

## Glycerol Esters of Fatty Acids

**Definition** Glycerol Esters of Fatty Acids are esters of fatty acids and glycerol or polyglycerol and their derivatives. Glycerol Esters of Fatty Acids include glycerol fatty acid ester, glycerol acetic acid fatty acid ester, glycerol lactic acid fatty acid ester, glycerol citric acid fatty acid ester, glycerol succinic acid fatty acid ester, glycerol diacetyl tartaric acid fatty acid ester, glycerol acetic acid ester, polyglycerol fatty acid ester, and polyglycerol condensed ricinoleic acid ester.

**Description** Glycerol Esters of Fatty Acids occur as colorless to brown powders, flakes, coarse powders, or granular or waxy lumps, or are a colorless to brown semi-fluids or liquids. They are odorless or have a characteristic odor.

**Identification** (1) To about 5 g of Glycerol Ester of Fatty Acid (1.5 g in case of glycerol acetic acid ester), add 50 ml of ethanolic potassium hydroxide TS, heat with a reflux condenser in a water bath for 1 hour, and evaporate the ethanol to an almost dry state. Add 50 ml of diluted hydrochloric acid (1 : 10), shake well, separate the produced fatty acid by extracting three times with 40 ml of petroleum ether - methyl ethyl ketone mixture (7 : 1) each time. Stir the water layer well, add sodium hydroxide solution (1 : 9) until it is almost neutral, and concentrate under reduced pressure in a water bath. Add 20 ml of methanol at about 40 °C, shake well, cool, filter, and evaporate the methanol of the filtrate in a water bath. Perform Thin-Layer Chromatography on 5 µl of the test solution, using a solution (1 : 10) of the residue dissolved in methanol as the test solution, a methanol-glycerol mixture (9 : 1) as the control solution, and an acetone - water mixture (9 : 1) as the developing solvent. In the case of glycerol esters, a brown spot is observed at the same position as the control solution; in the case of polyglycerol ester, a brown spot or a brown band-shaped spot is observed at a position below that of the control solution. For the thin layer plate, use silica gel for thin-layer chromatography dried at 110 °C for 1 hour as the support. Stop the development when the solvent front rises 15 cm above the original line, air-dry, heat at 110 °C for 10 minutes to remove the solvent, cool, spray the thymol - sulfuric acid TS, and heat at 110 °C for 20 minutes to develop the color.

(2) Except in the case of glycerol acetic acid ester, combine the petroleum ether - methyl ethyl ketone layers obtained by separation in test (1) above, and evaporate the solvent. An oily substance or a white to yellowish white solid remains. Add 5 ml of ether to 0.1 g of the residue, and shake. It dissolves.

(3) Except for glycerol fatty acid ester and polyglycerol ester, add 50 ml of water to 5 ml of the test solution of (1) above, and shake. This solution responds to all tests for Acetate in cases of glycerol acetic acid fatty acid ester and glycerol acetic acid ester, to the test for Lactate in the case of glycerol lactic acid fatty acid ester, to test (2) for Citrate in the case of glycerol citric acid fatty acid ester, to the test for Succinate in the case of glycerol succinic acid fatty acid ester, and to all tests for Acetate and for Tartrate in the case of glycerol diacetyl tartaric acid fatty acid ester, respectively.

(4) In the case of polyglycerol condensed ricinoleic acid ester, combine the petroleum ether - methyl ethyl ketone layers obtained by separation in test (1) above, wash this solution twice with 50 ml of water each time, and dehydrate with anhydrous sodium sulfate. Filter the dehydrated liquid and remove the solvent by warming under reduced pressure. Weigh accurately about 1 g of the residue, and proceed as directed under Hydroxyl Value in the Fats and Related Substances Tests. Use about 0.5 g of the residue to measure the acid value. The hydroxyl value is 150 - 170.

**Purity** (1) Acid value

Glycerol fatty acid ester: Not more than 6.0 (Fats and Related Substances Tests).

Glycerol acetic acid fatty acid ester: Not more than 6.0 (Fats and Related Substances Tests).

Glycerol lactic acid fatty acid ester: Not more than 6.0 (Fats and Related Substances Tests).

Glycerol acetic acid ester: Not more than 6.0 (Fats and Related Substances Tests).

Polyglycerol fatty acid ester: Not more than 12 (Fats and Related Substances Tests).

Polyglycerol condensed ricinoleic acid ester: Not more than 12 (Fats and Related Substances Tests).

Glycerol citric acid fatty acid ester: Not more than 100 (Fats and Related Substances Tests).

Glycerol succinic acid fatty acid ester: 60 - 120 (Fats and Related Substances Tests).

Glycerol diacetyl tartaric acid ester: 60 - 120 (Fats and Related Substances Tests).

(2) Heavy metals Not more than 10  $\mu\text{g/g}$  as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(3) Arsenic Not more than 4.0  $\mu\text{g/g}$  as  $\text{As}_2\text{O}_3$  (0.50 g, Method 3, Apparatus B).

(4) Polyoxyethylene Weigh 1.0 g of Glycerol Esters of Fatty Acids, transfer into a 200-ml flask, add 25 ml of ethanolic potassium hydroxide TS, and boil with a ground-glass reflux condenser on a water bath for 1 hour while shaking occasionally.

Evaporate the ethanol on a water bath or under reduced pressure until it becomes almost dry, add 20 ml of diluted sulfuric acid (3 : 100), and shake well while warming. Add 15 ml of ammonium thiocyanate - cobalt nitrate TS, shake well, add 10 ml of chloroform, shake again, and allow to stand. The color of the chloroform layer does not change to blue.

**Residue on Ignition** Not more than 1.5%.