solution and 0.5 mL of acetic acid (31), and allow to stand for 5 minutes: the solution has no more turbidity than the following control solution (not more than 150 ppm).

Control solution: Dissolve 0.181 g of potassium sulfate in water to make exactly 500 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and proceed in the same manner as above using this solution instead of the sample solution.

(3) Oxalic acid—Dissolve 0.80 g of Anhydrous Citric Acid in 4 mL of water, add 3 mL of hydrochloric acid and 1 g of zinc, and boil for 1 minute. After allowing to stand for 2 minutes, take the supernatant liquid, add 0.25 mL of a solution of phenylhydrazinium chloride (1 in 100), heat to boil, and then cool quickly. To this solution add the equal volume of hydrochloric acid and 0.25 mL of a solution of potassium hexacyanoferrate (III) (1 in 20), mix, and allow to stand for 30 minutes: the solution has no more color than the following control solution prepared at the same time (not more than 360 ppm expressed as oxalic anhydride).

Control solution: To 4 mL of a solution of oxalic acid dihydrate (1 in 10,000) add 3 mL of hydrochloric acid and 1 g of zinc, and proceed in the same manner as the test solution.

◆**(4)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Anhydrous Citric Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).◆

(5) Readily carbonizable substances—Place 1.0 g of Anhydrous Citric Acid in a Nessler tube, add 10 mL of sulfuric acid, immediately heat in a 90 ± 1°C water bath for 60 minutes, and cool quickly. Compare the color of 2.0 mL each of this solution and Matching Fluid K, using test tubes 12 mm in outside diameter, from a side against white background: the solution is not more colored than the matching fluid.

Water ⟨*2.48*⟩ Not more than 1.0% (2 g, volumetric titration, direct titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.55 g of Anhydrous Citric Acid, dissolve in 50 mL of water, and titrate with 1 mol/L sodium hydroxide VS (indicator: 1 drop of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS
= 64.04 mg of $C_6H_8O_7$

◆**Containers and storage** Containers—Tight containers.◆

Citric Acid Hydrate

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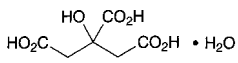


$C_6H_8O_7.H_2O$: 210.14
2-Hydroxypropane-1,2,3-tricarboxylic acid monohydrate
[*5949-29-1*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbol (◆ ◆).

Citric Acid Hydrate contains not less than 99.5% and not more than 100.5% of anhydrous citric acid ($C_6H_8O_7$: 192.12), calculated on the anhydrous basis.

◆**Description** Citric Acid Hydrate occurs as colorless crystals, white granules or crystalline powder.

It is very soluble in water, and freely soluble in ethanol (99.5).

It is efflorescent in dry air.◆

Identification Determine the infrared absorption spectrum of Citric Acid Hydrate, previously dried at 105°C for 2 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Clarity and color of solution—Dissolve 2.0 g of Citric Acid Hydrate in water to make 10 mL: the solution is clear and colorless or has no more color than the following control solutions (1), (2) or (3).

Control solution (1): To 1.5 mL of Cobalt (II) Chloride CS and 6.0 mL of Iron (III) Chloride CS add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

Control solution (2): To 2.5 mL of Cobalt (II) Chloride CS, 6.0 mL of Iron (III) Chloride CS and 1.0 mL of Copper (II) Sulfate CS add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

Control solution (3): To 0.15 mL of Cobalt (II) Chloride CS, 7.2 mL of Iron (III) Chloride CS and 0.15 mL of Copper (II) Sulfate CS add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

(2) Sulfates—Dissolve 2.0 g of Citric Acid Hydrate in water to make 30 mL, and use this solution as the sample solution. Separately, dissolve 0.181 g of potassium sulfate in diluted ethanol (3 in 10) to make exactly 500 mL. Pipet 5 mL of this solution, and add diluted ethanol (3 in 10) to make exactly 100 mL. To 4.5 mL of this solution add 3 mL of a solution of barium chloride dihydrate (1 in 4), shake, and allow to stand for 1 minute. To 2.5 mL of this solution add 15 mL of the sample solution and 0.5 mL of acetic acid (31), and allow to stand for 5 minutes: the solution has no more turbidity than the following control solution. (not more than 150 ppm).

Control solution: Dissolve 0.181 g of potassium sulfate in water to make exactly 500 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and proceed in the same manner as above using this solution instead of the sample solution.

(3) Oxalic acid—Dissolve 0.80 g of Citric Acid Hydrate in 4 mL of water, add 3 mL of hydrochloric acid and 1 g of zinc, and boil for 1 minute. After allowing to stand for 2 minutes, take the supernatant liquid, add 0.25 mL of a solution of phenylhydrazinium chloride (1 in 100), heat to boil, and then cool quickly. To this solution add the equal volume of hydrochloric acid and 0.25 mL of a solution of potassium hexacyanoferrate (III) (1 in 20), mix, and allow to stand for 30 minutes: the solution has no more color than the following control solution prepared at the same time (not more than 360 ppm expressed as oxalic anhydride).

Control solution: To 4 mL of a solution of oxalic acid dihydrate (1 in 10,000) add 3 mL of hydrochloric acid and 1 g of zinc, and proceed in the same manner as the test solution.

◆**(4)** Heavy metals <*1.07*>—Proceed with 2.0 g of Citric Acid Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).◆

(5) Readily carbonizable substances—Place 1.0 g of Citric Acid Hydrate in a Nessler tube, add 10 mL of sulfuric acid, immediately heat in a 90 ± 1°C water bath for 60 minutes, and cool quickly. Compare the color of 2.0 mL each of this solution and Matching Fluid K, using test tubes 12 mm in outside diameter, from a side against white background: the solution is not more colored than the matching fluid.

Water <*2.48*> Not less than 7.5% and not more than 9.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

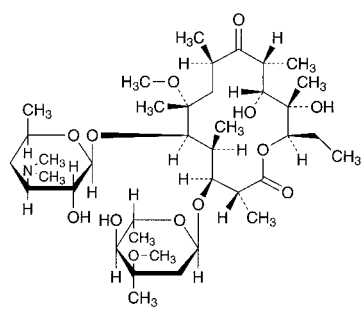
Assay Weigh accurately about 0.55 g of Citric Acid Hydrate, dissolve in 50 mL of water, and titrate <*2.50*> with 1 mol/L sodium hydroxide VS (indicator: 1 drop of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS
= 64.04 mg of $C_6H_8O_7$

◆**Containers and storage** Containers—Tight containers.◆

Clarithromycin

クラリスロマイシン



$C_{38}H_{69}NO_{13}$: 747.95
(2*R*,3*S*,4*S*,5*R*,6*R*,8*R*,10*R*,11*R*,12*S*,13*R*)-5-(3,4,6-Trideoxy-3-dimethylamino-*β*-D-*xylo*-hexopyranosyloxy)-3-(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-*α*-L-*ribo*-hexopyranosyloxy)-11,12-dihydroxy-6-methoxy-2,4,6,8,10,12-hexamethyl-9-oxopentadecan-13-olide
[*81103-11-9*]

Clarithromycin is a derivative of erythromycin.

It contains not less than 950 μg (potency) and not more than 1050 μg (potency) per mg, calculated on the anhydrous basis. The potency of Clarithromycin is expressed as mass (potency) of clarithromycin ($C_{38}H_{69}NO_{13}$).

Description Clarithromycin occurs as a white crystalline

powder and has a bitter taste.

It is soluble in acetone and in chloroform, slightly soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification **(1)** To 5 mg of Clarithromycin add 2 mL of sulfuric acid, and shake gently: a red-brown color develops.

(2) Dissolve 3 mg of Clarithromycin in 2 mL of acetone, and add 2 mL of hydrochloric acid: an orange color develops and changes immediately to red to deep purple.

(3) Determine the infrared absorption spectra of Clarithromycin and Clarithromycin RS as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Dissolve 10 mg each of Clarithromycin and Clarithromycin RS in 4 mL of chloroform, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of chloroform, methanol and ammonia water (28) (100:5:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly sulfuric acid on the plate, and heat at 105°C for 10 minutes: the principal spot from the sample solution and the spot from the standard solution show a dark purple color and have the same *R*f value.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -87 – $-97°$ (0.25 g calculated on the anhydrous basis, chloroform, 25 mL, 100 mm).

Melting point <*2.60*> 220 – 227°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Clarithromycin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic—Prepare the test solution with 1.0 g of Clarithromycin according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Weigh accurately about 0.1 g of Clarithromycin, dissolve in the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Clarithromycin RS, dissolve in the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the each peak area by the automatic integration method: the amount of each related substance calculated on the anhydrous basis is not more than 2.0%, and the total of them is not more than 5.0%. Exclude any peak with an area of less than 0.05%.

Amount (%) of each related substance calculated on the anhydrous basis
$= M_S/M_T \times A_T/A_S \times 100$

Total amount (%) of the related substances calculated on the anhydrous basis
$= M_S/M_T \times \Sigma A_T/A_S \times 100$

M_S: Amount (mg) of Clarithromycin RS taken
M_T: Amount (mg) of Clarithromycin taken, calculated on the anhydrous basis
A_S: Peak area of clarithromycin obtained with the standard solution
A_T: Peak area of each related substance obtained with the sample solution
ΣA_T: Total area of the peaks other than clarithromycin obtained with the sample solution

Operating conditions—
Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 5 times as long as the retention time of the main peak after 2 minutes of sample injection.

System suitability—
System performance: Proceed as directed in the system suitability in the Assay.

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL, and use this solution as the solution for system suitability test. Confirm that when the procedure is run with 10 μL of the solution for system suitability test, the peak area of clarithromycin is equivalent to 14 to 26% of that obtained with 10 μL of the standard solution.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of clarithromycin is not more than 3.0%.

Water <*2.48*> Not more than 2.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (2 g).

Assay Weigh accurately an amount of Clarithromycin and Clarithromycin RS, equivalent to about 0.1 g (potency), and dissolve in the mobile phase to make exactly 20 mL. Pipet 2 mL each of these solutions, add exactly 2 mL of the internal standard solution, add the mobile phase to make 20 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of clarithromycin to that of the internal standard.

Amount [μg (potency)] of clarithromycin ($C_{38}H_{69}NO_{13}$)
$= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Clarithromycin RS taken

Internal standard solution—A solution of butyl parahydroxybenzoate in the mobile phase (1 in 20,000).

Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of diluted 0.2 mol/L potassium dihydrogenphosphate TS (1 in 3) and acetonitrile (13:7).

Flow rate: Adjust so that the retention time of clarithromycin is about 8 minutes.

System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, clarithromycin and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of clarithromycin to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Well-closed containers.

Clarithromycin Tablets

クラリスロマイシン錠

Clarithromycin Tablets contain not less than 93.0% and not more than 107.0% of the labeled potency of clarithromycin ($C_{38}H_{69}NO_{13}$: 747.95).

Method of preparation Prepare as directed under Tablets, with Clarithromycin.

Identification Shake a quantity of powdered Clarithromycin Tablets, equivalent to 60 mg (potency) of Clarithromycin, with 40 mL of acetone for 10 minutes, and centrifuge at 4000 rpm for 5 minutes. Evaporate 30 mL of the supernatant liquid, and determine the infrared absorption spectrum of the residue so obtained as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 2980 cm^{-1}, 2940 cm^{-1}, 1734 cm^{-1}, 1693 cm^{-1}, 1459 cm^{-1}, 1379 cm^{-1} and 1171 cm^{-1}.

Uniformity of dosage units <6.02> Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Clarithromycin Tablets add exactly $V/20$ mL of the internal standard solution (1), then add the mobile phase so that each mL contains about 5 mg (potency) of clarithromycin ($C_{38}H_{69}NO_{13}$) to make V mL, and disperse to fine particles with the aid of ultrasonic waves for 20 minutes while occasional vigorous shaking. Centrifuge this solution at 4000 rpm for 15 minutes, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Then, proceed as directed in the Assay.

Amount [mg (potency)] of clarithromycin ($C_{38}H_{69}NO_{13}$)
$= M_S \times Q_T/Q_S \times V/10$

M_S: Amount [mg (potency)] of Clarithromycin RS taken

Internal standard solution (1)—A solution of butyl parahydroxybenzoate in the mobile phase (1 in 1000).
Internal standard solution (2)—To exactly 1 mL of the internal standard solution (1) add the mobile phase to make exactly 20 mL.

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 0.05 mol/L disodium hydrogen phosphate-citric acid buffer solution (pH 6.0) as the dissolution medium, the dissolution rates in 30 minutes of a 50-mg tablet and a 200-mg tablet are not less than 80% and not less than 75%, respectively.

Start the test with 1 tablet of Clarithromycin Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the mobile phase to make exactly V' mL so that each mL contains about 28 μg (potency) of Clarithromycin, and use this solution as the sample solution. Separately, weigh accurately an amount of Clarithromycin RS, equivalent to about 28 mg (potency), and dissolve in acetonitrile for liquid chromatography to make exactly 100 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of clarithromycin in each solution.

Dissolution rate (%) with respect to the labeled amount of clarithromycin ($C_{38}H_{69}NO_{13}$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount [mg (potency)] of Clarithromycin RS taken
C: Labeled amount [mg (potency)] of clarithromycin ($C_{38}H_{69}NO_{13}$) in 1 tablet

Operating conditions—
Proceed as directed in the operating conditions in the Assay.
System suitability—
System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clarithromycin are not less than 3000 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clarithromycin is not more than 2.0%.

Assay To not less than 5 Clarithromycin Tablets add diluted 0.2 mol/L potassium dihydrogen phosphate TS (1 in 3) so that each mL contains about 8 mg (potency) of clarithromycin ($C_{38}H_{69}NO_{13}$), disperse to fine particles with the aid of ultrasonic waves, add exactly 1 mL of the internal standard solution (1) per 100 mg (potency) of clarithromycin, then add acetonitrile for liquid chromatography so that each mL contains about 5 mg (potency) of clarithromycin ($C_{38}H_{69}NO_{13}$), and disperse to fine particles with the aid of ultrasonic waves for 10 minutes while occasional vigorous shaking. Centrifuge of this solution at 4000 rpm for 15 minutes, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, to 2 mL of the subsequent filtrate add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Clarithromycin RS, equivalent to about 50 mg (potency), and dissolve in the mobile phase to make exactly 10 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution (2) and the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of clarithromycin to that of the internal standard.

Amount [mg (potency)] of clarithromycin ($C_{38}H_{69}NO_{13}$)
$= M_S \times Q_T/Q_S \times 1/5$

M_S: Amount [mg (potency)] of Clarithromycin RS taken

Internal standard solution (1)—A solution of butyl parahydroxybenzoate in the mobile phase (1 in 1000).
Internal standard solution (2)—To exactly 1 mL of the internal standard solution (1) add the mobile phase to make

exactly 20 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of diluted 0.2 mol/L potassium dihydrogen phosphate TS (1 in 3) and acetonitrile for liquid chromatography (13:7).

Flow rate: Adjust so that the retention time of clarithromycin is about 8 minutes.

System suitability—

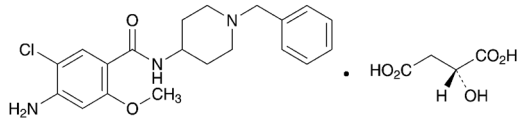
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, clarithromycin and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of clarithromycin to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Well-closed containers.

Clebopride Malate

クレボプリドリンゴ酸塩



and enantiomer

$C_{20}H_{24}ClN_3O_2.C_4H_6O_5$: 507.96

4-Amino-*N*-(1-benzylpiperidin-4-yl)-5-chloro-2-methoxybenzamide mono-(2*RS*)-malate

[*57645-91-7*]

Clebopride Malate, when dried, contains not less than 98.5% and not more than 101.0% of clebopride malate ($C_{20}H_{24}ClN_3O_2.C_4H_6O_5$).

Description Clebopride Malate occurs as a white crystalline powder.

It is freely soluble in acetic acid (100), soluble in methanol, sparingly soluble in water, and slightly soluble in ethanol (99.5).

A solution of Clebopride Malate in methanol (1 in 25) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Clebopride Malate in methanol (1 in 80000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Clebopride Malate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Clebopride Malate under Flame Coloration Test ⟨*1.04*⟩ (2): a green color appears.

Purity **(1)** Chloride ⟨*1.03*⟩—Dissolve 1.0 g of Clebopride Malate in 20 mL of acetic acid (100), add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS by adding 20 mL of acetic acid (100), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.009%).

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Clebopride Malate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.10 g of Clebopride Malate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 0.2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than clebopride obtained from the sample solution is not larger than the peak area of clebopride obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 3.85 g of ammonium acetate in water to make 500 mL, and filter through a membrane filter with a pore size not exceeding 0.5 μm. To 400 mL of the filtrate add 600 mL of methanol.

Flow rate: Adjust so that the retention time of clebopride is about 15 minutes.

Time span of measurement: About 2 times as long as the retention time of clebopride.

System suitability—

Test for required detectability: Pipet 10 mL of the standard solution, and add water to make exactly 100 mL. Confirm that the peak area of clebopride obtained from 10 μL of this solution is equivalent to 7 to 13% of that of clebopride obtained from 10 μL of the standard solution.

System performance: Dissolve 30 mg Clebopride Malate and 5 mg of propyl parahydroxybenzoate in the mobile phase to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, propyl parahydroxybenzoate and clebopride are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clebopride is not more than 2.5%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Clebopride Malate, previously dried, dissolve in 30 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same

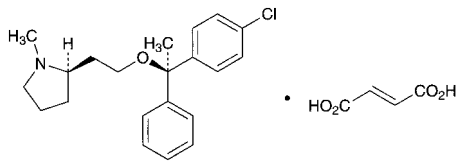
manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 50.80 mg of $C_{20}H_{24}ClN_3O_2.C_4H_6O_5$

Containers and storage Containers—Tight containers.

Clemastine Fumarate

クレマスチンフマル酸塩



$C_{21}H_{26}ClNO.C_4H_4O_4$: 459.96
(2*R*)-2-{2-[(1*R*)-1-(4-Chlorophenyl)-1-phenylethoxy]ethyl}-1-methylpyrrolidine monofumarate
[*14976-57-9*]

Clemastine Fumarate, when dried, contains not less than 98.5% of clemastine fumarate ($C_{21}H_{26}ClNO.C_4H_4O_4$).

Description Clemastine Fumarate occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in methanol and in acetic acid (100), slightly soluble in ethanol (95), very slightly soluble in diethyl ether, and practically insoluble in water.

Identification **(1)** To 5 mg of Clemastine Fumarate add 5 mL of sulfuric acid, and shake to dissolve: a yellow color develops. Slowly drop this solution into 10 mL of water: the yellow color immediately disappears.

(2) To 0.01 g of Clemastine Fumarate add 1 mL of fuming nitric acid, and evaporate on a water bath to dryness. Then add 2 mL of diluted hydrochloric acid (1 in 2) and 0.2 g of zinc powder, heat for 10 minutes on a water bath, cool, and filter. Add 20 mL of water to the filtrate. The solution responds to the Qualitative Tests <*1.09*> for primary aromatic amines.

(3) To 5 mL of a solution of Clemastine Fumarate (1 in 50,000), add 5 mL of 4-dimethylaminobenzaldehyde TS, and warm for 10 minutes: a red-purple color develops.

(4) Perform the test with Clemastine Fumarate as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

(5) Dissolve 0.04 g of Clemastine Fumarate and 0.01 g of fumaric acid for thin-layer chromatography in 2 mL each of a mixture of ethanol (95) and water (4:1) by gentle warming, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of isopropyl ether, formic acid and water (90:7:3) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spot with larger *R*f value from the sample solution has the same *R*f value as the spot from the standard solution.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +16 – +18° (after drying, 0.1 g, methanol, 10 mL, 100 mm).

Melting point <*2.60*> 176 – 180°C (with decomposition).

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Clemastine Fumarate in 10 mL of methanol by warming: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Perform the test with 1.0 g of Clemastine Fumarate according to Method 2. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <*1.11*>—Take 1.0 g of Clemastine Fumarate, prepare the test solution according to Method 3, and perform the test (not more than 2 ppm).

(4) Related Substances—Dissolve 0.10 g of Clemastine Fumarate in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 250 mL, and use this solution as the standard solution (1). Pipet 5 mL of the standard solution (1), add methanol to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (90:10:1) to a distance of about 15 cm, and air-dry the plate. After spraying evenly Dragendorff's TS on the plate, immediately spray evenly hydrogen peroxide TS: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (1), and not more than 2 spots from the sample solution are more intense than the spot from the standard solution (2).

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

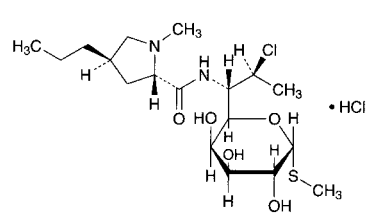
Assay Weigh accurately about 0.4 g of Clemastine Fumarate, previously dried, dissolved in 50 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 46.00 mg of $C_{21}H_{26}ClNO.C_4H_4O_4$

Containers and storage Containers—Tight containers.

Clindamycin Hydrochloride

クリンダマイシン塩酸塩



$C_{18}H_{33}ClN_2O_5S.HCl$: 461.44
Methyl 7-chloro-6,7,8-trideoxy-6-[(2*S*,4*R*)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-L-*threo*-α-D-*galacto*-octopyranoside monohydrochloride
[*21462-39-5*]

Clindamycin Hydrochloride is the hydrochloride of a derivative of lincomycin.

It contains not less than 838 μg (potency) and not more than 940 μg (potency) per mg, calculated on the anhydrous basis. The potency of Clindamycin Hydrochloride is expressed as mass (potency) of clindamycin

($C_{18}H_{33}ClN_2O_5S$: 424.98).

Description Clindamycin Hydrochloride occurs as white to grayish white, crystals or crystalline powder.

It is freely soluble in water and in methanol, and slightly soluble in ethanol (99.5).

Identification **(1)** Determine the infrared absorption spectrum of Clindamycin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Clindamycin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Clindamycin Hydrochloride (1 in 100) responds to the Qualitative Tests <*1.09*> (2) for chloride.

Optical rotation <*2.49*> $[\alpha]_D^{25}$: +135 – +150° (0.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Clindamycin Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Use the sample solution obtained in the Assay as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of clindamycin B, having the relative retention time of about 0.7 to clindamycin, and that of 7-epiclindamycin, having the relative retention time of about 0.8 to clindamycin, obtained from the sample solution are not larger than 2 times the peak area of clindamycin obtained from the standard solution, the area of the peak other than clindamycin and the peaks mentioned above from the sample solution is not larger than the peak area of clindamycin from the standard solution, and the total area of the peaks other than clindamycin from the sample solution is not larger than 4 times the peak area of clindamycin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of clindamycin, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of clindamycin obtained from 20 μL of this solution is equivalent to 7 to 13% of that of clindamycin obtained from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clindamycin are not less than 6000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clindamycin is not more than 2.0%.

Water <*2.48*> Not more than 6.0% (0.3 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Clindamycin Hydrochloride and Clindamycin Hydrochloride RS, equivalent to about 20 mg (potency), dissolve each in the mobile phase to make exactly 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of clindamycin in each solution.

Amount [μg (potency)] of clindamycin ($C_{18}H_{33}ClN_2O_5S$) $= M_S \times A_T/A_S \times 1000$

M_S: Amount [mg (potency)] of Clindamycin Hydrochloride RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 0.05 mol/L potassium dihydrogen phosphate TS add 8 mol/L potassium hydroxide TS to adjust the pH to 7.5. To 550 mL of this solution add 450 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust so that the retention time of clindamycin is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clindamycin are not less than 6000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clindamycin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Clindamycin Hydrochloride Capsules

クリンダマイシン塩酸塩カプセル

Clindamycin Hydrochloride Capsules contain not less than 93.0% and not more than 107.0% of the labeled potency of clindamycin ($C_{18}H_{33}ClN_2O_5S$: 424.98).

Method of preparation Prepare as directed under Capsules, with Clindamycin Hydrochloride.

Identification To an amount of the contents of Clindamycin Hydrochloride Capsules, equivalent to 10 mg (potency) of Clindamycin Hydrochloride, add 2 mL of methanol, shake well, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of Clindamycin Hydrochloride RS in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, toluene and ammonia solution (28) (140:60:3) to a distance of about 12

cm, and air-dry the plate. Spray evenly a mixture of 500 mL of a solution of L-tartaric acid (1 in 5) and 50 mL of bismuth subnitrate TS on the plate: the *R*f values of the principal spot with the sample solution and the spot with the standard solution are not different each other.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 capsule of Clindamycin Hydrochloride Capsules add a suitable amount of the mobile phase, shake for 30 minutes, and add the mobile phase to make exactly V mL so that each mL contains 0.75 mg (potency) of Clindamycin Hydrochloride. Centrifuge this solution, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

Amount [mg (potency)] of clindamycin ($C_{18}H_{33}ClN_2O_5S$) $= M_S \times A_T/A_S \times V/100$

M_S: Amount [mg (potency)] of Clindamycin Hydrochloride RS taken

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate of a 75-mg capsule in 15 minutes and that of a 150-mg capsule in 30 minutes are not less than 80%, respectively.

Start the test with 1 capsule of Clindamycin Hydrochloride Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' so that each mL contains about 83 μg (potency) of Clindamycin Hydrochloride, and use this solution as the sample solution. Separately, weigh accurately an amount of Clindamycin Hydrochloride RS, equivalent to about 17 mg (potency), dissolve in water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩, and determine the peak areas, A_T and A_S, of clindamycin in each solution.

Dissolution rate (%) with respect to the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$) $= M_S \times A_T/A_S \times V'/V \times 1/C \times 450$

M_S: Amount [mg (potency)] of Clindamycin Hydrochloride RS taken

C: Labeled amount [mg (potency)] of clindamycin ($C_{18}H_{33}ClN_2O_5S$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 7.5 with 8 mol/L potassium hydroxide TS. To 550 mL of this solution add 450 mL of acetonitrile.

Flow rate: Adjust so that the retention time of clindamycin is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clindamycin are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clindamycin is not more than 2.0%.

Assay Take out the contents of not less than 20 Clindamycin Hydrochloride Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 75 mg (potency) of Clindamycin Hydrochloride, add the mobile phase, shake for 30 minutes, and add the mobile phase to make exactly 100 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately an amount of Clindamycin Hydrochloride RS, equivalent to about 75 mg (potency), dissolve in the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of clindamycin in each solution.

Amount [mg (potency)] of clindamycin ($C_{18}H_{33}ClN_2O_5S$) $= M_S \times A_T/A_S$

M_S: Amount [mg (potency)] of Clindamycin Hydrochloride RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 0.05 mol/L of potassium dihydrogen phosphate TS add 8 mol/L potassium hydroxide TS to adjust the pH to 7.5. To 550 mL of this solution add 450 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust so that the retention time of clindamycin is about 7 minutes.

System suitability—

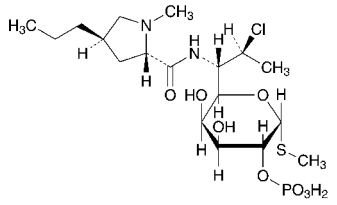
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clindamycin are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clindamycin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Clindamycin Phosphate

クリンダマイシンリン酸エステル



$C_{18}H_{34}ClN_2O_8PS$: 504.96
Methyl 7-chloro-6,7,8-trideoxy-6-[(2*S*,4*R*)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-L-*threo*-α-D-*galacto*-octopyranoside 2-dihydrogen phosphate
[*24729-96-2*]

Clindamycin Phosphate is a derivative of clindamycin.

It contains not less than 800 μg (potency) and not more than 846 μg (potency) per mg, calculated on the anhydrous basis. The potency of Clindamycin Phosphate is expressed as mass (potency) of clindamycin ($C_{18}H_{33}ClN_2O_5S$: 424.98).

Description Clindamycin Phosphate occurs as a white to pale yellowish white crystalline powder.

It is freely soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (95).

Identification Determine the infrared absorption spectrum of Clindamycin Phosphate, previously dried at 100°C for 2 hours, as directed in the paste method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Clindamycin Phosphate RS previously dried at 100°C for 2 hours: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +115 – +130° (0.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH <*2.54*> Dissolve 0.10 g of Clindamycin Phosphate in 10 mL of water. The pH of the solution is between 3.5 and 4.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Clindamycin Phosphate in 10 mL of freshly boiled and cooled water: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Clindamycin Phosphate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 5 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Clindamycin Phosphate according to Method 4, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.1 g of Clindamycin Phosphate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of clindamycin, having the relative retention time of about 1.8 to clindamycin phosphate, obtained from the sample solution is not larger than 1/2 times the peak area of clindamycin phosphate from the standard solution, and the total area of the peaks other than clindamycin phosphate from the sample solution is not larger than 4 times the peak area of clindamycin phosphate from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of clindamycin phosphate, beginning after the solvent peak.

System suitability—

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of clindamycin phosphate obtained from 20 μL of this solution is equivalent to 7 to 13% of that obtained from 20 μL of the standard solution.

Water <*2.48*> Not more than 6.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Clindamycin Phosphate and Clindamycin Phosphate RS, equivalent to about 20 mg (potency), add exactly 25 mL of the internal standard solution and the mobile phase to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of clindamycin phosphate to that of the internal standard.

Amount [μg (potency)] of clindamycin ($C_{18}H_{33}ClN_2O_5S$) $= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Clindamycin Phosphate RS taken

Internal standard solution—A solution of methyl parahydroxybenzoate in the mobile phase (3 in 50,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 10.54 g of potassium dihydrogen phosphate in 775 mL of water, adjust the pH to 2.5 with phosphoric acid, and add 225 mL of acetonitrile.

Flow rate: Adjust so that the retention time of clindamycin phosphate is about 8 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, clindamycin phosphate and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of clindamycin phosphate to that of the internal standard is not more than 2.5%.

Containers and storage Containers—Tight containers.

Clindamycin Phosphate Injection

クリンダマイシンリン酸エステル注射液

Clindamycin Phosphate Injection is an aqueous injection.

It contains not less than 90.0% and not more than 110.0% of the labeled potency of clindamycin phosphate ($C_{18}H_{34}ClN_2O_8PS$: 504.96).

Method of preparation Prepare as directed under Injections, with Clindamycin Phosphate.

Description Clindamycin Phosphate Injection is a clear, colorless or light yellow liquid.

Identification To a volume of Clindamycin Phosphate Injection, equivalent to 0.15 g (potency) of Clindamycin Phosphate, add 4 mL of water, 2 mL of 8 mol/L sodium hydroxide TS and 0.1 mL of sodium pentacyanonitrosylferrate (III) TS, mix, heat in a water bath for 10 minutes, and add 2 mL of hydrochloric acid: a blue-green color develops.

Osmotic pressure ratio Being specified separately when the drug is granted approval based on the Law.

pH ⟨*2.54*⟩ 6.0 – 7.0

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.1 EU/mg (potency).

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Clindamycin Phosphate Injection, equivalent to about 0.3 g (potency) of Clindamycin Phosphate, and add the mobile phase to make exactly 100 mL. Pipet 7 mL of this solution, add exactly 25 mL of the internal standard solution and the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Clindamycin Phosphate RS, equivalent to about 20 mg (potency), dissolve in exactly 25 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Clindamycin Phosphate.

Amount [mg (potency)] of clindamycin phosphate ($C_{18}H_{34}ClN_2O_8PS$)
$= M_S \times Q_T/Q_S \times 100/7$

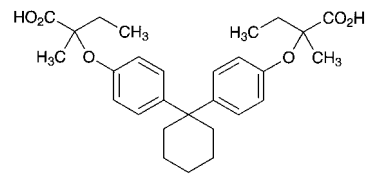
M_S: Amount [mg (potency)] of Clindamycin Phosphate RS taken

Internal standard solution—A solution of methyl parahydroxybenzoate in the mobile phase (3 in 50,000).

Containers and storage Containers—Hermetic containers.

Clinofibrate

クリノフィブラート



$C_{28}H_{36}O_6$: 468.58
2,2′-(4,4′-Cyclohexylidenediphenoxy)-2,2′-dimethyldibutanoic acid
[*30299-08-2*]

Clinofibrate, when dried, contains not less than 98.5% of clinofibrate ($C_{28}H_{36}O_6$).

Description Clinofibrate occurs as a white to yellowish white powder. It is odorless and has no taste.

It is freely soluble in methanol, in ethanol (99.5), in acetone and in diethyl ether, and practically insoluble in water.

A solution of Clinofibrate in methanol (1 in 20) shows no optical rotation.

Melting point: about 146°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Clinofibrate in ethanol (99.5) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Clinofibrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Clinofibrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Clinofibrate according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Clinofibrate in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetone to make exactly 50 mL. Pipet 5 mL of this solution, add acetone to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 50 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, cyclohexane and acetic acid (100) (12:5:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.2% (1 g).

Isomer ratio To 50 mg of Clinofibrate add 0.4 mL of

thionyl chloride, stopper tightly, heat on a water bath of 60°C for 5 minutes with occasional shaking, and evaporate the excess thionyl chloride at a temperature not exceeding 60°C under reduced pressure. Dissolve the residue in 2 mL of toluene previously dried with synthetic zeolite for drying, add 2 mL of a solution of D-(+)-α-methylbenzylamine in toluene previously dried with synthetic zeolite for drying (3 in 100), mix gently, allow to stand for 10 minutes, and evaporate the toluene at a temperature not exceeding 60°C under reduced pressure. Dissolve the residue in 5 mL of chloroform, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area, A_a, A_b and A_c, of three peaks appear in order near the retention time of 40 minutes: a value, $A_b/(A_a + A_b + A_c) \times 100$, is between 40 and 70.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 30 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of hexane and 2-propanol (500:3).

Flow rate: Adjust so that the retention time of the peak appearing first is about 35 minutes.

Selection of column: Proceed with 5 μL of the sample solution under the above operating conditions. Use a column giving a complete separation of the three peaks.

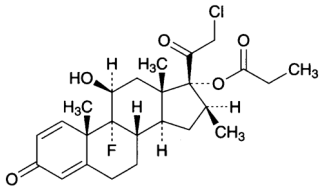
Assay Weigh accurately about 0.45 g of Clinofibrate, previously dried, dissolve in 40 mL of ethanol (99.5), add 30 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 23.43 mg of $C_{28}H_{36}O_6$

Containers and storage Containers—Tight containers.

Clobetasol Propionate

クロベタゾールプロピオン酸エステル



$C_{25}H_{32}ClFO_5$: 466.97

21-Chloro-9-fluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-propanoate [*25122-46-7*]

Clobetasol Propionate, when dried, contains not less than 97.0% and not more than 102.0% of clobetasol propionate ($C_{25}H_{32}ClFO_5$).

Description Clobetasol Propionate occurs as a white to pale yellowish white crystalline powder.

It is soluble in methanol and in ethanol (99.5), and practically insoluble in water.

It gradually turns yellow by light.

Melting point: about 196°C (with decomposition).

Identification Determine the infrared absorption spectra of Clobetasol Propionate as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Clobetasol Propionate RS: both spectra exhibit similar intensities of absorbance at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +109 – +115° (after drying, 0.1 g, methanol, 10 mL, 100 mm).

Purity **(1)** Heavy metals <1.07>—Proceed with 1.0 g of Clobetasol Propionate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 10 mg of Clobetasol Propionate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of these solutions by the automatic integration method: the area of the peak other than clobetasol propionate obtained from the sample solution is not larger than 2/5 times the peak area of clobetasol propionate obtained from the standard solution. Furthermore, the total area of the peaks other than clobetasol propionate from the sample solution is not larger than the peak area of clobetasol propionate from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of clobetasol propionate, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of clobetasol propionate obtained from 10 μL of this solution is equivalent to 2.8 to 5.2% of that obtained from 10 μL of the standard solution.

System performance: Dissolve 20 mg of Clobetasol Propionate in 20 mL of methanol. To 5 mL of this solution add 10 mL of a solution of beclometasone dipropionate in methanol (1 in 1000), and then add the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above conditions, clobetasol propionate and beclometasone dipropionate are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above conditions, the relative standard deviation of the peak area of clobetasol propionate is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 10 mg each of Clobetasol Propionate and Clobetasol Propionate RS, both previously dried, dissolve each in the mobile phase, add exactly 100 mL of the internal standard solution, add the mobile phase to make 250 mL, and use these solutions as the sample solution

and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of clobetasol propionate to that of the internal standard.

Amount (mg) of clobetasol propionate ($C_{25}H_{32}ClFO_5$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of Clobetasol Propionate RS taken

Internal standard solution—A solution of beclometasone dipropionate in the mobile phase (1 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust the pH to 2.5 with phosphoric acid, and then add water to make 1000 mL. To 425 mL of this solution add 475 mL of acetonitrile and 100 mL of methanol.

Flow rate: Adjust so that the retention time of clobetasol propionate is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above conditions, clobetasol propionate and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

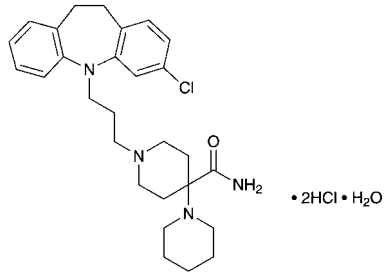
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above conditions, the relative standard deviation of the ratio of the peak area of clobetasol propionate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Clocapramine Hydrochloride Hydrate

クロカプラミン塩酸塩水和物



$C_{28}H_{37}ClN_4O.2HCl.H_2O$: 572.01

1′-[3-(3-Chloro-10,11-dihydro-5*H*-dibenz[*b*,*f*]azepin-5-yl)propyl]-1,4′-bipiperidine-4′-carboxamide dihydrochloride monohydrate

[*60789-62-0*]

Clocapramine Hydrochloride Hydrate, when dried, contains not less than 98.0% of clocapramine hydrochloride ($C_{28}H_{37}ClN_4O.2HCl$: 553.99).

Description Clocapramine Hydrochloride Hydrate occurs as white, crystals or crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in acetic acid (100), sparingly soluble in water and in methanol, slightly soluble in ethanol (95), in chloroform and in isopropylamine, and practically insoluble in acetic anhydride and in diethyl ether.

It is gradually colored by light.

Melting point: about 260°C (with decomposition, after drying).

Identification **(1)** To 5 mL of a solution of Clocapramine Hydrochloride Hydrate (1 in 2500) add 1 mL of nitric acid: a blue color develops at first, and rapidly changes to deep blue, and then changes to green to yellow-green.

(2) Determine the absorption spectrum of a solution of Clocapramine Hydrochloride Hydrate in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>,, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Clocapramine Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Dissolve 0.1 g of Clocapramine Hydrochloride Hydrate in 10 mL of water by warming, and after cooling, add 2 mL of ammonia TS, and filter. Acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests <1.09> (2) for chloride.

Purity **(1)** Sulfate <1.14>—Dissolve 0.5 g of Clocapramine Hydrochloride Hydrate in 40 mL of water by warming, after cooling, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Clocapramine Hydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.10 g of Clocapramine Hydrochloride Hydrate in 10 mL of a mixture of chloroform and isopropylamine (99:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and isopropylamine (99:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of diethyl ether, ethyl acetate, methanol and ammonia solution (28) (100:70:40:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> 2.0 – 3.5% (0.5 g, in vacuum at a pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

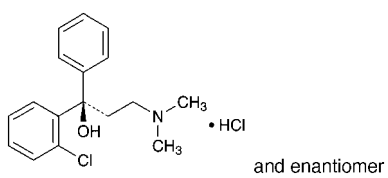
Assay Weigh accurately about 0.5 g of Clocapramine Hydrochloride Hydrate, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (6:1), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 27.70 mg of $C_{28}H_{37}ClN_4O.2HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Clofedanol Hydrochloride

クロフェダノール塩酸塩



and enantiomer

$C_{17}H_{20}ClNO.HCl$: 326.26
(1*RS*)-1-(2-Chlorophenyl)-3-dimethylamino-1-phenylpropan-1-ol monohydrochloride
[*511-13-7*]

Clofedanol Hydrochloride, when dried, contains not less than 98.5% of clofedanol hydrochloride ($C_{17}H_{20}ClNO.HCl$).

Description Clofedanol Hydrochloride occurs as white, crystals or crystalline powder.

It is freely soluble in methanol, in ethanol (95) and in acetic acid (100), sparingly soluble in water, and practically insoluble in diethyl ether.

A solution of Clofedanol Hydrochloride in methanol (1 in 20) does not show optical rotation.

Melting point: about 190°C (after drying, with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Clofedanol Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Clofedanol Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Clofedanol Hydrochloride (1 in 100) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Clofedanol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.05 g of Clofedanol Hydrochloride in 25 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 3 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than clofedanol from the sample solution is not larger than the peak area of clofedanol from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.34 g of potassium methanesulfonate in diluted phosphoric acid (1 in 1000) to make 1000 mL, and to 650 mL of this solution add 350 mL of methanol.

Flow rate: Adjust so that the retention time of clofedanol is about 9 minutes.

Selection of column: Dissolve 0.01 g each of Clofedanol Hydrochloride and ethyl parahydroxybenzoate in methanol to make 100 mL. Proceed with 3 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of clofedanol and ethyl parahydroxybenzoate in this order with the resolution of these peaks being not less than 4.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of clofedanol obtained from 3 μL of the standard solution composes between 20% and 50% of the full scale.

Time span of measurement: About three times as long as the retention time of clofedanol, beginning after the solvent peak.

Loss on drying ⟨*2.41*⟩ Not more than 2.0% (1 g, in vacuum, silica gel, 80°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

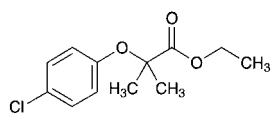
Assay Weigh accurately about 0.5 g of Clofedanol Hydrochloride, previously dried, dissolve in 15 mL of acetic acid (100), add 35 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.63 mg of $C_{17}H_{20}ClNO.HCl$

Containers and storage Containers—Tight containers.

Clofibrate

クロフィブラート



$C_{12}H_{15}ClO_3$: 242.70
Ethyl 2-(4-chlorophenoxy)-2-methylpropanoate
[*637-07-0*]

Clofibrate contains not less than 98.0% of clofibrate ($C_{12}H_{15}ClO_3$), calculated on the unhydrous basis.

Description Clofibrate occurs as a colorless or light yellow, clear, oily liquid. It has a characteristic odor and taste, which is bitter at first, and subsequently sweet.

It is miscible with methanol, with ethanol (95), with ethanol (99.5), with diethyl ether and with hexane, and practically insoluble in water.

It is gradually decomposed by light.

Identification **(1)** Determine the absorption spectrum of a solution of Clofibrate in ethanol (99.5) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1 or the spectrum of a solution of Clofibrate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Clofibrate in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2 or the spectrum of a solution of Clofibrate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Clofibrate as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Clofibrate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Refractive index <2.45> n_D^{20}: 1.500 – 1.505

Specific gravity <2.56> d_{20}^{20}: 1.137 – 1.144

Purity **(1)** Acidity—Dissolve 2.0 g of Clofibrate in 100 mL of neutralized ethanol, and add 1 drop of phenolphthalein TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: the solution is red in color.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Clofibrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—To 5.0 g of Clofibrate add 20 mL of nitric acid and 5 mL of sulfuric acid, and heat until white fumes are evolved. After cooling, if necessary, add further 5 mL of nitric acid, heat until white fumes are evolved, and repeat this procedure until the solution is colorless to light yellow. After cooling, add 15 mL of saturated ammonium oxalate solution, and heat again until white fumes are evolved. Cool, add water to make 25 mL, use 5 mL of this solution as the test solution, and perform the test.

Color standard: Prepare a solution according to the above procedure without using Clofibrate as the blank. Transfer 5 mL of the solution to a generator bottle, add 2.0 mL of Standard Arsenic Solution, and then proceed as directed in the test solution (not more than 20 ppm).

(4) *p*-Chlorophenol—To 1.0 g of Clofibrate add exactly 1 mL of the internal standard solution, then add the mobile phase to make 5 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of 4-chlorophenol in a mixture of hexane and 2-propanol (9:1) to make exactly 100 mL. Pipet 10 mL of this solution, and add a mixture of hexane and 2-propanol (9:1) to make exactly 50 mL. Pipet 6 mL of this solution, add exactly 4 mL of the internal standard solution, then add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of 4-chlorophenol to that of the internal standard: Q_T is not greater than Q_S.

Internal standard solution—A solution of 4-ethoxyphenol in the mobile phase (1 in 30,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 30 cm in length, packed with cyanopropylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of hexane, 2-propanol and acetic acid (100) (1970:30:1).

Flow rate: Adjust so that the retention time of clofibrate is about 2 minutes.

Selection of column: Dissolve 10.0 g of Clofibrate, 6 mg of 4-chlorophenol and 6 mg of 4-ethoxyphenol in 1000 mL of hexane. Proceed with 20 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of clofibrate, 4-chlorophenol and 4-ethoxyphenol in this order, with the resolution between the peaks of clofibrate and 4-chlorophenol is not less than 5, and with the resolution between the peaks of 4-chlorophenol and 4-ethoxyphenol is not less than 2.0.

Water <2.48> Not more than 0.2% (5 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Clofibrate, add exactly 50 mL of 0.1 mol/L potassium hydroxide-ethanol VS, and heat in a water bath under a reflux condenser with a carbon dioxide absorbing tube (soda-lime) for 2 hours with frequent shaking. Cool, and titrate <2.50> immediately the excess potassium hydroxide with 0.1 mol/L hydrochloric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 24.27 mg of $C_{12}H_{15}ClO_3$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Clofibrate Capsules

クロフィブラートカプセル

Clofibrate Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of clofibrate ($C_{12}H_{15}ClO_3$: 242.70).

Method of preparation Prepare as directed under Capsules, with Clofibrate.

Identification Cut and open Clofibrate Capsules, and use the contents as the sample. Determine the absorption spectrum of a solution of the sample in ethanol (99.5) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 278 nm and 282 nm, and it exhibits a maximum between 224 nm and 228 nm after diluting this solution 10 times with ethanol (99.5).

Purity *p*-Chlorophenol—Cut and open not less than 20 Clofibrate Capsules, and proceed with 1.0 g of the well-mixed contents as directed in the Purity (4) under Clofibrate.

Internal standard solution—A solution of 4-ethoxyphenol in

the mobile phase (1 in 30,000).

Assay Weigh accurately not less than 20 Clofibrate Capsules, cut and open the capsules, rinse the inside of the capsules with a small amount of diethyl ether after taking out the contents, evaporate the diethyl ether by allowing the capsules to stand at room temperature, and weigh the capsules accurately. Weigh accurately an amount of the contents, equivalent to about 0.1 g of clofibrate ($C_{12}H_{15}ClO_3$), dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Clofibrate RS, proceed in the same manner as directed for the sample solution, and use the solution so obtained as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of clofibrate to that of the internal standard.

$$\text{Amount (mg) of clofibrate } (C_{12}H_{15}ClO_3) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Clofibrate RS taken

Internal standard solution—A solution of ibuprofen in the mobile phase (1 in 100).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetonitrile and diluted phosphoric acid (1 in 1000) (3:2).

Flow rate: Adjust so that the retention time of clofibrate is about 10 minutes.

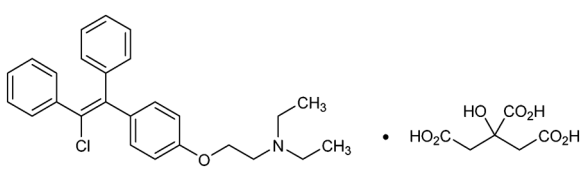
Selection of column: Dissolve 0.05 g of clofibrate and 0.3 g of ibuprofen in 50 mL of acetonitrile. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of ibuprofen and clofibrate in this order with the resolution between these peaks being not less than 6.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Clomifene Citrate

クロミフェンクエン酸塩



$C_{26}H_{28}ClNO.C_6H_8O_7$: 598.08

2-[4-(2-Chloro-1,2-diphenylvinyl)phenoxy]-*N*,*N*-diethylethylamine monocitrate

[*50-41-9*]

Clomifene Citrate, when dried, contains not less than 98.0% of clomifene citrate ($C_{26}H_{28}ClNO.C_6H_8O_7$).

Description Clomifene Citrate occurs as a white to pale yellowish white powder. It is odorless.

It is freely soluble in methanol and in acetic acid (100), sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

It gradually changes in color by light.

Melting point: about 115°C

Identification **(1)** To 2 mL of a solution of Clomifene Citrate in methanol (1 in 200) add 2 mL of Reinecke salt TS: a light red precipitate is produced.

(2) Determine the absorption spectrum of a solution of Clomifene Citrate in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Clomifene Citrate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) A solution of Clomifene Citrate in methanol (1 in 200) responds to the Qualitative Tests <*1.09*> (1) and (2) for citrate salt.

Purity **(1)** Clarity and color of solution—A solution of 1.0 g of Clomifene Citrate in 30 mL of methanol is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Clomifene Citrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Loss on drying <*2.41*> Not more than 1.0% (1 g, in vacuum, phosphorus (V) oxide, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Isomer ratio To 10 mg of Clomifene Citrate add 10 mL of water and 1 mL of sodium hydroxide TS, and shake to uniformly disperse. Add 10 mL of ethyl acetate, shake vigorously for 5 minutes, allow to stand for 5 minutes, and use the upper layer as the sample solution. Perform the test with 1 μL of the sample solution as directed under Gas Chromatography <*2.02*> according to the following conditions. Determine the areas of two adjacent peaks, A_a and A_b, having the retention time of about 8 minutes, where A_a is the peak area of shorter retention time and A_b is the peak area of longer retention time: $A_b/(A_a + A_b)$ is between 0.3 and 0.5.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.25 mm in inside diameter and 15 m in length, coated the inside surface with a layer about 0.1 μm thick of dimethylpolysiloxane for gas chromatography.

Column temperature: A constant temperature of about 230°C.

Injection port temperature: A constant temperature of about 270°C.

Detector temperature: A constant temperature of about 300°C.

Carrier gas: Helium.

Flow rate: Adjust so that the retention time of the first peak of clomifene citrate is about 7.5 minutes.

Split ratio: 1:50.

System suitability—

System performance: When the procedure is run with 1 μL of the sample solution under the above operating conditions, the resolution between the two adjacent peaks having the

retention time of about 8 minutes is not less than 5.

System repeatability: When the test is repeated 6 times with 1 μL of the sample solution under the above operating conditions, the relative standard deviation of the result of $A_b/(A_a + A_b)$ is not more than 1.0%.

Assay Weigh accurately about 1 g of Clomifene Citrate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 59.81 mg of $C_{26}H_{28}ClNO.C_6H_8O_7$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Clomifene Citrate Tablets

クロミフェンクエン酸塩錠

Clomifene Citrate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of the clomifene citrate ($C_{26}H_{28}ClNO.C_6H_8O_7$: 598.08).

Method of preparation Prepare as directed under Tablets, with Clomifene Citrate.

Identification Weigh a portion of powdered Clomifene Citrate Tablets, equivalent to 50 mg of Clomifene Citrate, shake vigorously with 50 mL of methanol for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of Clomifene Citrate RS in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, toluene and diethylamine (10:10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution and standard solution show the same *R*f value.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Clomifene Citrate Tablets add 10 mL of water, and shake until the tablets are disintegrated. To this solution add 50 mL of methanol, shake for 10 minutes, and add methanol to make exactly 100 mL. Centrifuge this solution, pipet 4 mL of the supernatant liquid, add methanol to make exactly *V* mL so that each mL contains about 20 μg of clomifene citrate ($C_{26}H_{28}ClNO.C_6H_8O_7$), and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of clomifene citrate ($C_{26}H_{28}ClNO.C_6H_8O_7$)
$= M_S \times A_T/A_S \times V/100$

M_S: Amount (mg) of Clomifene Citrate RS taken

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Clomifene Citrate Tablets is not less than 80%.

Start the test with 1 tablet of Clomifene Citrate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet *V* mL of the subsequent filtrate, add the dissolution medium to make exactly *V′* mL so that each mL contains about 28 μg of clomifene citrate ($C_{26}H_{28}ClNO.C_6H_8O_7$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Clomifene Citrate RS, previously dried in vacuum using phosphorus (V) oxide as a desiccant for 3 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 291 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using the dissolution medium as the blank.

Dissolution rate (%) with respect to the labeled amount of clomifene citrate ($C_{26}H_{28}ClNO.C_6H_8O_7$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount (mg) of Clomifene Citrate RS taken
C: Labeled amount (mg) of clomifene citrate ($C_{26}H_{28}ClNO.C_6H_8O_7$) in 1 tablet

Assay Weigh accurately, and powder not less than 20 Clomifene Citrate Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of clomifene citrate ($C_{26}H_{28}ClNO.C_6H_8O_7$), add 50 mL of methanol, shake for 10 minutes, and add methanol to make exactly 100 mL. Centrifuge a portion of this solution, pipet 4 mL of the supernatant liquid, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Clomifene Citrate RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 3 hours, and dissolve in methanol to make exactly 100 mL. Pipet 4 mL of this solution, and dilute with methanol to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and the standard solution, respectively, at 295 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

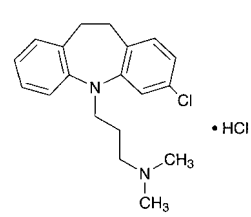
Amount (mg) of clomifene citrate ($C_{26}H_{28}ClNO.C_6H_8O_7$)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of Clomifene Citrate RS taken

Containers and storage Containers—Tight containers.

Clomipramine Hydrochloride

クロミプラミン塩酸塩



$C_{19}H_{23}ClN_2.HCl$: 351.31
3-(3-Chloro-10,11-dihydro-5*H*-dibenz[*b*,*f*]azepin-5-yl)-*N*,*N*-dimethylpropylamine monohydrochloride
[*17321-77-6*]

Clomipramine Hydrochloride, when dried, contains not less than 98.5% of clomipramine hydrochloride ($C_{19}H_{23}ClN_2.HCl$).

Description Clomipramine Hydrochloride occurs as a white to pale yellow, crystalline powder. It is odorless.

It is very soluble in acetic acid (100), freely soluble in water, in methanol and in chloroform, soluble in ethanol (95), sparingly soluble in acetic anhydride, slightly soluble in acetone, and practically insoluble in ethyl acetate and in diethyl ether.

Identification **(1)** Dissolve 3 mg of Clomipramine Hydrochloride in 1 mL of nitric acid: a deep blue color develops.

(2) Determine the absorption spectrum of a solution of Clomipramine Hydrochloride in 0.1 mol/L hydrochloric acid TS (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Take 1 g of Clomipramine Hydrochloride in a separator, dissolve in 10 mL of water, add 5 mL of sodium hydroxide TS, and extract with two 30-mL portions of diethyl ether [the water layer is used for Identification (4)]. Combine the diethyl ether extracts, add 20 mL of water, and shake. Take diethyl ether layer, dry with a small portion of anhydrous sodium sulfate, and filter. Evaporate the combined extracts by warming on a water bath, and proceed the test with the residue as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

(4) The solution neutralized by adding dilute nitric acid to the water layer obtained in (3) responds to the Qualitative Tests <*1.09*> for chloride.

pH <*2.54*> Dissolve 1.0 g of Clomipramine Hydrochloride in 10 mL of water: the pH of this solution is between 3.5 and 5.0.

Melting point <*2.60*> 192 – 196°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Clomipramine Hydrochloride in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Clomipramine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Clomipramine Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Clomipramine Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Separately, weigh 20 mg of Imipramine Hydrochloride, dissolve in methanol to make exactly 100 mL, and use this solution as the standard solution (1). Then pipet 1 mL of the sample solution, and add methanol to make exactly 50 mL. Pipet 5 mL of the solution, add methanol to make exactly 50 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone and ammonia solution (28) (15:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly potassium dichromate-sulfuric acid TS on the plate: the spot from the sample solution, corresponding to that from the standard solution (1), is not more intense than the spot from the standard solution (1). Each of the spots other than the principal spot and the spot mentioned above from the sample solution is not more intense than the spot from the standard solution (2).

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Clomipramine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

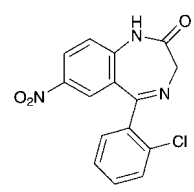
Each mL of 0.1 mol/L perchloric acid VS
= 35.13 mg of $C_{19}H_{23}ClN_2.HCl$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Clonazepam

クロナゼパム



$C_{15}H_{10}ClN_3O_3$: 315.71
5-(2-Chlorophenyl)-7-nitro-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one
[*1622-61-3*]

Clonazepam, when dried, contains not less than 99.0% of clonazepam ($C_{15}H_{10}ClN_3O_3$).

Description Clonazepam occurs as white to light yellow, crystals or crystalline powder.

It is sparingly soluble in acetic anhydride and in acetone, slightly soluble in methanol and in ethanol (95), very slightly soluble in diethyl ether, and practically insoluble in water.

It is gradually colored by light.

Melting point: about 240°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Clonazepam in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Clonazepam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Clonazepam as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Purity **(1)** Chloride <*1.03*>—To 1.0 g of Clonazepam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. Discard the first 20 mL portion of the filtrate, take the subsequent 20 mL portion of the filtrate, and add 6 mL of dilute nitric acid and water to make 50 mL. Use this solution as the test solution, and perform the test. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.022%).

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Clonazepam according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.25 g of Clonazepam in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 100 mL, then pipet 1 mL of this solution, add acetone to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of nitromethane and acetone (10:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.30% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Clonazepam, previously dried, dissolve in 70 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 31.57 mg of $C_{15}H_{10}ClN_3O_3$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Clonazepam Fine Granules

クロナゼパム細粒

Clonazepam Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of clonazepam ($C_{15}H_{10}ClN_3O_3$: 315.71).

Method of preparation Prepare as directed under Granules, with Clonazepam.

Identification Powder Clonazepam Fine Granules. To a portion of the powder, equivalent to 1 mg of Clonazepam, add an appropriate volume of methanol and shake for 10 minutes, add methanol to make 100 mL, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 307 nm and 311 nm.

Dissolution Being specified separately when the drug is granted approval based on the Law.

Assay Powder Clonazepam Fine Granules. Weigh accurately a portion of the powder, equivalent to about 2.4 mg of clonazepam ($C_{15}H_{10}ClN_3O_3$), add exactly 30 mL of a mixture of methanol and water (7:3), and shake for 15 minutes. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add a mixture of methanol and water (7:3) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of clonazepam for assay, previously dried at 105°C for 4 hours, dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of methanol and water (7:3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 15 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of clonazepam in each solution.

Amount (mg) of clonazepam ($C_{15}H_{10}ClN_3O_3$)
$= M_S \times A_T/A_S \times 3/25$

M_S: Amount (mg) of clonazepam for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 310 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, acetonitrile and methanol (4:3:3).

Flow rate: Adjust so that the retention time of clonazepam is about 5 minutes.

System suitability—

System performance: When the procedure is run with 15 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clonazepam are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 15 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clonazepam is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Clonazepam Tablets

クロナゼパム錠

Clonazepam Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of clonazepam ($C_{15}H_{10}ClN_3O_3$: 315.71).

Method of preparation Prepare as directed under Tablets, with Clonazepam.

Identification Powder Clonazepam Tablets. To a portion of the powder, equivalent to 1 mg of Clonazepam, add an appropriate volume of methanol and shake for 10 minutes, then add methanol to make 100 mL, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 307 nm and 311 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Clonazepam Tablets, add $V/10$ mL of methanol, shake for 15 minutes, add 2-propanol to make exactly V mL so that each mL contains about 10 μg of clonazepam ($C_{15}H_{10}ClN_3O_3$). Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of clonazepam for assay, previously dried at

105°C for 4 hours, dissolve in methanol to make exactly 200 mL. Pipet 10 mL of this solution, add 2-propanol to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 312 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a mixture of 2-propanol and methanol (9:1) as the control.

Amount (mg) of clonazepam ($C_{15}H_{10}ClN_3O_3$)
$= M_S \times A_T/A_S \times V/2000$

M_S: Amount (mg) of clonazepam for assay taken

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of 0.5-mg tablet and 1-mg tablet is not less than 80%, and that of 2-mg tablet is not less than 75%.

Start the test with 1 tablet of Clonazepam Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 0.56 μg of clonazepam ($C_{15}H_{10}ClN_3O_3$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of clonazepam for assay, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, and add methanol to make exactly 50 mL. Pipet 5 mL of this solution and add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of clonazepam in each solution.

Dissolution rate (%) with respect to the labeled amount of clonazepam ($C_{15}H_{10}ClN_3O_3$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/4$

M_S: Amount (mg) of clonazepam for assay taken
C: Labeled amount (mg) of clonazepam ($C_{15}H_{10}ClN_3O_3$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clonazepam are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clonazepam is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Clonazepam Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 2.5 mg of clonazepam ($C_{15}H_{10}ClN_3O_3$), add exactly 50 mL of a mixture of methanol and water (7:3), and shake for 15 minutes. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of clonazepam for assay, previously dried at 105°C for 4 hours, dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add a mixture of methanol and water (7:3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of clonazepam in each solution.

Amount (mg) of clonazepam ($C_{15}H_{10}ClN_3O_3$)
$= M_S \times A_T/A_S \times 1/10$

M_S: Amount (mg) of clonazepam for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 310 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, acetonitrile and methanol (4:3:3).

Flow rate: Adjust so that the retention time of clonazepam is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clonazepam are not less than 3000 and not more than 1.5, respectively.

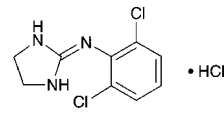
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clonazepam is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Clonidine Hydrochloride

クロニジン塩酸塩



$C_9H_9Cl_2N_3.HCl$: 266.55
2-(2,6-Dichlorophenylimino)imidazolidine monohydrochloride
[*4205-91-8*]

Clonidine Hydrochloride, when dried, contains not less than 99.0% of clonidine hydrochloride ($C_9H_9Cl_2N_3.HCl$).

Description Clonidine Hydrochloride occurs as white, crystals or crystalline powder.

It is freely soluble in methanol, soluble in water and in ethanol (95), slightly soluble in acetic acid (100), and practically insoluble in acetic anhydride and in diethyl ether.

Identification **(1)** To 5 mL of a solution of Clonidine Hydrochloride (1 in 1000) add 6 drops of Dragendorff's TS: an orange precipitate is formed.

(2) Determine the absorption spectrum of a solution of Clonidine Hydrochloride in 0.01 mol/L hydrochloric acid TS (3 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of

absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Clonidine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Clonidine Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 1.0 g of Clonidine Hydrochloride in 20 mL of water: the pH of this solution is between 4.0 and 5.5.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Clonidine Hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Clonidine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 0.5 g of Clonidine Hydrochloride according to Method 3, and perform the test (not more than 4 ppm).

(4) Related substances—Dissolve 0.20 g of Clonidine Hydrochloride in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 1 mL and 2 mL of this solution, to each add methanol to make exactly 20 mL, and use these solutions as the standard solution (1) and the standard solution (2), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of toluene, 1,4-dioxane, ethanol (99.5) and ammonia solution (28) (10:8:2:1) to a distance of about 12 cm, air-dry the plate, and then dry at 100°C for 1 hour. Spray evenly sodium hypochlorite TS on the plate, air-dry the plate for 15 minutes, and then spray evenly potassium iodide starch TS on the plate: the spots other than the principal spot and the spot of the starting point from the sample solution are not more intense than the spot from the standard solution (2), and the numbers of spots other than the principal spot and the spot of the starting point, which are more intense than the spot from the standard solution (1), are not more than 3.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

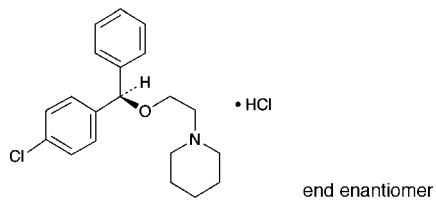
Assay Weigh accurately about 0.4 g of Clonidine Hydrochloride, previously dried, and dissolve in 30 mL of acetic acid (100) by warming. After cooling, add 70 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 26.66 mg of $C_9H_9Cl_2N_3$.HCl

Containers and storage Containers—Tight containers.

Cloperastine Hydrochloride

クロペラスチン塩酸塩



end enantiomer

$C_{20}H_{24}ClNO.HCl$: 366.32
1-{2-[(*RS*)-(4-Chlorophenyl)(phenyl)methoxy]ethyl}piperidine monohydrochloride
[*14984-68-0*]

Cloperastine Hydrochloride, when dried, contains not less than 98.5% of cloperastine hydrochloride ($C_{20}H_{24}ClNO.HCl$).

Description Cloperastine Hydrochloride occurs as white, crystals or crystalline powder.

It is very soluble in water, in methanol, in ethanol (95) and in acetic acid (100), and soluble in acetic anhydride.

A solution of Cloperastine Hydrochloride (1 in 10) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Cloperastine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Cloperastine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 62,500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cloperastine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Shake 10 mL of a solution of Cloperastine Hydrochloride (1 in 100) with 2 mL of ammonia TS and 20 mL of diethyl ether, separate the water layer, wash the water layer with 20 mL of diethyl ether, and filter. Acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests <1.09> for chloride.

Melting point <2.60> 148 – 152°C

Purity **(1)** Heavy metals <1.07>—Proceed with 1.0 g of Cloperastine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 40 mg of Cloperastine Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of both solutions by the automatic integration

method: The areas of two peaks corresponding to the relative retention times about 0.8 and about 3.0 to cloperastine obtained from the sample solution are not larger than the peak area of cloperastine obtained from the standard solution, respectively, and the area of the peak corresponding to the relative retention time about 2.0 is not larger than 5/3 times the peak area of cloperastine from the standard solution, and the areas of the peaks other than cloperastine and the peaks mentioned above from the sample solution are not larger than 3/5 times the peak area of cloperastine from the standard solution. The total area of these peaks is not larger than 2 times the peak area of cloperastine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 222 nm).

Column: A stainless steel column about 5 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol, 0.1 mol/L monobasic potassium phosphate TS and perchloric acid (500:250:1).

Flow rate: Adjust so that the retention time of cloperastine is about 7 minutes.

Selection of column: Dissolve 0.03 g of Cloperastine Hydrochloride and 0.04 g of benzophenone in 100 mL of the mobile phase. To 2.0 mL of this solution add the mobile phase to make 50 mL. Perform the test with 20 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of cloperastine and benzophenone in this order with the resolution between these peaks being not less than 6.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of cloperastine obtained from 20 μL of the standard solution is about 30% of the full scale.

Time span of measurement: About 4 times as long as the retention time of cloperastine, beginning after the solvent peak.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Cloperastine Hydrochloride, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

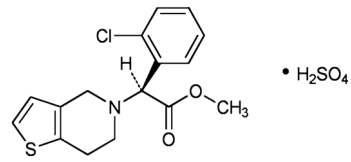
Each mL of 0.1 mol/L perchloric acid VS
= 36.63 mg of $C_{20}H_{24}ClNO.HCl$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Clopidogrel Sulfate

クロピドグレル硫酸塩



$C_{16}H_{16}ClNO_2S.H_2SO_4$: 419.90

Methyl (2*S*)-2-(2-chlorophenyl)-2-[6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl]acetate monosulfate

[*120202-66-6*]

Clopidogrel Sulfate contains not less than 97.0% and not more than 101.5% of clopidogrel sulfate ($C_{16}H_{16}ClNO_2S.H_2SO_4$), calculated on the anhydrous basis.

Description Clopidogrel Sulfate occurs as a white to pale yellowish white, crystalline powder or powder.

It is freely soluble in water and in methanol, and soluble in ethanol (99.5).

It gradually develops a brown color on exposure to light.

Melting point: about 177°C (with decomposition).

It shows crystal polymorphism.

Identification **(1)** Determine the absorption spectrum of a solution of Clopidogrel Sulfate in methanol (3 in 10,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Clopidogrel Sulfate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Clopidogrel Sulfate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Clopidogrel Sulfate RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Clopidogrel Sulfate, or each of Clopidogrel Sulfate and Clopidogrel Sulfate RS in ethanol (99.5), respectively. Then evaporate the ethanol to dryness, and repeat the test on the residues dried in vacuum.

(3) Perform the test with Clopidogrel Sulfate as directed under Flame Coloration Test ⟨*1.04*⟩ (2): a green color appears.

(4) A solution of Clopidogrel Sulfate in a mixture of water and methanol (1:1) (1 in 100) responds to the Qualitative Tests ⟨*1.09*⟩ (1) for sulfate.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Clopidogrel Sulfate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 65 mg of Clopidogrel Sulfate in 10 mL of a mixture of acetonitrile for liquid chromatography and mobile phase A (3:2), and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add a mixture of acetonitrile for liquid chromatography and the mobile phase A (3:2) to make exactly 100 mL. Pipet 2.5 mL of this solution, add a mixture of acetonitrile for liquid chromatography and the mobile phase A (3:2) to make exactly 50 mL, and use this solution as the standard

solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.5 and about 1.1 to clopidogrel, obtained from the sample solution is not larger than 2 times the peak area of clopidogrel obtained from the standard solution, the area of the peak other than clopidogrel and the peaks mentioned above from the sample solution is not larger than the peak area of clopidogrel from the standard solution, and the total area of the peaks other than clopidogrel from the sample solution is not larger than 5 times the peak area of clopidogrel from the standard solution.

Operating conditions—

Detector, column and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase A: Dissolve 0.87 g of sodium 1-pentanesufonate in 1000 mL of water, and adjust to pH 2.5 with phosphoric acid. To 950 mL of this solution add 50 mL of methanol.

Mobile phase B: A mixture of acetonitrile for liquid chromatography and methanol (19:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 3	89.5	10.5
3 – 48	89.5 → 31.5	10.5 → 68.5
48 – 68	31.5	68.5

Flow rate: 1.0 mL per minute.

Time span of measurement: For 68 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add a mixture of acetonitrile for liquid chromatography and the mobile phase A (3:2) to make exactly 20 mL. Confirm that the peak area of clopidogrel obtained with 10 μL of this solution is equivalent to 7 to 13% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clopidogrel are not less than 60,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clopidogrel is not more than 2.0%.

(3) Optical isomer—Dissolve 0.10 g of Clopidogrel Sulfate in 25 mL of ethanol (99.5) for liquid chromatography, add heptane for liquid chromatography to make 50 mL, and use this solution as the sample solution. Pipet 2.5 mL of the sample solution, and add a mixture of ethanol (99.5) for liquid chromatography and heptane for liquid chromatography (1:1) to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of ethanol (99.5) for liquid chromatography and heptane for liquid chromatography (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of the optical isomer, having the relative retention time of about 0.6 to clopidogrel, obtained from the sample solution is not larger than the peak area of clopidogrel obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with cellulose derivative-bonded silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of heptane for liquid chromatography and ethanol (99.5) for liquid chromatography (17:3).

Flow rate: Adjust so that the retention time of clopidogrel is about 18 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clopidogrel are not less than 3500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clopidogrel are not more than 2.0%.

Water <2.48> Not more than 0.5% (1 g, coulometric titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 45 mg each of Clopidogrel Sulfate and Clopidogrel Sulfate RS (separately, determine the water <2.48> in the same manner as Clopidogrel Sulfate), and dissolve them separately in the mobile phase to make exactly 50 mL. Take exactly 7 mL of each solution, add separately the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of clopidogrel in each solution.

Amount (mg) of clopidogrel sulfate ($C_{16}H_{16}ClNO_2S.H_2SO_4$) $= M_S \times A_T/A_S$

M_S: Amount (mg) of Clopidogrel Sulfate RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 0.87 g of sodium 1-pentanesufonate in 1000 mL of water, and adjust to pH 2.5 with phosphoric acid. To 950 mL of this solution add 50 mL of methanol. To 600 mL of this solution, add 400 mL of a mixture of acetonitrile for liquid chromatography and methanol (19:1).

Flow rate: Adjust so that the retention time of clopidogrel is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clopidogrel are not less than 4500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clopidogrel is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Clopidogrel Sulfate Tablets

クロピドグレル硫酸塩錠

Clopidogrel Sulfate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of clopidogrel ($C_{16}H_{16}ClNO_2S$: 321.82).

Method of preparation Prepare as directed under Tablets, with Clopidogrel Sulfate.

Identification To a quantity of powdered Clopidogrel Sulfate Tablets, equivalent to 75 mg of clopidogrel ($C_{16}H_{16}ClNO_2S$), add 50 mL of methanol, and after treating with ultrasonic waves with occasional shaking, add methanol to make 100 mL. To 10 mL of this solution add methanol to make 30 mL, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 269 nm and 273 nm, and between 276 nm and 280 nm.

Purity Related substances—Keep the sample solution and the standard solution at 5°C or below and use within 24 hours. Take a quantity of Clopidogrel Sulfate Tablets equivalent to 0.15 g of clopidogrel ($C_{16}H_{16}ClNO_2S$), add 120 mL of the mobile phase, treat with ultrasonic waves with occasional shaking until the tablets are disintegrated, and add the mobile phase to make 200 mL. Centrifuge this solution, to 10 mL of the supernatant liquid add the mobile phase to make 30 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention times of about 0.3, about 0.5 and about 0.9 to clopidogrel, obtained from the sample solution is not larger than 3/10 times the peak area of clopidogrel obtained from the standard solution. The area of the peak having the relative retention time of about 2.0 from the sample solution is not larger than 1.2 times the peak area of clopidogrel from the standard solution. The area of the peak other than clopidogrel and the peaks mentioned above from the sample solution is not larger than 1/10 times the peak area of clopidogrel from the standard solution. The total area of the peaks other than clopidogrel from the sample solution is not larger than 1.7 times the peak area of clopidogrel from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column of 4.6 mm in inside diameter and 15 cm in length, packed with ovomucoid-chemically bonded amino silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in 1000 mL of water, and to 750 mL of this solution add 250 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust so that the retention time of clopidogrel is about 6 minutes.

Time span of measurement: About 2.5 times as long as the retention time of clopidogrel, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add the mobile phase to make exactly 100 mL. Confirm that the peak area of clopidogrel obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clopidogrel are not less than 2500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clopidogrel is not more than 2.0%.

Uniformity of dosage units ⟨*6.02*⟩ Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Clopidogrel Sulfate Tablets add a suitable amount of the mobile phase, treat with ultrasonic waves with occasional shaking until the tablet is disintegrated, and add the mobile phase to make exactly 50 mL. Filter the solution through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add exactly $V/5$ mL of the internal standard solution, and add the mobile phase to make V mL so that each mL contains about 0.1 mg of clopidogrel ($C_{16}H_{16}ClNO_2S$). Use this solution as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of clopidogrel } (C_{16}H_{16}ClNO_2S) = M_S \times Q_T/Q_S \times V/10 \times 0.766$$

M_S: Amount (mg) of Clopidogrel Sulfate RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in the mobile phase (1 in 1500).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate of 25-mg tablet in 30 minutes is not less than 70%, and that of 75-mg tablet in 45 minutes is not less than 80%.

Start the test with 1 tablet of Clopidogrel Sulfate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 28 μg of clopidogrel ($C_{16}H_{16}ClNO_2S$), and use

this solution as the sample solution. Separately, weigh accurately about 30 mg of Clopidogrel Sulfate RS (separately determine the water <2.48> in the same manner as Clopidogrel Sulfate), dissolve in 5 mL of methanol, and add water to make exactly 100 mL. Pipet 6 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 240 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as a blank.

Dissolution rate (%) with respect to the labeled amount of clopidogrel ($C_{16}H_{16}ClNO_2S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 108 \times 0.766$

M_S: Amount (mg) of Clopidogrel Sulfate RS taken, calculated on the anhydrous basis
C: Labeled amount (mg) of clopidogrel ($C_{16}H_{16}ClNO_2S$) in 1 tablet

Assay To 20 tablets of Clopidogrel Sulfate Tablets add 400 mL of the mobile phase, treat with ultrasonic waves with occasional shaking until the tablets are disintegrated, add the mobile phase to make exactly 500 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add the mobile phase to make exactly V mL so that each mL contains about 0.5 mg of clopidogrel ($C_{16}H_{16}ClNO_2S$). Pipet 4 mL of this solution, add exactly 4 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 33 mg of Clopidogrel Sulfate RS (separately determine the water <2.48> in the same manner as Clopidogrel Sulfate), and dissolve in the mobile phase to make exactly 50 mL. Pipet 4 mL of this solution, add exactly 4 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of clopidogrel to that of the internal standard.

Amount (mg) of clopidogrel ($C_{16}H_{16}ClNO_2S$) in 1 tablet of Clopidogrel Sulfate Tablets
$= M_S \times Q_T/Q_S \times V/10 \times 0.766$

M_S: Amount (mg) of Clopidogrel Sulfate RS taken, calculated on the anhydrous basis

Internal standard solution—A solution of isopropyl parahydoxybenzoate in the mobile phase (1 in 1500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column of 3.9 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 0.87 g of sodium 1-pentanesulfonate in 1000 mL of water, and adjust to pH 2.5 with phosphoric acid. To 950 mL of this solution add 50 mL of methanol. To 600 mL of this solution add 400 mL of a mixture of acetonitrile for liquid chromatography and methanol (19:1).

Flow rate: Adjust so that the retention time of clopidogrel is about 8 minutes.

System suitability—

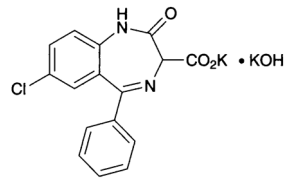
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and clopidogrel are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of clopidogrel to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Clorazepate Dipotassium

クロラゼプ酸二カリウム



$C_{16}H_{10}ClKN_2O_3.KOH$: 408.92
Monopotassium 7-chloro-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepine-3-carboxylate mono (potassium hydroxide)
[*57109-90-7*]

Clorazepate Dipotassium, when dried, contains not less than 98.5% and not more than 101.0% of clorazepate dipotassium ($C_{16}H_{10}ClKN_2O_3.KOH$).

Description Clorazepate Dipotassium occurs as white to light yellow, crystals or crystalline powder.

It is freely soluble in water, and very slightly soluble in ethanol (99.5).

It dissolves in acetic acid (100).

The pH of a solution obtained by dissolving 1 g of Clorazepate Dipotassium in 100 mL of water is between 11.5 and 12.5.

It gradually turns yellow on exposure to light.

Identification **(1)** Carefully and gradually ignite to redness 30 mg of Clorazepate Dipotassium with 50 mg of sodium. After cooling, add 3 drops of ethanol (99.5) and 5 mL of water, mix well, and filter: the filtrate responds to the Qualitative Tests <1.09> for chloride.

(2) Determine the absorption spectrum of a solution of Clorazepate Dipotassium (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Clorazepate Dipotassium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Clorazepate Dipotassium responds to the Qualitative Tests <1.09> (1) for potassium salt.

Purity **(1)** Chloride <1.03>—Dissolve 1.0 g of Clorazepate Dipotassium in 20 mL of water, add 20 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution as follows: To 0.40 mL of 0.01 mol/L hydrochloric acid VS add 20 mL of acetone, 6 mL of dilute nitric

acid and water to make 50 mL (not more than 0.014%).

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Clorazepate Dipotassium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Clorazepate Dipotassium according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 15 mg of Clorazepate Dipotassium in 25 mL of a mixture of water, potassium carbonate solution (97 in 1000) and acetonitrile (3:1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water, potassium carbonate solution (97 in 1000) and acetonitrile (3:1:1) to make exactly 200 mL, and use this solution as the standard solution. Prepare these solutions quickly and perform the test within 3 minutes. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of nordiazepam, having the relative retention time of about 3.0 to clorazepic acid, obtained from the sample solution is not larger than the peak area of clorazepic acid obtained from the standard solution, the area of the peak other than clorazepic acid and nordiazepam is not larger than 1/5 times the peak area of clorazepic acid from the standard solution, and the total area of the peaks other than clorazepic acid is not larger than 2 times the peak area of clorazepic acid from the standard solution. For the area of the peak of nordiazepam, multiply the relative response factor, 0.64.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 13.8 g of sodium dihydrogen phosphate dihydrate in 500 mL of water, and adjust to pH 8.0 with sodium hydroxide TS. To 100 mL of this solution add 400 mL of acetonitrile and 300 mL of water.

Flow rate: Adjust so that the retention time of clorazepic acid is about 1.3 minutes.

Time span of measurement: About 10 times as long as the retention time of clorazepic acid, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add the mixture of water, potassium carbonate solution (97 in 1000) and acetonitrile (3:1:1) to make exactly 25 mL. Confirm that the peak area of clorazepic acid obtained from 5 μL of this solution is equivalent to 15 to 25% of that obtained from 5 μL of the standard solution.

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clorazepic acid are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clorazepic acid is not more than 1.5%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 5 hours).

Assay Weigh accurately about 0.15 g of Clorazepate Dipotassium, previously dried, dissolve in 100 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of solution changes from violet to blue-green through blue (indicator: 3 drops of crystal violet TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 13.63 mg of $C_{16}H_{10}ClKN_2O_3.KOH$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Clorazepate Dipotassium Capsules

クロラゼプ酸二カリウムカプセル

Clorazepate Dipotassium Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of clorazepate dipotassium ($C_{16}H_{10}ClKN_2O_3.KOH$: 408.92).

Method of preparation Prepare as directed under Capsules, with Clorazepate Dipotassium.

Identification To 10 mL of the sample solution obtained in the Assay add water to make 20 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 228 nm and 232 nm.

Purity Related substances—Take out the contents of Clorazepate Dipotassium Capsules, and powder. To a portion of the powder, equivalent to 15 mg of Clorazepate Dipotassium, add a mixture of water, potassium carbonate solution (97 in 1000) and acetonitrile (3:1:1) to make 25 mL, and shake for 10 minutes. Filter the solution through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water, potassium carbonate solution (97 in 1000) and acetonitrile (3:1:1) to make exactly 200 mL, and use this solution as the standard solution. Then, proceed as directed in the Purity (4) under Clorazepate Dipotassium: the peak area of nordiazepam, having the relative retention time of about 3.0 to clorazepic acid, obtained from the sample solution is not larger than 3 times the peak area of clorazepic acid obtained from the standard solution, and the total area of the peaks other than clorazepic acid and nordiazepam is not larger than the peak area of clorazepic acid from the standard solution. For the peak area of nordiazepam, multiply the relative response factor, 0.64.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 capsule of Clorazepate Dipotassium Capsules add 70 mL of water, shake for 15 minutes, and add water to make exactly 100 mL. Centrifuge the solution, pipet V mL of the supernatant liquid, add water to make exactly V' mL so that each mL contains about 12 μg of clorazepate dipotassium ($C_{16}H_{10}ClKN_2O_3.KOH$), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of clorazepate dipotassium ($C_{16}H_{10}ClKN_2O_3.KOH$)
$= M_S \times A_T/A_S \times V'/V \times 2/25$

M_S: Amount (mg) of clorazepate dipotassium for assay taken

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Clorazepate Dipotassium Capsules is not less than 80%.

Start the test with 1 capsule of Clorazepate Dipotassium Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 8.3 μg of clorazepate dipotassium ($C_{16}H_{10}ClKN_2O_3$.KOH), and use this solution as the sample solution. Separately, weigh accurately about 21 mg of clorazepate dipotassium for assay, previously dried in vacuum over phosphorus (V) oxide at 60°C for 5 hours, and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 252 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of clorazepate dipotassium ($C_{16}H_{10}ClKN_2O_3$.KOH)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of clorazepate dipotassium for assay taken
C: Labeled amount (mg) of clorazepate dipotassium ($C_{16}H_{10}ClKN_2O_3$.KOH) in 1 capsule

Assay Carefully take out the contents of not less than 20 Clorazepate Dipotassium Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 15 mg of clorazepate dipotassium ($C_{16}H_{10}ClKN_2O_3$.KOH), add 70 mL of water, shake for 15 minutes, and add water to make exactly 100 mL. Centrifuge the solution, pipet 4 mL of the supernatant liquid, add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 15 mg of clorazepate dipotassium for assay, previously dried in vacuum over phosphorus (V) oxide at 60°C for 5 hours, and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and determine the absorbances, A_T and A_S, at 252 nm.

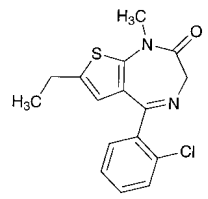
Amount (mg) of clorazepate dipotassium ($C_{16}H_{10}ClKN_2O_3$.KOH)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of clorazepate dipotassium for assay taken

Containers and storage Containers—Tight containers.

Clotiazepam

クロチアゼパム



$C_{16}H_{15}ClN_2OS$: 318.82
5-(2-Chlorophenyl)-7-ethyl-1-methyl-1,3-dihydro-2*H*-thieno[2,3-*e*][1,4]-diazepin-2-one
[*33671-46-4*]

Clotiazepam, when dried, contains not less than 98.5% of clotiazepam ($C_{16}H_{15}ClN_2OS$).

Description Clotiazepam occurs as white to light yellowish white, crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is very soluble in chloroform, freely soluble in methanol, in ethanol (95), in acetone, in acetic acid (100) and in ethyl acetate, soluble in diethyl ether, and practically insoluble in water.

It dissolves in 0.1 mol/L hydrochloric acid TS.

It is gradually colored by light.

Identification **(1)** Dissolve 0.01 g of Clotiazepam in 3 mL of sulfuric acid: the solution shows a light yellow fluorescence under ultraviolet light (main wavelength: 365 nm).

(2) Determine the absorption spectrum of a solution of Clotiazepam in 0.1 mol/L hydrochrolic acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Prepare the test solution with 0.01 g of Clotiazepam as directed under Oxygen Flask Combustion Method ⟨*1.06*⟩, using 10 mL of diluted hydrogen peroxide (30) (1 in 5) as the absorbing liquid. Apply a small amount of water to the upper part of the Apparatus A, pull out C carefully, wash C, B and the inner side of A with 15 mL of methanol, and use the obtained solution as the test solution. Add 0.5 mL of dilute nitric acid to 15 mL of the test solution: this solution responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride. The remaining test solution responds to the Qualitative Tests ⟨*1.09*⟩ (1) for sulfate.

Melting point ⟨*2.60*⟩ 106 – 109°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Clotiazepam in 10 mL of ethanol (95): the solution is clear and is not more colored than the following control solution.

Control solution: To 5 mL of Matching Fluid C add 0.01 mol/L hydrochloric acid TS to make 10 mL.

(2) Chloride ⟨*1.03*⟩—To 1.0 g of Clotiazepam add 50 mL of water, shake for 30 minutes, and filter. To 30 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.015%).

(3) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Clotiazepam according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g

of Clotiazepam, according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.25 g of Clotiazepam in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 20 mL, pipet 2 mL of this solution, add acetone to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and acetone (5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 80°C, 3 hours).

Residue on ignition <*2.44*> not more than 0.1% (1 g).

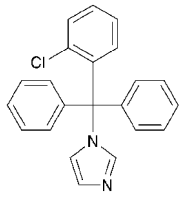
Assay Weigh accurately about 0.5 g of Clotiazepam, previously dried, dissolve in 80 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid (potentiometric titration). Perform a blank determination in, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 31.88 mg of $C_{16}H_{15}ClN_2OS$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Clotrimazole

クロトリマゾール



$C_{22}H_{17}ClN_2$: 344.84
1-[(2-Chlorophenyl)(diphenyl)methyl]-1*H*-imidazole
[*23593-75-1*]

Clotrimazole, when dried, contains not less than 98.0% of clotrimazole ($C_{22}H_{17}ClN_2$).

Description Clotrimazole occurs as a white, crystalline powder. It is odorless and tasteless.

It is freely soluble in dichloromethane and in acetic acid (100), soluble in *N*,*N*-dimethylformamide, in methanol and in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

Identification (1) To 0.1 g of Clotrimazole add 10 mL of 5 mol/L hydrochloric acid TS, dissolve by heating, and cool. To this solution add 3 drops of Reinecke salt TS: a light red precipitate is produced.

(2) Determine the absorption spectrum of a solution of Clotrimazole in methanol (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Clotrimazole, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Perform the test with Clotrimazole as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

Melting point <*2.60*> 142 – 145°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Clotrimazole in 10 mL of dichloromethane: the solution is clear and colorless.

(2) Chloride <*1.03*>—Dissolve 1.0 g of Clotrimazole in 40 mL of *N*,*N*-dimethylformamide, add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.60 mL of 0.01 mol/L hydrochloric acid VS, 40 mL of *N*,*N*-dimethylformamide, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(3) Sulfate <*1.14*>—Dissolve 0.5 g of Clotrimazole in 10 mL of methanol, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.05 mL of 0.005 mol/L sulfuric acid VS, 10 mL of methanol, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(4) Heavy metals <*1.07*>—Proceed with 2.0 g of Clotrimazole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Clotrimazole according to Method 3, and perform the test (not more than 2 ppm).

(6) Imidazole—Dissolve 0.10 g of Clotrimazole in exactly 10 mL of dichloromethane, and use this solution as the sample solution. Separately, dissolve 25 mg of imidazole for thin-layer chromatography in dichloromethane to make exactly 50 mL. Pipet 5 mL of this solution, add dichloromethane to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and chloroform (3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly sodium hypochlorite TS on the plate, and air-dry the plate for 15 minutes, then spray evenly potassium iodide-starch TS on the plate: the spot from the sample solution, corresponding to that from the standard solution, is not more intense than that from the standard solution.

(7) (2-Chlorophenyl)-diphenylmethanol—Dissolve 0.20 g of Clotrimazole in exactly 10 mL of dichloromethane, and use this solution as the sample solution. Separately, dissolve 10 mg of (2-chlorophenyl)-diphenylmethanol for thin-layer chromatography in dichloromethane to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and ammonia solution (28) (50:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution, corresponding to that from the standard solution, is not more intense than that from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.35 g of Clotrimazole, previously dried, and dissolve in 80 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

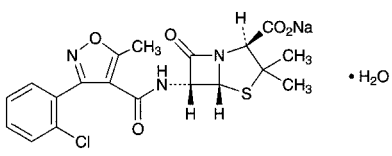
Each mL of 0.1 mol/L perchloric acid VS
= 34.48 mg of $C_{22}H_{17}ClN_2$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Cloxacillin Sodium Hydrate

クロキサシリンナトリウム水和物



$C_{19}H_{17}ClN_3NaO_5S.H_2O$: 475.88
Monosodium (2*S*,5*R*,6*R*)-6-{[3-(2-chlorophenyl)-5-methylisoxazole-4-carbonyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrate
[*7081-44-9*]

Cloxacillin Sodium Hydrate contains not less than 900 μg (potency) and not more than 960 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cloxacillin Sodium Hydrate is expressed as mass (potency) of cloxacillin ($C_{19}H_{18}ClN_3O_5S$: 435.88).

Description Cloxacillin Sodium Hydrate occurs as white to light yellowish white, crystals or crystalline powder.

It is freely soluble in water, in *N*,*N*-dimethylformamide and in methanol, and sparingly soluble in ethanol (95).

Identification **(1)** Determine the absorption spectrum of a solution of Cloxacillin Sodium Hydrate in methanol (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cloxacillin Sodium RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cloxacillin Sodium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Cloxacillin Sodium RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Cloxacillin Sodium Hydrate responds to the Qualitative Tests ⟨*1.09*⟩ (1) for sodium salt.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +163 – +171° (1 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 1.0 g of Cloxacillin Sodium Hydrate in 10 mL of water: the pH of the solution is between 5.0 and 7.5.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 1.0 g of Cloxacillin Sodium Hydrate in 10 mL of water is clear, and its absorbance at 430 nm, determined as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, is not more than 0.04.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Cloxacillin Sodium Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of Cloxacillin Sodium Hydrate according to Method 5, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Cloxacillin Sodium Hydrate in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than cloxacillin obtained from the sample solution is not larger than the peak area of cloxacillin obtained from the standard solution, and the total area of the peaks other than chloxacillin from the sample solution is not larger than 3 times the peak area of cloxacillin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of cloxacillin.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of cloxacillin obtained with 10 μL of this solution is equivalent to 7 to 13% of that obtained with 10 μL of the standard solution.

System performance: Dissolve about 50 mg of Cloxacillin Sodium RS in a suitable amount of the mobile phase, add 5 mL of a solution of guaifenesin in the mobile phase (1 in 200), then add the mobile phase to make 50 mL, and use this solution as the solution for system suitability test. When the procedure is run with 10 μL of the solution for system suitability test under the above operating conditions, guaifenesin and cloxacillin are eluted in this order with the resolution between these peaks being not less than 25.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of cloxacillin is not more than 1.0%.

Water ⟨*2.48*⟩ 3.0 – 4.5% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cloxacillin Sodium Hydrate and Cloxacillin Sodium RS, equivalent to about 50 mg (potency), dissolve each in a suitable amount of the mobile phase, add exactly 5 mL of the internal standard solution, then add the mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cloxacillin to that of the internal standard.

Amount [μg (potency)] of cloxacillin ($C_{19}H_{18}ClN_3O_5S$) = $M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Cloxacillin Sodium RS taken

Internal standard solution—A solution of guaifenesin in the mobile phase (1 in 200).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 4.95 g of diammonium hydrogen phosphate in 700 mL of water, add 250 mL of acetonitrile, adjust to pH 4.0 with phosphoric acid, and add water to make 1000 mL.

Flow rate: Adjust so that the retention time of cloxacillin is about 24 minutes.

System suitability—

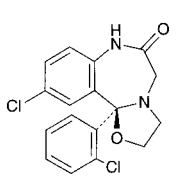
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, guaifenesin and cloxacillin are eluted in this order with the resolution between these peaks being not less than 25.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cloxacillin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cloxazolam

クロキサゾラム



and enantiomer

$C_{17}H_{14}Cl_2N_2O_2$: 349.21

(11b*RS*)-10-Chloro-11b-(2-chlorophenyl)-2,3,7,11b-tetrahydro[1,3]oxazolo[3,2-*d*][1,4]benzodiazepin-6(5*H*)-one

[*24166-13-0*]

Cloxazolam, when dried, contains not less than 99.0% of cloxazolam ($C_{17}H_{14}Cl_2N_2O_2$).

Description Cloxazolam occurs as white, crystals or crystalline powder. It is odorless and tasteless.

It is freely soluble in acetic acid (100), sparingly soluble in dichloromethane, slightly soluble in ethanol (99.5) and in diethyl ether, very slightly soluble in ethanol (95), and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

It is gradually colored by light.

Melting point: about 200°C (with decomposition).

Identification (1) Dissolve 0.01 g of Cloxazolam in 10 mL of ethanol (99.5) by heating, and add 1 drop of hydrochloric acid: the solution shows a light yellow color and a yellow-green fluorescence under ultraviolet light (main wavelength: 365 nm). Add 1 mL of sodium hydroxide TS to this solution: the color and fluorescence of this solution disappear immediately.

(2) Dissolve 0.01 g of Cloxazolam in 5 mL of dilute hydrochloric acid by heating in a water bath for 10 minutes. After cooling, 1 mL of this solution responds to the Qualitative Tests <*1.09*> for primary aromatic amines.

(3) Place 2 g of Cloxazolam in a 200-mL flask, add 50 mL of ethanol (95) and 25 mL of sodium hydroxide TS, and boil under a reflux condenser for 4 hours. After cooling, neutralize with dilute hydrochloric acid, and extract with 30 mL of dichloromethane. Dehydrate with 3 g of anhydrous sodium sulfate, filter, and evaporate the dichloromethane of the filtrate. Dissolve the residue in 5 mL of methanol by heating on a water bath, and cool immediately in an ice bath. Collect the crystals, and dry the crystals is vacuum at 60°C for 1 hour: it melts <*2.60*> between 87°C and 91°C.

(4) Determine the absorption spectrum of a solution of Cloxazolam in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(5) Proceed with Cloxazolam as directed under Flame Coloration Test <*1.04*> (2), and perform the test: a green color appears.

Absorbance <*2.24*> $E^{1\%}_{1\,cm}$ (244 nm): 390 – 410 (after drying, 1 mg, ethanol (99.5), 100 mL).

Purity (1) Chloride <*1.03*>—To 1.0 g of Cloxazolam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. To 25 mL of this filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Cloxazolam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <*1.11*>—Place 1.0 g of Cloxazolam in a Kjeldahl flask, add 5 mL of sulfuric acid and 5 mL of nitric acid, and heat gently. Repeat the addition of 2 to 3 mL of nitric acid at times, and continue heating until a colorless to light yellow solution is obtained. After cooling, add 15 mL of saturated ammonium oxalate solution, and heat the solution until dense white fumes are evolved, and evaporate to a volume of 2 to 3 mL. After cooling, dilute with water to 10 mL, and perform the test with this solution as the test solution (not more than 2 ppm).

(4) Related substances—Dissolve 0.05 g of Cloxazolam in 10 mL of dichloromethane, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dichloromethane to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Immediately after air-drying, develop the plate with a mixture of toluene and acetone (5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than that from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C,

3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

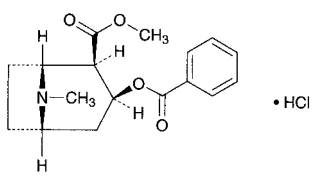
Assay Weigh accurately about 0.5 g of Cloxazolam, previously dried, and dissolve in 50 mL of acetic acid (100). Titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 2 drops of crystal violet TS). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 34.92 mg of $C_{17}H_{14}Cl_2N_2O_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Cocaine Hydrochloride

コカイン塩酸塩



$C_{17}H_{21}NO_4$.HCl: 339.81
(1*R*,2*R*,3*S*,5*S*)-2-Methoxycarbonyl-8-methyl-8-azabicyclo[3.2.1]oct-3-yl benzoate monohydrochloride
[*53-21-4*]

Cocaine Hydrochloride, when dried, contains not less than 98.0% of cocaine hydrochloride ($C_{17}H_{21}NO_4$.HCl).

Description Cocaine Hydrochloride occurs as colorless crystals or a white crystalline powder.

It is very soluble in water, freely soluble in ethanol (95) and in acetic acid (100), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Identification **(1)** Determine the absorption spectrum of a solution of Cocaine Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Cocaine Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cocaine Hydrochloride, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Cocaine Hydrochloride (1 in 50) responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −70 – −73° (after drying, 0.5 g, water, 20 mL, 100 mm).

Purity **(1)** Acidity—Dissolve 0.5 g of Cocaine Hydrochloride in 10 mL of water, add 1 drop of methyl red TS, and neutralize with 0.01 mol/L sodium hydroxide VS: the consumed volume is not more than 1.0 mL.

(2) Cinnamyl cocaine—Dissolve 0.10 g of Cocaine Hydrochloride in 5 mL of water, and add 0.3 mL of diluted sulfuric acid (1 in 20) and 0.10 mL of 0.02 mol/L potassium permanganate VS: the red color does not disappear within 30 minutes.

(3) Isoatropyl cocaine—Dissolve 0.10 g of Cocaine Hydrochloride in 30 mL of water in a beaker. Transfer 5 mL of this solution to a test tube, add 1 drop of ammonia TS, and mix. After the precipitate is coagulated, add 10 mL of water, and transfer the mixture to the former beaker, to which 30 mL of water has been added previously. Wash the test tube with 10 mL of water, combine the washings with the mixture in the beaker, add 3 drops of ammonia TS to the combined mixture, and mix gently: a crystalline precipitate is produced. Allow to stand for 1 hour: the supernatant liquid is clear.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.5 g of Cocaine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 33.98 mg of $C_{17}H_{21}NO_4$.HCl

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Cod Liver Oil

肝油

Cod Liver Oil is the fatty oils obtained from fresh livers and pyloric appendages of *Gadus macrocephalus* Tilesius or *Theragra chalcogramma* Pallas (*Gadidae*).

Cod Liver Oil contains not less than 2000 Vitamin A Units and not more than 5000 Vitamin A Units per g.

Description Cod Liver Oil is a yellow to orange oily liquid. It has a characteristic, slightly fishy odor and a mild taste.

It is miscible with chloroform.

It is slightly soluble in ethanol (95), and practically insoluble in water.

It is decomposed by air or by light.

Identification Dissolve 0.1 g of Cod Liver Oil in 10 mL of chloroform, and to 1 mL of this solution add 3 mL of antimony (III) chloride TS: a blue color develops immediately, but the color fades rapidly.

Specific gravity ⟨*1.13*⟩ d_{20}^{20}: 0.918 – 0.928

Acid value ⟨*1.13*⟩ Not more than 1.7.

Saponification value ⟨*1.13*⟩ 180 – 192

Unsaponifiable matter ⟨*1.13*⟩ Not more than 3.0%.

Iodine value ⟨*1.13*⟩ 130 – 170

Purity Rancidity—No unpleasant odor of rancid oil is perceptible on warming Cod Liver Oil.

Assay Proceed with about 0.5 g of Cod Liver Oil, accurately weighed, as directed in Method 2 under the Vitamin A

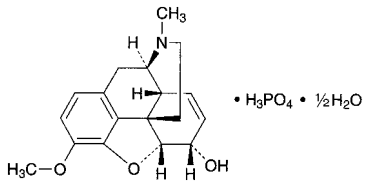
Determination <*2.55*>, and perform the test.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and almost well-filled, or under nitrogen atmosphere.

Codeine Phosphate Hydrate

コデインリン酸塩水和物



$C_{18}H_{21}NO_3.H_3PO_4.\frac{1}{2}H_2O$: 406.37

(5*R*,6*S*)-4,5-Epoxy-3-methoxy-17-methyl-7,8-didehydromorphinan-6-ol monophosphate hemihydrate [*41444-62-6*]

Codeine Phosphate Hydrate contains not less than 98.0% of codeine phosphate ($C_{18}H_{21}NO_3.H_3PO_4$: 397.36), calculated on the anhydrous basis.

Description Codeine Phosphate Hydrate occurs as white to yellowish white, crystals or crystalline powder.

It is freely soluble in water and in acetic acid (100), slightly soluble in methanol and in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of 1.0 g of Codeine Phosphate Hydrate in 10 mL of water is between 3.0 and 5.0.

It is affected by light.

Identification **(1)** Determine the absorption spectrum of a solution of Codeine Phosphate Hydrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Codeine Phosphate Hydrate, previously dried at 105°C for 4 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Codeine Phosphate Hydrate (1 in 20) responds to the Qualitative Tests <*1.09*> (1) for phosphate.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −98 – −102° (0.4 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

Purity **(1)** Chloride <*1.03*>—Perform the test with 0.5 g of Codeine Phosphate Hydrate. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(2) Sulfate <*1.14*>—Perform the test with 0.20 g of Codeine Phosphate Hydrate. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.240%).

(3) Related substances—Dissolve 0.20 g of Codeine Phosphate Hydrate in 10 mL of a mixture of 0.01 mol/L hydrochloric acid TS and ethanol (99.5) (4:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of 0.01 mol/L hydrochloric acid TS and ethanol (99.5) (4:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), toluene, acetone and ammonia solution (28) (14:14:7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <*2.48*> 1.5 – 3.0% (0.5 g, volumetric titration, direct titration).

Assay Dissolve about 0.5 g of Codeine Phosphate Hydrate, accurately weighed, in 70 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to greenish blue (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 39.74 mg of $C_{18}H_{21}NO_3.H_3PO_4$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

1% Codeine Phosphate Powder

コデインリン酸塩散 1%

1% Codeine Phosphate Powder contains not less than 0.90% and not more than 1.10% of codeine phosphate hydrate ($C_{18}H_{21}NO_3.H_3PO_4.\frac{1}{2}H_2O$: 406.37).

Method of preparation

Codeine Phosphate Hydrate	10 g
Lactose Hydrate	a sufficient quantity
	To make 1000 g

Prepare as directed under Granules or Powders, with the above ingredients.

Identification Determine the absorption spectrum of a solution of 1% Codeine Phosphate Powder (1 in 100) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 283 nm and 287 nm.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of 1% Codeine Phosphate Powder is not less than 85%.

Start the test with about 2 g of 1% Codeine Phosphate Powder, accurately weighed, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 28 mg of codeine phosphate hydrate for assay (separately determine the water <*2.48*> in the same manner as Codeine Phosphate Hydrate), and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to

the following conditions, and determine the peak areas, A_T and A_S, of codeine in each solution.

Dissolution rate (%) with respect to the labeled amount of codeine phosphate hydrate ($C_{18}H_{21}NO_3.H_3PO_4.{}^1/_2H_2O$)
$= M_S/M_T \times A_T/A_S \times 36/5 \times 1.023$

M_S: Amount (mg) of codeine phosphate hydrate for assay taken, calculated on the anhydrous basis
M_T: Amount (g) of 1% Codeine Phosphate Powder taken

Operating conditions—
Proceed as directed in the operating conditions in the Assay.
System suitability—
System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of codeine are not less than 3000 and not more than 2.0, respectively.
System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of codeine is not more than 2.0%.

Assay Weigh accurately about 5 g of 1% Codeine Phosphate Powder, dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of codeine phosphate hydrate for assay (previously determine the water <2.48> in the same manner as Codeine Phosphate Hydrate), dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of codeine to that of the internal standard.

Amount (mg) of codeine phosphate hydrate ($C_{18}H_{21}NO_3.H_3PO_4.{}^1/_2H_2O$)
$= M_S \times Q_T/Q_S \times 1.023$

M_S: Amount (mg) of codeine phosphate hydrate for assay taken, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (3 in 10,000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 280 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.
Flow rate: Adjust so that the retention time of codeine is about 10 minutes.
System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, codeine and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.
System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of codeine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

10% Codeine Phosphate Powder

コデインリン酸塩散 10%

10% Codeine Phosphate Powder contains not less than 9.3% and not more than 10.7% of codeine phosphate hydrate ($C_{18}H_{21}NO_3.H_3PO_4.{}^1/_2H_2O$: 406.37).

Method of preparation

Codeine Phosphate Hydrate	100 g
Lactose Hydrate	a sufficient quantity
To make	1000 g

Prepare as directed under Powders, with the above ingredients.

Identification Determine the absorption spectrum of a solution of 10% Codeine Phosphate Powder (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 283 nm and 287 nm.

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of 10% Codeine Phosphate Powder is not less than 85%.
Start the test with about 0.2 g of 10% Codeine Phosphate Powder, accurately weighed, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 28 mg of codeine phosphate hydrate for assay (separately determine the water <2.48> in the same manner as Codeine Phosphate Hydrate), and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of codeine in each solution.

Dissolution rate (%) with respect to the labeled amount of codeine phosphate hydrate ($C_{18}H_{21}NO_3.H_3PO_4.{}^1/_2H_2O$)
$= M_S/M_T \times A_T/A_S \times 18/25 \times 1.023$

M_S: Amount (mg) of codeine phosphate hydrate for assay taken, calculated on the anhydrous basis
M_T: Amount (g) of 10% Codeine Phosphate Powder

Operating conditions—
Proceed as directed in the operating conditions in the Assay.
System suitability—
System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of codeine are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of codeine is not more than 2.0%.

Assay Weigh accurately about 2.5 g of 10% Codeine Phosphate Powder, dissolve in water to make exactly 100 mL, then pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of codeine phosphate hydrate for assay (previously determine the water ⟨*2.48*⟩ in the same manner as Codeine Phosphate Hydrate), dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of codeine to that of the internal standard:

Amount (mg) of codeine phosphate hydrate ($C_{18}H_{21}NO_3.H_3PO_4.\frac{1}{2}H_2O$)
$= M_S \times Q_T/Q_S \times 5 \times 1.023$

M_S: Amount (mg) of codeine phosphate hydrate for assay taken, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (3 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust so that the retention time of codeine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, codeine and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of codeine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Codeine Phosphate Tablets

コデインリン酸塩錠

Codeine Phosphate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of codeine phosphate hydrate ($C_{18}H_{21}NO_3.H_3PO_4.\frac{1}{2}H_2O$: 406.37)

Method of preparation Prepare as directed under Tablets, with Codeine Phosphate Hydrate.

Identification To a quantity of powdered Codeine Phosphate Tablets, equivalent to 0.1 g of Codeine Phosphate Hydrate, add 20 mL of water, shake, and filter. To 2 mL of the filtrate add water to make 100 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 283 nm and 287 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Codeine Phosphate Tablets add $3V/25$ mL of water to disintegrate, add $2V/25$ mL of diluted dilute sulfuric acid (1 in 20), and treat with ultrasonic waves for 10 minutes. To this solution add exactly $2V/25$ mL of the internal standard solution, add water to make V mL so that each mL contains about 0.2 mg of codeine phosphate hydrate ($C_{18}H_{21}NO_3.H_3PO_4.\frac{1}{2}H_2O$), filter, and use the filtrate as the sample solution. Separately, weigh accurately about 50 mg of codeine phosphate hydrate for assay (separately, determine the water ⟨*2.48*⟩ in the same manner as Codeine Phosphate Hydrate), and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 2 mL of the internal standard solution, add water to make 25 mL, and use this solution as the standard solution. Proceed as directed in the Assay.

Amount (mg) of codeine phosphate hydrate ($C_{18}H_{21}NO_3.H_3PO_4.\frac{1}{2}H_2O$)
$= M_S \times Q_T/Q_S \times V/250 \times 1.023$

M_S: Amount (mg) of codeine phosphate hydrate for assay taken, calculated on the anhydrous basis

Internal standard solution—A solution of ethylefurin hydrochloride (3 in 2000).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Codeine Phosphate Tablets is not less than 80%.

Start the test with 1 tablet of Codeine Phosphate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 5.6 μg of codeine phosphate hydrate ($C_{18}H_{21}NO_3.H_3PO_4.\frac{1}{2}H_2O$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of codeine phosphate hydrate for assay (separately, determine the water ⟨*2.48*⟩ in the same manner as Codeine Phosphate Hydrate), dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of codeine in each solution.

Dissolution rate (%) with respect to the labeled amount of codeine phosphate hydrate ($C_{18}H_{21}NO_3.H_3PO_4.\frac{1}{2}H_2O$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18 \times 1.023$

M_S: Amount (mg) of codeine phosphate hydrate for assay taken, calculated on the anhydrous basis

C: Labeled amount (mg) of codeine phosphate hydrate ($C_{18}H_{21}NO_3.H_3PO_4.\frac{1}{2}H_2O$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of codeine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of codeine is not more than 2.0%.

Assay Weigh accurately and powder not less than 20 Codeine Phosphate Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of codeine phosphate hydrate ($C_{18}H_{21}NO_3.H_3PO_4.\frac{1}{2}H_2O$), add 30 mL of water, shake, add 20 mL of diluted dilute sulfuric acid (1 in 20), treat the mixture with ultrasonic waves for 10 minutes, and add water to make exactly 100 mL. Filter the solution, then pipet 5 mL of the filtrate, add exactly 10 mL of the internal standard solution and water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of codeine phosphate hydrate for assay (previously determine the water <2.48> in the same manner as Codeine Phosphate Hydrate), dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of codeine to that of the internal standard.

Amount (mg) of codeine phosphate hydrate ($C_{18}H_{21}NO_3.H_3PO_4.\frac{1}{2}H_2O$)
$= M_S \times Q_T/Q_S \times 2 \times 1.023$

M_S: Amount (mg) of codeine phosphate hydrate for assay taken, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (3 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust so that the retention time of codeine is about 10 minutes.

System suitability—

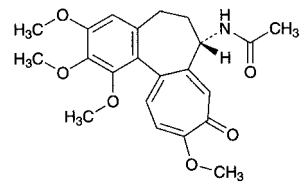
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, codeine and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of codeine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Colchicine

コルヒチン



$C_{22}H_{25}NO_6$: 399.44

N-[(7*S*)-(1,2,3,10-Tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl)]acetamide

[*64-86-8*]

Colchicine contains not less than 97.0% and not more than 102.0% of colchicine ($C_{22}H_{25}NO_6$), calculated on the anhydrous and residual ethyl acetate-free basis.

Description Colchicine occurs as a yellowish white powder.

It is very soluble in methanol, freely soluble in *N*,*N*-dimethylformamide, in ethanol (95) and in acetic anhydride, and sparingly soluble in water.

It is colored by light.

Identification **(1)** Determine the absorption spectrum of a solution of Colchicine in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) To 1 g of potassium bromide for infrared absorption spectrum add 0.5 mL of a solution of Colchicine in methanol (1 in 50), grind thoroughly, and dry in vacuum at 80°C for 1 hour. Determine the infrared absorption spectrum of this powder as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: −235 – −250° (0.1 g calculated on the anhydrous basis and corrected by the amount of ethyl acetate, ethanol (95), 10 mL, 100 mm).

Purity **(1)** Colchiceine—Dissolve 0.10 g of Colchicine in 10 mL of water, and to 5 mL of this solution add 2 drops of iron (III) chloride TS: no definite green color develops.

(2) Chloroform and ethyl acetate—Weigh accurately about 0.6 g of Colchicine, dissolve in exactly 2 mL of the internal standard solution, add *N*,*N*-dimethylformamide to make 10 mL, and use this solution as the sample solution. Separately, weigh 0.30 g of chloroform using a 100-mL volumetric flask containing about 20 mL of *N*,*N*-dimethylformamide, and add *N*,*N*-dimethylformamide to make exactly 100 mL. Pipet 2 mL of this solution, add *N*,*N*-dimethylformamide to make exactly 200 mL, and use this solution as the standard solution (1). Separately, weigh accurately about 1.8 g of ethyl acetate using a 100-mL volumetric flask containing about 20 mL of *N*,*N*-dimethylformamide, and add *N*,*N*-dimethylformamide to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution and *N*,*N*-dimethylformamide to make 10 mL, and use this solution as the standard solution (2). Perform the test with exactly 2 μL each of the sample solution and

standard solutions (1) and (2) as directed under Gas Chromatography <2.02> according to the following conditions: the peak area of chloroform from sample solution is not larger than that from the standard solution (1). Calculate the ratios of the peak area of ethyl acetate to that of the internal standard, Q_T and Q_S, of the sample solution and standard solution (2), and calculate the amount of ethyl acetate by the following formula: the amount of ethyl acetate is not more than 6.0%.

$$\text{Amount (\%) of ethyl acetate } (C_4H_8O_2) = M_S/M_T \times Q_T/Q_S \times 2$$

M_S: Amount (g) of ethyl acetate taken
M_T: Amount (g) of Colchicine taken

Internal standard solution—A solution of 1-propanol in *N*,*N*-dimethylformamide (3 in 200).
Operating conditions—
Detector: A hydrogen flame-ionization detector.
Column: A fused silica column 0.53 mm in inside diameter and 30 m in length, coated inside surface with polyethylene glycol 20 M for gas chromatography 1.0 μm in thickness.
Column temperature: 60°C for 7 minutes, then up to 100°C at a rate of 40°C per minute if necessary, and hold at 100°C for 10 minutes.
Injection port temperature: A constant temperature of about 130°C.
Detector temperature: A constant temperature of about 200°C.
Carrier gas: Helium.
Flow rate: Adjust so that the retention time of ethyl acetate is about 3 minutes.
Split ratio: 1:20.
System suitability—
Test for required detectability: Pipet 2 mL of the standard solution (2), and add *N*,*N*-dimethylformamide to make exactly 25 mL. Pipet 1 mL of this solution, and add *N*,*N*-dimethylformamide to make exactly 50 mL. Confirm that the peak area of ethyl acetate obtained from 2 μL of this solution is equivalent to 0.11 to 0.21% of that obtained from 2 μL of the standard solution (2).
System performance: To 1 mL of chloroform add *N*,*N*-dimethylformamide to make 10 mL. To 1 mL of this solution add 2 mL of ethyl acetate and *N*,*N*-dimethylformamide to make 100 mL. To 2 mL of this solution add 2 mL of the internal standard solution and *N*,*N*-dimethylformamide to make 10 mL. When the procedure is run with 2 μL of this solution under the above operating conditions, ethyl acetate, chloroform and the internal standard are eluted in this order with the resolution between the peaks of chloroform and the internal standard being not less than 2.0.
System repeatability: When the test is repeated 3 times with 2 μL of the standard solution (2) under the above operating conditions, the relative standard deviation of the ratio of the peak area of ethyl acetate to that of the internal standard is not more than 3.0%.

(3) Related substances—Dissolve 60 mg of Colchicine in 100 mL of diluted methanol (1 in 2). To 1 mL of this solution, add diluted methanol (1 in 2) to make 100 mL, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the total amount of the peaks other than colchicine by the area percentage method: not more than 5.0%.
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 254 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 25°C.
Mobile phase: To 450 mL of 0.05 mol/L potassium dihydrogen phosphate TS add methanol to make 1000 mL. Adjust the pH to 5.5 with diluted phosphoric acid (7 in 200).
Flow rate: Adjust so that the retention time of colchicine is about 7 minutes.
Time span of measurement: About 2 times as long as the retention time of colchicine, beginning after the solvent peak.
System suitability—
Test for required detectability: Pipet 1 mL of the sample solution, and add diluted methanol (1 in 2) to make exactly 50 mL. Confirm that the peak area of colchicine obtained from 20 μL of this solution is equivalent to 1.4 to 2.6% of that obtained from 20 μL of the sample solution.
System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of colchicine are not less than 6000 and not more than 1.5, respectively.
System repeatability: When the test is repeated 6 times with 20 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of colchicine is not more than 2.0%.

Water <2.48> Not more than 2.0% (0.5 g, volumetric titration, back titration).

Assay Weigh accurately about 0.4 g of Colchicine, dissolve in 25 mL of acetic anhydride, and titrate <2.50> with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

$$\text{Each mL of 0.05 mol/L perchloric acid VS} = 19.97 \text{ mg of } C_{22}H_{25}NO_6$$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Colestimide

コレスチミド

[*95522-45-5*]

Colestimide is an anion exchange resin, composed of a copolymer of 2-methylimidazole and 1-chloro-2,3-epoxypropane.

It contains not less than 18.0% and not more than 20.0% of chlorine (Cl: 35.45), calculated on the dried basis.

Each g of Colestimide, calculated on the dried basis, exchanges with not less than 2.0 g and not more than 2.4 g of cholic acid ($C_{24}H_{39}O_5$: 407.56).

Description Colestimide occurs as a white to pale yellowish white powder.
It is practically insoluble in water and in ethanol (99.5).
It is hygroscopic.

Identification Determine the infrared absorption spectrum of Colestimide, previously dried, as directed in the potas-

sium chloride disk method under Infrared Spectrophotometry ⟨2.25⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals ⟨1.07⟩—Take 2.0 g of Colestimide in a porcelain or platinum crucible, and carbonize by weakly heating. After cooling, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) and 5 mL of hydrogen peroxide (30), and ignite the ethanol. After cooling, add 1 mL of sulfuric acid, then, proceed according to Method 4, and perform the test. Prepare the control solution as follows: To 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) add 5 mL of hydrogen peroxide (30), and ignite the ethanol. After cooling, add 1 mL of sulfuric acid, then, proceed in the same manner as for the test solution, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 10 ppm).

(2) Related substances—To exactly 0.50 g of Colestimide add exactly 20 mL of water, shake for 1 hour, centrifuge, and use the supernatant liquid as the sample solution. Determine the absorbance of the sample solution at 210 nm as directed under Ultraviolet-visible Spectrophotometry ⟨2.24⟩: the absorbance is not more than 0.50.

Loss on drying ⟨2.41⟩ Not more than 10.0% (1 g, in vacuum, 105°C, 4 hours).

Residue on ignition ⟨2.44⟩ Not more than 0.1% (1 g).

Degree of swelling Weigh accurately about 1 g of Colestimide, put in a 25-mL glass stoppered measuring cylinder (about 11 mm in inside diameter), add 23 mL of water, shake for 2 minutes, and add water to make 25 mL. After standing for 2 hours, measure the volume of the resin layer, and determine the volume per g, calculated on the dried basis: the volume is 12 – 18 mL/g.

Assay **(1)** Chlorine—Weigh accurately about 0.2 g of Colestimide, add 50 mL of water, and shake. Add 1 mL of nitric acid and 25 mg of potassium nitrate, shake, and titrate ⟨2.50⟩ with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS
= 3.545 mg of Cl

(2) Exchange capacity—Weigh accurately about 0.45 g of sodium cholate hydrate (separately determine the water), dissolve in water to make exactly 100 mL, and use this solution as the sodium cholate standard stock solution. Separately, weigh accurately about 30 mg of Colestimide, add exactly 30 mL of the sodium cholate standard stock solution, shake for 1 hour, and centrifuge or filter through a membrane filter with a pore size not exceeding 0.8 μm. Pipet 5 mL of the supernatant liquid or the filtrate, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, pipet 5 mL of the sodium cholate standard stock solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨2.01⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cholic acid to that of the internal standard.

Exchanged amount (g) of cholic acid per g of Colestimide, calculated on the dried basis
$= M_S/M_T \times (Q_S - Q_T)/Q_S \times 3/10 \times 0.947$

M_S: Amount (mg) of sodium cholate hydrate taken, calculated on the anhydrous basis
M_T: Amount (mg) of Colestimide taken, calculated on the dried basis

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 80,000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 220 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 30°C.
Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (1:1).
Flow rate: Adjust so that the retention time of cholic acid is about 7 minutes.
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cholic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cholic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Colestimide Granules

コレスチミド顆粒

Colestimide Granules contain not less than 87.0% and not more than 113.0% of the labeled amount of colestimide.

Method of preparation Prepare as directed under Granules, with Colestimide.

Identification Determine the infrared absorption spectrum of powdered Colestimide Granules as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨2.25⟩: it exhibits absorption at the wave numbers of about 1587 cm^{-1}, 1528 cm^{-1} and 1262 cm^{-1}.

Uniformity of dosage units ⟨6.02⟩ Colestimide Granules in single-dose packages meet the requirement of the Mass variation test.

Disintegration ⟨6.09⟩ Carry out the test for 10 minutes with 0.09 – 0.11 g of Colestimide Granules in six glass tubes of the apparatus: it meets the requirement.

Assay Weigh accurately about 4.5 g of sodium cholate hydrate (separately determine the water), dissolve in water to make exactly 1000 mL, and use this solution as the sodium cholate standard stock solution. Take out the contents of not less than 20 single-dose packages of Colestimide Granules, weigh accurately an amount of the contents, equivalent to about 0.2 g of colestimide, add exactly 200 mL of the sodium cholate standard stock solution, shake for 1 hour, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Then, proceed as directed in

the Assay (2) under Colestimide.

Amount (mg) of colestimide
$= M_S \times (Q_S - Q_T)/Q_S \times 1/5 \times 1/2.2 \times 0.947$

M_S: Amount (mg) of sodium cholate hydrate taken, calculated on the anhydrous basis
2.2: Quantity (g) of the cholic acid exchange per mg of colestimide

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 80,000).

Containers and storage Containers—Tight containers.

Colestimide Tablets

コレスチミド錠

Colestimide Tablets contain not less than 87.0% and not more than 113.0% of the labeled amount of colestimide.

Method of preparation Prepare as directed under Tablets, with Colestimide.

Identification Powder Colestimide Tablets. Determine the infrared absorption spectrum of a portion of the powder as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩: it exhibits absorption at the wave numbers of about 1587 cm^{-1}, 1528 cm^{-1}, 1262 cm^{-1}, 1102 cm^{-1} and 1035 cm^{-1}.

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Disintegration ⟨*6.09*⟩ When carry out the test for 10 minutes, it meets the requirement.

Assay Weigh accurately about 0.45 g of sodium cholate hydrate (separately determine the water), dissolve in water to make exactly 100 mL, and use this solution as the sodium cholate standard stock solution. Separately, weigh accurately the mass of not less than 20 Colestimide Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 30 mg of colestimide, add exactly 30 mL of the sodium cholate standard stock solution, shake for 1 hour, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, pipet 5 mL of the sodium cholate standard stock solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cholic acid to that of the internal standard.

Amount (mg) of colestimide
$= M_S \times (Q_S - Q_T)/Q_S \times 3/10 \times 1/2.2 \times 0.947$

M_S: Amount (mg) of sodium cholate hydrate taken, calculated on the anhydrous basis
2.2: Exchanged amount (g) of cholic acid per g of colestimide, calculated on the dried basis

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 80,000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (1:1).

Flow rate: Adjust so that the retention time of cholic acid is about 7 minutes.

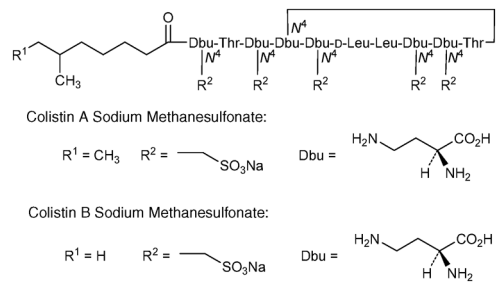
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cholic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cholic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Colistin Sodium Methanesulfonate

コリスチンメタンスルホン酸ナトリウム



[*8068-28-8*, Colistin Sodium Methanesulfonate]

Colistin Sodium Methanesulfonate is the sodium salt of colistin derivatives.

It is a mixture of colistin A sodium methanesulfonate and colistin B sodium methanesulfonate.

It, when dried, contains not less than 11,500 Units per mg. The unit of Colistin Sodium Methanesulfonate is expressed as mass of colistin A (R = 6-methyloctanic acid, R′ = H; $C_{53}H_{100}N_{16}O_{13}$: 1169.46).

Description Colistin Sodium Methanesulfonate occurs as a white to light yellowish white powder.

It is freely soluble in water, and practically insoluble in ethanol (95).

Identification **(1)** Dissolve 20 mg of Colistin Sodium Methanesulfonate in 2 mL of water, add 0.5 mL of sodium hydroxide TS, and add 5 drops of copper (II) sulfate TS while shaking: a blue-purple color develops.

(2) Dissolve 40 mg of Colistin Sodium Methanesulfonate in 1 mL of 1 mol/L hydrochloric acid TS, and add 0.5 mL of dilute iodine TS: the color of iodine disappears.

(3) Determine the infrared absorption spectrum of Colistin Sodium Methanesulfonate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of dried Colistin Sodium Methanesulfonate RS: both spectra exhibit similar in-

tensities of absorption at the same wave numbers.

(4) Colistin Sodium Methanesulfonate responds to the Qualitative Tests <1.09> (1) for sodium salt.

pH <2.54> Dissolve 0.1 g of Colistin Sodium Methanesulfonate in 10 mL of water, and allow to stand for 30 minutes: the pH of the solution is between 6.5 and 8.5.

Purity **(1)** Clarity and color of solution—Dissolve 0.16 g of Colistin Sodium Methanesulfonate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Colistin Sodium Methanesulfonate according to Method 4, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Colistin Sodium Methanesulfonate according to Method 4, and perform the test (not more than 2 ppm).

(4) Free colistin—Dissolve 80 mg of Colistin Sodium Methanesulfonate in 3 mL of water, add 0.05 mL of a solution of silicotungstic acid 26-water (1 in 10), and immediately compare the solution with the reference suspension described under Test Methods for Plastic Containers <7.02>: the turbidity is not greater than that of the reference suspension (not more than 0.25%).

Loss on drying <2.41> Not more than 3.0% (0.1 g, reduced pressure, 60°C, 3 hours).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Escherichia coli* NIHJ

(ii) Culture medium—To 10.0 g of peptone, 30.0 g of sodium chloride, 3.0 g of meat extract and 20.0 g of agar add 1000 mL of water, then add a suitable amount of sodium hydroxide TS so that the pH of the medium is being 6.5 to 6.6 after sterilization, sterile, and use this as the seeded agar medium and the agar medium for base layer.

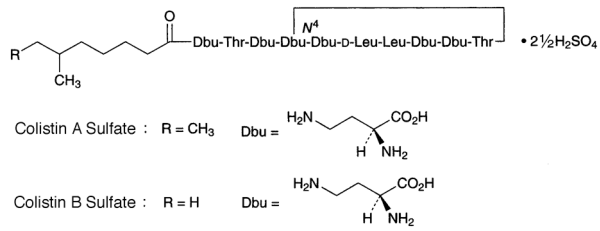
(iii) Standard solutions—Weigh accurately an amount of Colistin Sodium Methanesulfonate RS, previously dried, dissolve in phosphate buffer solution (pH 6.0) to make a solution containing 100,000 Units per mL, and use this solution as the standard stock solution. Keep the standard stock solution at 10°C or below and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, and add phosphate buffer solution (pH 6.0) to make solutions so that each mL contains 10,000 Units and 2500 Units, and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Colistin Sodium Methanesulfonate, previously dried, dissolve in phosphate buffer solution (pH 6.0) to make a solution containing about 100,000 Units per mL, and use this solution as the sample stock solution. Take exactly a suitable amount of the sample stock solution, add phosphate buffer solution (pH 6.0) to make solutions so that each mL contains 10,000 Units and 2500 Units, and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Colistin Sulfate

コリスチン硫酸塩



Colistin A Sulfate $C_{53}H_{100}N_{16}O_{13}.2\frac{1}{2}H_2SO_4$: 1414.66
Colistin B Sulfate $C_{52}H_{98}N_{16}O_{13}.2\frac{1}{2}H_2SO_4$: 1400.63
[*1264-72-8*]

Colistin Sulfate is the sulfate of a mixture of peptide substances having antibacterial activity produced by the growth of *Bacillus polymyxa* var. *colistinus*.

It, when dried, contains not less than 16,000 units per mg. The potency of Colistin Sulfate is expressed as unit calculated from the amount of colistin A ($C_{53}H_{100}N_{16}O_{13}$: 1169.46). One unit of Colistin Sulfate is equivalent to 0.04 μg of colistin A ($C_{53}H_{100}N_{16}O_{13}$).

Description Colistin Sulfate occurs as a white to light yellowish white powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

Identification **(1)** Dissolve 20 mg of Colistin Sulfate in 2 mL of water, add 0.5 mL of sodium hydroxide TS, then add 5 drops of copper (II) sulfate TS while shaking: a purple color develops.

(2) Dissolve 50 mg of Colistin Sulfate in 10 mL of diluted hydrochloric acid (1 in 2). Transfer 1 mL of this solution in a tube for hydrolysis, seal, and heat at 135°C for 5 hours. After cooling, open the tube, and evaporate the content to dryness until the odor of hydrochloric acid is no more perceptible. Dissolve the residue in 0.5 mL of water, and use this solution as the sample solution. Separately, dissolve 20 mg each of L-leucine, L-threonine, phenylalanine and L-serine in 10 mL of water, and use these solutions as the standard solution (1), (2), (3) and (4). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 1 μL each of the sample solution and standard solution (1), (2), (3) and (4) on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, acetic acid (100), water, pyridine and ethanol (99.5) (60:15:10:6:5) to a distance of about 10 cm, and dry the plate at 105°C for 10 minutes. Spray evenly ninhydrin TS on the plate, and heat at 110°C for 5 minutes: three principal spots are obtained from the sample solution, the *R*f values of two spots of them are the same with those of the corresponding spots obtained from the standard solution (1) and the standard solution (2), and the *R*f value of the rest principal spot is about 0.1. No spot is observed at the position corresponding to the spots obtained from the standard solution (3) and the standard solution (4).

(3) A solution of Colistin Sulfate (1 in 20) responds to the Qualitative Tests <1.09> (1) for sulfate.

Optical rotation <2.49> $[\alpha]_D^{20}$: -63 – $-73°$ (1.25 g, after drying, water, 25 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving

0.10 g of Colistin Sulfate in 10 mL of water is between 4.0 and 6.0.

Purity **(1)** Sulfuric acid—Weigh accurately about 0.25 g of previously dried Colistin Sulfate, dissolve in a suitable amount of water, adjust the pH to 11 with ammonia solution (28), and add water to make 100 mL. To this solution add exactly 10 mL of 0.1 mol/L barium chloride VS and 50 mL of ethanol (99.5), and titrate with <2.50> 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the blue-purple color of the solution disappears (indicator: 0.5 mg of phthalein purple): the amount of sulfuric acid (SO_4) is 16.0 to 18.0%.

Each mL of 0.1 mol/L barium chloride VS
= 9.606 mg of SO_4

(2) Related substances—Dissolve 50 mg of Colistin Sulfate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of pyridine, 1-butanol, water and acetic acid (100) (6:5:4:1) to a distance of about 10 cm, and dry the plate at 100°C for 30 minutes. Spray evenly ninhydrin-butanol TS on the plate, and heat at 100°C for about 20 minutes: the spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 6.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Escherichia coli* NIHJ

(ii) Culture medium—Dissolve 10.0 g of peptone, 30.0 g of sodium chloride, 3.0 g of meat extract and 15.0 g of agar in 1000 mL of water, adjust the pH with sodium hydroxide TS so that the solution will be 6.5 to 6.6 after sterilization, and use as the agar media for seed layer and for base layer.

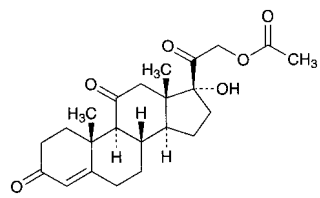
(iii) Standard solutions—Weigh accurately an amount of Colistin Sulfate RS, previously dried, equivalent to about 1,000,000 units, dissolve in phosphate buffer solution (pH 6.0) to make exactly 10 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 10°C, and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution (pH 6.0) to make solutions so that each mL contains 10,000 units and 2500 units, and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Colistin Sulfate, previously dried, equivalent to about 1,000,000 units, and dissolve in phosphate buffer solution (pH 6.0) to make exactly 10 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution (pH 6.0) to make solutions so that each mL contains 10,000 units and 2500 units, and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Cortisone Acetate

コルチゾン酢酸エステル



$C_{23}H_{30}O_6$: 402.48
17,21-Dihydroxypregn-4-ene-3,11,20-trione 21-acetate
[*50-04-4*]

Cortisone Acetate, when dried, contains not less than 97.0% and not more than 102.0% of cortisone acetate ($C_{23}H_{30}O_6$).

Description Cortisone Acetate occurs as white, crystals or crystalline powder.

It is sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

Melting point: about 240°C (with decomposition).

It shows crystal polymorphism.

Identification **(1)** To 2 mg of Cortisone Acetate add 2 mL of sulfuric acid, and allow to stand for a while: a yellowish green color is produced, and it gradually changes to yellow-orange. Examine the solution under ultraviolet light: the solution shows a light green fluorescence. Add carefully 10 mL of water to this solution: the color of the solution is discharged, and the solution remains clear.

(2) Determine the absorption spectrum of a solution of Cortisone Acetate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cortisone Acetate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Cortisone Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Cortisone Acetate RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Cortisone Acetate and Cortisone Acetate RS in acetone, respectively, then evaporate the acetone to dryness, and repeat the test on the residues.

Optical rotation <2.49> $[\alpha]_D^{20}$: +207 – +216° (after drying, 0.1 g, methanol, 10 mL, 100 mm).

Purity Related substances—Dissolve 25 mg of Cortisone Acetate in 10 mL of a mixture of acetonitrile, water and acetic acid (100) (70:30:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution add the mixture of acetonitrile, water and acetic acid (100) (70:30:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 15 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: each peak area other than cortisone acetate obtained with the sample solution is not larger than 1/2 times the peak area of cortisone acetate obtained with the standard solution, and the total area of the peaks other than

cortisone acetate is not larger than 1.5 times the peak area of cortisone acetate with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of water and acetonitrile (7:3).

Mobile phase B: A mixture of acetonitrile and water (7:3).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 5	90	10
5 – 25	90 → 10	10 → 90
25 – 30	10	90

Flow rate: About 1 mL per minute.

Time span of measurement: About 3 times as long as the retention time of cortisone acetate, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add a mixture of acetonitrile, water and acetic acid (100) (70:30:1) to make exactly 10 mL. Confirm that the peak area of cortisone acetate obtained with 15 μL of this solution is equivalent to 8 to 12% of that obtained with 15 μL of the standard solution.

System performance: When the procedure is run with 15 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cortisone acetate are not less than 10,000 and not more than 1.3, respectively.

System repeatability: When the test is repeated 3 times with 15 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cortisone acetate is not more than 5.0%.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (0.5 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (0.5 g).

Assay Dissolve about 10 mg each of Cortisone Acetate and Cortisone Acetate RS, previously dried and accurately weighed, in 50 mL of methanol, add exactly 5 mL each of the internal standard solution, then add methanol to make 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cortisone acetate to that of the internal standard.

$$\text{Amount (mg) of cortisone acetate } (C_{23}H_{30}O_6) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Cortisone Acetate RS taken

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (3 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (13:7).

Flow rate: Adjust so that the retention time of cortisone acetate is about 12 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cortisone acetate and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cortisone acetate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Absorptive Cream

吸水クリーム

Method of preparation

White Petrolatum	400 g
Cetanol	100 g
White Beeswax	50 g
Sorbitan Sesquioleate	50 g
Lauromacrogol	5 g
Ethyl Parahydroxybenzoate or Methyl Parahydroxybenzoate	1 g
Butyl Parahydroxybenzoate or Propyl Parahydroxybenzoate	1 g
Purified Water or Purified Water in Containers	a sufficient quantity
	To make 1000 g

Melt White Petrolatum, Cetanol, White Beeswax, Sorbitan Sesquioleate and Lauromacrogol by heating on a water bath, mix and maintain at about 75°C. Add Methyl Parahydroxybenzoate or Ethyl Parahydroxybenzoate and Propyl Parahydroxybenzoate or Butyl Parahydroxybenzoate to Purified Water or Purified Water in Containers, dissolve by warming at 80°C. Combine both solutions, mix to make emulsion, cool, and stir thoroughly until it congeals.

Description Absorptive Cream is white in color and is lustrous. It has a slightly characteristic odor.

Containers and storage Containers—Tight containers.

Hydrophilic Cream

親水クリーム

Method of preparation

White Petrolatum	250 g
Stearyl Alcohol	200 g
Propylene Glycol	120 g
Polyoxyethylene hydrogenated castor oil 60	40 g
Glycerin Monostearate	10 g
Methyl Parahydroxybenzoate	1 g
Propyl Parahydroxybenzoate	1 g
Purified Water or Purified Water in Containers	a sufficient quantity
To make	1000 g

Melt White Petrolatum, Stearyl Alcohol, polyoxyethylene hydrogenated castor oil 60 and Glycerin Monostearate by heating on a water bath, stir, and keep temperature of the mixture at about 75°C. To Propylene Glycol add Methyl Parahydroxybenzoate and Propyl Parahydroxybenzoate, melt by warming if necessary, dissolve in Purified Water or Purified Water in Containers, and warm to about 75°C. Add this solution to the above mixture, stir to form emulsion, cool, and stir thoroughly until it congeals.

Description Hydrophilic Cream is white in color. It has a slight, characteristic odor.

Containers and storage Containers—Tight containers.

Cresol

クレゾール

C_7H_8O: 108.14

Cresol is a mixture of isomeric cresols.

Description Cresol is a clear, colorless or yellow to yellow-brown liquid. It has a phenol-like odor.

It is miscible with ethanol (95) and with diethyl ether.

It is sparingly soluble in water.

It dissolves in sodium hydroxide TS.

A saturated solution of Cresol is neutral to bromocresol purple TS.

It is a highly refractive liquid.

It becomes dark brown by light or on aging.

Identification To 5 mL of a saturated solution of Cresol add 1 to 2 drops of dilute iron (III) chloride TS: a blue-purple color develops.

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: 1.032 – 1.041

Purity **(1)** Hydrocarbons—Dissolve 1.0 mL of Cresol in 60 mL of water: the solution shows no more turbidity than that produced in the following control solution.

Control solution: To 54 mL of water add 6.0 mL of 0.005 mol/L sulfuric acid VS and 1.0 mL of barium chloride TS, and after thorough shaking, allow to stand for 5 minutes.

(2) Sulfur compounds—Transfer 20 mL of Cresol in a 100-mL conical flask, place a piece of moistened lead (II) acetate paper on the mouth of the flask, and warm for 5 minutes on a water bath: the lead (II) acetate paper may develop a yellow color, but neither a brown nor a dark tint.

Distilling range ⟨*2.57*⟩ 196 – 206°C, not less than 90 vol%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cresol Solution

クレゾール水

Cresol Solution contains not less than 1.25 vol% and not more than 1.60 vol% of cresol.

Method of preparation

Saponated Cresol Solution	30 mL
Water, Purified Water or Purified Water in Containers	a sufficient quantity
To make	1000 mL

Prepare by mixing the above ingredients.

Description Cresol Solution is a clear or slightly turbid, yellow solution. It has the odor of cresol.

Identification Shake 0.5 mL of the oily layer obtained in the Assay with 30 mL of water, filter, and perform the following tests using this filtrate as the sample solution:

(1) To 5 mL of the sample solution add 1 to 2 drops of iron (III) chloride TS: a blue-purple color develops.

(2) To 5 mL of the sample solution add 1 to 2 drops of bromine TS: a light yellow, flocculent precipitate is produced.

Assay Transfer 200 mL of Cresol Solution, exactly measured, to a 500-mL distilling flask. Add 40 g of sodium chloride and 3 mL of dilute sulfuric acid, and connect the distilling apparatus with the distilling flask, and distil into a cassia flask which contains 30 g of powdered sodium chloride and 3 mL of kerosene, exactly measured, until the distillate measures 90 mL. Draw off the water from the condenser, and continue the distillation until water vapor begins to come out of the tip of the condenser. Shake often the cassia flask in warm water to dissolve the sodium chloride, and allow to stand for 15 minutes. After cooling to 15°C, add a saturated solution of sodium chloride, and allow to stand for more than 3 hours with occasional shaking. Allow to stand for 1 to 2 minutes with gentle shaking to combine the separated oil drops with the oil layer. The difference between the number of mL of the oil layer measured and 3 mL represents the amount (mL) of cresol.

Containers and storage Containers—Tight containers.

Saponated Cresol Solution

クレゾール石ケン液

Saponated Cresol Solution contains not less than 42 vol% and not more than 52 vol% of cresol.

Method of preparation

Cresol	500 mL
Fixed Oil	300 mL
Potassium Hydroxide	a suitable quantity
Water, Purified Water or Purified Water in Containers	a sufficient quantity
	To make 1000 mL

Dissolve Potassium Hydroxide, in required quantity for saponification, in a sufficient quantity of Water, Purified Water or Purified Water in Containers, add this solution to fixed oil, previously warmed, add a sufficient quantity of Ethanol, if necessary, heat in a water bath by thorough stirring, and continue the saponification. After complete saponification, add Cresol, stir thoroughly until the mixture becomes clear, and add sufficient Water, Purified Water or Purified Water in Containers to make 1000 mL. A corresponding amount of Sodium Hydroxide may be used in place of Potassium Hydroxide.

Description Saponated Cresol Solution is a yellow-brown to red-brown, viscous liquid. It has the odor of cresol.

It is miscible with water, with ethanol (95) and with glycerin.

It is alkaline.

Identification Proceed as directed in the Identification under Cresol, using the distillate in the Purity (3).

Purity **(1)** Alkalinity—Mix well 0.50 mL of Saponated Cresol Solution with 10 mL of neutralized ethanol, add 2 to 3 drops of phenolphthalein TS and 0.10 mL of 1 mol/L hydrochloric acid VS: no red color develops.

(2) Unsaponified matter—To 1.0 mL of Saponated Cresol Solution add 5 mL of water, and shake: the solution is clear.

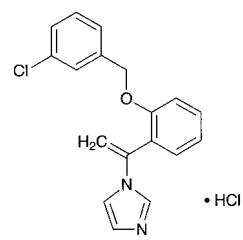
(3) Cresol fraction—Transfer 180 mL of Saponated Cresol Solution to a 2000-mL distilling flask, add 300 mL of water and 100 mL of dilute sulfuric acid, and distil with steam until the distillate becomes clear. Draw off the water from the condenser, and continue the distillation until water vapor begins to come out of the tip of the condenser. Cool the condenser again, and continue distillation for 5 minutes. Dissolve 20 g of sodium chloride per 100 mL of the distillate, allow to stand, and collect the separated clear oil layer. After adding about 15 g of powdered calcium chloride for drying in small portions with frequent shaking, allow to stand for 4 hours. Filter, and distil exactly 50 mL of the filtrate: the distillate is not less than 43 mL between 196°C and 206°C.

Assay Transfer 5 mL of Saponated Cresol Solution, exactly measured, to a 500-mL distilling flask, holding the pipet vertically for 15 minutes to draw off the solution into the flask. Add 200 mL of water, 40 g of sodium chloride and 3 mL of dilute sulfuric acid, connect the distilling apparatus with the distilling flask, and distil into a cassia flask which contains 30 g of powdered sodium chloride and exactly 3 mL of kerosene, until the distillate reaches 90 mL. Draw off the water from the condenser, and continue the distillation until water vapor begins to come out of the tip of the condenser. Allow the cassia flask to stand in warm water for 15 minutes to dissolve the sodium chloride with frequent shaking. Cool to 15°C, add a saturated solution of sodium chloride, and allow to stand for more than 3 hours with occasional shaking. Allow to stand for 1 to 2 minutes with gentle shaking, and combine the separated oil drops with the oil layer. The volume (mL) subtracted 3 (mL) from the oil layer measured represents the amount (mL) of cresol.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Croconazole Hydrochloride

クロコナゾール塩酸塩



$C_{18}H_{15}ClN_2O.HCl$: 347.24
1-{1-[2-(3-Chlorobenzyloxy)phenyl]vinyl}-1*H*-imidazole monohydrochloride
[*77174-66-4*]

Croconazole Hydrochloride, when dried, contains not less than 98.5% of croconazole hydrochloride ($C_{18}H_{15}ClN_2O.HCl$).

Description Croconazole Hydrochloride occurs as white to pale yellowish white, crystals or crystalline powder.

It is very soluble in water, freely soluble in methanol, in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

Identification **(1)** Determine the absorption spectrum of a solution of Croconazole Hydrochloride in methanol (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Croconazole Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.05 g of Croconazole Hydrochloride in 10 mL of water, add 2 mL of sodium hydroxide TS and 20 mL of diethyl ether, and shake. Wash the separated aqueous layer with two 10-mL portions of diethyl ether, and acidify the solution with 2 mL of dilute nitric acid: the solution responds to the Qualitative Tests <*1.09*> for chloride.

Melting point <*2.60*> 148 – 153°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Croconazole Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Croconazole Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution

as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane, methanol and ammonia solution (28) (30:15:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and the spot of the starting point from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 60°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Croconazole Hydrochloride, previously dried, dissolve in 10 mL of acetic acid (100), add 40 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS [indicator: 1 to 2 drops of a solution of malachite green oxalate in acetic acid (100) (1 in 100)] until the color of the solution changes from blue-green through green to yellow-green. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.72 mg of $C_{18}H_{15}ClN_2O.HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Crospovidone

クロスポビドン

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Crospovidone is a cross-linked polymer of 1-vinyl-2-pyrrolidone.

It contains not less than 11.0% and not more than 12.8% of nitrogen (N: 14.01), calculated on the dried basis.

Two types of Crospovidone are available, depending on the particle size: type A and type B.

◆The label states the type.◆

◆**Description** Crospovidone occurs as a white to pale yellowish powder.

It is practically insoluble in water, in methanol and in ethanol (99.5).

It is hygroscopic.◆

Identification **(1)** Suspend 1 g of Crospovidone in 10 mL of water, add 0.1 mL of iodine TS, shake for 30 seconds, then add 1 mL of starch TS, and shake: a blue color is not produced within 30 seconds.

(2) When add 0.1 g of Crospovidone to 10 mL of water, shake to suspend, and allow the suspension to stand, a clear liquid is not produced within 15 minutes.

Particle size Weigh accurately about 20 g of Crospovidone, place in a 1000-mL conical flask, add 500 mL of water, shake for 30 minutes, and pour onto an accurately tared No. 235 (63 μm) sieve, previously washed with hot water and dried at 105°C for a night, and wash the residue with water until the passing water is clear. Dry the residue together with the sieve in a drying machine at 105°C for 5 hours without air-circulation. After cooling down in a desiccator for 30 minutes, weigh the mass of the residue with sieve, and calculate the amount of the residue on the sieve by the following equation: Type A is more than 15%, and type B is not more than 15%.

Amount (%) of the residue of Crospovidone on No. 235 (63 μm) sieve
$= (M_1 - M_3)/M_2 \times 100$

M_1: The mass (g) of the residue with sieve after 5 hours drying
M_2: Amount (g) of Crospovidone taken, calculated on the dried basis
M_3: Mass (g) of the sieve

Purity ◆**(1)** Heavy metals <1.07>—Proceed with 2.0 g of Crospovidone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).◆

(2) Water-soluble substances—Place 25.0 g of Crospovidone in a 400-mL beaker, add 200 mL of water, and stir for 1 hour. Transfer the suspension to a 250-mL volumetric flask, rinsing with water, and dilute to volume with water. Allow the bulk of the solids to settle. Filter about 100 mL of the almost clear supernatant liquid through a 0.45 μm membrane filter, protected by superimposing a 3 μm membrane filter. Transfer exactly 50 mL of the clear filtrate to a tared 100-mL beaker, evaporate to dryness and dry at 105 – 110°C for 3 hours: the mass of the residue is not more than 75 mg.

(3) 1-Vinyl-2-pyrrolidone—To 1.250 g of Crospovidone add exactly 50 mL of methanol, and shake for 60 minutes. Leave bulk to settle, filter through a 0.2 μm membrane filter, and use the filtrate as the sample solution. Separately, dissolve 50 mg of 1-vinyl-2-pyrrolidone in methanol to make exactly 100 mL. Pipet 1 mL of this solution, and add methanol to make exactly 100 mL. To exactly 5 mL of this solution add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions: the peak area of 1-vinyl-2-pyrrolidone obtained from the sample solution is not larger than that obtained from the standard solution (not more than 10 ppm).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: Two stainless steel columns, one is 4 mm in inside diameter and 25 mm in length and the other is 4 mm in inside diameter and 250 mm in length, they are packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter), and used them as the pre-column and the separation column, respectively.

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (9:1).

Flow rate: 1.0 mL per minute.

Washing of pre-column: After each injection of the sample solution, wash the pre-column by passing the mobile phase backwards, at the same flow rate as applied in the test, for 30 minutes.

System suitability—

System performance: Dissolve 10 mg of 1-vinyl-2-pyrrolidone and 0.50 g of vinyl acetate in methanol to make 100 mL. To 1 mL of this solution add the mobile phase to make 100 mL. When the procedure is run with 50 μL of this solu-

tion under the above operating conditions, 1-vinyl-2-pyrrolidone and vinyl acetate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of 1-vinyl-2-pyrrolidone is not more than 2.0%.

(4) Peroxides—

Method 1: Apply to the sample labeled as type A. Suspend 4.0 g of Crospovidone in 100 mL of water, and use as the sample suspension. To 25 mL of the sample suspension add 2 mL of titanium (III) chloride-sulfuric acid TS, allow to stand for 30 minutes, and filter. Determine the absorbance of the filtrate at 405 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using the control, prepared by filtrating the sample suspension and adding 2 mL of diluted sulfuric acid (13 in 100) to 25 mL of this filtrate: not more than 0.35 (not more than 400 ppm expressed as hydrogen peroxide).

Method 2: Apply to the sample labeled as type B. Suspend 2.0 g of Crospovidone in 50 mL of water, and use as the sample suspension. To 10 mL of the sample suspension add water to make 25 mL, add 2 mL of titanium (III) chloride-sulfuric acid TS, allow to stand for 30 minutes, and filter. Determine the absorbance of the filtrate at 405 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using the control, prepared by filtrating the sample suspension, adding water to 10 mL of this filtrate to make 25 mL and 2 mL of diluted sulfuric acid (13 in 100): not more than 0.35 (not more than 1000 ppm expressed as hydrogen peroxide).

Loss on drying <*2.41*> Not more than 5.0% (0.5 g, 105°C, constant mass).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g of Crospovidone, place in a Kjeldahl flask, add 5 g of a powdered mixture of 33 g of potassium sulfate, 1 g of copper (II) sulfate pentahydrate and 1 g of titanium (IV) oxide, and 3 glass beads. Wash any adhering particles from the neck into the flask with a small quantity of water. Add 7 mL of sulfuric acid, allowing it to run down the inside wall of the flask. Gradually heat the flask until the solution has a clear, yellowish-green color, and the inside wall of the flask is free from carbonized material, and then heat for a further 45 minutes. After cooling, cautiously add 20 mL of water, and connect the flask to the distillation apparatus previously washed by passing steam through it. To the absorption flask add 30 mL of a solution of boric acid (1 in 25), 3 drops of bromocresol green-methyl red TS and sufficient water to immerse the lower end of the condenser tube. Add 30 mL of a solution of sodium hydroxide (21 in 50) through a funnel, cautiously rinse the funnel with 10 mL of water, immediately close the clamp attached to the rubber tube, then start the distillation with steam to obtain 80 – 100 mL of distillate. Remove the absorption flask from the lower end of the condenser tube, rinsing the end part with a small quantity of water, and titrate <*2.50*> the distillate with 0.025 mol/L sulfuric acid VS until the color of the solution changes from green through pale grayish-blue to pale grayish red-purple. Carry out a blank determination and make any necessary correction.

Each mL of 0.025 mol/L sulfuric acid VS
= 0.7003 mg of N

Containers and storage Containers—Tight containers.

Cyanamide

シアナミド

CH_2N_2: 42.04
Aminonitrile
[*420-04-2*]

Cyanamide contains not less than 97.0% and not more than 101.0% of cyanamide (CH_2N_2), calculated on the anhydrous basis.

Description Cyanamide occurs as white, crystals or crystalline powder.

It is very soluble in water, in methanol, in ethanol (99.5) and in acetone.

The pH of a solution of 1.0 g of Cyanamide in 100 mL of water is between 5.0 and 6.5.

It is hygroscopic.

Melting point: about 46°C

Identification **(1)** To 1 mL of a solution of Cyanamide (1 in 100) add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS and 0.2 mL of sodium hydroxide TS: a deep red color develops.

(2) Drop one or two drops of a solution of Cyanamide in acetone (1 in 100) onto a potassium bromide disk prepared as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and air-dry the disk. Determine the infrared absorption spectrum of the disk as directed in the film method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Cyanamide in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <*1.07*>—Proceed with 2.0 g of Cyanamide according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Water <*2.48*> Not more than 1.0% (1 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 1 g of Cyanamide, and dissolve in water to make exactly 250 mL. Pipet 15 mL of this solution, add 2 to 3 drops of dilute nitric acid, 10 mL of ammonia TS and exactly 50 mL of 0.1 mol/L silver nitrate VS, and allow to stand for 15 minutes with occasional shaking. Add water to make exactly 100 mL, filter, discard the first 20 mL of the filtrate, and pipet 50 mL of the subsequent filtrate. After neutralizing this solution with dilute nitric acid, add 3 mL of dilute nitric acid, and titrate <*2.50*> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

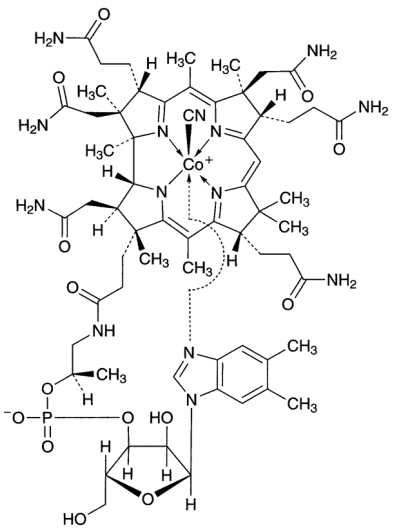
Each mL of 0.1 mol/L silver nitrate VS
= 2.102 mg of CH_2N_2

Containers and storage Containers—Tight containers.
Storage—In a cold place.

Cyanocobalamin

Vitamin B_{12}

シアノコバラミン



$C_{63}H_{88}CoN_{14}O_{14}P$: 1355.37
*Co*α-[α-(5,6-Dimethyl-1*H*-benzimidazol-1-yl)]-*Co*β-cyanocobamide
[*68-19-9*]

Cyanocobalamin contains not less than 96.0% and not more than 102.0% of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$), calculated on the dried basis.

Description Cyanocobalamin occurs as dark red, crystals or powder.

It is sparingly soluble in water, and slightly soluble in ethanol (99.5).

It is hygroscopic.

Identification **(1)** Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cyanocobalamin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Mix 1 mg of Cyanocobalamin with 50 mg of potassium hydrogen sulfate, and fuse by igniting. Cool, break up the mass with a glass rod, add 3 mL of water, and dissolve by boiling. Add 1 drop of phenolphthalein TS, then add dropwise sodium hydroxide TS until a light red color just develops. Add 0.5 g of sodium acetate trihydrate, 0.5 mL of dilute acetic acid and 0.5 mL of a solution of disodium 1-nitroso-2-naphthol-3,6-disulfonate (1 in 500): a red to orange-red color is immediately produced. Then add 0.5 mL of hydrochloric acid, and boil for 1 minute: the red color does not disappear.

(3) Transfer 5 mg of Cyanocobalamin to a 50-mL distilling flask, dissolve in 5 mL of water, and add 2.5 mL of hypophosphorous acid. Connect the flask with a short condenser, and dips its tip into a test tube containing 1 mL of a solution of sodium hydroxide (1 in 50). Heat gently for 10 minutes, then distil 1 mL into a test tube. To the test tube add 4 drops of a saturated solution of ammonium iron (II) sulfate hexahydrate, shake gently, then add about 30 mg of sodium fluoride, and heat the contents to boil. Immediately add dropwise diluted sulfuric acid (1 in 7) until a clear solution results, then add 3 to 5 drops more of diluted sulfuric acid (1 in 7): a blue to blue-green color develops.

pH <*2.54*> Dissolve 0.10 g of Cyanocobalamin in 20 mL of water: the pH of this solution is between 4.2 and 7.0.

Purity **(1)** Clarity and color of solution—Dissolve 20 mg of Cyanocobalamin in 10 mL of water: the solution is clear and red in color.

(2) Related substances—Conduct this procedure using light-resistant vessels. Dissolve 10 mg of Cyanocobalamin in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peak other than cyanocobalamin obtained from the sample solution is not larger than the peak area of cyanocobalamin obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 361 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 10 g of anhydrous disodium hydrogen phosphate in 1000 mL of water, and adjust to pH 3.5 with phosphoric acid. To 147 mL of this solution add 53 mL of methanol.

Flow rate: Adjust so that the retention time of cyanocobalamin is about 7 minutes.

Time span of measurement: About 4 times as long as the retention time of cyanocobalamin, beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution, add the mobile phase to make 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of cyanocobalamin obtained with 20 μL of this solution is equivalent to 7 to 13% of that obtained with 20 μL of the solution for system suitability test.

System performance: Perform this procedure quickly after the solution is prepared. To 25 mg of cyanocobalamin add 10 mL of water, and warm, if necessary, to dissolve. After cooling, add 0.5 mL of sodium toluenesulfonchloramide TS, 0.5 mL of 0.05 mol/L hydrochloric acid TS and water to make 25 mL, mix, and allow the solution to stand for 5 minutes. To 1 mL of the solution add the mobile phase to make 10 mL. When the procedure is run with 20 μL of the solution under the above operating conditions, two principal peaks appear with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of cyanocobalamin is not more than 3.0%.

Loss on drying <*2.41*> Not more than 12% (50 mg, in vacuum at a pressure not exceeding 0.67 kPa, phosphorus (V)

oxide, 100°C, 4 hours).

Assay Weigh accurately about 20 mg each of Cyanocobalamin and Cyanocobalamin RS (previously determine the loss on drying <2.41> under the same conditions as Cyanocobalamin), dissolve in water to make exactly 1000 mL, respectively, and use these solutions as the sample solution and the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution, at 361 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$) = $M_S \times A_T/A_S$

M_S: Amount (mg) of Cyanocobalamin RS taken, calculated on the dried basis

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Cyanocobalamin Injection

Vitamin B_{12} Injection

シアノコバラミン注射液

Cyanocobalamin Injection is an aqueous injection. It contains not less than 95.0% and not more than 115.0% of the labeled amount of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$: 1355.37).

Method of preparation Prepare as directed under Injections, with Cyanocobalamin.

Description Cyanocobalamin Injection is a clear, light red to red liquid.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 277 nm and 279 nm, between 360 nm, and 362 nm and between 548 nm and 552 nm. Determine the absorbances, A_1 and A_2, of this solution at the wavelengths of maximum absorption between 360 nm and 362 nm, and between 548 nm and 552 nm, respectively: the ratio A_2/A_1 is not less than 0.29 and not more than 0.32.

Bacterial endotoxins <4.01> Less than 0.30 EU/μg.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> It meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Cyanocobalamin Injection, equivalent to about 2 mg of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$), add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Cyanocobalamin RS (previously determine the loss on drying <2.41> under the same conditions as Cyanocobalamin), add water to make exactly 1000 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cyanocobalamin.

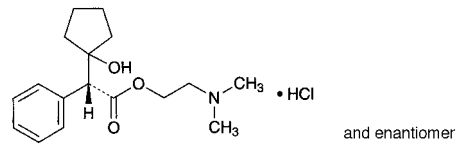
Amount (mg) of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$) = $M_S \times A_T/A_S \times 1/10$

M_S: Amount (mg) of Cyanocobalamin RS taken, calculated on the dried basis

Containers and storage Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant.

Cyclopentolate Hydrochloride

シクロペントラート塩酸塩



and enantiomer

$C_{17}H_{25}NO_3.HCl$: 327.85
2-(Dimethylamino)ethyl (2*RS*)-2-(1-hydroxycyclopentyl)phenylacetate monohydrochloride
[*5870-29-1*]

Cyclopentolate Hydrochloride, when dried, contains not less than 98.5% of cyclopentolate hydrochloride ($C_{17}H_{25}NO_3.HCl$).

Description Cyclopentolate Hydrochloride occurs as a white crystalline powder. It is odorless, or has a characteristic odor.

It is very soluble in water, freely soluble in ethanol (95), in acetic acid (100) and in chloroform, sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Identification **(1)** To 1 mL of a solution of Cyclopentolate Hydrochloride (1 in 100) add 1 mL of Reinecke salt TS: a light red precipitate is formed.

(2) Dissolve 0.2 g of Cyclopentolate Hydrochloride in 2 mL of water, add 2 mL of sodium hydroxide TS, and boil for 1 minute. After cooling, add 2 drops of nitric acid: a phenylacetic acid-like odor is perceptible.

(3) Determine the infrared absorption spectrum of Cyclopentolate Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Cyclopentolate Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 0.20 g of Cyclopentolate Hydrochloride in 20 mL of water: the pH of this solution is between 4.5 and 5.5.

Melting point <2.60> 135 – 138°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Cyclopentolate Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Cyclopentolate Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.20 g of Cyclopentolate Hydrochloride in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 20 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chro-

matography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, *n*-butyl acetate, water and ammonia solution (28) (100:60:23:17) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of sulfuric acid in ethanol (99.5) (1 in 10) on the plate, and heat at 120°C for 30 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.05% (1 g).

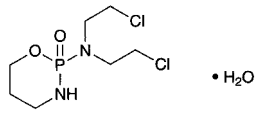
Assay Weigh accurately about 0.5 g of Cyclopentolate Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (4:1), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-green to yellow-green (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.79 mg of $C_{17}H_{25}NO_3.HCl$

Containers and storage Containers—Tight containers.

Cyclophosphamide Hydrate

シクロホスファミド水和物



$C_7H_{15}Cl_2N_2O_2P.H_2O$: 279.10
N,*N*-Bis(2-chloroethyl)-3,4,5,6-tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-amine 2-oxide monohydrate
[*6055-19-2*]

Cyclophosphamide Hydrate contains not less than 97.0% of cyclophosphamide hydrate ($C_7H_{15}Cl_2N_2O_2P.H_2O$).

Description Cyclophosphamide Hydrate occurs as white, crystals or crystalline powder. It is odorless.

It is very soluble in acetic acid (100), freely soluble in ethanol (95), in acetic anhydride and in chloroform, and soluble in water and in diethyl ether.

Melting point: 45 – 53°C

Identification **(1)** Dissolve 0.1 g of Cyclophosphamide Hydrate in 10 mL of water, and add 5 mL of silver nitrate TS: no precipitate is produced. Then boil this solution: a white precipitate is produced. Collect the precipitate, and add dilute nitric acid to a portion of this precipitate: it does not dissolve. Add excess ammonia TS to another portion of the precipitate: it dissolves.

(2) Add 1 mL of diluted sulfuric acid (1 in 25) to 0.02 g of Cyclophosphamide Hydrate, and heat until white fumes are evolved. After cooling, add 5 mL of water, and shake. Neutralize with ammonia TS, then acidify with dilute nitric acid: this solution responds to the Qualitative Tests <*1.09*> (2) for phosphate.

Purity **(1)** Clarity and color of solution—Dissolve 0.20 g of Cyclophosphamide Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Chloride <*1.03*>—Perform the test with 0.40 g of Cyclophosphamide Hydrate at a temperature not exceeding 20°C. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Cyclophosphamide Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Water <*2.48*> 5.5 – 7.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.3 g of Cyclophosphamide Hydrate, add 15 mL of hydrogen chloride-ethanol TS, and heat in a water bath under a reflux condenser for 3.5 hours while protecting from moisture. Distil the ethanol under reduced pressure. Dissolve the residue in 40 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> with 0.1 mol/L perchloric acid-dioxane VS (indicator: 2 drops of crystal violet TS) until the color of the solution changes from blue through green to yellow. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid-dioxane VS
= 13.96 mg of $C_7H_{15}Cl_2N_2O_2P.H_2O$

Containers and storage Containers—Tight containers.
Storage—Not exceeding 30°C.

Cyclophosphamide Tablets

シクロホスファミド錠

Cyclophosphamide Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of cyclophosphamide hydrate ($C_7H_{15}Cl_2N_2O_2P.H_2O$: 279.10).

Method of preparation Prepare as directed under Tablets, with Cyclophosphamide Hydrate.

Identification To Cyclophosphamide Tablets add 1 mL of water for every 53 mg of Cyclophosphamide Hydrate, shake vigorously for 5 minutes, add 6 mL of methanol for every 53 mg of Cyclophosphamide Hydrate, and shake vigorously for 10 minutes. To this solution add methanol so that each mL contains about 5.3 mg of Cyclophosphamide Hydrate, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard not less than 3 mL of the first filtrate, and use the subsequent filtrate as the sample solution. Separately, dissolve 53 mg of cyclophosphamide hydrate for assay in 10 mL of a mixture of methanol and water (9:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol and water (8:1) to a distance of about 10 cm, and air-dry the plate. Heat the plate at 130°C for 15 minutes. After cooling, spray evenly ninhydrin-butanol TS on the plate, and after air-drying heat at 130°C for 10 minutes: the principal spot obtained from the sample solution and the spot obtained from the standard solution show a red-purple color and the same *R*f value.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the

Content uniformity test.

To 1 tablet of Cyclophosphamide Tablets add $3V/5$ mL of a mixture of water and methanol (3:2), and shake vigorously to homogenously disperse the tablet. To this solution add a mixture of water and methanol (3:2) to make exactly V mL so that each mL contains about 1.1 mg of cyclophosphamide hydrate ($C_7H_{15}Cl_2N_2O_2P.H_2O$), and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of cyclophosphamide hydrate ($C_7H_{15}Cl_2N_2O_2P.H_2O$)
$= M_S \times A_T/A_S \times V/50$

M_S: Amount (mg) of cyclophosphamide hydrate for assay taken

Dissolution ⟨*6.10*⟩ When the test is performed at 100 revolutions per minute according to the Basket method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Cyclophosphamide Tablets is not less than 80%.

Start the test with 1 tablet of Cyclophosphamide Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 59 μg of cyclophosphamide hydrate ($C_7H_{15}Cl_2N_2O_2P.H_2O$) and use this solution as the sample solution. Separately, weigh accurately about 30 mg of cyclophosphamide hydrate for assay, and dissolve in water to make exactly 50 mL. Pipet 2 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions and determine the peak areas, A_T and A_S, of cyclophosphamide in each solution.

Dissolution rate (%) with respect to the labeled amount of cyclophosphamide hydrate ($C_7H_{15}Cl_2N_2O_2P.H_2O$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 180$

M_S: Amount (mg) of cyclophosphamide hydrate for assay taken
C: Labeled amount (mg) of cyclophosphamide hydrate ($C_7H_{15}Cl_2N_2O_2P.H_2O$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clophosphamide are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clophosphamide is not more than 2.0%.

Assay To 10 tablets of Cyclophosphamide Tablets add $13V/20$ mL of a mixture of water and methanol (3:2), and shake vigorously to homogenously disperse the tablets. To this solution add a mixture of water and methanol (3:2) to make exactly V mL so that each mL contains about 2.7 mg of cyclophosphamide hydrate ($C_7H_{15}Cl_2N_2O_2P.H_2O$), and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, pipet 4 mL of the subsequent filtrate, add a mixture of water and methanol (3:2) to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 53 mg of cyclophosphamide hydrate for assay, dissolve in a mixture of water and methanol (3:2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak area, A_T and A_S, of cyclophosphamide in each solution.

Amount (mg) of cyclophosphamide hydrate ($C_7H_{15}C_{l2}N_2O_2P.H_2O$)
$= M_S \times A_T/A_S \times V/200$

M_S: Amount (mg) of cyclophosphamide hydrate for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and methanol (3:2).

Flow rate: Adjust so that the retention time of cyclophosphamide is about 10 minutes.

System suitability—

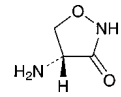
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cyclophosphamide are not less than 4000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cyclophosphamide is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cycloserine

サイクロセリン



$C_3H_6N_2O_2$: 102.09
(4*R*)-4-Aminoisoxazolidin-3-one
[*68-41-7*]

Cycloserine contains not less than 950 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the dried basis. The potency of Cycloserine is expressed as mass (potency) of cycloserine ($C_3H_6N_2O_2$).

Description Cycloserine occurs as white to light yellowish white, crystals or crystalline powder.

It is soluble in water, and sparingly soluble in ethanol (95).

Identification Determine the infrared absorption spectrum of Cycloserine, previously dried, as directed in the potassium

bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Cycloserine RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +108 – +114° (2.5 g calculated on the dried basis, 2 mol/L sodium hydroxide TS, 50 mL, 100 mm).

pH ⟨*2.54*⟩ Dissolve 1.0 g of Cycloserine in 20 mL of water: the pH of the solution is between 5.0 and 7.4.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Cycloserine according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Condensation products—Dissolve 20 mg of Cycloserine in sodium hydroxide TS to make exactly 50 mL, and determine the absorbance of this solution at 285 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: not more than 0.8.

Loss on drying ⟨*2.41*⟩ Not more than 1.5% (0.5 g, reduced pressure, 60°C, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.5% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 6.0 to 6.1 after sterilization.

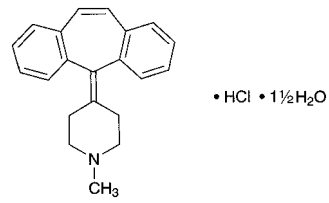
(iii) Standard solutions—Weigh accurately an amount of Cycloserine RS, previously dried at 60°C for 3 hours under reduced pressure of not exceeding 0.67 kPa, equivalent to about 40 mg (potency), dissolve in water to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 24 hours. Take exactly a suitable amount of the standard stock solution before use, and add phosphate buffer solution (pH 6.0) to make solutions so that each mL contains 100 μg (potency) and 50 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Cycloserine equivalent to about 40 mg (potency), dissolve in water to make exactly 100 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution (pH 6.0) to make solutions so that each mL contains 100 μg (potency) and 50 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

Containers and storage Containers—Well-closed containers.

Cyproheptadine Hydrochloride Hydrate

シプロヘプタジン塩酸塩水和物



$C_{21}H_{21}N.HCl.1\frac{1}{2}H_2O$: 350.88

4-(5*H*-Dibenzo[*a*,*d*]cycloheptene-5-ylidene)-1-methylpiperidine monohydrochloride sesquihydrate [*41354-29-4*]

Cyproheptadine Hydrochloride Hydrate, when dried, contains not less than 98.5% of cyproheptadine hydrochloride ($C_{21}H_{21}N.HCl$: 323.86).

Description Cyproheptadine Hydrochloride Hydrate occurs as a white to pale yellow crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in methanol and in acetic acid (100), soluble in chloroform, sparingly soluble in ethanol (95), slightly soluble in water, and practically insoluble in diethyl ether.

Identification **(1)** Dissolve 0.1 g of Cyproheptadine Hydrochloride Hydrate in 10 mL of methanol, apply 1 drop of this solution on filter paper, air-dry, and examine under ultraviolet light (main wavelength: 254 nm): the solution shows a pale blue fluorescence.

(2) Weigh 0.1 g of Cyproheptadine Hydrochloride Hydrate, transfer to a separator, dissolve in 5 mL of chloroform, add 4 mL of water and 1 mL of sodium carbonate TS, and shake. Transfer the chloroform layer to another separator, and wash with 4 mL of water by shaking well. Filter the chloroform layer through absorbent cotton moistened previously with chloroform, and evaporate the filtrate to dryness. Dissolve the residue in 8 mL of dilute ethanol by warming at 65°C. Rub the inner wall of the container with a glass rod while cooling until crystallization begins, and allow to stand for 30 minutes. Collect the crystals, and dry at 80°C for 2 hours: the crystals melt ⟨*2.60*⟩ between 111°C and 115°C.

(3) Determine the absorption spectrum of a solution of Cyproheptadine Hydrochloride Hydrate in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A saturated solution of Cyproheptadine Hydrochloride Hydrate responds to the Qualitative Tests ⟨*1.09*⟩ (2) for chloride.

Purity **(1)** Acidity—Dissolve 2.0 g of Cyproheptadine Hydrochloride Hydrate in 25 mL of methanol, and add 1 drop of methyl red TS and 0.30 mL of 0.1 mol/L sodium hydroxide VS: a yellow color develops.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Cyproheptadine Hydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying ⟨*2.41*⟩ 7.0 – 9.0% (1 g, in vacuum at a pres-

sure not exceeding 0.67 kPa, 100°C, 5 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

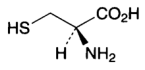
Assay Weigh accurately about 0.5 g of Cyproheptadine Hydrochloride Hydrate, previously dried, and dissolve in 20 mL of acetic acid (100) by warming at 50°C. After cooling, add 40 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.39 mg of $C_{21}H_{21}N.HCl$

Containers and storage Containers—Well-closed containers.

L-Cysteine

L-システイン



$C_3H_7NO_2S$: 121.16
(2*R*)-2-Amino-3-sulfanylpropanoic acid
[*52-90-4*]

L-Cysteine contains not less than 98.5% and not more than 101.0% of L-cysteine ($C_3H_7NO_2S$), calculated on the dried basis.

Description L-Cysteine occurs as white crystals or a white crystalline powder. It has a characteristic odor and a pungent taste.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

It dissolves in 1 mol/L hydrochloric acid TS.

Identification Determine the infrared absorption spectrum of L-Cysteine as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +8.0 – +10.0° (2 g calculated on the dried basis, 1 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH ⟨*2.54*⟩ The pH of a solution prepared by dissolving 1.25 g of L-Cysteine in 50 mL of water is 4.7 to 5.7.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of L-Cysteine in 20 mL of water: the solution is clear and colorless.

(2) Chloride ⟨*1.03*⟩—Dissolve 0.30 g of L-Cysteine in 10 mL of diluted nitric acid (1 in 4), add 10 mL of hydrogen peroxide (30), heat for 20 minutes in a boiling water bath, cool, and then add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.041%).

(3) Sulfate ⟨*1.14*⟩—Dissolve 0.6 g of L-Cysteine in 30 mL of water and 3 mL of dilute hydrochloric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.35 mL of 0.005 mol/L sulfuric acid VS add 3 mL of dilute hydrochloric acid and water to make 50 mL. Prepare the test solution and the control solution with 4 mL of barium chloride TS, respectively (not more than 0.028%).

(4) Ammonium ⟨*1.02*⟩—Perform the test with 0.25 g of L-Cysteine, using the distillation under reduced pressure. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of L-Cysteine according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Iron ⟨*1.10*⟩—Prepare the test solution with 1.0 g of L-Cysteine according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Dissolve 0.10 g of L-Cysteine in *N*-ethylmaleimide solution (1 in 50) to make exactly 10 mL, leave for 30 minutes, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 10 mL, pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution (1). Separately, dissolve 0.10 g of L-cystine in 0.5 mol/L hydrochloric acid TS to make exactly 20 mL. Pipet 1 mL of this solution, add water to make 100 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate for 30 minutes at 80°C. Spray the plate evenly with a solution of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) (1 in 100), and then heat at 80°C for 10 minutes: the spot obtained from the sample solution corresponding to the spot obtained from the standard solution (2) is not more intense than the spot from the standard solution (2). Also, the spots other than the principal spot and the spots mentioned above from the sample solution are not more intense than the spot from the standard solution (1).

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 3 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

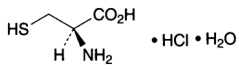
Assay Weigh accurately about 0.2 g of L-Cysteine, place it in a stoppered flask, and dissolve in 20 mL of water. Dissolve 4 g of potassium iodide in this solution, immediately place in ice cold water, add 5 mL of dilute hydrochloric acid and exactly 25 mL of 0.05 mol/L iodine VS, leave in a dark place for 20 minutes, and then titrate ⟨*2.50*⟩ an excess amount of iodine with 0.1 mol/L sodium thiosulfate VS (indicator: starch TS). Perform a blank determination using the same method.

Each mL of 0.05 mol/L iodine VS = 12.12 mg of $C_3H_7NO_2S$

Containers and storage Containers—Tight containers.

L-Cysteine Hydrochloride Hydrate

L-システイン塩酸塩水和物



$C_3H_7NO_2S.HCl.H_2O$: 175.63
(2*R*)-2-Amino-3-sulfanylpropanoic acid monohydrochloride monohydrate
[*7048-04-6*]

L-Cysteine Hydrochloride Hydrate contains not less than 98.5% and not more than 101.0% of L-cysteine hydrochloride ($C_3H_7NO_2S.HCl$: 157.62), calculated on the dried basis.

Description L-Cysteine Hydrochloride Hydrate occurs as white, crystals or crystalline powder. It has a characteristic odor and a strong acid taste.

It is very soluble in water, and soluble in ethanol (99.5).

It dissolves in 6 mol/L hydrochloric acid TS.

Identification **(1)** Determine the infrared absorption spectrum of L-Cysteine Hydrochloride Hydrate as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) To 10 mL of a solution of L-Cysteine Hydrochloride Hydrate (1 in 50) add 1 mL of hydrogen peroxide (30), heat on a water bath for 20 minutes, and cool: the solution responds to the Qualitative Tests <*1.09*> (2) for chloride.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +6.0 – +7.5° (2 g, calculated on the dried basis, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <*2.54*> The pH of a solution prepared by dissolving 1.0 g of L-Cysteine Hydrochloride Hydrate in 100 mL of water is between 1.3 and 2.3.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 1.0 g of L-Cysteine Hydrochloride Hydrate in 10 mL of water is clear and colorless.

(2) Sulfate <*1.14*>—Dissolve 0.8 g of L-Cysteine Hydrochloride Hydrate in 30 mL of water and 3 mL of dilute hydrochloric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.35 mL of 0.005 mol/L sulfuric acid VS add 3 mL of dilute hydrochloric acid and water to make 50 mL. To both of the test solution and the control solution add 4 mL of barium chloride TS (not more than 0.021%).

(3) Ammonium <*1.02*>—Perform the test with 0.25 g of L-Cysteine Hydrochloride Hydrate using the distillation under reduced pressure. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(4) Heavy metals <*1.07*>—Proceed with 1.0 g of L-Cysteine Hydrochloride Hydrate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Iron <*1.10*>—Prepare the test solution with 1.0 g of L-Cysteine Hydrochloride Hydrate according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(6) Related substances—Dissolve 0.10 g of L-Cysteine Hydrochloride Hydrate in *N*-ethylmaleimide solution (1 in 50) to make 10 mL, allow to stand for 30 minutes, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 10 mL. Pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-Layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Then develop with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) (1 in 100) on the plate, and then heat at 80°C for 10 minutes: the spot other than the principal spot obtained with the sample solution is not more intense than the spot obtained with the standard solution.

Loss on drying <*2.41*> 8.5 – 12.0% (1 g, in vacuum, phosphorus (V) oxide, 20 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

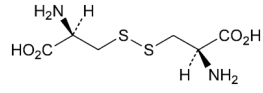
Assay Weigh accurately about 0.25 g of L-Cysteine Hydrochloride Hydrate, place in a glass-stoppered flask, and dissolve in 20 mL of water. Dissolve 4 g of potassium iodide in this solution, soak immediately in ice cold water, add 5 mL of dilute hydrochloric acid and exactly 25 mL of 0.05 mol/L iodine VS, allow to stand for 20 minutes in a dark place, titrate <*2.50*> the excess of iodine with 0.1 mol/L sodium thiosulfate VS (indicator: starch TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L iodine VS
= 15.76 mg of $C_3H_7NO_2S.HCl$

Containers and storage Containers—Tight containers.

L-Cystine

L-シスチン



$C_6H_{12}N_2O_4S_2$: 240.30
3,3′-Disulfanediylbis[(2*R*)-2-aminopropanoic acid]
[*56-89-3*]

L-Cystine, when dried, contains not less than 99.0% and not more than 101.0% of L-cystine ($C_6H_{12}N_2O_4S_2$).

Description L-Cystine occurs as white, crystals or crystalline powder.

It is practically insoluble in water and in ethanol (99.5).

It dissolves in 1 mol/L hydrochloric acid TS.

Identification Determine the infrared absorption spectrum of L-Cystine as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −215 – −225° (after drying, 1 g, 1 mol/L hydrochloric acid TS, 50 mL, 100 mm).

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 1.0 g of L-Cystine in 10 mL of 2 mol/L hydrochloric acid TS is clear and colorless.

(2) Chloride <*1.03*>—Dissolve 0.5 g of L-Cystine in 10 mL of dilute nitric acid, add 10 mL of hydrogen peroxide (30), and heat in a water bath for 10 minutes. After cooling, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <*1.14*>—Dissolve 0.6 g of L-Cystine in 5 mL of dilute hydrochloric acid, add water to make 45 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.35 mL of 0.005 mol/L sulfuric acid VS add 5 mL of dilute hydrochloric acid and water to make 45 mL. To both the test and control solutions add 5 mL of barium chloride TS (not more than 0.028%).

(4) Ammonium <*1.02*>—Perform the test with 0.25 g of L-Cystine, using the distillation under reduced pressure. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <*1.07*>—Proceed with 1.0 g of L-Cystine according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Iron <*1.10*>—Prepare the test solution with 1.0 g of L-Cystine according to Method 3, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Dissolve 0.20 g of L-Cystine in 20 mL of 1 mol/L hydrochloric acid TS, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 10 mL. Pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol and ammonia solution (28) (67:33) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) (1 in 100) on the plate, and heat at 80°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot obtained from the standard solution.

Loss on drying <*2.41*> Not more than 0.3% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

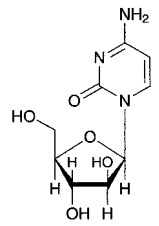
Assay Weigh accurately about 30 mg of L-Cystine, previously dried, and perform the test as directed under Nitrogen Determination <*1.08*>.

Each mL of 0.005 mol/L sulfuric acid VS
= 1.202 mg of $C_6H_{12}N_2O_4S_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Cytarabine

シタラビン



$C_9H_{13}N_3O_5$: 243.22
1-β-D-Arabinofuranosylcytosine
[*147-94-4*]

Cytarabine, when dried, contains not less than 98.5% and not more than 101.0% of cytarabine ($C_9H_{13}N_3O_5$).

Description Cytarabine occurs as white, crystals or crystalline powder.

It is freely soluble in water, soluble in acetic acid (100), and very slightly soluble in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS.

Melting point: about 214°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Cytarabine in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cytarabine as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +154 – +160° (after drying, 0.1 g, water, 10 mL, 100 mm).

pH <*2.54*> Dissolve 0.20 g of Cytarabine in 20 mL of water: the pH of this solution is between 6.5 and 8.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Cytarabine in 10 mL of water: the solution is clear and colorless.

(2) Chloride <*1.03*>—Perform the test with 1.0 g of Cytarabine. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.009%).

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Cytarabine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substances—Dissolve 0.10 g of Cytarabine in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution (1). Pipet 10 mL of the standard solution (1), add water to make exactly 25 mL and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with 1-butanol saturated with water to a distance of about 12 cm, and air-dry the

plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (1), and the number of them which are more intense than the spot from the standard solution (2) is not more than two. Spray evenly acidic potassium permanganate TS on the plate: any spot other than the principal spot does not appear.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.5% (1 g).

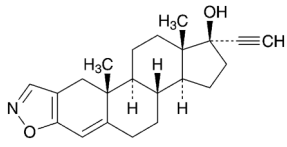
Assay Weigh accurately about 0.2 g of Cytarabine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 12.16 mg of $C_9H_{13}N_3O_5$

Containers and storage Containers—Tight containers.

Danazol

ダナゾール



$C_{22}H_{27}NO_2$: 337.46
17α-Pregna-2,4-dien-20-yno[2,3-*d*]isoxazol-17-ol
[*17230-88-5*]

Danazol, when dried, contains not less than 98.5% and not more than 101.0% of danazol ($C_{22}H_{27}NO_2$).

Description Danazol occurs as a white to pale yellow crystalline powder.

It is soluble in acetone, sparingly soluble in ethanol (99.5), and practically insoluble in water.

Melting point: about 225°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Danazol in ethanol (95) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Danazol RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Danazol as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of Danazol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: +8 – +11° (after drying, 0.25 g, ethanol (99.5), 50 mL, 100 mm).

Purity **(1)** Chloride ⟨*1.03*⟩—To 2.0 g of Danazol add 80 mL of water, shake well, and boil for 5 minutes. After cooling, add water to make 100 mL, and filter through a glass filter (G4). Discard the first 30 mL of the filtrate, take 40 mL of the subsequent filtrate, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Danazol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.20 g of Danazol in 4 mL of acetone, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add acetone to make exactly 200 mL. Pipet 4 mL of this solution, add acetone to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and ethyl acetate (3:2) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and the spot of the starting point obtained from the sample solution are not more intense than the spot obtained from the standard solution.

Loss on drying ⟨*2.41*⟩ Not more than 0.2% (1 g, in vacuum, phosphorous (V) oxide, 60°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 25 mg each of Danazol and Danazol RS, previously dried, dissolve separately in ethanol (95) to make exactly 50 mL. Pipet 2 mL each of these solutions, add ethanol (95) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and determine the absorbances, A_T and A_S, at 285 nm.

Amount (mg) of danazol ($C_{22}H_{27}NO_2$)
= $M_S \times A_T/A_S$

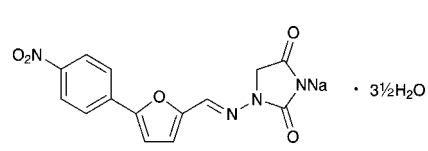
M_S: Amount (mg) of Danazol RS taken

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Dantrolene Sodium Hydrate

ダントロレンナトリウム水和物



$C_{14}H_9N_4NaO_5.3\frac{1}{2}H_2O$: 399.29
Monosodium 3-[5-(4-nitrophenyl)furan-2-ylmethylene]amino-2,5-dioxo-1,3-imidazolidinate hemiheptahydrate
[*14663-23-1*, anhydride]

Dantrolene Sodium Hydrate contains not less than 98.0% of dantrolene sodium ($C_{14}H_9N_4NaO_5$: 336.23), calculated on the anhydrous basis.

Description Dantrolene Sodium Hydrate occurs as a yellowish orange to deep orange, crystalline powder.