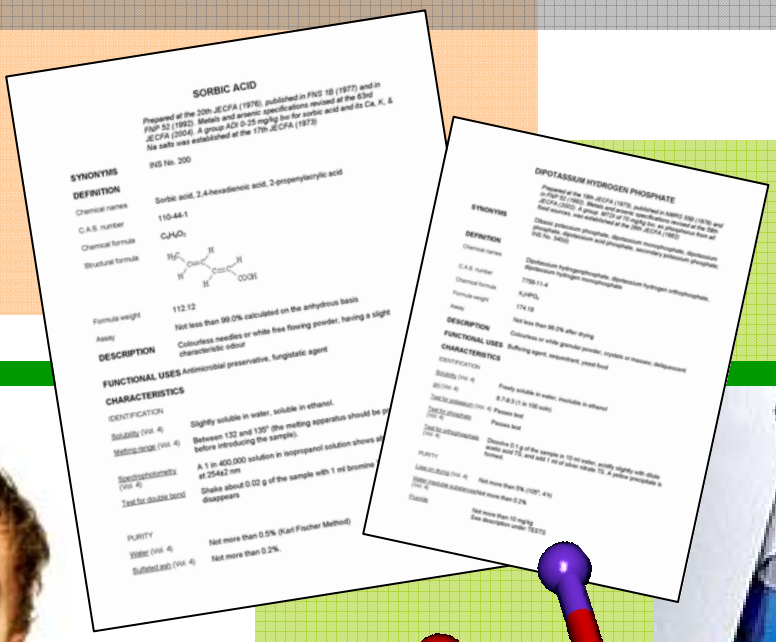
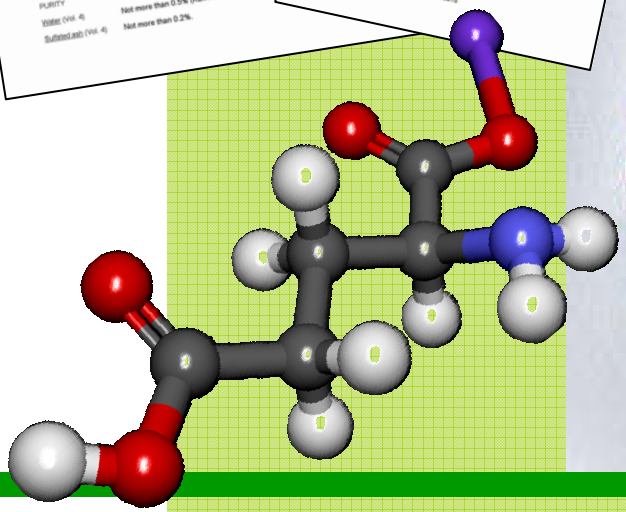
ข้อกำหนดคุณภาพหรือมาตรฐานวัตถุเจือปนอาหารตามประกาศสำนักงานคณะกรรมการอาหารและยาและมาตรฐานโคเด็กซ์ (Codex Advisory Specification for the Identity and Purity of Food Additives 2012)

เล่ม:2





สำนักอาหาร สำนักงานคณะกรรมการอาหารและยา

รายชื่อวัตถุเจือปนอาหารที่มีการกำหนดข้อกำหนดคุณภาพหรือมาตรฐาน (Specification)
โดย The Joint FAO/WHO Expert Committee on Food Additives (JECFA)

INS No.	รายชื่อวัตถุเจือปนอาหาร		ADI[a]	ปีที่กำหนดค่า ADI	หน้า
504(i)	MAGNESIUM CARBONATE	แมกนีเซียมคาร์บอเนต	Not limited	JECFA 1965	399
511	MAGNESIUM CHLORIDE	แมนีเซียมคลอไรด์	Not limited	JECFA 1979	401
625	MAGNESIUM Di-L- GLUTAMATE	แมกนีเซียมได-แอล-กลูตาเมต	Not specified	JECFA 1987	403
580	MAGNESIUM GLUCONATE	แมกนีเซียมกลูโคเนต	Not specified	JECFA 1998	405
528	MAGNESIUM HYDROXIDE	แมกนีเซียมไฮดรอกไซด์	Not limited	JECFA 1965	407
504(ii)	MAGNESIUM HYDROXIDE CARBONATE	แมกนีเซียมไฮดรอกไซด์ คาร์บอเนต	Not specified	JECFA 1979	409
329	MAGNESIUM L-LACTATE	แมกนีเซียมแอล-แลกเตต	Not limited	JECFA 1979	411
530	MAGNESIUM OXIDE	แมกนีเซียมออกไซด์	Not limited	JECFA 1965	413
553(i)	MAGNESIUM SILICATE,SYNTHETIC	แมกนีเซียมซิลิเคต, สังเคราะห์	Not specified	JECFA 1981	415 418
518	MAGNESIUM SULFATE	แมกนีเซียมซัลเฟต	Not specified	JECFA 2007	420
296	MALIC ACID, DL-	กรดมาลิก	Not specified	JECFA 1969	423
965(i)	MALTITOL	มอลทิทอล	Not specified	JECFA 1993	426
965(ii)	MALTITOL SYRUP	มอลทิทอลไซรัป	Not specified	JECFA 1997	429
421	MANNITOL	แมนนิทอล	Not specified	JECFA 1986	432
461	METHYL CELLULOSE	เมทิลเซลลูโลส	Not specified	JECFA 1989	434
465	METHYL ETHYL CELLULOSE	เมทิลเอทิลเซลลูโลส	Not specified	JECFA 1989	438
460(i)	MICROCRYSTALLINE CELLULOSE (CELLULOSE GEL)	ไมโครคริสตอลไลน์เซลลูโลส (เซลลูโลสเจล)	Not specified	JECFA 1998	441
905c	MICROCRYSTALLINE WAX	ไมโครคริสตอลไลน์แวกซ์	0-20	JECFA 1995	455
905a	MINERAL OIL, HIGH VISCOSITY	น้ำมันแร่ชนิดความหนืดสูง	0-20	JECFA 1995	457
905a	MINERAL OIL, MEDIUM AND LOW VISCOSITY, CLASS I	น้ำมันแร่ชนิดความหนืดปานกลางและต่ำ	0-10	JECFA 2002	464
	MODIFIED STARCHS				464
1422	ACETYLATED DISTARCH ADIPATE	อะซิติเลตเตตไดสตาร์ชอะดิเปต	Not specified	JECFA 2001	
1414	ACETYLATED DISTARCH PHOSPHATE	อะซิติเลตเตตไดสตาร์ชฟอสเฟต	Not specified	JECFA 2001	
1451	ACETYLATED OXIDIZED STARCH	อะซิติเลตเตตออกซิไดซ์สตาร์ช	Not specified	JECFA 2001	
1401	ACID TREATED STARCH	แอซิดทรีทเตตสตาร์ช	Not specified	JECFA 2001	
1402	ALKALINE TREATED STARCH	อัลกาไลทรีดเตตสตาร์ช	Not specified	JECFA 2001	
1403	BLEACHED STARCH	บลีชเซตสตาร์ช	Not specified	JECFA 1999	
1001	CHLORINE SALTS AND ESTERS	เกลือคลอรีนและเอสเทอร์	Not specified	JECFA 1999	
1400	DEXTRINS, ROASTED STARCH	เดกซตริน	Not specified	JECFA 2001	
1412	DISTARCH PHOSPHATE	ไดสตาร์ชฟอสเฟต	Not specified	JECFA 2001	

INS No.	รายชื่อวัตถุเจือปนอาหาร		ADI[a]	ปีที่กำหนดค่า ADI	หน้า
1442	HYDROXYPROPYL DISTARCH PHOSPHATE	ไฮดรอกซีโปรปิลไดสตาร์ชฟอสเฟต	Not specified	JECFA 2001	
1440	HYDROXYPROPYL STARCH		Not specified	JECFA 2001	
1410	MONOSTARCH PHOSPHATE	โมโนสตาร์ช ฟอสเฟต	Not specified	JECFA 2001	
1404	OXIDIZED STARCH	ออกซิไดส์ สตาร์ช	Not specified	JECFA 2001	
1413	Phosphated distarch phosphate	ฟอสเฟตเตต ไดสตาร์ชฟอสเฟต	Not specified	JECFA 2001	
1420	Starch acetate	สตาร์ชอะซีเตต	Not specified	JECFA 2001	
1405	Starches, enzyme treated	สตาร์ชเอนไซม์ทรีตเตต	Not specified	JECFA 2001	
1450	Starch sodium octenylsuccinate	สตาร์ชโซเดียมออคทีนิลซัคซีเนต	Not specified	JECFA 2001	
471	MONO- AND DI-GLYCERIDES	โมโนและไดกลีเซอไรด์	Not limited	JECFA 1973	483
624	MONOAMMONIUM L-GLUTAMATE	โมโนแอมโมเนียมแอล-กลูตาเมต	Not specified	JECFA 1987	485
622	MONOPOTASSIUM L-GLUTAMATE	โมโนโพแทสเซียมแอล-กลูตาเมต	Not specified	JECFA 1987	487
621	MONOSODIUM L-GLUTAMATE	โมโนโซเดียมแอล-กลูตาเมต	Not specified	JECFA 1987	489
235	NATAMYCIN (PIMARICIN)	นาตาไนซิน	0-0.3	JECFA 1976	491
961	NEOTAME	นีโอแตม	0-2	JECFA 2003	495
234	NISIN	ไนซิน	0-33000 units/kg bw	JECFA 1968	499
941	NITROGEN	ไนโตรเจน	No ADI necessary	JECFA 1980	503
942	NITROUS OXIDE	ไนตรัสออกไซด์	Acceptable	JECFA 1985	505
231	ORTHO-PHENYLPHENOLS	ออร์โธ-ฟีนิลฟินอล	0-0.2	JECFA 1964	508
1101(ii)	PAPAIN	ปาเปน	Not limited	JECFA 1971	510
440	PECTINS	เพกติน	Not specified	JECFA 1981	511
	PHOSPHATES	ฟอสเฟต			
338	Phosphoric acid	กรดฟอสฟอริก	70[d]	JECFA 1982	517
339(i)	Sodium dihydrogen phosphate	โซเดียม ไดไฮโดรเจนฟอสเฟต	70[d]	JECFA 1982	519
339(ii)	Disodium hydrogen phosphate	ไดโซเดียม ไฮโดรเจนฟอสเฟต	70[d]	JECFA 1982	521
339(iii)	Trisodium phosphate	ไตรโซเดียม ฟอสเฟต	70[d]	JECFA 1982	523
340(i)	Potassium dihydrogen phosphate	โพแทสเซียม ไดไฮโดรเจนฟอสเฟต	70[d]	JECFA 1982	525
340(ii)	Dipotassium hydrogen phosphate	ไดโพแทสเซียม ไฮโดรเจนฟอสเฟต	70[d]	JECFA 1982	528

INS No.	รายชื่อวัตถุเจือปนอาหาร		ADI[a]	ปีที่กำหนดค่า ADI	หน้า
340(iii)	Tripotassium phosphate	ไตรโพแทสเซียม ฟอสเฟต	70[d]	JECFA 1982	531
341(i)	Monocalcium dihydrogen phosphate	โมโนแคลเซียม ไดไฮโดรเจน ฟอสเฟต	70[d]	JECFA 1982	534
341(ii)	Calcium hydrogen phosphate	แคลเซียม ไฮโดรเจน ฟอสเฟต	70[d]	JECFA 1982	536
341(iii)	Tricalcium phosphate	ไตรแคลเซียม ฟอสเฟต	70[d]	JECFA 1982	538
342(i)	Ammonium dihydrogen phosphate	แอมโมเนียม ไดไฮโดรเจน ฟอสเฟต	70[d]	JECFA 1982	540
342(ii)	Diammonium hydrogen phosphate	ไดแอมโมเนียม ไฮโดรเจน ฟอสเฟต	70[d]	JECFA 1982	542
343(i)	Magnesium dihydrogen phosphate	แมกนีเซียม ไดไฮโดรเจน ฟอสเฟต	70[d]	JECFA 1982	543
343(ii)	Magnesium hydrogen phosphate	แมกนีเซียม ไฮโดรเจน ฟอสเฟต	70[d]	JECFA 1982	545
343(iii)	Trimagnesium phosphate	ไตรแมกนีเซียม ฟอสเฟต	70[d]	JECFA 1982	547
450(i)	Disodium diphosphate	ไดโซเดียม ไดฟอสเฟต	70[d]	JECFA 1982	549
450(ii)	Trisodium diphosphate	ไตรโซเดียม ไดฟอสเฟต	70[d]	JECFA 1982	551
450(iii)	Tetrasodium diphosphate	เตตระโซเดียม ไดฟอสเฟต	70[d]	JECFA 1982	553
450(v)	Tetrapotassium diphosphate	เตตระโพแทสเซียม ไดฟอสเฟต	70[d]	JECFA 1982	555
450(vi)	Dicalcium diphosphate	ไดแคลเซียม ไดฟอสเฟต	70[d]	JECFA 1982	557
450(vii)	Calcium dihydrogen diphosphate	แคลเซียม ไดไฮโดรเจน ไดฟอสเฟต	70[d]	JECFA 1982	559
451(i)	Pentasodium triphosphate	เพนตระโซเดียม ไตรฟอสเฟต	70[d]	JECFA 1982	561
451(ii)	Pentapotassium triphosphate	เพนตระโพแทสเซียม ไตรฟอสเฟต	70[d]	JECFA 1982	565
452(i)	Sodium polyphosphate	โซเดียม โพลีฟอสเฟต	70[d]	JECFA 1982	569
452(ii)	Potassium polyphosphate	โพแทสเซียม โพลีฟอสเฟต	70[d]	JECFA 1982	571
452(iii)	Sodium calcium polyphosphate	โซเดียม แคลเซียม โพลีฟอสเฟต	70[d]	JECFA 1982	574
452(iv)	Calcium polyphosphate	แคลเซียม โพลีฟอสเฟต	70[d]	JECFA 1982	576
452(v)	Ammonium polyphosphate	แอมโมเนียม โพลีฟอสเฟต	70[d]	JECFA 1982	578
542	Bone phosphate	โบน ฟอสเฟต	70[d]	JECFA 1982	580
1200	POLYDEXTROSES	โพลีเดกซ์โทรส	Not specified	JECFA 1987	584
900a	POLYDIMETHYLSILOXANE	โพลีไดเมทิลไซล็อกเซน	0-1.5	JECFA 2011	591
1521	POLYETHYLENE GLYCOL	โพลีเอทธิลีนไกลคอล	0-10	JECFA 1979	596
964	POLYGLYCITOL SYRUP	โพลีไกลซิทอลไซรัป	Not specified	JECFA 1998	607

INS No.	รายชื่อวัตถุเจือปนอาหาร		ADI[a]	ปีที่กำหนดค่า ADI	หน้า
	POLYSORBATES	โพลีซอร์เบต			
432	Polyoxyethylene (20) sorbitan monolaurate	โพลีออกซีเอทิลีน (20) ซอร์บิแทนโมโนลอเรต	0-25	JECFA 1973	610
433	Polyoxyethylene (20) sorbitan monooleate	โพลีออกซีเอทิลีน (20) ซอร์บิแทนโมโนลีเอต	0-25	JECFA 1973	612
434	Polyoxyethylene (20) sorbitan monopalmitate	โพลีออกซีเอทิลีน (20) ซอร์บิแทนโมโนปาล์มิเตต	0-25	JECFA 1973	614
435	Polyoxyethylene (20) sorbitan monostearate	โพลีออกซีเอทิลีน (20) ซอร์บิแทนโมโนสเตียเรต	0-25	JECFA 1973	616
436	Polyoxyethylene (20) sorbitan tristearate	โพลีออกซีเอทิลีน (20) ซอร์บิแทนไตรสเตียเรต	0-25	JECFA 1973	618
1203	POLYVINYL ALCOHOL	โพลีไวนิลแอลกอฮอล์	0-50	JECFA 2003	620
1201	POLYVINYLPYRROLIDONE	โพลีไวนิลไพโรลิโดน	0-50	JECFA 1986	625
124	PONCEAU 4R (COCHINEAL RED A)	ปองโซ 4 อาร์	0-4	JECFA 1983	629
632	POTASSIUM 5'-INOSINATE	โพแทสเซียม 5'- อินโนซิเนต	Not specified	JECFA 1985	632
261	POTASSIUM ACETATE	โพแทสเซียมอะซีเตต	Not limited	JECFA 1973	634
402	POTASSIUM ALGINATE	โพแทสเซียมอัลจิเนต	Not specified	JECFA 1992	636
501(i)	POTASSIUM CARBONATE	โพแทสเซียมคาร์บอเนต	0-6	JECFA 1965	639
508	POTASSIUM CHLORIDE	โพแทสเซียมคลอไรด์	Not limited	JECFA 1979	641
332(i)	POTASSIUM DIHYDROGEN CITRATE	โพแทสเซียมไดไฮโดรเจนซิเตรต	Not limited	JECFA 1979	643
577	POTASSIUM GLUCONATE	โพแทสเซียมกลูโคเนต	Not specified	JECFA 1998	645
501(ii)	POTASSIUM HYDROGEN CARBONATE	โพแทสเซียมไฮโดรเจนคาร์บอเนต	Not limited	JECFA 1965	647
525	POTASSIUM HYDROXIDE	โพแทสเซียมไฮดรอกไซด์	Not limited	JECFA 1965	648
326	POTASSIUM LACTATE	โพแทสเซียมแลกเตต	Not limited	JECFA 1979	650
283	POTASSIUM PROPIONATE	โพแทสเซียมโพรพิโอเนต	Not limited	JECFA 1973	652
515(i)	POTASSIUM SULFATE	โพแทสเซียมซัลเฟต	Not specified	JECFA 1985	654
460(ii)	POWDERED CELLULOSE	เซลลูโลสผง	Not specified	JECFA 1976	656
407a	PROCESSED EUCHEUMA SEAWEED	สาหร่ายยูชีม่าที่ผ่านกรรมวิธี	Not specified	JECFA 2001	658
280	PROPIONIC ACID	กรดโปรปิโอนิก	Not limited	JECFA 1973	663
310	PROPYL GALLATE	โปรปิลแกลเลต	0-1.4	JECFA 1996	665
477	PROPYLENE GLYCOL ESTERS OF FATTY ACIDS	โปรปิลีนไกลคอลเอสเตอร์ของกรดไขมัน	0-25	JECFA 1973	668

INS No.	รายชื่อวัตถุเจือปนอาหาร		ADI[a]	ปีที่กำหนดค่า ADI	หน้า
	PROTEASE	เอนไซม์โพรเตียส			
1101(i)	Protease from Aspergillus oryzae, var.	เอนไซม์โพรเตียสจากเชื้อแอสเพอจิลลัสโอไรเซ	Acceptable	JECFA 1987	674
1101(i)	Protease from Streptomyces fradiae	เอนไซม์โพรเตียสจากเชื้อสเตรปโตไมซีสฟราดิอี	Not specified	JECFA 1987	675
1204	PULLULAN	พอลาแลน	Not specified	JECFA 2011	676
	QUILLAIA EXTRACTS	สารสกัดจากควิลลาเรีย			
999(i)	Quillaia extract type I	สารสกัดจากควิลลาเรียชนิดที่ 1	0-1	JECFA 2005	679
999(ii)	Quillaia extract type 2	สารสกัดจากควิลลาเรียชนิดที่ 2	0-1	JECFA 2005	682
	RIBOFLAVINS	ไรโบฟลาวิน			
101(i)	Riboflavin, synthetic	ไรโบฟลาวิน (สังเคราะห์)	0-0.5	JECFA 1998	686
101(ii)	Riboflavin 5'-phosphate sodium	ไรโบฟลาวิน 5’-ฟอสเฟตโซเดียม	0-0.5	JECFA 1998	688
101(iii)	Riboflavin from *Bacillus subtilis*	ไรโบฟลาวินจาก *Bacillus subtilis*	0-0.5	JECFA 1998	692
	SACCHARINS	ซัคคาริน			
954(i)	Saccharin	ซัคคาริน	0-5	JECFA 1993	695
954(ii)	Calcium saccharin	แคลเซียม ซัคคาริน	0-5	JECFA 1993	697
954(iii)	Potassium saccharin	โพแทสเซียม ซัคคาริน	0-5	JECFA 1993	699
954(iv)	Sodium saccharin	โซเดียม ซัคคาริน	0-5	JECFA 1993	701
470	SALT OF FATTY ACIDS				703
470(i)	SALTS OF MYRISTIC, PALMITIC AND STEARIC ACIDS WITH AMMONIA, CALCIUM, POTASSIUM AND SODIUM	เกลือแอมโมเนียม แคลเซียม โพแทสเซียม และโซเดียมของกรดไมริสติก ปาล์มิติก และสเตียริก	Not specified	JECFA 1985	
470(ii)	SALTS OF OLEIC ACID WITH CALCIUM, POTASSIUM AND SODIUM	เกลือแคลเซียม โพแทสเซียมและโซเดียมของกรดโอลีอิก			
904	SHELLAC, BLEACHED	เชลแลค	Acceptable	JECFA 1992	712
551	SILICON DIOXIDE, AMORPHOUS	ซิลิคอนไดออกไซด์	Not specified	JECFA 1985	714
262(i)	SODIUM ACETATE	โซเดียมอะซีเตต	Not limited	JECFA 1973	716
401	SODIUM ALGINATE	โซเดียมอัลจิเนต	Not specified	JECFA 1992	718
554	SODIUM ALUMINOSILICATE	โซเดียมอลูมิเนียมซิลิเคต	1[b]	JECFA 2006	721

INS No.	รายชื่อวัตถุเจือปนอาหาร		ADI[a]	ปีที่กำหนดค่า ADI	หน้า
301	SODIUM ASCORBATE	โซเดียมแอสคอร์เบต	Not specified	JECFA 1981	723
500(i)	SODIUM CARBONATE	โซเดียมไบคาร์บอเนต	Not limited	JECFA 1965	725
466	SODIUM CARBOXYMETHYL CELLULOSE (Cellulose gum)	โซเดียมคาร์บอกซีเมทิลเซลลูโลส	Not specified	JECFA 1989	727
469	SODIUM CARBOXYMETHYL CELLULOSE, ENZYMATICALLY HYDROLYSED (Cellulose gum, enzymatically hydrolyzed)	โซเดียมคาร์บอกซีเมทิลเซลลูโลส, ไฮโดรไลส์ด้วยเอนไซม์	Not specified	JECFA 1989	732
331(i)	SODIUM DIHYDROGEN CITRATE	โซเดียมไดไฮโดรเจนซิเตรต	Not limited	JECFA 1979	736
350(ii)	SODIUM DL-MALATE	โซเดียมดีแอล-มาเลต	Not specified	JECFA 1979	738
365	SODIUM FUMARATES	โซเดียมฟูมาเรต	Not specified	JECFA 1989	740
576	SODIUM GLUCONATE	โซเดียมกลูโคเนต	Not specified	JECFA 1998	742
500(ii)	SODIUM HYDROGEN CARBONATE	โซเดียมไฮโดรเจนคาร์บอเนต	Not limited	JECFA 1965	744
350(i)	SODIUM HYDROGEN DL-MALATE	โซเดียมไฮโดรเจนดีแอล-มาเลต	Not specified	JECFA 1982	746
222	SODIUM HYDROGEN SULFITE	โซเดียมไฮโดรเจนซัลไฟต์	0-0.7 [d]	JECFA 1998	748
524	SODIUM HYDROXIDE	โซเดียมไฮดรอกไซด์	Not limited	JECFA 1965	750
325	SODIUM LACTATE	โซเดียมแลกเตต	Not limited	JECFA 1979	752
281	SODIUM PROPIONATE	โซเดียมโปรปิโอเนต	Not limited	JECFA 1973	754
500(iii)	SODIUM SESQUICARBONATE	โซเดียมเซสควิคาร์บอเนต	Not specified	JECFA 1981	756
514(i)	SODIUM SULFATE	โซเดียมซัลเฟต	Not specified	JECFA 2001	758
	SORBATES	ซอร์เบต			
200	Sorbic acid	กรดซอร์บิก	0-25	JECFA 1973	760
202	Potassium sorbate	โพแทสเซียม ซอร์เบต	0-25	JECFA 1973	762
203	Calcium sorbate	แคลเซียม ซอร์เบต	0-25	JECFA 1973	764
420(i)	SORBITOL	ซอร์บิทอล	Not specified	JECFA 1982	766
420(ii)	SORBITOL SYRUP	ซอร์บิทอลไซรัป	No ADI	JECFA 1988	769
512	STANNOUS CHLORIDE	สแตนัสคลอไรด์	0-14[b]	JECFA 1988	772
	STEAROYL LACTYLATES	สเตียโรอิล แลกติเลต			
481(i)	Sodium stearoyl lactylate	โซเดียม สเตียโรอิล แลกติเลต	0-20	JECFA 1973	774
482(i)	Calcium stearoyl lactylate	แคลเซียม สเตียโรอิล แลกติเลต	0-20	JECFA 1973	778
484	STEARYL CITRATE	สเตียริลซิเตรต	0-50	JECFA 1973	782
960	STEVIOL GLYCOSIDES	สตีวิโอไกลโคไซด์	0-4	JECFA 2008	784

INS No.	รายชื่อวัตถุเจือปนอาหาร		ADI[a]	ปีที่กำหนดค่า ADI	หน้า
955	SUCRALOSE (TRICHLOROGALACTOSUCROSE)	ซูคราโลส (ไตรคลอโรกาแลกโตซูโครส)	0-15	JECFA 1990	789
474	SUCROGLYCERIDES	ซูโครกลีเซอร์ไรด์	0-30	JECFA 2010	795
444	SUCROSE ACETATE ISOBUTYRATE	ซูโครสอะซีเตตไอโซบิวทิลเรต	0-20	JECFA 1996	801
	SULFITES	ซัลไฟต์			
220	Sulfur dioxide	ซัลเฟอร์ไดออกไซด์	0-0.7	JECFA 1998	804
221	Sodium sulfite	โซเดียม ซัลไฟต์	0-0.7	JECFA 1998	807
222	Sodium hydrogen sulfite	โซเดียม ไฮโดรเจน ซัลไฟต์	0-0.7	JECFA 1998	809
223	Sodium metabisulfite	โซเดียม เมตาไบซัลไฟต์	0-0.7	JECFA 1998	811
224	Potassium metabisulfite	โพแทสเซียม เมตาไบซัลไฟต์	0-0.7	JECFA 1998	813
225	Potassium sulfite	โพแทสเซียม ซัลไฟต์	0-0.7	JECFA 1998	815
539	Sodium thiosulfate	โซเดียม ไทโอซัลไฟต์	0-0.7	JECFA 1998	817
110	SUNSET YELLOW FCF	ซันเซ็ตเย็ลโลว์ เอ็ฟซีเอ็ฟ	0-4	JECFA 2011	819
553(iii)	TALC	ทาลคัม	Not specified	JECFA 1986	823
417	TARA GUM	ทารากัม	Not specified	JECFA 1986	825
	TARTRATES	ตาร์เตรต			
334	L(+)-Tartaric acid	กรดตาร์ตาริก	0-30	JECFA 1977	827
335(ii)	Sodium L(+)-tartrate	โซเดียม ตาร์เตรต	0-30	JECFA 1977	829
337	Potassium sodium L(+)-tartrate	โพแทสเซียม โซเดียม ตาร์เตรต	0-30	JECFA 1973	831
319	TERTIARY BUTYLHYDROQUINONE	เทอร์เชียรีบิวทิล ไฮโดรควิโนน	0-0.7	JECFA 1997	833
957	THAUMATIN	ทอมาติน	Not specified	JECFA 1985	838
479	THERMALLY OXIDIZED SOYA BEAN OIL INTERACTED WITH MONO- AND DIGLYCERIDES OF FATTY ACIDS	น้ำมันถั่วเหลืองที่ถูกออกซิไดซ์ที่อุณหภูมิสูง ที่ทำปฏิกิริยาเอสเตอริฟิเคชั่น	0-30	JECFA 1992	841
	THIODIPROPIONATES	ไทโอไดโปรปิโอเนต			
388	Thiodipropionic acid	กรดไทโอไดโปรปิโอนิก	0-3	JECFA 1973	847
389	Dilauryl thiodipropionate	ไดลอริลไทโอไดโปรปิโอเนต	0-3	JECFA 1973	849
171	TITANIUM DIOXIDE	ไททาเนียมไดออกไซด์	Not limited	JECFA 1969	851
	TOCOPHEROLS	โทโคเฟอรอล			
307a	d-alpha-Tocopherol	ดี-อัลฟ่า-โทโคเฟอรอล	0.15-2	JECFA 1986	857

INS No.	รายชื่อวัตถุเจือปนอาหาร		ADI[a]	ปีที่กำหนดค่า ADI	หน้า
307b	Tocopherol concentrate, mixed	โทโคเฟอรอลผสมชนิดเข้มข้น	0.15-2	JECFA 1986	861
307c	dl-alpha-Tocopherol	ดีแอล-อัลฟ่า-โทโคเฟอรอล	0.15-2	JECFA 1986	865
413	TRAGACANTH GUM	ทรากาแคนท์กัม	Not specified	JECFA 1985	868
1518	TRIACETIN	ไตรอซิติน	Not specified	JECFA 1975	871
380	TRIAMMONIUM CITRATE	ไตรแอมโมเนียมซิเทรต	Not limited	JECFA 1979	873
333(iii)	TRICALCIUM CITRATE	ไตรแคลเซียมซิเทรต	Not limited	JECFA 1973	875
1505	TRIETHYL CITRATE	ไตรเอทิลซิเตรต	0-20	JECFA 1984	877
332(ii)	TRIPOTASSIUM CITRATE	ไตรโพแทสเซียมซิเตรต	Not limited	JECFA 1973	879
331(iii)	TRISODIUM CITRATE	ไตรโซเดียมซิเตรต	Not limited	JECFA 1973	881
415	XANTHAN GUM	แซนแทนกัม	Not specified	JECFA 1986	882
967	XYLITOL	ไซลิทอล	Not specified	JECFA 1983	888

หมายเหตุ

[a] **Acceptable Daily Intake (ADI)** (มิลลิกรัม/กิโลกรัมน้ำหนักตัว)

[b] **Provisional Tolerable Weekly Intake (PTWI)** (มิลลิกรัม/กิโลกรัมน้ำหนักตัว)

[c] **Provisional Maximum Tolerable Daily Intake (PMTDI)** (มิลลิกรัม/กิโลกรัมน้ำหนักตัว)

[d] **Maximum Tolerable Daily Intake (MTDI)** (มิลลิกรัม/กิโลกรัมน้ำหนักตัว)

MAGNESIUM CARBONATE

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). An ADI 'not limited' was established at the 9th JECFA (1965)

SYNONYMS INS No. 504(i)

DEFINITION A basic hydrated or a normal hydrated magnesium carbonate or a mixture of the two

Chemical names Magnesium carbonate

C.A.S. number 546-93-0

Assay Not less than 24.0% and not more than 26.4% of Mg

DESCRIPTION Odourless, light, white friable masses or as a bulky white powder

FUNCTIONAL USES Anticaking agent, antibleaching agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Practically insoluble in water; insoluble in ethanol

Test for carbonate (Vol. 4) Passes test

Test for magnesium (Vol. 4) Passes test

PURITY

Acid insoluble substances Not more than 0.05%
Weigh 5 g of the sample to the nearest mg, and mix with 75 ml of water. Add hydrochloric acid in small portion, with agitation until no more of the sample dissolves, and boil for 5 min. If an insoluble residue remains, filter, wash well with water until the last washing is free from chloride, ignite, cool and weigh. Calculate as percentage.

Water soluble substances Not more than 1%
Weigh 2 g of the sample to the nearest mg. Add 100 ml of freshly boiled and cooled water, boil while stirring, cool and filter. Evaporate a 50 ml portion of the filtrate to dryness on a water bath, and dry the residue at 120^{o} for 3 h. Cool, weigh and calculate as percentage. (The weight of the residue should not exceed 10 mg).

Calcium Not more than 0.4%
Weigh 1 g of the sample to the nearest 0.1 mg and dissolve in a mixture of 3 ml of sulfuric acid and 22 ml of water. Add 50 ml of ethanol, and allow the mixture to stand overnight. If crystals of magnesium sulfate separate, warm the mixture to about 50^{o} to dissolve. Filter through a tared previously ignited

porcelain filter crucible and wash the precipitate several times with a mixture of 2 volumes of ethanol and 1 volume of dilute sulfuric acid TS. Ignite the crucible and contents at a dull red heat, cool and weigh. The weight of calcium sulfate obtained, multiplied by 0.294, gives the equivalent of calcium in the sample taken for the test.

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh 1 g of the sample to the nearest 0.1 mg, and transfer to a 250 ml conical flask. Pipette into the flask 50 ml of N sulfuric acid and swirl to dissolve. Titrate the excess acid with N sodium hydroxide solution, using methyl orange TS as indicator. Subtract from the volume of N sulfuric acid consumed the number of ml of N sulfuric acid corresponding to the weight of Ca in the sample taken, using as a factor 20.04 mg of Ca for each ml of N sulfuric acid. The difference is the volume of N sulfuric acid used to neutralize the magnesium carbonate and each ml is equivalent to 12.16 of Mg.

MAGNESIUM CHLORIDE

Prepared at the 27th JECFA (1983), published in FNP 28 (1983) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). An ADI 'Not limited' was established at the 23rd JECFA (1979).

SYNONYMS INS No. 511

DEFINITION

Chemical names	Magnesium chloride hexahydrate
C.A.S. number	7786-30-3
Chemical formula	$MgCl_2 \cdot 6H_2O$
Formula weight	203.30
Assay	Not less than 99.0% and not more than 105.0%

DESCRIPTION Colourless, odourless flakes, granules, lumps or crystals; it is very deliquescent

FUNCTIONAL USES Firming agent, colour retention agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Very soluble in water; freely soluble in ethanol
Test for chloride (Vol. 4)	Passes test
Test for magnesium (Vol. 4)	Passes test

PURITY

Ammonium	Not more than 50 mg/kg Dissolve 1 g of the sample in 90 ml of water, and slowly add 10 ml of a freshly boiled and cooled solution of sodium hydroxide (1 in 10 soln). Allow to settle, then decant 20 ml of the supernatant liquid into a colour comparison tube, dilute to 50 ml with water, and add 2 ml of Nessler's TS. Any colour does not exceed that produced by 10 µg of ammonium (NH_4) ion in 48 ml of water and 2 ml of the sodium hydroxide solution.
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the

specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Dissolve about 450 mg of the sample, accurately weighed, in 25 ml of water, add 5 ml of ammonia/ammonium chloride buffer TS and 0.1 ml of eriochrome black TS and titrate with 0.05 M disodium ethylenediaminetetraacetate until the solution is blue in colour. Each ml of 0.05 M disodium ethylenediaminetetra-acetate is equivalent to 10.16 mg of $MgCl_2 \cdot 6H_2O$.

MAGNESIUM di-L-GLUTAMATE

Prepared at the 31st JECFA (1987), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). A group ADI 'not specified' for glutamic acid and its Ammonium, Ca, K, Mg & Na salts, was established at the 31st JECFA (1987)

SYNONYMS Magnesium glutamate, INS No.625

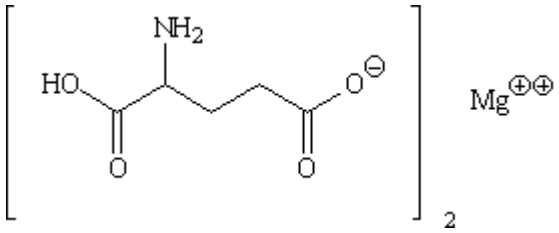
DEFINITION

Chemical names Monomagnesium di-L-glutamate tetrahydrate

C.A.S. number 18543-68-5

Chemical formula $C_{10}H_{16}MgN_2O_8 \cdot 4H_2O$

Structural formula



Formula weight 388.62

Assay Not less than 95.0% and not more than 105.0 % on the anhydrous basis

DESCRIPTION Odourless, white or off-white crystals or powder

FUNCTIONAL USES Flavour enhancer, salt substitute

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Very soluble in water; insoluble in ethanol

Test for glutamate (Vol. 4) Passes test

Test for magnesium (Vol. 4) Passes test

PURITY

Water (Vol. 4) Not more than 24% (Karl Fischer Method)

pH (Vol. 4) 6.4 - 7.5 (1 in 10 soln)

Specific rotation (Vol. 4) [alpha] 20, D: Between +23.8. and + 24.4^{o}(10% (w/v solution in 2N hydrochloric acid)

Chlorides (Vol. 4)	Not more than 0.2% Test 0.07 g of the sample as directed in the Limit Test using 0.4 ml of 0.01 N hydrochloric acid in the control
Sulfates (Vol. 4)	Not more than 0.2% Test 0.12 g of the sample as directed in the Limit Test using 0.5 ml of 0.01 N sulfuric acid in the control.
Pyrrolidone carboxylic acid (Vol. 4)	Passes test
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Dissolve about 250 mg of the sample, previously dried and weighed accurately, in 6 ml of formic acid, and add 100 ml of glacial acetic acid. Titrate with 0.1 N perchloric acid determining the end-point potentiometrically. Run a blank determination in the same manner and correct for the blank. Each ml of 0.1 N perchloric acid is equivalent to 7.914 mg of $C_{10}H_{16}MgN_2O_8$. Calculate the content on the anhydrous basis.

MAGNESIUM GLUCONATE

Prepared at the 53rd JECFA (1999) and published in FNP 52 Add 7 (1999), superseding tentative specifications prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998). Group ADI "Not specified" for glucono-delta-lactone and gluconates, established at the 51st JECFA in 1998.

SYNONYMS INS No. 580

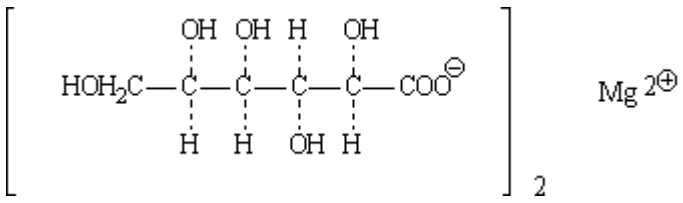
DEFINITION The material of commerce exists as anhydrous, dihydrate or a mixture of both

Chemical names: Magnesium di-D-gluconate

C.A.S. number: Anhydrous: 3632-91-5
Dihydrate: 59625-89-7

Chemical formula: $C_{12}H_{22}MgO_{14}$

Structural formula:



Formula weight: Anhydrous: 414.60
Dihydrate: 450.63

Assay: Not less than 98.0% and not more than 102.0% on the anhydrous basis

DESCRIPTION White to off white, odourless, fine powder

FUNCTIONAL USES Acidity regulator, firming agent, yeast nutrient, nutrient supplement

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Soluble in water; sparingly soluble in ethanol

Test for magnesium (Vol. 4): Passes test

Test for gluconate (Vol. 4): Passes test

PURITY

Water (Vol. 4): Between 3.0% and 12.0% (Karl Fischer Method)

Reducing substances: Not more than 1.0% calculated as D-glucose (Method I)

(Vol. 4)

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Dissolve about 0.6 g of the sample, accurately weighed, in 50 ml of water, add 10 ml of ammonia/ammonium chloride buffer solution and 5 drops of eriochrome black TS. Titrate with 0.05 M disodium ethylenediaminetetraacetate to a deep blue colour.

Calculate % magnesium gluconate, dihydrate (as is basis) from:

$$\frac{\text{ml of disodiumEDTA x M of disodiumEDTA x 45.07}}{\text{sampleweight (g)}}$$

where
45.07 = equivalence factor for magnesium gluconate, dihydrate

MAGNESIUM HYDROXIDE

Prepared at the 19th JECFA (1975), published in NMRS 55B (1976) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI not limited' was established at the 9th JECFA (1965)

SYNONYMS INS No. 528

DEFINITION

Chemical names	Magnesium hydroxide
C.A.S. number	1309-42-8
Chemical formula	$Mg(OH)_2$
Formula weight	58.32
Assay	Not less than 95.0%

DESCRIPTION Odourless, white bulky powder

FUNCTIONAL USES Alkali, colour adjunct

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Practically insoluble in water and in ethanol
Test for alkali	The sample is alkaline to moistened litmus paper
Test for magnesium (Vol. 4)	Passes test

PURITY

Loss on drying (Vol. 4)	Not more than 2% (105°, 2 h)
Loss on ignition (Vol. 4)	Not more than 30 - 33% (approx. 800° to constant weight)
Alkalis (free) and soluble salts	Boil 2 g of the sample with 100 ml of water for 5 min in a covered beaker and filter while hot. Add methyl red TS and titrate 50 ml of the cooled filtrate with 0.1 N sulfuric acid. Not more than 2 ml of the acid is required to reach the endpoint. Evaporate 25 ml of the filtrate to dryness and dry at 105° for 3 h. Not more than 10 mg of residue remains.
Calcium oxide	Not more than 1.5% Dissolve about 500 mg of the sample, accurately weighed, in a mixture of 3 ml of concentrated sulfuric acid and 22 ml of water. Add 50 ml of ethanol and allow the mixture to stand overnight. If crystals of magnesium sulfate separate, warm the mixture to about 50° to dissolve. Filter through a Gooch

crucible containing an asbestos mat previously washed with dilute sulfuric acid TS, water, and ethanol and ignited and weighed. Wash the crystals on the mat several times with a mixture of 3 volumes of ethanol and 1 volume of water. Ignite the crucible and contents at a dull red heat, cool and weigh. The weight of calcium sulfate obtained, multiplied by 0.4119, gives the equivalent of calcium oxide in the sample taken for the test.

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Transfer about 400 mg of the sample, previously dried at 105^{o} for 2 h and accurately weighed, into a conical flask. Add 25 ml of 1 N sulfuric acid and, after solution is complete, add methyl red TS and titrate the excess acid with 1 N sodium hydroxide. Subtract from the volume of 1 N sulfuric acid consumed in the assay the volume of 1 N sulfuric acid corresponding to the weight of CaO in the sample taken for the assay using as a factor 28.04 mg of CaO for each ml of 1 N sulfuric acid. Each ml of 1 N sulfuric acid used to neutralize the magnesium hydroxide is equivalent to 29.16 mg of $Mg(OH)_2$.

MAGNESIUM HYDROXIDE CARBONATE

Prepared at the 27th JECFA (1983), published in FNP 28 (1983) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002. An ADI 'not specified' was established at the 23rd JECFA (1979)

SYNONYMS Magnesium subcarbonate (light or heavy), hydrated basic magnesium carbonate, magnesium carbonate hydroxide; INS No. 504(ii)

DEFINITION

Chemical names: Magnesium carbonate hydroxide hydrated

Assay: Not less than 40.0% and not more than 45.0% of MgO

DESCRIPTION Odourless, light, white, friable masses, or a bulky-white powder

FUNCTIONAL USES Alkali, drying agent, colour-retention agent, carrier, anti-caking agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Practically insoluble in water; insoluble in ethanol

Alkalinity: Slurry shows slight alkalinity

Test for magnesium (Vol. 4): Passes test

PURITY

Soluble salts: Not more than 1.0%
Mix 2 g of the sample with 100 ml of a mixture of equal volumes of n-propanol and water. Heat the mixture to the boiling point with constant stirring, cool to room temperature, add water to make 100 ml and filter. Evaporate 50 ml of the filtrate on a steam bath to dryness, and dry at 105^{o} for 1 h. The weight of the residue does not exceed 10 mg.

Calcium: Not more than 1.0%
Dissolve about 1 g of the sample, accurately weighed, in a mixture of 3 ml of sulfuric acid and 22 ml of water. Add 50 ml of ethanol and allow the mixture to stand overnight. If crystals of magnesium sulfate separate, warm the mixture to about 50^{o} to dissolve them. Filter through a Gooch crucible containing an asbestos mat that previously has been washed with dilute sulfuric acid TS, water, and ethanol, and ignited and weighed. Wash the crystals on the mat several times with a mixture of 2 volumes of ethanol and 1 volume of dilute sulfuric acid TS. Ignite the crucible and contents to a dull red heat, cool, and weigh. The weight of calcium sulfate so obtained, multiplied by 0.2944 gives the equivalent of calcium in the sample taken for the test.

Acid insoluble matter

Not more than 0.05%
Mix 5 g of the sample with 75 ml of water, add hydrochloric acid in small portions, with agitation, until no more of the sample dissolves, and boil for 5 min. If an insoluble residue remains, filter, wash well with water until the last washing is free from chloride, ignite, cool and weigh.

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Dissolve about 1 g of the sample, accurately weighed, in 30 ml of 1 N sulfuric acid, add methyl orange TS, and titrate the excess acid with 1 N sodium hydroxide. From the volume of 1 N sulfuric acid consumed, deduct the volume of 1 N sulfuric acid corresponding to the content of calcium oxide in the weight of the sample taken for the assay. The difference is the volume of 1 N sulfuric acid equivalent to the magnesium oxide present. Each ml of 1 N sulfuric acid is equivalent to 20.15 mg of MgO.

MAGNESIUM L-LACTATE

Prepared at the 27th JECFA (1983), published in FNP 28 (1983) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). An ADI 'not limited' was established at the 23rd JECFA (1979)

SYNONYMS L-Lactic acid magnesium salt, magnesium di-L-lactate, INS No. 329

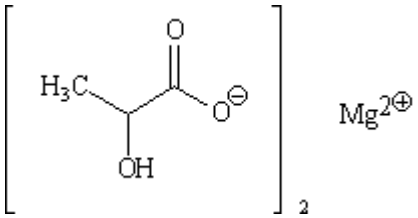
DEFINITION

Chemical names: Magnesium L(-)-2-hydroxypropionate dihydrate

C.A.S. number: 18917-93-6

Chemical formula: $Mg(C_3H_5O_3)_2 \cdot 2H_2O$

Structural formula:



Formula weight: 238.48

Assay: Not less than 97.5% and not more than 101.5% on the dried basis

DESCRIPTION White crystalline powder

FUNCTIONAL USES Buffering agent, dough conditioner, dietary supplement

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Soluble in water when shaking with water for 30 min; practically insoluble in ethanol

Specific rotation (Vol. 4): [alpha] 25, D : -7.5 to -8.8 (5% w/v aqueous solution)

Test for magnesium (Vol. 4): Passes test

Test for lactate (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4): 14.0 - 17.0% (120°, 24 h)

Chlorides (Vol. 4): Not more than 0.01%

Test 1 g of the sample as directed in the Limit Test using 0.3 ml of 0.01 N hydrochloric acid in the control

Microbiological criteria (Vol. 4)

Total aerobic microbial count: Max 1000/g
Total yeast and moulds: Max 100/g
E. coli: Absent in 1 g

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

METHOD OF ASSAY

Dissolve about 0.5 g of the dried sample, accurately weighed, in 25 ml of water, add 5 ml of ammonia/ammonium chloride buffer TS and 0.1 ml of ethylenediaminetetraacetate until the solution is blue in colour. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 10.12 mg of $Mg(C_3H_5O_3)_2$.

MAGNESIUM OXIDE

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). An ADI not limited' was established at the 9th JECFA (1965)

SYNONYMS INS No. 530

DEFINITION

Chemical names	Magnesium oxide
C.A.S. number	1309-48-4
Chemical formula	MgO
Formula weight	40.31
Assay	Not less than 96.0% after ignition at about 800^{o}

DESCRIPTION Very bulky white powder, known as light magnesium oxide, or as a relatively dense, white powder, known as heavy magnesium oxide. 5 g of light magnesium oxide occupy a volume of 40 to 50 ml, while 5 g of heavy magnesium oxide occupy a volume of 10 to 20 ml.

FUNCTIONAL USES Anticaking agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Practically insoluble in water; insoluble in ethanol
Alkalinity	The sample is alkaline to moistened litmus paper
Test for magnesium (Vol. 4)	Passes test

PURITY

Loss on drying (Vol. 4)	Not more than 5% after ignition at 800^{o} to 825^{o} to constant weight
Alkali (free) and soluble salts	Boil 2 g of the sample, weighed to the nearest mg, with 100 ml of water for 5 min in a covered beaker and filter while hot. Add methyl red TS and titrate 50 ml of the cooled filtrate with 0.1 N sulfuric acid. Not more than 2 ml of the acid should be consumed. Evaporate 25 ml of the filtrate to dryness and dry at 105^{o} for 1 h. Not more than 10 mg of residue should remain.
Calcium oxide	Not more than 1.5% Weigh 400 mg of the sample to the nearest 0.1 mg, and dissolve in a mixture of 3 ml of sulfuric acid and 22 ml of water. Add 50 ml of ethanol, and allow the mixture to stand overnight. If crystals of magnesium sulfate separate, warm the mixture to about 50^{o} to dissolve. Filter through a tared, previously ignited, porcelain filter crucible, and wash the precipitate several

times with a mixture of 2 volumes of ethanol and 1 volume of dilute sulfuric acid TS. Ignite the crucible and contents at a dull red heat, cool and weigh. The weight of calcium sulfate obtained, multiplied by 0.4119, gives the equivalent of calcium oxide in the sample taken for the test.

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Ignite about 400 mg of the sample to constant weight at 800° to 825° in a tared platinum crucible. Weigh the residue accurately, dissolve in 25.0 ml of N sulfuric acid, boil gently to remove any carbon dioxide and cool. Add methyl red TS and titrate the excess acid with N sodium hydroxide. Subtract from the volume of N sulfuric acid consumed the number of ml of N sulfuric acid corresponding to the weight of CaO in the sample taken, using as a factor 28.04 mg of CaO for each ml of N sulfuric acid. The difference is the volume of N sulfuric acid used to neutralize the magnesium oxide and each ml is equivalent to 20.16 mg MgO.

MAGNESIUM SILICATE, synthetic

Prepared at the 74th JECFA (2011) and published in FAO Monographs 11 (2011) superseding specifications prepared at the 61st JECFA (2003), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI 'not specified' was established at the 25th JECFA (1981).

SYNONYMS INS No. 553(i)

DEFINITION Magnesium silicate (synthetic) is manufactured by the precipitation reaction between sodium silicate and a soluble magnesium salt. The aqueous suspension of the precipitate is filtered and the collected solid washed, dried, classified for particle size and packaged. The finest material is intended for use as an anticaking agent and the coarser particles are for use as a filtering aid. The moisture content of the material meant for use as an anticaking agent is kept to less than 15%. Although magnesium silicate is of variable composition, the molar ratio of MgO to SiO_2 is approximately 2:5.

Chemical name: Magnesium silicate

C.A.S. number: 1343-88-0

Assay: Not less than 15% of MgO and not less than 67% of SiO_2, calculated on the ignited basis.

DESCRIPTION Very fine, white, odourless powder, free from grittiness

FUNCTIONAL USES Anticaking agent, filtering aid

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Insoluble in water

pH (Vol. 4): 7.0-11.0 (1 in 10 slurry)

Magnesium (Vol. 4): Mix about 0.5 g of the sample with 10 ml of dilute hydrochloric acid TS, filter, and neutralize the filtrate to litmus paper with ammonia TS. The neutralized filtrate gives a positive test for magnesium.

Silicate: Prepare a bead by fusing a few crystals of sodium ammonium phosphate on a platinum loop in the flame of a Bunsen burner. Place the hot, transparent bead in contact with the sample, and again fuse. Silica floats about in the bead, producing, upon cooling, an opaque bead with a web-like structure.

PURITY

Loss on drying (Vol. 4): Not more than 15% (for material used as an anticaking agent (105°, 2 h)).
Retain the dried sample for determination of loss on ignition.

Loss on ignition (Vol. 4)

Not more than 15% on the dried basis
Weigh to the nearest 0.1 mg, 1 g of the dried sample in a tared platinum crucible provided with a cover. Gradually apply heat to the crucible at first, then strongly ignite at 900/1000° for 20 min. Cool, weigh and calculate as percentage.

Free alkali

Not more than 1% (as NaOH)
Add 2 drops of phenolphthalein TS to 20 ml of dilute filtrate prepared in the test for Soluble salts (see below), representing 1 g of the sample. Not more than 2.5 ml of 0.1 N hydrochloric acid should be required to discharge the pink colour produced.

Soluble salts

Not more than 3%
Boil 10 g of the sample with 150 ml of water for 15 min. Cool to room temperature, and add water to restore the original volume. Allow the mixture to stand for 15 min, and filter until clear. Retain 20 ml of the filtrate for the test Free alkali. Evaporate 75 ml of the filtrate, representing 5 g of the sample in a tared platinum dish on a steam bath to dryness, and ignite gently to constant weight. Cool, weigh and calculate as percentage (the weight of the residue should not exceed 150 mg).

Fluoride (Vol. 4)

Not more than 10 mg/kg
Weigh 2.5 g of the sample to the nearest mg and proceed as directed in the Fluoride Limit Test (Method I or III).

Lead (Vol. 4)

Not more than 5 mg/kg
Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

METHOD OF ASSAY

Magnesium oxide and silicon dioxide: Weigh about 0.5 g of the sample to the nearest 0.1 mg, in a platinum or nickel crucible, add 5 g potassium hydroxide and 2 g boric acid, mix and melt completely using a torch burner (alkali fusion) and allow to stand at room temperature. Place the reaction product along with crucible in a 250-ml PTFE beaker, add 150 ml hot deionized water and dissolve residue by agitation. Wash the crucible with hot deionized water and remove it. Add 50 ml hydrochloric acid and transfer the contents into a 250-ml volumetric flask. Wash the beaker three times with hot deionized water, transfer the washings to the volumetric flask and make up to volume (Solution A). Prepare the test solution by diluting Solution A with 2% hydrochloric acid solution. Analyze magnesium and silica in the test solution by ICP-AES technique (Vol. 4). Set instrument parameters as specified by the instrument manufacturer and use the analytical lines for Si (251.611 nm) and Mg (279.553 nm). Read the concentration (as µg/ml) of Mg and Si from respective standard curves. Calculate the magnesium oxide and silicon dioxide content of the sample, on the ignited basis, using the formula:

$$\%MgO \text{ (on the ignited basis)} = \frac{4.1458 \times C \times DF}{W \times [100 - (\%LOD + \%LOI)]}$$

$$\%SiO_2 \text{ (on the ignited basis)} = \frac{5.3504 \times C \times DF}{W \times [100 - (\%LOD + \%LOI)]}$$

where

C is concentration of Mg or Si in the test solution, µg/ml;
DF is dilution factor (dilution of Solution A to get test solution);
W is weight of sample, g;
%LOD is % loss on drying; and
%LOI is % loss on ignition.

MAGNESIUM SULFATE

Prepared at the 68th JECFA (2007), published in FAO JECFA Monographs 4 (2007), superseding the specifications prepared at the 63rd JECFA (2004) and published the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI "not specified" was established at the 68th JECFA (2007).

SYNONYMS Epsom salt (heptahydrate); INS No.518

DEFINITION Magnesium sulfate occurs naturally in sea water, mineral springs and in minerals such as kieserite and epsomite. It is recovered from them or by reacting sulfuric acid and magnesium oxide. It is produced with one or seven molecules of water of hydration or in a dried form containing the equivalent of between 2 and 3 waters of hydration.

Chemical names: Magnesium sulfate

C.A.S. number:
Monohydrate: 14168-73-1
Heptahydrate: 10034-99-8
Dried: 15244-36-7

Chemical formula:
Monohydrate: $MgSO_4.H_2O$
Heptahydrate: $MgSO_4.7H_2O$
Dried: $MgSO_4.xH_2O$, where x is the average hydration value (between 2 and 3)

Formula weight:
Monohydrate: 138.38
Heptahydrate: 246.47

Assay: Not less than 99.0 % and not more than 100.5% on the ignited basis

DESCRIPTION Colourless crystals, granular crystalline powder or white powder. Crystals effloresce in warm, dry air.

FUNCTIONAL USES Nutrient; flavour enhancer; firming agent; and processing aid (fermentation aid in the production of beer and malt beverages)

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water, very soluble in boiling water, and sparingly soluble in ethanol.

Test for magnesium (Vol. 4): Passes test

Test for sulfate (Vol. 4): Passes test

PURITY

Loss on ignition (Vol. 4)	Monohydrate: between 13.0 and 16.0 %, Heptahydrate: between 40.0 and 52.0 %, Dried: between 22.0 and 32.0 % (105°, 2h, then 400° to constant weight)
pH (Vol. 4)	Between 5.5 and 7.5 (1 in 20 solution)
Chloride (Vol. 4)	Not more than 0.03% Test 1g of the sample as described under “Chloride Limit Test” using 0.9 ml of 0.01 N hydrochloric acid in the control
Arsenic (Vol. 4)	Not more than 3 mg/kg Determine by the atomic absorption hydride technique. Use Method I for sample preparation.
Iron (Vol. 4)	Not more than 20 mg/kg Use 1 ml of Iron Standard TS
Selenium (Vol. 4)	Not more than 30 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Lead (Vol. 4)	Not more than 2mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
METHOD OF ASSAY	Accurately weigh about 0.5 g of the ignited sample, dissolve in 5 ml of hydrochloric acid TS, Dilute, dilute with water to 100 ml, and mix. Transfer 50 ml of this solution into a 250-ml conical flask; add 10 ml of Ammonia/Ammonium Chloride Buffer TS and 0.1 ml of Eriochrome Black TS. Titrate with 0.05 M disodium EDTA until the colour of red-purple solution changes to blue. Each ml of 0.05 M disodium EDTA is equivalent to 12.04 mg of $MgSO_4$.

dl-MALIC ACID

Prepared at the 57th JECFA (1999) and published in FNP 52 Add 9 (2001), superseding specifications prepared at the 53rd JECFA (1999), published in FNP 52 Add 7 (1999). ADI "not specified", established at the 13th JECFA in 1969.

SYNONYMS 2-Hydroxybutanedioic acid; INS No. 296

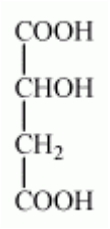
DEFINITION

Chemical names: dl-Malic acid, 2-Hydroxybutanedioic acid, Hydroxysuccinic acid

C.A.S. number: 617-48-1

Chemical formula: $C_4H_6O_5$

Structural formula:



Formula weight: 134.09

Assay: Not less than 99.0%

DESCRIPTION White or nearly white crystalline powder or granules

FUNCTIONAL USES Acidity regulator, flavouring agent (See 'Flavouring agents' monograph JECFA no. 619)

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Very soluble in water; freely soluble in ethanol

Melting range (Vol. 4): 127 - 132°

Test for malate (Vol. 4): Passes test
Test 5 ml of a 1 in 20 solution of the sample, neutralized with ammonia TS

PURITY

Sulfated ash (Vol. 4): Not more than 0.1%
Test 2 g of the sample (Method I)

Fumaric and maleic acid — Not more than 1.0% of fumaric acid and not more than 0.05% of maleic acid
See description under TESTS

Lead (Vol. 4) — Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Fumaric and maleic acid

Buffer solution A
In a 1000-ml volumetric flask dissolve 74.5 g of potassium chloride in 500 ml of water, add 100 ml of concentrated hydrochloric acid, and dilute to volume with water.

Buffer solution B
Dissolve 171.0 g of dipotassium hydrogen phosphate, $K_2HPO_4 \cdot \cdot H_2O$, in 1000 ml of water, and add potassium dihydrogen phosphate, KH_2PO_4, until the pH is exactly 7.0.

Maxima suppressor
Dissolve, with the aid of a magnetic stirrer, 1 g of gelatin in 65 ml of hot, boiled water. After cooling, add 35 ml of ethanol as a preservative.

Standard solution
Weigh out accurately about 20 g of the sample, 200 mg of fumaric acid and 10 mg of maleic acid, both of the highest purity available, and transfer into a 500-ml volumetric flask. Add 300 ml of sodium hydroxide TS, a few drops of phenolphthalein TS and continue adding sodium hydroxide TS to a faint pink colour that persists for at least 30 sec. Dilute to volume with water, and mix.

Sample solution
Transfer about 4 g of the sample, accurately weighed, to a 100-ml volumetric flask and dissolve in 25 ml of water. Add phenolphthalein TS, and neutralize with sodium hydroxide TS as directed for standard solution. Dilute to volume with water, and mix.

Procedure
Transfer two 25-ml portions of the "Sample solution" into separate 100-ml volumetric flasks. Dilute one flask (Sample A) to volume with "Buffer solution A". To the other flask (Sample B) add 50 ml of "Buffer solution B" and dilute to volume with water. Rinse a polarograph cell with a portion of "Sample A", add a suitable volume of the solution to the cell, immerse it in a water bath regulated at 24.5-25.5º, add 2 drops of the "Maxima suppressor", and then de-aereate by bubbling nitrogen through the solution for at least 5 min. Insert the dropping mercury electrode (negative polarity) of a suitable polarograph, adjust the current sensitivity as necessary, and record the polarogram from -0.1 to -0.8 volt at the rate of 0.2 volt per min,

using a saturated calomel electrode as the reference electrode. Transfer 25 ml of the "Standard solution" into a 100-ml volumetric flask, and dilute to volume with "Buffer solution A". Obtain the polarogram of this solution (Standard A) in the same manner as directed for "Sample A". In each polarogram, determine the height of the maleic acid plus fumaric acid wave occurring at the half-wave potential near -0.56 volt, recording that for sample as i_U and that for the standard as i_S. In the same manner, obtain polarograms from "Sample B" and a "Standard B", except record the polarogram from -1.05 to -1.7 volts at the rate of 0.1 volt per minute. In each polarogram, determine the height of the maleic acid wave occurring at the half-wave potential near -1.33 volts, recording that for the sample as i_U' and that for the standard as i_S'.

Calculation

Calculate the weight in mg, p, of combined maleic acid and fumaric acid in the sample taken by the formula:

$$500C \times [i_U/(i_S - i_U)]$$

where
C = the concentration, in mg per ml, of combined maleic acid and fumaric acid in the Standard solution.

Similarly, calculate the weight in mg, q, of maleic acid in the sample taken by the formula:

$$500C' \times [\, i_U'/(i_S' - i_U')]$$

where
C'= the concentration, in mg per ml of maleic acid in the Standard solution. Calculate the weight of fumaric acid in mg, r, in the sample taken from the difference in these values, i.e. (r = p - q).

Finally, calculate the percentage of fumaric and maleic acids present by multiplying r and q, respectively, by 0.025.

METHOD OF ASSAY

Dissolve about 2 g of the sample, accurately weighed, in 40 ml of recently boiled and cooled water, add 2 drops of phenolphthalein TS and titrate with 1 N sodium hydroxide to the first appearance of a faint pink colour which persists for at least 30 sec. Each ml of 1 N sodium hydroxide is equivalent to 67.04 mg of $C_4H_6O_5$.

MALTITOL

Prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 33rd JECFA (1988), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications were revised by JECFA in 2006. An ADI 'not specified' was established at the 41st JECFA (1993).

SYNONYMS D-Maltitol, hydrogenated maltose, INS No. 965(i)

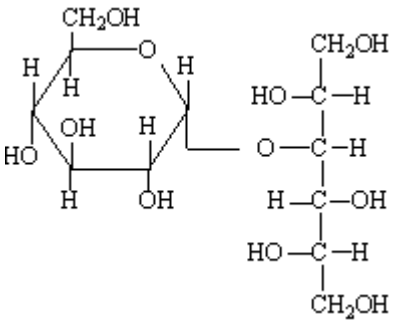
DEFINITION

Chemical names alpha-D-Glucopyranosyl-1,4-D-glucitol

C.A.S. number 585-88-6

Chemical formula $C_{12}H_{24}O_{11}$

Structural formula



Formula weight 344.31

Assay Not less than 98.0%

DESCRIPTION White crystalline powder

FUNCTIONAL USES Sweetener, humectant, stabilizer, bulking agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Very soluble in water, slightly soluble in ethanol

Melting range (Vol. 4) 148 - 151°

Thin layer chromatography (Vol. 4) Passes test
Proceed as directed under *Thin Layer Chromatography of Polyols*
Use the following:

Standard solution
Dissolve 50 mg of reference standard maltitol (available from US Pharmacopeial Convention, Inc. 12601 Twinbrook Parkway, Rockville, MD 20852, USA) in 20 ml water

Test solution
Dissolve 50 mg of the sample in 20 ml of water

PURITY

Water (Vol. 4)	Not more than 1.0% (Karl Fischer Method)
Specific rotation (Vol. 4)	[alpha] 20, D: Between +105.5 and +108.5^{o}(5% w/v solution)
Sulfated ash (Vol. 4)	Not more than 0.1% Test 2 g of sample (Method I)
Chlorides (Vol. 4)	Not more than 50 mg/kg Test 10 g of sample by the Limit Test using 1.5 ml of 0.01N hydrochloric acid in the control
Sulfates (Vol. 4)	Not more than 100 mg/kg Test 10 g of sample by the Limit Test using 2.0 ml of 0.01N sulfuric acid in the control
Nickel (Vol. 4)	Not more than 2 mg/kg Proceed as directed under *Nickel in Polyols*
Reducing sugars (Vol. 4)	Not more than 0.1% Proceed as directed under *Reducing Substances (as glucose*), Method II. The weight of cuprous oxide shall not exceed 20 mg
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Determine the maltitol content of the sample using *liquid chromatography* (see Volume 4)

Apparatus
Liquid chromatograph (HPLC)
Detection: Differential refractometer maintained at constant temperature
Integrator recorder
Column: AMINEX HPX 87 C (or equivalent resin in calcium form), length 30 cm, internal diameter 9 mm
Eluent: Double distilled degassed water (filtered through Millipore membrane filter 0.45 µm)
Chromatographic conditions
Column temperature: 85 ± 0.5^{o} ; Eluent flow rate: 0.5 ml/min

Standard preparation

Dissolve an accurately weighed quantity of standard reference maltitol in water to obtain a solution having known concentration of about 10.0 mg of maltitol per ml.

Sample preparation

Transfer about 1 g of the sample accurately weighed to a 50 ml volumetric flask, dilute with water to volume and mix.

Procedure

Separately inject equal volumes (about 20 µl) of the sample preparation and the standard preparation into the chromatograph. Record the chromatograms and measure the responses of the maltitol peak. Calculate the quantity, in mg, of maltitol in the portion of sample taken by the following formula:

$$50 \times C \times \frac{Ru}{Rs}$$

where

C = the concentration, in mg per ml, of maltitol in the standard preparation

R_U = the peak response of the sample preparation

R_S = the peak response of the standard preparation

MALTITOL SYRUP

Prepared at the 49th JECFA (1997), published in FNP 52 Add 5 (1997) superseding specifications prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996). An ADI 'not specified' was established at the 49th JECFA (1997)

SYNONYMS Hydrogenated high maltose-content glucose syrup, hydrogenated glucose syrup, dried maltitol syrup, maltitol syrup powder
INS No. 965(ii)

DEFINITION A mixture consisting of mainly maltitol with sorbitol and hydrogenated oligo- and polysaccharides. It is manufactured by the catalytic hydrogenation of high maltose-content glucose syrup. The article of commerce is typically supplied as a syrup. It may also be dried and supplied as a solid product

Assay Not less than 99.0% of total hydrogenated saccharides on the anhydrous basis and not less than 50.0% of maltitol on the anhydrous basis

DESCRIPTION Colourless and odourless, clear viscous liquids or white crystalline masses

FUNCTIONAL USES Sweetener, humectant, texturizer, stabilizer, bulking agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Very soluble in water, slightly soluble in ethanol

Thin layer chromatography (Vol. 4) Passes test
Proceed as directed under *Thin Layer Chromatography of Polyols*

Standard solution
Dissolve 50 mg of reference standard maltitol (available from US Pharmacopeial Convention, Inc., 12601 Twinbrook Parkway, Rockville, MD 20852, USA) in 20 ml water

Test solution
Dissolve 50 mg of sample in 20 ml of water

PURITY

Water (Vol. 4) Not more than 31% (Karl Fischer)

Sulfated ash (Vol. 4) Not more than 0.1 %
Test 3 g of the sample (Method I)

Chloride (Vol. 4)

Not more than 50 mg/kg
Test 10 g of the sample by the Limit Test using 1.5 ml of 0.01 N hydrochloric acid in the standard

Sulfate (Vol. 4)

Not more than 100 mg/kg
Test 10 g of the sample by the Limit Test using 2.0 ml of 0.0l N sulfuric acid in the standard

Nickel (Vol. 4)

Not more than 2 mg/kg
Proceed as directed under *Nickel in Polyols*

Reducing sugars (Vol. 4)

Not more than 0.3%
Proceed as described under *Reducing Substances (as glucose)*, Method II. The weight of cuprous oxide shall not exceed 50 mg

Lead (Vol. 4)

Not more than 1 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

METHOD OF ASSAY

Total hydrogenated saccharides (%)

$$\frac{100 - (\text{Water\%} + \text{Sulfated ash\%} + \text{Reducing sugars\%})}{100 - \text{Water\%}} \times 100$$

Determine the maltitol content using liquid chromatography.

Apparatus
-Detection: Differential refractometer maintained at constant temperature
-Integrator recorder
-Liquid chromatograph (HPLC)
-Column: AMINEX HPX 87 C (resin in calcium form), length 30 cm, internal diameter 9 mm
-Eluent: Double distilled degassed water (filtered through Millipore membrane filter 0.45 µm)

Chromatographic conditions
Column temperature: 85 ± 0.5°
Eluent flow rate: 0.5 ml/min

Standard preparation
Dissolve an accurately weighed quantity of standard reference maltitol (available from US Pharmacopeial Convention Inc., 12601 Twinbrook Parkway, Rockville, MD 20852, USA) in water to obtain a solution having known concentration of about 10.0 mg of maltitol per ml.

Sample preparation
Transfer about 1 g of the sample accurately weighed to a 50-ml volumetric flask, dilute with water to volume and mix.

Procedure

Separately inject equal volumes (about 20 µl) of the sample preparation and the standard preparation into the chromatograph. Record the chromatograms and measure the responses of each maltitol peak. Calculate the quantity, in mg, of maltitol in the syrup by the following formula:

$$50 \times C \times \frac{R_U}{R_S}$$

where

C = the concentration, in mg per ml, of maltitol in the standard preparation

R_U = the peak response of the sample preparation

R_S = the peak response of the standard preparation

MANNITOL

Prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 33rd JECFA (1988), published in FNP 38 (1988). Metals and arsenic specifications revised at the 57th JECFA (2001). An ADI 'not specified' was established at the 30th JECFA (1986)

SYNONYMS D-Mannitol, mannite, INS No. 421

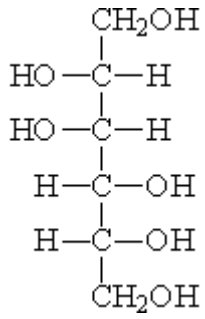
DEFINITION

Chemical names D-Mannitol

C.A.S. number 69-65-8

Chemical formula $C_6H_{14}O_6$

Structural formula



Formula weight 182.17

Assay Not less than 96.0% and not more than 102.0% on the dried basis

DESCRIPTION White, odourless, crystalline powder

FUNCTIONAL USES Sweetener, humectant, texturizer, stabilizer, bulking agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in water, very slightly soluble in ethanol; practically insoluble in ether

Melting range (Vol. 4) 164 - 169°

Thin layer chromatography (Vol. 4) Passes test
Proceed as directed under *Thin Layer Chromatography of Polyols*
Use the following:
Standard solution
Dissolve 50 mg of reference standard mannitol (available from US Pharmacopeial Convention, Inc. 12601 Twinbrook Parkway, Rockville, MD 20852, USA) in 20 ml water

	Test solution Dissolve 50 mg of the sample in 20 ml of water
PURITY	
Loss on drying (Vol. 4)	Not more than 0.3% (105°, 4 h)
Specific rotation (Vol. 4)	[alpha] 20, D: Between +23 and +25° Accurately weigh and dissolve 2.0 g of sample and 2.6 g of disodium tetraborate in about 20 ml of water previously heated to about 30°, shake continuously for 15-30 min without further heating. Dilute the resulting clear solution to 25 ml with water.
pH (Vol. 4)	Between 5 and 8 Add 0.5 ml of a saturated solution of potassium chloride to 10 ml of a 10% w/v solution of the sample, then measure the pH.
Sulfated ash (Vol. 4)	Not more than 0.1% Test 2 g of sample (Method I)
Chlorides (Vol. 4)	Not more than 70 mg/kg Test 10 g of sample by the Limit Test using 2.0 ml of 0.01N hydrochloric acid in the control
Sulfates (Vol. 4)	Not more than 100 mg/kg Test 10 g of sample by the Limit Test using 2.0 ml of 0.01N sulfuric acid in the control
Nickel (Vol. 4)	Not more than 2 mg/kg Proceed as directed under *Nickel in Polyols*
Reducing sugars(Vol. 4)	Not more than 0.3% Proceed as directed under *Reducing Substances (as glucose*), Method II. The weight of cuprous oxide shall not exceed 50 mg
Total sugars(Vol. 4)	Not more than 1.0% (as glucose) Transfer 2.1 g of the sample into a 250 ml flask fitted with a ground glass joint, add 40 ml of 0.1N hydrochloric acid, attach a reflux condenser, and reflux for 4 h. Transfer the solution to a 400 ml beaker, rinsing the flask with about 10 ml of water, neutralize with 6N sodium hydroxide and proceed as directed in the *General Method for Reducing Substances* (as glucose) Method II. The weight of the cuprous oxide shall not exceed 50 mg.
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF	Determine the mannitol content of the sample using *liquid chromatography*

ASSAY (see Volume 4)

Apparatus
Liquid chromatograph (HPLC)
Detection: differential refractometer maintained at constant temperature
Integrator recorder
Column: AMINEX HPX 87 C (resin in calcium form), length 30 cm, internal diameter 9 mm
Eluent: double distilled degassed water (filtered through Millipore membrane filter 0.45 µm)

Chromatographic conditions
Column temperature: 85±0.5°
Eluent flow rate: 0.5 ml/min

Standard preparation
Dissolve an accurately weighed quantity of standard reference mannitol in water to obtain a solution having known concentration of about 10.0 mg of mannitol per ml.

Sample preparation
Transfer about 1 g of the sample accurately weighed to a 50 ml volumetric flask, dilute with water to volume and mix.

Procedure
Separately inject equal volumes (about 20 µl) of the sample preparation and the standard preparation into the chromatograph. Record the chromatograms and measure the response of the mannitol peak. Calculate the quantity, in mg, of mannitol in the portion of sample taken by the following formula:

$$50 \times C \times \frac{Ru}{Rs}$$

where
C = the concentration, in mg per ml, of mannitol in the standard preparation
R_U = the peak response of the sample preparation
R_S = the peak response of the standard preparation.

METHYL CELLULOSE

Prepared at the 28th JECFA (1984), published in FNP 31/2 (1984) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). A group ADI 'not specified' for modified celluloses was established at the 35th JECFA (1989)

SYNONYMS Cellulose methyl ether; INS No. 461

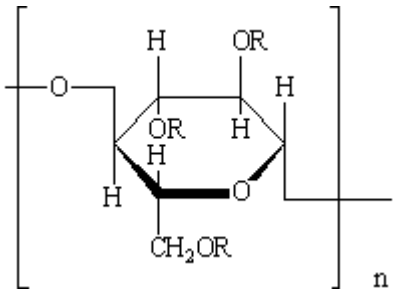
DEFINITION The methyl ether of cellulose, prepared from wood pulp or cotton by treatment with alkali and methylation of the alkali cellulose with methyl chloride. The article of commerce can be specified further by viscosity.

Chemical names: Methyl ether of cellulose; Cellulose methyl ether

C.A.S. number: 9004-67-5

Chemical formula: $[C_6H_7O_2(OH)_x(OCH_3)_y]_n$
where
x = 1.00 to 1.55
y = 2.00 to 1.45
x+y = 3.00
(y = degree of substitution)

Structural formula



where R = H or CH_3

Formula weight: Unsubstituted structural unit: 162.14
Structural unit with total degree of substitution of 1.45 : 182
Structural unit with total degree of substitution of 2.00 : 190
Macromolecules: from about 20 000 (n about 100) up to about 380 000 (n about 2,000)

Assay: Not less than 25% and not more than 33% of methoxyl groups (Some products of commerce designated "methyl cellulose" also contain components substituted with small amounts (max. 5%) of hydroxyethyl and/or hydroxypropyl groups. Development of separate specifications for these products should be considered).

DESCRIPTION Hygroscopic white or off-white, odourless fine granules, filaments or powder

FUNCTIONAL USES Thickening agent, emulsifier, stabilizer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Swelling in water, producing a clear to opalescent, viscous, colloidal solution; insoluble in ethanol, ether and chloroform; soluble in glacial acetic acid
Foam test	An 0.1% solution of the sample is shaken vigorously. A layer of foam appears. (This test permits the distinction of sodium carboxymethyl cellulose from other cellulose ethers.)
Precipitate formation	To 5 ml of an 0.5% solution of the sample, add 5 ml of a 5% solution of copper sulfate or of aluminium sulfate. No precipitate appears. (This test permits the distinction of sodium carboxymethyl cellulose from other cellulose ethers.)

PURITY

Loss on drying (Vol. 4)	Not more than 10% (105°, 3 h)
pH (Vol. 4)	5.0 - 8.0 (1 in 100 soln)
Sulfated ash (Vol. 4)	Not more than 1.5% Test 1 g of the sample (Method I)
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Determine the content of methoxyl groups by the method for *Ethoxyl and Methoxyl Group Determination* (see Volume 4).

METHYL ETHYL CELLULOSE

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). A group ADI 'not specified' for modified celluloses was established at the 35th JECFA (1989)

SYNONYMS MEC; INS No. 465

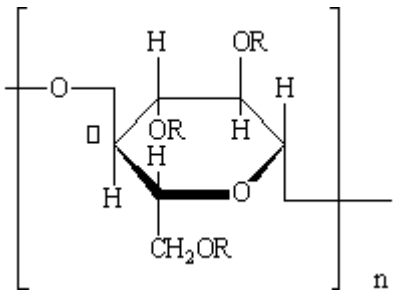
DEFINITION A mixed ether of cellulose, prepared from cellulose by treatment with alkali, dimethyl sulfate and ethyl chloride; both the methyl and ethyl groups are attached to the anhydroglucose units by ether linkages. The article of commerce can be specified further by viscosity.

Chemical names Ethyl methyl ether of cellulose

C.A.S. number 9004-69-7

Chemical formula $[C_6H_7O_2(OH)_x(OCH_3)_y(OC_2H_5)_z]_n$
where
z = 0.57 to 0.8
y = 0.2 to 0.4
x = 3 - (x + y)
(y + z = degree of substitution)

Structural formula



where R = H or CH_3 or C_2H_5

Formula weight Unsubstituted structural unit: 162.14
Structural unit with a total degree of substitution of 0.77: 181
Structural unit with a total degree of substitution of 1.2: 190
Macromolecules: 30 000 - 40 000 (n about 200)

Assay Methyl Ethyl Cellulose contains, on the dried basis, not less than 3.5% and not more than 6.5% of methoxyl groups ($-OCH_3$), not less than 14.5% and not more than 19.0% of ethoxyl groups ($-OCH_2CH_3$), and not less than 13.2% and not more than 19.6% of total alkoxyl groups, calculated as methoxyl (on the dry basis).

DESCRIPTION Hygroscopic and slightly yellowish odourless fibre or powder

FUNCTIONAL USES Emulsifier, stabilizer, thickening agent, foaming agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Swelling in water, producing a clear to opalescent, viscous, colloidal solution; insoluble in ethanol.
Foam test	A 0.1% solution of the sample is shaken vigorously. A layer of foam appears. (This test permits the distinction of sodium carboxymethyl cellulose from other cellulose ether and alginates and natural gums).
Precipitate formation	To 5 ml of an 0.5% solution of the sample add 5 ml of a 5% solution of copper sulfate or of aluminium sulfate. No precipitate appears. (This test permits the distinction of cellulose ethers from sodium carboxymethyl cellulose, gelatine, carob bean gum and tragacanth gum).
Substituents	Determine the substituents by *Gas Chromatography*

PURITY

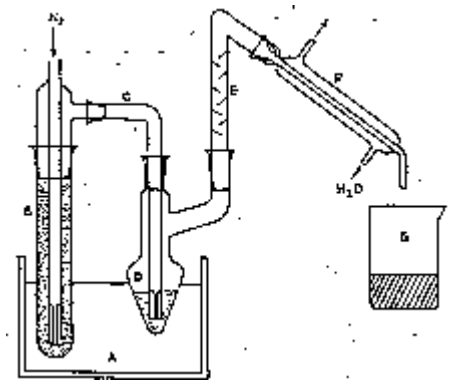
Loss on drying (Vol. 4)	Not more than 15% for the fibrous form, and not more than 10% for the powdered form, after drying to constant weight
Sulfated ash (Vol. 4)	Not more than 0.6% Test 1 g of the sample (Method I)
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Determination of the Ethoxyl group (Ethoxyl and methoxyl can be separately determined by gas chromatography (Cobler, Samsel and Beaver, Talanta, 9, 473, 1962)).

Apparatus

The apparatus for ethoxyl group determination is shown in the accompanying diagram. The boiling flask, D, is fitted with an aluminium foil-covered Vigreaux column, E, on the sidearm and with a bleeder tube through the neck and to the bottom of the flask for the introduction of steam and nitrogen. A steam generator, B, is attached to the bleeder tube through tube C, and a condenser, F, is attached to the Vigreaux column. The boiling flask and steam generator are immersed in an oil bath, A, equipped with a thermoregulator such that a temperature of 155^{o} and the desired heating rate may be maintained. The distillate is collected in a 150-ml beaker, G, or other suitable container.



Procedure

Transfer about 100 mg, weighed to the nearest 0.1 mg, of the sample, previously dried at 105° for 2 h, into the boiling flask, and add 10 ml of chromium trioxide solution (60 g in 140 ml of water). Immerse the steam generator and the boiling flask in the oil bath (at room temperature) to the level of the top of the chromium trioxide solution. Start cooling water through the condenser and pass nitrogen gas through the boiling flask at the rate of one bubble per second. Starting at room temperature, raise the temperature of the oil bath to 155° over a period of not less than 30 min, and maintain this temperature until the end of the determination. Distil until 50 ml of distillate is collected. Detach the condenser from the Vigreaux column, and wash it with water, collecting the washings in the distillate container. Titrate the combined washings and distillate with 0.02 N sodium hydroxide to a pH of 7.0, using a pH meter set at the expanded scale. (Note: Phenolphthalein TS may be used for this titration if it is also used for all standards and blanks.)

Record the volume, V_a, of the 0.02 N sodium hydroxide used. Add 500 mg of sodium bicarbonate and 10 ml of dilute sulfuric acid TS, and then, after evolution of carbon dioxide has ceased, add 1 g of potassium iodide. Stopper the flask, shake the mixture and allow it to stand in the dark for 5 min. Titrate the liberated iodine with 0.02 N sodium thiosulfate to the sharp disappearance of the yellow colour, confirming the end-point by the addition of a few drops of starch TS. Record the volume of 0.02 N sodium thiosulfate required as Y_a.

Make several reagent blank determinations, using only the chromium trioxide solution in the above procedure. The ratio of the sodium hydroxide titration (V_b), corrected for variation in normalities, will give the acidity-to-oxidizing ratio $V_b/Y_b = K$, for the chromium trioxide carried over in the distillation. The factor K should be constant for all determinations.
Make a series of blank determinations using 100 mg of methyl cellulose (containing no foreign material) in place of the sample, recording the average volume of 0.02 N sodium hydroxide required as V_m and the average volume of 0.02 N sodium thiosulfate required as Y_m.
Calculate the ethoxyl content of the sample, in mg, by the formula:

$$45.0 \times [N_1(V_a - V_m) - kN_2(Y_a - Y_m)]$$

where

N_1 = exact normality of the 0.02 N sodium hydroxide solution,
N_2 = exact normality of the 0.02 N sodium thiosulfate solution, and
$k = V_b N_1 / Y_b N_2$
Record the percentage of ethoxyl as B%.

Determination of the methoxyl content

Determine the methoxyl plus methoxyl content (Total alkoxyl content) as directed under *Ethoxyl and Methoxyl Group Determinations*. Then calculate the methoxyl content as follows:

$$\%\ Methoxyl = \frac{31}{45} x\ (A - B)$$

where
A = the total alkoxyl content expressed as % ethoxyl
B = the ethoxyl content expressed as %, as determined above.

Determination of total alkoxyl content (as methoxyl)

Each ml of 0.1 N sodium thiosulfate required in the determination of total alkoxyl content is equivalent to 0.517 mg of alkoxyl expressed as methoxyl.

MICROCRYSTALLINE CELLULOSE

Prepared at the 55th JECFA (2000) and published in FNP52 Add 8 (2000), superseding specifications prepared at the 51st JECFA (1998) and published in FNP 52 Add 6 (1998). An ADI "Not specified" was established at the 49th JECFA (1998).

SYNONYMS Cellulose gel; INS No. 460(i)

DEFINITION Purified, partially depolymerized cellulose prepared by treating alpha-cellulose, obtained as a pulp from fibrous plant material, with mineral acids. The degree of polymerization is typically less than 400. Not more than 10% of the particles have a diameter below 5 µm.

Chemical names: Cellulose

C.A.S. number: 9004-34-6

Chemical formula: $(C_6H_{10}O_5)_n$

Assay: Not less than 97% of carbohydrate calculated as cellulose on the dry basis.

DESCRIPTION Fine, white or almost white, odourless, free flowing crystalline powder.

FUNCTIONAL USES Emulsifier, stabilizer, anticaking agent, dispersing agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Insoluble in water, ethanol, ether and dilute mineral acids. Slightly soluble in sodium hydroxide solution

Infrared absorption: The infrared absorption spectrum of a potassium bromide dispersion of the sample corresponds to the infrared spectrum below.

Suspensoid: Mix 30 g of the sample with 270 ml of water in a high-speed (18,000 rpm) blender for 5 min. Transfer 100 ml of the mixture to a 100-ml graduated cylinder, and allow to stand for 3 h. A white, opaque, bubble-free dispersion that forms a supernatant, is obtained.

PURITY

Loss on drying (Vol. 4): Not more than 7.0% (105°, 3 h)

pH (Vol. 4): 5.0 - 7.5
Shake 5 g of the sample with 40 ml of water for 20 min and centrifuge. Determine the pH of the supernatant.

Sulfated ash (Vol. 4): Not more than 0.05%
Test 10 g of the sample (Method I)

Water soluble Substances

Not more than 0.24%.
Shake 5 g of the sample with approximately 80 ml of water for 10 min, filter through Whatman No. 42 or equivalent filter paper into a tared beaker, wash residue with 20 ml of water and evaporate to dryness on a steam bath. Dry at 105° for 1 h, cool, weigh and calculate as percentage.

Starch

Not detectable
To 20 ml of the dispersion obtained in the identification test for starch, add a few drops of iodine TS, and mix. No purplish to blue or blue colour should be obtained.

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

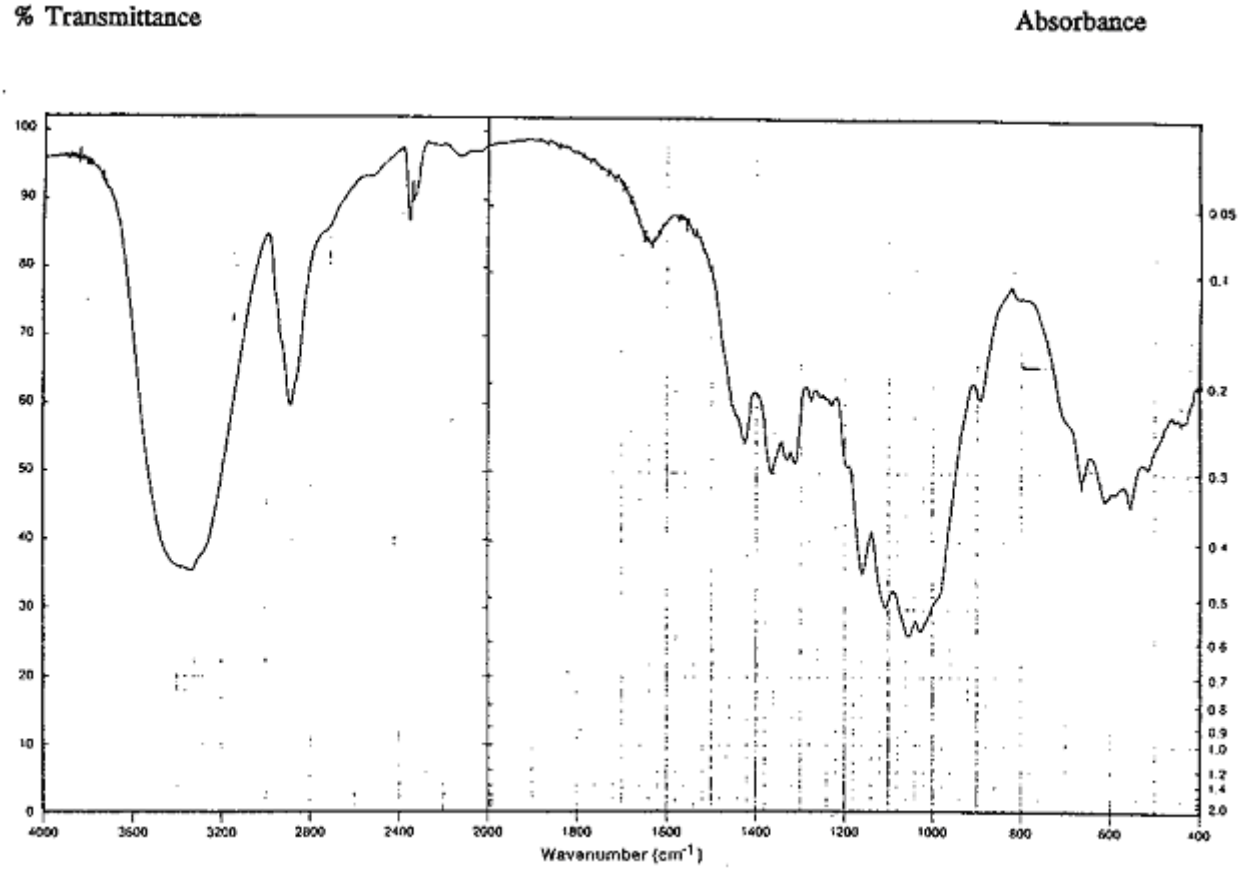
METHOD OF ASSAY

Transfer about 125 mg of the sample, accurately weighed, to a 300 ml Erlenmeyer flask, using about 25 ml of water. Add 50.0 ml of 0.5N potassium dichromate and mix. Carefully add 100 ml of sulfuric acid and heat to boiling. Remove from heat, allow to stand at room temperature for 15 min and cool in a water bath. Transfer the contents into a 250 ml volumetric flask, rinse flask with distilled water, add rinsings to the volumetric flask and dilute with water almost to volume. Allow the volumetric flask to reach room temperature (25°); then make up to volume with water and mix. Titrate a 50.0 ml aliquot with 0.1N ferrous ammonium sulfate using 2 or 3 drops of ortho-phenanthroline TS as the indicator and record the volume required as S in ml. Perform a blank determination and record the volume of 0.1N ferrous ammonium sulfate required as B in ml. Calculate the percentage of cellulose in the sample by the formula:

$$(B - S) \times \frac{338}{W} \%$$

where
W is the weight of sample taken, in mg, corrected for loss on drying.

Infrared Spectrum Microcrystalline cellulose



MICROCRYSTALLINE WAX

Prepared at the 55th JECFA (2000) and published in FNP 52 Add 8 (2000), superseding specifications prepared at the 49th JECFA (1997) and published in FNP 52 Add 5 (1997). A group ADI of 0-20 mg/kg bw was established at the 44th JECFA (1995).

SYNONYMS Petroleum wax; INS No. 905(c)

DEFINITION Microcrystalline Wax is a refined mixture of solid, saturated hydrocarbons, mainly branched paraffin, obtained from petroleum

DESCRIPTION Colourless or white, somewhat translucent, tasteless and odourless wax

FUNCTIONAL USES Chewing gum base, protective coating, defoaming agent, surface finishing agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water, very slightly soluble in ethanol, sparingly soluble in diethyl ether and hexane

Refractive index (Vol. 4) n (100, D): 1.434 - 1.448

Infrared absorption The infrared absorbance spectrum of the sample melted and prepared on a caesium or potassium bromide plate corresponds to the spectrum in the Appendix

PURITY

Viscosity, 100° Not less than 11 mm^2/sec
See description under TESTS

Carbon number at 5% distillation point Not more than 5% of molecules with carbon number less than 25
See description under TESTS

Average molecular weight Not less than 500
See description under TESTS

Residue on ignition Not more than 0.1%
See description under TESTS

Colour Passes test
See description under TESTS

Sulfur Not more than 0.4%
See description under TESTS

Lead (Vol. 4) Not more than 3 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described on

Volume 4, "Instrumental methods".

Polycyclic aromatic hydrocarbons

The sample shall meet the following ultraviolet absorbance limits when subjected to the analytical procedure described under the TESTS.

nm	max. absorbance per cm path length
280 - 289	0.15
290 - 299	0.12
300 - 359	0.08
360 - 400	0.02

TESTS

PURITY TESTS

Viscosity, 100°

(ASTM D 445 Adopted with permission from the Annual Book of ASTM Standards copyright American Society for Testing and Materials, 100 Harbor Drive, West Conshohocken, PA 19428. Copies of the complete ASTM standard may be purchased direct from ASTM, phone 610-832-9585, fax: 610-832-9555, e-mail: service@astm.org , website: http://www.astm.org)

Use a viscometer of the glass capillary type, calibrated and capable of measuring kinematic viscosity with a repeatability exceeding 0.35% only in one case in twenty. Immerse the viscometer in a liquid bath at the temperature required for the test ± 0.1° ensuring that at no time of the measurement will any portion of the sample in the viscometer be less than 20 mm below the surface of the bath. Charge the viscometer with sample in a manner directed by the design of the instrument. Allow the sample to remain in the bath for about 30 min. Where the design of the viscometer requires it, adjust the volume of sample to the mark. Use pressure to adjust the head level of the sample to a position in the capillary arm of the instrument about 5 mm ahead of the first mark. With the sample flowing freely, measure, in seconds (±0.2 sec), the time required for the meniscus to pass from the first to the second timing mark. If the time is less than 200 s, select a viscometer with a capillary of smaller diameter and repeat the operation. Make a second measurement of the flow time. If two measurements agree within 0.2%, use the average for calculating the kinematic viscosity. If the measurements do not agree, repeat the determination after thorough cleaning and drying the viscometer.

Viscosity, 100° (mm^2/sec) = C x t

where
C = calibration constant of the viscometer (mm^2/s)
t = flow time (s)

Carbon number at 5% distillation point

(ASTM D 5442 See TEST for Viscosity, 100° for Copyright permission) "Carbon number" is number of carbon atoms in a molecule. Determine the carbon number distribution of the sample by gas chromatography. Below is shown some typical working conditions for determination of up to carbon number 45.

Column length (m)	25	30	15
inside diameter (mm)	0.32	0.53	0.25
stationary phase	DB-1 methyl silicone	RTX-1 methyl silicone	DB-5 5% phenyl methyl silicone
film thickness (µm)	0.25	0.25	0.25
Carrier gas	helium	helium	helium
flow (ml/min)	1.56	5.0	2.3
Linear velocity (cm/sec)	33	35	60
Temperature program			
initial temperature	80°	80°	80°
rate (°/min)	10	8	5
final temperature	380°	340°	350°
Injection technique	cool on-column	cool on-column	cool on-column
Detector	FID	FID	FID
temperature	380°	340°	375°
Sample size (µl)	1.0	1.0	1.0

NOTE: By optimizing the length of separation column and/or column temperature, waxes with carbon number higher than 45 can also be included.

Standards for calibration and identification

Standard samples of normal paraffins covering the carbon number range of the sample of purity greater than 95%.

Linearity standard

Prepare a weighed mixture of n-paraffins covering the range between C_{16} to C_{65} and dissolve the mixture in cyclohexane. Use approximately equal amounts of each of the paraffins weighed with 1% accuracy. Solutions of 0.01 % (w/w) may be used. It is not necessary to include every n-paraffin in this mixture so long as it contains C_{16}, C_{44}, (C_{60} if determination of higher carbon numbers is relevant) and at least one of every fourth n-paraffin. This standard must be capped tightly to prevent solvent loss.

Internal standard solution

Prepare a stock solution containing 0.5% (w/w) n-C_{16} in n-hexane. (minimum purity of 98%) by accurately weighing approximately 0.4 g n-C_{16} into a 100 ml volumetric flask. Add 100 ml of cyclohexane and reweigh. Record the mass of n-C_{16} (W_{ISTD}) to within 0.001 g and the mass of the stock solution (Ws) to within 0.1 g. Prepare a dilute solution of internal standard by diluting one part of stock solution with 99 parts of cyclohexane. Calculate the concentration of internal standard using the following equation:

$$C_{INST} = \frac{W_{ISTD}}{Ws} \times \frac{100}{100} \text{ \% (w/w)}$$

Where

C_{INST} = concentration of n-C_{16} in the internal standard dilute solution in %

(w/w)
W_{ISTD} = weight of n-C_{16} used for the stock solution in g.
Ws = Weight of the stock solution in g

Check of solvent blank
Inject 1 µl of the solvent. No peaks must be detected within the retention time range over which the wax elutes.

Column resolution
Inject 1 µl of a solution of 0.05 % each of n-C_{20} and n-C_{24} in cyclohexane. The column resolution R not be less than 30 as calculated by the following equation:

$$R = \frac{2d}{1.699\ (W1 + W2)}$$

Where
d = the distance in mm between the peak maxima of n-C_{20} and n-C_{24}
W1 = the peak with in mm of half height of n-C_{20}
W2 = the peak with in mm of half height of n-C_{24}

Linearity
Analyze the linearity standard. The calculated mass response factors relative to hexadecane must be between 0.90 and 1.10.

Retention time repeatability
Analyze the linearity standard in duplicate. The retention times for duplicate analysis must not differ more than 0.10 min between duplicate runs.

Calibration for n-paraffin identification
Determine the retention time of each n-paraffin in the range from C_{16} to C_{44} (or C_{60} if determination of higher carbon numbers is relevant) by injecting small amounts of each paraffin either separately or in known mixtures.

Sampling
Heat the sample to 10° above the temperature at which the wax is completely molten. Mix well by stirring.. Using a clean eyedropper, transfer a few drops to the surface of a clean sheet of aluminium foil, allow to solidify and break into pieces. Aluminium foil usually contains a thin film of oil from processing. This oil must be removed by rinsing the foil with solvents such as hexane or mineral spirits, prior to use.
Procedure
Accurately weigh about 0.0100 g of the sample (W_{sample}) obtained as described under sampling into a glass vial of approximately 15 ml capacity. Add approximately 12 ml of dilute internal standard solution, cap the vial and determine the exact weight of the added dilute internal standard solution (W_{INSTD}). Agitate the vial until the wax is completely dissolved using gentle heating if necessary.

Inject 0.5 to 1.0 µl of the sample solution. Record the chromatogram and store the detector signal. The peak from the internal standard must be completely resolved from the wax sample area. Based on the retention time

as obtained under Calibration for n-paraffin identification, identify the normal paraffin peaks. Using a vertical drop to a horizontal baseline construction (see Figure 1), integrate the detector signal. Sum the area of all the peaks of each carbon number. By convention, the peaks assigned the carbon number n are those that elute between the valley immediately following the normal paraffin peak (C_{n-1}) and the corresponding valley following the next normal paraffin peak (C_n).

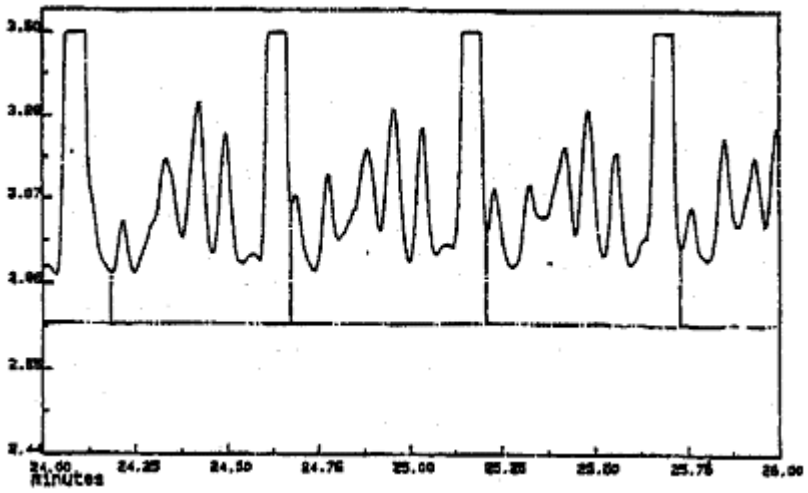


Figure 1. Carbon number summation (vertical drop to horizontal baseline)

Calculation

For each carbon number, calculate the content in % (w/w) by using the following equation:

$$C_i = \frac{A_i}{A_{ISTD}} \times RRF_i \times \frac{W_{ISTD}}{W_{sample}} \times C_{ISTD}$$

Where

C_i = content in % (w/w) of hydrocarbons with carbon number i

A_i = area sun of hydrocarbons with carbon number I

A_{ISTD} = the area of n-C_{16} internal standard peak

RRFi = the response factor relative to n-C_{16}

W_{INSTD} = the weight of dilute internal standard solution

Wsample = the weight of wax sample

C_{ISTD} = the concentration of n-C_{16} in the dilute internal standard solution

The combined contents of components with carbon number less than 25 is not more than 5%.

Average molecular weight

Using the carbon number distribution obtained in the test for "Carbon number at 5% distillation point" calculate the average molecular weight by the following formula:

Average molecular weight =

$$\frac{\sum_{i=1?}^{i=i} C_i (14i+2)}{100}$$

where
i = the carbon number
C_i = the content in % of components having a carbon number of i

Residue on ignition

Accurately weigh about 2 g of the sample in a tared porcelain or platinum dish and heat over a flame. The sample volatilizes without emitting an acrid odour. Ignite to not exceeding a very dull redness until free from carbon. Cool in a desiccator and weigh.

Colour

Melt about 10 g of the sample on a steam bath, and pour 5 ml of the liquid into a clear-glass, 16 x 150-mm bacteriological test tube: the warm, melted liquid is not darker than a solution made by mixing 3.8 ml of ferric chloride TS(FNP 5) and 1.2 ml of cobaltous chloride TS (FNP 5) in a similar tube, the comparison of the two being made in reflected light against a white background, the tubes being held directly against the background at such an angle that there is no fluorescence.

Sulfur

(ASTM D 2622 See TEST for Viscosity, 100° for Copyright permission)
Determine by X-ray spectrometry using the following conditions:

Apparatus
- X-ray spectrograph, equipped for soft ray detection in the 537 Å range
- Optical path of helium
- Pulse height analyzer or other means of energy discrimination
- Detector designed for detection of long wavelength X-rays

Analyzing crystal suitable for the dispersion of sulfur Kα X-rays within the angular range of the spectrometer employed. Pentaerythritol and germanium are the most popular although less reflective materials such as EDDT, ADP and quartz may be used.
X-ray tube of exiting sulfur Kα radiation.
X-ray tube with tungsten, platinum or chromium target

Sensitivity standards
Liquid petroleum materials containing sulfur in concentrations approximately in the middle of the calibration graph used for the test.

Calibration standards
Prepare calibration standards by careful weight dilution of di-n-butyl sulfide (high purity standard with a certified analysis, manufactured especially as a calibration material for this method, available from Philips Petroleum Co., Bartlesville, OK, USA) with white oil (containing less than 5 mg/kg). Exact standards of approximately 0.100, 0.250, 0.500 and 1.0 % (w/w) should be prepared.

Calibration curve
Measure the net emitted sulfur radiation from each of the calibration standards. Plot the intensity, in terms of net counts per sec, against sulfur concentration.

To maintain the validity of the calibration curve with slight changes in the instruments sensitivity, measure the sensitivity standard at frequent intervals during the course of the days run. Establish the counting rate of this standard by measuring its intensity at frequent intervals during the preparation of the calibration curve. Calculate the correction factor for changes in daily instrument sensitivity by using the following equation:

$$F = \frac{A}{B}$$

Where
A = the counting rate of the sensitivity standard determined at the time of calibration
B = the counting rate of the sensitivity standard determined at the time of analysis

Procedure

Place the sample in an appropriate cell. Although sulfur radiation will penetrate through only a small distance in the sample, scatter from the sample cup and the sample may vary to such an extent that a specific amount or a minimum amount of the sample must be used.
Place the sample in the X-ray beam and allow the X-ray optical atmosphere to come to equilibrium. Determine the intensity of the sulfur Kα radiation at 5.373 Å by making counting rate measurements at the precise angular settings for this wavelength. Measure background count-rate at 5.190 Å.

Calculation

Calculate the content of sulfur in the sample using the following equation:

$$R = \left[\frac{C_K}{S_1} - \frac{C_B \times F'}{S_2}\right] \times F$$

R = the corrected net counting rate
C_K = total counts collected at 5.373 Å for the sample
S_1 = the time in sec required to collect C_K counts
C_B = total counts collected at 5.190 Å for background
S_2 = the time in sec required to collect C_B counts
F' = count-rate at 5.373 Å / count-rate at 5.190 Å for a sample not containing sulfur
F = the correction factor for changes in daily instrument sensitivity
Using the corrected net counting rate, read the sulfur concentration from the calibration curve.

Polycyclic aromatic hydrocarbons

General Instructions

Because of the sensitivity of the test, the possibility of errors arising from contamination is great. It is of the greatest importance that all glassware be scrupulously cleaned to remove all organic matter such as oil, grease, detergent residues, etc. Examine all glassware, including stoppers and stopcocks, under ultraviolet light to detect any residual fluorescent contamination. As a precautionary measure it is a recommended practice to

rinse all glassware with purified isooctane immediately before use. No grease is to be used on stopcocks or joints. Great care to avoid contamination of wax samples in handling and to assure absence of any extraneous material arising from inadequate packaging is essential. Because some of the polynuclear hydrocarbons sought in this test are very susceptible to photo-oxidation, the entire procedure is to be carried out under subdued light.

Apparatus

- Separatory funnels: 250-ml, 500-ml, 1,000-ml, and preferably 2000-ml capacity, equipped with tetrafluoroethylene polymer stopcocks.
- Reservoir: 500 ml capacity, equipped with a 24/40 standard taper male fitting at the bottom and a suitable balljoint at the top for connecting to the nitrogen supply. The male fitting should be equipped with glass hooks.
- Chromatographic tube: 180 mm in length, inside diameter to be 15.7 mm ± 0.1 mm, equipped with a coarse, fritted-glass disc, a tetrafluoroethylene polymer stopcock, and a female 24/40 standard tapered fitting at the opposite end. (Overall length of the column with the female joint is 235 mm). The female 24/40 standard tapered fitting at the opposite end.
- Disc: Tetrafluoroethylene polymer 2-inch diameter disc approximately 3/16-inch thick with a hole bored in the center to closely fit the stem of the chromatographic tube.
- Heating jacket: Conical, for 500-ml separatory funnel. (Used with variable transformer heat control).
- Suction flask: 250-ml or 500-ml filter flask.
- Condenser: 24/40 joints, fitted with a drying tube, length optional.
- Evaporation flask (optional): 250-ml or 500-ml capacity all-glass flask equipped with standard taper stopper having inlet and outlet tubes permitting passage of nitrogen across the surface of the liquid to be evaporated.
- Vacuum distillation assembly: All glass (for purification of dimethyl sulfoxide); 2 litre distillation flask with heating mantle; Vigreaux vacuum-jacketed condenser (or equivalent) about 45 cm in length and distilling head with separable cold finger condenser. Use of tetrafluoroethylene polymer sleeves on the glass joints will prevent freezing. Do not use grease on stopcocks or joints.
- Spectrophotometric cells: Fused quartz cells, optical path length in the range of 5.000 ± 0.005 cm; also for checking spectrophotometer performance only, optical path length in the range 1.000 ± 0.005 cm. With distilled water in the cells, determine any absorbance differences.
- Spectrophotometer: Spectral range 250-400 nm with spectral slit width of 2 nm or less, under instrument operating conditions for these absorbance measurements, the spectrophotometer shall also meet the following performance requirements:

Absorbance repeatability: ±0.01 at 0.4 absorbance
Absorbance accuracy: ±0.05 at 0.4 absorbance
Wavelength repeatability: ±0.2 nm
Wavelength accuracy: ±1.0 nm

- Nitrogen cylinder: Water-pumped or equivalent purity nitrogen in cylinder equipped with regulator and valve to control flow at 5 p.s.i.g.

Reagents and materials

- Organic solvents: All solvents used throughout the procedure shall meet

the specifications and tests described in this specification. The isooctane, benzene, acetone, and methyl alcohol designated in the list following this paragraph shall pass the following test:

To the specified quantity of solvent in a 250-ml Erlenmeyer flask, add 1 ml of purified n-hexadecane and evaporate on the steam bath under a stream of nitrogen (a loose aluminium foil jacket around the flask will speed evaporation). Discontinue evaporation when not over 1 ml of residue remains. (To the residue from benzene add a 10 ml portion of purified isooctane, reevaporate, and repeat once to insure complete removal of benzene).

Alternatively, the evaporation time can be reduced by using the optional evaporation flask. In this case the solvent and n-hexadecane are placed in the flask on the steam bath, the tube assembly is inserted, and a stream of nitrogen is fed through the inlet tube while the outlet tube is connected to a solvent trap and vacuum line in such a way as to prevent any flow-back of condensate into the flask.

Dissolve the 1 ml of hexadecane residue in isooctane and make to 25 ml volume. Determine the absorbance in the 5 cm path length cells compared to isooactane as reference. The absorbance of the solution of the solvent residue (except for methyl alcohol) shall not exceed 0.01 per cm path length between 280 and 400 nm. For methyl alcohol this absorbance value shall be 0.00.

- Isooctane (2,2,4-trimethylpentane): Use 180 ml for the test described in the preceding paragraph. Purify, if necessary, by passage through a column of activated silica gel (Grade 12, Davison Chemical Company, Baltimore, Maryland, or equivalent) about 90 cm in length and 5 cm to 8 cm in diameter.
- Benzene, reagent grade: Use 150 ml for the test. Purify, if necessary, by distillation or otherwise.
- Acetone, reagent grade: Use 200 ml for the test. Purify, if necessary, by distillation.
- Eluting mixtures:
1. 10% benzene in isooctane: Pipet 50 ml of benzene into a 500-ml glass-stoppered volumetric flask and adjust to volume with isooctane, with mixing.
2. 20% benzene in isooctane: Pipet 50 ml of benzene into a 250-ml glass-stoppered volumetric flask, and adjust to volume with isooctane, with mixing.
3. Acetone-benzene-water mixture: Add 20 ml of water to 380 ml of acetone and 200 ml of benzene, and mix.
- n-Hexadecane, 99% olefin-free: Dilute 1.0 ml of n-hexadecane to 25 ml with isooctane and determine the absorbance in a 5-cm cell compared to isooctane as reference point between 280-400 nm. The absorbance per cm path length shall not exceed 0.00 in this range. Purify, if necessary, by percolation through activated silica gel or by distillation.
- Methyl alcohol, reagent grade: Use 10.0 ml of methyl alcohol. Purify, if necessary, by distillation.
- Dimethyl sulfoxide: Pure grade, clear, water-white, m.p. 18° minimum. Dilute 120 ml of dimethyl sulfoxide with 240 ml of distilled water in a 500-ml separatory funnel, mix and allow to cool for 5-10 min. Add 40 ml of

isooctane to the solution and extract by shaking the funnel vigorously for 2 min. Draw off the lower aqueous layer into a second 500 ml separatory funnel and repeat the extraction with 40 ml of isooctane. Draw off and discard the aqueous layer. Wash each of the 40 ml extractives three times with 50 ml portions of distilled water. Shaking time for each wash is 1 min. Discard the aqueous layers. Filter the first extractive through anhydrous sodium sulfate prewashed with isooctane (see Sodium sulfate under "Reagents and Materials" for preparation of filter), into a 250-ml Erlenmeyer flask, or optionally into the evaporating flask. Wash the first separatory funnel with the second 40 ml isooctane extractive, and pass through the sodium sulfate into the flask. Then wash the second and first separatory funnels successively with a 10 ml portion of isooctane, and pass the solvent through the sodium sulfate into the flask. Add 1 ml of n-hexadecane and evaporate the isooctane on the steam bath under nitrogen. Discontinue evaporation when not over 1 ml of residue remains. To the residue, add a 10 ml portion of isooctane and reevaporate to 1 ml of hexadecane. Again, add 10 ml of isooctane to the residue and evaporate to 1 ml of hexadecane to insure complete removal of all volatile materials. Dissolve the 1 ml of hexadecane in isooctane and make to 25 ml volume. Determine the absorbance in 5 cm path length cells compared to isooctane as reference. The absorbance of the solution should not exceed 0.02 per cm path length in the 280-400 nm range. (Note - Difficulty in meeting this absorbance specification may be due to organic impurities in the distilled water. Repetition of the test omitting the dimethyl sulfoxide will disclose their presence. If necessary to meet the specification, purify the water by redistillation, passage through an ion-exchange resin, or otherwise).
Purify, if necessary, by the following procedure: To 1.5 L of dimethyl sulfoxide in a 2 l glass-stoppered flask, add 6.0 ml of phosphoric acid and 50 g of Norit A (decolorizing carbon, alkaline) or equivalent. Stopper the flask, and with the use of a magnetic stirrer (tetrafluoroethylene polymer coated bar) stir the solvent for 15 min. Filter the dimethyl sulfoxide through four thicknesses of fluted paper (18.5 cm) (Schleicher & Schuell No. 597, or equivalent). If the initial filtrate contains carbon fines, refilter through the same filter until a clear filtrate is obtained. Protect the sulfoxide from air and moisture during this operation by covering the solvent in the funnel and collection flask with a layer of isooctane. Transfer the filtrate to a 2-l separatory funnel and draw off the dimethyl sulfoxide into the 2-l distillation flask of the vacuum distillation assembly and distil at approximately 3 mm Hg pressure or less. Discard the first 200 ml fraction of the distillate and replace the distillate collection flask with a clean one. Continue the distillation until approximately 1 litre of the sulfoxide has been collected. At completion of the distillation, the reagent should be stored in glass-stoppered bottles since it is very hygroscopic and will react with some metal containers in the presence of air.
- Phosphoric acid, 85% reagent grade
- Sodium borohydride, 98%
- Magnesium oxide (Sea Sorb 43, Food Machinery Company, Westvaco Division, distributed by chemical supplier firms, or equivalent): Place 100 g of the magnesium oxide in a large beaker, add 700 ml of distilled water to make a thin slurry, and heat on a steam bath for 30 min with intermittent stirring. Stir well initially to insure that all the absorbent is completely wetted. Using a Buchner funnel and a filter paper* of suitable diameter, filter with suction. Continue suction until water no longer drips from the funnel.

Transfer the absorbent to a glass trough lined with aluminium foil (free from rolling oil). Break up the magnesia with a clean spatula and spread out the absorbent on the aluminium foil in a layer about 1-2 cm thick. Dry at 160±1° for 24 h. Pulverize the magnesia with mortar and pestle. Sieve the pulverized absorbent between 60-180 mesh. Use the magnesia retained on the 180-mesh sieve.
- Celite 545 (Johns-Manville Company, diatomaceous earth, or equivalent)
- Magnesium oxide-Celite 545 mixture (2+1) by weight: Place the magnesium oxide (60-180 mesh) and the Celite 545 in 2 to 1 proportions, respectively, by weight in a glass-stoppered flask large enough for adequate mixing. Shake vigorously for 10 min. Transfer the mixture to a glass trough lined with aluminium foil (free from rolling oil) and spread it out on a layer about 1 to 2 cm thick. Reheat the mixture at 160±1° for 2 h, and store in a tightly closed flask.
- Sodium sulfate, anhydrous, reagent grade, preferably in granular form: For each bottle of sodium sulfate reagent used, establish as follows the necessary sodium sulfate prewash to provide such filters required in the method: Place approximately 35 g of anhydrous sodium sulfate in a 30 ml coarse, fritted-glass funnel or in a 65 ml filter funnel with glass wool plug; wash with successive 15 ml portions of the indicated solvent until a 15 ml portion of the wash shows 0.00 absorbance per cm path length between 280 nm and 400 nm when tested as prescribed under "Organic solvents." Usually three portions of wash solvent are sufficient.

Procedure

Before proceeding with the analysis of a sample, determine the absorbance in a 5 cm path cell between 250 nm and 400 nm for the reagent blank by carrying out the procedure, without a wax sample, at room temperature, recording the spectra after the extraction stage and after the complete procedure as prescribed. The absorbance per centimeter path length following the extraction stage should not exceed 0.040 in the wavelength range from 250 to 400 nm; the absorbance per cm path length following the complete procedure should not exceed 0.070 in the wavelength range from 250 to 299 nm, inclusive, nor 0.045 in the wavelength range from 300 nm to 400 nm. If in either spectrum the characteristic benzene peaks in the 250-260 nm region are present, remove the benzene by the procedure under "Organic solvents" and record absorbance again.

Place 300 ml of dimethyl sulfoxide in a 1liter separatory funnel and add 75 ml of phosphoric acid. Mix the contents of the funnel and allow to stand for 10 min. (The reaction between the sulfoxide and the acid is exothermic. Release pressure after mixing, then keep funnel stoppered). Add 150 ml of isooctane and shake to preequilibrate the solvents. Draw off the individual layers and store in glass-stoppered flasks.

Place a representative 1 kg sample of wax, or if this amount is not available, the entire sample, in a beaker of a capacity about three times the volume of the sample and heat with occasional stirring on a steam bath until the wax is completely melted and homogenous. Weigh four 25 ± 0.2 g portions of the melted wax in separate 100 ml beakers. Reserve three of the portions for later replicate analyses as necessary. Pour one weighed portion immediately after remelting (on the steam bath) into a 500 ml separatory funnel containing 100 ml of the preequilibrated sulfoxide-

phosphoric acid mixture that has been heated in the heating jacket at a temperature just high enough to keep the wax melted. (Note: In preheating the sulfoxide-acid mixture, remove the stopper of the separatory funnel at intervals to release the pressure).

Promptly complete the transfer of the sample to the funnel in the jacket with portions of the preequilibrated isooctane, warming the beaker, if necessary, and using a total volume of just 50 ml of the solvent. If the wax comes out of solution during these operations, let the stoppered funnel remain in the jacket until the wax redissolves. (Remove stopper from the funnel at intervals to release pressure).

When the wax is in solution, remove the funnel from the jacket and shake it vigorously for 2 min. Set up three 250 ml separatory funnels with each containing 30 ml of preequilibrated isooctane. After separation of the liquid phases, allow to cool until the main portion of the wax-isooctane solution begins to show a precipitate. Gently swirl the funnel when precipitation first occurs on the inside surface of the funnel to accelerate this process. Carefully draw off the lower layer, filter it slowly through a thin layer of glass wool fitted loosely in a filter funnel into the first 250 ml separatory funnel, and wash in tandem with the 30 ml portions of isooctane contained in the 250 ml separatory funnels. Shaking time for each wash is 1 min. Repeat the extraction operation with two additional portions of the sulfoxide-acid mixture, replacing the funnel in the jacket after each extraction to keep the wax in solution and washing each extractive in tandem through the same three portions of isooctane.

Collect the successive extractives (300 ml total) in a separatory funnel (preferably 2 liter), containing 480 ml of distilled water, mix, and allow to cool for a few minutes after the last extractive has been added. Add 80 ml of isooctane to the solution and extract by shaking the funnel vigorously for 2 min. Draw off the lower aqueous layer into a second separatory funnel (preferably 2 litre) and repeat the extraction with 80 ml of isooctane. Draw off and discard the aqueous layer. Wash each of the 80 ml extractives three times with 100 ml portions of distilled water. Shaking time for each wash is 1 min. Discard the aqueous layers. Filter the first extractive through anhydrous sodium sulfate prewashed with isooctane (see Sodium Sulfate under "Reagents and Materials" for preparation of filter) into a 250-ml Erlenmeyer flask (or optionally into the evaporation flask). Wash the first separatory funnel with the second 80 ml isooctane extractive and pass through the sodium sulfate. Then wash the second and first separatory funnels successively with a 20 ml portion of isooctane and pass the solvent through the sodium sulfate into the flask. Add 1 ml of n-hexadecane and evaporate the isooctane on the steam bath under nitrogen. Discontinue evaporation when not over 1 ml of residue remains. To the residue, add a 10 ml portions of isooctane, reevaporate to 1 ml of hexadecane, and repeat this operation once more.

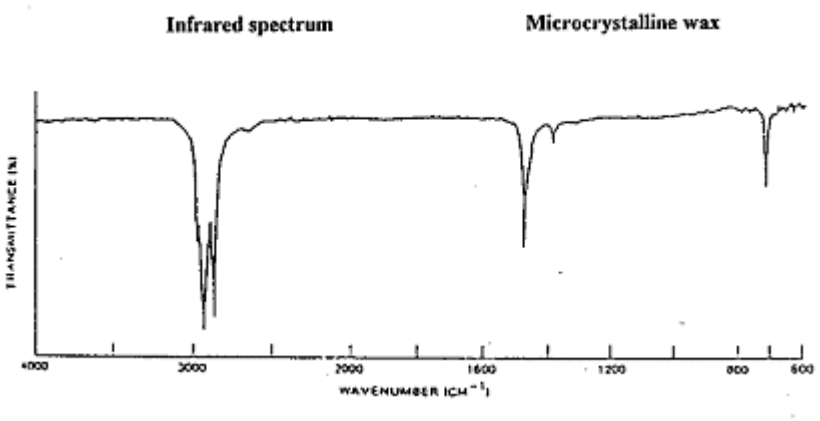
Quantitatively transfer the residue with isooctane to a 25 ml volumetric flask, make to volume, and mix. Determine the absorbance of the solution in the 5 cm path length cells compared to isooctane as reference between 280-400 nm (take care to lose none of the solution in filling the sample cell). Correct the absorbance values for any absorbance derived from reagents

as determined by carrying out the procedure without a wax sample. If the corrected absorbance does not exceed the limits prescribed in the Characteristics, the wax meets the ultraviolet absorbance specifications. If the corrected absorbance per centimeter path length exceeds the limits prescribed in the Characteristics, proceed as follows:
Quantitatively transfer the isooctane solution to a 125 ml flask equipped with 24/40 joint and evaporate the isooctane on the steam bath under a stream of nitrogen to a volume of 1 ml of hexadecane. Add 10 ml of methyl alcohol and approximately 0.3 g of sodium borohydride (Minimize exposure of the borohydride to the atmosphere. A measuring dipper may be used). Immediately fit a water-cooled condenser equipped with a 24/40 joint and with a drying tube into the flask, mix until the borohydride is dissolved, and allow to stand for 30 min at room temperature, with intermittent swirling. At the end of this period, disconnect the flask and evaporate the methyl alcohol on the steam bath under nitrogen until the sodium borohydride begins to come out of the solution. Then add 10 ml of isooctane and evaporate to a volume of about 2-3 ml. Again, add 10 ml of isooctane and concentrate to a volume of approximately 5 ml. Swirl the flask repeatedly to assure adequate washing of the sodium borohydride residues.

Fit the tetrafluoroethylene polymer disc on the upper part of the stem of the chromatographic tube, then place the tube with the disc on the suction flask and apply the vacuum (approximately 135 mm Hg pressure). Weigh out 14 g of the 2+1 magnesium oxide-Celite 545 mixture and pour the adsorbent mixture into the chromatographic tube in approximately 3 cm layers. After the addition of each layer, level off the top of the adsorbent with a flat glass rod or metal plunger by pressing down firmly until the adsorbent is well packed. Loosen the topmost few ml of each adsorbent layer with the end of a metal rod before the addition of the next layer. Continue packing in this manner until all the 14 g of the adsorbent is added to the tube. Level off the top of the adsorbent by pressing down firmly with a flat glass rod or metal plunger to make the depth of the adsorbent bed approximately 12.5 cm in depth. Turn off the vacuum and remove the suction flask. Fit the 500 ml reservoir onto the top of the chromatographic column and prewet the column by passing 100 ml of isooctane through the column. Adjust the nitrogen pressure so that the rate of descent of the isooctane coming off of the column is between 2-3 ml per min. Discontinue pressure just before the last of the isooctane reaches the level of the adsorbent. (Caution: Do not allow the liquid level to recede below the adsorbent level at any time). Remove the reservoir and decant the 5 ml isooctane concentrate solution onto the column and with slight pressure again allow the liquid level to recede to barely above the adsorbent level. Rapidly complete the transfer similarly with two 5 ml portions of isooctane, swirling the flask repeatedly each time to assure adequate washing of the residue. Just before the final 5 ml wash reaches the top of the adsorbent, add 100 ml of isooctane to the reservoir and continue the percolation at the 2-3 ml per minute rate. Just before the last of the isooctane reaches the adsorbent level, add 100 ml of 10% benzene in isooctane to the reservoir and continue the percolation at the aforementioned rate. Just before the solvent mixture reaches adsorbent level, add 25 ml of 20% benzene in isooctane to the reservoir and continue the percolation at 2-3 ml per minute until all this solvent mixture has been removed from the column. Discard all the elution solvents collected up to this point. Add 300 ml of the acetone-benzene-water mixture to the

reservoir and percolate through the column to elute the polynuclear compounds. Collect the eluate in a clean 1-l separatory funnel. Allow the column to drain until most of the solvent mixture is removed. Wash the eluate three times with 300 ml portions of distilled water, shaking well for each wash. (The addition of small amounts of sodium chloride facilitates separation). Discard the aqueous layer after each wash. After the final separation, filter the residual benzene through anhydrous sodium sulfate prewashed with benzene (see *Sodium sulfate* under "Reagents and Materials" for preparation of filter) into a 250-ml Erlenmeyer flask (or optionally into the evaporation flask). Wash the separatory funnel with two additional 20 ml portions of benzene which are also filtered through the sodium sulfate. Add 1 ml of n-hexadecane and completely remove the benzene by evaporation under nitrogen, using the special procedure to eliminate benzene as previously described under "Organic Solvents". Quantitatively transfer the residue with isooctane to a 25 ml volumetric flask and adjust the volume. Determine the absorbance of the solution in the 5 cm path length cells compared to isooctane as reference between 250 - 400 nm. Correct for any absorbance derived from the reagents as determined by carrying out the procedure without a wax sample. If either spectrum shows the characteristic benzene peaks in the 250 - 260 nm region, evaporate the solution to remove benzene by the procedure under "Organic Solvents". Dissolve the residue, transfer quantitatively, and adjust to volume in isooctane in a 25 ml volumetric flask. Record the absorbance again. If the corrected absorbance does not exceed the limits prescribed in the Characteristics the wax meets the ultraviolet absorbance specifications.

Appendix



MINERAL OIL (HIGH VISCOSITY)

Prepared at the 44th JECFA (1995), published in FNP 52 Add 3 (1995) superseding specifications for Mineral oil prepared at the 37th JECFA (1990), published in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). An ADI of 0-20 mg/kg bw was established at the 44th JECFA (1995).

SYNONYMS Liquid paraffin, liquid petrolatum, food grade mineral oil, white mineral oil; INS No. 905a

DEFINITION A mixture of highly refined paraffinic and naphthenic liquid hydrocarbons with boiling point above 350°; obtained from mineral crude oils through various refining steps (e.g. distillation, extraction and crystallization) and subsequent purification by acid and/or catalytic hydrotreatment; may contain antioxidants approved for food use.

C.A.S. number 8012-95-1

DESCRIPTION Colourless, transparent, oily liquid, free from fluorescence in daylight; odourless

FUNCTIONAL USES Release agent, lubricant, protective coating

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water, sparingly soluble in ethanol, soluble in ether

Burning Burns with bright flame and with paraffin-like characteristic smell

PURITY

Viscosity, 100° (Vol. 4) Not less than 11 cSt

Carbon number at 5% distillation point (Vol. 4) Not less than 28
The boiling point at the 5% distillation point is higher than 422°.

Average molecular weight (Vol. 4) Not less than 500

Acidity or alkalinity To 10 ml of the sample add 20 ml of boiling water and shake vigorously for 1 min. Separate the aqueous layer and filter. To 10 ml of the filtrate, add 0.1 ml of phenolphthalein solution TS. The solution is colourless. Not more than 0.1 ml of 0.1N sodium hydroxide is required to change the colour to pink

Readily carbonizable substances Place 5 ml of the sample in a glass-stoppered test tube that has previously been rinsed with chromic acid cleaning mixture. Add 5 ml of sulfuric acid TS, and heat in a boiling water bath for 10 min. After the test tube has been in the bath for 30 sec, remove it quickly, and while holding the stopper in place, give three vigorous vertical shakes over an amplitude of about 10 cm. Repeat every 30 sec. Do not keep the test

	tube out of the bath longer than 3 sec for each shaking period. At the end of 10 min from the time when first placed in the water bath, remove the test tube. The sample remains unchanged in colour, and the acid does not become darker than standard colour produced by mixing in a similar test tube 3 ml of ferric chloride TSC, 1.5 ml of cobaltous chloride TSC, and 0.5 ml of cupric sulfate TSC, this mixture being overlaid with 5 ml of mineral oil.
Polycyclic aromatic hydrocarbons (Vol. 4)	Passes test
Solid paraffins	Dry a suitable quantity of the substance to be examined by heating at 100^{o} for 2 h and cool in a desiccator over concentrated sulfuric acid. Place in a glass tube with an internal diameter of about 25 mm, close the tube and immerse in a bath of iced water. After 4 h the liquid is sufficiently clear for a black line, 0.5 mm wide against a white background held vertically behind the tube, to be easily seen.
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

MINERAL OIL (MEDIUM AND LOW VISCOSITY)

Prepared at the 59th JECFA (2002), published in FNP 52 Add 10 (2002) superseding specifications prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998). At the 59th JECFA for mineral oils of Class I an ADI of 0-10 mg/kg bw and for minerals oils of Class II and Class III a temporary group ADI of 0-0.01 mg/kg bw were established.

SYNONYMS Liquid paraffin, liquid petrolatum, food grade mineral oil, white mineral oil INS No. 905a

DEFINITION A mixture of highly refined paraffinic and naphthenic liquid hydrocarbons with boiling point above 200°; obtained from mineral crude oils through various refining steps (eg. distillation, extraction and crystallisation) and subsequent purification by acid and/or catalytic hydrotreatment; may contain antioxidants approved for food use.
The oil is divided into three classes having different viscosities and average molecular weights as specified below.

C.A.S. number 8012-95-1

DESCRIPTION Colourless, transparent, oily liquid, free from fluorescence in daylight; odourless

FUNCTIONAL USES Release agent, glazing agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water, sparingly soluble in ethanol, soluble in ether

Burning Burns with bright flame and with paraffin-like characteristic smell

PURITY

Viscosity, 100° Class I: 8.5-11 mm^2/s
Class II: 7.0-8.5 mm^2/s
Class III:3.0-7.0 mm^2/s
See description under TESTS

Carbon number at 5% distillation point Class I: Not less than 25
Class II: Not less than 22
Class III: Not less than 17
The boiling point at the 5% distillation point is higher than: 391° for Class I substances, 356° for Class II substances and 287° for Class III substances.
See description under TESTS

Average molecular weight Class I: 480-500
Class II: 400-480
Class III:300-400
See description under TESTS

Acidity or alkalinity

To 10 ml of the sample add 20 ml of boiling water and shake vigorously for 1 min. Separate the aqueous layer and filter. To 10 ml of the filtrate, add 0.1 ml of phenolphthalein solution TS. The solution is colourless. Not more than 0.1 ml of 0.1N sodium hydroxide is required to change the colour to pink

Readily carbonizable substances

Place 5 ml of the sample in a glass-stoppered test tube that has previously been rinsed with chromic acid cleaning mixture. Add 5 ml of sulfuric acid TS, and heat in a boiling water bath for 10 min. After the test tube has been in the bath for 30 sec, remove it quickly, and while holding the stopper in place, give three vigorous vertical shakes over an amplitude of about 10 cm. Repeat every 30 sec. Do not keep the test tube out of the bath longer than 3 sec for each shaking period. At the end of 10 min from the time when first placed in the water bath, remove the test tube. The sample remains unchanged in colour, and the acid does not become darker than standard colour produced by mixing in a similar test tube 3 ml of ferric chloride TSC, 1.5 ml of cobaltous chloride TSC, and 0.5 ml of cupric sulfate TSC, this mixture being overlaid with 5 ml of mineral oil.

Polycyclic aromatic hydrocarbons

Transfer 25.0 ml of sample to a 125 ml separating funnel with unlubricated ground-glass parts (stopper, stopcock). Add 25 ml of hexane which has been previously shaken twice with one-fifth its volume of dimethyl sulfoxide. Mix and add 5.0 ml of dimethyl sulfoxide. Shake vigorously for 1 min and allow to stand until two clear layers are formed. Transfer the lower layer to a second separating funnel, add 2 ml of hexane and shake the mixture vigorously. Allow to stand until two clear layers are formed. Separate the lower layer and measure its absorbance between 260 nm and 420 nm, using as the compensation liquid the clear lower layer obtained by vigorously shaking 5.0 ml of dimethyl sulfoxide with 25 ml of hexane for 1 min. Prepare a reference solution in trimethylpentane counting 7.0 mg of naphthalene per litre and measure the absorbance of the solution at the maximum at 275 nm, using trimethylpentane as the compensation liquid. At no wavelength between 260 nm and 420 nm does the absorbance of the test solution exceed one-third that of the reference solution at 275 nm. Use hexane, dimethyl sulfoxide and trimethylpentane in quality specified for ultraviolet spectrometry.

Solid paraffins

Dry a suitable quantity of the substance to be examined by heating at 100^{o} for 2 h and cool in a desiccator over concentrated sulfuric acid. Place in a glass tube with an internal diameter of about 25 mm, close the tube and immerse in a bath of iced water. After 4 h the liquid is sufficiently clear for a black line, 0.5 mm wide against a white background held vertically behind the tube, to be easily seen.

Lead (Vol. 4)

Not more than 1 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and the method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods".

TESTS

PURITY TESTS

Viscosity, 100°

ASTM D 445
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Copies of the complete ASTM standard may be purchased directly from ASTM, phone: +1 610-832-9585, fax: +1 610-832-9555 e-mail: service@astm.org

http://www.astm.org

Use a viscometer of the glass capillary type, calibrated and capable of measuring kinematic viscosity with a repeatability exceeding 0.35% only in one case in twenty. Immerse the viscometer in a liquid bath at the temperature required for the test ± 0.1° ensuring that at no time of the measurement will any portion of the sample in the viscometer be less than 20 mm below the surface of the bath liquid or less than 20 mm above the bottom of the bath. Charge the viscometer with sample in the manner dictated by the design of the instrument. Allow the sample to remain in the bath for about 30 min. Where the design of the viscometer requires it, adjust the volume of sample to the mark. Use pressure to adjust the head level of the sample to a position in the capillary arm of the instrument about 5 mm ahead of the first mark. With the sample flowing freely, measure, in seconds (±0.2 sec), the time required for the meniscus to pass from the first to the second timing mark. If the time is less than 200 s, select a viscometer with a capillary of smaller diameter and repeat the operation. Make a second measurement of the flow time. If two measurements agree within 0.2%, use the average for calculating the kinematic viscosity. If the measurements do not agree, repeat the determination after thoroughly cleaning and drying the viscometer.

Viscosity, 100° (mm^2/sec) = C x t
Where

C = calibration constant of the viscometer (mm^2/sec^2)
t = flow time (sec)

Carbon number

ASTM D 2887
See TEST for Viscosity for Copyright permission.

"Carbon number" is number of carbon atoms in a molecule. Determine the boiling point distribution of the sample by *gas chromatography* using the following conditions:

The system must have the following performance characteristics:

Sensitivity
: 1% dodecane must be detected with a peak height of at least 10% of full scale under the conditions prescribed below.

Stability
: when operated at the required sensitivity level, the baseline drift is not more than 1% of full scale per hour

Repeatability of retention times
: 6 sec for each component of the calibration mixture.
Resolution (R)
: determined for a solution of 1% of each of hexadecane and octadecane in n-octane is not less than three and not more than eight, using the following formula:

$$R = \frac{2d}{W1 + W2}$$

where
d = distance in mm between the peak maxima of hexadecane and octadecane
W1 = the peak width in mm at the baseline of hexdecane
W2 = the peak width in mm at the baseline of octadecane
Typical conditions which may be used:

Column
length: 1.5m
outside diameter: 3.2 mm
liquid phase: SE - 30.5 %
support material: Chromosorb G, mesh 60/80

Column temperature
: initial: 10°
final: 350°
rate: 6.5° / min.

Carrier
: gas: helium
flow: 30 ml/min.

Detector
:FID
Detector temperature: 370°

Injection temperature
: 370°

Sample size
: 0.3 µl

Calibration mixture
: Prepare a mixture of hydrocarbons of known boiling points covering the range of the sample. At least one compound must have a boiling point lower than the initial boiling point of the sample.

Procedure
: Calibration: Cool the column to the selected starting temperature (the retention time for the initial boiling point must be at least 1 min) and inject the calibration mixture. Record the retention time of each peak maximum and the peak areas for each component. Plot the retention time of each peak versus the corresponding normal boiling point of that component in degrees Celsius to obtain a calibration curve.

Sample analysis
: Using the exact conditions used in the calibration run, inject the sample. Record the area of each time segment at fixed time intervals not greater than 1% of the retention time equivalent to a boiling point of 538° obtained from the calibration curve.

Calculation
: Sum the area segments to obtain the cumulative area at each time

interval during the run. At the point of the chromatogram, where the baseline at the end first becomes steady, observe the cumulative area counts. Move back along the record until a cumulative area equal to 99.5% of the total at the steady point appears. Mark this point as the final boiling point. Observe the area counts at the start of the run until the point is reached, where the cumulative area count is equal to 0.5% of the total area. Mark this point as the initial boiling point of the sample. Divide the cumulative area at each interval between the initial and final boiling points by the total cumulative area and multiply by 100. This will give the cumulative percent of the sample recovered at each time interval. Tabulate the cumulative percent recovered at each interval and the retention time at the end of the interval. Using linear interpolation, if necessary, determine the retention time associated with 5% and read the corresponding boiling temperature from the calibration curve.
The boiling point at the 5% distillation point is higher than: 391° for Class I substances, 356° for Class II substances and 287° for Class III substances.

Average molecular weight

ASTM D 2502
See TEST for Viscosity for Copyright permission

Determine the kinematic viscosity of the sample at 37.8 and 98.9° as described in the method for Viscosity, 100°. Read the value of H that corresponds to the measured viscosity at 37.8° by the use of table 1; linear interpolation between adjacent columns may be required. Read a viscosity -molecular weight chart for H and 98.9° viscosity (the chart is available from the American Society for Testing and Materials (ASTM). A simplified version is shown in Figure 1 for illustration purposes only. Interpolate where necessary between adjacent lines of 98.89° viscosity. After locating the point corresponding to the value of H (ordinate) and the 98.89°viscosity (superimposed lines), read the molecular weight along the abscissa.

Readily carbonizable substances

Place 5g of the sample in a glass-stoppered test tube that has previously been cleaned with a chromic acid cleaning solution, rinsed with water and dried in an oven (105°, 1h). Add 5 ml of sulfuric acid TS, and place in a boiling water bath. After the test tube has been in the bath for 30 sec, remove quickly, and while holding the stopper in place, give three vigorous vertical shakes over an amplitude of about 10 cm. Repeat every 30 sec. Do not keep the test tube out of the bath longer than 3 sec for each shaking period. At the end of 10 min from the time when first placed in the water bath, remove the test tube. The sample remains unchanged in colour, and the acid does not become darker than a very slight straw colour (Matching Fluid E , see FNP 5). No black material occurs at the interface between the two layers.

Table 1 - Tabulation of H Function					
Kinematic viscosity, mm²/s at 37.8°	H				
	0	0.2	0.4	0.6	0.8
2	-176	-151	-126	-104	-85
3	-67	-52	-38	-25	-13
4	-1	9	19	28	36
5	44	52	59	66	73
6	79	85	90	96	101
7	106	111	116	120	124
8	128	132	136	140	144
9	147	151	154	157	160
10	163	166	169	172	175
11	178	180	183	185	188
12	190	192	195	197	199
13	201	203	206	208	210
14	211	213	215	217	219
15	212	222	224	226	227
16	229	231	232	234	235
17	237	238	240	241	243
18	244	245	247	248	249
19	2551	252	253	255	256
20	257	258	259	261	262
21	263	264	265	266	267
22	269	270	271	272	273
23	274	275	276	277	278
24	279	280	281	281	282
25	283	284	285	286	287
26	288	289	289	290	291
27	292	293	294	294	295
28	296	297	298	298	299
29	300	301	301	302	303
30	304	304	305	306	306
31	307	308	308	309	310
32	310	311	312	312	313
33	314	314	315	315	316
34	317	317	318	319	319
35	320	320	321	322	322
36	323	323	324	325	325
37	325	326	327	327	328
38	328	329	329	330	331
39	331	332	332	333	333

Table 1 – Tabulation of H Function (cont'd)

Kinematic viscosity, mm²/s at 37.8°	H									
	0	1	2	3	4	5	6	8	9	10
40	334	336	339	341	343	345	347	349	352	354
50	356	357	359	361	363	364	366	368	369	371
60	371	374	375	377	378	380	381	382	384	385
70	386	387	388	390	391	392	393	394	395	397
80	398	399	400	401	402	403	404	405	406	407
90	409	409	410	410	411	412	413	414	415	416
100	416	417	418	419	420	420	421	422	423	423
110	424	425	425	426	427	428	428	429	430	430
120	431	432	432	433	433	434	435	435	436	437
130	437	438	438	439	439	440	441	441	442	442
140	443	443	444	444	445	446	446	447	447	448
150	448	449	449	450	450	450	451	451	452	452
160	453	453	454	454	455	455	456	456	456	457
170	457	458	458	459	459	460	460	460	461	461
180	461	462	462	463	463	463	464	464	465	465
190	465	466	466	466	467	467	468	468	468	469
	H									
	0	10	20	30	40	50	60	10	80	90
200	469	473	476	479	482	485	487	490	492	495
300	497	499	501	503	505	507	509	511	512	514
400	515	517	518	520	521	523	524	525	527	528
500	529	530	531	533	534	535	536	537	538	539
600	540	541	542	543	544	545	546	547	547	548
700	549	550	551	551	552	553	554	554	555	556
800	557	557	558	559	559	560	561	562	562	563
900	563	564	565	565	566	566	567	567	568	569
	H									
	0	100	200	300	400	500	600	700	800	900
1000	569	574	578	583	587	591	594	597	600	603
2000	605	608	610	614	616	618	620	621	623	625
3000	626	626	628	629	631	632	633	634	636	637
4000	638	639	640	641	642	643	644	645	646	647
5000	648	649	650	651	652	652	653	654	655	656
6000	656	657	658	658	659	660	660	661	662	662
7000	663	664	664	665	665	666	666	667	667	668
8000	668	669	670	670	671	671	671	672	672	673
9000	673	674	674	675	675	676	676	677	677	677
	H									
	0	1000	2000	3000	4000	5000	6000	7000	8000	9000
10000	678	681	684	688	691	694	696	699	701	703
20000	705	707	709	711	712	716	715	717	718	719
30000	720	722	723	724	725	726	727	728	729	730
40000	731	732	732	733	734	735	736	736	737	738
50000	739	739	740	741	741	742	743	743	744	744
60000	746	746	746	747	747	748	748	749	749	750
70000	750	751	751	752	752	753	753	753	754	754
80000	755	755	756	756	756	757	757	758	758	758
90000	759	759	759	760	760	760	761	761	761	762
100000	762	762	763	763	763	764	764	764	764	765

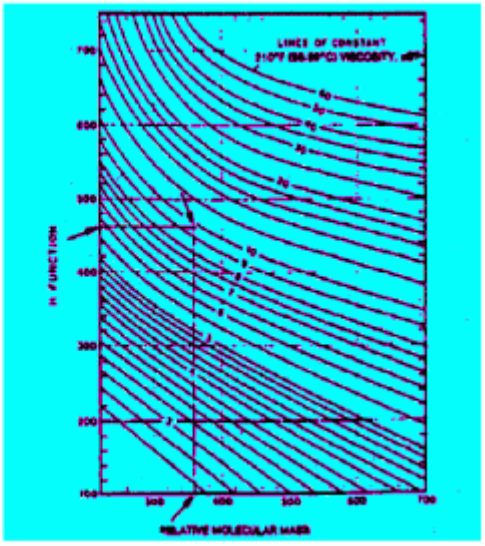


Figure 1 – Lines of constant viscosity (mm²/s) at 98.89°

MODIFIED STARCHES

Prepared at the 74th JECFA (2011) and published in FAO JECFA Monographs 11 (2011), superseding specifications prepared at the 71st JECFA (2009), published in FAO JECFA Monographs 7 (2009). An ADI "not specified" was established at the 26th JECFA (1982) for all modified starches listed below except for acetylated oxidized starch for which an ADI "not specified" was established at the 57th JECFA (2001).

Modified starches comprise the following:

Dextrin roasted starch: INS No. 1400
Acid treated starch: INS No. 1401
Alkaline treated starch: INS No. 1402
Bleached starch: INS No. 1403
Oxidized starch: INS No. 1404
Enzyme-treated starch: INS No. 1405
Monostarch phosphate: INS No. 1410
Distarch phosphate: INS No. 1412
Phosphated distarch phosphate: INS No. 1413
Acetylated distarch phosphate: INS No. 1414
Starch acetate: INS No. 1420
Acetylated distarch adipate: INS No. 1422
Hydroxypropyl starch: INS No. 1440
Hydroxypropyl distarch phosphate: INS No. 1442
Starch sodium octenylsuccinate: INS No. 1450
Acetylated oxidized starch: INS No. 1451

DEFINITION

Food starches which have one or more of their original characteristics altered by treatment in accordance with good manufacturing practice by one of the procedures listed in Table 1. In the case of starches treated with heat in the presence of acid or with alkali, the alteration is a minor fragmentation. When the starch is bleached, the change is essentially in the colour only. Oxidation involves the deliberate production of carboxyl groups. Acetylation results in substitution of hydroxyl groups with acetyl esters. Treatment with reagents such as orthophosphoric acid results in partial substitution in the 2, 3- or 6- position of the anhydroglucose unit unless the 6-position is occupied for branching. In cases of cross-linking, where a polyfunctional substituting agent, such as phosphorus oxychloride, connects two chains, the structure can be represented by: Starch-O-R-O-Starch, where R = cross-linking group and Starch refers to the linear and/or branched structure. The article of commerce can be specified by the parameter specific for the particular type of modification as indicated in Column 3 of Table 1, and may also be further specified as to the loss on drying, sulfated ash, protein and fat.

C.A.S. numbers

Starch acetate:	9045-28-7
Acetylated distarch adipate:	68130-14-3
Hydroxypropyl starch:	9049-76-7
Hydroxypropyl distarch phosphate:	53124-00-8

Acetylated oxidized starch: 68187-08-6

DESCRIPTION

Most modified starches are white or off-white, odourless powders. According to the drying method these powders can consist of whole granules having the appearance of the original native starch, or aggregates consisting of a number of granules (pearl starch, starch-grits) or, if pre-gelatinized, of flakes, amorphous powder or coarse particles.

FUNCTIONAL USES

Thickener, stabilizer, binder, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Insoluble in cold water (if not pre-gelatinized); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol.
Microscopy	Passes test See description under TESTS
Iodine stain	Passes test See description under TESTS
Copper reduction	Passes test See description under TESTS
Differentiation test	Passes test for type of starch See description under TESTS for: 1. Hypochlorite oxidized starch 2. Specific reaction for acetyl groups 3. Positive test for ester groups

PURITY

Sulfur dioxide	Not more than 50 mg/kg for modified cereal starches Not more than 10 mg/kg for other modified starches unless otherwise specified in Table I See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Additional purity specifications for individual chemically modified starches	See column 3 of Table I See description under TESTS

TESTS

IDENTIFICATION TESTS

Microscopy

Modified starches which have not been pre-gelatinized retain their granular structure and can be identified as starches by microscopic observation. Shape, size and sometimes striations are characteristics of the botanical origin. In polarized light under cross nicol prisms the typical polarization cross will be observed

Iodine stain

Add a few drops of 0.1 N potassium tri-iodide to an aqueous suspension of the sample. These starches stain with iodine in the same way as native starches. The colour can range from dark blue to red

Copper reduction

Place about 2.5 g of the sample previously washed with water, in a boiling flask, add 10 ml of dilute hydrochloric acid (3%) and 70 ml of water, mix, reflux for about three hours and cool. Add 0.5 ml of the resulting solution to 5 ml of hot alkaline cupric tartrate TS. A copious red precipitate is produced

Differentiation test

To differentiate between various treated starches perform the following tests:

1. Test for hypochlorite-oxidized starch (not for slightly oxidized potato starch)

Principle

Because of the carboxyl group content, hypochlorite-oxidized starch has anionic properties. It can be dyed with positively charged dyes such as methylene blue.

Procedure

50 mg of the sample are kept in suspension for 5-10 min in 25 ml of a 1% aqueous dye solution and stirred occasionally. After decantation of the excess solution, the starch is washed with distilled water. Microscopic inspection clearly shows colouring, if the sample is hypochlorite-oxidized starch. By this test hypochlorite-oxidized starch is distinguished from native and acid modified starch of the same botanical origin.

2. Specific reaction of acetyl groups

Principle

Acetate is liberated upon saponification of acetylated starch. After concentration the acetate is converted to acetone by heating with calcium hydroxide. The acetone thus produced stains blue with o-nitrobenzaldehyde.

Procedure
About 10 g of the sample is suspended in 25 ml water to which is added 20 ml of 0.4 N NaOH. After shaking for 1 h the starch is filtered off and the filtrate evaporated in an oven at 110°. The residue is dissolved in a few drops of water and transferred to a test tube. Add calcium hydroxide and heat the tube. If the sample is acetylated starch, acetone vapours are produced. These produce a blue colour on a paper strip soaked in a fresh saturated solution of o-nitrobenzaldehyde in 2 N NaOH. The blue colour is more distinct when the original yellow colour of the reagents is removed with 1 drop of a 1 in 10 solution of hydrochloric acid.

3. Positive test for ester groups
The infrared spectrum of a thin film gives a typical absorption band at about 1720 cm-1 which is an indication for ester groups. The limit of detection is about 0.5% acetyl, adipyl or succinyl groups in the product.

PURITY TESTS

Sulfur dioxide

Scope
The method is applicable, with minor modifications, to liquid or solid samples even in the presence of other volatile sulfur compounds.

Principle
The sulfur dioxide is released from the sample in a boiling acid medium and is removed by a stream of carbon dioxide. The separated gas is collected in dilute hydrogen peroxide where it is oxidized to sulfuric acid and titrated with standard alkali. Alternatively, the sulfuric acid may be determined gravimetrically as barium sulfate.

Apparatus
“Monier-Williams" apparatus for the determination of sulfurous acid, constructed with standard-taper glass connections, can be obtained from any reliable scientific glass apparatus store. It is customary, however, to construct the apparatus with regular laboratory glassware using stopper connections (see Figure 1).

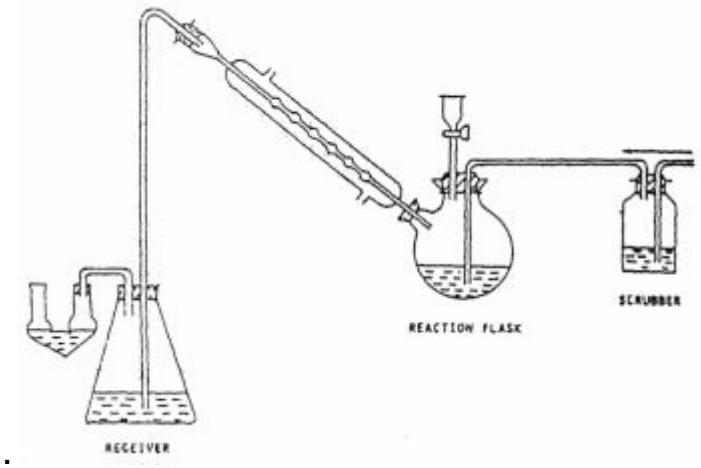


Figure 1

The assembly consists of a 1000-ml two-neck round-bottom boiling flask to which a gas-inlet tube, a 60-ml dropping funnel having a 2-

mm bore stopcock, and a sloping Allihn reflux condenser are attached. A delivery tube connects the upper end of the condenser to the bottom of a 250-ml conical receiving flask, which is followed by a Peligot tube.
In operation, carbon dioxide is passed through the scrubber and bubbled through the heated reaction mixture, sweeping sulfur dioxide through the condenser and into the receivers where it is absorbed quantitatively.

Preparation of solutions
Sodium carbonate solution: Dissolve approximately 15 g of Na_2CO_3 or 40 g of $Na_2CO_3 \cdot 10H_2O$ in distilled water, and dilute to 100 ml.
Hydrogen peroxide, 3%: Dilute 10 ml of C.P. (Chemical Purity) neutral 30% hydrogen peroxide (H_2O_2) with distilled water to 100 ml.

Procedure
Pass carbon dioxide from a generator or cylinder through the sodium carbonate scrubber solution to remove chlorine, thence into the gas-inlet tube of the boiling flask. Place 15 ml of the 3% hydrogen peroxide in the receiving flask and 5 ml in the Peligot tube. Connect the apparatus and introduce into the boiling flask, by means of the dropping funnel, 300 ml of distilled water and 20 ml of concentrated hydrochloric acid. Boil the contents approximately 10 min in a current of carbon dioxide. Weigh, to the nearest g, 100 g of the sample and disperse in approximately 300 ml of recently-boiled distilled water. Transfer the slurry to the boiling flask by means of dropping funnel, regulating the sample addition rate and the gas flow rate through the apparatus to prevent drawback of hydrogen peroxide, inclusion of air, or burning of sample. Boil the mixture gently for 1 h in a slow current of carbon dioxide. Stop the flow of water in the condenser just before the end of the distillation. When the delivery tube just above the receiving flask becomes hot, remove the tube from the condenser immediately. Wash the delivery tube and the Peligot tube contents into the receiving flask, and titrate with 0.1 N sodium hydroxide, using bromphenol blue indicator (see NOTE).

Perform a blank determination on the reagents, and correct results accordingly.

$$\% \text{ sulfur dioxide} = \frac{(S - B) \times 0.0032 \times 100}{W}$$

where
S is ml of 0.1 N sodium hydroxide used for the sample;
B is ml of 0.1 N sodium hydroxide used for the blank; and
W is the weight (in grams) of the sample.

NOTE: A gravimetric determination may be made after titration. Acidify with HCl, precipitate with $BaCl_2$, settle, filter, wash, ignite, and weigh as $BaSO_4$.

Table 1. Additional purity specifications for individual chemically modified starches
(All percentages calculated on dry substance)

Modification	Process limitations	End-product specifications
Dextrin roasted starch	Dry heat treatment with hydrochloric acid or ortho-phosphoric acid	Final pH 2.5-7.0

Modification	Process limitations	End-product specifications
Acid treated starch	Treatment with hydrochloric acid or ortho-phosphoric acid or sulfuric acid	Final pH 4.8-7.0
Alkaline treated starch	Treatment with sodium hydroxide or potassium hydroxide	Final pH 5.0-7.5
Bleached starch	Treatment with peracetic acid and/or hydrogen peroxide, or sodium hypochlorite or sodium chlorite, or sulfur dioxide or alternative permitted forms of sulfites, or potassium permanganate or ammonium persulfate	Added carbonyl group not more than 0.1% No residual reagent Residual sulfur dioxide not more than 50 mg/kg Residual manganese not more than 50 mg/kg
Enzyme-treated starch	Treatment in an aqueous solution at a temperature below the gelatinization point with one or more food-grade amyolytic enzymes	Residual sulfur dioxide not more than 50 mg/kg
Oxidized starch	Treatment with sodium hypochlorite	Carboxyl groups not more than 1.1% Residual sulfur dioxide not more than 50 mg/kg
Monostarch phosphate	Esterification with ortho-phosphoric acid, or sodium or potassium ortho-phosphate, or sodium tripolyphosphate	Phosphate calculated as phosphorus not more than 0.5% for potato or wheat, and not more than 0.4% for other starches
Distarch phosphate	Esterification with sodium trimetaphosphate or phosphorus oxychloride	Phosphate calculated as phosphorus not more than 0.5% for potato and wheat, and not more than 0.4% for other starches
Phosphated distarch phosphate	Combination of treatments for Monostarch phosphate and Distarch phosphate	Phosphate calculated as phosphorus not more than 0.5% for potato and wheat, and not more than 0.4% for other starches

Modification	Process limitations	End-product specifications
Acetylated distarch phosphate	Esterification with sodium trimetaphosphate or phosphorus oxychloride combined with esterification with acetic anhydride or vinyl acetate	Acetyl groups not more than 2.5%; phosphate calculated as phosphorus not more than 0.14% for potato and wheat, and 0.04% for other starches; and vinyl acetate not more than 0.1 mg/kg
Starch acetate	Esterification with acetic anhydride or vinyl acetate	Acetyl groups not more than 2.5%
Acetylated distarch adipate	Esterification with acetic anhydride and adipic anhydride	Acetyl groups not more than 2.5% and adipate groups not more than 0.135%
Hydroxypropyl starch	Esterification with propylene oxide	Hydroxypropyl groups not more than 7.0%; propylene chlorohydrin not more than 1 mg/kg
Hydroxypropyl distarch phosphate	Esterification with sodium trimetaphosphate or phosphorus oxychloride combined with etherification by propylene oxide	Hydroxypropyl groups not more than 7.0%; propylene chlorohydrin not more than 1 mg/kg; and residual phosphate calculated as phosphorus not more than 0.14% for potato and wheat, and not more than 0.04% for other starches
Starch sodium octenylsuccinate	Esterification with octenylsuccinic anhydride	Octenylsuccinyl groups not more than 3%; and residual octenylsuccinic acid not more than 0.3%

Modification	Process limitations	End-product specifications
Acetylated oxidized starch	Treatment with sodium hypochlorite followed by esterification with acetic anhydride	Acetyl groups not more than 2.5 % and carboxyl groups not more than 1.3 %

METHODS FOR ADDITIONAL PURITY SPECIFICATIONS

pH (Vol. 4)

As specified in Column 3 of Table 1
Suspend 20 g of the sample with 80 ml of water, and agitate continuously at a moderate rate for 5 min (In the case of pre-gelatinized starches, 3 g should be suspended in 97 ml of water).

Carboxyl groups

As specified in Column 3 of Table 1.

Principle
The carboxyl containing starch is equilibrated with mineral acid to convert carboxyl salts to the acid form. Cations and excess acid are removed by washing with water. The washed sample is gelatinized in water and titrated with standard alkali.

NOTE: Native phosphate groups present in potato starch increase the titre found in this method (See NOTE 6).

Reagents
Hydrochloric Acid Solution, 0.10 N : Standardization unnecessary
Sodium Hydroxide Solution, 0.10 N : Standardized
Phenolphthalein Indicator, 1%

Procedure
If necessary, grind sample completely through a laboratory cutting mill to 20 mesh or finer, taking precautions to prevent any significant change in moisture, and mix thoroughly.

Weigh accurately a sample containing not more than 0.25 milliequivalents of carboxyl (Note 1), and transfer quantitatively to a 150-ml beaker. Add 25 ml of 0.1 N hydrochloric acid and stir occasionally over a period of 30 min. Vacuum filter the slurry through a medium porosity fritted-glass crucible or small funnel, using a fine stream of water from a wash bottle to aid quantitative transfer of the sample. Wash the sample with distilled water (300 ml usually sufficient) until the filtrate is free from chloride determined by silver nitrate test (NOTE 2).

Transfer the demineralized sample quantitatively to a 600-ml beaker with the aid of distilled water, and slurry the sample in 300 ml of distilled water. Heat sample dispersion in a steam bath or boiling water bath (NOTE 3), stirring continuously until the starch gelatinizes, and continue heating for 15 min to ensure complete gelatinization (NOTE 4).

Remove sample from bath and titrate while hot with standard 0.10 N sodium hydroxide solution to a phenolphthalein end-point. The end-

point may be detected electrometrically at pH 8.3. A blank determination is run on the original sample to correct for native acid substances (Note 5). Weigh the same quantity of starch as taken for carboxyl titration, and slurry in 10 ml of distilled water. Stir at about 5-min intervals for 30 min.
Vacuum filter the slurry quantitatively through a medium porosity fritted-glass crucible or small funnel, and wash sample with 200 ml of distilled water. Transfer, gelatinize, and titrate the sample with standard 0.10 N sodium hydroxide in the same manner as the demineralized sample.

Calculation:

$$\text{Carboxyl groups (\%)} = \frac{(\text{ml } 0.10\text{N NaOH} - \text{Blank}) \times 0.0045 \times 100}{\text{Sample weight (g)}}$$

Notes and Precautions

1. Sample size should not exceed 5.0 g for a mildly oxidized or less than 0.15 g for a highly oxidized commercial starch.

2. Add 1 ml of 1% aqueous silver nitrate solution to 5 ml of filtrate. Turbidity or precipitation occurs within 1 min if chloride is present.

3. Heating on a hot plate or over a Bunsen burner is not recommended. Over-heating or scorching in amounts too small to be visible will cause sample decomposition and apparent high carboxyl results.

4. Thorough gelatinization facilitates rapid titration and accurate end-point detection.

5. A blank titration is run on a water-washed sample to correct for acidic components which are not introduced by oxidation or derivatization. Free fatty acids complexed with amylose in common corn starch are the principal contributors to the blank titre.

6. A correction for phosphate content in potato starch (deduction) should be made after determining the phosphorus content of the sample being examined.

The deduction is calculated:

$$\frac{2 \times 45.02 \times P}{30.97} = 2.907 \times P$$

where
P is the phosphorus content (%).

Manganese (Vol. 4)

As specified in Column 3 of Table 1.

Instrumentation
Atomic absorption spectrophotometer with manganese hollow cathode lamp.
Preparation of solutions
Standard solution: Prepare a solution containing 0.5 mg/l of manganese.
Sample solution: Transfer 10.000 g of the sample into a 200-ml Kohlrausch volumetric flask, previously rinsed with 0.5 N hydrochloric acid, add 140 ml of 0.5 N hydrochloric acid, and shake vigorously for 15 min, preferably with a mechanical shaker. Dilute to volume with 0.5 N hydrochloric acid, and shake. Centrifuge approximately 100 ml of the mixture in a heavy-walled centrifuge tube or bottle at 650xg for 5 min, and collect the supernatant liquid. This supernatant comprises the "sample solution".

Procedure
Follow manufacturer's instructions for operating the atomic absorption spectrophotometer and aspirate distilled water through the air-acetylene burner for 5 min to obtain a base-line reading at 279.5 nm. In the same manner aspirate a portion of the "Standard solution" and note the reading. Finally, aspirate the "Sample solution" and compare the reading with the reading for the "Standard solution", and multiply this value by 20 to obtain mg per kg of manganese in the original sample taken for analysis.

Phosphorus (Vol. 4)

As specified in the Column 3 of Table 1.
Reagents

- Ammonium Molybdate Solution (5%): Dissolve 50 g of ammonium molybdate tetrahydrate, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, in 900 ml of warm water, cool to room temperature, dilute to 1000 ml with water, and mix.
- Ammonium Vanadate Solution (0.25%): Dissolve 2.5 g of ammonium metavanadate, NH4VO3, in 600 ml of boiling water, cool to 60 - 70o, and add 20 ml of nitric acid. Cool to room temperature, dilute to 1000 ml with water, and mix.
- Zinc Acetate Solution (10%): Dissolve 120 g of zinc acetate dihydrate, Zn(C2H3O2)2·2H2O, in 880 ml of water, and filter through Whatman No. 2V or equivalent filter paper before use.
- Nitric Acid Solution (29%): Add 300 ml of nitric acid (sp. gr 1.42) to 600 ml of water, and mix.
- Standard Phosphorus Solution: (100 µg P in 1 ml): Dissolve 438.7 mg of monobasic potassium phosphate, KH2PO4, in water in a 1000-ml volumetric flask, dilute to volume with water, and mix.

Standard Curve
Pipet 5.0, 10.0, and 15.0 ml of the Standard Phosphorus Solution into separate 100-ml volumetric flasks. To each of these flasks, and to a fourth blank flask, add in the order stated 10 ml of Nitric Acid Solution, 10 ml of Ammonium Vanadate Solution, and 10 ml of Ammonium Molybdate Solution, mixing thoroughly after each addition. Dilute to volume with water, mix, and allow to stand for 10 min. Determine the absorbance of each standard solution in a 1 cm cell at 460 nm, with a suitable spectrophotometer, using the blank to set the instrument at

zero. Prepare a standard curve by plotting the absorbance of each solution versus its concentration, in mg P per 100 ml.

Sample pre-treatment

Place 20 to 25 g of the starch sample in a 250-ml beaker, add 200 ml of a 7 to 3 methanol-water mixture, disperse the sample, and agitate mechanically for 15 min. Recover the starch by vacuum filtration in a 150 ml medium-porosity fritted-glass or Buchner funnel, and wash the wet cake with 200 ml of the methanol-water mixture. Reslurry the wet cake in the solvent, and wash it a second time in the same manner. Dry the filter cake in an air oven at a temperature below 50°, then grind the sample to 20-mesh or finer, and blend thoroughly. Determine the amount of dry substance by drying a 5 g portion in a vacuum oven, not exceeding 100 mm of Hg, at 120° for 5 h. (NOTE: The treatment outlined above is satisfactory for starch products that are insoluble in cold water.

For pregelatinized starch and other water-soluble starches, prepare a 1% to 2% aqueous paste, place it in a cellophane tube, and dialyze against running distilled water for 30 to 40 h. Precipitate the starch by pouring the solution into 4 volumes of acetone per volume of paste, while stirring. Recover the starch by vacuum filtration in a medium-porosity fritted-glass or Buchner funnel, and wash the filter cake with absolute ethanol. Dry the filter cake, and determine the amount of dry substance as directed for water-insoluble starches).

Sample preparation

Transfer about 10 g of the Treated Sample, calculated on the dry-substance and accurately weighed, into a Vycor dish, and add 10 ml of Zinc Acetate Solution in a fine stream, distributing the solution uniformly in the sample. Carefully evaporate to dryness on a hot plate, then increase the heat, and carbonize the sample on the hot plate or over a gas flame. Ignite in a muffle furnace at 550° until the ash is free from carbon (about 1 to 2 h), and cool. Wet the ash with 15 ml of water and wash slowly down the sides of the dish with 5 ml of Nitric Acid Solution. Heat to boiling, cool, and quantitatively transfer the mixture into a 200-ml volumetric flask, rinsing the dish with three 20-ml portions of water and adding the rinsings to the flask. Dilute to volume with water, and mix. Transfer an accurately measured aliquot (V, in ml) of this solution, containing not more than 1.5 mg of phosphorus, into a 100-ml volumetric flask and add 10 ml of Nitric Acid Solution, 10 ml of Ammonium Vanadate Solution, and 10 ml of Ammonium Molybdate Solution, mixing thoroughly after each addition. Dilute to volume with water, mix, and allow to stand for 10 min.

Procedure

Determine the absorbance of the Sample Preparation in a 1 cm cell at 460 nm, with a suitable spectrophotometer, using the blank to set the instrument at zero. From the Standard Curve, determine the mg of phosphorus in the aliquot taken, recording this value as a. Calculate the amount in mg/kg of Phosphorus (P) in the original sample by the formula:

$$\frac{a \times 200 \times 1000}{V \times W}$$

where

W is the weight of the sample taken, in g.

Acetyl groups

As specified in Column 3 of Table 1.

Accurately weigh about 5 g of the sample and transfer into a 250 ml conical flask. Suspend in 50 ml of water, add a few drops of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide to a permanent pink end-point. Add 25.0 ml of 0.45 N sodium hydroxide, stopper the flask, and shake vigorously for 30 min, preferably with a mechanical shaker. (NOTE: the temperature should not exceed 30^{o} as some starches may gelatinize). Remove the stopper, wash the stopper and sides of the flask with a few ml of water, and titrate the excess alkali with 0.2 N hydrochloric acid to the disappearance of the pink colour. Record the volume, in ml of 0.2 N hydrochloric acid required as S.(S).
Perform a blank titration on 25.0 ml of 0.45 N sodium hydroxide, and record the volume, in ml, of 0.2 N hydrochloric acid required as B.— (B).

$$\text{Acetyl groups (\%)} = \frac{(B - S) \times N \times 0.043 \times 100}{W}$$

where
N is the normality of hydrochloric acid solution; and
W is the weight of sample, in grams.

Vinyl acetate

- Headspace Gas Chromatographic method

Chromatographic system
Use a gas chromatograph equipped with a 2 m x 2 mm (i.d.) glass column containing Porapak Q, 80-100 mesh (or equivalent) fitted with a flame ionization detector, under the following conditions:
- Carrier gas flow (nitrogen): 20 ml/min
- injection port temperature: 200°
- column temperature: 50
- detector temperature: 200°
Standard preparation: Accurately weigh 150 mg vinyl acetate (reagent grade) into a 100 ml volumetric flask. Dissolve and make up to volume with distilled water. Place 1 ml of this solution in a 10-ml volumetric flask and make up to volume with distilled water. Add 1 ml of this dilute solution to 30 g unmodified starch of the same botanical origin as the test substance in a 100-ml flask with a septum-liner. Seal the flask immediately with the septum-liner. This provides a standard starch preparation with a vinyl acetate content of 5 mg/kg.
Procedure
Weigh 30 g of the test substance into a 100-ml flask with a septum-liner. Seal the flask. Place the flask containing the test substance and the flask containing the standard preparation in a constant temperature water bath at 70° for 30 min. Withdraw 2.0 ml from the headspace volume of the flask containing the standard preparation using a gas-tight syringe, inject directly into the injection port of the gas chromatograph and record the peak height of the chromatogram. Similarly inject 2.0 ml of the headspace volume from the flask containing the test substance into the chromatograph. Calculate the content of vinyl acetate in the test substance from a comparison of the peak heights of the two chromatograms.

Adipate groups

As specified in Column 3 of Table 1.

Reagents and Solutions

N,N-Bis-trimethylsilyltrifluoroacetamide (BSTFA): Macherey-Nagel, D 5160 Dueren, Germany or equivalent.
Glutaric acid solution: Dissolve 1.00 g of glutaric acid (Merck or equivalent) in water and dilute to 1000 ml.
Adipic acid solution: Dissolve 1.00 g of adipic acid (UCB, Brussels, Belgium or equivalent) in 900 ml of warm water, cool to room temperature, dilute to 1000 ml and mix.

Apparatus

Chromatograph: Hewlett Packard Model 7620A gas chromatograph or equivalent equipped with flame ionization detector and Model 3370Aintegrator. (Hewlett-Packard Model 7620A, with integrator Model 3370A or equivalent)

Column parameters: 2-m stainless steel, 1.83 mm id, packed with 5% OV-17 on 80-100 mesh Chromosorb GAW-DMCS (Alltech Europe, Inc., B 9731 Eke, Belgium); precondition column 24 h at 350° with nitrogen carrier gas at 40 ml/min. Operating gas flow rates (ml/min): nitrogen carrier 30, hydrogen 40, air 400. Temperature: injection 280°, detector 250°, column 140°. Retention times (min): glutaric acid 2.83, adipic acid 4.50.

Calibration

Weigh 1.0 g waxy corn starch into each of four 250-ml Erlenmeyer flasks. To each flask add 50 ml water and 1.0 ml of an aqueous solution containing 1.0 mg glutaric acid/ml. Add, to one flask, 0.25 ml of an aqueous solution containing 1.0 mg adipic acid per ml; to the other three, add 0.50 ml, 0.75 ml, and 1.0 ml, respectively. Each flask then contains 1.0 mg glutaric acid and, respectively, 0.25, 0.50, 0.75 and 1.0 mg adipic acid. Agitate flasks manually to disperse the starch fully and add 50 ml 4N sodium hydroxide. Continue agitation another 5 min, place each flask in water bath at ambient temperature, and carefully add 20 ml 12 N hydrochloric acid to each. When each flask is cool quantitatively transfer contents to 250 ml separatory funnel. Extract with 100 ml reagent grade ethyl acetate. Drain bottom aqueous layer into beaker and collect upper organic layer in 500-ml Erlenmeyer flask containing 20 g anhydrous sodium sulphate. Transfer aqueous portion back to separatory funnel and repeat ethyl acetate extraction twice more. Shake flasks periodically during 10 min and then filter contents through Whatman No. 1 paper into 1-litre round-bottom flasks. Rinse flasks and insoluble residues in filters twice with 50 ml of ethyl acetate. Under vacuum, (50 mm Hg) at temperature not exceeding 40°, evaporate total organic extraction and washings of each flask until completely dry.

The evaporation of ethyl acetate should be effected as quickly as possible because some hydrolysis takes place on standing. The products of hydrolysis cause deterioration in the resolution of adipic acid in the chromatographic separation.
Successively add 2 ml pyridine and 1 ml N,N-bis-trimethylsilyltrifluoro-acetamide to the dry contents. Close each of the round-bottom flasks with stopper and rinse internal surfaces thoroughly by swirling. Let flasks stand 1 h; then transfer ca 2 ml from each to small glass vials

and immediately seal. Inject 4 µl into gas chromatograph.

Calculations

Establish retention times for each acid and determine peak height for glutaric acid and for each level of adipic acid represented. A plot of peak height ratio of adipic acid to glutaric acid against amount of adipic acid is linear. This calibration curve may be used, but it is simpler to use a response factor (RF):

$$RF = \frac{H_I \times W_S}{H_S}$$

where

H_S and H_I is the peak heights of the standard adipic acid and glutaric acid, respectively; and

W_S is the weight of the standard adipic acid.

RF should be verified weekly.

Total adipate

Accurately weigh about 1.0 g of the sample into a 250 ml Erlenmeyer flask, and add 50 ml water and 1.0 ml of an aqueous solution containing 1.0 mg glutaric acid/ml. Proceed as in Calibration, beginning "Agitate flasks manually...".

Free adipic acid

Accurately weigh about 5.0 g of the sample into a 250 ml Erlenmeyer flask, add 100 ml water and 1.0 ml of the glutaric acid solution. Agitate for 1 h, filter through a 0.45 µm Millipore filter, add 1 ml concentrated hydrochloric acid to the filtrate and transfer it quantitatively to a 250-ml separating funnel. Proceed as in Calibration, beginning "Extract with 100 ml..."

Calculation

For both preparations ("Total adipate content" and "Free adipic acid content") record peak heights for adipic acid and glutaric acid (internal standard). Calculate the amounts of total adipate and free adipic acid, respectively, contained in the sample as follows:

$$A = \frac{H_X \times RF}{H_{IX} \times S \times 10}$$

where

A is the content of total adipate or free adipic acid respectively (%);

H_X is the peak height of adipic acid in the actual sample preparation;

H_{IX} is the peak height of glutaric acid in the actual sample preparation;

RF is the response factor for adipic acid; and

S is the weight of sample in the actual preparation (g).

Adipate groups (%) is equal to content of total adipate (%) - content of free adipic acid (%).

Hydroxypropyl groups — As specified in Column 3 of Table 1

Ninhydrin reagent
A 3% solution of 1,2,3,-triketohydrindene crystals in 5% aqueous sodium bisulfite solution.

Procedure
Accurately weigh 50 - 100 mg of the sample into a 100-ml volumetric flask and add 25 ml of 1 N sulfuric acid. Prepare a sample of unmodified starch of the same source (i.e. corn or potato) in the same manner. Place the flasks in a boiling water bath and heat until the samples are in solution. Cool and dilute the contents to 100 ml with water. If necessary, dilute the sample further to assure the presence of no more than 4 mg of hydroxypropyl group per 100 ml, and then dilute the blank starch in the same proportion. Pipet 1 ml of the solutions into 25-ml graduated test tubes with glass stoppers and, with the tubes immersed in cold water, add dropwise 8 ml of concentrated sulfuric acid to each. Mix well and place the tubes in a boiling water bath for exactly 3 min. Immediately transfer the tubes to an ice bath until the solution is chilled. Add 0.6 ml of ninhydrin reagent, carefully allowing the reagent to run down the walls of the test tubes. Immediately shake well, and place the tubes in a 25° water bath for 100 min. Adjust the volume in each tube to 25 ml with concentrated sulfuric acid and mix by inverting the tubes several times. (Do not shake). Immediately transfer portions of the solutions to 1-cm cells and after exactly 5 min, measure the absorption (A) at 590 nm, using the starch blank as the reference. Prepare a calibration curve with 1-ml aliquots of standard aqueous solutions, containing 10, 20, 30, 40 and 50 µg of propylene glycol per ml.

Calculation

$$\text{Hydroxypropyl groups (\%)} = \frac{C \times 0.7763 \times 10 \times F}{W}$$

where
C is the amount of propylene glycol in the sample solution read from the calibration curve (µg/ml);
F is the dilution factor (if a further dilution has been necessary); and
W is the weight of sample (mg).

Propylene chlorohydrin — As specified in Column 3 of Table 1.
Determine by gas chromatography

Gas Chromatographic system
Use a Hewlett Packard model 5750 or equivalent.. A dual-column and a flame-ionization detector is recommended.. An integrator should be part of the recording system

Gas Chromatography column: Use a stainless steel column, 3 m x 3.2 mm (o.d.), packed with 10% Carbowax 20 M on 80/100-mesh Gas Chrom 2, or equivalent. After packing and prior to use, condition the column overnight at 200°, using a helium flow of 25 ml per min.
Concentrator: Use a Kuderna-Danish concentrator having a 500-ml flask, available from Kontes Glass Co., Vineland, N.J., USA,

(Catalogue No. K-57000), or equivalent.

Pressure Bottles: Use 200-ml pressure bottles, with a Neoprene washer, glass stopper, and attached wire clamp, available from Fisher Scientific Co., Pittsburgh, PA, USA (Vitro 400, Catalogue No. 3-100), or equivalent.

Reagents

Diethyl ether: Use anhydrous, analytical reagent-grade, diethyl ether.
Florisil: Use60/100 mesh material,, available from Floridin Co., 3 Penn Center, Pittsburgh, PA 15235, USA, or an equivalent product.
Propylene chlorohydrins: Use Eastman No. P 13251-Chloro-2-propanol Practial containing 25% 2-chloro-1-propanol available from Eastman Kodak Co., Rochester, N.Y. 14650, USA or equivalent).

Standard preparation

Draw 25 µl of mixed propylene chlorohydrin isomers containing 75% of 1-chloro-2-propanol and 25% of 2-chloro-propanol into a 50-µl syringe. Accurately weigh the syringe and discharge the contents into a 500-ml volumetric flask partially filled with water. Reweigh the syringe, and record the weight of the chlorohydrins taken. Dilute to the volume with water, and mix. This solution contains about 27.5 mg of mixed chlorohydrins, or about 55 µg per ml. Prepare this solution fresh on the day of use.

Sample preparation

Transfer a blended representative 50.0 g sample into a Pressure Bottle, and add 125 ml of 2 N sulfuric acid. Clamp the top in place, and swirl the contents until the sample is completely dispersed. Place the bottle in a boiling water bath, heat for 10 min, then swirl the bottle to mix the contents, and heat in the bath for an additional 15 min. Cool in air to room temperature, then neutralize the hydrolyzed sample to pH 7 with 25% sodium hydroxide solution, and filter through Whatman No. 1 paper, or equivalent, in a Buchner funnel, using suction. Wash the bottle and filter paper with 25 ml of water, and combine the washings with the filtrate. Add 30 g of anhydrous sodium sulfate, and stir with a magnetic stirring bar for 5 to 10 min, or until the sodium sulfate is completely dissolved. Transfer the solution into a 500-ml separator equipped with a teflon plug, rinse the flask with 25 ml of water, and combine the washings with the sample solution. Extract with five 50 ml portions of diethyl ether, allowing at least 5 min in each extraction for adequate phase separation. Transfer the combined ether extracts in a Concentrator, place the graduated receiver of the concentrator in a water bath maintained at 50 - 55°, and concentrate the extract to a volume of 4 ml.
(NOTE: Ether extracts of samples may contain foreign residues that interfere with the analysis and/or the interpretation of the chromatograms. These residues are believed to be degradation products arising during the hydrolysis treatment. Analytical problems created by their presence can be avoided through application of a clean-up treatment performed as follows: Concentrate the ether extract to about 8 ml, instead of 4 ml specified above. Add 10 g of Florisil, previously heated to 130° for 16 h just before use, to a chromatographic tube of suitable size, then tap gently, and add 1 g of anhydrous sodium sulfate to the top of the column. Wet the column with 25 ml of diethyl ether, and quantitatively transfer the concentrated

extract to the column with the aid of small portions of the ether. Elute with three 25-ml portions of the ether, collect all of the eluate, transfer it to a concentrator, and concentrate to a volume of 4 ml).
Cool the extract to room temperature; transfer it quantitatively to a 5.0 ml volumetric flask with the aid of small portions of diethyl ether, dilute to volume with the ether, and mix.

Control preparation

Transfer 50.0 g portions of unmodified (underivatized) waxy corn starch into five separate pressure bottles, and add 125 ml of 2 N sulfuric acid to each bottle. Add 0.0, 0.5, 1.0, 2.0, and 5.0 ml of the Standard Preparation to the bottles, respectively, giving propylene chlorohydrin concentrations, on the starch basis, of 0, 0.5, 1.0, 2.0, and 5.0 mg/kg, respectively. Calculate the exact concentration in each bottle from the weight of propylene chlorohydrins used in making the Standard Preparation. Clamp the tops in place, swirl until the contents of each bottle are completely dissolved, and proceed with the hydrolysis, neutralization, filtration, extraction, extract concentration, and final dilution as directed under Sample Preparation.

Procedure

The operating conditions may be varied, depending upon the particular instrument used, but a suitable chromatogram is obtained with the Hewlett-Packard Model 5750 using a column oven temperature of 110°, isothermal; injection port temperature of 210°; detector temperature of 240°; and hydrogen (30 ml per min), helium (25 ml per min), or air (350 ml per min) as the carrier gas. A 1.0 mV full-scale recorder is recommended; range, attenuation, and chart speed should be selected to optimize signal characteristics. Inject 2.0 µl aliquots of each of the concentrated extracts, prepared as directed under Control preparation, allowing sufficient time between injections for signal peaks corresponding to the two chlorohydrin isomers to be recorded (and integrated) and for the column to be purged. Record and sum the signal areas (integrator outputs) from the two chlorohydrin isomers for each of the controls. Using identical operating conditions, inject a 2.0 µl aliquot of the concentrated extract prepared as directed under Sample preparation, and record and sum the signal areas (integrator outputs) from the sample.

Calculation

Prepare a calibration plot on linear coordinate graph paper by plotting the summed signal areas for each of the controls against the calculated propylene chlorohydrin concentrations, in mg/kg, derived from the actual weight of chlorohydrin isomers used. Using the summed signal areas corresponding to the 1-chloro-2-propanol and 2-chloro-1-propanol from the sample, determine the concentration of mixed propylene chlorohydrins, in mg/kg, in the sample by reference to the calibration plot derived from the control samples. After gaining experience with the procedure and demonstrating that the calibration plot derived from the control samples is linear and reproducible, the number of controls can be reduced to one containing about 5 mg/kg of mixed propylene chlorohydrin isomers. The propylene chlorohydrin level in the sample can then be calculated as follows:

$$\text{Propylene chlorohydrins (mg/kg)} = \frac{C \times a}{A}$$

where

C is the concentration, in mg/kg, of propylene chlorohydrins (sum of isomers) in the control;

a is the sum of signal areas produced by the propylene chlorohydrin isomers in the sample; and

A is the sum of the signal areas produced by the propylene chlorohydrin isomers in the control.

Degree of substitution of starch sodium octenyl succinate

The degree of esterification is determined by the amount of alkali consumed after acidification and thorough washing of the sample.

Procedure

Weigh 5.0 g (to nearest 0.1 mg), of the sample in a 150-ml beaker and wet it with a few ml of isopropanol. Pipette 25.0 ml of 2.5 N hydrochloric acid in isopropanol and stir the mixture with a magnetic stirrer for 30 min. Using a graduated measuring cylinder, add 100 ml of 90% isopropanol in water and stir for another 10 min. Filter the sample through a Buchner funnel and wash the filter cake with 90% isopropanol in water until the filtrate is negative for chloride (check using 0.1 N silver nitrate). Transfer the filtrate to a 600-ml beaker, rinse the Buchner flask and bring to a 300-ml volume with distilled water. Place the beaker on the top of a boiling water bath for 10 min, while stirring. While hot, titrate with 0.1 N sodium hydroxide using phenolphthalein TS as an indicator.

Calculation

$$\text{Degree of substitution} = \frac{0.162 \times A}{1 - 0.210 \times A}$$

where

A is milliequivalents of sodium hydroxide required per 1g of starch octenyl succinate.

Residual octenyl succinic acid in starch sodium octenyl succinate

Determine by HPLC on the 2-bromoacetophenone-derivatised methanolic extract of the sample.

Extraction and Preparation of Sample Solution

Accurately weigh 500 mg (to nearest 0.1 mg) of the sample in a 25 ml Erlenmeyer flask, add 15 ml of methanol, stopper the flask and shake it on a shaker overnight. Filter the extract using a filter paper, wash the residue, three times with 7 ml portions of methanol and combine the filtrate (about 80% of the OSA residues is extracted by this procedure). Add 1 ml of 0.16 N KOH in methanol to the combined filtrate. Dry the extract using a flash evaporator at 30° and dissolve the residue in 2 ml of methanol. Pipette 0.5 ml of this solution into a reaction vial, add 0.5 ml of derivatisation reagent [2.8 g of 2-p-dibromoacetophenone and 0.28 g of 1,4,7,10,13,16-hexaoxacyclooctadecane (18-Crown-6) in 50 ml CH_3CN]. Add 2 ml CH_3CN to the reaction vial, cap the vial and heat at 80° for 30 min. Allow the vial to reach room temperature and analyse by HPLC within 24 h.

HPLC Conditions:
Column: μ-Bondapack C18 or equivalent
Mobile Phase: Methanol and Water with gradient elution: 70% to 80% of methanol in water in 5 min
Flow rate: 1.5 ml/min
Detector: UV at 254 nm
Injection volume: 5 μl

Preparation of Standard Curve
Prepare a 105.14 mg/ml solution of octenyl succinic acid anhydride (available from Milliken Chemicals) in methanol (Solution A). Using a syringe draw 0.25 ml of Solution A, transfer into a 25-ml volumetric flask and dilute to mark with methanol (Solution B).

Prepare three working standards (Solution C1, C2 and C3) by transferring 0.5, 1 and 2 ml each of Solution B into three 50-ml round bottom flasks, add 1 ml of 0.16 N KOH in methanol to each flask, dry the solution using a flash evaporator at 30° and dissolve the residue in 2.0 ml of methanol. To 0.5 ml each of these solutions in reaction vials, add 0.5 ml each of derivatisation reagent [2.8 g of 2-p-dibromoacetophenone and 0.28 g of 1,4,7,10,13,16-hexaoxacyclooctadecane (18-Crown-6) in 50 ml of CH_3CN]. Add 2 ml of CH_3CN to each vial, cap the vials and heat for 30 min at 80°. Allow the vials to reach room temperature and analyze by HPLC immediately. The amount of octenyl succinic acid in each 5-μl injection is as follows:

Solution C1: 0.2375 μg
Solution C2: 0.4750 μg
Solution C3: 0.9500 μg

Construct the standard curve using peak height against the amount of standard in the injection.

Inject 5-μl of prepared sample solution and read the amount of octenyl succinic acid in the injection from the standard curve.

Calculation

$$\% \text{ Residual octenyl succinic acid } = \frac{300 \times V}{W}$$

where
V is the amount of OSA in the injected volume; and
W is the weight of the sample (mg).

NOTE: The formula is corrected to 100% recovery by dividing with 0.80, so that 240/0.80 = 300.

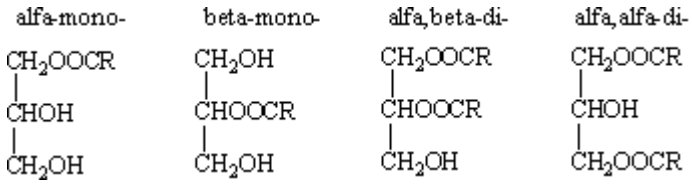
MONO- AND DIGLYCERIDES

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 55th JECFA (2000). An ADI not limited' was established at the 17th JECFA (1973)

SYNONYMS Glyceryl monostearate, glyceryl monopalmitate, glyceryl monooleate, etc; monostearin, monopalmitin, monoolein, etc.; GMS (for glyceryl monostearate); INS No. 471

DEFINITION A mixture of mono- and diglyceryl esters of long chain, saturated and unsaturated fatty acids that occur in food fats; contain not less than 30% of alpha-monoglycerides and may also contain other isomeric monoglycerides, as well as di- and triglycerides, free glycerol, free fatty acids, soap and moisture; usually manufactured by the glycerolysis of edible fats and oils, but may also be prepared by esterification of fatty acids with glycerol, with or without molecular distillation of the product.

Structural formula



where -OCR represents the fatty acid moiety

Formula weight

Glyceryl monostearate: 358.6
Glyceryl distearate: 625.0
These are two major components of commercial products

DESCRIPTION White or cream coloured hard fats of waxy appearance, plastic products or viscous liquids

FUNCTIONAL USES Emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water; soluble in ethanol, chloroform and benzene

Infrared absorption The infrared spectrum of the sample is characteristic of a partial fatty acid ester of a polyol

Tests for fatty acids (Vol. 4) Passes tests

Test for glycerol (Vol. 4) Passes tests

PURITY

Water (Vol. 4) Not more than 2.0% (Karl Fischer Method)

Acid value (Vol. 4)	Not more than 6
Free glycerol (Vol. 4)	Not more than 7%
Soap	Not more than 6%, calculated as a sodium oleate Add 10.00 g of the sample to a mixture of 60 ml of acetone and 0.15 ml of bromophenol blue solution (0.5%), previously neutralized with 0.1 N hydrochloric acid or 0.1 N sodium hydroxide. Warm gently on a water bath until solution is complete, and titrate with 0.1 N hydrochloric acid until the blue colour is discharged. Allow to stand for 20 min, warm until any solidified matter has re-dissolved and, if the blue colour reappears, continue the titration. Each ml of 0.1 N hydrochloric acid is equivalent to 0.0304 g of $C_{18}H_{33}O_2Na$.
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHODS OF ASSAY	Determine as described under *alpha-Monoglyceride and Free Glycerol Contents* in Volume 4

MONOAMMONIUM L-GLUTAMATE

Prepared at the 31st JECFA (1987), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). A group ADI 'not specified' for glutamic acid and its ammonium, Ca, K, Mg & Na salts, was established at the 31st JECFA (1987)

SYNONYMS Ammonium glutamate, INS No. 624

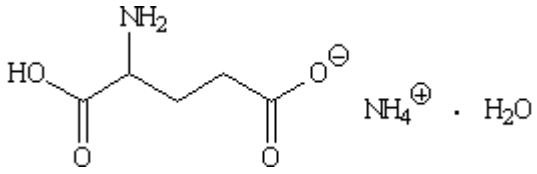
DEFINITION

Chemical names: Monoammonium L-glutamate monohydrate

C.A.S. number: 7558-63-6

Chemical formula: $C_5H_{12}N_2O_4 \cdot H_2O$

Structural formula:



Formula weight: 182.18

Assay: Not less than 99.0% on the dried basis

DESCRIPTION White, practically odourless crystals or crystalline powder

FUNCTIONAL USES Flavour enhancer, salt substitute

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water

Test for glutamate (Vol. 4): Passes test

Test for ammonium (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4): Not more than 0.5% (50°, 4 h)

pH (Vol. 4): 6.0 - 7.0 (1 in 20 soln)

Specific rotation (Vol. 4): [alpha] 20, D: Between +25.4 and +26.4° (10% (w/v) solution in 2N hydrochloric acid)

Sulfated ash (Vol. 4): Not more than 0.1%.
Test 1 g of the sample (Method I)

Pyrrolidone carboxylic acid (Vol. 4) — Passes test

Lead (Vol. 4) — Not more than 1 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Dissolve about 200 mg of the sample, previously dried and weighed accurately, in 6 ml of formic acid, and add 100 ml of glacial acetic acid. Titrate with 0.1 N perchloric acid determining the end-point potentiometrically. Run a blank determination in the same manner and correct for the blank. Each ml of 0.1 N perchloric acid is equivalent to 9.106 mg of $C_5H_{12}N_2O_4 \cdot H_2O$.

MONOPOTASSIUM L-GLUTAMATE

Prepared at the 31st JECFA (1987), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). A group ADI 'not specified' for glutamic acid and its Ammonium, Ca, K, Mg & Na salts, was established at the 31st JECFA (1987)

SYNONYMS Potassium glutamate, MPG; INS No.622

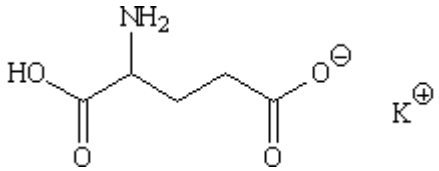
DEFINITION

Chemical names: Monopotassium L-glutamate monohydrate,

C.A.S. number: 19473-49-5

Chemical formula: $C_5H_8KNO_4 \cdot H_2O$

Structural formula:



Formula weight: 203.24

Assay: Not less than 99.0% on the dried basis

DESCRIPTION

FUNCTIONAL USES Flavour enhancer, salt substitute

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water; slightly soluble in ethanol

Test for glutamate (Vol. 4): Passes test

Test for potassium (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4): Not more than 0.2% (80°, 5 h)

pH (Vol. 4): 6.7 - 7.3 (1 in 50 soln)

Specific rotation (Vol. 4): [alpha] 20, D: Between +22.5 and +24.0° (10% (w/v) solution in 2N hydrochloric acid

Chlorides (Vol. 4): Not more than 0.2%
Test 0.07 g of the sample as directed in the Limit Test using 0.4 ml of 0.01

N hydrochloric acid in the control

Pyrrolidone carboxylic acid (Vol. 4) — Passes test

Lead (Vol. 4) — Not more than 1 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Dissolve about 200 mg of the sample, previously dried and weighed accurately, in 6 ml of formic acid, and add 100 ml of glacial acetic acid. Titrate with 0.1 N perchloric acid determining the end-point potentiometrically. Run a blank determination in the same manner and correct for the blank. Each ml of 0.1 N perchloric acid is equivalent to 10.162 mg of $C_5H_8KNO_4 \cdot H_2O$

MONOSODIUM L-GLUTAMATE

Prepared at the 31st JECFA (1987), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). A group ADI 'not specified' for glutamic acid and its Ammonium, Ca, K, Mg & Na salts, was established at the 31st JECFA (1987)

SYNONYMS Sodium glutamate, MSG, INS No. 621

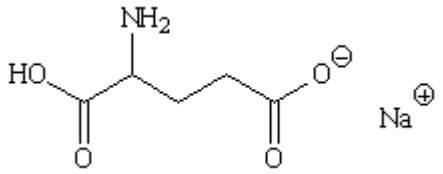
DEFINITION

Chemical names: Monosodium L-glutamate monohydrate, glutamic acid monosodium salt monohydrate

C.A.S. number: 6106-04-3

Chemical formula: $C_5H_8NNaO_4 \cdot H_2O$

Structural formula:



Formula weight: 187.13

Assay: Not less than 99.0% on the dried basis

DESCRIPTION White, practically odourless crystals or crystalline powder

FUNCTIONAL USES Flavour enhancer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water; sparingly soluble in ethanol; practically insoluble in ether

Test for glutamate (Vol. 4): Passes test

Test for sodium (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4): Not more than 0.5% (98°, 5 h)

pH (Vol. 4)	6.7 - 7.2 (1 in 50 soln)
Specific rotation (Vol. 4)	[alpha] 20, D: : Between +24.8 and +25.3° (10% (w/v) solution in 2N hydrochloric acid)
Chlorides (Vol. 4)	Not more than 0.2% Test 0.07 g of the sample as directed in the Limit Test using 0.4 ml of 0.01 N hydrochloric acid in the control
Pyrrolidone carboxylic acid (Vol. 4)	Passes test
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Dissolve about 200 mg of the sample, previously dried and weighed accurately, in 6 ml of formic acid, and add 100 ml of glacial acetic acid. Titrate with 0.1 N perchloric acid determining the end-point potentiometrically. Run a blank determination in the same manner and correct for the blank. Each ml of 0.1 N perchloric acid is equivalent to 9.356 mg of $C_5H_8NNaO_4 \cdot H_2O$

NATAMYCIN

Prepared at the 61st JECFA (2003) and published in FNP 52 Add 11 (2003) superseding specifications prepared at the 57th JECFA (2001) and published in FNP 52 Add 9 (2001) superseding specifications for pimaricin prepared at the 20th JECFA (1976), published in FNP 52 (1992). An ADI 0-0.3mg/kg bw was established at the 20th JECFA (1976).

SYNONYMS Pimaricin; INS No. 235

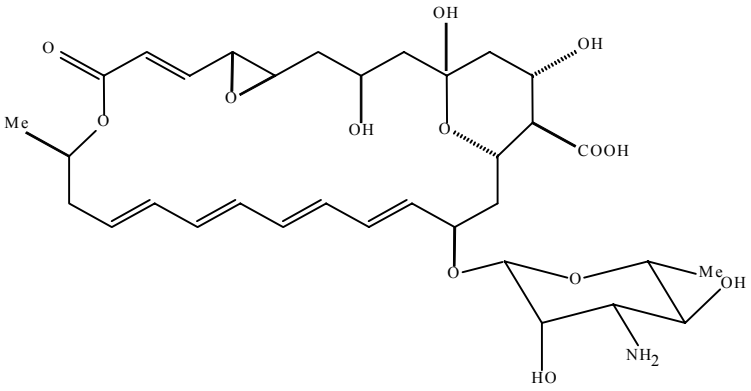
DEFINITION A fungicidal antimycotic of the polyene macrolide group. It is produced by several species of *Streptomyces*. The commercial product may contain up to three moles of water.

Chemical names A stereoisomer of 22-(3-Amino-3,6-dideoxy-β-D-mannopyranosyloxy)-1,3,26-trihydroxy-12-methyl-10-oxo-6,11,28-trioxatricyclo[22.3.1.$0^{5,7}$]octacosa-8,14,16,18,20-pentaene-25-carboxylic acid

C.A.S. number 7681-93-8

Chemical formula $C_{33}H_{47}NO_{13}$

Structural formula



Formula weight 665.74

Assay Not less than 95.0% calculated on the dried basis

DESCRIPTION White to creamy-white, almost odourless, crystalline powder

FUNCTIONAL USES Fungicidal preservative

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Practically insoluble in water, in lipid and in mineral oils; slightly soluble in methanol; soluble in glacial acetic acid and dimethylformamide.

Colour reaction On adding a few crystals of the sample, on a spot plate, to a drop of
- concentrated hydrochloric acid, a blue colour develops;

- concentrated phosphoric acid, a green colour develops, which changes into pale-red after a few minutes

Infrared absorption — The infrared spectrum of a potassium bromide dispersion of the sample corresponds with the reference infrared spectrum in Appendix A.

Ultraviolet absorption — A solution of 5mg/l of the sample in 0.1% glacial acetic acid in methanol has absorption maxima at about 290, 303 and 318 nm, a shoulder at about 280 nm and exhibits minima at about 250, 295.5 and 311 nm. See Appendix B.

PURITY

Loss on drying (Vol. 4) — Not more than 8.0% (60°, over P_2O_5, pressure less than 5 mm Hg)

Specific rotation (Vol. 4) — $[\alpha]_D^{20}$: + 250° to + 295° (1% w/v solution in glacial acetic acid)

pH (Vol. 4) — 5.0 - 7.5 (1.0% w/v suspension in demineralised water)

Sulfated ash (Vol. 4) — Not more than 0.5%
Test 2 g of the sample (Method I)

Lead (Vol. 4) — Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4,"Instrumental Methods"

METHOD OF ASSAY

High Performance Liquid Chromatography

(Note: Throughout this *Assay*, protect from direct light all solutions containing natamycin)

Mobile phase: Dissolve 3.0 g of ammonium acetate and 1.0 g of ammonium chloride in 760 ml of water, and mix. Add 5.0 ml of tetrahydrofuran and 240 ml of acetonitrile, mix, and filter through a 0.5-μm or finer porosity filter. Make adjustments if necessary to meet the system suitability requirements.

Standard preparation: Transfer about 20 mg of natamycin Reference Standard, accurately weighed, to a 100-ml volumetric flask. Add 5.0 ml of tetrahydrofuran, and sonicate for 10 min. Add 60 ml of methanol, and swirl to dissolve. Add 25 ml of water, and mix. Allow to cool to room temperature. Dilute with water to volume, mix, and filter through a membrane filter of 5-μm or finer porosity.

Resolution solution: To prepare a mixture of natamycin and natamycin methyl ester, dissolve 20 mg of natamycin in a mixture of 99 ml of methanol and 1 ml of 0.1 N hydrochloric acid, and allow to stand for 2 h.

Note: use this solution within 1 h.

Assay preparation: Transfer about 20 mg of natamycin, accurately weighed, to a 100-ml volumetric flask. Proceed as directed under "Standard preparation", beginning with "add 5.0 ml of tetrahydrofuran...."

Chromatographic system (see High-Performance Liquid Chromatography, (see Volume 4):

Use a high performance liquid chromatograph equipped with an ultraviolet detector measuring at 303 nm and a 4.6-mm x 25-cm column packed with octadecylsilanized silica (Supelcosil LC 18 or equivalent). The flow rate is about 3 ml/min. Chromatograph the "standard preparation", and record the peak responses. The column efficiency should not be less than 3000 theoretical plates and the tailing factor should be between 0.8 and 1.3. The relative standard deviation for three replicate injections of the standard preparation is not more than 1.0 %.

Chromatograph the "resolution solution". The relative retention times are about 0.7 for Natamycin and 1.0 for its methyl ester. The resolution (R) between Natamycin and its methyl ester is not less than 2.5:

$$R = 2(t_2-t_1)/(W_2+W_1)$$

where:
t_2 and t_1 are the retention times of natamycin methyl ester and natamycin, respectively

W_2 and W_1 are the width of the corresponding peaks at their bases extrapolated to the baseline.

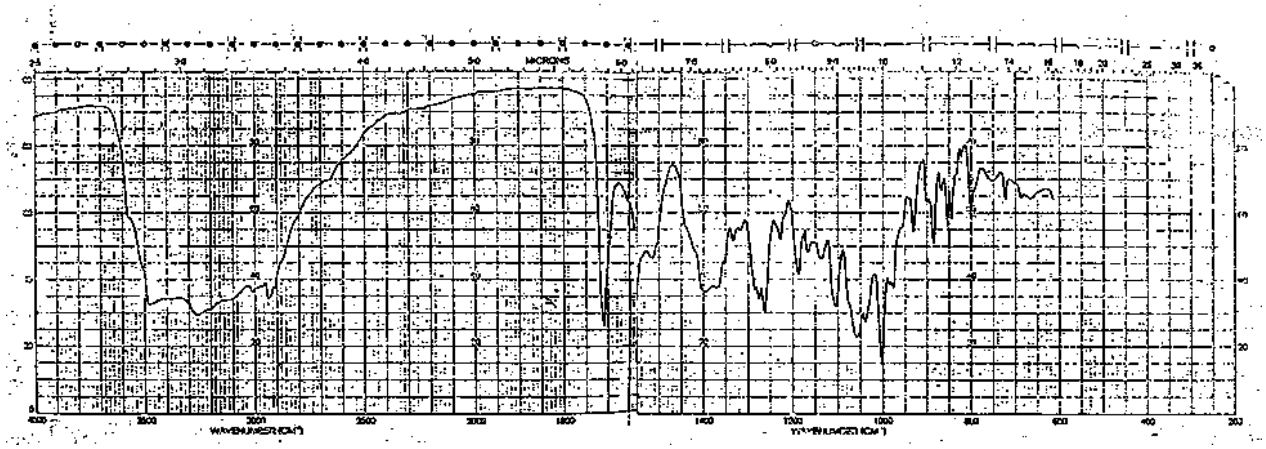
Procedure: Separately inject about 20 µl for each of the "standard preparation" and the "assay preparation" into the chromatograph, and record the peak areas of the major peaks. Calculate the percentage of Natamycin in the portion taken by the formula:

$$0.1(W_sP_s/W_u)(r_u/r_s)$$

in which W_s is the weight, in mg, of Natamycin Reference Standard taken to prepare the "Standard preparation); P_s is the stated content, in µg/ml, of Natamycin Reference Standard; W_u is the weight, in mg, of Natamycin taken to prepare the "Assay preparation"; and r_u and r_s are the peak area responses obtained with the "Assay preparation" and the "Standard preparation", respectively.

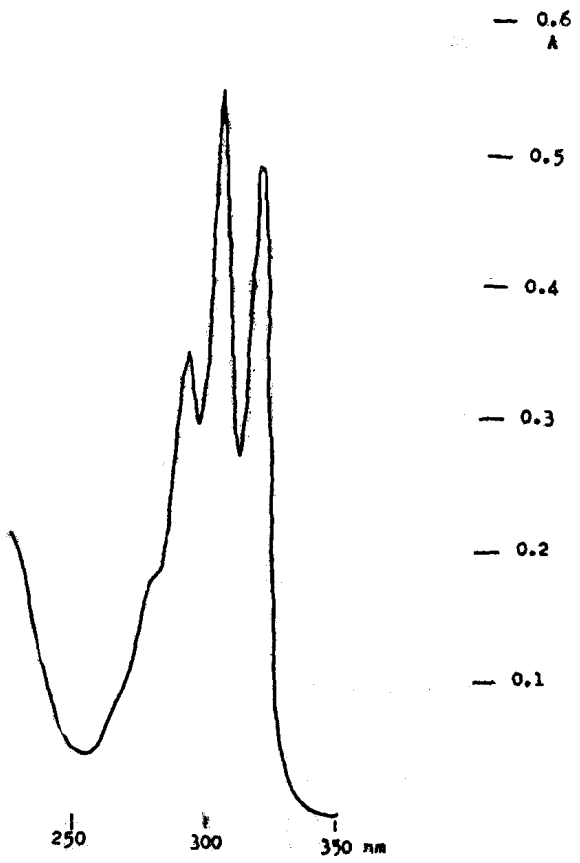
Appendix A

Reference Infrared Spectrum (1.3 mg solid in 300 mg potassium bromide) for natamycin



Appendix B

Ultraviolet absorption spectrum of natamycin
Concentration: 5 µg/ml in methanol/glacial acetic acid mixture



NEOTAME

New specifications prepared at the 61st JECFA (2003) and published in FNP 52 Add 11(2003). An ADI of 0 – 2 mg/kg bw was established at the 61st JECFA (2003).

SYNONYMS INS No. 961

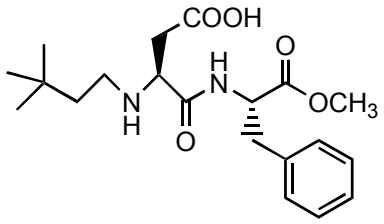
DEFINITION Neotame is manufactured in single process in which aspartame and 3,3-dimethylbutyraldehyde are reacted together in a methanol solution in the presence of hydrogen. Neotame is isolated by removal of methanol, followed by washing and drying.

Chemical names N-[N-(3,3-Dimethylbutyl)-L-α-aspartyl]- L-phenylalanine 1-methyl ester

C.A.S. number 165450-17-9

Chemical formula $C_{20}H_{30}N_2O_5$

Structural formula



Formula weight 378.47

Assay Not less than 97.0% and not more than 102.0% on the anhydrous basis

DESCRIPTION White to off-white powder

FUNCTIONAL USES Sweetener, flavour enhancer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Sparingly soluble in water, very soluble in ethanol

Infrared spectrum The infrared spectrum of a potassium bromide dispersion of the sample corresponds to the standard infrared spectrum in Appendix A.

PURITY

pH (Vol. 4) 5.0 – 7.0 (0.5 % solution)

Melting range (Vol. 4) 81° - 84°

Water (Vol. 4) Not more than 5.0% in a sample size of 25±5 mg (Karl Fischer)

N-[N-(3,3-Dimethylbutyl)-α-aspartyl]-L-phenylalanine

Not more than 1.5%
See under METHOD OF ASSAY

Other related substances

Not more than 2.0% based on the results of the Method of Assay using the following formula:

$$100 \times A/(A+B)$$

where
A = the sum of the peak areas for all secondary peaks other than those for neotame and N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine and
B = the sum of the peak areas for neotame and N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine.

Sulfated ash (Vol. 4)

Not more than 0.2%

Specific rotation (Vol. 4)

$[\alpha]_D^{20}$: Between –40.0° and –43.3° (0.5 % solution) calculated on the anhydrous basis

Lead (Vol. 4)

Not more than 1 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles described in Volume 4, "Instrumental Methods".

METHOD OF ASSAY

Neotame

Determine by HPLC using the following conditions:
Mobile phase: 25% acetonitrile and 75% buffer (final pH of 3.7). The buffer is composed of 0.02 M heptanesulfonic acid sodium salt and 0.5% v/v triethylamine at pH 3.5.
Preparation of sample: Dissolve the sample in mobile phase solution to a concentration of 1 mg/ml.
Preparation of standard: Dissolve the neotame standard (NutraSweet Kelco) in mobile phase solution to a concentration of 1 mg/ml
HPLC Conditions:

Column:	Partisil 5 ODS3 (4.6 x 100 mm length) or equivalent.
Column temperature:	45°
Pump:	Isocratic
Solvent:	25% acetonitrile and 75% buffer adjusted to a pH of 3.7).
Flow rate:	1.5 ml/min
Injection:	25 µl
Detection:	UV 210 nm
Run Time:	approximately 18 min.

Calculation: Compare the area of the neotame peak in the sample (A_{sample}) to that in the standard ($A_{standard}$). Calculate the percentage content of the sample, on the dry basis, from the formula

$$\% \text{ neotame} = (A_{sample}/A_{standard}) \times 100 \times F$$

Where
F= 100/(100 - % water in sample)

N-[N-(3,3-Dimethylbutyl)-α-aspartyl]-L-phenylalanine

This is determined using the same HPLC method:
Preparation of sample: Dissolve the sample in mobile phase solution to a concentration of 2 mg/ml.
Preparation of standard: Dissolve the N-[N-(3,3-dimethylbutyl)-α-aspartyl]-L-phenylalanine standard (NutraSweet Kelco) in mobile phase solution to concentrations of 75, 45, 15, 3 and 0.9 µg/ml.

Calculation: The retention time for N-[N-(3,3-dimethylbutyl)-α-aspartyl]-L-phenylalanine is approximately 4.4 min compared with approximately 12.2 min for neotame.
Determine the area response of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine from the sample preparation. Prepare a full fit linear regression standard curve by plotting the area response of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine in the standard solution on the ordinate scale versus its respective concentration in µg/ml. From the slope and intercept of the standard curve, calculate the concentration, C_1 (µg/ml), of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine in the sample using the equation:

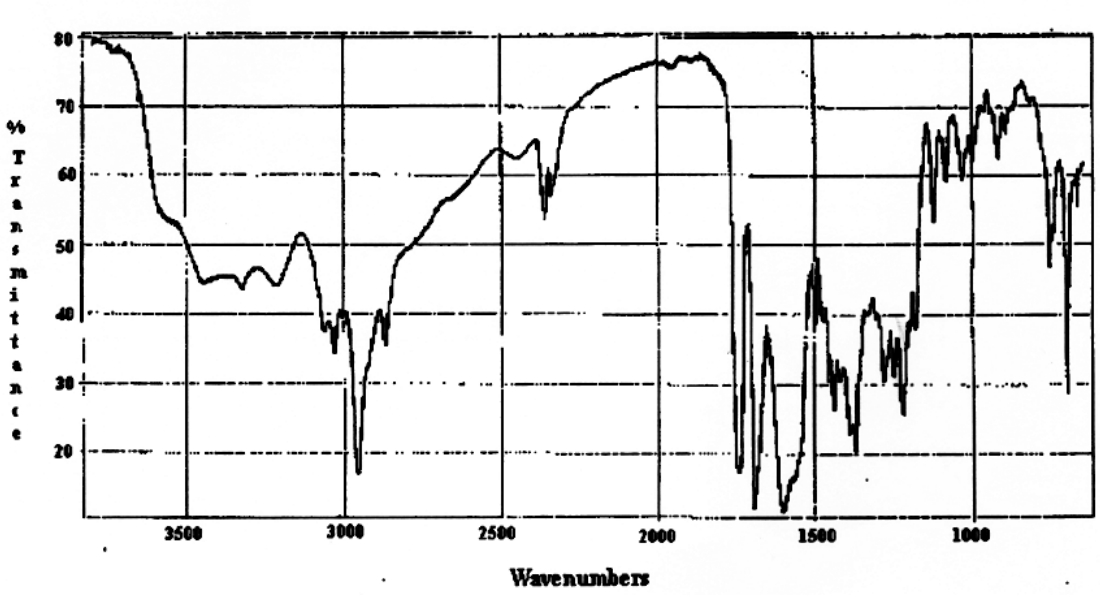
$$C_1 = (A_{sample} - \text{intercept})/\text{slope of curve}$$

Calculate the percentage of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine in the sample using the equation

% N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine = $(C_1/C_2) \times 100$
where C_2 is the concentration of the sample.
See Appendix B for examples of chromatograms obtained using the method.

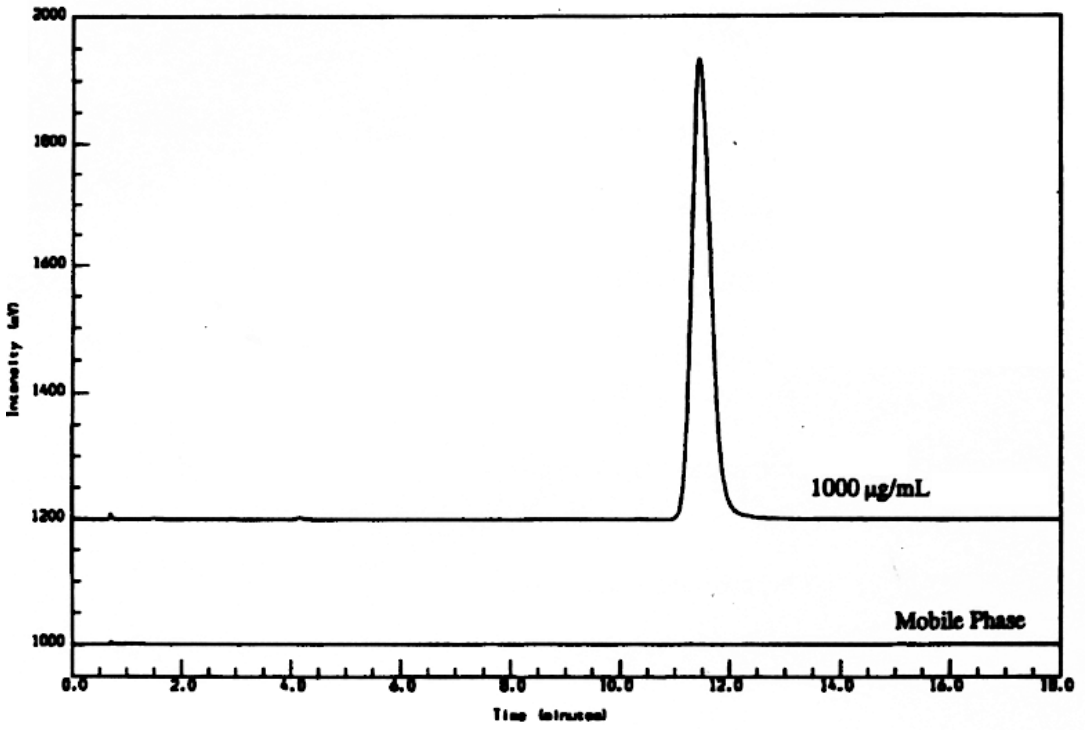
Appendix A

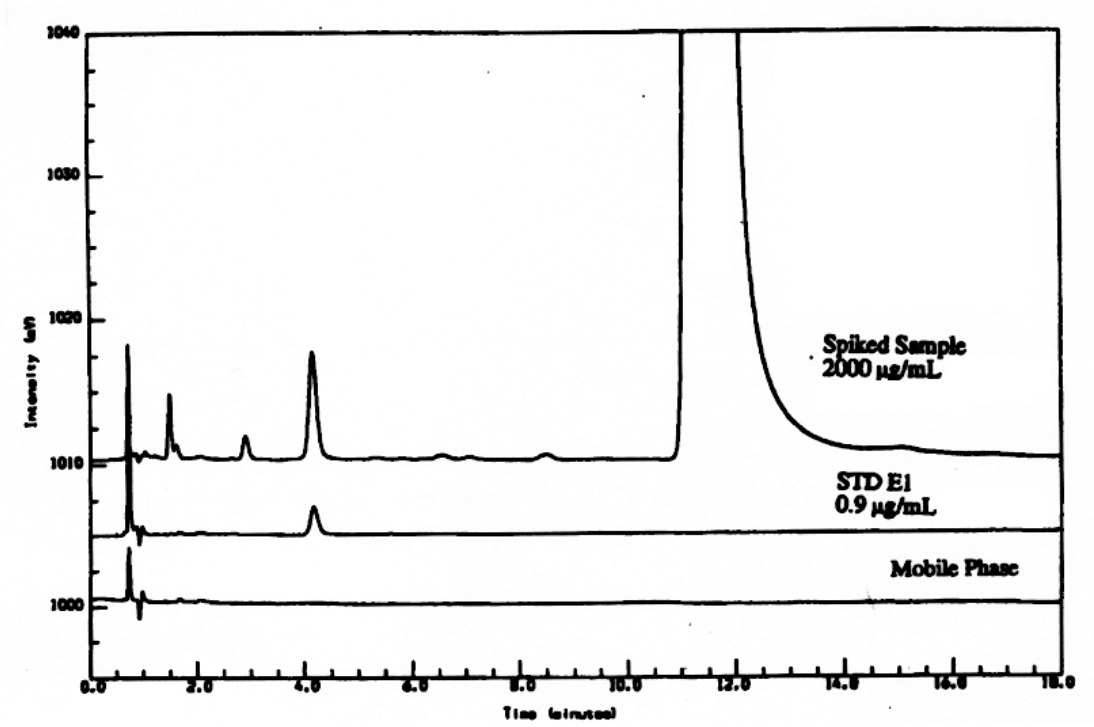
IR Spectrum of neotame standard



Appendix B

Chromatograms for neotame and N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine (Std E1)





NISIN

Prepared at the 71st JECFA (2009), published in FAO JECFA Monographs 7 (2009), superseding specifications for Nisin Preparation prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007). An ADI of 0-33,000 units/kg bw was established at the 12th JECFA (1968).

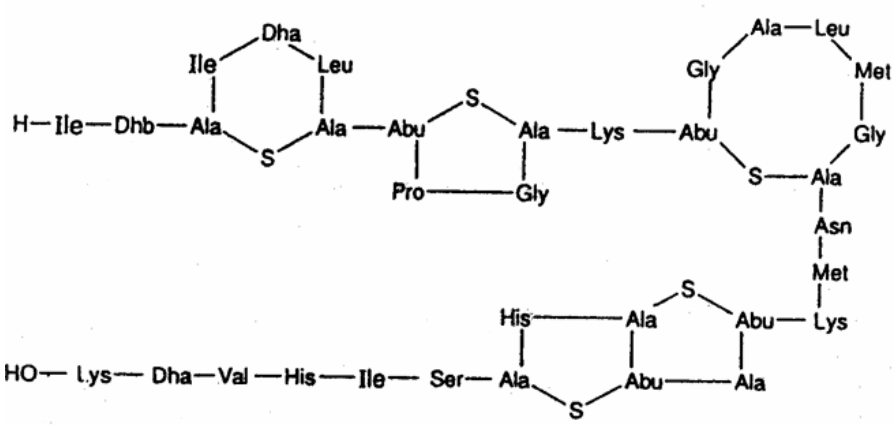
SYNONYMS Nisin preparation; INS No. 234

DEFINITION Nisin is a mixture of closely related antimicrobial polypeptides produced by strains of *Lactococcus lactis* subsp. *lactis*. The major polypeptide is Nisin A. Nisin is produced in a sterilized medium of non-fat milk solids or of a non-milk-based fermentation source, such as yeast extract and carbohydrate solids. Nisin can be recovered from the fermentation medium by various methods, such as injecting sterile, membrane filtration; acidification; salting out; and spray-drying. Non-fat milk solids or solids from other fermentation sources are present in the product. Nisin is available in the commerce as a preparation consisting of Nisin and sodium chloride and is stable at ambient temperatures and upon heating under acid conditions (maximum stability at pH 3).

C.A.S. number 1414-45-5 (Nisin A)

Chemical formula $C_{143}H_{230}N_{42}O_{37}S_7$ (Nisin A)

Structural formula



Abu=alpha-aminobutyric acid, Dha=dehydroalanine, Dhb=dehydrobutyrine
(Nisin A)

Formula weight 3354.12 (Nisin A)

Assay Not less than 900 IU of nisin per milligram and not less than 50% w/w sodium chloride

DESCRIPTION White to light brown micronized powder

FUNCTIONAL USES Antimicrobial preservative

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water and insoluble in non-polar solvents
Differentiation from other antimicrobial substances	Passes tests See description under TESTS

PURITY

Loss on drying (Vol. 4)	Not more than 3.0% (105°, 2 h) (See under “General methods, Inorganic Components.”)
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under "General Methods, Metallic Impurities").
Microbiological criteria (Vol. 4)	*Salmonella* species: Absent in 25 g of sample Total coliforms: Not more than 30 per gram *Escherichia coli*: Absent in 25 g sample (See Volume 4 under “General Methods, Microbiological Analyses.”)

TESTS

IDENTIFICATION TESTS

Differentiation from other antimicrobial substances

Stability to acid

Suspend a 100-mg sample in 0.02 N hydrochloric acid as described in “Standard stock solution” under Method of Assay. Boil this solution for 5 min and determine the nisin activity as directed under Method of Assay. No significant loss of activity is noted following this heat treatment. The calculated nisin concentration of the boiled sample is 100% (+/- 5%) of the assay value. Adjust the pH of the nisin solution to 11.0 by adding 5 N sodium hydroxide. Heat the solution at 65° for 30 min, and then cool. Adjust the pH to 2.0 by adding hydrochloric acid dropwise. Again determine the nisin concentration as directed under Method of Assay. Complete loss of the antimicrobial activity of nisin is observed following this treatment.

Tolerance of *Lactococcus lactis* to high concentrations of nisin

Prepare cultures of *L. lactis* (ATCC 11454, NCIMB 8586) in sterile skim (<1% fat) milk by incubating for 18 h at 30°. Prepare one or more flasks containing 100 ml of litmus milk, and sterilize at 121° for 15 min. Suspend 0.1 g of sample in the sterilized litmus milk, and allow to stand at room temperature for 2 h. Add 0.1 ml of the *L. lactis* culture, and incubate at 30° for 24 h. *L. lactis* will grow in this concentration of sample (about 1000 IU/ml); however, it will not grow in similar concentrations of other antimicrobial substances. (NOTE: This test will not differentiate nisin from subtilin.)

METHOD OF ASSAY **Determination of nisin activity**

Preparation of the test organism

*Lactococcus lactis subsp. cremoris (*ATCC 14365, NCDO 495) is subcultured daily in sterile separated milk by transferring one loopful to a McCartney bottle of litmus milk and incubating at 30°. Prepare inoculated milk for the assay by inoculating a suitable quantity of sterile skim milk with 2 percent of a 24 h culture, and place it in a water-bath at 30° for 90 min. Use immediately.

Standard stock solution

Dissolve an accurately weighed quantity of standard nisin in 0.02 N hydrochloric acid to give a solution containing 5,000 units/ml. Immediately before use, dilute the solution further with 0.02 N hydrochloric acid to give 50 units/ml. (NOTE: Nisin containing 2.5% nisin, minimum potency of 10^6 IU/g, obtainable from Sigma, St Louis, USA or Fluka, Buchs, Switzerland, may be used for the Standard stock solution, as well as the preparation under the name of Nisaplin, available from Danisco, Copenhagen, Denmark).

Sample solution

Weigh an amount of sample sufficient to ensure that corresponding tubes of the sample and standard series match, i.e., within close limits, the nisin content in the sample and standard is the same. Dilute the sample solution in 0.02 N hydrochloric acid to give an approximate concentration of 50 units of nisin per ml.

Resazurin solution

Prepare a 0.0125% w/v solution of resazurin in water immediately before use.

Procedure

Pipet graded volumes (0.60, 0.55, 0.50, 0.45, 0.41, 0.38, 0.34, 0.31, 0.28, 0.26 ml) of the 50 unit per ml sample and standard solutions into rows of 10 dry 6-inches x 5/8-inch bacteriological test tubes. Add 4.6 ml of the inoculated milk to each by means of an automatic pipetting device. (NOTE: The addition of inoculated milk is made in turn across each row of tubes containing the same nominal concentration, not along each row of ten tubes.) Place the tubes in a water bath at 30° for 15 min, then cool in an ice-water bath while adding 1 ml resazurin solution to each. Make the addition in the same order as for the addition of inoculated milk, using an automatic pipetting device. Thoroughly mix the contents of the tubes by shaking. Continue incubation at 30° in a water bath for a further 3-5 min.

Examine the tubes under fluorescent light in a black matt-finish cabinet. Compare the sample tube of the highest concentration that shows the first clear difference in colour (i.e., has changed from blue to mauve) with tubes of the standard row of tubes to find the nearest match in colour. Make further matches at the next two lower concentrations of the sample and standard. Interpolation of matches may be made at half dilution steps. As the standard tubes contain known amounts of nisin, calculate the concentration of nisin in the sample solution. Obtain three readings of the solution and average them. Calculate the activity in terms of IU per gram of product.

Determination of sodium chloride

Transfer about 200 mg of the sample, accurately weighed, into a glass-stoppered flask containing 50 ml of water. Agitate the flask to dissolve the sample while adding 3 ml of nitric acid, 5 ml of nitrobenzene, 50.0 ml of standardized 0.1 N silver nitrate, and 2 ml of ferric ammonium sulfate TS. Shake the solution well, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate. The titration endpoint is indicated by the appearance of a red colour. Each ml of reacted 0.1 N silver nitrate is equivalent to 5.844 mg of NaCl. Calculate the percentage of sodium chloride in the sample taken by the equation:

$$\text{Sodium chloride \% (w/w)} = 100 \times 58.44(50 \times A - V \times B)/(W)$$

where

A is the concentration of the silver nitrate solution;
B is the concentration of the ammonium thiocyanate solution;
V is the volume (ml) of the ammonium thiocyanate consumed;
W is the weight of the sample (mg); and
58.44 is the formula weight of sodium chloride.

NITROGEN

Prepared at the 53rd JECFA (1999) and published in FNP 52 Add 7 (1999), superseding specifications prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998). ADI "No ADI necessary" established at the 24th JECFA in 1980.

SYNONYMS INS No. 941

DEFINITION This monograph defines nitrogen with a maximum oxygen content of 1%, which is suitable only for some food applications. There are food applications that require a higher purity (i.e., lower oxygen content).

Chemical names	Nitrogen
C.A.S. number	7727-37-9
Chemical formula	N_2
Formula weight	28.0
Assay	Not less than 99.0% v/v

DESCRIPTION Colourless, odourless gas or liquid

FUNCTIONAL USES Freezing agent, propellant, packaging gas

CHARACTERISTICS

IDENTIFICATION

Flame test	A flame is extinguished in an atmosphere of the sample

PURITY

Oxygen	As declared by the vendor but not more than 1% v/v See description under TESTS
Carbon monoxide	Not more than 10 µl/l See description under TESTS

TESTS

PURITY TESTS

Oxygen

Use an oxygen analyser with a detector scale ranging from 0 µl/l to 100 µl/l and equipped with an electrochemical cell. The gas to be examined is passed through a detection cell containing an aqueous solution, generally potassium hydroxide. Oxygen in the sample gas produces variation in the electrical signal recorded at the outlet of the cell that is proportional to the oxygen content.

Calibrate the analyzer according to the instructions of the manufacturer.

Pass the gas through the analyser using a suitable pressure regulator and airtight metal tubes and operating at the prescribed flow-rates until constant readings are obtained.

Carbon monoxide

Pass 1050 ± 50 ml of the gas sample through a carbon monoxide detector tube at the rate specified for the tube. The indicator change corresponds to not more than 10 µl/l.

METHOD OF ASSAY

Determine by Gas chromatography using the following conditions:

Column:
- material: stainless steel
- length: 2 m
internal diameter: 2 mm
packing material: appropriate molecular sieve capable of absorbing molecules with diameters up to 0.5 nm.

Carrier:
- gas: helium (not less than 99.995 % (v/v) of He)
- flow: 40 ml/min
Detector: thermal conductivity detector
Injector: loop injector
Column temperature: 50°
Detector temperature: 130°
Reference gas (a): ambient air
Reference gas (b): Nitrogen (not less than 99.999 % (v/v) of N_2, less than 1 ppm CO, less than 5 ppm O_2)

Procedure:
Inject reference gas (a). Adjust the injected volumes and operating conditions so that the height of the peak due to nitrogen in the chromatogram is at least 35 % of full scale of the recorder. The assay is not valid unless the chromatograms obtained show a clear separation of oxygen and nitrogen.

Inject the gas to be examined and the reference gas (b). In the chromatogram obtained with the gas to be examined, the area of the principal peak is at least 99.0 % of the area of the principal peak in the chromatogram obtained with reference gas (b).

NITROUS OXIDE

Prepared at the 74th JECFA (2011) and published in FAO Monographs 11(2011), superseding the tentative specifications prepared at the 71st JECFA (2009), published in FAO JECFA Monographs 7 (2009). An ADI "Acceptable" was established at the 29th JECFA (1985).

SYNONYMS Dinitrogen oxide; Dinitrogen monoxide; INS No. 942

DEFINITION Nitrous oxide, a colourless and non-flammable gas, commonly known as laughing gas, is manufactured by the controlled heating of ammonium nitrate, at temperatures 170-240°, either using superheated steam or other thermal decomposition processes. The hot, corrosive mixture of gases are cooled to condense the steam and filtered to remove higher oxides of nitrogen. The gas is further purified in a train of three gas washes with base, acid and base again. Nitric oxide impurity, if present, is chelated out with ferrous sulfate, or reduced with iron metal, or oxidised and absorbed in a base as a higher oxide.

Chemical names	Nitrous oxide
C.A.S. number	10024-97-2
Chemical formula	N_2O
Formula weight	44.01
Assay	Not less than 99 % (v/v)

DESCRIPTION Colourless, odourless gas

FUNCTIONAL USES Propellant, antioxidant, packaging gas, foaming agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	1 volume dissolves in 1.4 volumes of water (20° 760 mm Hg). Freely soluble in alcohol; soluble in ether and in oils.
Infrared absorption	The infrared absorption band of the sample corresponds with the typical infrared absorption band of nitrous oxide
Carbon dioxide test	Passes test See description under TESTS

PURITY

Carbon monoxide	Not more than 10 µl/l See description under TESTS
Nitric oxide	Not more than 1 µl/l See description under TESTS
Nitrogen dioxide	Not more than 1 µl/l See description under TESTS
Halogens (as Cl)	Not more than 5 µl/l See description under TESTS
Ammonia	Not more than 25 µl/l See description under TESTS

TESTS

NOTE 1: The carbon dioxide identification test and all of the purity tests are referenced from the Food Chemicals Codex, 7th Edition, 2011, p. 719-720.
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NOTE 2: The identification and purity tests given below are designed to reflect the quality of nitrous oxide in both its vapour and its liquid phases, which are present in previously unopened cylinders. Reduce the sample cylinder pressure with a regulator. Withdraw the samples for the tests with the least possible release of sample gas consistent with proper purging of the sample apparatus. Measure the gases with a gas volume meter downstream from the detector tubes to minimize contamination of or change to the samples. Perform the tests in the sequence in which they are listed below.

NOTE 3: Detector tubes referenced under identification and purity tests are available from National Draeger Inc., P.O. Box 120, Pittsburgh, PA 15205-0120, USA.

IDENTIFICATION TESTS

Carbon dioxide test	Pass 100 ml of sample gas released from the vapour phase of the contents of the sample gas cylinder through a carbon dioxide detector tube (Draeger CH 30801 or equivalent) at the rate specified for the tube. No colour change occurs.

PURITY TESTS

Carbon monoxide	Pass 1000 ml of sample gas released from the vapour pressure of the contents of the sample gas cylinder, through a carbon monoxide detector tube (Draeger CH 25601 or equivalent) at the rate specified for the tube. The indicator change corresponds to not more than 10 µl of carbon monoxide.

Nitric oxide

Pass 500 ml of sample gas, released from the vapour phase of the contents of the sample gas cylinder, through a nitric oxide-nitrogen dioxide detector tube (Draeger CH 29401 or equivalent) at the rate specified for the tube. The indicator change corresponds to not more than 0.5 µl of nitrogen monoxide.

Nitrogen dioxide

Arrange a sample gas cylinder so that when its valve is opened, a portion of the liquid phase of the contents is released through a piece of tubing of sufficient length to allow all of the liquid to vaporize during passage through it and to prevent frost from reaching the inlet of the detector tube. Release a flow of liquid into the tubing sufficient provide 500 ml of the vaporized sample plus any excess necessary to ensure adequate flushing of air from the system.

Pass 500 ml of sample gas, released from the vapour phase of the contents of the sample gas cylinder, through a nitric oxide-nitrogen dioxide detector tube (Draeger CH 29401 or equivalent) at the rate specified for the tube. The indicator change corresponds to not more than 0.5 µl of nitrogen dioxide.

Halogens (as Cl)

Pass 1000 ml of sample gas, released from the vapour phase of the contents of the sample gas cylinder, through a chlorine detector tube (Draeger CH 24301 or equivalent) at the rate specified for the tube. The indicator change corresponds to not more than 5 µl.

Ammonia

Pass 1000 ml of sample gas, released from the vapour phase of the contents of the sample gas cylinder, through an ammonia detector tube (Draeger CH 20501 or equivalent) at the rate specified for the tube. The indicator change corresponds to not more than 25 µl.

METHOD OF ASSAY

Determine using an Infrared gas analyzer or conventional IR/FTIR fitted with a suitable gas cell to analyze nitrous oxide in the range of 85% to 100% v/v.

Reference gas standards: (a) nitrous oxide standard (99.9%) and (b) a mixture containing 5%v/v nitrogen and 95%v/v nitrous oxide.

Method using Infrared gas analyzer:
Infrared gas analyzers consist of a light source emitting broad band radiation, an optical device, a gas sample cell and a detector. Set up the instrument and select the filter for nitrous oxide. Calibrate the instrument using reference gas standards (a) and (b). Flush the sample cell using the gas to be examined and read the nitrous oxide concentration from the analyzer.

Method using conventional IR/FTIR:
Set up the instrument, following manufacturer's instructions, and set the wave number at the highest absorption band (2218 cm^{-1}). Construct standard curve, using a set of standard gases containing 5% v/v of nitrogen in 95% nitrous oxide to pure nitrous oxide (>99.9% nitrous oxide). Flush the sample cell using the gas to be examined and read the nitrous oxide concentration from the standard curve.

o-PHENYLPHENOL

Prepared at the 8th JECFA (1964), published in NMRS 38A (1965) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). An ADI of 0-0.2 mg/kg bw was established at the 8th JECFA (1964)

SYNONYMS Orthoxenol; INS No. 231

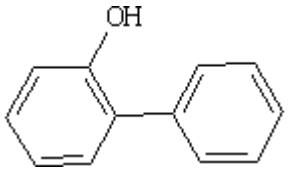
DEFINITION

Chemical names (1,1'-Biphenyl)-2-ol, 2-hydroxydiphenyl, *o*-hydroxydiphenyl

C.A.S. number 90-43-7

Chemical formula $C_{12}H_{10}O$

Structural formula



Formula weight 170.20

Assay Not less than 98%

DESCRIPTION White, slightly yellow or pink flaky crystals or solid, having a mild characteristic odour

FUNCTIONAL USES For the post-harvest treatment of fruits and vegetables to protect against microbial damage

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Practically insoluble in water, very soluble in ethanol

Melting point (Vol. 4) About 57°

Test for phenol An ethanolic solution (1 g in 10 ml) produces a green colour upon addition of 10% ferric chloride solution

PURITY

Total ash (Vol. 4) Not more than 0.05%

Lead (Vol. 4) Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh 2.000 g of o-phenylphenol, dissolve in 10 ml of 10% sodium hydroxide solution by warming and dilute to 500.0 ml. Pipette 25.0 ml into a 250-ml iodine flask and add 30.0 ml of 0.1 N bromide-bromate TS and 50 ml of anhydrous methanol. Place the stopper in the flask and add 10 ml of dilute (1 to 1) hydrochloric acid to the well. Raise the stopper slightly to allow the acid to flow down the sides inside the flask, but retain a small amount of the acid in the well to act as a seal. Mix the contents by swirling and allow it to react for exactly 30 sec at 25±5°. Immediately add 10 ml of 20% potassium iodide solution to the well and allow it to drain into the assay flask as for the acid. Mix well, allow the solution to stand for 5 min, shaking occasionally. Wash the stopper and the sides of the flask with water. Titrate the liberated iodine with 0.1 N sodium thiosulfate adding starch TS as the endpoint is approached. Each ml of 0.1 N bromide-bromate TS consumed is equivalent to 4.255 mg of $C_{12}H_{10}O$.

PAPAIN

Prepared at the 15th JECFA (1971), published in NMRS 50B (1972) and in FNP 52 (1992). An ADI 'not limited' was established at the 15th JECFA (1971)

SYNONYMS INS No.1101(ii)

SOURCES Purified proteolytic substances derived from the fruit of *Carica papaya* (L) (Fam. *Caricaceae*).

Active principles
1. Papain (papaya peptidase I, cystein proteinase)
2. Chymopapain (cystein proteinase)

Systematic names and numbers
1. None (EC 3.4.22.2)
2. None (EC 3.4.22.6)

Reactions catalyzed: These enzymes hydrolyze polypeptides, amides and esters, especially at linkages involving basic amino acids, or leucine or glycine, yielding peptides of lower molecular weight.

DESCRIPTION White to light tan amorphous powder or liquids; soluble in water, the solutions being colourless to light yellow and somewhat opalescent; practically insoluble in alcohol, chloroform and ether

FUNCTIONAL USES Enzyme preparation
Used in the chillproofing of beer, tenderizing of meat, preparation of precooked cereals, and production of protein hydrolysates

GENERAL SPECIFICATIONS Must conform to the *General Specifications for Enzyme Preparations used in Food Processing* (see Volume 1, Introduction)

CHARACTERISTICS

IDENTIFICATION

Papain activity (Vol. 4): The sample shows plant proteolytic activity

PECTINS

Prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs 7 (2009), superseding specifications prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007). A group ADI "not specified" was established for pectins and amidated pectins, singly or in combination at the 25th JECFA in 1981.

SYNONYMS INS No. 440

DEFINITION Consists mainly of the partial methyl esters of polygalacturonic acid and their sodium, potassium, calcium and ammonium salts; obtained by extraction in an aqueous medium of appropriate edible plant material, usually citrus fruits or apples; no organic precipitants shall be used other than methanol, ethanol and isopropanol; in some types a portion of the methyl esters may have been converted to primary amides by treatment with ammonia under alkaline conditions. Sulfur dioxide may be added as a preservative.

The commercial product is normally diluted with sugars for standardization purposes. In addition to sugars, pectins may be mixed with suitable food-grade buffer salts required for pH control and desirable setting characteristics. The article of commerce may be further specified as to pH value, gel strength, viscosity, degree of esterification, and setting characteristics.

C.A.S. number 9000-69-5

DESCRIPTION White, yellowish, light greyish or light brownish powder

FUNCTIONAL USES Gelling agent, thickener, stabilizer, emulsifier

CHARACTERISTICS

IDENTIFICATION

Test for pectins
Passes test
See description under TESTS

Test for amide group
Passes test (amidated pectins only)
Add 2 ml of concentrated hydrochloric acid and 50 ml of 60% ethanol to 0.5 g of the sample, and stir well for 20 min. Transfer to a fritted glass filter tube wash with six 10 ml portions of the HCl-60% ethanol mixture. Dissolve in 100 ml distilled water; it may be necessary to add a few drops 0.1 mol/L sodium hydroxide to achieve solution. Transfer 4 ml of this solution into a test tube (recommended dimensions 15.5 mm inner diameter and 146 mm length). Add 1 ml 5 mol/L sodium hydroxide and mix. The mixture will form a gel. Fill a small glass tube (recommended dimensions 7.8 mm inner diameter and 79 mm length) with 2.5 ml boric acid TS and let glide into the test tube. Close with parafilm and incubate overnight at 30°. In case of presence of amide groups the indicator changes its colour from red to green, due to release of ammonia.

PURITY

Loss on drying (Vol. 4)	Not more than 12% (105°, 2 h)
Sulfur dioxide	Not more than 50mg/kg See description under TESTS
Residual solvents (Vol. 4)	Not more than 1% methanol, ethanol and 2-propanol, singly or in combination See description under TESTS
Acid-insoluble ash (Vol. 4)	Not more than 1%
Total insolubles	Not more than 3% See description under TESTS
Nitrogen content (Vol. 4)	Not more than 2.5% after washing with acid and ethanol
Galacturonic acid	Not less than 65% calculated on the ash-free and dried basis See description under TESTS
Degree of amidation	Not more than 25% of total carboxyl groups of pectin See description under TESTS
Lead (Vol. 4)	Not more than 5 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under “General Methods, Metallic Impurities.”)

TESTS

IDENTIFICATION TESTS

Test for Pectins

Moisten 0.05 g of the sample with 2-propanol. Add 50 ml of water on a magnetic stirrer. Adjust pH to 12 using 0.5 mol/l sodium hydroxide and let the solution remain without stirring for 15 min. Reduce pH to 7.0 with 0.5 mol/l hydrochloric acid. Adjust to 100.0 ml with water. Make up samples in 1 cm quartz cuvettes as follows:

	Buffer pH 7.0 *)	Sample soln	Water	Enzyme soln **)
Enzyme blank	0.5 ml	1.0 ml	1.0 ml	-
Sample blank	0.5 ml	-	1.5 ml	0.5 ml
Sample	0.5 ml	1.0 ml	0.5 ml	0.5 ml

*) Dissolve 6.055 g of tris(hydroxymethyl)aminomethane (e.g. TRIZMA Base, Sigma) and 0.147 g of calcium chloride dihydrate in water to 1 l. Adjust pH to 7.0 with 1 mol/l hydrochloric acid

**) Dilute pure pectate lyase 1:100 with buffer pH 7.0
Shake the solutions well, and measure the absorbance at 235 nm at

0 and 10 min.

Calculations

A_0 = absorbance at 0 min = Sample - (enzyme blank + sample blank)

A_{10} = absorbance at 10 min = Sample - (enzyme blank + sample blank)

The amount of unsaturated product produced is proportional to the change in absorbance (A_{10} - A_0). This value should be greater than 0.023. This distinguishes pectins from other gums, which show essentially no change.

PURITY TESTS

Sulfur dioxide

Suspend 100 g of the sample in 500 ml of methanol in a 1000-ml round-bottom flask, which is provided with a gas inlet tube reaching almost the bottom and connected to the neck with a reflux condenser. Prepare a glass joint connection from the condenser to an absorption flask or U-tube containing 10 ml of 3% hydrogen peroxide solution neutralized to methyl red TS. Connect the gas inlet tube with an oxygen-free source of carbon dioxide or nitrogen, and maintain a gas stream so as to cause steady bubbling. As soon as the apparatus is flushed free of air, pour 30 ml of hydrochloric acid solution (10 ml conc. HCl + 20 ml H_2O) into the reflux condenser, and immediately connect the absorption flask or U-tube. Heat slowly until methanol starts refluxing, and reflux gently for 2 h. Disconnect the apparatus and titrate the hydrogen peroxide solution against methyl red TS with 0.01 mol/l sodium hydroxide. Each ml of 0.01 mol/l sodium hydroxide corresponds to 0.32 mg of SO_2.

Total insolubles

Dry a 70 mm glass fiber filter paper (GF/B (Whatman code 1821 070) in an oven with fan set at 105° for about 1 h. Transfer the filter paper to a desiccator containing silica gel and allow to cool. Weigh the paper (M_1). Weigh about 1 g (= S) of the sample into a 250-ml beaker. Add 5 ml of 2-propanol to disperse the sample. While stirring magnetically, add 100 ml of 0.03 mol/l sodium hydroxide containing 0.1% (w/w) ethylene diamine tetra-acetic acid (Na salt), which has been filtered through GF/B paper. Stir for about 30 min at room temperature, then heat to boiling (remove heat if excessive foaming occurs). Filter the hot solution through the glass fiber paper under vacuum using, e.g. a vacuum filtration kit with 3 piece Hartley funnel (70 cm), with heat resistant plate. Rinse the beaker five times and filter the rinsings with 100 ml of warm (about 50°) water that has been filtered through GF/B paper.
Dry the filter paper with the residue at 105° for 1 h. Transfer to desiccator containing silica gel and leave to cool. Weigh the paper (M_2). Calculate the percentage of total insolubles from

Total insolubles (%) = $[(M_2 - M_1)/S] \times 100$

Galacturonic acid and Degree of amidation

Weigh 5 g of the sample to the nearest 0.1 mg, and transfer to a suitable beaker. Stir for 10 min with a mixture of 5 ml of hydrochloric acid TS, and 100 ml of 60% ethanol. Transfer to a fritted-glass filter tube (30 to 60 ml capacity) and wash with six 15-ml portions of the HCl-60% ethanol mixture, followed by 60% ethanol until the filtrate

is free of chlorides. Finally wash with 20 ml of ethanol, dry for 2.5 h in an oven at 105°, cool and weigh. Transfer exactly one-tenth of the total net weight of the dried sample (representing 0.5 g of the original unwashed sample) to a 250-ml conical flask and moisten the sample with 2 ml of ethanol TS. Add 100 ml of recently boiled and cooled distilled water, stopper and swirl occasionally until a complete solution is formed. Add 5 drops of phenolphthalein TS, titrate with 0.1 mol/l sodium hydroxide and record the results as the initial titre (V_1).

Add exactly 20 ml of 0.5 mol/l sodium hydroxide TS, stopper, shake vigorously and let stand for 15 min. Add exactly 20 ml of 0.5 mol/l hydrochloric acid and shake until the pink colour disappears. Titrate with 0.1 mol/l sodium hydroxide to a faint pink colour which persists after vigorous shaking; record this value as the saponification titre (V_2).
Quantitatively transfer the contents of the conical flask into a 500-ml distillation flask fitted with a Kjeldahl trap and a water-cooled condenser, the delivery tube of which extends well beneath the surface of a mixture of 150 ml of carbon dioxide-free water and 20.0 ml of 0.1 mol/L hydrochloric acid in a receiving flask. To the distillation flask add 20 ml of a 1-in-10 sodium hydroxide solution, seal the connections, and then begin heating carefully to avoid excessive foaming. Continue heating until 80-120 ml of distillate has been collected. Add a few drops of methyl red TS to the receiving flask, and titrate the excess acid with 0.1 mol/l sodium hydroxide recording the volume required, in ml, as S. Perform a blank determination on 20.0 ml of 0.1 mol/l hydrochloric acid, and record the volume required, in ml, as B. The amide titre is (B - S).

Transfer exactly one-tenth of total net weight of the dried sample (representing 0.5 g of the original unwashed sample) and wet with about 2 ml ethanol in a 50-ml beaker. Dissolve the pectin in 30 ml of 0.1 mol/l sodium hydroxide. Let the solution stand for 1 h with agitation at room temperature. Transfer quantitatively the saponified pectin solution to a 50-ml measuring flask and dilute to the mark with distilled water. Transfer 25 ml of the diluted pectin solution to a distillation apparatus and add 20 ml of Clark's solution, which consists of 100 g of magnesium sulfate heptahydrate and 0.8 ml of concentrated sulphuric acid and distilled water to a total of 180 ml. This apparatus consists of a steam generator connected to a round-bottom flask to which a condenser is attached. Both steam generator and round-bottom flask are equipped with heating mantles.

Start the distillation by heating the round-bottom flask containing the sample. Collect the first 15 ml of distillate separately in a measuring cylinder. Then start the steam supply and continue distillation until 150 ml of distillate have been collected in a 200-ml beaker. Add quantitatively the first 15 ml distillate and titrate with 0.05 mol/l sodium hydroxide to pH 8.5 and record volume required, in ml, as A.

Perform a blank determination on 25 ml distilled water. Record the required volume, in ml, as A_0. The acetate ester titre is ($A - A_0$). Calculate degree of amidation (as % of total carboxyl groups) by the formula:

$$100 \times \frac{B - S}{V_1 + V_2 + (B - S) - (A - A_0)}$$

Calculate mg of galacturonic acid by the formula:

$$19.41 \times [V_1 + V_2 + (B - S) - (A - A_0)]$$

The mg of galacturonic acid obtained in this way is the content of one-tenth of the weight of the washed and dried sample. To calculate % galacturonic acid on a moisture- and ash-free basis, multiply the number of mg obtained by 1000/x, x being the weight in mg of the washed and dried sample.

NOTE 1: If the pectin is known to be of the nonamidated type, only V1 and V2 need to be determined and (B - S) may be regarded as zero.
NOTE 2: For pectins from apple or citrus $(A - A_0)$ is usually insignificant in calculating galacturonic acid and degree of amidation.
NOTE 3: If desired, calculate degree of esterification (as % of total carboxyl groups) by the formula:

$$100 \times \frac{V_2 - (A - A_0)}{V_1 + V_2 + (B - S) - (A - A_0)}$$

NOTE 4: If desired, calculate degree of acetate ester (as % of total carboxylic groups from galacturonic acid) by the formula:

$$100 \times \frac{A - A_0}{V_1 + V_2 + (B - S) - (A - A_0)}$$

Residual solvents (Vol. 4) Apply Method I in Volume 4, General Methods, Organic Components.

Standard stock solution: To 500 ml of water in a 1000-ml volumetric flask, add about 5 g each of methanol, ethanol and 2-propanol, accurately weighed. Make up to the mark with water.

Internal standard solution: To 500 ml of water in a 1000-ml volumetric flask, add about 5 g of 2-butanol ($W_{standard}$), accurately weighed. Make up to the mark with water.

Blank Solution: Omit the blank determination

Samples: Store the sample in a cool, dry place. Mix the sample thoroughly before analysis.

Weigh accurately about 1 g of sample (W_{sample}) in a 100 ml beaker and mix with about 5 g of sucrose. Into a 100-ml Erlenmeyer flask with magnetic stirrer bar, add 95 ml water and 1.0 ml internal standard solution. While stirring fast, slowly add the pectin-sucrose mixture. Stopper the flask and stir for 2 h. The pectin must be completely dissolved. Accurately weigh about 1 g of this solution

(M_{sample}) into a headspace vial for GC analysis.

Calibration solution: Pipette 2.0 ml of standard stock solution and 2.0 ml of internal standard solution into a 200-ml volumetric flask and make up to the mark with water. Accurately weigh about 1 g of this solution ($M_{standard}$) is filled into a head space vial and used for GC analysis.

Procedure

Continue the analysis as described in Vol.4 'Residual solvents', using the given conditions except for the sample heating temperature, which should be 70°, and syringe temperature, which should be 80°.

Calculation

Calculate the concentration of each residual solvent using the following equation:

$$\%\ \text{of solvent} = \frac{R_{sample} \times W_{standard} \times M_{standard}}{R_{standard} \times W_{sample} \times M_{sample} \times 1000} \times 100$$

where

R_{sample}	is the relative peak area of the sample;
$R_{standard}$	is the relative peak area of the standard;
W_{sample}	is the weight of sample (g);
$W_{standard}$	is the weight of solvent used for the standard stock solution;
M_{sample}	is the weight of sample solution used for the GC analysis; and
$M_{standard}$	is the weight of Calibration solution used for the GC analysis.

PHOSPHORIC ACID

Prepared at the 44th JECFA (1995), published in FNP 52 Add 3 (1995) superseding specifications prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). No ADI was established, but a group MTDI of 70 mg/kg bw, expressed as phosphorus from all food sources, was established at the 26th JECFA (1982).

SYNONYMS Orthophosphoric acid, INS No. 338

DEFINITION

Chemical names	Phosphoric acid, orthophosphoric acid
C.A.S. number	7664-38-20
Chemical formula	H_3PO_4
Formula weight	98.00
Assay	Not less than 75%, and not less than the minimum or within the range of percent claimed by the vendor

DESCRIPTION Clear, colourless, odourless, viscous liquid

FUNCTIONAL USES Acidulant, sequestrant, synergist for antioxidants

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Miscible with water and with ethanol
Test for acid	Strongly acid, even at high dilution
Test for phosphate	Neutralize a few ml of phosphoric acid and add dilute nitric acid TS. Then add an equal volume of ammonium molybdate TS and warm. A bright canary-yellow precipitate is obtained which is soluble in dilute ammonia TS.

PURITY

Nitrates	Not more than 5 mg/kg Dilute 3.48 g of the sample to 10 ml with water and add 5 mg of sodium chloride, 0.1 ml of indigo carmine TS, and 10 ml of sulfuric acid. The blue colour shall not disappear entirely within 5 min.
Volatile acids	Not more than 10 mg/kg as acetic acid Dilute 60.05 g of the sample with 75 ml of freshly boiled and cooled water in a distilling flask with a spray trap, and distil 50 ml. To the distillate add phenolphtalein TS and titrate with 0.1N sodium hydroxide. Not more than 0.1 ml of 0.1N sodium hydroxide should be required for neutralization.

Chlorides (Vol. 4)	Not more than 200 mg/kg as chlorine Test 1.78 g of the sample as directed in the Limit Test using 1.0 ml of 0.01 N hydrochloric acid in the control
Sulfates (Vol. 4)	Not more than 0.15% 1.25 g of the sample meets the requirements of the Limit Test
Fluoride (Vol. 4)	Not more than 10 mg/kg
Arsenic (Vol. 4)	Not more than 3 mg/kg A solution of 1.5 g of sample in 35 ml of water meets the requirements of the Limit Test (Method II) using as control a mixture of 3 ml of Standard Arsenic Solution (3 µg As) and 1.5 g reagent grade phosphoric acid.
Lead (Vol. 4)	Not more than 4 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Weigh 1.00 g of the sample into a glass-stoppered flask, dilute with about 100 ml of water, add 0.5 ml of thymolphthalein TS, and titrate with 1N sodium hydroxide. Each ml of 1N sodium hydroxide is equivalent to 0.049 g of H_3PO_4.

SODIUM DIHYDROGEN PHOSPHATE

Prepared at the 7th JECFA (1963), published in NMRS 35 (1964) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). A group MTDI of 70 mg/kg bw, as phosphorus from all food sources, was established at the 26th JECFA (1982)

SYNONYMS Monobasic sodium phosphate, monosodium monophosphate sodium acid phosphate, sodium biphosphate; INS No. 339(i)

DEFINITION

Chemical names	Sodium dihydrogenphosphate, monosodium dihydrogenortho- phosphate, monosodium dihydrogen monophosphate
C.A.S. number	7558-80-7
Chemical formula	Anhydrous: NaH_2PO_4 Monohydrate: $NaH_2PO_4 \cdot H_2O$ Dihydrate: $NaH_2PO_4 \cdot 2H_2O$
Formula weight	Anhydrous: 119.98 Monohydrate: 138.00 Dihydrate: 156.01
Assay	Not less than 97% after drying

DESCRIPTION White odourless, slightly deliquescent powder, crystals, or granules

FUNCTIONAL USES Acidity regulator, sequestrant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Freely soluble in water; insoluble in ethanol, ether or chloroform
pH (Vol. 4)	4.2 - 4.6 (1 in 100 soln)
Test for sodium (Vol. 4)	Passes test
Test for phosphate (Vol. 4)	Passes test
Test for orthophosphate	To a 1% solution of the sample add silver nitrate TS; the yellow precipitate formed is soluble in dilute nitric acid TS.

PURITY

Loss on drying (Vol. 4)	Anhydrous: Not more than 2% (60^o, 1 h, then 105^o, 4 h) Monohydrate: Not more than 15% (60^o, 1 h, then 105^o, 4 h) Dihydrate: Not more than 25% (60^o, 1 h, then 105^o, 4 h)
Free acid and disodium	2.00 g of the sample dissolved in 40 ml of water require for neutralization

phosphate	not more than 0.3 ml of either N sodium hydroxide or N sulfuric acid, using methyl orange TS as indicator.
Fluoride (Vol. 4)	Not more than 10 mg/kg
Arsenic (Vol.4)	Not more than 3 mg/kg (Method II)
Lead (Vol. 4)	Not more than 4 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Transfer 0.7000 g of the dried sample to a 250-ml beaker, add 50 ml of 0.1 N hydrochloric acid, and stir until the sample is completely dissolved. Place the electrodes of a suitable pH meter in the solution and add slowly from a burette, with constant stirring, 0.1 N sodium hydroxide until a pH of 3.3 is attained. Continue to add sodium hydroxide solution until the next ml or 0.5-ml graduation mark on the burette is reached. Record the burette reading under column 1 of a suitable data sheet and record the pH under column 2. Continue the addition of 0.1 N sodium hydroxide in 0.5-ml increments until a pH of 6.0 is attained, recording the burette reading and the pH after the addition of each increment. Then proceed with the titration in the usual manner until a pH of 0.8 is attained, and again continue to add the solution until the next ml or 0.5-ml graduation mark is reached before recording the burette reading and the pH. Then continue adding 0.1 N sodium hydroxide in 0.5-ml increments until the pH is 10.0, again recording the burette reading and the pH after the addition of each increment. In column 3 of the data sheet, note the values for "delta"pH obtained by subtracting each pH value recorded from the net higher value. In column 4, note the values for "delta"$_2$pH, i.e., the differences between successive "delta"pH values, recording them as plus or minus depending upon whether the value of "delta"pH is higher or lower than the preceding one. The end-point lies in the 0.5-ml increment of sodium hydroxide that gives the highest value for "delta"pH, its exact position being calculated by adding 0.5 b/B to the next lower burette reading, where b is the last "delta"$_2$pH value having a plus sign and B is the sum, without regard to sign, of the last "delta"$_2$pH value having a plus sign and the first "delta"$_2$pH value having a minus sign. Two end-points are calculated, that occurring between pH 3.3 and pH 6.0 being designated F and that between pH 8.5 and pH 10.0 being designated T. The volume of sodium hydroxide used in the titration is obtained by subtracting F from T. Each ml of 0.1 N sodium hydroxide is equivalent to 0.0120 g of NaH_2PO_4.

DISODIUM HYDROGEN PHOSPHATE

Prepared at the 19th JECFA (1975), published in NMRS 55B (1976) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). A group MTDI of 70 mg/kg bw, as phosphorus from all food sources, was established at the 26th JECFA (1982)

SYNONYMS Dibasic sodium phosphate, disodium phosphate, disodium acid phosphate, secondary sodium phosphate; INS No. 339(ii)

DEFINITION

Chemical names	Disodium hydrogen phosphate, disodium hydrogen orthophosphate, disodium hydrogen monophosphate
C.A.S. number	7558-79-4
Chemical formula	Anhydrous: Na_2HPO_4 Hydrated: $Na_2HPO_4 \cdot x\ H_2O$
Formula weight	141.98 (anhydrous)
Assay	Not less than 98.0% after drying

DESCRIPTION Anhydrous: White, hygroscopic, odourless powder
Dihydrate: White crystalline, odourless solid
Heptahydrate: White, odourless, efflorescent crystals or granular powder
Dodecahydrate: White, efflorescent, odourless powder or crystals

FUNCTIONAL USES Emulsifier, texturizer, buffer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Freely soluble in water; insoluble in ethanol
pH (Vol. 4)	9.0- 9.6 (1 in 100 soln)
Test for sodium (Vol. 4)	Passes test
Test for phosphate (Vol. 4)	Passes test
Test for orthophosphate (Vol. 4)	Dissolve 0.1 g of the sample in 10 ml water, acidify slightly with dilute acetic acid TS, and add 1 ml of silver nitrate TS. A yellow precipitate is formed.

PURITY

Loss on drying (Vol. 4)	Anhydrous: Not more than 5.0% (40^o, 3 h, then 105^o, 5 h) Dihydrate: Not more than 22.0% (40^o, 3 h, then 105^o, 5 h) Heptahydrate: Not more than 50.0% (40^o, 3 h, then 105^o, 5 h) Dodecahydrate: Not more than 61.0% (40^o, 3 h, then 105^o, 5 h)

Water insoluble substances (Vol. 4)	Not more than 0.2%
Fluoride (Vol. 4)	Not more than 50 mg/kg (Method I or III)
Arsenic (Vol. 4)	Not more than 3 mg/kg (Method II)
Lead (Vol. 4)	Not more than 4 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Into a 250-ml beaker transfer about 6.5 g of the dried sample accurately weighed. Add 50 ml of 1N hydrochloric acid and 50 ml of water, and stir until the sample is completely dissolved. Place the electrodes of a suitable pH meter in the solution and titrate the excess acid with 1N sodium hydroxide to the inflection point occurring at about pH 4. Record the buret reading and calculate the volume (A) of 1N hydrochloric acid consumed by the sample. Continue the titration with 1N sodium hydroxide until the inflection point occurring at about pH 8.8 is reached, record the buret reading, and calculate the volume (B) of 1N sodium hydroxide required in the titration between the two inflection points (pH 4 to pH 8.8). When (A) is equal to, or less than, (B), each ml of the volume (A) of 1N hydrochloric acid is equivalent to 142.0 mg of Na_2HPO_4. When (A) is greater than (B), each ml of the volume 2(B) -(A) of 1N sodium hydroxide is equivalent to 142.0 mg of Na_2HPO_4

TRISODIUM PHOSPHATE

Prepared at the 19th JECFA (1975), published in NMRS 55B (1976) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). A group MTDI of 70 mg/kg bw, as phosphorus from all food sources, was established at the 26th JECFA (1982).

SYNONYMS Tribasic sodium phosphate, sodium phosphate; INS No. 339(iii)

DEFINITION

Chemical names: Trisodium orthophosphate, trisodium phosphate, trisodium monophosphate

C.A.S. number: 7601-54-9

Chemical formula: Anhydrous: Na_3PO_4
Hydrated: $Na_3PO_4 \cdot xH_2O$

Formula weight: Anhydrous: 163.94

Assay: Anhydrous, hemihydrate and monohydrate: Not less than 97.0% calculated on the dried basis
Dodecahydrate: Not less than 92.0% calculated on the ignited basis

DESCRIPTION White odourless crystals, granules or a crystalline powder; hydrated forms available include hemi- and monohydrates, hexahydrate, octahydrate, decahydrate and dodecahydrate; the dodecahydrate contains 1/4 mol of sodium hydroxide.

FUNCTIONAL USES Buffer, sequestrant, emulsion stabilizer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water; insoluble in ethanol

pH (Vol. 4): 11.5 - 12.5 (1 in 100 soln)

Test for sodium: To 5 ml of a 1 in 20 solution of the sample add 1 ml of acetic acid TS and 1 ml of uranyl zinc acetate TS. A yellow crystalline precipitate is formed within a few min.

Test for phosphate: To 5 ml of a 1 in 100 solution of the sample add 1 ml of concentrated nitric acid and 5 ml of ammonium molybdate TS and warm. A bright canary-yellow precipitate is obtained.

Test for orthophosphate: Dissolve 0.1 g of the sample in 10 ml water, acidify slightly with dilute acetic acid TS, and add 1 ml of silver nitrate TS. A yellow precipitate is formed.

PURITY

Loss on ignition (Vol. 4)	Anhydrous: Not more than 2% (120^o, 2 h, then 800^o, 30 min) Monohydrate: Not more than 11% (120^o, 2 h, then 800^o, 30 min) Dodecahydrate: 45-58% (120^o, 2 h, then 800^o, 30 min)
Water insoluble substances (Vol. 4)	Not more than 0.2%
Fluoride (Vol. 4)	Not more than 50 mg/kg (Method I or III)
Arsenic (Vol. 4)	Not more than 3 mg/kg (Method II)
Lead (Vol. 4)	Not more than 4 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

METHOD OF ASSAY

Dissolve an accurately weighed quantity of the sample, equivalent to between 5.5 and 6 g of anhydrous Na_3PO_4, in 40 ml of water in a 400-ml beaker, and add 100 ml of 1 N hydrochloric acid. Pass a stream of carbon dioxide-free air, in fine bubbles, through the solution for 30 min to expel carbon dioxide, covering the beaker loosely to prevent loss by spraying. Wash the cover and sides of the beaker with a few ml of water, and place the electrodes of a suitable pH meter in the solution. Titrate the solution with 1 N sodium hydroxide to the inflection point occurring at about pH 4, then calculate the volume (A) of 1 N hydrochloric acid consumed. Protect the solution from absorbing carbon dioxide from the air, and continue the titration with 1 N sodium hydroxide until the inflection point occurring at about pH 8.8 is reached. Calculate the volume (B) of 1 N sodium hydroxide consumed in the titration. When (A) is equal to, greater than, 2(B), each ml of the volume (B) of 1 N sodium hydroxide is equivalent to 163.9 mg of Na_3PO_4. When (A) is less than 2(B), each ml of the volume (A) - (B) of 1 N sodium hydroxide is equivalent to 163.9 mg of Na_3PO_4.

POTASSIUM DIHYDROGEN PHOSPHATE

Prepared at the 19th JECFA (1975), published in NMRS 55B (1976) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). No ADI was established, but a group MTDI of 70 mg/kg bw, expressed as phosphorus from all food sources, was established at the 26th JECFA (1982).

SYNONYMS Monobasic potassium phosphate, monopotassium monophosphate potassium acid phosphate, potassium biphosphate; INS No. 340(i)

DEFINITION

Chemical names	Potassium dihydrogenphosphate, monopotassium dihydrogen-orthophosphate, monopotassium dihydrogen monophosphate
C.A.S. number	7778-77-0
Chemical formula	KH_2PO_4
Formula weight	136.09
Assay	Not less than 98.0% after drying

DESCRIPTION Odourless, colourless crystals or white granular or crystalline powder

FUNCTIONAL USES Buffer, neutralizing agent, sequestrant, yeast food

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Freely soluble in water; insoluble in ethanol
pH (Vol. 4)	4.2 - 4.7 (1 in 100 soln)
Test for potassium (Vol. 4)	Passes test
Test for phosphate (Vol. 4)	Passes test
Test for orthophosphate	To 5 ml of a 1 in 100 soln of the sample, add silver nitrate TS. A yellow precipitate is obtained.

PURITY

Loss on drying (Vol. 4)	Not more than 2% (105°, 4 h)
Water insoluble substances (Vol. 4)	Not more than 0.2%
Fluoride	Not more than 10 mg/kg See description under TESTS

Arsenic (Vol. 4) — Not more than 3 mg/kg (Method II)

Lead (Vol. 4) — Not more than 4 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Fluoride

Place 5 g of the sample, 25 ml of water, 50 ml of perchloric acid, 5 drops of silver nitrate solution (1 in 2), and a few glass beads in a 250-ml distilling flask connected with a condenser and carrying a thermometer and a capillary tube, both of which must extend into the liquid. Connect a small dropping funnel, filled with water, or a steam generator, to the capillary tube. Support the flask on an asbestos mat with a hole which exposes about one-third of the flask to the flame. Distil into a 250-ml flask until the temperature reaches 135^{o}. Add water from the funnel or introduce steam through the capillary to maintain the temperature between 135^{o} and 140^{o}. Continue the distillation until 225-240 ml has been collected, then dilute to 250 ml with water, and mix. Place a 50-ml aliquot of this solution in a 100-ml Nessler tube. In another similar Nessler tube place 50 ml of water as a control. Add to each tube 0.1 ml of a filtered solution of sodium alizarinsulfonate (1 in 1000) and 1 ml of freshly prepared hydroxylamine solution (1 in 4000), and mix well. Add, dropwise, and with stirring, 0.05 N sodium hydroxide to the tube containing the distillate until its colour just matches that of the control, which is faintly pink. Then add to each tube exactly 1 ml of 0.1 N hydrochloric acid, and mix well. From a buret, graduated in 0.05 ml, add slowly to the tube containing the distillate enough thorium nitrate solution (1 in 4000) so that, after mixing, the colour of the liquid just changes to a faint pink. Note the volume of the solution added, add exactly the same volume to the control, and mix. Now add to the control sodium fluoride TS (10 µg F per ml) from a buret to make the colours of the two tubes match after dilution to the same volume. Mix well, and allow all air bubbles to escape before making the final colour comparison. Check the end-point by adding 1 or 2 drops of sodium fluoride TS to the control. A distinct change in colour should take place. Note the volume of sodium fluoride added. The volume of sodium fluoride TS required for the control solution should not exceed 1 ml.

METHOD OF ASSAY

Transfer about 5 g of the dried sample, accurately weighed, into a 250-ml beaker. Add 100 ml of water and 5 ml of 1 N hydrochloric acid, and stir until the sample is completely dissolved. Place the electrodes of a suitable pH meter in the solution, and slowly titrate the excess acid, stirring constantly, with 1 N sodium hydroxide to the inflection point occurring at about pH 4. Record the buret reading, and calculate the volume (A), if any, of 1 N hydrochloric acid consumed by the sample. Continue the titration with 1 N sodium hydroxide until the inflection point occurring at about pH 8.8 is reached, record the buret reading, and calculate the volume (B) of 1 N sodium hydroxide required in the titration between the two inflection

points (pH 4 and pH 8.8). Each ml of the volume (B) - (A) of 1 N sodium hydroxide is equivalent to 136.1 mg of KH_2PO_4.

DIPOTASSIUM HYDROGEN PHOSPHATE

Prepared at the 19th JECFA (1975), published in NMRS 55B (1976) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). A group MTDI of 70 mg/kg bw, as phosphorus from all food sources, was established at the 26th JECFA (1982)

SYNONYMS Dibasic potassium phosphate, dipotassium monophosphate, dipotassium phosphate, dipotassium acid phosphate, secondary potassium phosphate; INS No. 340(ii)

DEFINITION

Chemical names	Dipotassium hydrogenphosphate, dipotassium hydrogen orthophosphate, dipotassium hydrogen monophosphate
C.A.S. number	7758-11-4
Chemical formula	K_2HPO_4
Formula weight	174.18
Assay	Not less than 98.0% after drying

DESCRIPTION Colourless or white granular powder, crystals or masses; deliquescent

FUNCTIONAL USES Buffering agent, sequestrant, yeast food

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Freely soluble in water, insoluble in ethanol
pH (Vol. 4)	8.7-9.3 (1 in 100 soln)
Test for potassium (Vol. 4)	Passes test
Test for phosphate (Vol. 4)	Passes test
Test for orthophosphate (Vol. 4)	Dissolve 0.1 g of the sample in 10 ml water, acidify slightly with dilute acetic acid TS, and add 1 ml of silver nitrate TS. A yellow precipitate is formed.

PURITY

Loss on drying (Vol. 4)	Not more than 5% (105°, 4 h)
Water insoluble substances (Vol. 4)	Not more than 0.2%
Fluoride	Not more than 10 mg/kg See description under TESTS

Arsenic (Vol. 4) — Not more than 3 mg/kg

Lead (Vol. 4) — Not more than 4 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Fluoride — Place 5 g of the sample, 25 ml of water, 50 ml of perchloric acid, 5 drops of silver nitrate solution (1 in 2), and a few glass beads in a 250-ml distilling flask connected with a condenser and carrying a thermometer and a capillary tube, both of which must extend into the liquid. Connect a small dropping funnel, filled with water, or a steam generator, to the capillary tube. Support the flask on an asbestos mat with a hole which exposes about one-third of the flask to the flame. Distil into a 250-ml flask until the temperature reaches 135^{o}. Add water from the funnel or introduce steam through the capillary to maintain the temperature between 135^{o} and 140^{o}. Continue the distillation until 225-240 ml has been collected, then dilute to 250 ml with water, and mix. Place a 50-ml aliquot of this solution in a 100-ml Nessler tube. In another similar Nessler tube place 50 ml of water as a control. Add to each tube 0.1 ml of a filtered solution of sodium alizarinsulfonate (1 in 1000) and 1 ml of freshly prepared hydroxylamine solution (1 in 4000), and mix well. Add, drop wise, and with stirring, 0.05 N sodium hydroxide to the tube containing the distillate until its colour just matches that of the control, which is faintly pink. Then add to each tube exactly 1 ml of 0.1 N hydrochloric acid, and mix well. From a buret, graduated in 0.05 ml, add slowly to the tube containing the distillate enough thorium nitrate solution (1 in 4000) so that, after mixing, the colour of the liquid just changes to a faint pink. Note the volume of the solution added, add exactly the same volume to the control, and mix. Now add to the control sodium fluoride TS (10 µg F per ml) from a buret to make the colours of the two tubes match after dilution to the same volume. Mix well, and allow all air bubbles to escape before making the final colour comparison. Check the end-point by adding 1 or 2 drops of sodium fluoride TS to the control. A distinct change in colour should take place. Note the volume of sodium fluoride added. The volume of sodium fluoride TS required for the control solution should not exceed 1 ml.

METHOD OF ASSAY

Into a 250-ml beaker transfer about 6.5 g of the dried sample, accurately weighed. Add 50 ml of 1 N hydrochloric acid and 50 ml of water, and stir until the sample is completely dissolved. Place the electrodes of a suitable pH meter in the solution and titrate the excess acid with 1N sodium hydroxide to the inflection point occurring at about pH 4. Record the buret reading and calculate the volume (A) of 1N hydrochloric acid consumed by the sample. Continue the titration with 1N sodium hydroxide until the inflection point occurring at about pH 8.8 is reached, record the buret reading, and calculate the volume (B) of 1 N sodium hydroxide required in the titration between the two inflection points (pH 4 to pH 8.8). When (A) is

equal to, or less than, (B), each ml of the volume (A) of 1N hydrochloric acid is equivalent to 174.2 mg of K_2HPO_4. When (A) is greater than (B), each ml of the volume 2(B) - (A) of 1N sodium hydroxide is equivalent to 174.2 mg of K_2HPO_4.

TRIPOTASSIUM PHOSPHATE

Prepared at the 19th JECFA (1975), published in NMRS 55B (1976) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). A group MTDI of 70 mg/kg bw, as phosphorus from all food sources, was established at the 26th JECFA (1982)

SYNONYMS Tribasic potassium phosphate, potassium phosphate; INS No. 340(iii)

DEFINITION

Chemical names	Tripotassium phosphate, tripotassium orthophosphate, tripotassium monophosphate
C.A.S. number	7778-53-2
Chemical formula	Anhydrous: K_3PO_4 Hydrated: $K_3PO_4 \cdot xH_2O$
Formula weight	212.27 (anhydrous)
Assay	Not less than 97.0% of K_3PO_4, calculated on the ignited basis

DESCRIPTION Colourless or white, odourless hygroscopic crystals or granules; hydrated forms available include the monohydrate and trihydrate

FUNCTIONAL USES Buffer, emulsion stabilizer, sequestrant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Freely soluble in water; insoluble in ethanol
pH (Vol. 4)	11.5 - 12.5 (1 in 100 soln)
Test for potassium	To a 1 in 100 solution of the sample add 1 volume of saturated sodium hydrogen tartrate solution and 1 volume of ethanol and shake. A white crystalline precipitate is formed.
Test for phosphate	To 5 ml of a 1 in 100 solution of the sample add 1 ml of concentrated nitric acid and 5 ml of ammonium molybdate TS and warm. A bright canary-yellow precipitate is obtained.
Test for orthophosphate	Dissolve 0.1 g of the sample in 10 ml water, acidify slightly with dilute acetic acid TS, and add 1 ml of silver nitrate TS. A yellow precipitate is formed.

PURITY

Loss on ignition (Vol. 4)	Anhydrous: Not more than 3% (120°, 2 h, then 800°, 30 min) Hydrated: Not more than 23% (120°, 2 h, then 800°, 30 min)

Water insoluble substances (Vol. 4) — Not more than 0.2%

Fluoride (Vol. 4) — Not more than 10 mg/kg
See description under TESTS

Arsenic (Vol. 4) — Not more than 3 mg/kg (Method II)

Lead (Vol. 4) — Not more than 4 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Fluoride

Place 5 g of the sample, 25 ml of water, 50 ml of perchloric acid, 5 drops of silver nitrate solution (1 in 2), and a few glass beads in a 250-ml distilling flask connected with a condenser and carrying a thermometer and a capillary tube, both of which must extend into the liquid. Connect a small dropping funnel, filled with water, or a steam generator, to the capillary tube. Support the flask on an asbestos mat with a hole which exposes about one-third of the flask to the flame. Distil into a 250-ml flask until the temperature reaches 135°. Add water from the funnel or introduce steam through the capillary to maintain the temperature between 135° and 140°. Continue the distillation until 225-240 ml has been collected, then dilute to 250 ml with water, and mix. Place a 50-ml aliquot of this solution in a 100-ml Nessler tube. In another similar Nessler tube place 50 ml of water as a control. Add to each tube 0.1 ml of a filtered solution of sodium alizarinsulfonate (1 in 1000) and 1 ml of freshly prepared hydroxylamine solution (1 in 4000), and mix well. Add, dropwise, and with stirring, 0.05 N sodium hydroxide to the tube containing the distillate until its colour just matches that of the control, which is faintly pink. Then add to each tube exactly 1 ml of 0.1 N hydrochloric acid, and mix well. From a buret, graduated in 0.05 ml, add slowly to the tube containing the distillate enough thorium nitrate solution (1 in 4000) so that, after mixing, the colour of the liquid just changes to a faint pink. Note the volume of the solution added, add exactly the same volume to the control, and mix. Now add to the control sodium fluoride TS (10 µg F per ml) from a buret to make the colours of the two tubes match after dilution to the same volume. Mix well, and allow all air bubbles to escape before making the final colour comparison. Check the end-point by adding 1 or 2 drops of sodium fluoride TS to the control. A distinct change in colour should take place. Note the volume of sodium fluoride added. The volume of sodium fluoride TS required for the control solution should not exceed 1 ml.

METHOD OF ASSAY

Dissolve an accurately weighed quantity of the sample, equivalent to about 8 g of anhydrous K_3PO_4, in 40 ml of water in a 400-ml beaker, and add 100 ml of 1 N hydrochloric acid. Pass a stream of carbon dioxide free air, in fine bubbles, through the solution for 30 min to expel carbon dioxide, covering the beaker loosely to prevent loss by spraying. Wash the

cover and sides of the beaker with a few ml of water, and place the electrodes of a suitable pH meter in the solution. Titrate the solution with 1 N sodium hydroxide to the inflection point occurring at about pH 4, then calculate the volume (A) of 1 N hydrochloric acid consumed. Protect the solution from absorbing carbon dioxide from the air, and continue the titration with 1 N sodium hydroxide until the inflection point occurring at about pH 8.8 is reached. Calculate the volume (B) of 1 N sodium hydroxide consumed in this titration. When (A) is equal to, or greater than, 2(B), each ml of the volume (B) of 1 N sodium hydroxide is equivalent to 212.3 mg of K_3PO_4. When (A) is less than 2(B), each ml of the volume (A) - (B) of 1 N sodium hydroxide is equivalent to 212.3 mg of K_3PO_4.

CALCIUM DIHYDROGEN PHOSPHATE

Prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 9th JECFA (1965), published in NMRS 40ABC (1967) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). A group MTDI of 70 mg/kg bw, as phosphorus from all food sources, was established at the 26th JECFA (1982)

SYNONYMS Monobasic calcium phosphate, monocalcium orthophosphate, monocalcium phosphate, calcium biphosphate, acid calcium phosphate, INS No. 341(i)

DEFINITION

Chemical names	Calcium dihydrogen phosphate
C.A.S. number	Anhydrous: 7758-23-8 Monohydrate: 10031-30-8
Chemical formula	Anhydrous: $Ca(H_2PO_4)_2$ Monohydrate: $Ca(H_2PO_4)_2,H_2O$
Formula weight	Anhydrous: 234.05 Monohydrate: 252.07
Assay	Anhydrous: Not less than 16.8% and not more than 18.3% of Ca Monohydrate: Not less than 15.9% and not more than 17.7% of Ca

DESCRIPTION Hygroscopic white crystals or granules, or granular powder

FUNCTIONAL USES Buffering agent, firming agent, sequestrant, leavening agent, dough conditioner, texturizer, yeast food, and nutrient

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Sparingly soluble in water, insoluble in ethanol
Test for calcium (Vol. 4)	Passes test
Test for phosphate (Vol. 4)	Passes test

PURITY

Loss on drying (Vol. 4)	Monohydrate: Not more than 1% (60^o, 3 h)
Loss on ignition (Vol. 4)	Anhydrous: Between 14.0 and 15.5% (800^o, 30 min)
Fluoride (Vol. 4)	Not more than 50 mg/kg Anhydrous: Determine as directed in Method II Monohydrate: Proceed as directed under Method IV

Arsenic (Vol. 4) — Not more than 3 mg/kg (Method II)

Lead (Vol. 4) — Not more than 4 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh accurately a portion of the sample equivalent to about 475 mg of the anhydrous salt and dissolve it in 10 ml of hydrochloric acid TS. Add a few drops of methyl orange TS, and boil for 5 min, keeping the volume and pH of the solution constant during the boiling period by adding hydrochloric acid or water, if necessary. Add 2 drops of methyl red TS and 30 ml of ammonium oxalate TS, then add dropwise, with constant stirring, a mixture of equal volumes of 6N ammonia solution and water until the pink colour of the indicator just disappears. Digest on a steam bath for 30 min, cool to room temperature, allow the precipitate to settle, and filter the supernatant liquid through a sintered-glass crucible, using gentle suction. Wash the precipitate in the beaker with about 30 ml of cold (below 200) wash solution, prepared by diluting 10 ml of ammonium oxalate TS to 1000 ml. Allow the precipitate to settle, and pour the supernatant liquid through the filter. Repeat this washing by decantation three more times. Using the wash solution, transfer the precipitate as completely as possible to the filter. Finally, wash the beaker and the filter with two 10 ml portions of cold (below 200C) water. Place the sintered-glass crucible in the beaker, and add 100 ml of water and 50 ml of cold dilute sulfuric acid (1 in 6). Add from a buret 35 ml of 0.1N potassium permanganate, and stir until the colour disappears. Heat to about 700, and complete the titration with 0.1N potassium permanganate. Each ml of 0.1N potassium permanganate is equivalent to 2.004 mg of Ca.

CALCIUM HYDROGEN PHOSPHATE

Prepared at the 19th JECFA (1975), published in NMRS 55B (1976) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). A group MTDI of 70 mg/kg bw, as phosphorus from all food sources, was established at the 26th JECFA (1982)

SYNONYMS Dibasic calcium phosphate, dicalcium phosphate, INS No. 341 (ii)

DEFINITION

Chemical names	Calcium monohydrogen phosphate, calcium hydrogen orthophosphate, secondary calcium phosphate
C.A.S. number	7757-93-9
Chemical formula	Anhydrous: $CaHPO_4$ Dihydrate: $CaHPO_4 \cdot 2H_2O$
Formula weight	Anhydrous: 136.06 Dihydrate: 172.09
Assay	Not less than 98.0% and not more than the equivalent of 102.0% after drying

DESCRIPTION White crystals or granules, granular powder or powder

FUNCTIONAL USES Dough conditioner, yeast food

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Sparingly soluble in water; insoluble in ethanol
Test for calcium (Vol. 4)	Passes test
Test for phosphate (Vol. 4)	Passes test

PURITY

Loss on drying (Vol. 4)	Anhydrous: Not more than 2% (200°, 3 h) Dihydrate: Not less than 18% and not more than 22% (200°, 3 h)
Fluoride (Vol. 4)	Not more than 50 mg/kg (Method I or III)
Arsenic (Vol. 4)	Not more than 3 mg/kg (Method II).
Lead (Vol. 4)	Not more than 4 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be

based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh accurately about 0.3 g of the sample, previously dried for 3 h at 200°. Dissolve in 10 ml of dilute hydrochloric acid TS, add about 120 ml of water and a few drops of methyl orange TS, and boil for 5 min, keeping the volume and pH of the solution in the beaker constant during the boiling period by adding hydrochloric acid or water as necessary. Add 2 drops of methyl red TS and 30 ml of ammonium oxalate TS. Then add dropwise, with constant stirring, a mixture of equal volumes of ammonia TS and water until the pink colour of the indicator just disappears.

Digest on a steam bath for 30 min, cool to room temperature, allow the precipitate to settle, and filter the supernatant liquid through an asbestos mat in a Gooch crucible, using gentle suction. Swirl the precipitate in the beaker with about 30 ml of a cold (below 20°) wash solution prepared by diluting 10 ml of ammonium oxalate TS to 1000 ml. Allow the precipitate to settle, and pass the supernatant through the filter. Repeat this washing by decantation three more times. Using the wash solution, transfer the precipitate as completely as possible to the filter. Finally, wash the beaker and the filter with to 10 ml portions of cold (below 20°) water. Place the Gooch crucible in the beaker, and add 100 ml of water and 50 ml of cold dilute sulfuric acid (1 in 6). Add from a buret 35 ml of 0.1 N potassium permanganate, and stir until the colour disappears. Heat to about 70°, and complete the titration with 0.1 N potassium permanganate. Each ml of 0.1 N potassium permanganate is equivalent to 6.803 mg of $CaHPO_4$.

TRICALCIUM PHOSPHATE

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). A group MTDI of 70 mg/kg bw, as phosphorus from all food sources, was established at the 26th JECFA (1982)

SYNONYMS Calcium phosphate, tribasic; precipitated calcium phosphate; INS No. 341(iii)

DEFINITION Consists of a variable mixture of calcium phosphates having and approximate composition of $10CaO \cdot 3P_2O_5 \cdot H_2O$. The article of commerce can be specified further as to titration value.

Assay Not less than the equivalent of 90% of $Ca_3(PO_4)_2$, calculated on the ignited basis

DESCRIPTION White, odourless powder which is stable in air

FUNCTIONAL USES Anticaking agent, buffer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Practically insoluble in water; insoluble in ethanol, soluble in dilute hydrochloric and nitric acid

Test for phosphate To a warm solution of the sample in a slight excess of nitric acid add ammonium molybdate TS. A yellow precipitate forms.

Test for calcium Dissolve about 100 mg of the sample by warming with 5 ml of dilute hydrochloric acid TS and 5 ml of water. Add 1 ml of ammonia TS, dropwise, with shaking, and then add 5 ml of ammonium oxalate TS. A white precipitate forms.

PURITY

Loss on ignition (Vol. 4) Not more than 10% after ignition at 825° to constant weight.

Fluoride (Vol. 4) Not more than 50 mg/kg
Weigh 1 g of the sample to the nearest mg and proceed as directed in the Fluoride Limit Test (Method I or III).

Lead (Vol. 4) Not more than 4 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY Weigh 200 mg of the sample to the nearest 0.1 mg and dissolve in a mixture of 25 ml of water and 10 ml of dilute nitric acid TS. Filter, if necessary, wash any precipitate, add sufficient ammonia TS to the filtrate to produce a slight precipitate, then dissolve the precipitate with the addition of

1 ml of dilute nitric acid TS. Adjust the temperature to about 50°, add 75 ml of ammonium molybdate TS, and maintain the temperature at about 50° for 30 min, stirring occasionally. Wash the precipitate once or twice with water by decantation, using from 30 to 40 ml each time. Transfer the precipitate to a filter, and wash with a 1 in 100 potassium nitrate solution until the last washing is not acid to litmus paper. Transfer the precipitate and filter to the precipitation vessel, add 40.0 ml of N sodium hydroxide, agitate until the precipitate is dissolved, add 3 drops of phenolphthalein TS, and then titrate the excess alkali with N sulfuric acid. Each ml of N sodium hydroxide corresponds to 6.743 mg of $Ca_3(PO_4)_2$.

AMMONIUM DIHYDROGEN PHOSPHATE

Prepared at the 27th JECFA (1983), published in FNP 28 (1983) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002) A group MTDI of 70 mg/kg bw, as phosphorus from all food sources, was established at the 27th JECFA (1983)

SYNONYMS Monobasic ammonium phosphate, monoammonium phosphate, acid ammonium phosphate, primary ammonium phosphate; INS No. 342(i)

DEFINITION

Chemical names: Ammonium dihydrogen phosphate, ammonium dihydrogen tetraoxophosphate, monoammonium monophosphate, ammonium dihydrogen orthophosphate

C.A.S. number: 7722-76-1

Chemical formula: $NH_4H_2PO_4$

Formula weight: 115.03

Assay: Not less than 96% and not more than 102%

DESCRIPTION Colourless or white crystals, a white crystalline powder or granules

FUNCTIONAL USES Buffering agent, dough conditioner, leavening agent

CHARACTERISTICS

IDENTIFICATION

Solubility: Freely soluble in water

pH (Vol. 4): 4.3 - 5.0 (1 in 100 soln)

Test for ammonium (Vol. 4): Passes test

Test for phosphate (Vol. 4): Passes test

PURITY

Fluoride (Vol. 4): Not more than 10 mg/kg (Method I or III)

Arsenic (Vol. 4): Not more than 3 mg/kg

Lead (Vol. 4): Not more than 4 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Dissolve about 500 mg of the sample, accurately weighed, in 50 ml of water, and titrate to a pH of 8.0 with 0.1 N sodium hydroxide. Each ml of 0.1 N sodium hydroxide is equivalent to 11.50 mg of $NH_4H_2PO_4$.

DIAMMONIUM HYDROGEN PHOSPHATE

Prepared at the 26th JECFA (1982), published in FNP 25 (1982) and FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). A group MTDI of 70 mg/kg bw, as phosphorus from all food sources, was established at the 26th JECFA (1982)

SYNONYMS Dibasic ammonium phosphate, diammonium phosphate; INS No. 342(ii)

DEFINITION

Chemical names	Diammonium hydrogen phosphate, diammonium hydrogen tetraoxophosphate, diammonium hydrogen orthophosphate
C.A.S. number	7783-54-0
Chemical formula	$(NH_4)_2HPO_4$
Formula weight	132.06
Assay	Not less than 96.0% and not more than 102.0%

DESCRIPTION White crystals or crystalline powder

FUNCTIONAL USES Acidity regulator, dough conditioner, raising agent, yeast food

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water
pH (Vol. 4)	7.6 - 8.4 (1 in 100 soln)
Test for ammonium (Vol. 4)	Passes test
Test for phosphate (Vol. 4)	Passes test

PURITY

Fluoride (Vol. 4)	Not more than 10 mg/kg (Method I or III)
Arsenic (Vol. 4)	Not more than 3 mg/kg (Method II)
Lead (Volume 4)	Not more than 4 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY Dissolve about 600 mg of the sample, accurately weighed, in 40 ml of water and titrate to a pH of 8.0 with 0.1 N sulfuric acid. Each ml of 0.1 N sulfuric acid is equivalent to 13.21 mg of $(NH_4)_2HPO_4$.

MONOMAGNESIUM PHOSPHATE

Prepared at the 69th JECFA (2008), published in FAO JECFA Monographs 5 (2008), based on the previously withdrawn tentative specifications prepared at the 61st JECFA and published in FNP 52, Add 11 (2003). A group MTDI of 70 mg/kg bw, expressed as phosphorus from all food sources, was established at the 26th JECFA (1982).

SYNONYMS Monomagnesium orthophosphate, Magnesium dihydrogen phosphate; Magnesium phosphate, monobasic; Magnesium biphosphate; Acid magnesium phosphate; INS No. 343(i)

DEFINITION Monomagnesium phosphate is manufactured by partial neutralization of phosphoric acid with magnesium oxide and drying of the resultant product.

Chemical names: Monomagnesium dihydrogen phosphate

C.A.S. number: 13092-66-5 (Anhydrous)
15609-87-7 (Dihydrate)

Chemical formula: $Mg(H_2PO_4)_2 \cdot x\ H_2O$ (x = 0 to 4)

Formula weight: 218.3 (Anhydrous)
254.3 (Dihydrate)
290.3 (Tetrahydrate)

Assay: Not less than 96% and not more than 102% as $Mg_2P_2O_7$ on the ignited basis

DESCRIPTION White, odourless, crystalline powder

FUNCTIONAL USES Acidity regulator, nutrient

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Slightly soluble in water

Magnesium (Vol. 4): Passes test

Phosphate (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4): Anhydrous: Not more than 1.5 % (105°, 4 h)

Loss on ignition (Vol. 4): Anhydrous: Not more than 18.5 %
Dihydrate: Not more than 33 %
Tetrahydrate: Not more than 43%

Accurately weigh about 2 g of sample, and ignite, preferably in a muffle furnace at about 800° for 30 min. Allow the crucible to cool in a desiccator to constant weight. Save the residue for the Assay.

Fluoride (Vol. 4)

Not more than 10 mg/kg
See description under TESTS

Arsenic (Vol. 4)

Not more than 3 mg/kg
Determine by the atomic absorption hydride technique. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under “General Methods, Metallic Impurities”).

Lead (Vol. 4)

Not more than 4 mg/kg
Determine using an atomic absorption/ICP technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under “General Methods, Metallic Impurities”).

TESTS

PURITY TESTS

Fluoride (Vol. 4)

Use Method III. The standard curve constructed in Method III may not be suitable for samples containing low fluoride levels. Therefore, it will be necessary to prepare standard solutions with concentrations other than those specified for Method III for the construction of a standard curve and to choose a sample size that will bring the fluoride concentration within the standard curve.

METHOD OF ASSAY

Accurately weigh 200 mg of the residue obtained in the test for Loss on ignition in a high 250 ml beaker. Dissolve the residue in 2 ml of hydrochloric acid (16 %) and add 100 ml of water. Heat the solution to 50° to 60° and add 10 ml of 0.1 M disodium EDTA from a buret. Add a magnetic stirring bar and, while stirring, adjust with 1 N sodium hydroxide to pH 10. Add 10 ml of ammonia-ammonium chloride buffer TS (Vol. 4), 12 drops of Eriochrome black TS and continue the titration with 0.1 M disodium EDTA until the red colour changes to green. [NOTE: The solution must be clear when the end point is reached] Calculate the weight (mg) of $Mg_2P_2O_7$ in the residue taken by the formula

$$9.14 \times V$$

where V is the volume (ml) of 0.1 M disodium EDTA required in the titration.

MAGNESIUM HYDROGEN PHOSPHATE

Prepared at the 26th JECFA (1982), published in FNP 25 (1982) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). A group MTDI of 70 mg/kg bw, as phosphorus from all food sources, was established at the 26th JECFA (1982)

SYNONYMS Magnesium phosphate, dibasic; secondary magnesium phosphate; dimagnesium phosphate; INS No. 343(ii).

DEFINITION

Chemical names	Magnesium hydrogen orthophosphate trihydrate, magnesium salt of phosphoric acid (1:1)
C.A.S. number	7757-86-0
Chemical formula	$MgHPO_4 \cdot 3H_2O$
Formula weight	174.33
Assay	Not less than 96.0% on the ignited basis

DESCRIPTION Odourless white crystalline powder

FUNCTIONAL USES Nutrient adjunct, dietary supplement, nutrient agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Slightly soluble in water, soluble in dilute acids, but insoluble in ethanol
Test for phosphate (Vol. 4)	Passes test
Test for magnesium (Vol. 4)	Dissolve 100 mg in 0.5 ml of diluted acetic acid TS and 20 ml of water. Add 1 ml of ferric chloride TS, let stand for 5 min and filter. The filtrate gives a positive test for Magnesium.

PURITY

Loss on ignition	Not less than 29% and not more than 36% (800±25^{o} to constant weight)
Fluoride (Vol. 4)	Not more than 10 mg/kg (Method III) Use 10 ml of N hydrochloric acid to dissolve the sample
Arsenic (Vol. 4)	Not more than 3 mg/kg
Lead (Vol. 4)	Not more than 4 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh accurately about 500 mg of the residue obtained in the test for Loss on Ignition, and dissolve it by heating in a mixture of 50 ml of water and 2 ml of hydrochloric acid. Cool, dilute to 100.0 ml with water, and mix. Transfer 50.0 ml of this solution into a 400-ml beaker, add 100 ml of water, and heat to 55^{o} to 60^{o}. From a buret add 15 ml of 0.1 M disodium EDTA, add a magnetic stirring bar, and adjust with sodium hydroxide TS to pH 10 while stirring. Add 10 ml of ammonia-ammonium chloride buffer TS and 12 drops of eriochrome black TS, and continue the titration with 0.1 M disodium EDTA until the wine-red colour changes to pure blue. Calculate the weight, in mg, of $Mg_2P_2O_7$ in the residue taken by the formula:

$$2 \times 11.13 \times V$$

where
V = the volume, in ml of 0.1 M disodium EDTA required in the titration of the 50.0-ml aliquot.

TRIMAGNESIUM PHOSPHATE

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). A group MTDI of 70 mg/kg bw, as phosphorus from all food sources, was established at the 26th JECFA (1982).

SYNONYMS Magnesium phosphate, tribasic, tertiary magnesium phosphate; INS No. 343(iii)

DEFINITION May contain 4, 5 or 8 molecules of water of hydration. The article of commerce can be specified further as to titration value.

Chemical names Trimagnesium orthophosphate

C.A.S. number 7757-87-1

Chemical formula $Mg_3(PO_4)_2$ (various hydrates)

Formula weight 262.86 (anhydrous)

Assay Not less than 98% of $Mg_3(PO_4)_2$ after ignition at 425^o

DESCRIPTION White, odourless crystalline powder

FUNCTIONAL USES Anticaking agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Practically insoluble in water; insoluble in ethanol, soluble in dilute mineral acids

Test for phosphate To a warm solution of the sample in a slight excess of nitric acid add ammonium molybdate TS. A yellow precipitate of ammonium phosphomolybdate forms which is soluble in ammonia TS.

Test for magnesium Dissolve about 100 mg of the sample in 0.7 ml of dilute acetic acid TS and 20 ml of water. Add 1 ml of ferric chloride TS, let stand for 5 min., and filter. Add ammonium chloride TS and ammonium carbonate TS. No precipitated is formed Add sodium phosphate TS. A white crystalline precipitate is formed which is insoluble in ammonia TS.

PURITY

Loss on ignition (Vol. 4) Tetrahydrate: Between 15% and 23% (425^o to constant weight)
Pentahydrate: Between 20% and 27% (425^o to constant weight)
Octahydrate: Between 30% and 37% (425^o to constant weight)

Fluoride (Vol. 4)

Not more than 5 mg/kg
See description under TESTS

Lead (Vol. 4)

Not more than 4 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Fluoride (Vol. 4)

Weigh 5 g of the sample to the nearest mg, and transfer into a 200-ml distilling flask connected with a condenser and carrying a thermometer and a dropping funnel equipped with a stopcock. Dissolve in 25 ml of a 1 in 2 sulfuric acid solution, add 6 glass beads, and connect the apparatus for distillation, using a 600-ml beaker to collect the distillate. Add 40 ml of the diluted sulfuric acid to the flask through the dropping funnel, then fill the funnel with water, heat the solution to boiling, and continue heating until the temperature reaches 165°. Adjust the stopcock of the dropping funnel so that the temperature is maintained at 165±5°, and continue the distillation until about 300 ml has been collected. Rinse the condenser and condenser arm with water, collecting the rinsings in the beaker. Add sodium hydroxide TS to the distillate to make it alkaline to litmus paper, and then add 5 ml in excess. Add 5 ml of 30% hydrogen peroxide and 6 glass beads to the beaker, boil until a volume of about 30 ml is reached, and cool. Transfer the condensed distillate, including the glass beads, into a 125 ml distilling flask connected with a condenser and carrying a thermometer and a capillary tube, both of which must extend into the liquid. Add 30 ml of perchloric acid and continue as directed under the Fluoride Limit Test (Method I Thorium Nitrate Colorimetric method), beginning with "Connect a small dropping funnel or a steam generator to the capillary tube."

METHOD OF ASSAY

Weigh to the nearest 0.1 mg, 200 mg of the ignited sample. Dissolve in a mixture of 25 ml of water and 10 ml of dilute nitric acid TS. Filter, if necessary, wash any precipitate, then dissolve the precipitate by the addition of 1 ml of dilute nitric acid TS. Adjust the temperature to about 50°, add 75 ml of ammonium molybdate TS, and maintain the temperature at about 50° for 30 min., stirring occasionally. Wash the precipitate once or twice with water by decantation, using from 30 to 40 ml each time. Transfer the precipitate to a filter, and wash with a 1 in 100 potassium nitrate solution until the last washing is not acid to litmus paper. Transfer the precipitate and filter to the precipitation vessel, add 40.0 ml of N sodium hydroxide, agitate until the precipitate is dissolved, add 3 drops of phenolphthalein TS and then titrate the excess alkali with N sulfuric acid. Each ml of N sodium hydroxide corresponds to 5.715 mg of $Mg_3(PO_4)_2$.

DISODIUM PYROPHOSPHATE

Prepared at the 41st JECFA (1993), published in FNP 52 Add 2 (1993) superseding specifications prepared at the 37th JECFA (1990), published in FNP 52 (1992). Metals and arsenic specifications revised at the 55th JECFA (2000). A group MTDI of 70 mg/kg bw, as phosphorus from all food sources, was established at the 26th JECFA (1982)

SYNONYMS Disodium dihydrogen diphosphate, disodium dihydrogen pyrophosphate, acid sodium pyrophosphate, INS No. 450 (i)

DEFINITION

Chemical names	Disodium dihydrogen diphosphate, disodium dihydrogen pyrophosphate
C.A.S. number	7758-16-9
Chemical formula	$Na_2H_2P_2O_7$
Formula weight	221.94
Assay	Not less than 95.0%

DESCRIPTION White, crystalline powder, or granules

FUNCTIONAL USES Raising agent, buffering agent, sequestrant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water
Test for phosphate (Vol. 4)	Passes test
Test for sodium (Vol. 4)	Passes test
pH (Vol. 4)	3.7 - 5.0 (1 in 100 soln)

PURITY

Loss on drying (Vol. 4)	Not more than 0.5% (105°, 4 h)
Water-insoluble matter	Not more than 1% Dissolve 10 g of the sample in 100 ml of hot water, and filter through a tared filtering crucible. Wash the insoluble residue with hot water, dry at 105° for 2 h, cool and weigh.
Fluoride (Vol. 4)	Not more than 10 mg/kg (Method I or III)
Arsenic (Vol. 4)	Not more than 3 mg/kg (Method II)
Lead (Vol. 4)	Not more than 4 mg/kg

Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh accurately about 400 mg of the sample, previously dried at 105° for 4 h, and dissolve in 100 ml of water in a 400-ml beaker. Adjust the pH of the solution to 3.8 with dilute hydrochloric acid TS or sodium hydroxide TS, using a pH meter, then add 50 ml of a 1 in 8 solution of zinc sulfate (125 g of $ZnSO_4 \cdot 7\ H_2O$ dissolved in water, diluted to 1000 ml, filtered, and adjusted to pH 3.8) and allow to stand for 2 min. Titrate the liberated acid with 0.1 N sodium hydroxide until a pH of 3.8 is again reached. After each addition of sodium hydroxide near to the end-point, time should be allowed for any precipitated zinc hydroxide to redissolve. Each ml of 0.1 N sodium hydroxide is equivalent to 11.10 mg of $Na_2H_2P_2O_7$.

TRISODIUM DIPHOSPHATE

Prepared at the 69th JECFA (2008), published in FAO JECFA Monographs 5 (2008), based on the previously withdrawn tentative specifications prepared at the 61st JECFA and published in FNP 52, Add 11 (2003). A group MTDI of 70 mg/kg bw, expressed as phosphorus from all food sources, was established at the 26th JECFA (1982).

SYNONYMS Acid trisodium pyrophosphate, trisodium monohydrogen diphosphate; INS No. 450(ii)

DEFINITION Trisodium diphosphate is manufactured by calcining sodium orthophosphate having a $Na_2O:P_2O_5$ ratio of 3:2

Chemical names: Trisodium monohydrogen diphosphate

C.A.S. number: 14691-80-6 (Anhydrous)
26573-04-6 (Monohydrate)

Chemical formula: $Na_3HP_2O_7 \cdot x\ H_2O$ (x = 0 or 1)

Formula weight: 243.93 (Anhydrous)
261.95 (Monohydrate)

Assay: Not less than 57% and not more than 59% expressed as P_2O_5 on the dried basis

DESCRIPTION White powder or grains

FUNCTIONAL USES Stabilizer, leavening agent, emulsifier, nutrient

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Soluble in water

Sodium (Vol. 4): Passes test

Phosphate (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4): Anhydrous: Not more than 0.5 % (105°, 4 h)
Monohydrate: Not more than 1.0 % (105°, 4 h)

Loss on ignition (Vol. 4): Anhydrous: Not more than 4.5%
Monohydrate: Not more than 11.5%

Water-insoluble matter (Vol. 4): Not more than 0.2 %

Fluoride (Vol. 4): Not more than 10 mg/kg
See description under TESTS

Arsenic (Vol. 4)

Not more than 3 mg/kg
Determine by the atomic absorption hydride technique. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under “General Methods, Metallic Impurities”).

Lead (Vol. 4)

Not more than 4 mg/kg
Determine using an atomic absorption/ICP technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under “General Methods, Metallic Impurities”).

TESTS

PURITY TESTS

Fluoride (Vol. 4)

Use Method III. The standard curve constructed in Method III may not be suitable for samples containing low fluoride levels. Therefore, it will be necessary to prepare standard solutions with concentrations other than those specified in Method III for the construction of the standard curve and to choose a sample size that will bring the fluoride concentration within the standard curve.

METHOD OF ASSAY

Using a previously dried sample, proceed as directed under *Phosphate Determination as P_2O_5*, *Method I,* Inorganic components (Volume 4). Each ml of 1N sodium hydroxide consumed is equivalent to 3.088 mg of P_2O_5 or 5.307 mg of trisodium monohydrogen diphosphate on the dried basis.

TETRASODIUM PYROPHOSPHATE

Prepared at the 41st JECFA (1993), published in FNP 52 Add 2 (1993) superseding specifications prepared at the 37th JECFA (1990), published in FNP 52 (1992). Metals and arsenic specifications revised at the 55th JECFA (2000). A group MTDI of 70 mg/kg bw, as phosphorus from all food sources, was established at the 26th JECFA (1982)

SYNONYMS Tetrasodium diphosphate, sodium pyrophosphate; INS No 450 (iii)

DEFINITION

Chemical names	Tetrasodium diphosphate, tetrasodium pyrophosphate
C.A.S. number	7722-88-5
Chemical formula	Anhydrous: $Na_4P_2O_7$ Decahydrate: $Na_4P_2O_7 \cdot 10H_2O$
Formula weight	Anhydrous: 265.94 Decahydrate: 446.09
Assay	Not less than 95.0% on the ignited basis

DESCRIPTION Colourless or white crystals, or a white crystalline or granular powder; the decahydrate effloresces slightly in dry air

FUNCTIONAL USES Emulsifier, buffering agent, emulsifying agent, sequestrant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water; insoluble in ethanol
pH (Vol. 4)	9.9 - 10.8 (1 in 100 soln)
Test for phosphate (Vol. 4)	Passes test
Test for sodium (Vol. 4)	Passes test

PURITY

Loss on ignition (Vol. 4)	Not more than 0.5% for anhydrous, 38-42% for decahydrate (105º, 4h then 550º, 30 min)
Water insoluble matter	Not more than 0.2% Dissolve 10 g of the sample in 100 ml of hot water, and filter through a tared filtering crucible. Wash the insoluble residue with hot water, dry at 105° for 2 h, cool and weigh.
Fluoride	Not more than 10 mg/kg (Method I or III)

Arsenic(Vol. 4)	Not more than 3 mg/kg
Lead (Vol. 4)	Not more than 4 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Dissolve an accurately weighed quantity of the sample, equivalent to about 500 mg of anhydrous $Na_4P_2O_7$, in 100 ml of water in a 400-ml beaker. Adjust the pH of the solution to 3.8 with hydrochloric acid, using a pH meter, then add 50 ml of a 1 in 8 solution of zinc sulfate (125 g of $ZnSO_4 \cdot 7H_2O$ dissolved in water, diluted to 1000 ml, filtered, and adjusted to pH 3.8) and allow to stand for 2 min. Titrate the liberated acid with 0.1 N sodium hydroxide until a pH of 3.8 is again reached. After each addition of sodium hydroxide near the end-point, time should be allowed for any precipitated zinc hydroxide to redissolve. Each ml of 0.1 N sodium hydroxide is equivalent to 13.30 mg of $Na_4P_2O_7$.

TETRAPOTASSIUM PYROPHOSPHATE

Prepared at the 24th JECFA (1980), published in FNP 17 (1980) and in FNP 52 (1992). Metals and arsenic specifications revised at the 55th JECFA (2000). A group MTDI of 70 mg/kg bw, as phosphorus from all food sources, was established at the 26th JECFA (1982)

SYNONYMS Tetrapotassium diphosphate, potassium pyrophosphate; INS No. 450(v)

DEFINITION

Chemical names	Tetrapotassium diphosphate, tetrapotassium pyrophosphate, tetrapotassium salt of diphosphoric acid
C.A.S. number	7320-34-5
Chemical formula	$K_4P_2O_7$
Formula weight	330.34
Assay	Not less than 95% on the ignited basis

DESCRIPTION Colourless or white crystals, or a white crystalline or granular powder, powder of granular solid; hygroscopic

FUNCTIONAL USES Emulsifier, texturizer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water; insoluble in ethanol
pH (Vol. 4)	10.0 - 10.7 (1 in 100 soln)
Test for phosphate (Vol. 4)	Passes test
Test for potassium (Vol. 4)	Passes test

PURITY

Loss on ignition (Vol. 4)	Not more than 2% (105°, 4 h; then 550°, 30 min)
Water insoluble matter (Vol. 4)	Not more than 0.2%
Fluoride	Not more than 10 mg/kg See description under TESTS
Arsenic (Vol. 4)	Not more than 3 mg/kg (Method II)
Lead (Vol. 4)	Not more than 4 mg/kg

Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Fluoride

Place 5 g of the sample, 25 ml of water, 50 ml of perchloric acid, 5 drops of silver nitrate solution (1 in 2), and a few glass beads in a 250-ml distilling flask connected with a condenser and carrying a thermometer and capillary tube, both of which must extend into the liquid. Connect a small dropping funnel, filled with water, or a steam generator to the capillary tube. Support the flask on an asbestos mat with a hole which exposes about one-third of the flask to the flame. Distil into a 250-ml flask until the temperature reaches 135°.

Add water from the funnel or introduce steam through the capillary to maintain the temperature between 135° and 140°. Continue the distillation until 225-240 ml has been collected, then dilute to 250 ml with water, and mix.

Place a 50-ml aliquot of this solution in a 100-ml Nessler tube. In another similar Nessler tube place 50 ml of water as a control. Add to each tube 0.1 ml of filtered solution of sodium alizarinsulfonate (1 in 1,000) and 1 ml of freshly prepared hydroxylamine hydrochloride solution (1 in 4,000), and mix well. Add, dropwise, and with stirring, 0.05 N sodium hydroxide to the tube containing the distillate until its colour just matches that of the control, which is faintly pink. Then add to each tube exactly 1 ml of 0.1 N hydrochloric acid, and mix well. From a buret, graduated in 0.05-ml, add slowly to the tube containing the distillate enough thorium nitrate solution (1 in 4,000) so that, after mixing, the colour of the liquid just changes to a faint pink. Note the volume of the solution added, add exactly the same volume to the control, and mix. Now add to the control sodium fluoride TS (10 µg F per ml) from a buret to make the colours of the two tubes match after dilution to the same volume. Mix well, and allow all air bubbles to escape before making the final colour comparison. Check the end-point by adding 1 or 2 drops of sodium fluoride TS to the control. A distinct change in colour should take place. Note the volume of sodium fluoride added. The volume of sodium fluoride TS required for the control solution should not exceed 1.0 ml.

METHOD OF ASSAY

Dissolve about 600 mg of the sample, accurately weighed, in 100 ml of water in 400-ml beaker, and adjust the pH of the solution to exactly 3.8 with hydrochloric acid, using a pH meter. Add 50 ml of a 1 in 8 solution of zinc sulfate (125 g of $ZnSO_4 \cdot 7H_2O$ dissolved in water, diluted to 1000 ml, filtered, and adjusted to pH 3.8) and allow to stand for 2 min. Titrate the liberated acid with 0.1 N sodium hydroxide until a pH of 3.8 is again reached. After each addition of sodium hydroxide near the end-point, time should be allowed for any precipitated zinc hydroxide to redissolve. Each ml of 0.1 N sodium hydroxide is equivalent to 16.52 mg of $K_4P_2O_7$.

DICALCIUM PYROPHOSPHATE

Prepared at the 24th JECFA (1980), published in FNP 17 (1980) and FNP 52 (1992). Metals and arsenic specifications revised at the 55th JECFA (2000). A group MTDI of 70 mg/kg bw, as phosphorus from all food sources, was established at the 26th JECFA (1982)

SYNONYMS Calcium pyrophosphate; INS No. 450(vi)

DEFINITION

Chemical names	Dicalcium diphosphate, dicalcium pyrophosphate
C.A.S. number	7790-76-3
Chemical formula	$Ca_2P_2O_7$
Formula weight	254.12
Assay	Not less than 96%

DESCRIPTION Fine, white, odourless powder

FUNCTIONAL USES Buffering agent, neutralizing agent, yeast food

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Insoluble in water; soluble in dilute hydrochloric and nitric acids
pH (Vol. 4)	5.5-7.0 (1 in 10 slurry)
Test for phosphate (Vol. 4)	Passes test
Test for calcium (Vol. 4)	Passes test

PURITY

Loss on ignition (Vol. 4)	Not more than 1.5% Weigh accurately about 1 g of the sample, and ignite, preferably in a muffle furnace, at 800-825^o for 30 min.
Fluoride (Vol. 4)	Not more than 50 mg/kg (Method I or III)
Arsenic (Vol. 4)	Not more than 3 mg/kg (Method II)

Lead (Vol. 4)

Not more than 4 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Dissolve about 200 mg of the sample accurately weighed in 10 ml of dilute hydrochloric acid TS. Add about 120 ml of water and cool for 30 min, keeping the volume and pH of the solution constant during the cooling period by adding dilute hydrochloric acid or water if necessary. After cooling add 25 ml of 0.05 M disodium ethylenediamine tetraacetate and dilute to 200 ml with water. Neutralize with strong ammonia TS. Add 10 ml of buffer solution (pH 10) and a few drops of eriochrome black TS. Titrate with 0.05 M zinc sulfate. Each ml of 0.05 M disodium ethylenediamine tetraacetate is equivalent to 6.352 mg of $Ca_2P_2O_7$.

CALCIUM DIHYDROGEN DIPHOSPHATE

New specifications prepared at the 57th JECFA (2001) and published in FNP 52 Add 9 (2001). No ADI, but a group MTDI of 70 mg/kg bw, expressed as phosphorus from all food sources, was established at the 26th JECFA (1982).

SYNONYMS Acid calcium pyrophosphate, monocalcium dihydrogen pyrophosphate; INS No. 450 (vii)

DEFINITION

Chemical names	Monocalcium dihydrogen diphosphate
C.A.S. number	14866-19-4
Chemical formula	$CaH_2P_2O_7$
Formula weight	215.97
Assay	Not more than 64% expressed as P_2O_5 on dried basis.

DESCRIPTION White crystals or powder

FUNCTIONAL USES Stabilizer, leavening agent, emulsifier, nutrient

CHARACTERISTICS

IDENTIFICATION

Test for calcium (Vol. 4)	Passes test
Test for phosphate (Vol. 4)	Passes test

PURITY

Loss on drying (Vol. 4)	Anhydrous: Not more than 1% (105°, 4 h)
Acid insoluble matter (Vol. 4)	Not more than 0.4%
Fluoride (Vol. 4)	Not more than 30 mg/kg Method III; use an appropriate sample size and appropriate volumes of standard solution for construction of the calibration curve.
Arsenic (Vol. 4)	Not more than 3 mg/kg
Lead (Vol. 4)	Not more than 4 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental

Methods."

METHOD OF ASSAY

Weigh accurately about 200 mg of the sample, dissolve in 25 ml of water and 10 ml of diluted nitric acid TS and boil for 30 min. Filter if necessary, and wash any precipitate, then dissolve the precipitate by the addition of 1 ml diluted nitric acid TS. Adjust the temperature to about 50^{o}, add 75 ml of ammonium molybdate TS, and maintain the temperature at about 50^{o} for 30 min, stirring occasionally. Allow to stand for 16 h or overnight at room temperature. Decant the supernate, through a filter paper, wash the precipitate once or twice with water by decantation using 30 to 40 ml each time, and pour the washings through the same filter. Transfer the precipitate to the same filter, and wash with potassium nitrate solution (1 in 100) until the filtrate is no longer acid to litmus paper. Transfer the precipitate with filter paper to the original precipitation vessel, add 50.0 ml of 1N sodium hydroxide, agitate until the precipitate is dissolved, add 3 drops of phenolphthalein TS and titrate the excess alkali with 1N sulfuric acid. Each ml of 1N sodium hydroxide consumed is equivalent to 3.088 mg of P_2O_5.

PENTASODIUM TRIPHOSPHATE

Prepared at the 55th JECFA (2000) and published in FNP 52 Add 8 (2000), superseding tentative specifications prepared at the 20th JECFA (1976) and published in FNS 1B (1977) and in FNP 52 (1992). No ADI was established, but a group MTDI of 70 mg/kg bw, expressed as phosphorus from all food sources, was established at the 26th JECFA (1982).

SYNONYMS Pentasodium tripolyphosphate, Sodium triphosphate, Sodium tripolyphosphate, Triphosphate; INS No. 451(i)

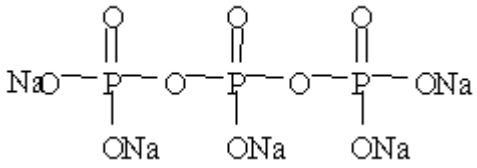
DEFINITION

Chemical names: Pentasodium triphosphate, pentasodium tripolyphosphate

C.A.S. number: 7758-29-4

Chemical formula: $Na_5O_{10}P_3 \cdot x\ H_2O$ (x = 0 or 6)

Structural formula:



Formula weight: Anhydrous: 367.86
Hexahydrate: 475.94

Assay: Anhydrous: not less than 85.0% of $Na_5O_{10}P_3$ and not less than 56.0% and not more than 58.0% of P_2O_5

Hexahydrate: not less than 65.0% of $Na_5O_{10}P_3$ and not less than 43.0% and not more than 45.0% of P_2O_5

DESCRIPTION White, slightly hygroscopic granules or powder

FUNCTIONAL USES Sequestrant, texturizer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water; insoluble in ethanol

pH (Vol. 4): 9.1 - 10.1 (1 % soln)

Test for phosphate (Vol. 4): Passes test

Test for sodium (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4): Anhydrous: not more than 0.7% (105°, 1 h)

Hexahydrate: not more than 23.5% (60 °, 1 h, followed by 105°, 4 h)

Water-insoluble matter (Vol. 4) — Not more than 0.1%

Higher polyphosphates — Not detectable
See description under TESTS

Fluoride (Vol. 4) — Not more than 50 mg/kg (Method I or III)

Arsenic (Vol. 4) — Not more than 3 mg/kg (Method II)

Lead (Vol. 4) — Not more than 4 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods”.

TESTS

PURITY TESTS

Higher polyphosphates

Chromatographic solvent
Mix 75 ml of isopropanol, 10 ml of water, 20 ml of 20% trichloroacetic acid and 0.3 ml of 20% ammonia. Make fresh every week.

Chromatographic spray
Dissolve 1 g of ammonium molybdate in 85 ml of water, 10 ml of N hydrochloric acid and 5 ml of 60% perchloric acid.

Sample solution
Dissolve 1 g of the sample in 50 ml of water.

Reference solution
Dissolve 1 g of a standard sample of pentasodium triphosphate in 50 ml of water.

Procedure
Place 0.01 ml of the sample solution and 0.01 ml of reference solution on the starting line of the chromatographic paper and allow to dry in a stream of warm air. Use ascending chromatography at 18-20° until the solvent has ascended about 25 cm from the starting line (12 - 15 h). Dry at 60° in an oven and spray with the chromatographic spray. Place the paper under an ultraviolet lamp and irradiate until the phosphates are visible as blue spots (about 2 min).

Three spots (one from the monophosphate (R_f = 0.69), a second from the diphosphate (R_f = 0.44) and the third from the triphosphate (R_f = 0.29) are observed, and no other spot is observed.

METHOD OF ASSAY

1. Determination of $Na_5O_{10}P_3$

Reagents and solutions
- Potassium acetate buffer (pH 5.0): Dissolve 78.5 g of potassium acetate

in 1000 ml of water and adjust the pH of the solution to 5.0 with acetic acid. Add a few mg of mercuric iodide to inhibit mould growth.
- 0.3 M Potassium chloride: Dissolve 22.35 g of potassium chloride in water, add 5 ml of potassium acetate buffer, dilute with water to 1000 ml, and mix. Add a few mg of mercuric iodide.
- 0.6 M Potassium chloride: Dissolve 44.7 g of potassium chloride in water, add 5 ml of potassium acetate buffer, dilute with water to 1000 ml, and mix. Add a few mg of mercuric iodide.
- 1 M Potassium chloride: Dissolve 74.5 g of potassium chloride in water, add 5 ml of potassium acetate buffer, dilute to 1000 ml with water, and mix. Add a few mg of mercuric iodide.

Chromatographic Column

Use a standard chromatographic column 20 to 40 cm length, 20 to 28 cm inside diameter, with a sealed-in, coarse porosity fritted disk. If a stopcock is not provided, attach a stopcock having a 3 to 4 mm, diameter bore to the outlet of the column with a short length of flexible vinyl tubing.

Procedure

Close the column stopcock, fill the space between the fritted disk and the stopcock with water, and connect a vacuum line to the stopcock. Prepare a 1:1 water slurry of Dowex F x 8, or equivalent, chloride form, 100-200 or 200-400 mesh, styrenedivinylbenzene ion exchange resin, and decant off any fine particles and any foam. Do this two or three times or until no more finely suspended material or foaming is observed. Fill the column with the slurry, and open the stopcock to allow the vacuum to pack the resin bed until the water level is slightly above the top of the resin, then immediately close the stopcock. Do not allow the liquid level to fall below the resin level at any time. Repeat this procedure until the packed resin column is 15 cm above the fritted disk. Place one circle of tightly fitting fibre filter paper on top of the resin bed, then place a perforated polyethylene disk on top of the paper. Alternatively, a loosely packed plug of glass wool may be placed on top of the bed. Close the top of the column with a rubber stopper in which a 7.6 cm length of capillary tubing (1.5 mm i.d., 7 mm o.d.) has been inserted through the centre, so that about 12 mm of the tubing extends through the bottom of the stopper. Connect the top of the capillary tubing to the stem of a 500-ml separator with flexible vinyl tubing, and clamp the separator to a ring stand above the column. Wash the column by adding 100 ml of water to the separator with all stopcocks closed. First open the separator stopcock, then open the column stopcock. The rate of flow should be about 5 ml per min. When the separator is empty, close the stopcock on the column then close the separator stopcock.

Transfer about 500 mg of the sample (a), accurately weighed, into a 250 ml volumetric flask, dissolve and dilute to volume with water, and mix. Transfer 10 ml of this solution into the separator, open both stopcocks and allow the solution to drain into the column, rinsing the separator with 20 ml of water. Discard the eluate. Add 370 ml of 0.3 M Potassium Chloride to the separator, and allow this solution to pass through the column, discarding the eluate. Add 250 ml of 0.6 M Potassium Chloride to the column, allow the solution to pass through the column, and receive the eluate in a 400-ml beaker. (To ensure a clean column for the next run, pass 100 ml of 1 M Potassium Chloride through the column, followed by 100 ml of water. Discard all washings.) To the beaker add 15 ml of nitric acid, mix, and boil

for 15 to 20 min. Add methyl orange TS, and neutralize the solution with stronger ammonia TS. Add 1 g of ammonium nitrate crystals, stir to dissolve, and cool. Add 15 ml of ammonium molybdate TS, with stirring, and stir vigorously for 3 min or allow to stand with occasional stirring for 10 to 15 min. Filter the contents of the beaker with suction through a 6-7 mm paper pulp filter pad supported in a 25 mm porcelain disk. The filter pad should be covered with a suspension of a filtering aid. After the contents of the beaker have been transferred to the filter, wash the beaker with five 10 ml portions of a 1% solution of sodium or potassium nitrate, passing the washings through the filter, then wash the filter with five 5-ml portions of the wash solution. Return the filter pad and the precipitate to the beaker, wash the funnel thoroughly with water into the beaker, and dilute to about 150 ml. Add 0.1 N sodium hydroxide from a buret until the yellow precipitate is dissolved, then add 5 to 8 ml in excess. Add phenolphthalein TS, and titrate the excess alkali with 0.1 N nitric acid. Finally, titrate with 0.1 N sodium hydroxide to the first appearance of the pink colour. The difference between the total volume of 0.1 N sodium hydroxide added and the volume of nitric acid required represents the volume, V, in ml, of 0.1 N sodium hydroxide consumed by the phosphomolybdate complex.

Calculate the $Na_5O_{10}P_3$ content of the sample in % by the formula

$$\%\,Na_5O_{10}P_3 = \frac{0.533 \times 25 \times V}{a} \times 100$$

where
a = the weight of the sample (mg)
2. Determination of P_2O_5

Accurately weigh about 20 g of the sample into a beaker. Add 150 ml water and 20 ml concentrated nitric acid. Introduce anti-bumping granules, cover the beaker with a watch glass and boil gently for 1 h. Cool to room temperature. Quantitatively transfer the solution to a 500-ml volumetric flask, dilute with water, mix well and dilute to the mark with water. Transfer 20.0 ml of the solution to a plastic beaker, dilute to about 50 ml with water and place the beaker in an automatic titrator equipped with a pH meter. Adjust the pH of the solution to between 2.5 and 2.8 with 5 mol/l sodium hydroxide. Titrate the solution with 0.5 mol/l sodium hydroxide. Record the consumed volumes at the inflection points at about pH 4 (V1) and about pH 9 (V2).

Calculate the P_2O_5 content of the sample in % by the formula

% P_2O_5 = [(V2 - V1)/2000] x f x 70.97 x (500/20) x (100/w)
= [(V2 - V1)/w] x f x 88.71

where
w = weight of the sample (g)
f = factor of 0.5 mol/l sodium hydroxide (= actual molarity/0.5)

PENTAPOTASSIUM TRIPHOSPHATE

Prepared at the 29th JECFA (1985), published in FNP 34 (1986) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). A group MTDI of 70 mg/kg bw, as phosphorus from all food sources, was established at the 26th JECFA (1982)

SYNONYMS Pentapotassium tripolyphosphate, potassium triphosphate, potassium tripolyphosphate; INS No. 451(ii)

DEFINITION

Chemical names	Pentapotassium triphosphate, pentapotassium tripolyphosphate
C.A.S. number	13845-36-8
Chemical formula	$K_5O_{10}P_3$
Formula weight	448.42
Assay	Not less than 85% of $K_5O_{10}P_3$ on the dried basis, the remainder being principally other potassium phosphates

DESCRIPTION Hygroscopic white granules or powder

FUNCTIONAL USES Texturizer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Very soluble in water
pH (Vol. 4)	9.2 - 10.1 (1 in 100 soln)
Test for phosphate (Vol. 4)	Passes test
Test for potassium (Vol. 4)	Passes test

PURITY

Water insoluble matter (Vol. 4)	Not more than 2%
Loss on ignition (Vol. 4)	Not more than 0.4% after drying (105°, 4 h), followed by ignition at 550° for 30 min.
P_2O_5 content	Not less than 46.5% and not more than 48.0% Proceed as directed in the *Phosphate Determination as P_2O_5* using about 1.5 g of the dried sample accurately weighed
Fluoride	Not more than 10 mg/kg

See description under TESTS

Arsenic (Vol. 4) — Not more than 3 mg/kg (Method II)

Lead (Vol. 4) — Not more than 4 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Fluoride

Place 5 g of the sample, 25 ml of water, 50 ml of perchloric acid, 5 drops of silver nitrate solution (1 in 2), and a few glass beads in a 250-ml distilling flask connected with a condenser and carrying a thermometer and a capillary tube, both of which must extend into the liquid. Connect a small dropping funnel, filled with water, or a steam generator to the capillary tube. Support the flask on an asbestos mat with a hole which exposes about one-third of the flask to the flame. Distil into a 250-ml flask until the temperature reaches 135^{o}. Add water from the funnel or introduce steam through the capillary to maintain the temperature between 135 and 140^{o}. Continue the distillation until 225-240 ml has been collected, then dilute to 250 ml with water, and mix. Place a 50-ml aliquot of this solution in a 100-ml Nessler tube. In another similar Nessler tube place 50 ml of water as a control. Add to each tube 0.1 ml of a filtered solution of sodium alizarin-sulfonate (1 in 1000) and 1 ml of freshly prepared hydroxylamine hydrochloride solution (1 in 4000), and mix well. Add, dropwise, and with stirring, 0.05 N sodium hydroxide to the tube containing the distillate until its colour just matches that of the control, which is faintly pink. Then add to each tube exactly 1 ml of 0.01 N hydrochloric acid, and mix well. From a buret, graduated in 0.05 ml, add slowly to the tube containing the distillate enough thorium nitrate solution (1 in 4000) so that, after mixing, the colour of the liquid just changes to a faint pink. Note the volume of the solution added, add exactly the same volume to the control, and mix. Now add to the control sodium fluoride TS (10 µg, F per ml) from a buret to make the colours of the two tubes match after dilution to the same volume. Mix well, and allow all air bubbles to escape before making the final drops of sodium fluoride TS to the control. A distant change in colour should take place. Note the volume of sodium fluoride added. The volume of sodium fluoride TS required for the control solution should not exceed 10 ml.

METHOD OF ASSAY

Reagents and Solutions
- Potassium acetate buffer (pH 5.0): Dissolve 78.5 g of potassium acetate in 1000 ml of water. and adjust the pH of the solution to 5.0 with acetic acid. Add a few mg of mercuric iodide to inhibit mould growth.
- 0.3 M Potassium chloride: Dissolve 22.35 g of potassium chloride in water, add 5 ml of potassium acetate buffer, dilute with water to 1000 ml, and mix. Add a few mg of mercuric iodide.
- 0.6 M Potassium chloride: Dissolve 44.7 g of potassium chloride in water, add 5 ml of potassium acetate buffer, dilute with water to 1000 ml, and mix. Add a few mg of mercuric iodide.

- 1 M Potassium chloride: Dissolve 74.5 g of potassium chloride in water, add 5 ml of potassium acetate buffer, dilute to 1000 ml with water, and mix. Add a few mg of mercuric iodide.

Chromatographic Column:
Use a standard chromatographic column 20 to 40 cm in length, 20 to 28 cm in inside diameter, with a sealed-in, coarse porosity fritted disk. If a stopcock is not provided, attach a stopcock having a 3 to 4 mm, diameter bore to the outlet of the column with a short length of flexible vinyl tubing.

Procedure:
Close the column stopcock, fill the space between the fritted disk and the stopcock with water, and connect a vacuum line to the stopcock. Prepare a 1:1 water slurry of Dowex F x 8, or equivalent, chloride form, 100-200 or 200-400 mesh, styrenedivinylbenzene ion exchange resin, and decant off any fine particles and any foam. Do this two or three times or until no more finely suspended material or foaming is observed. Fill the column with the slurry, and open the stopcock to allow the vacuum to pack the resin bed until the water level is slightly above the top of the resin, then immediately close the stopcock. Do not allow the liquid level to fall below the resin level at any time. Repeat this procedure until the packed resin column is 15 cm above the fritted disk. Place one circle of tightly fitting fiber filter paper on top of the resin bed, then place a perforated polyethylene disk on top of the paper. Alternatively, a loosely packed plug of glass wool may be placed on top of the bed. Close the top of the column with a rubber stopper in which a 7.6 cm length of capillary tubing (1.5 mm i.d., 7 mm 0.d.) has been inserted through the centre, so that about 12 mm of the tubing extends through the bottom of the stopper. Connect the top of the capillary tubing to the stem of a 500 ml separator with flexible vinyl tubing, and clamp the separator to a ring stand above the column. Wash the column by adding 100 ml of water to the separator with all stopcocks closed. First open the separator stopcock, then open the column stopcock. The rate of flow should be about 5 ml per min. When the separator is empty, close the stopcock on the column then close the separator stopcock.
Transfer about 500 mg of the sample previously dried at 105º for 4 h and accurately weighed, into a 250 ml volumetric flask, dissolve and dilute to volume with water, and mix. Transfer 10 ml of this solution into the separator, open both stopcocks and allow the solution to drain into the column, rinsing the separator with 20 ml of water. Discard the eluate. Add 370 ml of 0.3 M Potassium Chloride to the separator, and allow this solution to pass through the column, discarding the eluate. Add 250 ml of 0.6 M Potassium Chloride to the column, allow the solution to pass through the column, and receive the eluate in a 400 ml beaker. (To ensure a clean column for the next run, pass 100 ml of 1 M Potassium Chloride through the column, followed by 100 ml of water. Discard all washings.) To the beaker add 15 ml of nitric acid, mix, and boil for 15 to 20 min. Add methyl orange TS, and neutralize the solution with stronger ammonia TS. Add 1 g of ammonium nitrate crystals, stir to dissolve, and cool. Add 15 ml of ammonium molybdate TS, with stirring, and stir vigorously for 3 min or allow to stand with occasional stirring for 10 to 15 min. Filter the contents of the beaker with suction through a 6-7 mm paper pulp filter pad supported in a 25 mm porcelain disk. The filter pad should be covered with a suspension of infusorial earth. After the contents of the beaker have been transferred to

the filter, wash the beaker with five 10 ml portions of a 1 in 100 solution of sodium or potassium nitrate, passing the washings through the filter, then, wash the filter with five 5-ml portions of the wash solution. Return the filter pad and the precipitate to the beaker, wash the funnel thoroughly with water into the beaker, and dilute to about 150 ml. Add 0.1 N sodium hydroxide from a buret until the yellow precipitate is dissolved, then add 5 to 8 ml in excess. Add phenolphthalein TS, and titrate the excess alkali with 0.1 N, nitric acid. Finally, titrate with 0.1 N sodium hydroxide to the first appearance of the pink colour. The difference between the total volume of 0.1 N sodium hydroxide added and the volume of nitric acid required represents the volume, V, in ml, of 0.1 N sodium hydroxide consumed by the phosphomolybdate complex. Calculate the quantity, in mg, of $K_5O_{10}P_3$ in the sample taken by the formula 0.650 x 25V.

SODIUM POLYPHOSPHATES, GLASSY

Prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared by the 20th JECFA (1976), published in FNS 1B (1977) and in FNP 52 (1992). Metals and arsenic specifications revised at the 55th JECFA (2000). A group MTDI of 70 mg/kg bw, as phosphorus from all food sources, was established at the 26th JECFA (1982)

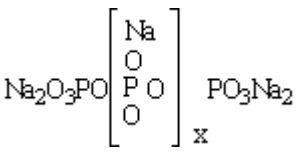
SYNONYMS Sodium hexametaphosphate, sodium tetrapolyphosphate, Graham's salt; INS No 452(i)

DEFINITION Obtained by fusion and subsequent chilling of sodium orthophosphates; a class of compounds consisting of several amorphous, water-soluble polyphosphates composed of linear chains of metaphosphate units, $(NaPO_3)_x$ where x=2, terminated by Na_2PO_4 groups; usually identified by their Na_2O/P_2O_5 ratio or their P_2O_5 content. The Na_2O/P_2O_5 ratios vary from about 1.3 for sodium tetrapolyphosphate, where x = approximately 4; to about 1.1 for Graham's salt, commonly called sodium hexametaphosphate, where x = 13 to 18; and to about 1.0 for the higher molecular weight sodium polyphosphates, where x = 20 to 100 or more. The pH of their solutions varies from about 3 to 9.

Chemical names Sodium tetrapolyphosphate, sodium hexametaphosphate, sodium polyphosphate

C.A.S. number 68915-31-1, 10124-56-8, 10361-03-2

Structural formula



Assay Not less than 60.0% and not more than 71.0% of P_2O_5

DESCRIPTION Colourless or white, transparent platelets, granules, or powders

FUNCTIONAL USES Emulsifier, sequestrant, texturizer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Very soluble in water

Test for sodium (Vol. 4) A 1 in 20 solution passes test

Test for orthophosphate Dissolve 0.1 g of the sample in 5 ml of hot dilute nitric acid TS. Warm on a steam bath for 10 min, and cool. Neutralize to litmus with sodium hydroxide TS, and add silver nitrate TS. A yellow precipitate is formed which is soluble in dilute nitric acid TS.

PURITY

Loss on ignition (Vol. 4) — Not more than 1.0%

Insoluble substances — Not more than 0.1%
Dissolve about 10 g of the sample, accurately weighed, in 100 ml of hot water, and filter through a tared filtering crucible. Wash the insoluble residue with hot water, dry at 105° for 2 h, and weigh.

Fluoride (Vol. 4) — Not more than 10 mg/kg (Method I or III)

Arsenic (Vol. 4) — Not more than 3 mg/kg

Lead (Vol. 4) — Not more than 4 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Transfer about 800 mg of the sample, accurately weighed, into a 400-ml beaker. Add 100 ml of water and 25 ml of nitric acid, cover with a watch glass, and boil for 10 min on a hot plate. Rinse any condensate from the watch glass into the beaker; cool the solution to room temperature; transfer it quantitatively to a 500-ml volumetric flask; dilute to volume with water; and mix thoroughly. Pipet 20.0 ml of this solution into a 500-ml Erlenmeyer flask, add 100 ml of water, and heat just to boiling. Add with stirring 50 ml of quimociac TS, then cover with a watch glass, and boil for 1 min in a well-ventilated hood. Cool to room temperature, swirling occasionally while cooling, then filter through a tared, sintered-glass filter crucible of medium porosity, and wash with five 25-ml portions of water. Dry at about 225° for 30 min, cool, and weigh. Each mg of precipitate thus obtained is equivalent to 32.074 µg of P_2O_5.

POTASSIUM POLYPHOSPHATES

Prepared at the 26th JECFA (1982), published in FNP 25 (1982) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003). No ADI was established, but a group MTDI of 70 mg/kg bw, expressed as phosphorus from all food sources, was established at the 26th JECFA (1982).

SYNONYMS Potassium metaphosphate; INS No 452(ii)

DEFINITION A heterogeneous mixture of potassium salts of linear condensed polyphosphoric acids of general formula $H_{n+2}P_nO_{3n+1}$ where "n" is not less than 2

Chemical names: Potassium metaphosphate, potassium polymetaphosphate, potassium polyphosphate

C.A.S. number: 7790-53-6

Assay: Not less than 53.5% and not more than 61.5% of P_2O_5 on the ignited basis

DESCRIPTION Odourless, colourless or white glassy masses, fragments, crystals or powder

FUNCTIONAL USES Emulsifier, moisture-retaining agent, sequestrant, texturizer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): 1 g dissolves in 100 ml of a 1 in 25 soln of sodium acetate

Gel formation: Finely powder about 1 g of the sample, and add it slowly to 100 ml of a 1 in 50 solution of sodium chloride while stirring vigorously. A gelatinous mass is formed.

Test for potassium (Vol. 4): Mix 0.5 g of the sample with 10 ml of nitric acid and 50 ml of water, boil for about 30 min, and cool. The resulting solution is used for the test

Test for phosphate (Vol. 4): Mix 0.5 g of the sample with 10 ml of nitric acid and 50 ml of water, boil for about 30 min and cool. The resulting solution is used for the test

PURITY

Loss on ignition (Vol. 4): Not more than 2 % after drying (105°, 4 h) followed by ignition at 550° for 30 min

Cyclic phosphate (Vol. 4): Not more than 8.0%

Fluoride: Not more than 10 mg/kg
See description under TESTS

Arsenic (Vol. 4) — Not more than 3 mg/kg (Method II)

Lead (Vol. 4) — Not more than 4 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Fluoride

Place 5 g of the sample, 25 ml of water, 50 ml of perchloric acid, 5 drops of silver nitrate solution (1 in 2), and a few glass beads in a 250-ml distilling flask connected with a condenser and carrying a thermometer and capillary tube, both of which must extend into the liquid. Connect a small dropping funnel, filled with water, or a steam generator to the capillary tube. Support the flask on an asbestos mat with a hole which exposes about one-third of the flask to the flame. Distil into a 250-ml flask until the temperature reaches 135°.

Add water from the funnel or introduce steam through the capillary to maintain the temperature between 135° and 140°. Continue the distillation until 225-240 ml has been collected, then dilute to 250 ml with water, and mix.

Place a 50-ml aliquot of this solution in a 100-ml Nessler tube. In another similar Nessler tube place 50 ml of water as a control. Add to each tube 0.1 ml of filtered solution of sodium alizarinsulfonate (1 in 1,000) and 1 ml of freshly prepared hydroxylamine hydrochloride solution (1 in 4,000), and mix well. Add, dropwise, and with stirring, 0.05 N sodium hydroxide to the tube containing the distillate until its colour just matches that of the control, which is faintly pink. Then add to each tube exactly 1 ml of 0.1 N hydrochloric acid, and mix well. From a buret, graduated in 0.05-ml, add slowly to the tube containing the distillate enough thorium nitrate solution (1 in 4,000) so that, after mixing, the colour of the liquid just changes to a faint pink. Note the volume of the solution added, add exactly the same volume to the control, and mix. Now add to the control sodium fluoride TS (10 µg F per ml) from a buret to make the colours of the two tubes match after dilution to the same volume. Mix well, and allow all air bubbles to escape before making the final colour comparison. Check the end-point by adding 1 or 2 drops of sodium fluoride TS to the control. A distinct change in colour should take place. Note the volume of sodium fluoride added. The volume of sodium fluoride TS required for the control solution should not exceed 1.0 ml.

METHOD OF ASSAY

Mix about 300 mg of the sample, accurately weighed, with 15 ml of nitric acid and 30 ml of water, boil for 30 min, and dilute with water to about 100 ml. Heat at 60°, add an excess of ammonium molybdate TS, and heat at 50° for 30 min. Filter, and wash the precipitate with dilute nitric acid (1 in 36 soln), followed by potassium nitrate solution (1 in 100 soln) until the filtrate is no longer acid to litmus. Dissolve the precipitate in 50 ml of 1 N sodium

hydroxide, add phenolphthalein TS, and titrate the excess sodium hydroxide with 1 N sulfuric acid. Each ml of 1 N sodium hydroxide is equivalent to 3.086 mg of P_2O_5.

SODIUM CALCIUM POLYPHOSPHATE

New specifications prepared at the 57th JECFA (2001) and published in FNP 52 Add 9 (2001). No ADI, but a group MTDI of 70 mg/kg bw, expressed as phosphorus from all food sources, was established at the 26th JECFA (1982).

SYNONYMS Sodium calcium polyphosphate, glassy; INS No. 452 (iii)

DEFINITION

Chemical names: Sodium calcium polyphosphate

Chemical formula: $(NaPO_3)_n$_CaO where n is typically 5

Assay: Not less than 61% and not more than 69 % expressed as P_2O_5 on dried basis

DESCRIPTION White glassy crystals, spheres

FUNCTIONAL USES Stabilizer, leavening agent, emulsifier, nutrient

CHARACTERISTICS

IDENTIFICATION

Test for sodium (Vol. 4): Passes test

Test for calcium (Vol. 4): Passes test

Test for phosphate (Vol. 4): Passes test

PURITY

Arsenic (Vol. 4): Not more than 3 mg/kg

Lead (Vol. 4): Not more than 4 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation, may be based on the principles of the method described in FNP 5, “Instrumental Methods”.

Fluoride (Vol. 4): Not more than 10 mg/kg
Method III; use an appropriate sample size and appropriate volumes of standard solution for construction of the calibration curve.

METHOD OF ASSAY Weigh accurately about 200 mg of the sample, dissolve in 25 ml of water and 10 ml of diluted nitric acid TS and boil for 30 min. Filter if necessary, and wash any precipitate, then dissolve the precipitate by the addition of 1 ml diluted nitric acid TS. Adjust the temperature to about 50°, add 75 ml of ammonium molybdate TS, and maintain the temperature at about 50° for 30

min, stirring occasionally. Allow to stand for 16 h or overnight at room temperature. Decant the supernate, through a filter paper, wash the precipitate once or twice with water by decantation using 30 to 40 ml each time, and pour the washings through the same filter. Transfer the precipitate to the same filter, and wash with potassium nitrate solution (1 in 100) until the filtrate is no longer acid to litmus paper. Transfer the precipitate with filter paper to the original precipitation vessel, add 50.0 ml of 1N sodium hydroxide, agitate until the precipitate is dissolved, add 3 drops of phenolphthalein TS and titrate the excess alkali with 1N sulfuric acid.

Each ml of 1N sodium hydroxide consumed is equivalent to 3.088 mg of P_2O_5.

CALCIUM POLYPHOSPHATE

Prepared at the 26th JECFA (1982), published in FNP 25 (1982) and in FNP 52 (1992 Metals and arsenic specifications revised at the 55th JECFA (2000) A group MTDI of 70 mg/kg bw, as phosphorus from all food sources, was established at the 26th JECFA (1982)

SYNONYMS INS No. 452(iv)

DEFINITION A heterogeneous mixture of calcium salts of polyphosphoric acids of general formula $H_{n+2}P_nO_{n+1}$.

Assay Not less than 50.0 and not more than 71.0% of P_2O_5 on the ignited basis

DESCRIPTION Odourless, colourless crystals or powder

FUNCTIONAL USES Emulsifier, moisture-retaining agent, sequestrant, texturizer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Usually incompletely soluble in water; soluble in acid medium

Test for phosphate (Vol. 4) Mix 0.5 g of the sample with 10 ml of nitric acid and 50 ml of water, boil for about 30 min and cool. The resulting solution is used for the test

Test for calcium (Vol. 4) The solution of the test for phosphate gives positive tests for calcium

PURITY

Loss on ignition (Vol. 4) Not more than 2% after drying (105°, 4 h) followed by ignition (550°, 30 min)

Cyclic phosphate (Vol. 4) Not more than 8% calculated on P_2O_5 content

Fluoride (Vol. 4) Not more than 10 mg/kg

Arsenic (Vol. 4) Not more than 3 mg/kg
Dissolve 1 g of the sample in 15 ml dilute hydrochloric acid TS, add 20 ml of water. Test this solution as directed in the Limit Test (Method II).

Lead (Vol. 4) Not more than 4 mg/kg.
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY Mix about 300 mg of the sample, accurately weighed, with 15 ml of nitric acid and 30 ml of water, boil for 30 min and dilute with water to about 100 ml. Heat at 60°, and add excess of ammonium molybdate TS, and heat at 50° for 30 min. Filter, and wash the precipitate with dilute nitric acid (1 in 36), followed by potassium nitrate solution (1 in 100) until the filtrate is no longer acid to litmus.

Dissolve the precipitate in 50 ml of 1 N sodium hydroxide, add phenolphtalein TS, and titrate the excess of sodium hydroxide with 1 N sulfuric acid. Each ml of 1 N sodium hydroxide is equivalent to 3.086 mg of P_2O_5.

AMMONIUM POLYPHOSPHATE

Prepared at the 41st JECFA (1993), published in FNP 52 Add 2 (1993) superseding specifications prepared at the 26th JECFA (1982), published in FNP 25 (1982). Metals and arsenic specifications revised at the 55th JECFA (2000)
A group MTDI of 70 mg/kg bw, as phosphorus from all food sources, was established at the 26th JECFA (1982)

SYNONYMS INS No. 452(v)

DEFINITION

Chemical names: Ammonium polyphosphate

C.A.S. number: 6833-79-9

Chemical formula: Heterogeneous mixture of ammonium salts of linear condensed polyphosphoric acids of general formula $H_{n+2}P_nO_{3n+1}$

Assay: Not less than 55.0% and not more than 75.0% on an anhydrous basis, calculated as P_2O_5

DESCRIPTION Aqueous solution

FUNCTIONAL USES Sequestrant, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility: Freely soluble in water

pH (Vol. 4): 4.0-9.0 (1 in 100 soln)

Test for ammonium (Vol. 4): Passes test

Test for phosphate (Vol. 4): Passes test
Test a solution resulting from mixing 0.5 g of sample with 10 ml of nitric acid and 50 ml of water, boiled for 30 min and cooled

PURITY

Cyclic phosphate (Vol. 4): Not more than 8% calculated as P_2O_5 content

Fluoride (Vol. 4): Not more than 10 mg/kg

Arsenic (Vol. 4): Not more than 3 mg/kg

Lead: Not more than 4 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be

based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Proceed as directed under *Phosphate Determination as P_2O_5,* Method I, in Volume 4.

BONE PHOSPHATE

Prepared at the 33rd JECFA (1988), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003)
A group MTDI of 70 mg/kg bw, as phosphorus from all food sources, was established at the 29th JECFA (1985)

SYNONYMS Edible bone phosphate, INS No. 542

DEFINITION A heterogeneous residual mixture of calcium phosphates, principally $3Ca_3(PO_4)_2 \cdot Ca(OH)_2$, obtained by the grinding of bones which have been treated with hot water and steam under pressure; may contain unextracted fat and proteins.

Assay Not less than 30% and not more than 40% of Ca, and not less than 32% of P_2O_5.

DESCRIPTION White to pale cream coloured, odourless powder

FUNCTIONAL USES Emulsifier, moisture retaining agent, sequestrant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in ethanol and water

Test for phosphate (Vol. 4) Passes test
Use a solution obtained by dissolving 1 g of the sample by warming in 50 ml diluted hydrochloric acid.

Test for calcium (Vol. 4) Passes test
Use a solution obtained by dissolving 1 g of the sample by warming in 50 ml diluted hydrochloric acid.

PURITY

Loss on drying (Vol. 4) Not more than 2%

Loss on ignition (Vol. 4) Not more than 20%

Fluoride (Vol. 4) Total not more than 1000 mg/kg
Dissolve an amount of ash (obtained from the test for Loss on ignition) equal to 0.1 g of the sample, and proceed as described under the Limit Test, Method IV using buffer solution C.

Copper Not more than 25 mg/kg
See description under TESTS

Zinc Not more than 250 mg/kg
See description under TESTS

Arsenic (Vol. 4)	Not more than 3 mg/kg
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
Fat residue	Not more than 2% Accurately weigh 5-10 g sample. Without previous drying, extract in soxhlet or other suitable container with petroleum ether (40-60^{o}) for about 6 h. Filter extract through small hardened paper into weighed vessel, washing paper into weighed vessel, washing paper finally with small portion of hot fresh solvent. Distil or evaporate solvent at temperature ca 100^{o} and dry vessel containing residue in air oven for 1h at 100-105^{o}C. Weigh the dried residue and calculate percentage of the sample.
Protein residue (Vol. 4)	Not more than 10% (N x 6.25) Proceed as directed under *Nitrogen Determination (Kjeldahl Method)* Method II
Microbiological criteria (Vol. 4)	Total aerobic microbial count: Max 1000 in 1 g *Salmonella*: Absent in 50 g *E. coli*: Absent in 10 g

TESTS

PURITY TESTS

Copper and zinc

General precautions

Because of the min amounts of metals involved special care must be taken to reduce the reagent blanks to as low a value as possible and to avoid contamination during the test. All apparaturs should be thoroughly cleaned with a mixture of hot dilute acids (1 part hydrochloric acid, 1 part concentrated nitric acid, and 3 parts water) followed by thorough washing with water immediately before use. The methods of preparation described should be followed exactly.

Apparatus

Atomic absorption spectrophotometer equipped with air/acethylene flame and lamps for copper and zinc determination.

Reagents

Reagents shall be of an order of purity higher than accepted analytical reagent grade quality. Metal-free water (see below) shall be used throughout.

- Sulfuric acid, 98% H_2SO_4
- Nitric acid, sp.gr. 1.42
- Hydrochloric acid, sp. gr. 1.16-1.18 (conc.)
- Hydrochloric acid 5 M solution prepared by dilution of hydrochloric acid (conc.) with water
- Hydrochloric acid 0.5 M solution prepared by dilution of hydrochloric acid 5 M with water
- Water, metal free. Distilled water may be re-distilled from an all glass

apparatus or may be passed down a column of cation exchange resin, e.g., Amberlite IR 120 (H).

Standards

Standard copper solution: Dissolve 3.928 g of pure copper sulfate $CuSO_4$. $5H_20$ in distilled water, dilute to 1000 ml at 20^o with distilled water in a one-mark graduated flask. Dilute 10 ml to 100 ml with water in a one-mark graduated flask as required. 1 ml = 100 µg Cu.

Standard zinc solution: Dissolve 1.000 g of pure zinc powder in a mixture of 10 ml distilled water and 5 ml hydrochloric acid special reagent (d) and dilute to 1000 ml at 20^o with distilled water, in a one-mark graduated flask. Dilute 10 ml to 100 ml with water in a one-mark graduated flask as required. 1 ml = 100 µg Zn.

Sample preparation

Place about 2.5 g of the sample, accurately weighed, in a suitable crucible, add sufficient sulfuric acid to wet the sample, and carefully ignite at a low temperature until thoroughly charred, covering the crucible loosely with a suitable lid during the ignition. After the substance is thoroughly carbonized, add 2 ml of nitric acid and 5 drops of sulfuric acid, and cautiously heat until white fumes are evolved, then ignite, preferably in a muffle furnace, at 500^o to 600^o until all the carbon is burned off. Cool, add 4 ml of hydrochloric acid 5 M, cover, and digest on a steam bath for 10 to 15 min. Uncover, and slowly evaporate on a steam bath to dryness. Finally cool, add 10 ml 5 M hydrochloric acid and boil gently for a few min. Cool and transfer the solution to a 50-ml one-mark graduated flask washing out the Kjeldahl flask with small portions of water. Add the washings to the graduated flask and dilute to the mark with water (Solution A). To a 100 ml one mark volumetric flask pipet 10 ml of solution A and dilute to the mark with hydrochloric acid 0.5 M (Solution B). Prepare a reagent blanks using the same quantities of reagents as used in the sample preparation for obtaining solutions A and B (Blank A and Blank B).

Preparation of standard curve solutions

To a series of 100-ml one-mark volumetric flasks pipet 0, 1, 2, 3, 4 and 5 ml of each of the two standard solutions to (e) and dilute to about 50 ml. Add 20 ml of hydrochloric acid 5 M and dilute to the mark with metal-free water. These solutions then contain 0, 1.0, 2.0, 3.0, 4.0 and 5.0 µg per ml of copper and zinc.

Instrumental Conditions

Select the wavelength to be used for the particular element under consideration 324.7 nm for copper; 213.9 for zinc. The recommended settings for the various instrumental parameters vary from model to model, and certain parameters require optimization at the time of use to obtain the best results. Instruments should therefore be adjusted as described in the manufacturer's instructions using wavelength settings specified above.

Set the atomic absorption spectrophotometer to the appropriate conditions. Aspirate the strongest standard containing the element to be determined and optimize the instrument settings to give full-scale or maximum deflection on the chart recorder. Measure the absorbances of the other standards and plot a graph showing the net absorbance against the concentration of the element in

the standard solutions. Aspirate Solution A and the corresponding Blank A for determination of copper or Solution B and the corresponding Blank B for determination of zinc and determine the net absorbance. Using the graph prepared above, determine the concentration of the element in the sample solution.

Calculate the content of copper and zinc, respectively from:

$$Copper\ (mg / kg) = \frac{c \times 50}{w}$$

$$Zinc\ (mg / kg) = \frac{c \times 50}{w}$$

where
c = concentration of element (µg/ml) in the sample solution
w = the weight (g) of sample taken

METHOD OF ASSAY

Calcium:
Weigh accurately about 0.150 g of the sample. Dissolve, with the aid of gentle heat if necessary, in a mixture of 5 ml of hydrochloric acid and 3 ml of water contained in a 250 ml beaker equipped with a magnetic stirrer, and cautiously add 125 ml of water. With constant stirring, add, in the following order, 0.5 ml of triethanolamine, 300 mg of hydroxynaphthol blue indicator, and, from a 50 ml buret, about 23 ml of 0.05 M disodium ethylenediamine tetraacetate. Add sodium hydroxide solution (45 in 100) until the initial red colour changes to clear blue, then continue to add it drop wise until the colour changes to violet, then add an additional 0.5 ml. The pH is between 12.3 and 12.5. Continue the titration drop wise with the 0.05 M disodium ethylenediamine tetraacetate until the appearance of a clear blue endpoint that persists for not less than 60 sec. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 2.004 mg of Ca.

P_2O_5: Proceed as directed in the *Phosphate Determination as P_2O_5,* Method II (see Volume 4).

POLYDEXTROSES

Prepared at the 51st JECFA (1998) and published in FNP 52 Add 6 (1998) superseding specifications prepared at the 44th JECFA (1995), published in FNP 52 Add 3 (1995). An ADI "not specified" was established at the 31st JECFA in 1987.

SYNONYMS Modified polydextroses; INS No. 1200

DEFINITION Randomly bonded condensation polymers of glucose with some sorbitol end-groups, and with citric acid or phosphoric acid residues attached to the polymers by mono or diester bonds. They are obtained by melting and condensation of the ingredients which consist of approximately 90 parts D-glucose, 10 parts sorbitol and up to 1 part citric acid or 0.1 part phosphoric acid. The 1,6-glucosidic linkage predominates in the polymers but other linkages are present. The products contain small quantities of free glucose, sorbitol, levoglucosan (1,6-anhydro-D-glucose) and citric acid and may be neutralized with any food-grade base and/or decolourized and deionized for further purification. The products may also be partially hydrogenated with Raney nickel catalyst to reduce residual glucose. Polydextrose-N is neutralized Polydextrose.

C.A.S. number 68424-04-4

Assay Not less than 90.0% of polymer on the ash-free and water-free bases

DESCRIPTION White to light tan-coloured solid. Polydextroses dissolve in water to give clear, colourless to straw-coloured solutions

FUNCTIONAL USES Bulking agent, humectant, stabilizer, thickener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Very soluble in water

Test for sugar To 1 drop of 1 in 10 solution of the sample, add 4 drops of 5% phenol solution, then rapidly add 15 drops of sulfuric acid TS. A deep yellow to orange colour is produced.

Solubility in acetone With vigorous swirling add 1 ml of acetone to 1 ml of a 1 in 10 solution of the sample. The solution remains clear. With vigorous swirling add 2 ml of acetone to the solution. A heavy, milky turbidity develops immediately.

Test for reducing sugar To 1 ml of a 1 in 50 solution of the sample, add 4 ml of alkaline cupric citrate TS. Boil vigorously 2-4 min. Remove from heat and let precipitate (if any) settle. The supernatant is blue or blue-green.

PURITY

Water (Vol. 4) Not more than 4.0% (Karl Fischer Method)

pH (Vol. 4)	2.5 - 7.0 (for Polydextrose) (1 in 10 soln) 5.0 - 6.0 (for Polydextrose-N) (1 in 10 soln)
Sulfated ash (Vol. 4)	Not more than 0.3% (for Polydextrose) Not more than 2.0% (for Polydextrose-N)
Nickel (Vol. 4)	Not more than 2 mg/kg for hydrogenated polydextroses Use method *Nickel* for polyols
1,6-Anhydro-D-glucose	Not more than 4.0% on the ash-free and the dried bases See description under TESTS
Glucose and sorbitol	Not more than 6.0% combined on the ash-free and the dried bases; glucose and sorbitol are determined separately See description under TESTS
Molecular weight limit	Negative to test for polymer of molecular weight greater than 22,000 See description under TESTS
5-Hydroxymethylfurfural	Not more than 0.1% in Polydextrose Not more than 0.05% in Polydextrose-N See description under TESTS
Lead (Vol. 4)	Not more than 0.5 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Lead (Vol. 4)

Apparatus:
Use a suitable atomic absorption spectrophotometer (Perkin-Elmer Model 6000, or equivalent), a graphite furnace containing a L'vov platform (Perkin-Elmer Model HGA-500, or equivalent), and an autosampler (Perkin-Elmer Model AS-40, or equivalent).
Use a lead hollow cathode lamp (lamp current of 10 mA), a slit width of 0.7 mm (set low), the wavelength set at 283.3 nm, and a deuterium arc lamp for background correction. Use argon as the carrier gas. (Note: For this test use reagent-grade chemicals with as low a lead content as practicable, as well as high-purity water and gases. Before use, rinse all glassware and plasticware twice with 10% nitric acid and twice with 10% hydrochloric acid, and then rinse thoroughly with high-purity water.)

Lead Nitrate Stock Solution:
Dissolve 159.8 mg of reagent-grade lead nitrate in 1000.0 ml of water. Prepare and store this solution in glass containers that are free from lead salts. Each ml of this solution contains the equivalent of 100 µg of lead ion.

Standard Lead Solution:

On the day of use, dilute 10.0 ml of Lead Nitrate Stock Solution with water to 100.0 ml, and mix. Each ml of Standard Lead Solution contains the equivalent of 10 µg of lead ion.

Standard Solutions:
Prepare a series of lead standard solutions serially diluted from the Standard Lead Solution. Into separate 100 ml volumetric flasks, pipet 0.2, 0.5, 1, 2 ml, and 5 ml, respectively, of Standard Lead Solution, dilute to volume with water, and mix. The Standard Solutions contain, respectively, 0.02, 0.05, 0.1, 0.2, and 0.5 µg of lead per ml.

Matrix Modifier:
Transfer 100.0 mg of ammonium phosphate, dibasic ($(NH_4)_2HPO_4$) to a 10 ml volumetric flask, dilute to volume with water, and mix.
Sample Solution: Transfer about 1 g of the sample, accurately weighed, to a 10 ml volumetric flask, add 5 ml of water, and mix. Dilute to volume with water, and mix.

Spiked (fortified) Sample Solution:
Prepare a solution as directed under Sample Solution, but add 100 µl of the Standard Lead Solution, dilute to volume with water, and mix. This solution contains 0.1 µg/ml of added lead.

Procedure:
With the use of an autosampler, atomize 10-µl aliquots of the five Standard Solutions, using the following sequence of conditions: step (1) dry at 130° with a 20-sec ramp period, a 40-sec hold time, and a 300 ml/min argon flow rate; step (2) char at 800° with a 20-sec ramp period, a 40-sec hold time, and a 300 ml/min argon flow rate; step (3) atomize at 2400° for 6 sec with a 50 ml/min argon flow rate, and read; step (4) clean at 2600° with a 1-sec ramp period, a 5-sec hold time, and a 300 ml/min argon flow rate; and step (5) recharge at 20° with a 2-sec ramp period, a 20-sec hold time, and a 300 ml/min argon flow rate. Atomize 10 µl of the Matrix Modifier in combination with 10 µl of the Sample Solution under identical conditions used for the Standard Solutions. Repeat with 10 µl of the Matrix Modifier in combination with 10 µl of the Spiked Sample Solution.

Plot a standard curve using the concentration, in µg/ml, of each Standard Solution versus its maximum absorbance value compensated for background correction, and draw the best straight line. From the Standard Curve, determine the concentrations C_S and C_A in µg/ml, of the Sample Solution and the Spiked Sample Solution, respectively. Calculate the quantity, in mg/kg, of lead in the sample by the formula:

$$\frac{10 \times C_S}{W}$$

where
W = the weight, in g, of the sample taken.
Calculate the recovery by the formula:

$$\frac{C_S - C_A}{0.1} \times 100$$

where
0.1 = the amount of lead, in µg/ml, added to the Spiked Sample Solution.

1,6-Anhydro-D-glucose, glucose and sorbitol

Gas chromatography
- Octadecane Solution: Accurately weigh 50 mg of n-octadecane into a 100-ml volumetric flask and make up to volume with pyridine.
- Monomer Standard Solution: Weigh accurately 50 mg reagent grade alpha-D-glucose, 40 mg anhydrous D-sorbitol (min. 97% purity), and 35 mg of reagent grade (1,6-anhydro-D-glucose), into a 100-ml volumetric flask and make up to volume with pyridine.

Silylation of Monomer Standard Solution
Transfer 1.0 ml of Monomer Standard Solution to a screw-cap vial and add 1 ml of Octadecane Solution and 0.5 ml of N-trimethylsilylimidazole. Cap the vial and immerse in an ultrasonic bath at 70° for 60 min.

Gas Chromatograph conditions
Glass column, 2.44 m by 2 mm i.d. packed with 3% OV-1 on Gas Chrom Q 100/120 mesh. Flame ionization detector. Temperatures: column 175°; injection port 210°; detector 230°. Retention times (min): 1,6-anhydro-D-glucose, pyranose form 3.7; 1,6-anhydro-D-glucose furanose form (not present in standard) 4.3; n-octadecane 5.1; alpha-D-glucose 8.7; D-sorbitol 11.3; beta-D-glucose 13.3.

Procedure
Accurately weigh 20 mg of the sample into a screw-cap vial and add 1.0 ml of Octadecane Solution, 1 ml of pyridine, and 0.5 ml of N-trimethylsilylimidazole. Cap the vial and immerse in an ultrasonic bath at 70° for 60 min. Prior to sample analysis, inject 3 µl of the silylated Monomer Standard Solution into the gas chromatograph. Repeat two times, then inject 3 µl of the sample solution. Calculate the percentage of each monomer by the formula:

$$\frac{R \times W_S}{R_S \times W}$$

where
W = the weight of the sample in mg, adjusted for ash and moisture
W_S = the weight in mg of the monomer in the Monomer Standard Solution
R = the ratio of the area of the monomer peak to the area of the octadecane peak in the sample injection
R_S = the mean ratio of the area of the monomer peak to the area of the octadecane peak in the standard injections.
In the case of glucose, the peak areas for the alpha- and beta-epimers and in the case of 1,6-anhydro-D-glucose the peak areas for the pyranose form and furanose form are combined.

Molecular weight limit

Apparatus
Use a suitable high-pressure liquid chromatograph (HPLC) equipped with a differential refractometer, either a loop injector or suitable autosampler,

a column heating block or oven and a computing integrator, or computer data handling system with molecular weight determination capabilities. Use a Waters Ultrahydrogel 250 A size exclusion column, or equivalent. The column is maintained at 45^{o}, and the HPLC pump supplies eluent to it at 0.8 ml/min reproducible to 0.5%. The differential refractometer should be set at a sensitivity of 4×10^{-6} refractive index units full scale, and the plotter of the integrator should be set to 64 millivolts full scale. Maintain the detector cell at 35 ± 0.1^{o}. Noise attributable to the detector and electronics should be less than 0.1% full scale.

Eluent

The eluent is 0.1 N sodium nitrate containing 0.025% sodium azide. Dissolve 35.0 g of sodium nitrate and 1.0 g of sodium azide in 100 ml of HPLC-grade water. Filter through a 0.45-µm filter into a 4-l flask. Dilute to volume with HPLC-grade water. De-gas by applying an aspirator vacuum for 30 min.

Standard Solution

Transfer 20 mg each of dextrose, stachyose, 5800, 23,700, and 100,000 MW pullulan standards into a 10-ml volumetric flask. Dissolve in and dilute to volume with Eluent. Filter through a 0.45 µm syringe filter into a suitable autosampler vial, and seal (All components of the Standard Solution are available from Polymer Laboratories, Inc., Technical Center, Amherst Fields Research Park, 160 Old Farm Road, Amherst, MA 01002, USA).

Column Equilibration

After installation of a new column in the HPLC, pump Eluent through it overnight at 0.3 ml/min. Before calibration or analysis, increase the flow slowly to 0.8 ml/min over a 1-min period, then pump at 0.8 ml/min for at least 1 h before the first injection. Check the flow gravimetrically, and adjust it if necessary. Reduce the flow to 0.1 ml/min when the system is not in use.

Data System Setup

Set the integrator or computerized data handling system as their respective manuals instruct for normal gel permeation chromatographic determinations. Set the integration time to 15 min.

Column Standardization

After the HPLC system has been equilibrated at a flow rate of 0.8 ml/min for at least 1 h, inject 50 µl of the Standard Solution five times, allowing 15 min between injections. Record the retention times of the various components in the Standard Solution. Retention times for each component should agree within ± 2 sec. Insert the average retention time along with the molecular weight of each component into the calibration table of the molecular weight distribution software.

System Suitability

Check the regression results for a cubic fit of the calibration points. They should have an R^2 value of 0.9999+. Dextrose and stachyose should be baseline-resolved from one another and from the 5800 MW pullulan standard. Elevated valleys are usually observed between the 5800,

23,700 and 100,000 MW pullulan standards.

Sample Preparation
Transfer 50 mg of sample, accurately weighed, into a 10-ml volumetric flask. Dissolve in and dilute to volume with Eluent. Filter through a 0.45-µm syringe filter into a suitable autosampler vial.

Procedure
Inject 50 µl of the Sample Preparation, following the same conditions and procedure as described under Column Standardization. Using the Formula weight Distribution software of the data reduction system, generate a molecular weight distribution curve of the sample. There is no measurable peak above a molecular weight of 22,000.

5-Hydroxymethylfurfural (HMF)

Principle
HMF solutions absorb light in the ultraviolet region. The maximum absorption occurs at 283 nm and the molar extinction coefficient is 16,830 at that wavelength. The HMF concentration in polydextrose solutions is determined from the optical density at 283 nm and the application of the Beer-Lambert law.

Apparatus
- Standard laboratory equipment
- Ultraviolet spectrophotometer
- Spectrophotometer cells (quartz), 1.00 cm path length

Procedure
Accurately weigh 1.00±0.01 g of the sample into a 100-ml volumetric flask and make up to volume with distilled water (for polydextrose-N 70% solution use 1.43 ± 0.01 g sample). Read the optical density of this solution against a water blank at 283 nm in a 1.00 cm quartz cell in the spectrophotometer according to the directions supplied with the instrument. Under these conditions, the % HMF in the original sample is 0.0749 x optical density, on the dried basis.

Calculation

$$C = \frac{100 \times M \times D}{10 \times L \times E}$$

where
C = % HMF in the original polydextrose sample
M = HMF molecular weight
D = optical density of the solution
L = the path length of the spectrophotometer cell
E = the molar extinction coefficient for HMF
The numbers 100 and 10 are factors to convert solution concentration in mg/l to % HMF in the original sample, on the dried basis.

METHOD OF ASSAY

Phenol Solution
Add 20 ml of water to 80 g of phenol.

Glucose Standard Solutions

Weigh accurately 100 mg of alpha-D-glucose (minimum 97% purity) into a 500-ml volumetric flask and make up to volume with distilled water. Dilute five aliquots of the solution with distilled water to obtain the following concentrations of standard: 50, 40, 20, 10 and 5 µg/ml.

Standard Curve

Run each analysis in triplicate. On a daily basis, pipet 2.0 ml of each of the Glucose Standard Solutions into 4-dram (14.8 ml) acetone-free screw-cap vials. Add 0.12 ml of the phenol solution and mix gently. Uncap vials and add rapidly 5 ml of sulfuric acid TS. Immediately recap the vials and shake vigorously.

CAUTION: Rubber gloves and safety shield should be used in the sulfuric acid addition step.

Let the vials stand at room temperature for 45 min. Determine absorbances at 490 nm in a suitable spectrophotometer, using a Phenol Solution-sulfuric acid mixture as a blank in the reference cell. Plot mean absorbances versus concentrations in µg/ml.

Procedure

In triplicate, weigh accurately about 250 mg of the sample into a 250-ml volumetric flask and make up to volume with distilled water. Transfer a 10 ml aliquot to a 250-ml volumetric flask and make up to volume with distilled water. Proceed as in Standard Curve. Calculate the percentage of polymer by the formula:

$$\text{Polymer (\%)} = 1.05 \times \frac{100 \times A}{S \times C} - P_G - 1.11 \times P_1$$

where

A = the sample absorbance

S = the slope of absorbance versus glucose concentration in µg/ml obtained from the Standard Curve

C = the concentration of the sample solution in µg/ml (adjusted for ash and moisture)

P_G and P_1 = the percentages of glucose and 1,6-anhydro-D-glucose, respectively, determined by the tests for monomers (see Purity tests for 1,6-Anhydro-D-glucose, glucose and sorbitol as described above).

POLYDIMETHYLSILOXANE

Prepared at the 69th JECFA (2008), published in FAO JECFA Monographs 5 (2008), superseding specifications prepared at the 37th JECFA (1990), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI of 0-1.5 mg/kg bw was established at the 74th JECFA (2011).

SYNONYMS Poly(dimethylsiloxane); dimethylpolysiloxane; dimethylsilicone fluid; dimethylsilicone oil; dimethicone; INS No. 900a

DEFINITION Polydimethylsiloxane consists of fully methylated linear siloxane polymers containing repeating units of the formula $[(CH_3)_2SiO]$ with trimethylsiloxy end-blocking units of the formula $(CH_3)_3SiO$-. The additive is produced by hydrolysis of a mixture of dimethyldichlorosilane and a small quantity of trimethylchlorosilane. The average molecular weights of the linear polymers range from approximately 6,800 to 30,000.

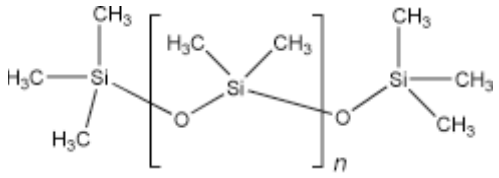
(NOTE: In commerce, polydimethylsiloxane is frequently used in preparations usually containing silica gel. The pure substance described in this monograph can be isolated from silica gel-containing liquids by centrifuging at about 20,000 rpm. Before testing the Polydimethylsiloxane for *Identification, Refractive index*, *Specific gravity*, and *Viscosity*, any silica gel present must be removed by centrifugation.)

(NOTE: This monograph does not apply to aqueous formulations of Polydimethylsiloxane containing emulsifying agents and preservatives, in addition to silica gel.)

Chemical names α-(Trimethylsilyl)-ω-methylpoly(oxy(dimethylsilylene))

C.A.S. number 9006-65-9

Structural formula



n ranges from 90 to 410

Assay Silicon content not less than 37.3% and not more than 38.5% of the total

DESCRIPTION Clear, colourless, viscous liquid.

FUNCTIONAL USES Antifoaming agent, anticaking agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Insoluble in water and in ethanol; soluble in most aliphatic and aromatic hydrocarbon solvents
Specific gravity (Vol. 4)	d^{25}_{25} : 0.964 - 0.977
Refractive index (Vol. 4)	n^{25}_{D} : 1.400 - 1.405
Infrared absorption	The infrared absorption spectrum of a liquid film of the sample between two sodium chloride plates exhibits relative maxima at the same wavelengths as those of a similar preparation of USP Dimethylpolysiloxane Reference Standard (available through http://www.usp.org/referenceStandards/catalog.html or by mail to USP 12601 Twinbrook Pkwy, Rockville, MD 20852 USA).

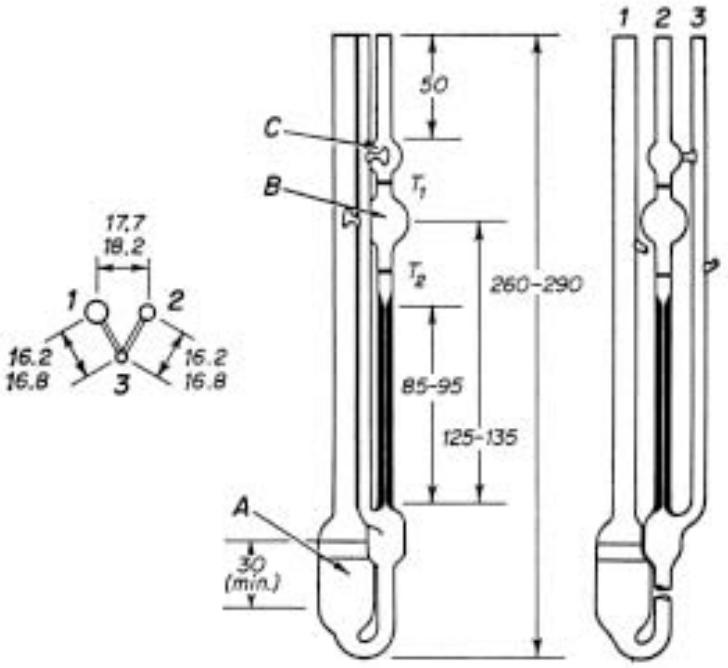
PURITY

Loss on drying (Vol.4)	Not more than 0.5% (150°, 4h)
Viscosity	100 - 1500 cSt at 25 ° See description under TESTS
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

TESTS

PURITY TESTS

Viscosity	The Ubbelohde suspended level viscometer, shown in the accompanying diagram, is preferred for the determination of the viscosity.



(Dimensions in mm)

For use in the range of 100 to 1,500 centistokes, a No. 3 size viscometer, having a capillary diameter of 2.00 + 0.04 mm, is required. The viscometer should be fitted with holders that satisfy the dimensional positions of the separate tubes as shown in the diagram, and that hold the viscometer vertical. Filling lines in bulb *A* indicate the minimum and maximum volumes of liquid to be used for convenient operation. The volume of bulb *B* is approximately 5 ml.

Calibration of the viscometer

Determine the viscosity constant, *k*, for each viscometer by using an oil of known viscosity. [NOTE: Choose an oil with a viscosity as close as possible to that of the sample to be tested.] Charge the viscometer by tilting the instrument about 30 degrees from the vertical, with bulb *A* below the capillary, and then introduce enough of the sample into tube *1* to bring the level up to the lower filling line. The level should not be above the upper filling line when the viscometer is returned to the vertical position and the sample has drained from tube *1.* Charge the viscometer in such a manner that the U-tube at the bottom fills completely without trapping air.

After the viscometer has been in a constant-temperature bath long enough for the sample to reach temperature equilibrium, place a finger over tube *3* and apply suction to tube *2* until the liquid reaches the centre of bulb *C*. Remove suction from tube *2*, then remove the finger from tube *3* and place it over tube *2* until the sample drops away from the lower end of the capillary. Remove the finger from tube *2,* and measure the time, to the nearest 0.1 sec required for the meniscus to pass from the first time mark (T_1) to the second (T_2). In order to obtain accurate results within a reasonable time, the apparatus should be adjusted to give an elapsed time of from 80 to 100 sec.

Calculate the viscometer constant k by the equation

$$k = v/t_1$$

where
v is the viscosity, in centistokes; and
t_1 is the efflux time, in sec, for the standard liquid.

Viscosity determination of Polydimethylsiloxane
Charge the viscometer with the sample in the same manner as described for the calibration procedure; determine the efflux time, t_2; and calculate the viscosity of the sample, v_s, by the equation

$$v_s = kt_2$$

METHOD OF ASSAY

Principle
Silicon in the sample is converted to a soluble form by fusion with sodium peroxide. Soluble silicon is measured in the percent range as total silicon by atomic absorption spectrophotometry.

Apparatus
- Fusion apparatus: Parr-type fusion cup; 500-ml nickel beaker; and nickel lid for beaker - or equivalent (avoid use of glassware during fusion and solubilization).
- Instrument: atomic absorption spectrophotometer with silicon hollow cathode lamp; nitrous oxide - acetylene burner, or equivalent.

Reagents
- Sodium peroxide, glacial acetic acid, silica (of known purity for use as standard).

Procedure
[CAUTION: Normal safe laboratory practices for Parr-type bomb fusion should be followed.]

Equivalent fusions must be performed on sample(s), reagent blank(s) and silica standards for each series of samples. For each sample weigh a portion (W) not to exceed 0.3 g into a Parr-type fusion cup (use gelatine capsules for liquid samples). Add 15.0±0.5 g of sodium peroxide.

Assemble the fusion apparatus and place it in a protective ignition rack. Fill the cavity above the cap with water and keep it full during ignition to prevent the gasket from melting. Heat the bottom of the cup with a blast lamp until the cup becomes cherry red about 100 mm up from the bottom within 90 sec. Quench the apparatus in ice water and disassemble the apparatus. Place the cup in the nickel beaker containing 150 to 200 ml of distilled water. Rinse any material adhering to the inside of the assembly cap into the beaker with distilled water. Cover the beaker with the nickel lid. When dissolution is complete and the solution has cooled, remove the cup from the beaker and rinse it with distilled water into the beaker. Add 55.0 ml of reagent grade glacial acetic acid to the beaker. Cool the solution to room temperature and transfer it to a 500 ml volumetric flask. Dilute to volume with distilled water. The solution should contain about 100 µg

silicon/ml for a sample weight of about 0.13 g. This method performs best if the silicon concentration of the final analysis solution is 1 to 200 µg/ml. Prepare a series of standards using the same fusion technique that brackets the sample.

Measure the absorbance of sample(s), reagent blank and standards at 251.6 nm with the spectrophotometer according to the manufacturer's operating instructions to obtain optimum analysis conditions for maximum lamp output and fuel and oxidant flow rate to the burner (or equivalent procedures for other vaporizing techniques). Adjust the zero absorbance while aspirating the solvent blank (water) used to dilute the samples. Measure the absorbance of sample(s), reagent blank and standards. Estimate the concentration of silicon in the sample solution from the standards, correcting for the reagent blank. Calculate the percent total silicon in the sample by the equation

$$\%\text{Silicon} = 0.05 \times C/W$$

where

C is the silicon concentration of the sample solution, µg/ml; and
W is the weight of sample taken, g.

POLYETHYLENE GLYCOLS

Prepared at the 31st JECFA (1987), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003). An ADI of 0-10 mg/kg bw was established at the 23rd JECFA (1979)

SYNONYMS PEG, Macrogol; INS No. 1521

DEFINITION Addition polymers of ethylene oxide and water usually designated by a number roughly corresponding to the molecular weight.

Chemical names alpha-Hydro-omega-hydroxypoly (oxy-1,2-ethanediol)

C.A.S. number 25322-68-3

Chemical formula $(C_2H_4O)_{n+1}H_2O$

Structural formula $HOCH_2 - (CH_2 - O - CH_2)_n - CH_2OH$

Formula weight 200-9500

DESCRIPTION PEG's below 700 molecular weight occur as clear to slightly hazy, colourless, slightly hygroscopic liquids with a slight characteristic odour. PEG's between 700-900 are semi-solid. PEG's over 1000 molecular weight are creamy white waxy solids, flakes, or free-flowing powders.

FUNCTIONAL USES Carrier solvent, excipient

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Polyethylene glycols having a molecular weight of 1000 or above are freely soluble in water; polyethylene glycols are soluble in many organic solvents, including aliphatic ketones and alcohols, chloroform, glycol ethers, esters, and aromatic hydrocarbons; they are insoluble in ether and in most aliphatic hydrocarbons; with increased molecular weight, water solubility and solubility in organic solvents decrease

Molecular weight PEG's having molecular weight below 1000: not less than 95.0% and not more than 105.0% of the declared value
PEG's having molecular weight between 1000 and 7000: not less than 90.0% and not more than 110.0% of the declared value
PEG's having molecular weight above 7000: not less than 87.5% and not more than 112.5% of the declared value
See description under TESTS

Viscosity The viscosity ranges at 100±0.3°, in cSt for PEG's of various molecular weight should be:

Average MW	Viscosity range, cSt

200	4.1-4.8
300	5.4-6.4
400	6.8-8.0
500	8.3-9.6
600	9.9-11.3
700	11.5-13.0
800	12.5-14.5
900	15.0-17.0
1000	16.0-19.0
1100	18.0-22.0
1200	20.0-24.5
1300	22.0-27.0
1400	24.0-30.0
1450	25.0-32.0
1500	26.0-33.0
1600	28.0-36.0
1700	31.0-39.0
1800	33.0-42.0
1900	35.0-45.0
2000	38.0-49.0
2100	40.0-53.0
2200	43.0-56.0
2300	46.0-60.0
2400	49-65
2500	51-70
2600	54-74
2700	57-78
2800	60-83
2900	64-88
3000	67-93
3250	73-105
3350	76-110
3500	87-123
3750	99-140
4000	110-158
4250	123-177
4500	140-200
4750	150-228
5000	170-250
5500	206-315
6000	250-390
6500	295-480
7000	350-590
7500	405-735
8000	470-900

For PEG's not listed in the table, calculate the limits by interpolation. See description under TESTS

PURITY

pH (Vol. 4) 4.5 - 7.5 (1 in 20 soln)

Sulfated ash (Vol. 4)	Not more than 0.1% w/w Test 5 g of the sample
Acidity	Not more than 0.05% w/w (as acetic acid) Transfer 6 g of the sample into a 250-ml Erlenmeyer flask, add phenolphthalein TS and 50 ml neutral ethanol and titrate with 0.1 N ethanolic potassium hydroxide to a pink end-point that persists for at least 15 sec. Not more than 0.5 ml is required.
1,4-Dioxane	Not more than 10 mg/kg. Proceed as directed in the Limit Test using *Gas chromatography*. See also Headspace gas chromatography method described below under the test method for Ethylene oxide.
Ethylene oxide	Not more than 0.02% See description under TESTS
Ethylene glycol and diethylene glycol	Total not more than 0.25% w/w individually or in combination See description under TESTS
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

IDENTIFICATION TESTS

Molecular weight

Phthalic anhydride solution

Place 49 g of phthalic anhydride in an amber bottle and dissolve it in 300 ml of pyridine that has been freshly distilled over phthalic anhydride. Shake the bottle vigorously until solution is effected, and allow to stand overnight before using.

Sample preparation for liquid polyethylene glycols

Carefully introduce 25 ml of the Phthalic anhydride solution into a clean, dry, heat-resistant pressure bottle. To the bottle add an accurately weighed amount of the sample equivalent to its expected molecular weight divided by 160. (Thus, a sample of about 1.3 g would be taken for PEG 200, or about 3.8 g for PEG 600). Stopper the bottle, and wrap it securely in a fabric bag.

Sample preparation for solid polyethylene glycols

Carefully introduce 25 ml of the Phthalic anhydride solution into a clean, dry, heat-resistant pressure bottle. To the bottle add an accurately weighed amount of the sample, previously melted, equivalent to its expected molecular weight divided by 160; because of limited solubility, however, do not use more than 25 g of any sample. Add 25 ml of pyridine, freshly distilled over phthalic anhydride, swirl to effect solution, stopper the bottle, and wrap it securely in a fabric bag.

Procedure

Immerse the sample bottle in a water bath, maintained between 96° and 100°, to the same depth as that of the mixture in the bottle. Heat it in the water bath for 30 to 60 min., then remove the bottle from the bath and allow it to cool to room temperature. Uncap the bottle carefully to release any pressure, remove the bottle from the fabric bag, add 5 drops of a 1 in 100 solution of phenolphthalein in pyridine, and titrate with 0.5 N sodium hydroxide to the first pink colour that persists for 15 sec, recording the volume, in ml of 0.5 N sodium hydroxide required as S. Perform a blank determination on 25 ml of the Phthalic anhydride solution plus any additional pyridine added to the sample bottle, and record the volume, in ml of 0.5 N sodium hydroxide required as B. Calculate the molecular weight of the sample by the formula:

$$Molecular\ weight = \frac{2000\,W}{(B - S)\,N}$$

where
W = weight of the sample in g
B = volume of 0.5 N NaOH consumed by the blank, in ml
S = volume of 0.5 N NaOH consumed by the sample, in ml
N = exact normality of NaOH

Alternative Tentative method using size exclusion chromatography (gel permeation chromatography)

1. Polyethylene glycols having nominal molecular weight below 1000

Apparatus

Use a suitable HPLC chromatograph equipped with a differential refractometer fitted with a 0.60 m x 7.7 mm (inside diameter) column packed with PL gel 10 µm 50 Å with tetrahydrofuran as the mobile phase.

Operating Conditions

The operating parameters may vary depending upon the particular instrument used but a suitable chromatogram may be obtained using the following conditions:
-Mobile phase flow rate: 1 ml/min
- Pressure: 35 bars
- Injected volume: 20 µl of a 1% (w/v) solution
- Temperature of detection: 25° ± 0.1°
The procedure allows the identification of PEG by comparison with a standard and to examine mixtures of PEG.

2. Polyethylene glycols having a nominal molecular weight of 1000 and higher

The determination is carried out with the same procedure but with a mobile phase: Tetrahydrofuran/n-heptane (80/20).
The elution volumes of PEG are approximately as follows depending on the particular instrument and operating conditions.

Molecular Mass	Elution Volume ml
35 000	21.2

10 000	22.8
6 000	24.2
4 000	25.1
2 000	26.8
1 000	28.4

Viscosity

Apparatus

The Ubbelohde suspended level viscometer, shown in the Figure is efficient for the determination of viscosity in the case of polyethylene glycols. This apparatus is preferred for the determination of viscosity. For use in the range of 300 to 600 centistrokes, a number 3 size viscometer, having a capillary diameter of 2.00±0.04 mm, is required. The viscometer should be fitted with holders which satisfy the dimensional positions of the separate tubes as shown in the diagram, and which hold the viscometer vertical. Filling lines in bulb A indicate the minimum and maximum volumes of liquid to be used for convenient operation. The volume of bulb B is approximately 5 ml.

Calibration of the Viscometer

Determine the viscosity constant (C) for each viscometer by using an oil of known viscosity. Charge the viscometer by tilting the instrument about 30 degrees from the vertical, with bulb A below the capillary, and then introduce enough of the sample into tube 1 to bring the level up to the lower filling line. The level should not be above the upper filling line when the viscometer is returned to the vertical position and the sample has drained from tube 1. Charge the viscometer in such a manner that the U-tube at the bottom fills completely without trapping air. After the viscometer has been in a constant temperature bath long enough for the sample to reach temperature equilibrium, place a finger over tube 3 and apply suction to tube 2 until the liquid reaches the center of bulb C. Remove suction from tube 2, then remove the finger from tube 3 and place it over tube 2 until the sample drops away from the lower end of the capillary. Remove the finger from tube 2, and measure the time, to the nearest 0.1 sec., required for the meniscus to pass from the first timing mark (T_1) to the second (T_2). In order to obtain accurate results within a reasonable time, the apparatus should be adjusted to give an elapsed time of from 80 to 100 sec.

Calculate the viscometer constant C by the equation $C = cSt/t_1$ in which cSt is the viscosity, in centistokes, and t_1 is the efflux time, in sec, for the standard liquid.

Determine the viscosity of the sample, maintaining the constant temperature bath at 100±0.3^o and using a capillary viscometer having a flow time of at least 200 sec for the PEG being tested. The viscosity must be within the limits specified in the table, or interpolated from the table.

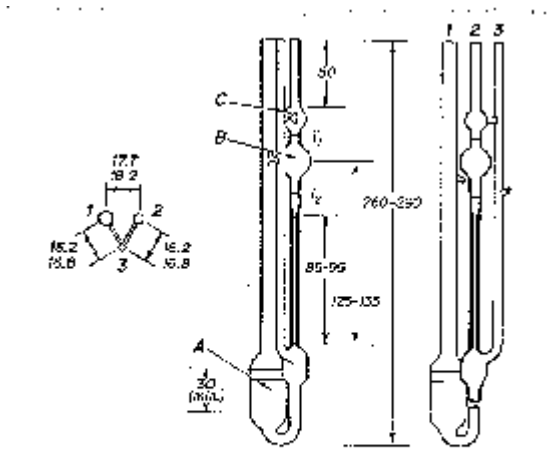


Figure. Ubbelohde Viscometer (all dimensions are in mm)

PURITY TESTS

Ethylene oxide

Morpholine solution
Mix one part of redistilled morpholine with nine parts of anhydrous methanol.

Mixed indicator
Weigh 0.050 g of 4,4'bis-(amino-1-naphthylazo-2,2'-stilbenedisulfonic acid) and 0.010 g of brilliant yellow into a 60-ml vial. Pipet 1.5 ml of 0.1 N sodium hydroxide into the vial, and mix. Add 3.5 ml of water, and mix. Transfer the mixture to a storage bottle with the aid of 45 ml of methanol as a rinse, and mix.

Standard methanolic hydrochloric acid
Mix 8.5 ml of hydrochloric acid and 1000 ml of anhydrous methanol, and standardize by titrating 9.00 ml with 0.1 N sodium hydroxide TS to a phenolphthalein end-point. Restandardize if this solution is used more than 48 h after standardization.

Procedure
Place 50 ml of anhydrous methanol into a 250-ml conical flask. Add 4 to 6 drops of Mixed indicator, and titrate with Standard methanolic hydrochloric acid to a clear blue colour.

Transfer to the flask about 25 g of the sample, accurately weighed, to provide the specimen blank, swirl to effect complete solution. Titrate with Standard methanolic hydrochloric acid to a clear blue colour, approaching the end-point carefully using small increments of titrant. Place 50 ml of Morpholine solution into a heat-resistant pressure bottle, and place an equal amount into a similar bottle to provide the reagent blank. To the first bottle add about 25 g of the sample, accurately weighed, and swirl to effect complete solution. Wrap the bottles securely in a cloth bag, and place them

close together in a water bath maintained at 98±2° for 30 min, keeping the water level in the bath just above the liquid level in the bottles. Remove the bottles from the bath, and allow them to cool in air to room temperature. When the bottles have cooled, loosen the wrappers, uncap to release any pressure, and remove the wrappers. Slowly add 20 ml of acetic anhydride to each bottle, and swirl to effect complete solution. Allow to stand at room temperature for 15 min. If the bottles are still warm, cool them to room temperature. To each bottle add 4 to 6 drops of Mixed indicator and titrate with Standard methanolic hydrochloric acid to a clear blue colour, adding very small increments when approaching the end-point.

Calculate the percentage of ethylene oxide by the formula:

$$4.41 \times N \times \frac{(A - B)}{W_1 - \frac{C}{W_2}}$$

where
N = the normality of the Standard methanolic hydrochloric acid
A, B, and C = the volumes (ml) required in the titration of the specimen, the reagent blank, and the specimen blank, respectively
W_1 and W_2 = the weights (g) of the sample taken for the reaction and the specimen blank, respectively

Headspace gas chromatography:
Alternative tentative method for 1,4-Dioxane and Ethylene oxide

Principle:
After addition of water to the sample, ethylene oxide and 1,4-dioxane are analysed by headspace gas chromatography.

Standard Solutions
- 1,4-Dioxane Standard Stock Solution
Standard solutions of 1,4-dioxane in water are prepared by weighing out about 1.00 g 1,4-dioxane/100 ml distilled water (stock solution) with successive dilutions. Two standard solutions of about 20 and 100 µg 1,4-dioxane/ml water are used.
- Ethylene Oxide (EO) Standard Stock Solution
In a 25 ml multidose injection vial, introduce 25 ml of distilled water. Close the vial with septum and cap with a gas tight syringe. Introduce slowly into the liquid 20 ml of ethylene oxide gas (about 40 mg). Determine the exact amount of ethylene oxide added by weighing the vial before and after the introduction of the gas (stock solution).

Prepare two working standard solutions by dilution with about 2 and 10 µg ethylene oxide per ml of water by successive dilutions.

Apparatus
Use a suitable gas chromatograph equipped with a flame-ionization detector (FID) containing a 30 m fused silica capillary column coated with dimethylpolysiloxane, internal diameter 0.25 mm, film thickness 1.0 µm.
Operating Conditions

The operating conditions may vary depending upon the particular instrument used, but the suitable chromatogram can be obtained using the following conditions:

Headspace Sampler Setting
- Temperature equilibration time: 45 min
- Temperature: 70°
- Transfer line temperature: 150°
- Pressurization time: 30 sec
- Injection time: 6 sec
- Analysis time: 45 min

Gas chromatography conditions
- Temperature: Column, 50° (5 min isothermal), then 5°/min to 180°
Detector (FID), 250°
Carrier gas: Helium, 1 ml/min
Carrier pressure: 0.7 bar
Split ratio: 40 : 1
Hydrogen and air: for FID

Sample Preparations
Transfer about 1 g of the sample accurately weighed (± 0.1 mg) in a headspace vial and add 1 ml of distilled water. Seal the vial and insert it into the headspace analyser for equilibration 45 min at 70°.
Prepare in the same conditions 2 vials with each 1 g sample accurately weighed (±0.1 mg) and 1 ml of standard stock solutions of 1,4-dioxane and EO.

Standard Solutions for Work
Two standard solutions A and B (for spiking) are prepared as follows:

A. 1 ml EO stock solution with ca. 200 mg EO/100 ml + 2 ml 1,4-dioxane stock solution with ca. 1000 mg dioxane/100 ml + water make up to 100 ml. This solution will be diluted 1 : 10 with water to yield a concentration of about 2 µg EO/ml and 20 µg 1,4-dioxane/ml.

B. 1 ml EO stock solution with ca. 500 mg EO/100 ml + 5 ml 1.4 dioxane stock solution with ca. 1000 mg dioxane/100 ml + water make up to 100 ml. This solution will be diluted 2 : 10 with water to yield a concentration of about 10 µg EO/ml and 100 µg 1.4-dioxane/ml.

Calculation
The concentration of compound i can be calculated by the following formula:

$$\mu g\,compound\,i\,/\,g = \frac{W_i \times A_i}{A_{is}}$$

where
µg compound i/g = Mass portion of 1,4-dioxane or EO in the sample [µg/g]
W_i = Mass of spiked compound i normalized to 1 g sample [µg/g]
A_i = Peak area of compound i in the sample, normalized to 1 g sample
A_{is} = Peak area of compound i in the spiked sample, normalized to 1 g

sample

Ethylene glycol and diethylene glycol

Polyethylene glycols having molecular weights below 450

Apparatus

Use a suitable gas chromatograph equipped with a hydrogen flame ionization detector, containing a 1.5 m x 3 mm (inside diameter) stainless steel column packed with sorbitol 12%, by weight, on 60/80 mesh non-acid-washed diatomaceous earth (Chromosorb W, or equivalent).

Operating conditions

The operating parameters may vary, depending upon the particular instrument used, but a suitable chromatogram may be obtained using the following conditions: Column temperature: 165º; Inlet temperature: 260º; Carrier gas: nitrogen (or suitable inert gas); flowing at a rate of 70 ml per min; Recorder: -0.5 to 1.05 mv, full span, 1 sec. full response time; Hydrogen and air flow to burner, optimize to give maximum sensitivity.

Standard solutions

Prepare chromatographic standards by dissolving accurately weighed amounts of commercial ethylene glycol and diethylene glycol, previously purified by distillation if necessary, in water. Suitable concentrations range from 1 to 6 mg of each glycol per ml.

Sample preparation

Transfer about 4 g of the sample, accurately weighed, into a 10-ml volumetric flask, dilute to volume with water and mix.

Procedure

Inject a 2 µl portion of each of the Standard solutions into the chromatograph, and obtain the chromatogram for each solution. Under the stated conditions, the elution time is approximately 2 min for ethylene glycol and 6.5 min for diethylene glycol. Measure the peak heights, and record the values as follows:

A = height, in mm, of the ethylene glycol peak;
B = weight, in mg, of ethylene glycol per ml of the Standard solution;
C = height, in mm, of the diethylene glycol peak; and
D = weight, in mg, of diethylene glycol per ml of the Standard solution

Similarly, inject a 2 µl portion of the Sample preparation into the chromatograph, and obtain the chromatogram, recording the height of the ethylene glycol peak as E and that of the diethylene glycol peak as F.

Calculation

Calculate the percent of ethylene glycol in the sample by the formula: (E x B) / A x sample weight in g.

Calculate the percent of diethylene glycol in the sample by the formula: (F x D) / C x sample weight in g.

Polyethylene glycols having molecular weights of 450 or higher

Sample preparation
Dissolve 50 g of the sample in 75 ml of diphenyl ether in a 250-ml distillation flask. Slowly distil at a pressure of 1-2 mm of mercury into a receiver graduated to 100 ml in 1-ml subdivisions, until 25 ml of distillate has been collected. Add 25 ml of water to the distillate, shake vigorously, and allow the layers to separate. Cool the container in an ice bath to solidify the diphenyl ether and to facilitate its removal. Filter the water layer through filter paper into a 50-ml glass-stoppered graduated cylinder, and to the filtrate add an equal volume of freshly distilled acetonitrile.

Standard preparation
Transfer 50 mg of diethylene glycol to a 25-ml volumetric flask, dilute to volume with a 1:1 mixture of freshly distilled acetonitrile and water, and mix.

Procedure
Transfer 10 ml of each of the Sample preparation and of the Standard preparation into separate 50-ml flasks each containing 15 ml of ceric ammonium nitrate TS, and mix. Within 2 to 5 min., determine the absorbance of each solution in a 1-cm cell at 450 nm, with a suitable spectrophotometer, using a blank, consisting of 15 ml of ceric ammonium nitrate TS and 10 ml of a 1:1 mixture of acetonitrile and water. The absorbance of the solution from the Sample preparation does not exceed that from the Standard preparation.

Alternative tentative method for the determination of mono and diethylene glycol

Test solution
In a 100 ml volumetric flask weigh 5.0 g of the substance to be examined, dissolve in acetone and dilute to 100.0 ml with acetone.

Reference solution
In a 100 ml volumetric flask weigh 100 mg of monoethylene glycol and 500 mg of diethylene glycol . Dilute to 100.0 ml with acetone. Dilute 1.0 ml of this solution to 10.0 ml with acetone.

Procedure
Gas chromatographic procedure may be carried out using:
- Glass column 1.8 m and 2 mm internal diameter, packed with diatomaceous earth for gas chromatography, washed and silanised (Chromosorb G.AW.DMCS 100-125 mesh is suitable), impregnated with 4% (m/m) of polyethylene glycol 20.000 (Carbowax 20 M is suitable).
- Nitrogen as carrier gas with a flow rate of 30 ml/min,
- Flame ionization detector

If necessary, preconditioning of the column may be carried out by heating at 200^{o} for about 15 h.

Adjust the initial temperature to obtain a retention time of 14 min to 16 min for diethylene glycol. Lower the temperature of the column to 140^{o}. Inject the solutions and raise the temperature of the column to 170^{o}, at a rate of 2^{o} per min. Maintain the temperature of the injection port at 250^{o} and that

of the detector at 250°. Inject 2.0 µl of the test solution and of the reference solution. Verify after five injections the repeatability of the response.

Calculation

Measure the peak areas of the mono and diethyleneglycol in the test and reference solutions. Calculate the concentration of the mono and diethylene glycol in the test solution from the peak areas.

POLYGLYCITOL SYRUP

Prepared at the 51st JECFA (1998) and published in FNP 52 Add 6 (1998). Group ADI "not specified" for polyglycitol and maltitol syrups, established at the 51st JECFA in 1998.

SYNONYMS Hydrogenated starch hydrolysate, polyglucitol

DEFINITION A mixture consisting mainly of maltitol and sorbitol and lesser amounts of hydrogenated oligo and polysaccharides and maltrotriitol. Manufactured by the catalytic hydrogenation of a mixture consisting of glucose, maltose, and higher glucose polymers; typically supplied as a syrup; may also be dried and supplied as a solid product

Assay Not less than 99.0% of total hydrogenated saccharides on the anhydrous basis and not more than 50.0% of maltitol and not more than 20.0% of sorbitol on the anhydrous basis.

DESCRIPTION Colourless and odourless, clear viscous liquids or white crystalline masses

FUNCTIONAL USES Sweetener, humectant, texturizer, stabilizer, bulking agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Very soluble in water; slightly soluble in ethanol

Test for maltitol
Passes test for maltitol
as directed under *Thin Layer Chromatography of Polyols*
Use the following:
Standard solution: Dissolve 50 mg of reference standard maltitol (available from US Pharmacopeial Convention, Inc. 12601 Twinbrook Parkway, Rockville, MD 20852, USA) in 20 ml water
Test solution: Dissolve 50 mg of the sample in 20 ml of water

Test for sorbitol To 5 g of the sample add 7 ml of methanol, 1 ml of benzaldehyde, and 1 ml of hydrochloric acid. Mix and shake in a mechanical shaker until crystals appear. Filter the crystals and dissolve in 20 ml of boiling water containing 1 g of sodium bicarbonate. Filter the hot solution and allow to cool until crystals are formed. Filter the crystals, wash with 5 ml of a water-methanol mixture (1 in 2), and dry in air. The crystals of the monobenzylidine derivative of sorbitol so obtained melt between 173 and 179º.

PURITY

Water (Vol. 4) Not more than 31% (Karl Fischer Method)

Sulfated ash (Vol. 4) Not more than 0.1%
Test 3 g of the sample (Method I)

Chlorides (Vol. 4) Not more than 50 mg/kg
Test 10 g of sample by the Limit Test using 1.5 ml of 0.01N hydrochloric acid in the control

Sulfates (Vol. 4)

Not more than 100 mg/kg
Test 10 g of sample by the Limit Test using 2.0 ml of 0.01N sulfuric acid in the control

Nickel (Vol. 4)

Not more than 2 mg/kg
Proceed as directed under *Nickel in Polyols*

Reducing sugars

Not more than 0.3%
Proceed as directed under *Reducing Substances (as glucose*), Method II. The weight of cuprous oxide shall not exceed 50 mg

Lead (Vol. 4)

Not more than 1 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

METHOD OF ASSAY

Total hydrogenated saccharides (%):

$$\frac{100 - (\text{Water\%} + \text{Sulfated ash\%} + \text{Reducing sugars\%})}{100 - \text{Water\%}} \times 100$$

Determine the maltitol and sorbitol content using liquid chromatography.

Apparatus
Liquid chromatograph (HPLC)
- Detection: Differential refractometer maintained at constant temperature
- Integrator recorder
- Column: AMINEX HPX 87 C or equivalent, (resin in calcium form), length 30 cm, internal diameter 9 mm
- Eluent: Double distilled degassed water (filtered through Millipore membrane filter or equivalent, 0.45 µm)
Chromatographic conditions
Column temperature: 85±0.5°
Eluent flow rate: 0.5 ml/min

Standard preparation:
Dissolve accurately weighed quantities of standard reference maltitol and sorbitol (available from US Pharmacopeial Convention, Inc., 12601 Twinbrook Parkway, Rockville, MD 20852, USA) in water to obtain a solution having known concentration of about 10.0 mg of maltitol and 5.0 mg of sorbitol per ml.

Sample preparation:
Transfer about 1 g of the sample accurately weighed to a 50-ml volumetric flask, dilute with water to volume and mix. Filter through a 0.2 micron filter.

Procedure:
Separately inject equal volumes (about 20 µl) of the sample preparation and the standard preparation into the chromatograph. Record the chromatograms and measure the responses of each polyol (maltitol,

sorbitol) peak. Calculate separately the quantities, in mg, of maltitol and sorbitol in the portion of syrup taken by the following formula:

$$50 \times C \times \frac{R_U}{R_S}$$

where
C = the concentration, in mg per ml, of the corresponding polyol in the standard preparation
R_U = the peak response of the polyol in the sample preparation
R_S = the peak response of the corresponding polyol in the standard preparation.

POLYOXYETHYLENE (20) SORBITAN MONOLAURATE

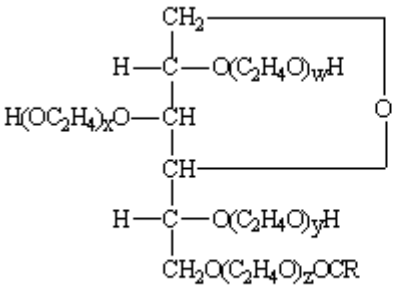
Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 55th JECFA (2000). An ADI of 0-25 mg/kg bw was established at the 17th JECFA (1973).

SYNONYMS Polysorbate 20; INS No. 432

DEFINITION Consists of a mixture of the partial esters of sorbitol and its mono- and dianhydrides (which have an acid value below 7 and a water content below 0.2%) with edible commercial lauric acid and condensed with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides.

Structural formula

Nominal formula and approximate composition:



where w + x + y + z = approx. 20 and RCO- is the fatty acid moiety

Assay

Not less than 70.0 and not more than 74.0% of oxyethylene groups, equivalent to not less than 97.3 and not more than 103.0% of polyoxyethylene (20) sorbitan monolaurate calculated on the anhydrous basis

DESCRIPTION Lemon to amber coloured oily liquid at 25°, with a faint characteristic odour

FUNCTIONAL USES Emulsifier, dispersing agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)

Soluble in water, ethanol, methanol, ethyl acetate and dioxane. Insoluble in mineral oil and petroleum ether

Infrared absorption

The infrared spectrum of the sample is characteristic of a partial fatty acid ester of a polyoxyethylated polyol

Colour reaction

To 5 ml of a 5% (w/v) aqueous solution of the sample add 10 ml of ammonium cobaltothiocyanate solution and 5 ml of chloroform, shake well

	and allow to separate; a blue colour is produced in the chloroform layer. (Ammonium cobaltothiocyanate solution: 37.5 g of cobalt nitrate and 150 g of ammonium thiocyanate made up to 100 ml with water - freshly prepared).
Test for fatty acids	To 5 ml of a 5% (w/v) aqueous solution of the sample add 5 ml sodium hydroxide TS. Boil for a few min, cool, and acidify with dilute hydrochloric acid. The solution is strongly opalescent, owing to the fatty acids liberated.
Saponification (Vol. 4)	100 g of the sample yields approximately 16 g of fatty acids and 81 g of polyol
PURITY	
Water (Vol. 4)	Not more than 3% (Karl Fischer Method)
Sulfated ash (Vol. 4)	Not more than 0.25% Test 5 g of the sample
Acid value (Vol. 4)	Not more than 2
Saponification value (Vol. 4)	Not less than 40 and not more than 50
Hydroxyl value (Vol. 4)	Not less than 96 and not more than 108
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Determine the content of *Oxyethylene groups*.

POLYOXYETHYLENE (20) SORBITAN MONOOLEATE

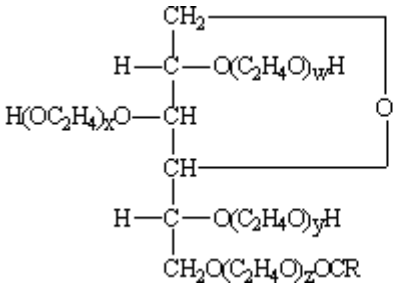
Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 55th JECFA (2000). An ADI of 0-25 mg/kg bw was established at the 17th JECFA (1973)

SYNONYMS Polysorbate 80; INS No. 433

DEFINITION Consists of a mixture of the partial esters of sorbitol and its mono- and dianhydrides (which have an acid value below 7.5 and a water content below 0.2%) with edible commercial oleic acid and condensed with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides.

C.A.S. number 9005-65-6

Structural formula Nominal formula and approximate composition:



where w + x + y + z = approx. 20 and RCO- is the fatty acid moiety

Assay Not less than 65.0 and not more than 69.5% of oxyethylene groups, equivalent to not less than 96.5 and not more than 103.5% of polyoxyethylene (20) sorbitan monooleate, calculated on the anhydrous basis.

DESCRIPTION Lemon to amber coloured oily liquid at 25°, with a faint characteristic odour

FUNCTIONAL USES Emulsifier, dispersing agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in water, ethanol, methanol, ethyl acetate and toluene; insoluble in mineral oil and petroleum ether

Infrared absorption The infrared spectrum of the sample is characteristic of a partial fatty acid ester of a polyoxyethylated polyol

Colour reaction To 5 ml of a 5% (w/v) aqueous solution of the sample add 10 ml of ammonium cobaltothiocyanate solution and 5 ml of chloroform, shake well and allow to separate; a blue colour is produced in the chloroform layer. (Ammonium cobaltothiocyanate solution: 37.5 g of cobalt nitrate and 150 g

	of ammonium thiocyanate made up to 100 ml with water - freshly prepared).
Test for fatty acids	To 5 ml of a 5% (w/v) aqueous solution of the sample add 5 ml sodium hydroxide TS. Boil for a few min, cool, and acidify with dilute hydrochloric acid. The solution is strongly opalescent, owing to the fatty acids liberated.
Test for unsaturation	To a solution of the sample (1 in 20) add bromine TS dropwise. The bromine is decolourized
Gelatinization	A mixture of 60 parts by volume of the sample and 40 parts of water yields a gelatinous mass at or below room temperature
Saponification (Vol. 4)	100 g of the sample yields approximately 23 g of fatty acids and 75 g of polyols
PURITY	
Water (Vol. 4)	Not more than 3% (Karl Fischer Method)
Sulfated ash (Vol. 4)	Not more than 0.25% Test 5 g of the sample
Acid value (Vol. 4)	Not more than 2
Saponification value (Vol. 4)	Not less than 45 and not more than 55
Hydroxyl value (Vol. 4)	Not less than 65 and not more than 80
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Determine the content of *Oxyethylene groups*.

POLYOXYETHYLENE (20) SORBITAN MONOPALMITATE

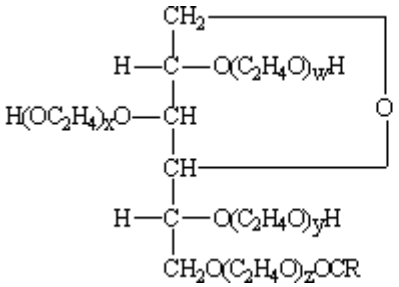
Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 55th JECFA (2000). An ADI of 0-25 mg/kg bw was established at the 17th JECFA (1973)

SYNONYMS Polysorbate 40; INS No. 434

DEFINITION Consists of a mixture of the partial esters of sorbitol and its mono- and dianhydrides (which have an acid value below 7.5 and a water content below 0.2%) with edible commercial palmitic acid condensed with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides.

C.A.S. number 9005-66-7

Structural formula Nominal formula and approximate composition:



where w + x + y + z = approx. 20 and RCO- is the fatty acid moiety

Assay Not less than 66.0 and not more than 70.5% of oxyethylene groups, equivalent to not less than 97.0 and not more than 103.0% of polyoxyethylene (20) sorbitan monopalmitate calculated on the anhydrous basis.

DESCRIPTION Lemon to orange coloured, oily liquid or semi-gel at 25°, with a faint characteristic odour

FUNCTIONAL USES Emulsifier, dispersing agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in water, ethanol, methanol, ethyl acetate and acetone; insoluble in mineral oil

Infrared absorption The infrared spectrum of the sample is characteristic of a partial fatty acid ester of a polyoxyethylated polyol

Colour reaction To 5 ml of a 5% (w/v) aqueous solution of the sample add 10 ml of ammonium cobaltothiocyanate solution and 5 ml of chloroform, shake well

	and allow to separate; a blue colour is produced in the chloroform layer. (Ammonium cobaltothiocyanate solution: 37.5 g of cobalt nitrate and 150 g of ammonium thiocyanate made up to 100 ml with water - freshly prepared).
Test for fatty acids	To 5 ml of a 5% (w/v) aqueous solution of the sample add 5 ml sodium hydroxide TS. Boil for a few min, cool, and acidify with dilute hydrochloric acid. The solution is strongly opalescent, owing to the fatty acids liberated.
Gelatinization	A mixture of 60 parts by volume of the sample and 40 parts of water yields a gelatinous mass at or below room temperature
Saponification (Vol. 4)	100 g of the sample yields approximately 20 g of fatty acids and 78 g of polyols
PURITY	
Water (Vol. 4)	Not more than 3% (Karl Fischer Method)
Sulfated ash (Vol. 4)	Not more than 0.25% Test 5 g of the sample
Acid value (Vol. 4)	Not more than 2
Saponification value (Vol. 4)	Not less than 41 and not more than 52
Hydroxyl value (Vol. 4)	Not less than 90 and not more than 107
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Determine the content of *Oxyethylene groups*.

POLYOXYETHYLENE (20) SORBITAN MONOSTEARATE

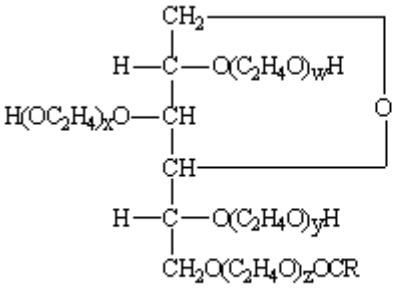
Prepared at the 25th JECFA (1981), published in FNP 19 (1981) and in FNP 52 (1992). Metals and arsenic specifications revised at the 55th JECFA (2000). An ADI of 0-25 mg/kg bw was established at the 17th JECFA (1973).

SYNONYMS Polysorbate 60; INS No. 435

DEFINITION Consists of a mixture of the partial esters of sorbitol and its mono- and dianhydrides (which have an acid value below 10 and a water content below 0.2%) with the edible commercial stearic acid and condensed with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides.

C.A.S. number 9005-07-6

Structural formula Nominal formula and approximate composition:



where w + x + y + z = approx. 20 and RCO- is the fatty acid moiety

Assay Not less than 65.0 and not more than 69.5% of oxyethylene groups, equivalent to not less than 97.0 and not more than 103.0% of polyoxyethylene (20) sorbitan monostearate, on the anhydrous basis

DESCRIPTION Lemon to orange coloured oily liquid or semi-gel at 25°, with a faint characteristic odour

FUNCTIONAL USES Emulsifier, dispersing agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in water, ethyl acetate, aniline and toluene; insoluble in mineral oil and vegetable oils

Infrared absorption The infrared spectrum of the sample is characteristic of a partial fatty acid ester of a polyoxyethylated polyol

Colour reaction	To 5 ml of a 5% (w/v) aqueous solution of the sample add 10 ml of ammonium cobaltothiocyanate solution and 5 ml of chloroform, shake well and allow to separate; a blue colour is produced in the chloroform layer. (Ammonium cobaltothiocyanate solution: 37.5 g of cobalt nitrate and 150 g of ammonium thiocyanate made up to 100 ml with water - freshly prepared).
Test for fatty acids	To 5 ml of a 5% (w/v) aqueous solution of the sample add 5 ml sodium hydroxide TS. Boil for a few min, cool, and acidify with dilute hydrochloric acid. The solution is strongly opalescent, owing to the fatty acids liberated.
Gelatinization	A mixture of 60 parts by volume of the sample and 40 parts of water yields a gelatinous mass at or below room temperature
Saponification (Vol. 4)	100 g of the sample yields approximately 25 g of fatty acids and 77 g of polyols
PURITY	
Water (Vol. 4)	Not more than 3% (Karl Fischer Method)
Sulfated ash (Vol. 4)	Not more than 0.25% Test 5 g of the sample
Acid value (Vol. 4)	Not more than 2
Saponification value (Vol. 4)	Not less than 41 and not more than 52
Hydroxyl value (Vol. 4)	Not less than 90 and not more than 107
1,4-Dioxane (Vol. 4)	Not more than 10 mg/kg
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Determine the content of *Oxyethylene groups*.

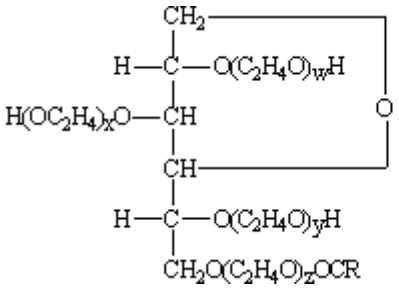
POLYOXYETHYLENE (20) SORBITAN TRISTEARATE

Prepared at the 25th JECFA (1981), published in FNP 19 (1981) and in FNP 52 (1992). Metals and arsenic specifications revised at the 55th JECFA (2000). An ADI of 0-25 mg/kg bw was established at the 17th JECFA (1973)

SYNONYMS Polysorbate 65; INS No. 436

DEFINITION Consists of a mixture of the partial esters of sorbitol and ist mono- and dianhydrides (which have an acid value below 15 and a water content below 0.2%) with edible commercial stearic acid and condensed with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides.

Structural formula Nominal formula and approximate composition:



where w + x + y + z = approx. 20 and RCO- is the fatty acid moiety

Assay Not less than 46.0 and not more than 50.0% of oxyethylene groups, equivalent to not less than 96.0 and not more than 104.0% of polyoxyethylene (20) sorbitan tristearate on the anhydrous basis

DESCRIPTION Tan coloured, waxy solid at 25°, with a faint characteristic odour

FUNCTIONAL USES Emulsifier, dispersing agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Dispersible in water; soluble in mineral oil, vegetable oils, petroleum ether, acetone, ether, dioxane, ethanol and methanol

Congealing range (Vol. 4) 29 - 33°

Infrared absorption The infrared spectrum of the sample is characteristic of a partial fatty acid ester of a polyoxyethylated polyol

Colour reaction To 5 ml of a 5% (w/v) aqueous solution of the sample add 10 ml of ammonium cobaltothiocyanate solution and 5 ml of chloroform, shake well and allow to separate; a blue colour is produced in the chloroform layer. (Ammonium cobaltothiocyanate solution: 37.5 g of cobalt nitrate and 150 g

	of ammonium thiocyanate made up to 100 ml with water - freshly prepared).
Test for fatty acids	To 5 ml of a 5% (w/v) aqueous solution of the sample add 5 ml sodium hydroxide TS. Boil for a few min, cool, and acidify with dilute hydrochloric acid. The solution is strongly opalescent, owing to the fatty acids liberated.
Saponification (Vol. 4)	100 g of the sample yields approximately 43 g of fatty acids and 56 g of polyols
PURITY	
Water (Vol. 4)	Not more than 3% (Karl Fischer Method)
Sulfated ash (Vol. 4)	Not more than 0.25% Test 2 g of the sample (Method I)
Acid value (Vol. 4)	Not more than 2
Saponification value (Vol. 4)	Not less than 88 and not more than 98
Hydroxyl value (Vol. 4)	Not less than 40 and not more than 60
1,4-Dioxane (Vol. 4)	Not more than 10 mg/kg
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Determine the content of *Oxyethylene groups*.

POLYVINYL ALCOHOL

Prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007), superseding specifications prepared at the 63rd JECFA (2004) and published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI of 50 mg/kg bw was established at 61st JECFA (2003).

SYNONYMS Vinyl alcohol polymer, PVOH, INS No. 1203

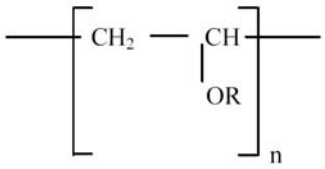
DEFINITION Polyvinyl alcohol is a synthetic resin prepared by the polymerization of vinyl acetate, followed by partial hydrolysis of the ester in the presence of an alkaline catalyst. The physical characteristics of the product depend on the degree of polymerization and the degree of hydrolysis.

Chemical names: Ethenol homopolymer

C.A.S. number: 9002-89-5

Chemical formula: $(C_2H_3OR)_n$ where R=H or $COCH_3$ (randomly distributed)

Structural formula



Where R=H or $COCH_3$ (randomly distributed)

DESCRIPTION Odourless, translucent, white or cream-coloured granular powder.

FUNCTIONAL USES Coating, binder, sealing agent and surface-finishing agent.

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Soluble in water, sparingly soluble in ethanol.

pH (Vol. 4): 5.0 – 6.5 (1 in 25)

Infrared spectrum (Vol. 4): The infrared absorption spectrum of a potassium bromide dispersion of the sample corresponds to that of a polyvinyl alcohol standard (see Appendix).

Colour reaction A: Dissolve 0.01 g of the sample in 100 ml of water with warming and let the solution cool to room temperature. To 5 ml of the solution, add one drop of iodine TS and a few drops of boric acid solution (1 in 25). A blue colour is produced.

Colour reaction B: Dissolve 0.5 g of the sample in 10 ml of water with warming and let the solution cool to room temperature. Add 1 drop of iodine TS to 5 ml of solution and allow to stand. A dark red to blue colour is produced.

Precipitation reaction: Add 10 ml of ethanol to the remaining 5 ml of solution prepared for Colour

reaction B. A white, turbid or flocculent precipitate is formed.

PURITY

Loss on drying (Vol. 4)	Not more than 5.0% (105°, 3 h)
Residue on ignition (Vol. 4)	Not more than 1.0%
Water insoluble substances (Vol. 4)	Not more than 0.1% Substitute a 100-mesh screen for the sintered-glass filter specified in Volume 4
Particle size	Not less than 99.0% material to pass through a 100 mesh sieve Determine by sieving for 30 min 100g of sample through a 100 mesh sieve and weigh the material passing through the sieve.
Methanol and methyl acetate	Not more than 1.0 % of each See description under TESTS
Acid value	Not more than 3.0 See description under TESTS
Ester value	Between 125 and 153 mg KOH/g See description under TESTS
Degree of hydrolysis	Between 86.5 and 89.0% See description under TESTS
Viscosity	4.8 - 5.8 mPa•s (4% solution at 20°) See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Vol. 4 (under "General Methods, Metallic Impurities).

TESTS

PURITY TESTS

Methanol and methyl acetate

Place 2.0 g of the sample into a 100 ml screw-cap bottle, and add a magnetic stirrer. Add 98 ml of water and 30 µl of acetone. Close the bottle tightly with the screw cap and heat in a water-bath, stirring continuously. Once the solution becomes clear, remove the bottle from the water bath and allow it to cool to room temperature.
Prepare a standard by taking 2 ml of a mixed solution of methanol and methyl acetate (1.2 % v/v solution), 98 ml of water and 30µl acetone; proceed as above starting from "close the bottle…Temperature".

GC Conditions:

Column:	Sunpak A (3.2 mm i.d. x 3 m) or equivalent
Column temperature:	160°
Injector temperature:	160°
Detector temperature:	160°

Inject 0.4 ± 0.1µl of the standard solution into the gas chromatograph and record the peak areas (PAs) for methanol, methyl acetate and acetone.
Inject 0.4 ± 0.1 µl of the sample solution and record the peak areas (PAs) for methanol, methyl acetate, and acetone.
Calculate the methanol and methyl acetate content using the formulae:

Methanol (wt%) = PA(methanol)/PA(acetone) x PR_1 x 0.024 x 100/2

Methyl acetate (wt %) = PA(methyl acetate)/ PA(acetone) x PR_2 x 0.024 x 100/2

where

0.024 is the conversion factor to obtain the masses of methanol and methyl acetate added to 30 µl acetone (density = 0.8) for the methanol/methyl acetate standard; and

PR_1 and PR_2 are the peak area ratios PA(acetone)/PA(methanol) and PA(acetone)/PA(methyl acetate), respectively, of the standard 1.2% methanol and methyl acetate aqueous solutions.

Acid value

Add 200 ml of water and a stir bar into a 500-ml round-bottom flask, attach a reflux condenser and begin heating in a boiling water bath. Add 10.0 g of the sample and continue heating for 30 min while stirring continuously. Remove the flask from the water bath and continue stirring until the solution reaches room temperature. Quantitatively transfer this solution to a 250-ml volumetric flask and dilute to volume with water. Take 50 ml of the solution, add 1 ml of phenolphthalein TS and titrate with 0.05 M potassium hydroxide until the pink colour persists for 15 sec; record the titre in ml (V). Calculate the acid value, A:

$$A = 5.0(56.1 \times V \times M)/W$$

where

56.1 is the formula weight of KOH,
M is the molarity of the KOH solution, and
W is the weight of sample (g).

Ester value

Accurately weigh about 1.0 g of sample into a 250-ml round-bottom flask, add 25 ml 0.5 M alcoholic potassium hydroxide, 25.0 ml of water and a few glass beads. Attach a condenser and allow the contents to reflux for 30 minutes in a boiling water-bath. Let cool to room temperature, remove the condenser, add 1 ml of phenolphthalein TS and titrate immediately with 0.5 M hydrochloric acid; record the titre in ml (V_1).

Carry out a blank test under the same conditions. Titrate with 0.5 M hydrochloric acid and record the titre in ml (V_2). Calculate the saponification value, S:

$$S = 56.1(V_2 - V_1) \times M/W$$

where

56.1 is the formula weight of KOH,
M is the molarity of the hydrochloric acid solution, and
W is the weight of the sample in (g).

Calculate the ester value, E:

$$E = S - A$$

where

S is the saponification value and
A is acid value.

Degree of hydrolysis

Convert the saponification value obtained during the determination of the ester value to the "dried basis" (S_{db}):

$$S_{db} = (S \times 100)/(100 - LOD)$$

where

LOD is Loss on Drying

The degree of hydrolysis is:

$$100 - [7.84\ S_{db}/(100 - 0.075\ S_{db})]$$

Viscosity

Calibration of *capillary-type* viscometers

An oil of known viscosity is used to determine the viscometer constant (k*)*. *Ostwald-Type Viscometer*: Fill the tube with the exact amount of oil (adjusted to 20.0±0.1°), as specified by the manufacturer. Use either pressure or suction to adjust the meniscus of the column of liquid in the capillary tube to the level of the top graduation line. Allow the liquid to flow into the reservoir against atmospheric pressure by opening both the filling and capillary tubes. If either tube is not open, false values might be obtained. Record the time (seconds), for the liquid to flow from the upper mark to the lower mark of the capillary tube (efflux time).

Ubbelohde-Type Viscometer: Place a quantity of the oil (adjusted to 20.0 ± 0.1°) in the filling tube, and transfer to the capillary tube by gentle suction. Keep the air vent tube closed in order to prevent bubble formation in the liquid. Adjust the meniscus of the column of liquid in the capillary tube to the level of the top graduation line. Allow the liquid to flow into the reservoir against atmospheric pressure by opening both the filling and capillary tubes. If either tube is not open, false values might be obtained. Record the efflux time (seconds).

The viscosity constant for *capillary-type* viscometers is given by:

$$k = v/dt,$$

where v is the known viscosity (mPa•s) of the oil used for viscometer calibration; d is the density (g/ml) of the liquid tested at 20°/20°; and t (seconds) is the efflux time.

Procedure

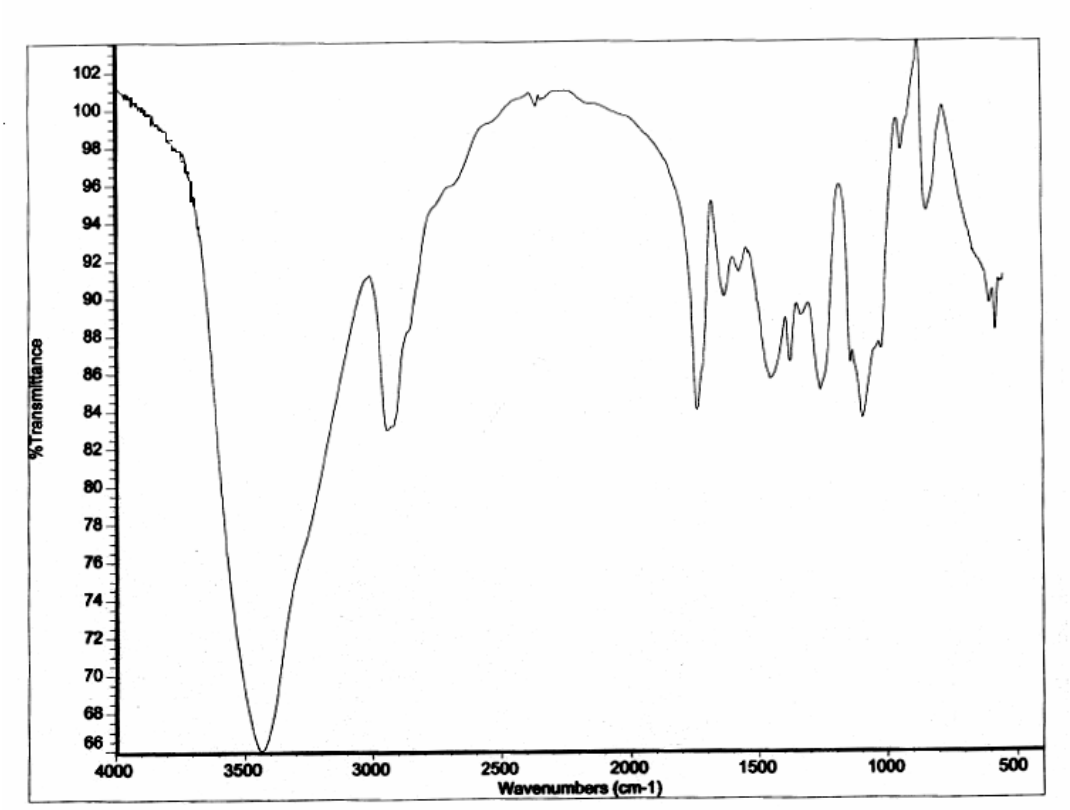
Weigh a quantity of undried sample equivalent to 6.00 g on the dried basis. Into a tared 250-ml flask containing a magnetic stir bar and approximately 140 ml of water, quickly (seconds) transfer the sample, while simultaneously stirring slowly and continuously. Once the sample appears thoroughly saturated, slowly increase the stirring rate to minimize the entrainment of air in the mixture. Heat the mixture to 90°, and maintain it at this temperature for approximately 5 minutes; discontinue heating and continue stirring for 1 hour. Add water in small amounts to attain a total mixture weight of 150 g, and resume stirring until the mixture appears homogenous. Filter the mixture through a tared 100-mesh screen into a 250 ml conical flask, cool the filtrate to about 15°, mix, and determine its

viscosity at 20° using an appropriate viscometer (follow the manufacturer's instructions). NOTE: The temperature at which the viscosity measurement is made must be strictly controlled.
For measurements using *capillary-type* viscometers, the viscosity is given by:

$$v = kdt$$

where t is the efflux time for the sample solution and d is its density at 20°.

Appendix



POLYVINYL ALCOHOL

POLYVINYLPYRROLIDONE

Prepared at the 30th JECFA (1986), published in FNP 37 (1986) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). An ADI of 0-50 mg/kg bw was established at the 30th JECFA (1986)

SYNONYMS Povidone, PVP; INS No. 1201

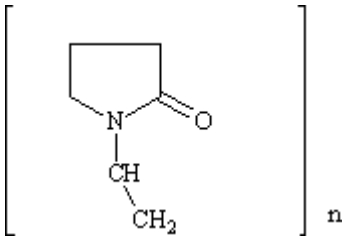
DEFINITION

Chemical names: Polyvinylpyrrolidone, poly-[1-(2-oxo-1-pyrrolidinyl)- ethylene]

C.A.S. number: 9003-39-8

Chemical formula: $(C_6H_9NO)_n$

Structural formula:



Formula weight: Lower molecular weight range product: about 40 000
Higher molecular weight range product: about 360 000

Assay: Not less than 12.2% and not more than 13.0% of Nitrogen (N) on the anhydrous basis

DESCRIPTION White to tan powder; supplied in two molecular weight forms; the molecular weight value is an average molecular weight for the two forms

FUNCTIONAL USES Clarifying agent, stabilizer, bodying agent, tableting adjunct, dispersing agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Soluble in water, in ethanol and in chloroform; insoluble in ether

pH (Vol. 4): 3.0 - 7.0 (5% soln)

Precipitate formation: To 5 ml of a 1 in 50 solution of the sample add 5 ml of dilute hydrochloric acid TS, 5 ml of water and 2 ml of 1 in 10 solution of potassium dichromate. A yellow precipitate forms.

Add 5 ml of a 1 in 50 solution of the sample to 75 mg of cobalt nitrate and 0.3 g of ammonium thiocyanate dissolved in 2 ml of water, mix and acidify with dilute hydrochloric acid TS. A pale blue precipitate forms.

To 5 ml of a 1 in 50 solution of the sample add 1 ml of 25% hydrochloric acid and 5 ml of 5% barium chloride solution and 1 ml of 5% phosphomolybdotungstic acid solution. A voluminous white precipitate is formed which becomes gradually blue on standing in daylight. (Note: The blue colouration on exposure to light distinguishes polyvinylpyrrolidone from polyethylene oxide adducts which give similar precipitates with the same reagents but which retain their white colour in light).

PURITY

Water (Vol. 4)	Not more than 5% (Karl Fischer Method)
Relative viscosity	Between 1.188 and 1.325 for lower molecular weight product, and between 3.225 and 5.662 for higher molecular weight product See description under TESTS.
Total ash (Vol. 4)	Not more than 0.02% Test 10 g of the sample
Aldehyde	Not more than 0.2% (as acetaldehyde) See description under TESTS
Monomer content	Not more than 1% (as vinylpyrrolidone) See description under TESTS
Hydrazine	Not more than 1 mg/kg See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Relative viscosity	Transfer an accurately weighed portion of the sample, equivalent to 1 g of anhydrous polyvinylpyrrolidone, into a 250 ml conical flask, and calculate the amount of water to be added to make a 1% solution. Allow the mixture to stand at room temperature, with occasional swirling, until solution is complete, and then allow to stand for 1 h. longer. Filter through a dry sintered-glass filter funnel of coarse porosity, then pipet 10 ml of the filtrate into a Cannon-Fenske viscometer, or equivalent, and place the viscometer in a water bath maintained at 25±0.05°. After allowing the sample solution and pipet to warm in the water bath for 10 min., draw the solution by means of very gentle suction up through the capillary until the meniscus is formed from 3 to 4 mm above the upper etched mark. Release the vacuum, and, when the meniscus reaches the upper etched mark, begin timing the flow through the capillary. Record the exact time when the meniscus reaches the lower etched mark, and calculate the flow time to the nearest 0.1 sec. Repeat this operation until at least three readings are obtained. The

readings must agree within 0.3 sec.; if not, repeat the determination with additional 10 ml portions of the sample solution after recleaning the viscosity pipet with sulfuric acid-dichromate cleaning solution.

Calculate the average flow time for the sample solution, and then obtain the average flow time in similar manner for 10 ml of filtered water for the same viscosity pipet. Calculate the relative viscosity of the sample by dividing the average flow time for the sample solution by that of the water sample.

Aldehyde

Transfer about 10 g of the sample, accurately weighed and dissolved in 300 ml of water, into a 1000 ml round-bottom flask containing 80 ml of 25% sulfuric acid, reflux for about 45 min. under a water-cooled condenser, and then distil about 100 ml into a receiver containing 20 ml of 1 N hydroxylamine hydrochloride previously adjusted to pH 3.1. Titrate the contents of the receiver with 0.1 N sodium hydroxide to a pH of 3.1, using a pH meter. Perform a blank determination and make any necessary correction. Each ml of 0.1 N sodium hydroxide is equivalent to 4.405 mg of C_2H_4O.

Monomer content

Dissolve about 4 g of the sample, accurately weighed, in 30 ml of water in a 125 ml round-bottom flask, add 0.5 g of sodium acetate, mix and begin titrating with 0.1 N iodine. When the iodine colour no longer fades, add additional 3.0 ml of the titrant, and allow the solution to stand for 5 to 10 min. Add starch TS, and titrate the excess iodine with 0.1 N sodium thiosulfate. Perform a blank determination, using the same volume of 0.1 N iodine, accurately measured, as was used for the sample. Each ml of 0.1 N iodine is equivalent to 5.56 mg of vinylpyrrolidone.

Hydrazine

Transfer 2.5 g of the sample into a 50-ml centrifuge tube, add 25 ml of a 1 in 20 solution of salicylaldehyde in methanol, swirl, and heat in a water bath at 60^{o} for 15 min. Allow to cool, add 2.0 ml of toluene, insert a stopper in the tube, shake vigorously for 2 min, and centrifuge. On a suitable thin-layer chromatographic plate, coated with a 0.25-mm layer of dimethylsilanized chromatographic silica gel mixture, apply 10 µl of the clear upper toluene layer in the centrifuge tube and 10 µl of a Standard solution of salicylaldazine in toluene containing 9.38 µg per ml. Allow the spots to dry, and develop the chromatogram in a solvent system of methanol and water (2:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under ultraviolet light at a wavelength of 365 nm: salicylaldazine appears as a fluorescent spot having an R_f value of about 0.3, and the fluorescence of any salicylaldazine spot from the test specimen is not more intense than that produced by the spot obtained from the Standard solution (1 ppm of hydrazine).

Preparation of Salicylaldazine Standard:

Dissolve 300 mg of hydrazine sulfate in 5 ml of a freshly prepared 20% (v/v) solution of salicylaldehyde in isopropyl alcohol, mix, and allow to stand until a yellow precipitate forms. Extract the mixture with two 15-ml portions of methylene chloride. Combine the methylene chloride extracts, and dry over anhydrous sodium sulfate. Decant the methylene chloride solution, and evaporate it to dryness. Recrystallize the residue of salicyclaldazine from a mixture of warm toluene and methanol (60:40) with cooling; filter and dry the

crystals in vacuum. The crystals have a melting range of 213° to 214°.

METHOD OF ASSAY

Determine as directed under *Nitrogen Determination* in Volume 4, using about 1 g of the sample, accurately weighed.

PONCEAU 4R

Prepared at the 74th JECFA (2011) and published in FAO JECFA Monographs 11 (2011), superseding specifications prepared at the 28th JECFA (1984), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI of 0-4 mg/kg bw was established at the 27th JECFA (1983) and maintained at the 74thJECFA (2011).

SYNONYMS CI Food Red 7; Cochineal Red A; New Coccine; Brilliant Scarlet; CI (1975) No. 16255; INS No. 124

DEFINITION Ponceau 4R consists essentially of trisodium 2-hydroxy-1-(4-sulfonato-1-naphthylazo)-6,8-naphthalenedisulfonate, and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.

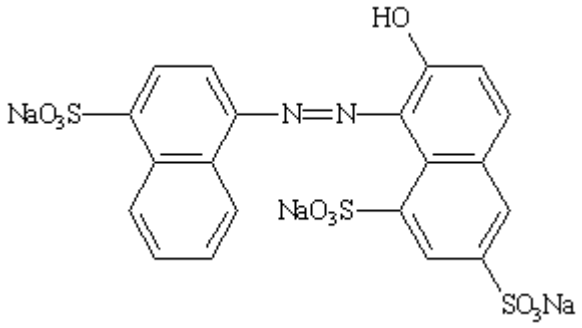
May be converted to the corresponding aluminium lake, in which case only the *General Specifications for Aluminium Lakes of Colouring Matters* apply.

Chemical names Trisodium 2-hydroxy-1-(4-sulfonato-1-naphthylazo)-6,8-naphthalenedisulfonate

C.A.S. number 2611-82-7

Chemical formula $C_{20}H_{11}N_2Na_3O_{10}S_3$

Structural formula



Formula weight 604.48

Assay Not less than 80% total colouring matters

DESCRIPTION Reddish powder or granules

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water; sparingly soluble in ethanol
Spectrophotometry	Maximum wave length: Between 505 and 510 nm Determine the UV-visible absorption spectrum of the sample solution dissolved in 0.02 mol/l ammonium acetate.

PURITY

Loss on drying (Vol. 4)	Not more than 20% at 135° together with chloride and sulfate calculated as sodium salts Determine using Loss on Drying under “GENERAL METHODS”, Chloride as Sodium Chloride and Sulfate as Sodium Sulfate under “SPECIFIC METHODS, Food Colours” in Volume 4.
Water-insoluble matter (Vol. 4)	Not more than 0.2%
Subsidiary colouring matters	Not more than 1% See description under TESTS
Organic compounds other than colouring matters (Vol. 4)	Not more than 0.5% of sum of 4-amino-1-naphthalenesulfonic acid, 7-hydroxy-1,3-naphthalenedisulfonic acid, 3-hydroxy-2,7-naphthalenesulfonic acid, 6-hydroxy-2-naphthalenesulfonic acid, and 7-hydroxy-1,3,6-naphthalenetrisulfonic acid. (See Volume 4 under “SPECIFIC METHODS, Food Colours”) Proceed as directed under *Determination by High Performance Liquid Chromatography* using the conditions of Subsidiary colouring matters except detector wavelength (238 nm).
Unsulfonated primary aromatic amines (Vol. 4)	Not more than 0.01% calculated as aniline (See Volume 4 under “SPECIFIC METHODS, Food Colours”)
Ether-extractable matter (Vol. 4)	Not more than 0.2% (See Volume 4 under “SPECIFIC METHODS, Food Colours, Method II”) Use 2 g of sample for the test.
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under “General Methods, Metallic Impurities”).

TESTS

PURITY TESTS

Subsidiary colouring matters Determine by HPLC using the following conditions:

Chromatography conditions
- HPLC system with a UV/VIS detector or a diode array detector
- Detector wavelength: 510 nm
- Column: C18 on silica gel (250 x 4.6 mm, 5 µm)
- Mobile phase: solvent A: 0.02 mol/l ammonium acetate and solvent B: acetonitrile:water (7:3 v/v)
- Gradient elution: A:B 100:0 v/v to A:B 40:60 v/v (0-30 min); hold at A:B 40:60 v/v (30-35 min).
- Column temperature: 40°
- Flow rate: 1.0 ml/min

Procedure
Accurately weigh 10 mg of the sample into a 100-ml volumetric flask. Dissolve and make to volume with 0.02 mol/l ammonium acetate. Filter through a 0.45 µm membrane filter. Inject 20 µl of the sample solution into HPLC.

Calculation
Calculate the percentage of subsidiary colouring matters from;

$$\text{Subsidiary colouring matters } (\%) = \left(\frac{A_{total} - A_{main}}{A_{total}} \right) \times D \times 100$$

where
D is the total colouring matters content of sample (%);
A_{total} is the sum of the area of all the peaks in the chromatogram between 2 and 40 min; and
A_{main} is the area of main peak.

METHOD OF ASSAY Proceed as directed under *Colouring Matters Content by Titration with Titanous Chloride* in Volume 4 (under "Specific Methods, Food Colours), using the following:

Weight of sample: 0.7-0.8 g
Buffer: 10 g sodium citrate
Weight (D) of colouring matters equivalent to 1.00 ml of 0.1 N $TiCl_3$: 0.01511 g

DIPOTASSIUM 5'-INOSINATE

Prepared at the 29th JECFA (1985), published in FNP 34 (1986) and FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). A group ADI 'not specified' for inosinic acid and its Ca, K & Na salts, was established at the 29th JECFA (1985)

SYNONYMS Potassium inosinate, potassium 5'-inosinate, INS No. 632

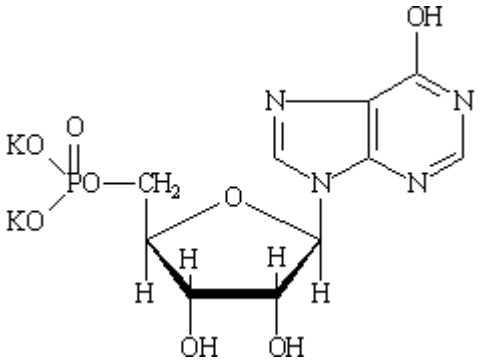
DEFINITION

Chemical names: Dipotassium inosine-5'-monophosphate

C.A.S. number: 20262-26-4

Chemical formula: $C_{10}H_{11}K_2N_4O_8P$

Structural formula:



Formula weight: 424.39

Assay: Not less than 97.0% and not more than 102.0% on the anhydrous basis

DESCRIPTION Odourless, colourless or white crystals, or a white crystalline powder

FUNCTIONAL USES Flavour enhancer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water; practically insoluble in ethanol

Spectrophotometry (Vol. 4): A 1 in 50,000 solution of the sample in 0.01 N hydrochloric acid exhibits an absorbance maximum at 250±2 nm. The ratio A250/A260 is between 1.55 and 1.65, and the ratio A280/260 is between 0.20 and 0.30.

Test for potassium (Vol. 4): Passes test

Test for ribose (Vol. 4): Passes test

Test for organic phosphate (Vol. 4): Passes test
Test 5 ml of a 1 in 20 solution

PURITY

Water (Vol. 4)	Not more than 10% (Karl Fischer Method)
pH (Vol. 4)	7.0 - 8.5 (1 in 20 soln)
Related foreign substances (Vol. 4)	Chromatographically not detectable Test 1 µl of a 1 in 200 soln
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh accurately about 0.5 g of the sample, dissolve in and make to 1,000 ml with 0.01 N hydrochloric acid. Take 10 ml of this solution and dilute with 0.01 N hydrochloric acid to 250 ml. Determine the absorbance A of the solution in a 1-cm cell at the wave length of 250 nm using 0.01 N hydrochloric acid as the reference. Calculate the content of $C_{10}H_{11}K_2N_4O_8P$, in % in the sample by the formula:

$$\frac{A}{286.5} \times \frac{250,000}{\text{weight of sample (mg)}} \times \frac{100}{100 - \text{water \%}} \times 100$$

POTASSIUM ACETATE

Prepared at the 18th JECFA (1974), published in NMRS 54B (1975) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). A group ADI 'Not limited' for acetic acid and its K & Na salts was established at the 17th JECFA (1973).

SYNONYMS INS No. 261

DEFINITION

Chemical names	Potassium acetate
C.A.S. number	127-08-2
Chemical formula	$C_2H_3KO_2$
Structural formula	CH_3-COOK
Formula weight	98.14
Assay	Not less than 99.0% after drying

DESCRIPTION Colourless, deliquescent crystals or a white, crystalline powder, odourless or with a faint acetic odour

FUNCTIONAL USES Buffer, antimicrobial preservative

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Very soluble in water; freely soluble in ethanol
pH (Vol. 4)	7.5 - 9.0 (1 in 20 soln)
Test for potassium (Vol. 4)	Passes test
Test for acetate (Vol. 4)	Passes test

PURITY

Loss on drying (Vol. 4)	Not more than 8.0% (150°, 2 h)
Alkalinity	Dissolve 1 g of the sample in 20 ml of freshly boiled and cooled water, and add 3 drops of phenolphthalein TS. If a pink colour is produced, not more than 0.5 ml of 0.1 N hydrochloric acid should be required to discharge it.

Test for sodium (Vol. 4)	Negative test
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Dissolve about 200 mg of the dried sample, accurately weighed, in 25 ml of glacial acetic acid. Add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid in glacial acetic acid. Perform a blank determination, and make any necessary correction. Each ml of 0.1 N perchloric acid is equivalent to 9.814 mg of $C_2H_3KO_2$

POTASSIUM ALGINATE

Prepared at the 49th JECFA (1997) , published in FNP 52 Add 5 (1997) superseding specifications prepared at the 44th JECFA (1995), published in FNP 52 Add 3 (1995). An ADI 'not specified' was established at the 39th JECFA (1992)

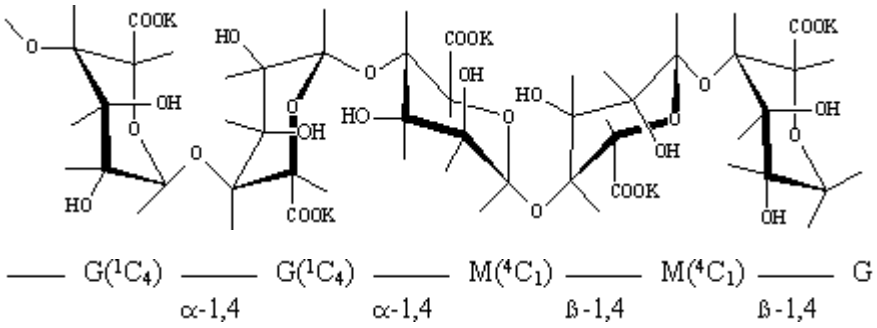
SYNONYMS INS No. 402

DEFINITION Potassium salt of alginic acid

C.A.S. number 9005-36-1

Chemical formula $(C_6H_7KO_6)_n$

Structural formula Structural formula from Phillips, Wedlock, Williams: Gums and Stabilizers for the Food Industry 5 (1990) by permission of Oxford University Press.



The number and sequence of the Mannuronate and Glucuronate residues shown above vary in the naturally occurring alginate. The associated water molecules are not shown.

Formula weight Structural unit: 214.22 (theoretical) , 238 (actual average)
Macromolecule: 10,000 - 600,000 (typical average)

Assay Yields, on the dried basis, not less than 16.5% and not more than 19.5% of carbon dioxide (CO_2), equivalent to not less than 89.2% and not more than 105.5% of potassium alginate $(C_6H_7KO_6)_n$.

DESCRIPTION White to yellowish brown filamentous, grainy, granular or powdered forms

FUNCTIONAL USES Stabilizer, thickener, gelling agent, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Dissolves slowly in water forming a viscous solution; insoluble in ethanol and ether

Precipitate formation with To a 0.5% solution of the sample in sodium hydroxide TS add one-fifth of its

calcium chloride	volume of a 2.5% solution of calcium chloride. A voluminous, gelatinous precipitate is formed. This test distinguishes potassium alginate from gum arabic, sodium carboxymethyl cellulose, carrageenan, gelatin, gum ghatti, karaya gum, carob bean gum, methyl cellulose and tragacanth gum.
Precipitate formation with ammonium sulfate	To a 0.5% solution of the sample in sodium hydroxide TS add one-half of its volume of a saturated solution of ammonium sulfate. No precipitate is formed. This test distinguishes potassium alginate from agar, sodium carboxymethyl cellulose, carrageenan, de-esterified pectin, gelatin, carob bean gum, methyl cellulose and starch.
Test for alginate (Vol. 4)	Passes test
Potassium (Vol. 4)	Passes test
PURITY	
Loss on drying (Vol. 4)	Not more than 15% (105°, 4 h)
Water-insoluble matter	Not more than 2% on the dried basis See description under TESTS
Arsenic (Vol. 4)	Not more than 3 mg/kg (Method II)
Lead (Vol. 4)	Not more than 5 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
Microbiological criteria (Vol. 4)	Total plate count: Not more than 5,000 colonies per gram. Initially prepare a 10^{-1} dilution by adding a 50 g sample to 450 ml of Butterfield’s phosphate buffered dilution water and homogenizing in a high speed blender. Yeasts and moulds: Not more than 500 colonies per gram Coliforms: Negative by test Salmonella: Negative by test

TESTS

IDENTIFICATION TESTS

Water-insoluble matter	Disperse 2 g of the sample, weighed to the nearest 0.1 mg, in 800 ml of water in a 2,000-ml flask. Neutralize to pH 7 with sodium hydroxide TS and then add 3 ml in excess. Add 40 ml of hydrogen peroxide solution containing 30% by weight H_2O_2, cover the flask and boil for 1 h with frequent stirring. Filter while hot through a tared Gooch crucible provided with a glass fibre filter (2.4 cm, No. 934 AH, Reeve Angel & Co., Clifton, N.Y., or equivalent filter). If slow filtration is caused by high viscosity of the sample solution, boil until the viscosity is reduced enough to permit filtration. Wash the crucible thoroughly with hot water, dry the crucible and its contents at 105° for 1 h, cool and weigh. Calculate as percentage of the dry weight.

METHOD OF ASSAY

Proceed as directed under *Carbon Dioxide Determination by Decarboxylation*. Each ml of 0.25 N sodium hydroxide consumed is equivalent to 5.5 mg of carbon dioxide (CO_2) or 29.75 mg of potassium alginate (equivalent weight 238).

POTASSIUM CARBONATE

Prepared at the 19th JECFA (1975), published in NMRS 55B (1976) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI of 0-6 mg/kg bw was established at the 9th JECFA (1965).

SYNONYMS INS No. 501 (i)

DEFINITION

Chemical names	Potassium carbonate, potassium salt of carbonic acid
C.A.S. number	584-08-7
Chemical formula	Anhydrous: K_2CO_3 Hydrated: $K_2CO_3 \cdot 1½H_2O$
Formula weight	138.21 (anhydrous)
Assay	Not less than 99.0% after drying

DESCRIPTION White, odourless, very deliquescent powder; the hydrated form occurs as small, white, translucent crystals or granules

FUNCTIONAL USES Alkali

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Very soluble in water; insoluble in ethanol
Test for potassium (Vol. 4)	Passes test
Test for carbonate (Vol. 4)	Passes test

PURITY

Loss on drying (Vol. 4)	Anhydrous: Not more than 5% (180^o, 4 h) Hydrated forms: Between 10% and 18% (180^o, 4 h)
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh accurately about 1 g of the dried sample. Dissolve carefully in 50 ml of 1 N sulfuric acid, add methyl orange TS and titrate the excess acid with 1 N sodium hydroxide. Each ml of 1 N sulfuric acid is equivalent to 69.11 mg of K_2CO_3.

POTASSIUM CHLORIDE

Prepared at the 23rd JECFA (1979), published in FNP 12 (1979) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). A group ADI 'not limited' for hydrochloric acid and its ammonium, Mg, K salts was established at the 23rd JECFA (1979)

SYNONYMS Sylvine, sylvite; INS No. 508

DEFINITION

Chemical names	Potassium chloride
C.A.S. number	7447-40-7
Chemical formula	KCl
Formula weight	74.56
Assay	Not less than 99.0% on the dried basis

DESCRIPTION Colourless, elongated, prismatic, or cubital crystals, or white granular powder; odourless

FUNCTIONAL USES Seasoning agent, gelling agent, yeast food

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Freely soluble in water; insoluble in ethanol
Test for potassium (Vol. 4)	Passes test
Test for chloride (Vol. 4)	Passes test

PURITY

Loss on drying (Vol. 4)	Not more than 1% (105°, 2 h)
Acidity or alkalinity	To a solution of 5 g of the sample in 50 ml of recently boiled and cooled water add 3 drops of phenolphthalein TS. No pink colour is produced. Then add 0.3 ml of 0.02 N sodium hydroxide. A pink colour is produced.
Iodide or bromide	Dissolve 2 g of the sample in 6 ml of water, add 1 ml of chloroform, and then add, dropwise and with constant agitation, 5 ml of a mixture of equal parts of chlorine TS and water. The chloroform is free from even a transient violet or permanent orange colour.
Test for sodium (Vol. 4)	Negative test
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample

preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Dissolve about 250 mg of the dried sample, accurately weighed in 50 ml of water in a glass-stoppered flask. Add, while agitating, 50 ml of 0.1 N silver nitrate, 3 ml of nitric acid, and 5 ml of nitrobenzene, shake vigorously, add 2 ml of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate. Each ml of 0.1 N silver nitrate is equivalent to 7.456 mg of KCl.

POTASSIUM DIHYDROGEN CITRATE

Prepared at the 31st JECFA (1987), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). Group ADI 'not limited' for citric acid and its salts was established at the 23rd JECFA (1979)

SYNONYMS Monopotassium citrate, potassium citrate monobasic; INS No. 332(i)

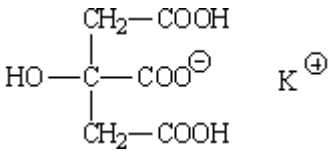
DEFINITION

Chemical names: Potassium dihydrogen citrate, monopotassium salt of 2-hydroxy-propan-1,2,3-tricarboxylic acid

C.A.S. number: 866-83-1

Chemical formula: $C_6H_7KO_7$

Structural formula:



Formula weight: 230.21

Assay: Not less than 99.0% and not more than the equivalent of 101.0% on the dried basis

DESCRIPTION Odourless, transparent crystals or white powder

FUNCTIONAL USES Buffering agent, sequestrant, yeast food

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water; very slightly soluble in ethanol

pH (Vol. 4): 3.5 - 3.9 (1 in 10 soln)

Test for citrate (Vol. 4): Passes test

Test for potassium (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4): Not more than 0.5% (105°, 4 h)

Oxalate (Vol. 4): Not more than 0.04%

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Dissolve about 180 mg of the dried sample, accurately weighed in 25 ml of water and titrate with 0.1 N sodium hydroxide (potentiometric end-point determination). Each ml of 0.1 N sodium hydroxide is equivalent to 11.511 mg of $C_6H_7KO_7$.

POTASSIUM GLUCONATE

Prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998) superseding specifications prepared at the 23rd JECFA (1979), published in FNP 12 (1979). Group ADI "not specified" for glucono-delta-lactone and gluconates, excluding ferrous gluconate, established at the 51st JECFA in 1998.

SYNONYMS Potassium D-gluconate; INS No. 577

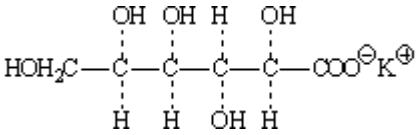
DEFINITION

Chemical names: Potassium D-gluconate

C.A.S. number: 299-27-4 (Anhydrous)
35398-15-3 (Monohydrate)

Chemical formula: $C_6H_{11}KO_7$

Structural formula:



Formula weight: 234.25 (Anhydrous)
252.26 (Monohydrate)

Assay: Not less than 97.0% and not more than 103.0% on dried basis

DESCRIPTION Odourless, free flowing white to yellowish white, crystalline powder or granules

FUNCTIONAL USES Acidity regulator, nutrient supplement, yeast food

CHARACTERISTICS

IDENTIFICATION

Test for potassium (Vol. 4): Passes test

Test for gluconate (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4): Anhydrous: Not more than 3% (105°, 4 h, under vacuum)
Monohydrate: Not less than 6% and not more than 7.5% (105°, 4 h, under vacuum)

Reducing substances (Vol. 4): Not more than 1.0% calculated as D-glucose (Method I)

Lead (Vol. 4): Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the

specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Transfer about 175 mg of the sample, accurately weighed, into a clean, dry 200-ml Erlenmeyer flask, add 75 ml of glacial acetic acid and dissolve by heating on a hot plate. Cool, add quinaldine red TS, and titrate with 0.1 N perchloric acid in glacial acetic acid, using a 10-ml microburet, to a colourless end point. Each ml of 0.1 N perchloric acid is equivalent to 23.42 mg of $C_6H_{11}KO_7$.

POTASSIUM HYDROGEN CARBONATE

Prepared at the 19th JECFA (1975), published in NMRS 55B (1976) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI not limited' was established at the 9th JECFA (1965)

SYNONYMS Potassium bicarbonate; INS No. 501(ii)

DEFINITION

Chemical names	Potassium hydrogen carbonate, potassium acid carbonate
C.A.S. number	298-14-6
Chemical formula	$KHCO_3$
Formula weight	100.11
Assay	Not less than 99.0% and not more than 101% calculated on the dried basis

DESCRIPTION Odourless, colourless crystals or white powder or granules

FUNCTIONAL USES Alkali, leavening agent, buffer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Freely soluble in water; insoluble in ethanol
Test for potassium (Vol. 4)	Passes test
Test for carbonate (Vol. 4)	Passes test

PURITY

Loss on drying (Vol. 4)	Not more than 0.25% (over silica gel, 4 h)
Normal carbonate	Dissolve 1 g of the sample without agitation in 20 ml of water at a temperature not above 5°. Add 2 ml of 0.1 N hydrochloric acid and 2 drops of phenolphthalein TS and observe immediately. The colour of the solution is not deeper than a faint pink.
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY Dissolve about 4 g of the sample, accurately weighed, in 25 ml of water, add methylorange TS and titrate with 1 N sulfuric acid. Each ml of 1 N sulfuric acid is equivalent to 100.1 mg of $KHCO_3$.

POTASSIUM HYDROXIDE

Prepared at the 19th JECFA (1975), published in NMRS 55B (1976) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI not limited' was established at the 9th JECFA (1965)

SYNONYMS Caustic potash, Potassium hydrate; INS No. 525

DEFINITION

Chemical names	Potassium hydroxide
C.A.S. number	1310-58-3
Chemical formula	KOH
Formula weight	56.11
Assay	Not less than 85.0% of total alkali calculated as KOH

DESCRIPTION White or nearly white pellets, flakes, sticks, fused masses or other forms

FUNCTIONAL USES Alkali

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Very soluble in water; freely soluble in ethanol
Test for alkali	A 1 in 100 solution of the sample is strongly alkaline
Test for potassium (Vol. 4)	Passes test

PURITY

Water insoluble substances	A 1 in 20 solution of the sample is complete, clear, and colourless
Carbonate	Not more than 3.5% (as potassium carbonate) Each ml of 1 N sulfuric acid required between the phenolphthalein and methyl orange endpoints in the "METHOD OF ASSAY" is equivalent to 138.2 mg of K_2CO_3.
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY Dissolve about 1.5 g of the sample, accurately weighed, in 40 ml of recently boiled and cooled water, cool to 15^{o}, add phenolphthalein TS and titrate with 1 N sulfuric acid. At the discharge of the pink colour, record the volume

of acid required, then add methyl orange TS and continue to titrate to a persistent pink colour. Record the total volume of acid required for the titration. Each ml of 1 N sulfuric acid is equivalent to 56.11 mg of total alkali, calculated as KOH.

POTASSIUM LACTATE (SOLUTION)

Prepared at the 18th JECFA (1974), published in NMRS 54B (1975) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003). An ADI 'not limited' for lactic acid and its salts was established at the 23rd JECFA (1979)

SYNONYMS INS No. 326

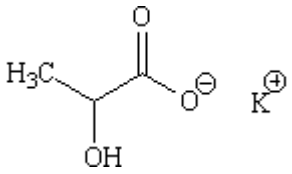
DEFINITION

Chemical names: Potassium lactate, potassium 2-hydroxypropanoate

C.A.S. number: 996-31-6

Chemical formula: $C_3H_5KO_3$

Structural formula:



Formula weight: 128.17 (anhydrous)

Assay: Not less than 95% and not more than 110% of the labelled amount; this specification is based on a 60% w/w solution of in water.

DESCRIPTION Slightly viscous, almost odourless clear liquid; odourless, or with a slight, characteristic odour

FUNCTIONAL USES Antioxidant, synergist

CHARACTERISTICS

IDENTIFICATION

Ignition: Ignite to an ash. The ash is alkaline, and an effervescence occurs when acid is added

Colour reaction: Overlay 2 ml of the sample on 5 ml of a 1 in 100 solution of catechol in sulfuric acid. A deep red colour is produced at the zone of contact

Test for potassium (Vol. 4): Passes test

Test for lactate (Vol. 4): Passes test

PURITY

Acidity: Dissolve 1 g of the sample in 20 ml of water, add 3 drops of phenolphthalein TS and titrate with 0.1 N sodium hydroxide. Not more than 0.2 ml should be required

Reducing substances (Vol. 4)	The sample shall not cause any reduction of Fehling's solution
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh accurately about 0.6 g of the sample into a small beaker, and evaporate to dryness. Add to the residue 60 ml of a 1 in 5 mixture of acetic anhydride in glacial acetic acid, and stir until the residue is completely dissolved. Add crystal violet TS, and titrate with 0.1 N perchloric acid to a blue end-point. Perform a blank determination, and make any necessary correction. Each ml of 0.1 N perchloric acid is equivalent to 12.82 mg of $C_3H_5KO_3$.

POTASSIUM PROPIONATE

Prepared at the 49th JECFA (1997) , published in FNP 52 Add 5 (1997) superseding specifications prepared at the 44th JECFA (1995), published in FNP 52 Add 3 (1995). An ADI not limited' was established at the 17th JECFA (1973)

SYNONYMS Potassium propanoate, INS No. 283

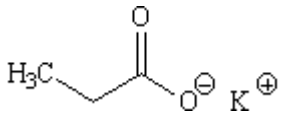
DEFINITION

Chemical name: Potassium propionate

C.A.S. number: 327-62-8

Chemical formula: $C_3H_5KO_2$

Structural formula:



Formula weight: 112.17

Assay: Not less than 99.0 % on the dried basis

DESCRIPTION White or colourless crystals

FUNCTIONAL USES Preservative, antimould and antirope agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water, soluble in ethanol

Positive test for potassium (Vol. 4): Passes test

Positive test for propionate: Warm the sample with sulfuric acid. The propionic acid evolved may be recognized by its odour.

Positive test for alkali salt of organic acid: Ignite the sample at a relatively low temperature. The alkaline residue effervesces with acid.

PURITY

Loss on drying (Vol. 4): Not more than 4% (105°, 2 h)

pH (Vol. 4): 7.5 - 10.5 (1 in 10 soln)

Water-insoluble matter: Not more than 0.1 %
Weigh 5 g of the sample to the nearest mg, transfer into a 100-ml beaker and add 50 ml of water. Stir until all the sample appears to be completely

dissolved. Filter through a Gooch crucible, tared to an accuracy of ± 0.2 mg. Rinse the beaker with 20 ml of water. Dry the crucible with its contents in a 60° oven to constant weight. Cool in a desiccator, weigh, and calculate as percentage.

Iron (Vol. 4)

Not more than 30 mg/kg
Test 0.5 g of the sample as described in the Limit Test using 1.5 ml of Iron Standard Solution (15 µg), in the control.

Lead (Vol. 4)

Not more than 5 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh, to the nearest mg, 3 g of the sample previously dried at 105° for 2 h into a distillation flask and add 200 ml of 50% phosphoric acid. Boil for 2 h and collect the distillate. During distillation keep the volume in the flask at about 200 ml by adding water using dropping funnel. Titrate the distillate with 1N sodium hydroxide using phenolphthalein TS as indicator. Each ml of 1N sodium hydroxide corresponds to 112.17 mg of $C_3H_5KO_2$.

POTASSIUM SULFATE

Prepared at the 29th JECFA (1985), published in FNP 34 (1986) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). An ADI 'not specified' was established at the 29th JECFA (1985).

SYNONYMS INS No. 515(i)

DEFINITION

Chemical names	Potassium sulfate
C.A.S. number	7778-80-5
Chemical formula	K_2O_4S
Structural formula	K_2SO_4
Formula weight	174.25
Assay	Not less than 99.0%

DESCRIPTION Colourless or white crystals or crystalline powder

FUNCTIONAL USES Salt substitute, acidity regulator

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Freely soluble in water, insoluble in ethanol
pH (Vol. 4)	5.5 - 8.5 (1 in 20 solution)
Test for potassium (Vol. 4)	Passes test
Test for sulfate (Vol. 4)	Passes test

PURITY

Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Selenium (Vol. 4)	Not more than 30 mg/kg Test 0.2 g of the sample as directed in the Limit Test (Method II)

METHOD OF ASSAY Weigh accurately about 0.5 g of the sample, dissolve in 200 ml of water, add 1 ml of hydrochloric acid, and heat to boiling. Gradually add, in small portions and while stirring constantly, an excess of hot barium chloride TS (about 8 or 9 ml), and heat the mixture on a steam bath for 1 h. Collect the precipitate on a filter, wash until free from chloride, dry, ignite, and weigh. The weight of the barium sulfate so obtained, multiplied by 0.7466, indicates its equivalent of K_2SO_4.

POWDERED CELLULOSE

Prepared at the 20th JECFA (1976), published in FNS 1B (1977) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). An ADI 'not specified' was established at the 20th JECFA (1976)

SYNONYMS INS No. 460(ii)

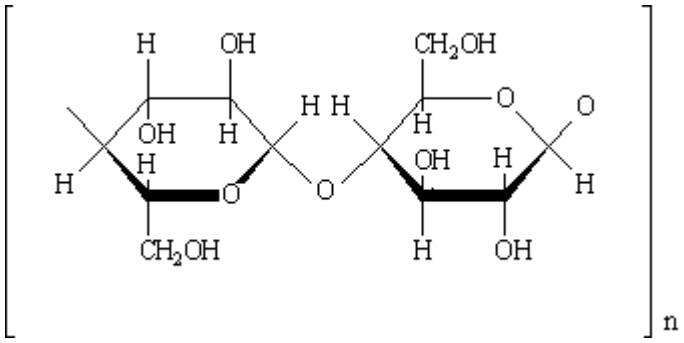
DEFINITION

Chemical names Cellulose, linear polymer of 1:4 linked glucose residues

C.A.S. number 9004-34-6

Chemical formula $(C_{12}H_{20}O_{10})_n$

Structural formula



Formula weight $(324)_n$ (n is predominantly 500 and greater)
Of the order of 1.6×10^5 and greater

Assay Not less than 92% $(C_{12}H_{20}O_{10})_n$

DESCRIPTION Purified, mechanically disintegrated cellulose prepared by processing alpha cellulose obtained as a pulp from fibrous plant materials; occurs as a white, odourless substance consisting of fibrous particles which may be compressed into self-binding tablets which disintegrate rapidly in water; exists in various grades exhibiting degrees of fineness ranging from a dense free flowing powder to a coarse, fluffy non-flowing material.

FUNCTIONAL USES Anticaking agent, dispersing agent, texturizing agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water, ethanol, ether and dilute mineral acids. Slightly soluble in sodium hydroxide solution

Suspension formation Mix 30 g of the sample with 270 ml of water in a high-speed (12,000 rpm) power blender for 5 min. The resultant mixture will be either a free-flowing suspension or a heavy, lumpy suspension which flows poorly, if at all, settles only slightly and contains many trapped air bubbles. If a free flowing

suspension is obtained, transfer 100 ml into a 100-ml graduated cylinder and allow to stand for 1 h. The solids settles and a supernatant liquid appears.

PURITY

Loss on drying (Vol. 4)	Not more than 7% after drying (105°, 3 h)
pH (Vol. 4)	5.0 - 7.5 Mix 10 g of the dried sample, accurately weighed, with 90 ml water and allow to stand with occasional stirring for 1 h.
Water soluble substances	Not more than 1.5% Mix about 6 g of the sample, previously dried, with 90 ml of recently boiled and cooled water and allow to stand with occasional stirring for 10 min. Filter, discard the first 10 ml of filtrate and pass the filtrate through the same filter a second time if necessary to obtain a clear filtrate. Evaporate a 15 ml portion of the filtrate to dryness in a tared evaporation dish on a steam bath, dry at 105° for 1 h. Not more than 15 mg of residue is obtained.
Total ash (Vol. 4)	Not more than 0.3% (at approximately 800° to constant weight).
Starch	Not detectable To 20 ml of the mixture obtained in the Identification Test B add a few drops of iodine TS and mix. No purplish-to-blue or blue colour is produced.
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

PROCESSED EUCHEUMA SEAWEED

Prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007), superseding specifications prepared at the 57th JECFA (2001)and published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A group ADI "not specified" for carrageenan and processed Eucheuma *seaweed was established at the 57th JECFA (2001).*

SYNONYMS PES, PNG-carrageenan, semi-refined carrageenan; INS No. 407a

DEFINITION A substance with hydrocolloid properties obtained from either *Eucheuma cottonii* or *E. spinosum* (from the *Rhodophyceae* class of red seaweeds). In addition to carrageenan polysaccharides, processed eucheuma seaweed may contain up to 15% of insoluble algal cellulose and minor amounts of other insoluble matter. Articles of commerce may include sugars for standardization purposes or salts to obtain specific gelling or thickening characteristics. It is distinguished from carrageenan (INS No. 407) by its higher content of cellulosic matter and by the fact that it is not solubilized and precipitated during processing.

The functional component of the product obtained from *E. cottonii* is kappa-carrageenan (a copolymer of D-galactose-4-sulfate and 3,6-anhydro-D-galactose). From *E. spinosum* it is iota-carrageenan (a copolymer of D-galactose-4-sulfate and 3,6-anhydro-D-galactose-2-sulfate).

Processing consists of soaking the cleaned seaweed in alkali for a short time at elevated temperatures. The material is then thoroughly washed with water to remove residual salts followed by purification, drying, and milling to a powder. Alcohols that may be used during purification are restricted to methanol, ethanol, and isopropanol.

DESCRIPTION Light tan to white coarse to fine powder

FUNCTIONAL USES Thickener, gelling agent, stabilizer, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Forms cloudy viscous suspensions in water; insoluble in ethanol
A 1 g sample disperses and partially dissolves in 100 ml of water at 80º giving a cloudy opalescent solution. (The sample disperses in water more readily if first moistened with alcohol, glycerol, or a saturated solution of glucose or sucrose in water).

Test for sulfate Dissolve a 100-mg sample in 20 ml of water. Heat to boiling, cool to room temperature, and add 3 ml of barium chloride TS and 5 ml of hydrochloric acid, dilute TS. Filter the mixture. Boil the filtrate for 5 min. A white,

	crystalline precipitate appears.
Test for galactose and anhydrogalactose (Vol.4)	Proceed as directed in Volume 4 (under "General Methods, Organic Components, Gum Constituents Identification") using the following as reference standards: galactose, rhamnose, galacturonic acid, 3,6-anhydrogalactose, mannose, arabinose and xylose. Galactose and 3,6-anhydrogalactose should be present.
Identification of hydrocolloid and predominant type of copolymer	Add 4 g of sample to 200 ml of water, and heat the mixture in a water bath at 80º, with constant stirring until dissolved. Replace any water lost by evaporation, and allow the solution to cool to room temperature. The solution becomes viscous and may form a gel. To 50 ml of the solution or gel, add 200 mg of potassium chloride, then reheat, mix well, and cool. A short-textured ("brittle") gel indicates a carrageenan of a predominantly kappa-type. A compliant ("elastic") gel indicates a predominantly iota-type.
Infrared absorption	Passes test See description under TESTS
PURITY	
Loss on drying (Vol. 4)	Not more than 12% (105º to constant weight)
pH (Vol. 4)	Between 8 and 11 (1 in 100 suspension)
Viscosity	Not less than 5 cp at 75º (1.5% solution) See description under TESTS
Sulfate	Not less than 15% and not more than 40% (as SO_4^{2-}) on the dried basis See description under TESTS
Total ash	Not less than 15% and not more than 30% on the dried basis See description under TESTS
Acid-insoluble ash (Vol. 4)	Not more than 1% Use the ash from the Total ash test
Acid-insoluble matter (Vol. 4)	Not less than 8% and not more than 15% on the dried basis Use 2 g of sample obtained from part (a) of the procedure for sulfate determination
Residual solvents (Vol. 4)	Not more than 0.1% of ethanol, isopropanol, or methanol, singly or in combination See description under TESTS
Microbiological criteria (Vol. 4)	Initially prepare a 10^{-1} dilution by adding a 50 g sample to 450 ml of Butterfield's phosphate-buffered dilution water and homogenizing the mixture in a high speed blender. Total (aerobic) plate count: Not more than 5000 cfu/g

Salmonella spp.: Negative per test
E. coli: Negative in 1 g

Arsenic (Vol. 4) — Not more than 3 mg/kg
Determine by the atomic absorption hydride technique. Use Method II for sample preparation.

Lead (Vol. 4) — Not more than 5 mg/kg
Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under "General Methods, Metallic Impurities").

Cadmium (Vol.4) — Not more than 2 mg/kg
Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under "General Methods, Metallic Impurities").

Mercury (Vol.4) — Not more than 1 mg/kg
Determine by the cold vapour atomic absorption technique

TESTS

IDENTIFICATION TESTS

Infrared absorption — Prepare a 0.2% aqueous solution of the sample. Cast films of 0.5 mm thickness (when dry) on a suitable non-sticking surface such as Teflon and obtain the infrared absorption spectrum of each film. (Alternatively, the spectra may be obtained using films cast on potassium bromide plates if care is taken to avoid moisture).

Iota- and kappa-carrageenan have strong, broad absorption bands, typical of all polysaccharides, in the 1000 to 1100 cm^{-1} region. Other characteristic absorption bands and their intensities relative to the absorbance at 1050 cm^{-1} are as follows:

Wave number (cm^{-1})	*Molecular Assignment*	*Absorbance Relative to 1050 cm^{-1}*	
		Kappa	Iota
1220-1260	ester sulfate	0.6-1.0	0.9-1.2
928-933	3,6-anhydrogalactose	0.3-0.6	0.2-0.6
840-850	galactose-4-sulfate	0.3-0.5	0.2-0.4
800-805	3,6-anhydrogalactose-2-sulfate	0.0-0.2	0.2-0.4

PURITY TESTS

Sulfate

Principle:
Hydrolysed sulfate groups are precipitated as barium sulfate.

Procedure:
(a) Disperse an accurately weighed 15 g sample of commercial product into 500 ml of 60% w/w isopropanol/water at room temperature. Stir gently for 4 h. Filter through ash-free filter paper. Discard the filtrate. Wash the material remaining on the filter paper with two 15-ml portions of 60% isopropanol/water. Dry the material at 105º to constant weight. Approximately 1 g of the dried matter is to be used for part (b). The remainder should be retained for determination of Total ash, Acid-insoluble matter, and viscosity.

(b) Accurately weigh a 1 g sample (W_1) obtained from part (a), Transfer the sample to a 100-ml long-neck round-bottom flask and add 50 ml of 0.2 N hydrochloric acid. Fit a condenser, preferably one with at least 5 condensing bulbs, to the flask and reflux for 1 h. Add 25 ml of a 10% (by volume) hydrogen peroxide solution and resume refluxing for about 5 h or until the solution becomes completely clear. Transfer the solution to a 600-ml beaker, bring to a boil, and add dropwise 10 ml of a 10% barium chloride solution. Heat the reaction mixture for 2 h on a boiling water bath. Filter the mixture through ash-free slow-filtration filter paper. Wash with boiling distilled water until the filtrate is free from chloride. Dry the filter paper and contents in a drying oven. Gently burn and ash the paper at 800° in a tared porcelain or silica crucible until the ash is white. Cool in a desiccator.

Weigh the crucible containing the ash. Calculate the percentage sulfate from the weight in g (W_2) of the ash (barium sulfate) using the formula:

$(W_2/W_1) \times 100 \times 0.4116$.

Total ash

Accurately weigh 2 g of the dried sample (W_1) obtained from part (a) under the procedure for Sulfate determination. Transfer to a previously ignited, tared, silica or platinum crucible. Heat the sample with a suitable infrared lamp, increasing the intensity gradually, until the sample is completely charred; continue heating for an additional 30 min. Transfer the crucible with charred sample into a muffle furnace and ignite at about 550º for 1 h. Cool in a desiccator and weigh. Repeat the ignition in the muffle furnace until a constant weight (W_2) is obtained. If a carbon-free ash is not obtained after the first ignition, moisten the charred spot with a 1 in 10 solution of ammonium nitrate and dry under an infrared lamp. Repeat the ignition step. Calculate the percentage of total ash of the sample:

$(W_2/W_1) \times 100$.

Retain the ash for the Acid-insoluble ash test.

Viscosity

Transfer 7.5 g of the dried sample obtained from part (a) under the procedure for sulfate determination into a tared, 600-ml tall-form (Berzelius) beaker, and disperse with agitation for 10 to 20 min in 450 ml of deionized water. Add sufficient water to bring the final weight to 500 g and heat in a water bath, with continuous agitation, until a temperature of 80º is reached (20-30 min). Add 7.5 g of diatomaceous earth or perlite filter aid.

Stir for two minutes. Add water to adjust for loss by evaporation. Filter the solution through a Büchner funnel (pre-heated with hot water to 80º) equipped with a coarse filter paper. Place the filter assembly in a vacuum receiver bottle.

Filter 200 ml of solution. Cool to 76-77º, and heat in a constant temperature bath at 75º. Pre-heat the bob and guard of a Brookfield LVF viscometer to approximately 75º in water. Dry the bob and guard and attach them to the viscometer, which should be equipped with a No. 1 spindle (19 mm in diameter, approximately 65 mm in length) and capable of rotating at 30 rpm. Adjust the height of the bob in the sample solution, start the viscometer rotating at 30 rpm and, after six complete revolutions of the viscometer, take the viscometer reading on the 0-100 scale.

If the viscosity is very low, increased precision may be obtained by using the Brookfield UL (ultra low) adapter or equivalent.

Record the results in centipoises, obtained by multiplying the reading on the scale by the factor given by the Brookfield manufacturer.

Residual solvents (Vol. 4)

See Method 1 under Vol. 4. General Methods, Organic Components, Residual Solvents.

Prepare standard, blank, and calibration solutions as directed under Method1.

Sample Preparation

Disperse 1 ml of a suitable antifoam emulsion, such as Dow-Corning G-10 or equivalent, in 200 ml of water contained in a 1000-ml 24/40 round-bottom distilling flask. Add about 5 g of the sample, accurately weighed, and shake for 1 h on a wrist -action mechanical shaker. Connect the flask to a fractionating column and distil about 100 ml, adjusting the heat so that the foam does not enter the column. Quantitatively transfer the distillate to a 200-ml volumetric flask, fill to the mark with water and shake the flask to mix. Weigh accurately 8.0 g of this solution into an injection vial. Add 1.0 ml of the internal standard solution. Heat at 60º for 10 min and shake vigorously for 10 sec.

PROPIONIC ACID

Prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998) superseding specifications prepared at the 49th JECFA (1997), published in FNP 52 Add 5 (1997). ADI "not limited" established at the 17th JECFA in 1973.

SYNONYMS Propanoic acid, ethylformic acid, methylacetic acid, INS No. 280

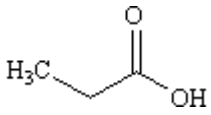
DEFINITION

Chemical names: Propionic acid

C.A.S. number: 79-09-4

Chemical formula: $C_3H_6O_2$

Structural formula:



Formula weight: 74.08

Assay: Not less than 99.5% on dried basis

DESCRIPTION An oily liquid with a slightly pungent odour

FUNCTIONAL USES Preservative, antimould, antirope agent, flavouring agent (see "Flavouring agents" monograph JECFA no. 84)

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Miscible with water and ethanol

Specific gravity (Vol. 4): d^{20}_{20}: 0.993-0.997

PURITY

Distillation range (Vol. 4): 138.5 - 142.5°

Non-volatile residue (Vol. 4): Not more than 0.01% when dried at 140° to constant weight

Formic acid: Not more than 0.1%
See description under TESTS

Aldehydes: Not more than 0.2% (as propionaldehyde)
See description under TESTS

Lead (Vol. 4): Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the

specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Formic acid

Dissolve 15 g of sodium hydroxide in 50 ml of water, cool, add 6 ml of bromine, stirring to effect complete solution, and dilute to 2,000 ml with water. Transfer 25.0 ml of this solution, into a 250 ml, glass-stoppered Erlenmeyer flask containing 100 ml of water, and add 10 ml of a 1 in 5 solution of sodium acetate and 10.0 ml of the sample. Allow to stand for 15 min, add 5 ml of a 1 in 4 solution of potassium iodide and 10 ml of hydrochloric acid, and titrate with 0.1 N sodium thiosulfate just to the disappearance of the brown colour. Perform a blank determination. The difference between the volume of 0.1 N sodium thiosulfate required for the blank and that required for the sample is not more than 4.4 ml.

Aldehydes

Transfer 10.0 ml of the sample into a 250-ml glass-stoppered conical flask containing 50 ml of water and 10.0 ml of a 1 in 8 solution of sodium bisulfite. Stopper the flask, and shake vigorously. Allow the mixture to stand for 30 min, then titrate with 0.1N iodine to the same brownish yellow end-point obtained with a blank treated with the same quantities of the same reagents. The difference between the volume of 0.1N iodine required for the blank and that required for the sample is not more than 7 ml.

METHOD OF ASSAY

Mix 3 g of the sample, weighed to the nearest 0.1 mg, with 50 ml of water in a 250-ml flask. Add phenolphthalein TS, and titrate with 1N sodium hydroxide to the first appearance of a faint pink end-point which persists for at least 30 sec. Each ml of 1N sodium hydroxide is equivalent to 74.08 mg of $C_3H_6O_2$.

PROPYL GALLATE

Prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 41st JECFA (1993), published in FNP 52 Add 2 (1993). Metals and arsenic specifications revised at the 61st JECFA (2003). An ADI of 0-1.4 mg/kg bw was established at the 46th JECFA (1996)

SYNONYMS INS No. 310

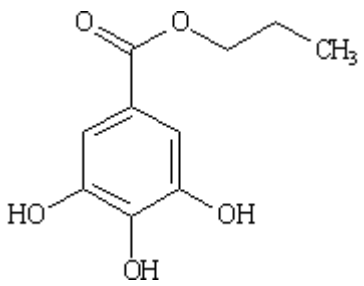
DEFINITION

Chemical names: Propyl gallate, propyl ester of gallic acid, n-propyl ester of 3,4,5-trihydroxybenzoic acid, propyl 3,4,5-trihydroxybenzoate

C.A.S. number: 121-79-9

Chemical formula: $C_{10}H_{12}O_5$

Structural formula:



Formula weight: 212.20

Assay: Not less than 98.0% and not more than 102.0% on the dried basis

DESCRIPTION White or creamy-white, crystalline odourless solid

FUNCTIONAL USES Antioxidant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Slightly soluble in water; freely soluble in ethanol, ether and propane-1,2-diol

Melting range (Vol. 4): 146 - 150° after drying

Gallic acid: Dissolve about 0.5 g of the sample in 10 ml of sodium hydroxide TS and boil for 30 min under nitrogen. Maintaining a stream of nitrogen, cool the mixture and acidify to pH 2-3 with sulfuric acid TS. Filter the precipitate through a sintered glass crucible, wash with a minimum amount of water and then dry at 110ºfor 2 h. The melting point of the gallic acid so obtained is about 240º, with decomposition.

TLC-separation of gallate esters

Use a thin-layer plate prepared with silica gel G. Prepare a sample solution by dissolving 10 mg of sample in 10 ml ethanol. Prepare control solution A by dissolving 10 mg of propyl gallate in 10 ml ethanol and control solution B by dissolving 10 mg of propyl gallate and 10 mg of octyl gallate in 10 ml ethanol.

Place 5 µl of each solution on the plate. Develop the chromatogram to about 15 cm from the starting point using a developing solvent containing glacial acetic acid, petroleum ether and toluene (1:2:2).

Dry the plate in air. Spray the plate with an indicator solution, containing 20% w/v phosphomolybdic acid in ethanol until a yellow colouration persists.

Examine in daylight. After a few min there is a progressive change to blue colouration. After 5 to 10 min expose the plate to ammonia vapours until the background is white.

Examine in daylight. The principal spot of the sample solution corresponds with that for propyl gallate in the control solutions. Suitable resolution of propyl and octyl gallate spots is determined from control solution B.

PURITY

Loss on drying (Vol. 4)

Not more than 0.5% (110^{o}, 4 h)
Note: Ventilation during drying is advisable

Sulfated ash (Vol. 4)

Not more than 0.1%
Test 2 g of the sample (Method I)

Chlorinated organic compounds

Not more than 100 mg/kg as chlorine
Dissolve 1 g of the sample in 10 ml of 0.1N sodium hydroxide. Acidify with nitric acid TS and filter off the precipitate. Mix the precipitate with 2 g of calcium carbonate, dry the mixture and then ignite. Take up the ignition residue in 20 ml of dilute nitric acid TS and filter. Mix the solution with 0.5 ml of 0.1 N silver nitrate. The turbidity should not be more than that obtained in 20 ml of dilute nitric acid TS containing 0.5 ml of 0.1N silver nitrate and 0.3 ml of 0.01N hydrochloric acid.

Free acid

Not more than 0.5% as gallic acid
To a mixture of 50 ml of carbon dioxide-free water and 50 ml of acetone, add 5 drops of bromocresol green TS and titrate with 0.005N hydrochloric acid to match a buffer (pH 5) TS containing the same amount of indicator. Dissolve 0.4 g of the sample in 50 ml of acetone and add 50 ml of carbon dioxide-free water, 5 drops of bromocresol green TS and the amount of 0.005N hydrochloric acid found in the preliminary test to bring the solvent to pH 5. Titrate the solution back to pH 5 with 0.05N sodium hydroxide, matching against the buffer (pH 5) TS. Each ml of 0.05N sodium hydroxide is equivalent to 8.506 mg of gallic acid.

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample

preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh accurately about 0.2 g of the dried sample into a 400-ml beaker. Add 150 ml of water and heat to boiling. Then with constant and vigorous stirring add 50 ml of bismuth nitrate TS (II). Continue stirring for a few min more until precipitation is complete, then allow the solution to cool to room temperature. Filter the yellow precipitate onto a weighed sintered-glass crucible, wash first with cold 0.05N nitric acid and then with ice-cold water, until free from acid. Dry at 110^{o} to constant weight. Proceed as directed in the specifications for dodecyl gallate. Calculate the propyl gallate content by the formula:

$$\%\ Propyl\ gallate = \frac{Weight\ of\ precipitate\ x\ 48.63}{Weight\ of\ sample}$$

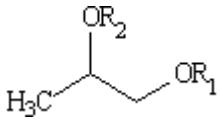
PROPYLENE GLYCOL ESTERS of FATTY ACIDS

Prepared at the 49th JECFA (1997) , published in FNP 52 Add 5 (1997) superseding specifications prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996). Metals and arsenic specifications revised at the 55th JECFA (2000). An ADI of 0-25 mg/kg bw was established at the 17th JECFA (1973)

SYNONYMS Propane-1,2-diol esters of fatty acids, INS No. 477

DEFINITION Propylene glycol esters of fatty acids are mixtures of propylene glycol mono- and diesters of saturated and unsaturated fatty acids derived from edible oils and fats. The products are produced either by direct esterification of propylene glycol with fatty acids or by transesterification of propylene glycol with oils or fats. When prepared by transesterification, the product may contain residual mono- and diglycerides and glycerol. The process may be followed by molecular distillation to separate the monoesters.

Structural formula



where R_1 and R_2 represent one fatty acid moiety and hydrogen in the case of mono-esters and two fatty acid moieties in the case of di-esters

Assay Not less than 85% total fatty acid esters

DESCRIPTION White or cream coloured solids of waxy appearance, plastic products or viscous liquids

FUNCTIONAL USES Emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water, soluble in ethanol and ethyl acetate

Positive test for fatty acids (Vol. 4) Passes test

Positive test for propylene glycol (Vol. 4) Passes test
Glycerol may also be detectable by TLC

PURITY

Sulfated ash (Vol. 4) Not more than 0.5% Test 5 g of the sample (Method I, if the sample is solid; Method II, if liquid)

Acid value (Vol. 4) Not more than 4

Acids (Vol. 4) Acids other than fatty acids shall not be detectable

Dimer and trimer of propylene glycol	Not more than 0.5%
Soap	Not more than 7% (as potassium stearate) See description under TESTS
Free propylene glycol	Not more than 1.5% (soap free) See description under TESTS
Total propylene glycol	Not less than 11% (soap free) See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Soap

Prepare a solvent mixture consisting of equal parts, by volume, of toluene and methanol, add bromophenol blue TS, and neutralize with 0.5N hydrochloric acid, or use neutralized acetone as the solvent. Weigh accurately about 5 g of the sample, dissolve it in 100 ml of the neutralized solvent mixture, and titrate with 0.5N hydrochloric acid to a definite yellow endpoint. Calculate the percentage of soap in the sample by the formula:

$$\frac{V \times N \times e}{W}$$

where V and N are the volume and normality, respectively, of the hydrochloric acid, W is the weight of the sample, in g, and e is the equivalence factor, e = 31.0.

Free propylene glycol

Reagents and Solutions:

Periodic Acid solution: Dissolve 5.4 g of periodic acid, H_5IO_6 in 100 ml of water, add 1900 ml of glacial acetic acid, and mix. Store in a light-resistant, glass-stoppered bottle or in a clear, glass-stoppered bottle protected from light.

Chloroform:

Use chloroform meeting the following test: To each of three 500-ml flasks add 50.0 ml of Periodic Acid Solution, then add 50 ml of chloroform and 10 ml of water to two of the flasks and 50 ml of water to the third. To each flask add 20 ml of potassium iodide TS, mix gently, and allow to stand at least 1 min, but no longer than 5 min, before titrating. Add 100 ml of water, and titrate with 0.1N sodium thiosulfate, using a magnetic stirrer to keep the solution thoroughly mixed, to the disappearance of the brown iodine colour, then add 2 ml of starch TS and continue the titration to the disappearance of the blue colour. The

difference between the volume of 0.1N sodium thiosulfate required in the titrations with and without the chloroform is not greater than 0.5 ml.

Procedure:
Melt the sample, if not liquid, at a temperature not higher than 100º above its melting point, and mix thoroughly. Transfer an accurately weighed portion of the sample, equivalent to about 30 mg of propylene glycol into a 100 ml beaker, and dissolve in 25 ml of chloroform. Transfer the solution with the aid of an additional 25 ml of chloroform, into a separator, wash the beaker with 25 ml of water, and add the washing to the separator. Stopper the separator tightly, shake vigorously for 30 to 60 sec, and allow the layers to separate. (Add 1 to 2 ml of glacial acetic acid to break emulsions formed due to the presence of soap.) Collect the aqueous layer in a 500-ml glass-stoppered Erlenmeyer flask, and extract the chloroform solution again using two 25-ml portions of water. To the combined aqueous extracts add 50.0 ml of Periodic Acid Solution. Run two blanks by adding 50.0 ml of this reagent solution to two 500 ml glass-stoppered Erlenmeyer flasks, each containing 75 ml of water and allow to stand for at least 30 min, but no longer than 90 min. To each flask, add 20 ml of potassium iodide TS, and allow to stand at least 1 min, but no longer than 5 min, before titrating. Add 100 ml of water, and titrate with 0.1N sodium thiosulfate, using a magnetic stirrer to keep the solution thoroughly mixed, to the disappearance of the brown iodine colour, then add 2 ml of starch TS and continue the titration to the disappearance of the blue colour.

Calculation:
Calculate the percentage of free propylene glycol by the formula:

$$\frac{(b - S) \times N \times 3.81}{W}$$

where "b" is the number of ml of sodium thiosulfate consumed in the blank determination; "S" is the number of ml required in the titration of the aqueous extracts from the sample; "N" is the exact normality of the sodium thiosulfate; "W" is the weight, in g, of the original sample taken and 3.81 is the molecular weight of propylene glycol divided by 20.

Note:
If the aqueous extract contains more than 30 mg of propylene glycol, dilute the extract in a volumetric flask and transfer a suitable aliquot into a 500 ml glass-stoppered Erlenmeyer flask before proceeding with the test. The weight of the sample should be corrected in the calculation.

Total propylene glycol and glycerol

Sample preparation
Transfer about 15 g of sample, accurately weighed, into a 500-ml flask, add 250 ml of ethanol and 7.5 g of potassium hydroxide and mix. Reflux the solution for 2 h, transfer into an 800-ml beaker rinsing the flask with about 100 ml of water and adding the rinse water to the beaker. Heat on a steam or water bath, adding water occasionally to replace the ethanol and evaporate until the odour of ethanol can no longer be detected.

Adjust the volume to about 250 ml with hot water, neutralize with diluted sulfuric acid (1 in 2), add a slight excess of acid, heat with gentle stirring until the fatty acid layer separates. Transfer the fatty acids into a warm 500-ml separatory funnel, wash with four 20-ml portions of hot water and combine the washings with the original aqueous layer from the saponification. Extract the combined aqueous layer with three 20 ml portions of petroleum ether. Neutralize the aqueous layer with sodium hydroxide TS to pH 7. Transfer the solution to a 500-ml volumetric flask and dilute to the mark with water.

Determination of apparent propylene glycol

Pipette 5.0 ml of the solution into a 125 ml Erlenmeyer flask, add 5.0 ml of 1M periodic acid, swirl and let stand 15 min. Add 10 ml of a saturated solution of sodium bicarbonate, followed by 15.0 ml of 0.1N sodium arsenite and 1 ml of potassium iodide solution (1 in 20) and mix. Add enough sodium bicarbonate so that at the end point some remains undissolved, and titrate with 0.1N iodine, using a 10-ml microburette and continuing the titration to a faint yellow colour. Perform a blank determination and make the appropriate corrections. Each ml of 0.1N iodine is equivalent to 3.805 mg of propylene glycol.

Calculate the apparent propylene glycol content (g /100 g of esters) from the formula:

$$\frac{38.05 \times \text{ml } 0.1\text{N iodine solution}}{\text{sample weight (g)}}$$

If the qualitative test for glycerol included under Identification Test (Positive test for propylene glycol) showed the product to contain glycerol, it becomes necessary to correct for the glycerol content of the polyol solution obtained after saponification and separation of liberated fatty acids.

Determination of glycerol content

Pipette 50 ml of the solution prepared in “A. Sample preparation” into a 600-ml beaker, add bromothymol blue TS and acidify with 0.2N sulfuric acid to a definite greenish-yellow colour. Neutralize with 0.05N sodium hydroxide to a definite blue end point free of green colour. Prepare a blank containing 50 ml of water and neutralize in the same manner. Pipette 50 ml of sodium periodate TS into each beaker, mix by swirling, cover with a watch glass and allow to stand for 30 min at room temperature (not above 35^{o}) in the dark or in subdued light. Add 10 ml of a mixture of equal volumes of ethylene glycol and water and allow to stand 20 min. Dilute each solution to about 300 ml and titrate with 0.1N sodium hydroxide to pH 8.1±0.1 using a calibrated pH meter. Each ml of 0.1N sodium hydroxide, after correction for the blank, is equivalent to 9.210 mg of glycerol.

Calculate the glycerol content (g/100 g of esters) from the formula:

$$\frac{9.210 \times \text{ml } 0.1\text{N NaOH}}{\text{sample weight (g)}}$$

The true propylene glycol content (in g/100 g of esters) is equal to the apparent propylene glycol content (in g/100 g of esters) minus 1.65 x the glycerol content (in g/100 g of esters).

METHOD OF ASSAY

Determine by gas chromatography using the following: Gas chromatograph, with split injection or on-column injection, oven temperature programming and flame ionisation detector. For split injection an injection port with programmable temperature is preferable. For on-column injection, the reaction mixture is diluted 1:50 with pyridine prior to injection.

Column:
Fused silica capillary column, surface fully deactivated by silylation agent, 12-25 m, 0.25-0.35 mm i.d., coating 95% methyl- 5% phenyl silicone (or other phase with similar polarity), film thickness 0.1-0.2 µm.

Injection:
Volume 1-5µl: split injection (Split ratio 1:10-1:50); direct injection (hold for 1 min)

Temperatures:
Injection port 320º (or 60º for on-column injection); column initial 50º (or 60º for on-column injection); programme rate 10º/ min; final temperature 350º, hold 1 min; detector 400º; carrier gas flow 2-5 ml He/ min (at 80º)
N.B. The precise temperature conditions will be dependent on the details of the equipment used.

Reagents:
N,N - bis(trimethylsilyl)fluoroacetamide (BSTFA)
Trimethylchlorosilane (TMCS)
Pyridine, analytical grade, kept over KOH
n-Heptadecane, analytical grade, 99% minimum

Reference materials:
Propylene glycol, propylene glycol monostearate.
Internal standard solution: Accurately weigh approximately 100 mg internal standard, n-heptadecane into a 100-ml volumetric flask, dilute with pyridine to the mark.

Reference solution:
Accurately weigh approximately 100 mg propylene glycol monostearate into a 25-ml volumetric flask adding internal standard solution to the mark. When pure reference material of other components such as propylene glycol and di-fatty acid esters of propylene glycol are available, the method is suitable for these also.

Procedure:
Accurately weigh approximately 100 mg of the homogenised sample into a 25-ml volumetric flask and dilute to volume with the internal standard solution. Transfer 0.8 ml of the sample solution to a 2.5-ml screw cap vial with Teflon faced septa or 2.0-ml vial for auto sampler. Add 0.3 ml BSTFA and 0.1 ml TMCS. Close the vial and shake vigorously. Heat the

reaction mixture in a heating device at 70º for approximately 20 min, inject 1 to 5 µl of the reaction mixture into the gas chromatograph showing a stable baseline. Do not delay GC analysis. Repeat the reaction with a further 100 mg sample. Make two injections per reaction sample. Transfer 0.8 ml of reference solution to a vial and add the silylating agents, 0.3 ml BSTFA and 0.1 ml TMCS . Heat the reaction mixture and inject into the gas chromatograph as described above.

Identification:
Analyse reference solution using the same operating conditions as for the sample solution. Identify peaks by comparison of retention time with known substances or apply coupled GC/MS.

Calculation and expression of results:
Calculate response factor R_x of the reference substance X vs. internal standard using the reference solution chromatogram. The value of the response factor is given by the formula:

$$R_x = (m_{is}/m_x) \times (A_x/A_{is})$$

where:
m_{is} = mass of internal standard in mg
m_x = mass of reference substance X in mg
A_x = peak area of reference substance X
A_{is} = peak area of internal standard

Calculate percentage of mass content m'_x of component X in the sample by the formula:

$$m'_x = 1/R_x \times (m'_{is}/m'_s) \times (A'_x / A'_{is})\%$$

where:
m'_{is} = mass of internal standard in sample in mg
m'_s = mass of sample in mg
A'_x = peak area of component X in the sample
A'_{is} = peak area of internal standard in sample

When calculating the total content of propylene glycol monoesters in the sample the response factor of propylene monostearate is used for all propylene glycol monoesters in the sample. The FID response of propylene glycol monostearate does not differ significantly from that of other fatty acid monoesters of propylene glycol.

PROTEASE from *ASPERGILLUS ORYZAE*, var.

Prepared at the 55th JECFA (2000) and published in FNP 52 Add 8 (2000), superseding tentative specifications prepared at the 31st JECFA (1987) and published in FNP 38 (1988) and in FNP 52 (1992). An ADI "Acceptable" was established at the 31st JECFA (1987).

SYNONYMS INS No.1101(i)

SOURCES Produced by the controlled fermentation of non-toxicogenic and non-pathogenic strains of *Aspergillus oryzae* and isolated from the growth medium.

Active principles: Endo- and exopeptidases

Systematic names and numbers:
1. Aminopeptidases (EC 3.4.11)
2. Serine endopeptidases (EC 3.4.21)
3. Aspartic endopeptidases (EC 3.4.23)

Reactions catalyzed:
1. Hydrolysis of proteins at the N-terminal, liberating amino acids
2. Hydrolysis of proteins containing serine peptide bonds
3. Hydrolysis of proteins containing aspartic acid bonds

Secondary enzyme activities: alpha-Amylase (EC 3.2.1.1)

DESCRIPTION Off-white to tan amorphous powders dispersed in food-grade diluents or carriers; may contain stabilizers and preservatives; soluble in water and practically insoluble in ethanol and ether

FUNCTIONAL USES Enzyme preparation
Used in the preparation of and/or in meat and fish products, beverages, soup and broths, dairy and bakery products

GENERAL SPECIFICATIONS Must conform to the General Specifications for Enzyme Preparations Used in Food Processing (see Volume Introduction)

CHARACTERISTICS

IDENTIFICATION

Proteolytic activity (Vol. 4) The sample shows proteolytic activity; use method Proteolytic activity, Fungal (HUT)

PROTEASE from *STREPTOMYCES FRADIAE*

Prepared at the 26th JECFA (1982), published in FNP 25 (1982) and in FNP 52 (1992). A temporary ADI 'not specified' was established at the 26th JECFA (1982) and was withdrawn at the 28th JECFA (1984)

SYNONYMS INS No. 1101(i)

SOURCES Produced by the controlled fermentation of *Streptomyces fradiae*.

Active principles: Proteinase (peptide hydrolase)

Systematic names and numbers: Proteinase of unknown catalytic mechanism
None (EC 3.4.99)

Reactions catalyzed: The enzyme preparations hydrolyzes proteins and peptides with no clear specificity, yielding peptides of lower molecular weight.

DESCRIPTION Off-white to tan amorphous powders, soluble in water and practically insoluble in ethanol, chloroform and ether

FUNCTIONAL USES Enzyme preparation
Used in the preparation of beverages

GENERAL SPECIFICATIONS Must conform to the *General Specifications for Enzyme Preparations used in Food Processing* (see Volume Introduction)

CHARACTERISTICS

IDENTIFICATION

Proteinase activity (Vol. 4): The sample shows proteinase activity

PULLULAN

Prepared at the 74th JECFA (2011) and published in FAO JECFA Monographs 11 (2011), superseding specifications prepared at the 65th JECFA (2005), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI 'not specified' was established at the 65th JECFA (2005) and maintained at the 74th JECFA (2011).

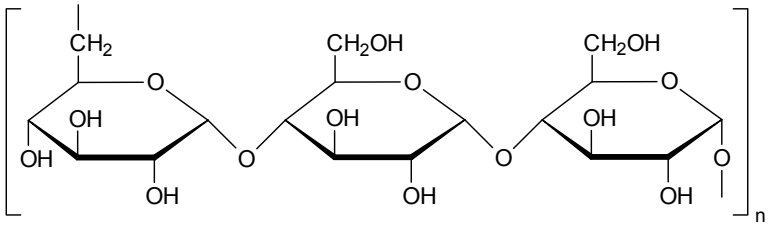
SYNONYMS INS No. 1204

DEFINITION Linear, neutral glucan consisting mainly of maltotriose units connected by α-1,6 glycosidic bonds. It is produced by fermentation from a food grade hydrolysed starch using a non-toxigenic strain of *Aureobasidium pullulans*. After completion of the fermentation, the fungal cells are removed by microfiltration, the filtrate is heat-sterilized and pigments and other impurities are removed by adsorption and ion exchange chromatography.

C.A.S. number 9057-02-7

Chemical formula $(C_6H_{10}O_5)_x$

Structural formula



Assay Not less than 90% of glucan on the dried basis

DESCRIPTION White to off-white odourless powder

FUNCTIONAL USES Glazing agent, film-forming agent, thickener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in water, practically insoluble in ethanol

pH (Vol. 4) 5.0 - 7.0 (10% solution)

Precipitation with polyethylene glycol 600 Add 2 ml of polyethylene glycol 600 to 10 ml of a 2% aqueous solution of pullulan. A white precipitate is formed.

Depolymerization with pullulanase Prepare two test tubes each with 10 ml of a 10% pullulan solution. Add 0.1 ml pullulanase solution having activity 10 units/g (refer to pullulanase activity, under Methods for enzyme preparations in Volume 4) to one test tube, and 0.1 ml water to the other. After incubation at about 25° for 20 min, the viscosity of the pullulanase-treated solution is visibly lower than that of the untreated solution.

PURITY

Loss on drying (Vol. 4)	Not more than 6% (90°, pressure not more than 50 mm Hg, 6 h)
Mono-, di- and oligosaccharides	Not more than 10% (expressed as glucose) See description under TESTS
Viscosity	100-180 mm^2/s (10% w/w aqueous solution at 30°) See description under TESTS
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under "General Methods, Metallic Impurities").
Microbiological criteria (Vol. 4)	Yeast and moulds: Not more than 100 CFU/g Coliforms: Negative in 25 g Salmonella: Negative in 25 g

TESTS

PURITY TESTS

Mono-, di- and oligosaccharides

Principle

The soluble mono-, di- and oligosaccharides of pullulan are measured using the anthrone-sulfuric acid method after pullulan has been precipitated with methanol and KCl.

Equipment

Spectrophotometer capable of measuring absorbance at 620 nm

Procedure

*Preparation of standard: W*eigh accurately 0.2 g glucose, dissolve in water and make up to 1 l.

Measurement of mono-, di- and oligosaccharides:
Weigh accurately 0.8 g sample and dissolve in water to make 100 ml (stock solution).
Place 1 ml of the stock solution in a centrifuge tube. Add 0.1 ml saturated potassium chloride solution. Add 3 ml methanol and mix vigorously for 20 sec. Centrifuge at 11000 rpm for 10 minutes. Add 0.2 ml of the supernatant to 5 ml modified anthrone solution (0.2 g anthrone in 100 g 75% (v/v) sulfuric acid, freshly prepared). Add 0.2 ml of glucose standard solution and 0.2 ml water (blank control) to separate 5 ml portions of modified anthrone solution. Mix rapidly. Place samples in a 90° water bath and incubate for 15 min. Measure absorbance of the test solution at 620 nm.

Calculate the percent of mono-, di- and oligosaccharides expressed as glucose, *C*, in the sample:

$$C(\%) = [(A_t - A_b)\times 0.41\times G\times 100]/(A_s - A_b)\times W$$

where

A_t is absorbance of the test solution;
A_b is absorbance of the water blank;
A_s is absorbance of the standard solution;
G is weight of the glucose (g); and
W is weight of the sample (g).

Viscosity

Dry the sample for 6 h at 90º under reduced pressure (50 mm Hg). Weigh 10.0 g of the sample and dissolve in water to yield 100 g of solution.

Use an Ubbelohde-type (falling-ball) viscometer. Charge the viscometer with sample in the manner dictated by the design of the instrument. Immerse the viscometer vertically in the thermostatic tank at 30 ± 0.1° and allow to stand for 20 min so that the sample equilibrates with the temperature in the tank. Adjust the meniscus of the column of liquid in the capillary tube to a position about 5 mm above of the first mark. With the sample flowing freely, measure, in seconds, the time required for the meniscus to pass from the first to the second mark. Calculate the viscosity, V:

$$V\ (mm^2/s) = C \times t$$

where

C is the calibration constant of the viscometer (mm^2/s^2); and
t is the flow time (s).

METHOD OF ASSAY

Calculate the percentage of pullulan on dried basis, P, as the difference between 100% and the sum of the percentages of known impurities (mono-, di- and oligosaccharides and water).

$$P(\%) = 100 - (L+C)$$

where

L is loss on drying; and
C is taken from the calculation for mono-, di- and oligosaccharides.

QUILLAIA EXTRACT (TYPE 1)

Specifications prepared at the 61st JECFA (2003) and published in FNP 52 Add 11 (2003). The previous tentative specifications for Quillaia extracts prepared at the 57th JECFA (2001), published in FNP 52 Add 9 (2001), are replaced by these and by separate specifications for "Quillaia extract (Type 2)". A group ADI of 0-1 mg quillaia saponins /kg bw for Quillaia ExtractsTypes 1 & 2 was established at 65th JECFA (2005)

SYNONYMS Quillaja extract, Soapbark extract, Quillay bark extract, Bois de Panama, Panama bark extract, Quillai extract; INS No. 999(i)

DEFINITION Quillaia extract (Type 1) is obtained by aqueous extraction of the milled inner bark or of the wood of pruned stems and branches of *Quillaja saponaria* Molina (family *Rosaceae*). It contains triterpenoid saponins (quillaia saponins, QS) consisting predominantly of glycosides of quillaic acid. Polyphenols and tannins are major components and some sugars and calcium oxalate will be present.
Quillaia extract (Type 1) is available commercially as liquid product or as spray-dried powder that may contain carriers such as lactose, maltitol or maltodextrin. The liquid product is usually preserved with sodium benzoate or ethanol.

C.A.S. number 68990-67-0

Formula weight Monomeric saponins range from ca. 1800 to ca. 2300, consistent with a triterpene with 8-10 monosaccharide residues

Assay Saponin content: not less than 20 % and not more than 26 % on the dried basis

DESCRIPTION Red-brownish liquid or light brown powder with a pink tinge

FUNCTIONAL USES Emulsifier, foaming agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Very soluble in water, insoluble in ethanol, acetone, methanol and butanol

Foam Dissolve 0.5 g of powder extract in 9.5 g of water or 1 ml of liquid extract in 9 ml of water. Add 1 ml of this mixture to 350 ml of water in a 1000-ml graduated cylinder. Cover the cylinder, vigorously shake it 30 times, and allow settling. Record the foam level (ml) after 30 min. Typical values are 150 ml of foam

Chromatography Determine as in METHOD OF ASSAY. The retention time of major peak of the sample corresponds to the major saponin peak (QS-18) of the standard.

Colour and turbidity Powder form only: Dissolve 0.5 g in 9.5 g of water. The solution is not turbid. Determine the absorbance of the solution against water at 520 nm. The absorbance is less than 1.2.

PURITY

Water (Vol. 4)	Powder form: not more than 6% (Karl Fischer Method)
Loss on drying (Vol. 4)	Liquid form: 50 to 80% (2 g, 105°, 5 h)
pH (Vol. 4)	3.7 -5.5 (4 % solution)
Ash (Vol. 4)	Not more than 14% on a dried basis (use 1.0 g for powder samples; for liquid samples, use the residue from loss on drying)
Tannins	Not more than 8% on a dried basis See description under TESTS
Lead (Vol. 4)	Not more 2 mg/kg. Determine using an atomic absorption technique appropriate to the specified level. The selection of the sample size and method of sample preparation may be based on the principles of the method described in FNP 5, "Instrumental Methods".

TESTS

PURITY TESTS

Tannins

Weigh either 3.0 g of the powder form or an equivalent amount of liquid sample, accounting for solids content determined from loss on drying. Dissolve in 250 ml of water. Adjust the pH to 3.5 with acetic acid. Dry 25 ml of this solution at 105° for 5 h and determine the weight of the dried solid, in g (W_i). Mix 50 ml of the solution with 360 mg of polyvinyl polypyrrolidone. Stir the solution for 30 min at room temperature; then centrifuge at 800 × g. Recover the supernatant and dry this solution at 105° (5 h).Weigh the recovered solid (W_f, in g). The percentage of tannins in the sample is:

$$\% \text{ tannins (dried basis)} = 100 \times (W_i - W_f/2) / W_i$$

METHOD OF ASSAY

Principle:

The saponins QS-7, QS-17, QS-18 and QS-21 are separated by reversed phase HPLC and their quantitation is used as an indicator for total saponins levels in Quillaia extract (Type 1).

Sample preparation:

Powders: Weigh 0.5 g of sample and dissolve in 9.5 g of water. Filter through a 0.2 µm filter.

Aqueous extracts (~ 550 mg solids/ml): Weigh 1 g of sample and dilute with 9 g of water. Filter through a 0.2 µm filter.

In either case, the sample volume is ca. 10 ml.

Standard preparation:

Weigh 1.5 g of purified saponins (SuperSap, Natural Response, Chile; Quil-A, Superfos, Denmark or similar, containing a known saponin content) and dissolve in 100 ml of water. Filter through a 0.2 µm filter.

High performance liquid chromatography (HPLC):

HPLC conditions:

Column: Vydac 214TP54 (4.6 x 250 mm length, 5 µm pore) or equivalent
Column temperature: room temperature
Pump: gradient
Solvent A: 0.15% trifluoroacetic acid in HPLC-grade water.
Solvent B: 0.15% trifluoroacetic acid in HPLC-grade acetonitrile.
Gradient:

Time(min)	% solvent A	% solvent B
0	70	30
40	55	45
45	70	30

Flow rate: 1 ml/min
Detection wavelength: 220 nm
Injection volume: 20 µl

Calculation:

The concentration of saponins, C_{sap}, in mg/ml, in the solution prepared as directed under sample preparation is:

$$C_{sap} = (A_{sample}/A_{standard})C_{Standard}$$

where $C_{Standard}$ (mg/ml) is the saponins concentration of the standard injected (e.g., $C_{Standard}$ = 13.5 mg/ml if the saponin content of 1.5 g of standard sample is 90 %) and A_{sample} and $A_{standard}$ are the sums of the peak areas attributed to the four principle saponins in the sample preparation and in the standard preparation, respectively, as noted in the figure. (Tannins and Polyphenols will elute before the saponins. The peaks due to the saponins will appear after the major peak due to the polyphenols - see figure.)

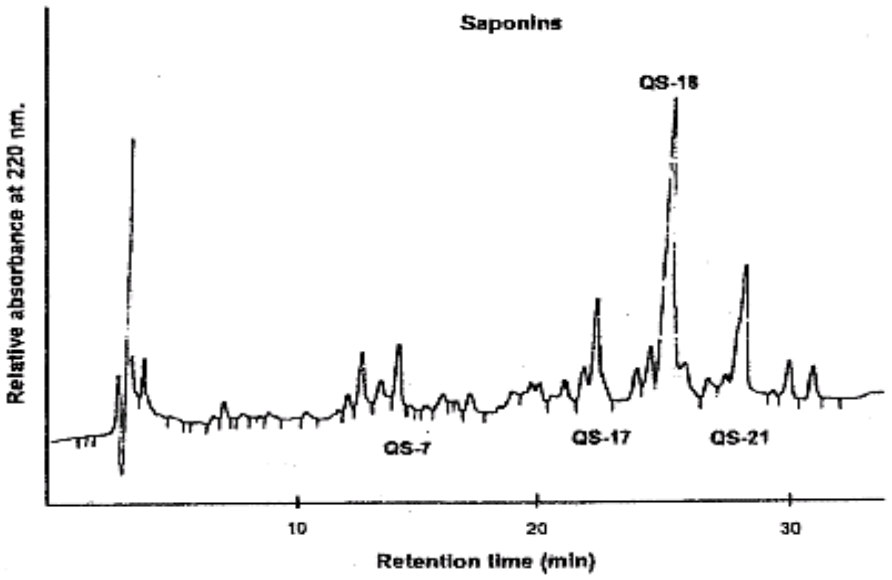
The percentage of saponins in the test sample is:

$$\% \text{ Saponins} = 100 \times C_{sap}/(0.1W_{sample})$$

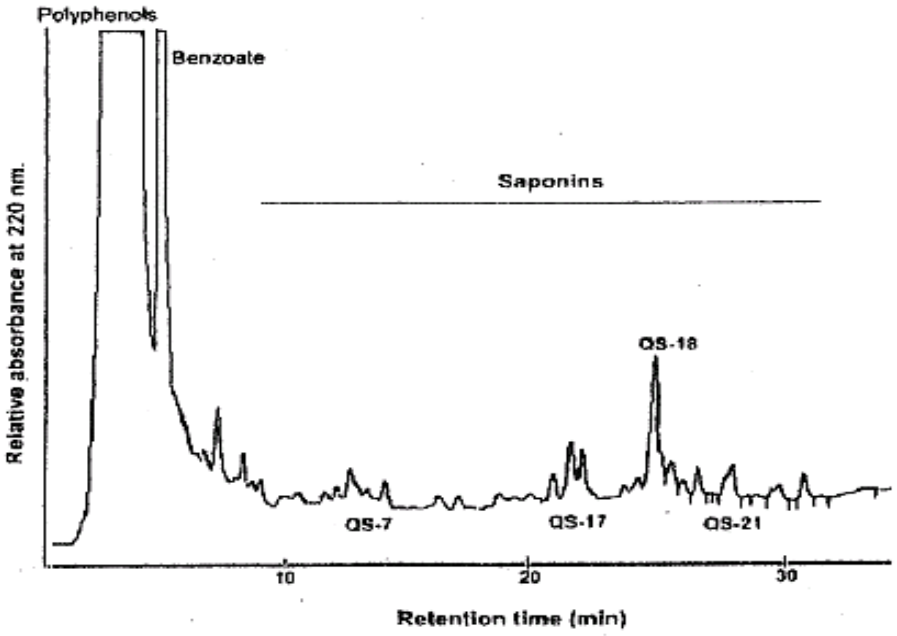
where W_{sample} is the weight of the sample (mg) taken for the sample preparation and 0.1 is the inverse of the sample volume, 10 ml.

Appendix

Chromatogram of Standard (15 mg solids/ml equivalent to 13.5 mg saponins/ml).



Chromatogram of Quillaia extract (Type 1) (55 mg solids/ml)



QUILLAIA EXTRACT (TYPE 2)

Revised specifications prepared at the 65th JECFA and published in FNP 52 Add 13 (2005), superseding specification prepared at the 61st JECFA (2003) and published in FNP 52 Add 11 (2003). A group ADI of 0-1 mg quillaia saponins /kg bw for Quillaia ExtractsTypes 1 & 2 was established at 65th JECFA (2005)

SYNONYMS Quillaja extract, Soapbark extract, Quillay bark extract, Bois de Panama, Panama bark extract, Quillai extract; INS No. 999(ii)

DEFINITION Quillaia extract (Type 2) is obtained either by chromatographic separation or ultrafiltration of the aqueous extraction of the milled inner bark or of the wood of pruned stems and branches of *Quillaja saponaria* Molina (family *Rosaceae*). It contains triterpenoid saponins (quillaia saponins, QS) consisting predominantly of glycosides of quillaic acid. Polyphenols and tannins are minor components. Some sugars and calcium oxalate will also be present.

Quillaia extract (Type 2) is available commercially as a liquid product or as a spray-dried powder that may contain carriers such as lactose, maltitol or maltodextrin. The liquid product is usually preserved with sodium benzoate or ethanol.

C.A.S. number 68990-67-0

Formula weight Monomeric saponins range from ca. 1800 to ca. 2300, consistent with a triterpene with 8-10 monosaccharide residues

Assay Saponin content:
not less than 65 % and not more than 90 % on the dried basis

DESCRIPTION Light red-brownish liquid or powder

FUNCTIONAL USES Emulsifier, foaming agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Very soluble in water, insoluble in ethanol, acetone, methanol, and butanol

Foam Dissolve 0.5 g of the powder form in 9.5 ml of water or 1 ml of the liquid form in 9 ml of water. Add 1 ml of this solution to 350 ml of water in a 1000-ml graduated cylinder. Cover the cylinder, vigorously shake it 30 times, and allow settling. Record the foam volume (ml) after 30 min. Typical volumes are about 260 ml.

Chromatography Determine as in METHOD OF ASSAY. The retention time of major sample peak corresponds to the major saponin peak (QS-18) of the standard.

Colour and turbidity Powder form only: Dissolve 0.5 g in 9.5 ml of water. The solution shall not turbid Determine the absorbance of the solution against water at 520 nm. The absorbance shall be less than 0.7.

PURITY

Water (Vol. 4) Powder form: not more than 6% (Karl Fischer Method)

Loss on drying (Vol. 4)	Liquid form: 50 to 80% (2 g, 105°, 5 h)
pH (Vol. 4)	3.7 -5.5 (4 % solution)
Ash (Vol. 4)	Not more than 5% on a dried basis (use 1.0 g for powder samples; for liquid samples, use the residue from Loss on drying)
Tannins	Not more than 8% on a dried basis See description under TESTS
Lead (Vol. 4)	Not more 2 mg/kg. Determine using an atomic absorption technique appropriate to the specified level. The selection of the sample size and method of sample preparation may be based on the principles of the method described in FNP 5, "Instrumental Methods".

TESTS

PURITY TESTS

Tannins

Weigh either 3.0 g of the powder form or an equivalent amount of liquid sample, accounting for solids content determined from loss on drying. Dissolve in 250 ml of water. Adjust the pH to 3.5 with acetic acid. Dry 25 ml of this solution at 105° for 5 h and determine the weight of the dried solid, in g (W_i). Mix 50 ml of the solution with 360 mg of polyvinyl polypyrrolidone. Stir the solution for 30 min at room temperature; then centrifuge at 800 × g. Recover the supernatant and dry this solution at 105° (5 h).Weigh the recovered solid (W_f, in g). The percentage of tannins in the sample is:

$$\% \text{ tannins (dried basis)} = 100 \times (W_i - W_f/2) / W_i$$

METHOD OF ASSAY

Principle:
The saponins QS-7, QS-17, QS-18 and QS-21 are separated by reversed phase HPLC and their quantitation is used as an indicator for total saponins levels in Quillaia extract (Type 2).

Sample preparation:
Powders: Weigh 0.5 g of sample and dissolve in 9.5 ml of water. Filter through a 0.2 µm filter.
Aqueous extracts (~ 550 mg solids/ml): Weigh 1 g of sample and dilute with 9 ml of water. Filter through a 0.2 µm filter.
In each case, the sample volume is ca. 10 ml.

Standard preparation:
Weigh 1.5 g of purified saponins (SuperSap, Natural Response, Chile; Quil-A, Superfos, Denmark or similar, containing a known saponin content) and dissolve in 100 ml of water. Filter through a 0.2 µm filter.

High performance liquid chromatography (HPLC):
HPLC conditions:
Column: Vydac 214TP54 (4.6 x 250 mm length, 5 µm particle size) or equivalent
Column temperature: Room temperature
Pump: Gradient
Solvent A: 0.15% trifluoroacetic acid in HPLC-grade water.
Solvent B: 0.15% trifluoroacetic acid in HPLC-grade acetonitrile.

Gradient:

Time(min)	% solvent A	% solvent B
0	70	30
40	55	45
45	70	30

Flow rate: 1 ml/min
Detection wavelength: 220 nm
Injection volume: 20 µl

Calculation:
The concentration of saponins, C_{sap}, in mg/ml, in the solution prepared as directed under sample preparation is:

$$C_{sap} = (A_{sample}/A_{standard})C_{Standard}$$

where $C_{Standard}$ (mg/ml) is the saponins concentration of the standard injected (e.g., $C_{Standard}$ = 13.5 mg/ml if the saponin content of 1.5 g of standard sample is 90 %) and A_{sample} and $A_{standard}$ are the sums of the peak areas attributed to the four principle saponins in the sample preparation and in the standard preparation, respectively, as noted in the figure. (Tannins and polyphenols will elute before the saponins. The peaks corresponding to the saponins will appear after the major peak corresponding to the polyphenols)

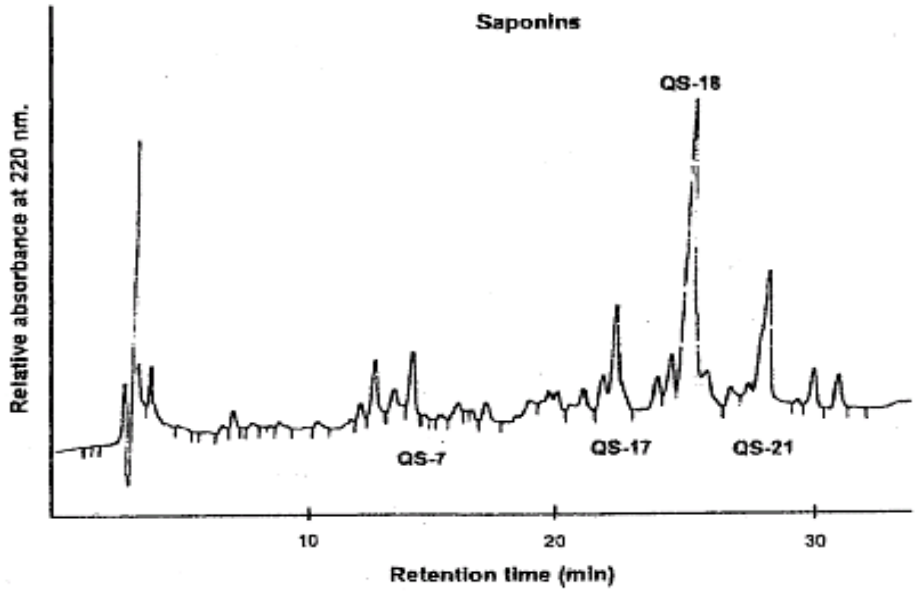
The percentage of saponins in the test sample is:

$$\% \text{ Saponins} = 100 \times C_{sap}/(0.1W_{sample})$$

where W_{sample} is the weight of the sample (mg) taken for the sample preparation and 0.1 is the inverse of the sample volume, 10 ml.

Appendix

Chromatogram of Standard (15 mg solids/ml equivalent to 13.5 mg saponins/ml).



RIBOFLAVIN

Prepared at the 31st JECFA (1987), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). A group ADI of 0-0.5 mg/kg bw for riboflavin from Bacillus subtilis, synthetic riboflavin and riboflavin-5-phosphate was established at the 51st JECFA (1998).

SYNONYMS Vitamin B_2, lactoflavin; INS No. 101(i)

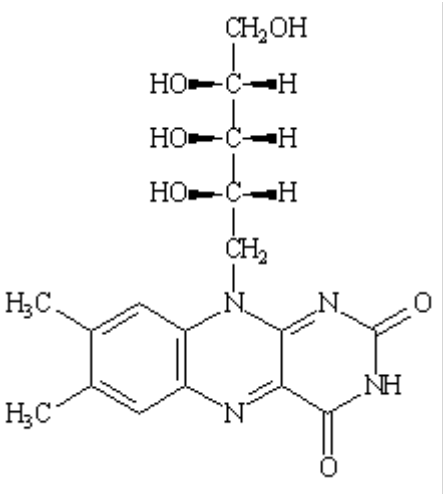
DEFINITION

Chemical names Riboflavin; 3,10-dihydro-7,8-dimethyl-10-[(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl]benzo-[g]pteridine-2,4-dione; 7,8-dimethyl-10-(1'-D-ribityl)isoalloxazine,

C.A.S. number 83-88-5

Chemical formula $C_{17}H_{20}N_4O_6$

Structural formula



Formula weight 376.37

Assay Not less than 98%

DESCRIPTION Yellow to orange-yellow crystalline powder, with slight odour

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Very slightly soluble in water; practically insoluble in alcohol, chloroform, acetone and ether; very soluble in dilute alkali solutions

Spectrophotometry (Vol. 4)

Using the aqueous solution from the Assay, determine the absorbance (A) at 267 nm, 375 nm and 444 nm. The ratio A_{375}/A_{267} is between 0.31 and 0.33. The ratio A_{444}/A_{267} is between 0.36 and 0.39.

Specific rotation

[alpha] 20, D: Between -115° and -140°
Dry the sample at 100° for 4 h. Dissolve 50.0 mg in 0.05 N sodium hydroxide free from carbonate and dilute to 10.0 ml with the same solvent. Measure the optical rotation within 30 min of dissolution.

Colour reaction

Dissolve about 1 mg of sample in 100 ml of water. The solution has a pale greenish-yellow colour by transmitted light, and by reflected light has an intense yellowish-green fluorescence which disappears on the addition of mineral acids and alkalis.

PURITY

Loss on drying (Vol. 4)

Not more than 1.5% (105°, 4 h)

Sulfated ash (Vol. 4)

Not more than 0.1%
Test 2 g of the sample (Method I)

Subsidiary colouring matters

Prepare the standard for this test for the absence of lumiflavin by diluting 3 ml of 0.1 N potassium dichromate with water to 1000 ml. Pour some chloroform through an alumina column to remove any ethanol. To 10 ml of this chloroform add 35 mg of the sample, shake for 5 min and filter. The colour of the filtrate should be no more intense than that of 10 ml of the standard when viewed in identical containers.

Primary aromatic amines (Vol. 4)

Not more than 100 mg/kg calculated as aniline

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Carry out the assay in subdued light. In a brown glass 500 ml volumetric flask, suspend 65.0 mg of the sample in 5 ml of water, ensuring that it is completely wetted, and dissolve in 5 ml of 2 N sodium hydroxide solution. As soon as dissolution is complete, add 100 ml of water and 2.5 ml of glacial acetic acid and dilute to 500.0 ml with water. Place 20.0 ml of this solution in a brown glass 200 ml volumetric flask, add 3.5 ml of a 1.4% w/v solution of sodium acetate and dilute to 200.0 ml with water. Measure the absorbance (A) at the maximum at 444 nm.

$$\%\ Riboflavin = \frac{A \times 5000}{328 \times W} \times 1.367$$

where
A = absorbance of the sample solution at 444 nm
W = weight of sample in g

RIBOFLAVIN 5'-PHOSPHATE SODIUM

Prepared at the 31st JECFA (1987), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). Add 6. A group ADI 0-0.5 mg/kg bw for riboflavin from Bacillus subtilis, synthetic riboflavin and riboflavin-5-phosphate was established at the 51st JECFA (1998).

SYNONYMS Riboflavin 5'-phosphate ester monosodium salt, Vitamin B_2 phosphate ester monosodium salt; INS No. 101(ii)

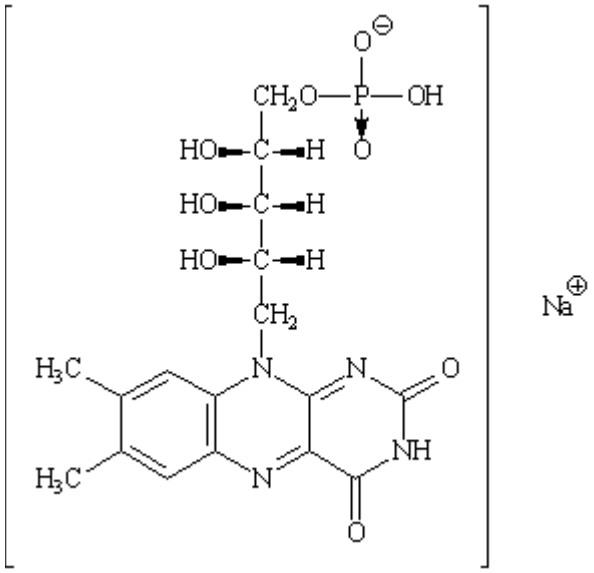
DEFINITION These specifications apply to riboflavin 5'-phosphate sodium together with minor amounts of free riboflavin and riboflavin diphosphate sodium.

Chemical names Monosodium (2R,3R,4S)-5-(3')10'-dihydro-7',8'-dimethyl-2',4'-dioxo-10'-benzo[g]pteridinyl)-2,3,4-trihydroxypentyl phosphate; monosodium salt of 5'-monophosphate ester of riboflavin.

C.A.S. number 130-40-5

Chemical formula $C_{17}H_{20}N_4NaO_9P \cdot 2H_2O$

Structural formula



Formula weight 514.36

Assay Not less than 95% of total colouring matters calculated as $C_{17}H_{20}N_4NaO_9P \cdot 2H_2O$

DESCRIPTION Yellow to orange crystalline hygroscopic powder, with slight odour

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water; insoluble in ethanol
Spectrophotometry (Vol. 4)	Using the aqueous solution from the Assay, determine the absorbance (A) at 267 nm, 375 nm and 444 nm. The ratio A_{375}/A_{267} is between 0.30 and 0.34. The ratio A_{444}/A_{267} is between 0.35 and 0.40.
Specific rotation	[alpha] 20, D: Between +38° and +42° (1.5% w/v solution of dried sample in 20% w/v hydrochloric acid)
Test for sodium (Vol. 4)	Passes test Use the sulfated ash for the test

PURITY

Loss on drying (Vol. 4)	Not more than 8% (100°, 5 h in a vacuum over phosphorus pentoxide)
Sulfated ash (Vol. 4)	Not more than 25% Test 0.5 g of the sample
Inorganic phosphate	Not more than 1% calculated as PO_4 on a dried basis See description under TESTS
Subsidiary colouring matters	Not more than 6% of each of free riboflavin and riboflavine disphosphate See description under TESTS Passes test for absence of lumiflavin
Primary aromatic amines (Vol. 4)	Not more than 70 mg/kg calculated as aniline
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Inorganic phosphate	Standard preparation: Transfer 220.0 mg of monobasic potassium phosphate KH_2PO_4, to a 1000 ml volumetric flask, dissolve in and dilute to volume with water and mix. Transfer 20.0 ml of this solution to a 100 ml volumetric flask, dilute to volume with water and mix. Test preparation: Transfer 300.0 mg of the sample to a 100 ml volumetric flask, dissolve in

and dilute to volume with water, and mix.

Acid molybdate solution:

Dilute 25 ml of ammonium molybdate solution (7 g of $(NH_4)_6Mo_7O_{24}.4H_2O$ in sufficient water to make 100 ml) to 200 ml with water, and then add slowly 25 ml of 7.5 N sulfuric acid.

Ferrous sulfate solution:

Just before use, prepare a 10% aqueous ferrous sulfate solution containing 2 ml of 7.5 N sulfuric acid per 100 ml of final solution.

Procedure:

Transfer 10.0 ml each of the Standard preparations and of the Test preparation into separate 50 ml Erlenmeyer flasks, add 10.0 ml of Acid molybdate solution and 5.0 ml of Ferrous sulfate solution to each flask, and mix. Determine the absorbance of each solution in a 1 cm cell at 700 nm with a suitable spectrophotometer, using as the blank a mixture of 10.0 ml of water, 10.0 ml of Acid molybdate solution, and 5.0 ml of Ferrous sulfate solution. The absorbance of the solution from the Test preparation is not greater than that of the Standard preparation.

Subsidiary colouring matters

Free riboflavin and riboflavine disphosphate

Standard preparation:

Transfer 35.0 mg of Riboflavin reference standard into a 250 ml Erlenmeyer flask, add 20 ml of pyridine and 75 ml of water, and dissolve the riboflavin by frequent shaking. Transfer the solution to a 1000 ml volumetric flask, dilute to volume with water, and mix. Transfer 20.0 ml of this solution to a second 1000 ml volumetric flask, adjust the pH to 6.0 by the addition of 8 ml of 0.1 N sulfuric acid, dilute to volume with water, and mix. Finally, transfer 25.0 ml of the last solution into a 100 ml volumetric flask, dilute to volume with dioxane-water mixture (1:3), and mix. This solution contains 0.175 µg of riboflavin per ml.

pH Buffer solution:

Dissolve 15.6 g of monobasic phosphate ($NaH_2PO_4.2H_20$) in about 100 ml of water, add 59.3 ml of 1 N sodium hydroxide TS, and dilute to 2000 ml with water. Check the pH with a pH meter, and adjust to 7.0 if necessary.

Test preparation:

Dissolve 100.0 g of the sample in 10.0 ml of pH 7 Buffer solution. Prepare a strip of Whatman chromatography paper, Type 3 mm, medium flow rate, or other equivalent paper suitable for electrophoresis, and saturate the paper with pH 7 Buffer solution. Using a micropipette, apply 0.01 ml of the sample solution along a narrow line of the cathode side of the paper strip contained in a suitable paper electrophoresis chamber. Apply a potential of approximately 250 V, allow electrophoresis to continue for 6 h, and then remove the paper from the chamber. Detect any free riboflavin and/or riboflavin diphosphate by observing the strip in daylight or under ultraviolet light. Free riboflavin, if present, will appear as a band nearest to the starting line, and riboflavin diphosphate will appear farthest from the starting line.

CAUTION:

The riboflavin will be destroyed if exposed to the ultraviolet light for more

than a few sec.
Cut off the respective bands, place them in separate 250 ml Erlenmeyer flasks containing 35.0 ml of dioxane-water mixture (1:3), and allow to stand until the spots are completely eluted from the strips.

Procedure:
Using a suitable fluorometer, determine the intensity of the fluorescence of each sample solution and of the Standard preparation at about 530 nm, using an excitation wavelength of about 460 nm. The fluorescence of the sample solution containing the eluted riboflavin band and riboflavin diphosphate band, respectively, is not greater than that produced by the Standard preparation.

Lumiflavin
Prepare the standard for this limit test for the absence of lumiflavin by diluting 3 ml of 0.1 N potassium dichromate with water to 1000 ml. Pour some chloroform through an alumina column to remove any ethanol. To 10 ml of this chloroform add 35 mg of the sample, shake for 5 min and filter. The colour of the filtrate should be no more intense than that of 10 ml of the standard when viewed in identical containers.

METHOD OF ASSAY

Carry out the assay in subdued light. In a brown glass 500 ml volumetric flask, dissolve 100 mg of the sample in 100 ml of water and add 2.5 ml of glacial acetic acid and dilute to 500.0 ml with water. Place 20.0 ml of this solution in a brown glass 200 ml volumetric flask, add 3.5 ml of a 1.4% w/v solution of sodium acetate and dilute to 200.0 ml with water. Measure the absorbance (A) at the maximum at 444 nm.

$$\% \ Total \ colouring \ matters = \frac{A \times 5000}{328 \times W} \times 1.367$$

where
A = absorbance of the sample solution at 444 nm.
W = weight of sample in g

RIBOFLAVIN from *BACILLUS SUBTILIS*

Prepared at the 53rd JECFA (1999) and published in FNP 52 Add 7 (1999), superseding specifications prepared at the 51st JECFA (1998), published in FNP 52 Add 6. Group ADI 0-0.5 mg/kg bw for riboflavin from Bacillus subtilis, synthetic riboflavin and riboflavin-5-phosphate established at the 51st JECFA in 1998.

SYNONYMS Vitamin B_2; lactoflavin; INS No. 101(iii)

SOURCE Prepared by submerged fermentation by *Bacillus subtilis* genetically modified for riboflavin overproduction. The strain is non-pathogenic and non-toxicogenic.

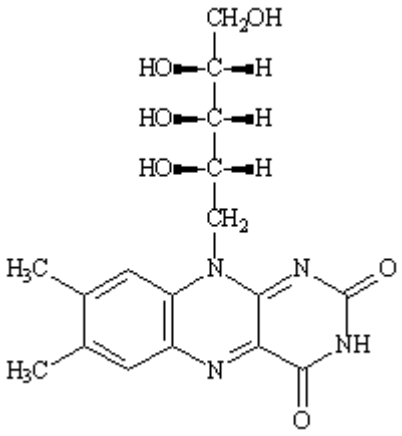
DEFINITION

Chemical names Riboflavin; 3,10-dihydro-7,8-dimethyl-10-[(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl]benzo-[g]pteridine-2,4-dione; 7,8-dimethyl-10-(1'-D-ribityl)isoalloxazine

C.A.S. number 83-88-5

Chemical formula $C_{17}H_{20}N_4O_6$

Structural formula



Formula weight 376.37

Assay Not less than 98.0% and not more than 101.0%, calculated on the dried basis

DESCRIPTION Yellow to orange-yellow crystalline powder

FUNCTIONAL USES Colour, nutrient supplement

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Practically insoluble in ethanol, acetone and diethyl ether; very soluble in dilute alkali solutions
Spectrophotometry (Vol. 4)	Using the aqueous solution from the Assay, determine the absorbance (A) at 267 nm, 375 nm and 444 nm. The ratio A_{375}/A_{267} is between 0.31 and 0.33. The ratio A_{444}/A_{267} is between 0.36 and 0.39.
Specific rotation	$[\alpha]^{20}_{D}$: Between -120 and -135° Dry the sample at 100° for 4 h. Dissolve 50.0 mg in 0.05 N sodium hydroxide free from carbonate and dilute to 10.0 ml with the same solvent. Measure the optical rotation within 30 min of dissolution.
Colour reaction	Dissolve about 1 mg of sample in 100 ml of water. The solution has a pale greenish-yellow colour by transmitted light, and by reflected light has an intense yellowish-green fluorescence, which disappears on the addition of mineral acids and alkalis.

PURITY

Loss on drying (Vol. 4)	Not more than 2.0% (105°, 4 h)
Sulfated ash (Vol. 4)	Not more than 0.1% Test 2 g of the sample (Method I)
Lumiflavin (Vol. 4)	Not more than 0.025% See description under TESTS
Primary aromatic amines (Vol. 4)	Not more than 100 mg/kg calculated as aniline
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Lumiflavin (Vol. 4)	Reference Solution: Dissolve 25 mg of lumiflavin in 50.0 ml of chloroform. Dilute 1.0 ml of this solution with chloroform to 20.0 ml, and dilute 2.5 ml of the resultant solution to 100 ml. This solution contains 0.625 µg lumiflavin per ml. Test Solution: Shake 25 mg of the sample with 10.0 ml chloroform for 5 min and filter.

Thin Layer Chromatography:
Stationary phase: Precoated HPTLC plates of silica gel $WRF_{254,}$ 10 x 20 cm, layer thickness 0.1 mm (Merck Cat No 1.12363)
Mobile phase: Water
Run length: approx. 6 cm
Elution time: approx. 20 min
Application volumes: 10 µl of Reference Solution and 10 µl of Test Solution

Detection: Dry the plate in a current of cold air and evaluate the fluorescence at 366 nm
Any spot in the chromatogram of the Test Solution, which corresponds to the main spot of the Reference Solution, shall not be larger or more intensely coloured than the Reference Solution spot.

METHOD OF ASSAY

Carry out the assay in subdued light. In a brown-glass 500-ml volumetric flask, suspend 65.0 mg of the sample in 5 ml of water, ensuring that it is completely wetted, and dissolve in 5 ml of 2 N sodium hydroxide solution. As soon as dissolution is complete, add 100 ml of water and 2.5 ml of glacial acetic acid and dilute to 500.0 ml with water. Place 20.0 ml of this solution in a brown glass 200-ml volumetric flask, add 3.5 ml of a 1.4% w/v solution of sodium acetate and dilute to 200.0 ml with water. Measure the absorbance (A) at the maximum, 444 nm.

$$\%\,\text{Riboflavin} = \frac{A \times 5000}{328 \times W} \times 1.367$$

where A = absorbance of the sample solution at 444 nm
W = weight of sample in g

SACCHARIN

Prepared at the 33rd JECFA (1988), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). An ADI of 0-5 mg/kg bw for saccharin and its Ca, K, Na salts was established at 41st JECFA (1993)

SYNONYMS

INS No. 954(i)

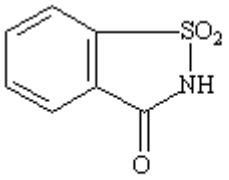
DEFINITION

Chemical names: 1,2-Benzisothiazole-3(2H)-one-1,1-dioxide, 3-oxo-2,3-dihydrobenzo[d]isothiazol-1,1-dioxide

C.A.S. number: 81-07-2

Chemical formula: $C_7H_5NO_3S$

Structural formula:



Formula weight: 183.18

Assay: Not less than 99% and not more than 101.0% on the dried basis

DESCRIPTION

White crystals or a white, crystalline powder, odourless or with a faint, aromatic odour

FUNCTIONAL USES

Sweetener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Slightly soluble in water; soluble in basic solutions; sparingly soluble in ethanol

Acidity: A saturated aqueous solution is acidic

Derivation to salicylic acid: Dissolve about 0.1 g of the sample in 5 ml of 5% sodium hydroxide solution. Evaporate to dryness and gently fuse the residue over a small flame until it no longer evolves ammonia. After the residue has cooled, dissolve it in 20 ml of water, neutralize the solution with dilute hydrochloric acid TS and filter.

	The addition of a drop of ferric chloride TS to the filtrate produces a violet colour.
Derivation to fluorescent substance	Mix 20 mg of the sample with 40 mg of resorcinol, add 10 drops of sulfuric acid, and heat the mixture in a liquid bath at 200º for 3 min. After cooling, add 10 ml of water and an excess of sodium hydroxide TS. A fluorescent green liquid is produced.
PURITY	
Loss on drying (Vol. 4)	Not more than 1% (105°, 2 h)
Melting range (Vol. 4)	226 - 230°
Sulfated ash (Vol. 4)	Not more than 0.2% Test 2 g of the sample (Method I)
Benzoic and salicylic acid	Add ferric chloride TS dropwise to 10 ml of a hot, saturated solution of the sample. No precipitate or violet colour appears.
Readily carbonizable substances (Vol. 4)	Dissolve 0.2 g of the sample in 5 ml of sulfuric acid TS. Keep at 48° to 50° for 10 min. The colour should not be darker than a very light brownish-yellow (*Matching Fluid A).*
Toluenesulfonamides (Vol. 4)	Not more than 25 mg/kg
Selenium (Vol. 4)	Not more than 30 mg/kg Test 0.2 g of the sample as directed in the Limit Test (Method I)
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Dissolve about 0.5 g of previously dried sample, accurately weighed, in 75 ml of hot water. Cool quickly, add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide. Each ml of 0.1 N sodium hydroxide is equivalent to 18.32 mg of $C_7H_5NO_3S$.

CALCIUM SACCHARIN

Prepared at the 24th JECFA (1980), published in FNP 17 (1980) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). An ADI of 0-5 mg/kg bw for saccharin and its calcium, potassium and sodium salts was established at the 41st JECFA (1993).

SYNONYMS

INS No. 954(ii)

DEFINITION

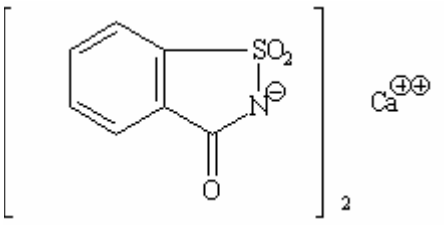
Chemical names

Calcium salt hydrate (2:7) of 1,2-benzisothiazole-3-one-1,1-dioxide, 3-oxo-2,3-dihydrobenzo[d]isothiazol-1,1- dioxide, 2,3-dihydro-3-oxobenziso-sulfonazole; calcium o-benzosulfimide.

Chemical formula

$C_{14}H_8CaN_2O_6S_2 \cdot 3½H_2O$

Structural formula



Formula weight

467.48

Assay

Not less than 99% after drying

DESCRIPTION

White crystals or a white, crystalline powder, odourless or with a faint, aromatic odour

FUNCTIONAL USES

Sweetener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)

Freely soluble in water, soluble in ethanol

Melting range of saccharin derived from the sample (Vol. 4)

226 - 230°
To 10 ml of a 1 in 10 solution add 1 ml of hydrochloric acid. A crystalline precipitate of saccharin is formed. Wash the precipitate well with cold water and dry at 105° for 2 h.

Derivation to salicylic acid

Dissolve about 0.1 g of the sample in 5 ml of 5% sodium hydroxide solution. Evaporate to dryness and gently fuse the residue over a small flame until it no longer evolves ammonia. After the residue has cooled, dissolve it in 20 ml of water, neutralize the solution with dilute hydrochloric

acid TS and filter. The addition of a drop of ferric chloride TS to the filtrate produces a violet colour.

Derivation to fluorescent substance — Mix 20 mg of the sample with 40 mg of resorcinol, add 10 drops of sulfuric acid, and heat the mixture in a liquid bath at 200º for 3 min. After cooling, add 10 ml of water and an excess of sodium hydroxide TS. A fluorescent green liquid is produced.

Test for calcium (Vol. 4) — Passes test

PURITY

Loss on drying (Vol. 4) — Not more than 15% (120°, 4 h)

Benzoic and salicylic acid — Add ferric chloride TS dropwise to 10 ml of a hot, saturated solution of the sample. No precipitate or violet colour appears.

Readily carbonizable substances (Vol. 4) — Dissolve 0.2 g of the sample in 5 ml of sulfuric acid TS. Keep at 48° to 50° for 10 min. The colour should not be darker than a very light brownish-yellow (Matching Fluid A).

Toluenesulfonamides (Vol. 4) — Not more than 25 mg/kg

Selenium (Vol. 4) — Not more than 30 mg/kg

Lead (Vol. 4) — Not more than 1 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh accurately about 0.5 g of the sample and transfer quantitatively to a separator with the aid of 10 ml of water. Add 2 ml of dilute hydrochloric acid TS, and extract the precipitated saccharin first with 30 ml, then with five 20 ml portions, of a liquid composed of 9 volumes of chloroform and 1 volume of ethanol. Filter each extract through a small filter paper moistened with the solvent mixture. Evaporate the combined filtrates on a steam bath to dryness with the aid of a current of air. Dissolve the residue in 75 ml of hot water, cool, add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide. Perform a blank determination, and make any necessary correction. Each ml of 0.1 N sodium hydroxide is equivalent to 20.22 mg of $C_{14}H_8CaN_2O_6S_2$.

POTASSIUM SACCHARIN

Prepared at the 28th JECFA (1984), published in FNP 31/2 (1984) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). An ADI of 0-5 mg/kg bw for saccharin and its Ca, K, Na salts was established at 41st JECFA (1993)

SYNONYMS INS No. 954(iii)

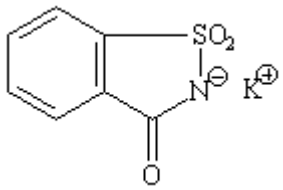
DEFINITION

Chemical names: Potassium salt of 1,2-benzisothiazole-3(2H)-one-1,1-dioxide monohydrate, 3-oxo-2,3-dihydrobenzo[d]isothiazol-1,1-dioxide monohydrate, 2,3-dihydro-3-oxobenzisо-sulfonazole monohydrate; potassium o-benzosulfimide

C.A.S. number: 10332-51-1

Chemical formula: $C_7H_4KNO_3S \cdot H_2O$

Structural formula:



Formula weight: 239.77

Assay: Not less than 99% and not more than 101% on the dried basis

DESCRIPTION White crystals or a white, crystalline powder, odourless or with a faint, aromatic odour

FUNCTIONAL USES Sweetener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water; sparingly soluble in ethanol

Melting range of saccharin derived from the sample: 226 - 230°
To 10 ml of a 1 in 10 solution add 1 ml of hydrochloric acid. A crystalline precipitate of saccharin is formed. Wash the precipitate well with cold water and dry at 105° for 2 h.

Derivation to salicylic acid: Dissolve about 0.1 g of the sample in 5 ml of 5% sodium hydroxide solution. Evaporate to dryness and gently fuse the residue over a small

flame until it no longer evolves ammonia. After the residue has cooled, dissolve it in 20 ml of water, neutralize the solution with dilute hydrochloric acid TS and filter. The addition of a drop of ferric chloride TS to the filtrate produces a violet colour.

Derivation to fluorescent substance — Mix 20 mg of the sample with 40 mg of resorcinol, add 10 drops of sulfuric acid, and heat the mixture in a liquid bath at 200º for 3 min. After cooling, add 10 ml of water and an excess of sodium hydroxide TS. A fluorescent green liquid is produced.

Test for potassium (Vol. 4) — Passes test
Test the residue obtained by igniting 2 g of the sample.

PURITY

Loss on drying (Vol. 4) — Not more than 8% (120°, 4 h)

Acidity and alkalinity — Dissolve 1 g of the sample in 10 ml of freshly boiled and cooled water. Add a drop of phenolphthalein TS. No pink colour should appear. Add a drop of 0.1 N sodium hydroxide. A pink colour should appear.

Benzoic and salicylic acid — To 10 ml of a 1 in 20 solution, previously acidified with 5 drops of acetic acid, add 3 drops of ferric chloride TS. No precipitate or violet colour appears.

Readily carbonizable substances (Vol. 4) — Dissolve 0.2 g of the sample in 5 ml of sulfuric acid TS. Keep at 48° to 50° for 10 min. The colour should not be darker than a very light brownish-yellow (Matching Fluid A).

Toluenesulfonamides (Vol. 4) — Not more than 25 mg/kg

Selenium (Vol. 4) — Not more than 30 mg/kg (0.2 g sample, Method II)

Lead (Vol. 4) — Not more than 1 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY — Dissolve about 0.3 g of the dried sample, accurately weighed, in 20 ml of glacial acetic acid. Add 2 drops of crystal violet-glacial acetic acid TS as indicator, and titrate with 0.1 N perchloric acid. End-point is where violet colour of the solution changes to green, via blue. Perform a blank determination, and make any necessary correction. Each ml of 0.1 N perchloric acid is equivalent to 22.18 mg of $C_7H_4KNO_3S$.

SODIUM SACCHARIN

Prepared at the 24th JECFA (1980), published in FNP 17 (1980) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). An ADI of 0-5 mg/kg bw for saccharin and its Ca, K, Na salts was established at 41st JECFA (1993)

SYNONYMS Soluble saccharin, INS No. 954(iv)

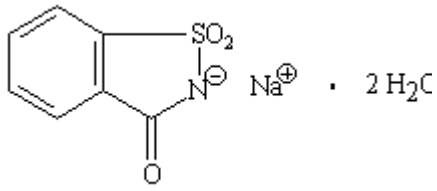
DEFINITION

Chemical names: Sodium salt dihydrate of 1,2-Benzisothiazolin-3(2H)-one-1,1-dioxide, 3-oxo-2,3-dihydrobenzo[d]isothiazole-1,1-dioxide; sodium o-benzosulfimide.

C.A.S. number: 128-44-9

Chemical formula: $C_7H_4NNaO_3S \cdot 2H_2O$

Structural formula:



Formula weight: 241.19

Assay: Not less than 99% and not more than 101% on the dried basis

DESCRIPTION White crystals or a white, crystalline efflorescent powder, odourless or with a faint, aromatic odour

FUNCTIONAL USES Sweetener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water; sparingly soluble in ethanol

Melting range of saccharin derived from the sample: 226 - 230°
To 10 ml of a 1 in 10 solution add 1 ml of hydrochloric acid. A crystalline precipitate of saccharin is formed. Wash the precipitate well with cold water and dry at 105° for 2 h.

Derivation to salicylic acid: Dissolve about 0.1 g of the sample in 5 ml of 5% sodium hydroxide solution. Evaporate to dryness and gently fuse the residue over a small

	flame until it no longer evolves ammonia. After the residue has cooled, dissolve it in 20 ml of water, neutralize the solution with dilute hydrochloric acid TS and filter. The addition of a drop of ferric chloride TS to the filtrate produces a violet colour.
Derivation to fluorescent substance	Mix 20 mg of the sample with 40 mg of resorcinol, add 10 drops of sulfuric acid, and heat the mixture in a liquid bath at 200° for 3 min. After cooling, add 10 ml of water and an excess of sodium hydroxide TS. A fluorescent green liquid is produced.
Test for sodium (Vol. 4)	Passes test
PURITY	
Loss on drying (Vol. 4)	Not more than 15% (120°, 4 h)
Acidity and alkalinity	Dissolve 1 g of the sample in 10 ml of freshly boiled and cooled water. Add a drop of phenolphthalein TS. No pink colour should appear. Add a drop of 0.1 N sodium hydroxide. A pink colour should appear.
Benzoic and salicylic acid	Add ferric chloride TS dropwise to 10 ml of a hot, saturated solution of the sample. No precipitate or violet colour appears.
Readily carbonizable substances (Vol. 4)	Dissolve 0.2 g of the sample in 5 ml of sulfuric acid TS. Keep at 48° to 50° for 10 min. The colour should not be darker than a very light brownish-yellow (Matching Fluid A).
Toluenesulfonamides (Vol. 4)	Not more than 25 mg/kg
Selenium (Vol. 4)	Not more than 30 mg/kg
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Dissolve about 0.3 g of previously dried sample, accurately weighed, in 20 ml of glacial acetic acid. Add 2 drops of crystal violet-glacial acetic acid TS as indicator, and titrate with 0.1 N perchloric acid. End-point is where violet colour of the solution changes to green, via blue. Perform a blank determination, and make any necessary correction. Each ml of 0.1 N perchloric acid is equivalent to 20.52 mg of $C_7H_4NNaO_3S$.

SALTS of FATTY ACIDS

Prepared at the 33rd JECFA (1988), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 55th JECFA (2000). An ADI 'not specified' was established at the 33rd JECFA (1988)

SYNONYMS INS No. 470

DEFINITION These products consist of calcium, potassium or sodium salts of commercial myristic, oleic, palmitic, stearic, acids or mixtures of these acids from edible fats and oils. The article of commerce can be further specified by:
- saponification value,
- solidification point for the fatty acids obtained from the salts,
- iodine value,
- residue on ignition including assay of the cation, and
- moisture content

Assay Not less than 95% total fatty acid salts, dry weight basis

DESCRIPTION Hard, white or faintly yellowish, somewhat glossy and crystalline solids or semi-solids or white or yellowish-white powder

FUNCTIONAL USES Anticaking agent, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Potassium and sodium salts are soluble in water and ethanol; calcium salts are insoluble in water, ethanol and ether

Test for cations Heat 1 g of the sample with a mixture of 25 ml of water and 5 ml of hydrochloric acid. Fatty acids are liberated, floating as a solid or oil layer on the surface which is soluble in hexane. After cooling, aqueous layer is decanted and evaporated to dryness. Dissolve the residue in water and *test for the appropriate cation*.

Fatty acid composition Using the Method of Assay, identify the individual fatty sample. The fatty acid(s) in primary abundance should conform to those declared on the label of the product

PURITY

Free fatty acids Not more than 3%
Measure free fatty acids as directed in the method *Free Fatty Acids.*
Compute free fatty acid content using an equivalence factor (e) equal to 1/10th the molecular weight of the salt.

Unsaponifiable matter Not more than 2%
See description under TESTS

Lead (Vol. 4) Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the

specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Unsaponifiable matter

Unsaponifiable matter is the whole of the products present in a fatty substance which, after saponification thereof with an alkaline hydroxide and extraction by a specified solvent, remains non-volatile under defined conditions of the test.

It includes lipids of natural origin such as sterols, higher aliphatic alcohols, pigments and hydrocarbons as well as any foreign matter non-volatile at 103^{o} which may be present.

Weigh to the nearest 0.01 g about 5 g of the well-mixed sample into a 250 ml round-bottomed flask. Add 50 ml of approximately 0.5N potassium hydroxide solution and some pumice, attach a reflux condenser, and boil gently for 1 h. Stop heating. Add 100 ml of distilled water through the top of the condenser and swirl.

After cooling, transfer the solution to a separatory funnel. Rinse the flask and the pumice several times with diethyl ether (100 ml in all) and pour this into the separatory funnel. Stopper and shake vigorously for 1 min, periodically releasing pressure by inverting the separating funnel and opening the stopcock.

Allow to stand until there is complete separation of the two phases. Then draw off the soap solution as completely as possible into a second separating funnel.

Extract the aqueous ethanolic soap solution twice more, each time in the same way with 100 ml of diethyl ether. Combine the three ether extracts in one separating funnel containing 40 ml of water.

Gently rotate the separating funnel containing the combined extracts and the 40 ml water. Violent agitation at this stage may result in troublesome emulsions. Allow the layers to separate completely and draw off the lower aqueous layer. Wash the ethereal solution twice more with 40 ml portions of water, shaking vigorously each time and discarding the lower aqueous layers after separation. Draw off each washing solution up to 2 ml, then rotate the separating funnel around its axis, wait some min to give the last remainders the opportunity for collection and draw off the collected remainders, close stopcock when ether starts to pass the bore of the stopcock.
Wash the ethereal solution successively with 40 ml of 0.5 N potassium hydroxide solution, 40 ml of water, and again with 40 ml of potassium hydroxide solution, then at least twice more with 40 ml of water. Continue to wash with water until the wash-water no longer gives a pink colour on the addition of a drop of phenolphthalein solution.

Transfer the ethereal solution quantitatively a little at a time through the top of the separating funnel into a flask previously dried and weighed to the nearest 0.0001 g.

Evaporate the solvent by distillation on a boiling-water bath.
Add 5 ml of acetone and remove the volatile solvent completely in a gentle current of air, holding the flask obliquely while turning it in a boiling-water bath.

Dry the residue at 103±2^{o} for 15 min, placing the flask in an almost horizontal position. Cool in a desiccator and weigh to the nearest 0.0001 g. Repeat the drying for successive 15 min periods until the loss of weight between two successive weighings is less than 0.0015 g.
Note: If constant mass is not obtained after three periods of drying, the unsaponifiable matter is probably contaminated.

After weighing the residue dissolve it in 4 ml of diethyl ether and then add 20 ml of ethanol previously neutralized to a faint pink colour, using phenolphthalein TS as indicator. Titrate with accurately standardized 0.1N ethanolic potassium hydroxide solution (prepare an approx. 1 N ethanolic solution by dissolving 60 g of potassium hydroxide in 50 ml of water and making up to 1 liter with ethanol; dilute this solution 1:10 with ethanol) to the same final colour.

Correct the weight of the residue for the free acidity content of the blank. Calculate the unsponifiable matter, in % (m/m) from:

$$\frac{100 \times (m_1 - 0.281 \times T \times V)}{m}$$

where
m = the mass, in g, of the test portion
m_1 = the mass, in g, of the residue
V = the number of ml of the standardized potassium hydroxide solution used
T = the exact normality of the potassium hydroxide solution used

METHOD OF ASSAY

Principle:
Saponification of the salts and esterification by methanol of the fatty acids in the presence of boron trifluoride, alkaline methanol. Gas liquid chromatography of the fatty acid methyl esters.

Part A - Preparation of the fatty acid methyl esters

Apparatus
- 50 and 100 ml ground-necked round-bottom flasks.
- Reflux condenser, 20 to 30 cm effective length, with ground joint appropriate to the flask.
- 250 ml separating funnels.
- Inlet tube for passing nitrogen.
- Test tubes with ground glass stoppers.
- Graduated pipette, capacity at least 10 ml, fitted with a rubber bulb or automatic pipette.

- Boiling chips (fat-free).

Reagents
- Heptane, chromatographic quality (Notes 2 and 4)
- Redistilled light petroleum (b.p. 40-60°), bromine value less than 1, residue free, or hexane (Note 2)
- Sodium sulfate, anhydrous
- Sodium hydroxide, appropriately 0.5 N methanolic solution: Dissolve 2 g of sodium hydroxide in 100 ml methanol containing not more than 0.5% (m/m) of water. When the solution has to be stored for a considerable time, a small amount of white precipitate of sodium may be formed; this has no effect on the preparation of the methyl esters.
- Boron trifluoride, 12 to 25% (m/m) methanolic solution. 14 and 50% solutions are available commercially (Note 2).
Caution: Boron trifluoride is poisonous. For this reason it is not recommended that the analyst prepare the methanolic solution of boron trifluoride from methanol and boron trifluoride (Note 3).
- Sodium chloride, saturated aqueous solution
- Methyl red, 1 g/l solution in 60% (v/v) ethanol
- Nitrogen, containing less than 5 mg/kg of oxygen

Procedure
Because of the toxic character of boron trifluoride, the following operations are best performed under a ventilated hood. All glassware must be washed with water immediately after use
Dry the sample at 105° to constant weight, using 2 hour increments of heating. Accurately weigh about 350 mg of dried sample. Sample sizes larger or smaller than 350 mg may be taken, however, the size of flask and quantities of reagents should conform to the following table:

weight of sample (mg)	*Flask capacity (ml)*	*0.5N NaOH (ml)*	*Methanolic soln. of BF_3 (ml)*
100-250	50	4	5
250-500	50	6	7
500-700	100	8	9
750-1000	100	10	12

Place the desired amount of prepared fatty acids in the appropriate flask. Add the appropriate amount of methanolic boron trifluoride solution. Boil for 2 min.

Add 2 to 5 ml of heptane (Note 4) (the precise amount does not affect the reaction) to the boiling mixture through the top of the condenser and continue boiling for 1 minute.
Withdraw the source of heat, and then remove the condenser. Add a small portion of saturated sodium chloride solution and shake the flask gently by rotating it several times.

Add more saturated sodium chloride solution to the flask in order to bring the level of liquid into the neck of the flask. Allow to separate and transfer about 1 ml of the upper layer (heptane solution) into a ground-necked test tube and add a little anhydrous sodium sulfate to remove any trace of water. If the sample taken was 350 mg, this solution contains about 7-17 percent

of methyl esters and may be injected directly onto the column for gas-liquid chromatography. In the other cases dilute the heptane solution to obtain a 5-10% concentration of methyl esters (Note 6).

In order to recover the whole of the dry esters, transfer the saline solution and the heptane layer into a separating funnel. Separate the layers. Extract the saline solution twice with 50 ml portions of light petroleum. Combine the heptane solution and the two extracts, and wash them with 20-ml portions of water until free from acid (methyl red indicator). Dry over anhydrous sodium sulfate, filter and evaporate the solvent over a boiling-water bath in a current of nitrogen (Notes 6 and 7). For samples under 500 mg it is desirable to reduce proportionately the volumes of solvent and water used.
Alternative methods which do not involve the use of boron trifluoride are available. In the general method the methylation reagents, 0.5 N methanolic sodium hydroxide and 12-25 % methanolic boron trifluoride, may be substituted with:

- 1 N Methanolic potassium hydroxide, (reacted with fatty substance in the presence of excess low moisture content methanol);
- Sodium methylate solution (prepared by dissolving 1 g of sodium metal in 100 ml of low-moisture content methanol).

Notes

1. If the unsaponifiable matter interferes, dilute the saponified solution with water and eliminate the unsaponifiable matter by extraction with diethyl ether or hexane. Acidify the aqueous soap solution and separate the fatty acids. Prepare the methyl esters from these as described.

2. In the course of the gas-liquid chromatography of the methyl esters, certain reagents, particularly the methanolic boron trifluoride solution may produce adventitious peaks on the graph (in the region of C_{20}-C_{22} esters in the case of methanolic boron trifluoride solutions). Consequently any new batch of reagent should be checked by preparing the methyl esters of pure oleic acid, and chromatographing them; if an extraneous peak appears, the reagent should be rejected. The various reagents must not give peaks interfering with those of methyl esters of fatty acids during the gas-liquid chromatography.
The methanolic solutions of boron trifluoride must be stored in a refrigerator.

3. If it is absolutely unavoidable to prepare a solution of boron trifluoride from gaseous boron trifluoride, the recommended method is:
Weigh a 2 l flask containing 1 l of methanol. Cool in an ice bath, and with the flask still in bath, bubble BF_3 from a cylinder through a glass tube into the methanol until 125 g BF_3 is absorbed. Perform the operation in a fume-cupboard. BF_3 must be flowing through the glass tube before it is placed in and until it is removed from methanol to prevent liquid from being drawn into the gas cylinder valve system. Gas should not flow so fast that white fumes emerge from flask.
This reagent is stable for 2 years.

4. If fatty acids containing twenty or more carbon atoms are absent, hexane may be substituted for heptane (mixture of pure C_7 isomers tested by gas-liquid chromatography).

5. If the suggested amount of sample is not available, 10 mg, or even less, may be used, provided that the amounts of reagents and the size of the containers are reduced proportionally.

6. Preferably, the solutions of methyl esters should be analysed as soon as possible. If necessary, the heptane solution containing the methyl esters may be stored under an inert gas in a refrigerator. In the case of prolonged storage, it is desirable to protect the methyl esters from autoxidation by adding to the solution an antioxidant in such a concentration as will not interfere with the subsequent analysis, e.g. 0.05 g/l of EHT (2,6-di-tertbutyl 4-methyl phenol).
If necessary, the dry and solvent-free methyl esters may be stored 24 h under inert gas in a refrigerator, or longer in sealed tube under vacuum in a deep-freeze.

7. There is some risk of losing part of the most volatile methyl esters if the evaporation of the solvent is prolonged, or if the current of nitrogen is too vigorous.
For infrared spectroscopy, elimination of the solvent must be as complete as possible.
For gas liquid chromatography remove solvent.

Part B - Gas-liquid chromatography of fatty acid methyl esters

Apparatus
The instruction given relate to the ordinary equipment used for gas-liquid chromatography, employing a packed column and a flame-ionization detector (Note 1). Any apparatus giving efficiency and resolution for the specific fatty substance is suitable.

Gas liquid chromatography
Injection system: The injection system should have the least dead space possible. If possible, it should be heated to a temperature 20 to 50^{o} higher than that of the column.
Oven: The oven should be capable of heating the column to at least 220^{o} and of maintaining the desired temperature to within 1^{o}.
If programmed heating is to be employed, an apparatus with a twin column is recommended.
Packed column:
- Column: The column must be constructed of a material inert to the substances to be analysed: glass or, failing this, stainless steel (Note 2); Length: 1 to 3 m, a relative short column should be used when long-chain acids (C_{20+}) are present. For the determination of C_4 and C_6 fatty acids, a 2-m column is recommended; Internal diameter: 2 to 4 mm.

Packing
- Support: Acid-washed and silanized diatomaceous earth, or other suitable inert support with a narrow range (25 µm) of grain size between the limits 125-200 µm, the average grain size being related to the internal diameter and length of the column.
- Stationary phase: Polyester type of polar liquid (e.g. diethylene glycol polysuccinate, butanediol polysuccinate, ethylene glycol polyadipate ...) or

any other liquid (e.g. cyanosilicones ...) meeting the requirements below. The stationary phase should amount to 5 to 20% of the packing. A non-polar stationary phase can be used for certain separations
- Conditioning the newly prepared column: The column being disconnected from the detector, if possible, heat the oven gradually to 185^{o} and pass a current of inert gas through the freshly prepared column at a rate of 20 - 60 ml/min for at least 16 h at this temperature, and for 2 h more at 195^{o}.
Detector : The manipulations described below relate to the use of a flame ionization detector (Note 1).
Syringe: Syringe, maximum capacity 10 µl, graduated in 0.1 µl.

Recorder
If the recorder curve is to be used to calculate the composition of the mixture analysed, an electronic recorder of high precision is required. It should be compatible with the apparatus used. The characteristics of the recorder should be:
- Rate of response below 1.5 sec, preferentially below 1 sec (the rate of response is the time taken for the recording pen to pass from 0 to 90% following the momentary introduction of a 100% signal)
- Breadth of the paper: 25 cm minimum
- Paper speed: 25-100 cm/h

Integrator or Calculator (Optional)
Rapid and accurate calculation can be performed with the help of an electronic integrator or calculator. This must give a linear response with adequate sensitivity, and the correction for deviation of the base-line must be satisfactory.

Reagents
- Carrier gas: Inert gas (nitrogen, helium, argon ...) thoroughly dried and containing less than 10 mg/kg of oxygen.
- Auxiliary gases: Hydrogen (99.9% min.) free from organic impurities, air or oxygen.
- Reference standards: A mixture of methyl esters, or the methyl esters of an oil, of known composition, preferably similar to that of the fatty matter to be analysed.
Procedure

Conditions of Test
Determining optimal operating conditions
As a rule, the figures shown in Table 1 and 2 below, will lead to the desired results.

Table 1

Internal diameter of column	Carrier gas supply
2 mm	15-25 ml/min
3 mm	20-40 ml/min
4 mm	40-60 ml/min

Table 2

Concentration of stationary phase	Temperature

5 %	175°
10 %	180°
15 %	180°
20 %	185°

When the apparatus allows it, the injector should be at a temperature of about 200°C and the detector at a temperature equal to, or higher than, that of the column.

The flow of hydrogen to the flame-ionization detector is, as a rule, about half that of the carrier gas, and the flow of oxygen about 5 to 10 times that of the hydrogen.

Determining the efficiency and the resolution (Optional)
Carry out the analysis of a standard of methyl stearate. Choose the size of the sample, the temperature of the column and the carrier gas flow so that the maximum of the methyl stearate peak is recorded about 15 min after the solvent peak, and rises to three-quarters of the full scale
- Analysis
The sample for examination shall be 0.1 to 2 µl of the heptane solution of methyl esters obtained according to Part A. In the case of esters not in solution, prepare an approximate 10% solution in heptane and inject 0.1 to 1 µl of this.
As a rule, the operating conditions will be those defined above. Nevertheless, it is possible to work with a lower column temperature where the determination of acids below C_{12} is required, or at higher temperature when determining fatty acids above C_{20}.
On occasion, it is possible to employ temperature programming in both the previous cases. If the sample contains the methyl esters of fatty acids below C_{12}, it is necessary to inject the sample at 100° (or at 50-60° if butyric acid is present) and immediately to raise the temperature at a rate of 4-8°/min to the optimum. In some cases the two procedures can be combined: after the programmed heating, continue the elution at a constant temperature until all the components have been eluted. If the instrument does not operate with programme heating, work at two fixed temperatures between 100° and 195°.

Expression of the results

Qualitative Analysis
Analyse the reference standard mixture of known composition in the same operating conditions as those employed for the sample, and measure the retention distances (or retention times) for the constituent fatty esters. Using a semi-logarithmic paper, construct the graphs showing the logarithm of the retention distance (or retention time) as a function of the number of carbon atoms of the acids; in isothermal conditions the graphs for straight chain esters of the same degree of unsaturation should be straight lines. These straight lines are approximately parallel.
Identify the peaks for the sample from these graphs, if necessary by interpolation. It is necessary to avoid conditions such that "masked peaks" exist, i.e. where the resolution is insufficient to separate two components.

Quantitative Analysis

Determination of the composition
Apart from exceptional cases, use the method of area normalization, i.e. assume that the whole of the components of the sample are represented on the chromatogram, so that the total of the areas under the peaks represents 100 percent of the constituents (total elution).
By appropriate standardization procedures (using a reference standard mixture or an internal standard), determine the total weight of fatty acids in the dried sample. Calculate the content of Fatty Acid Salts for the specific cation(s) in the sample. the content of total Fatty Acid Salts shall be not less than 95% of the dried sample. In addition if there are specifications on the label of the Fatty Acid Salt for content of individual fatty acids, the sample must comply with these specifications.

Notes
1. A gas-liquid chromatograph employing a catharometer (working on thermal conductivity changes) may be used. Operating conditions must then be modified as follows:

Column
- length: 2 to 4 m
- internal diameter: 4 mm
- support: grain size between 160 and 200 µm
- stationary phase: 15 to 25%
Carrier gas: helium, or failing this, hydrogen, with a content of oxygen as low as possible. No auxiliary gases.
Flow rate: usually between 60 and 80 ml/min
Temperatures
- Injector: 40^{o} to 60^{o} above that of the column
- Column: 180^{o} to 200^{o}

Quantitative analysis: correction of factors derived from the analysis of a reference mixture of esters of known composition, determined under operating conditions identical with those used for the sample, must be used.

2. If polyunsaturated components with more than three double bonds are present, they may decompose in a stainless-steel column.

SHELLAC, BLEACHED

Prepared at the 55th JECFA (2000) and published in FNP 52 Add 8 (2000), superseding specifications prepared at the 51st JECFA (1998) and published in FNP 52 Add 6 (1998). An ADI "Acceptable, present uses (as a coating, glazing, and surface-finishing agent externally applied to food) not of toxicological concern" was established at the 39th JECFA (1992).

SYNONYMS INS No. 904

DEFINITION Shellac is a polyester resin obtained from lac, the resinous secretion of the insect *Laccifer* (*Tachardia*) lacca Kerr (Fam. *Coccidae*). Bleached shellac is obtained by dissolving the lac in aqueous sodium carbonate, followed by bleaching with sodium hypochlorite, precipitation of the bleached lac with dilute sulfuric acid solution, and drying; wax-free bleached shellac is prepared by further treatment whereby the wax is removed by filtration.

C.A.S. number 9000-59-3

DESCRIPTION Bleached shellac: off-white to tan, amorphous granular resin; wax-free bleached shellac: light yellow, amorphous, granular resin

FUNCTIONAL USES Coating agent, glazing agent, surface finishing agent

CHARACTERISTICS

IDENTIFICATION

Colour reaction — To 50 mg of the sample add a few drops of a solution of 1 g ammonium molybdate in 3 ml of sulfuric acid. A green colour is produced, changing to lilac when the solution is neutralized with 6 N ammonium hydroxide.

Solubility (Vol. 4) — Insoluble in water; freely (though very slowly) soluble in ethanol; slightly soluble in acetone and ether

Acid value — Between 60 and 89
See description under TESTS

PURITY

Loss on drying (Vol. 4) — Not more than 6% (40°, 4 h, then room temperature over silica gel, 15 h)

Rosin — Dissolve 2 g of the sample in 10 ml of dehydrated ethanol, and add slowly, with shaking, 50 ml of solvent hexane. Transfer to a separator, wash with two 50-ml portions of water, and discard the washings. Filter the solvent layer, evaporate it to dryness, and to the residue add 2 ml of a mixture of 1 volume of liquefied phenol and 2 volumes of methylene chloride. Stir and transfer a portion of the mixture to a cavity of a colour-reaction plate. Fill an adjacent cavity with a mixture of 1 volume of bromine and four volumes of methylene chloride, and cover both cavities with an inverted watch glass. No purple or deep indigo blue colour is produced in or above the liquid containing the sample residue.

Wax — Bleached shellac: not more than 5.5%; wax-free bleached shellac, not more than 0.2%
See description under TESTS

Lead (Vol. 4) — Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods”.

TESTS

IDENTIFICATION TESTS

Acid value — Dissolve about 1 g of finely ground sample, accurately weighed, in 50 ml of alcohol previously neutralized to phenolphthalein with sodium hydroxide, and titrate with 0.1 N potassium hydroxide. Determine the end point using phenolphthalein TS or a potentiometer. If phenolphthalein is used, titrate until the pink colour persists for at least 30 sec. Calculate the acid value by the formula 56.1V x N/W, in which V is the exact volume, in ml, N is the exact normality of the sodium hydroxide solution, and W is the weight, in g, of sample taken, calculated on the dry basis.

PURITY TESTS

Wax — Transfer about 10 g of finely ground sample, accurately weighed, and 2.5 g of sodium carbonate to a 200-ml tall-form beaker. Add 150 ml of hot water, immerse the beaker in a boiling water bath, and stir until the sample is dissolved. Cover the beaker with a watch glass, heat for 3 h without agitation, and cool in a cold water bath. When the wax has floated to the surface, filter the mixture through medium-speed quantitative ashless filter paper, transferring the wax to the paper, and wash the filter with water. Pour 5 to 10 ml of ethanol onto the filter to accelerate drying. Wrap the paper loosely in a large piece of filter paper, bind with a piece of fine wire, and dry with the aid of gentle heat. Extract with chloroform in a suitable continuous extraction apparatus for 2 h, using a previously dried and accurately weighed flask to receive the extracted wax and solvent. Evaporate the solvent, dry the wax at 105^{o} to constant weight, and calculate the percentage of wax.

SILICON DIOXIDE, AMORPHOUS

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). An ADI 'not specified' for silicon dioxide and certain silicates, was established at the 29th JECFA (1985)

SYNONYMS Silica; INS No. 551

DEFINITION The products included under this specification are: silica aerogel (precipitated silicon dioxide), hydrated silica, "silicic acid", and dehydrated silica gel. The article of commerce can be further specified as to loss on drying and soluble ionizable salts.

Chemical names: Silicon dioxide

C.A.S. number: 7631-86-9

Chemical formula: $(SiO_2)_x$

Formula weight: 60.09 (SiO_2)

Assay: Silica aerogel: not less than 90% of SiO_2 on the ignited basis.
Hydrated silica: not less than 89% of SiO_2 on the ignited basis.

DESCRIPTION Silica aerogel: a microcellular silica occurring as a fluffy powder or granules
Hydrated silica: a precipitated, hydrated silicon dioxide occurring as a fine, white, amorphous powder, or as beads or granules

FUNCTIONAL USES Anticaking agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Insoluble in water and ethanol; soluble in hydrofluoric acid and alkalies (80° - 100°). (CAUTION: hydrofluoric acid is toxic, corrosive, must not contact skin. Work under fume hood).

Test for silica: Test for volatility of SiF_4 -see Method of Assay

PURITY

Loss on ignition (Vol. 4): Not more than 6% on the dried basis (105° to constant weight), after igniting at 600° (for silica aerogel) or at 900° (for hydrated silica) to constant weight.

Lead (Vol. 4): Not more than 5mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF Weigh 2 g of the sample to the nearest 0.1 mg, and transfer into a tared

ASSAY

platinum crucible. Ignite at 1000^{o} for 1 h, cool in a desiccator and weigh. Moisten the residue with 7 or 8 drops of ethanol, add 3 drops of concentrate sulfuric acid, and, with CAUTION, add enough hydrofluoric acid to cover the wetted sample. Evaporate to dryness on a hot-plate, using medium heat (95-105^{o}), then add 5 ml of hydrofluoric acid, swirl the dish carefully to wash down the sides, and again evaporate to dryness. Ignite the dried residue to a red heat over a Meker burner, cool in a desiccator, and weigh. The difference between the total weight loss and the weight loss after ignition at 1000^{o} represents the weight of SiO_2 in the sample taken.

SODIUM ACETATE

Prepared at the 18th JECFA (1974), published in NMRS 54B (1975) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI not limited' was established at the 17th JECFA (1973)

SYNONYMS INS No. 262(i)

DEFINITION

Chemical names	Sodium acetate
C.A.S. number	127-09-3
Chemical formula	$C_2H_3NaO_2 \cdot nH_2O$ (n = 0 or 3)
Structural formula	$CH_3COONa \cdot nH_2O$ (n = 0 or 3)
Formula weight	Anhydrous: 82.03 Trihydrate: 136.08
Assay	Not less than 98.5% after drying

DESCRIPTION Anhydrous: White, odourless, granular, hygroscopic powder
Trihydrate: Colourless, transparent crystals or a granular crystalline powder, odourless or with a faint, acetic odour. Effloresces in warm, dry air.

FUNCTIONAL USES Buffer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Very soluble in water; soluble in ethanol
pH (Vol. 4)	8.0 - 9.5 (1 in 100 soln)
Test for sodium (Vol. 4)	Passes test
Test for acetate (Vol. 4)	Passes test
Heat test	Anhydrous: When heating the sample slowly, it first fuses gradually and boils, and later decomposes evolving an unpleasant odour of acetone. A solution of the residue gives alkaline reaction with litmus paper. Trihydrate: When heating the sample slowly, it liquefies. Then water evaporates, and a powder forms. By heating more strongly, the powder fuses, and becomes lumpy and later decomposes evolving an odour of acetone. A solution of the residue gives alkaline reaction with litmus paper.

PURITY

Loss on drying (Vol. 4)	Anhydrous: Not more than 2.0% (120^o, 4 h) Trihydrate: Between 36 and 42% (120^o, 4 h)

Test for potassium (Vol. 4)	Negative test
Acidity and alkalinity	Anhydrous: Dissolve 1.2 g of the sample in 20 ml of freshly boiled and cooled water. Add 2 drops of phenolphthalein TS, and keep the solution at 10^{o}. If a colourless solution is produced, not more than 0.1 ml of 0.1 N sodium hydroxide should be required to give a pink colour. If a pink colour is produced, not more than 0.1 N hydrochloric acid should be required to discharge it. Trihydrate: Weigh 2 g of the sample and proceed as directed under Anhydrous above.
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Weigh accurately about 200 mg of the sample obtained in the test for "Loss on drying". Dissolve in 40 ml of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid in glacial acetic acid. Perform a blank determination, and make any necessary correction. Each ml of 0.1 N perchloric acid is equivalent to 8.203 mg of $C_2H_3NaO_2$.

SODIUM ALGINATE

Prepared at the 49th JECFA (1997) , published in FNP 52 Add 5 (1997) superseding specifications prepared at the 44th JECFA (1995), published in FNP 52 Add 3 (1995). An ADI 'not specified' was established at the 39th JECFA (1992)

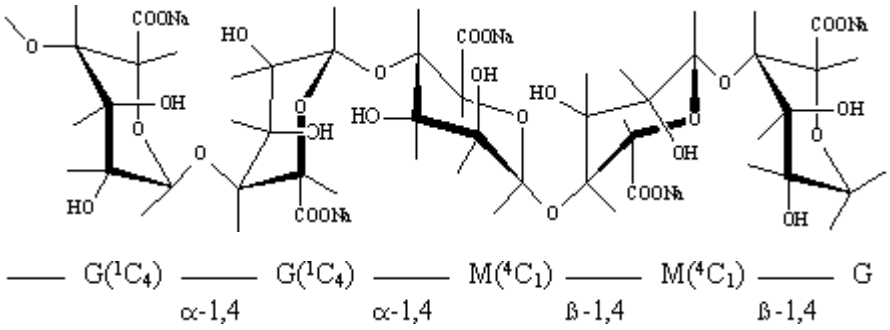
SYNONYMS INS No. 401

DEFINITION Sodium salt of alginic acid

C.A.S. number 9005-38-3

Chemical formula $(C_6H_7NaO_6)_n$

Structural formula Structural formula from Phillips, Wedlock and Williams: Gums and Stabilizers for the Food Industry 5 (1990) by permission of Oxford University Press.



The number and sequence of the Mannuronate and Glucuronate residues shown above vary in the naturally occurring alginate. The water molecules associated with the alginate molecule are not shown in the above structural formula.

Formula weight Structural unit : 198.11 (theoretical), 222 (actual average)
Macromolecule : 10,000 - 600,000 (typical average)

Assay Yields, on the dried basis, not less than 18.0% and not more than 21.0% of carbon dioxide (CO_2), equivalent to not less than 90.8% and not more than 106.0% of sodium alginate $(C_6H_7NaO_6)_n$.

DESCRIPTION White to yellowish brown filamentous, grainy, granular or powdered forms

FUNCTIONAL USES Stabilizer, thickener, gelling agent, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Dissolves slowly in water, forming a viscous solution; insoluble in ethanol and ether

Precipitate formation with calcium chloride	To a 0.5% solution of the sample in sodium hydroxide TS add one-fifth of its volume of a 2.5% solution of calcium chloride. A voluminous, gelatinous precipitate is formed. This test distinguishes sodium alginate from gum arabic, sodium carboxymethyl cellulose, carrageenan, gelatin, gum ghatti, karaya gum, carob bean gum, methyl cellulose and tragacanth gum.
Precipitate formation with ammonium sulphate	To a 0.5% solution of the sample in sodium hydroxide TS add one-half of its volume of a saturated solution of ammonium sulfate. No precipitate is formed. This test distinguishes sodium alginate from agar, sodium carboxymethyl cellulose, carrageenan, de-esterified pectin, gelatin, carob bean gum, methyl cellulose and starch.
Test for alginate (Vol. 4)	Passes test
Test for sodium (Vol. 4)	Passes test
PURITY	
Loss on drying (Vol. 4)	Not more than 15% (105^{o}, 4 h)
Water-insoluble matter	Not more than 2% on the dried basis See description under TESTS
Arsenic (Vol. 4)	Not more than 3 mg/kg (Method II)
Lead (Vol. 4)	Not more than 5 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
Microbiological criteria (Vol. 4)	Total plate count: Not more than 5,000 colonies per gram. Initially prepare a 10^{-1} dilution by adding a 50 g sample to 450 ml of Butterfield’s phosphate buffered dilution water and homogenizing in a high speed blender. Yeasts and moulds: Not more than 500 colonies per gram Coliforms: Negative by test Salmonella: Negative by test

TESTS

PURITY TESTS

Water-insoluble matter	Disperse 2 g of the sample, weighed to the nearest 0.1 mg, in 800 ml of water in a 2,000-ml flask. Neutralize to pH 7 with sodium hydroxide TS and then add 3 ml in excess. Add 40 ml of hydrogen peroxide solution containing 30% by weight H_2O_2, cover the flask and boil for 1 h with frequent stirring. Filter while hot through a tared Gooch crucible provided with a glass fibre filter (2.4 cm, No 934 AH, Reeve Angel & Co, Clifton, N.Y.,USA, or equivalent). If slow filtration is caused by high viscosity of the sample solution, boil until the viscosity is reduced enough to permit filtration. Wash the crucible thoroughly with hot water, dry the crucible and its contents at 105^{o} for 1 h, cool and weigh. Calculate as percentage of the

dry weight.

METHOD OF ASSAY

Proceed as directed under *Carbon Dioxide Determination by Decarboxylation* (see Volume 4). Each ml of 0.25 N sodium hydroxide consumed is equivalent to 5.5 mg of carbon dioxide (CO_2) or 27.75 mg of sodium alginate (equivalent weight 222).

SODIUM ALUMINOSILICATE

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). A group PTWI of 1 mg/kg bw for aluminium and its salts was established at the 67th JECFA (2006).

SYNONYMS Sodium silicoaluminate; INS No. 554

DEFINITION A series of hydrated sodium aluminium silicates. The article of commerce may be specified further as to silicon dioxide, aluminium oxide, and sodium oxide content, loss on drying, loss on ignition and pH of a slurry in water.

Chemical names Sodium aluminosilicate

DESCRIPTION Odourless, fine, white amorphous powder, or as beads.

FUNCTIONAL USES Anticaking agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water and ethanol, partially soluble in strong acids and alkali hydroxides

Test for sodium (Vol. 4) Passes test

Test for aluminium (Vol. 4) Passes test

Test for silicate Passes test
See description under TESTS

PURITY

Lead (Vol. 4) Not more than 5 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

IDENTIFICATION TESTS

Test for silicate Mix about 500 mg of the sample with about 200 mg of anhydrous sodium carbonate and 2 g of anhydrous potassium carbonate, and heat the mixture in a platinum or nickel crucible until it melts completely. Cool, add 5 ml of

water, and allow to stand for 3 min. Heat the bottom of the crucible gently, detach the melt, and transfer it to a beaker with the aid of about 50 ml of water. Add gradually hydrochloric acid until no effervescence is observed, then add 10 ml more of the acid, and evaporate the mixture on a steam bath to dryness. Cool, add 20 ml of water, boil and filter the mixture through an ash-free filter paper. An insoluble residue of silica remains. (Note. Retain the filtrate for the test for aluminium). Transfer the gelatinous residue into a platinum dish, and cautiously add 5 ml of hydrofluoric acid (warning: toxic, corrosive, must not contact skin; work under fume hood). The precipitate dissolves. (If it does not dissolve, repeat the evaporation with hydrofluoric acid.) Heat and hold in the vapours a glass stirring rod with a drop of water on the tip. The drop becomes turbid.

SODIUM ASCORBATE

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003). A group ADI 'not specified' was established for ascorbic acid and its Ca, K and Na salts at the 25th JECFA (1981).

SYNONYMS INS No. 301

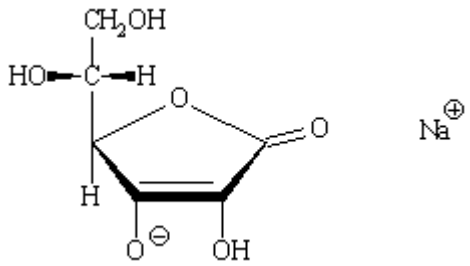
DEFINITION

Chemical names: Sodium ascorbate, sodium L-ascorbate, 2,3-didehydro-L-threo-hexono-1,4-lactone sodium enolate; 3-keto-L-gulofurano-lactone sodium enolate

C.A.S. number: 134-03-2

Chemical formula: $C_6H_7O_6Na$

Structural formula:



Formula weight: 198.11

Assay: White or almost white, odourless crystalline powder which darkens on exposure to light

DESCRIPTION Not less than 99% after drying

FUNCTIONAL USES Antioxidant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water; very slightly soluble in ethanol
See description under TESTS

Test for ascorbate Vol. 4): Passes test

Test for sodium (Vol. 4): Passes test
Test a solution of previously ignited sample, acidified with dilute acetic acid TS, filtered if necessary

Reducing reaction	A solution of the sample will decolourize a solution of 2,6-dichloro-phenolindophenol TS

PURITY

Loss on drying (Vol. 4)	Not more than 0.25% (vacuum desiccator over sulfuric acid, 24 h)
pH (Vol. 4)	6.5 - 8.0 (1 in 10 soln)
Specific rotation (Vol. 4)	[alpha] 25,D: Between +103^{o} and +108^{o} (10% (w/v) aqueous solution)
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Dissolve about 0.400 g of the dried sample in a mixture of 100 ml of carbon dioxide-free water and 25 ml of dilute sulphuric acid TS. Titrate the solution at once with 0.1 N iodine, adding a few drops of starch TS as indicator as the end-point is approached. Each ml of 0.1 N iodine is equivalent to 9.905 mg of $C_6H_7O_6Na$.

SODIUM CARBONATE

Prepared at the 19th JECFA (1975), published in NMRS 55B (1976) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI 'not limited' was established at the 9th JECFA (1965).

SYNONYMS Soda ash; INS No.500(i)

DEFINITION

Chemical names: Sodium carbonate, sodium salt of carbonic acid

C.A.S. number: 497-19-8

Chemical formula: Anhydrous: Na_2CO_3
Hydrated: $Na_2CO_3 \cdot xH_2O$

Formula weight: 106.00 (anhydrous)

Assay: Not less than 99.0% after drying

DESCRIPTION Colourless crystals or white, granular or crystalline powder; the anhydrous form is hygroscopic; hydrated forms available include the monohydrate and the decahydrate; the latter is efflorescent.

FUNCTIONAL USES Alkali

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water; insoluble in ethanol

Test for sodium (Vol. 4): Passes test

Test for carbonate (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4): Anhydrous: Not more than 2%
Monohydrate: Not more than 15%
Decahydrate: 55 - 65%
For all forms, heat the sample first at about 70°, then gradually raise the temperature and finally dry at 250-300° to constant weight.

Lead (Vol. 4): Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the

specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh accurately about 2 g of the dried sample. Dissolve carefully in 50 ml of 1 N sulfuric acid, add methyl orange TS and titrate the excess acid with 1 N sodium hydroxide.

Each ml of 1 N sulfuric acid is equivalent to 53.0 mg of Na_2CO_3.

SODIUM CARBOXYMETHYL CELLULOSE

Prepared at the 74th JECFA (2011) and published in FAO JECFA Monographs 11 (2011), superseding specifications prepared at the 55th JECFA (2000), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI 'not specified' was established for modified celluloses at the 35th JECFA (1989).

SYNONYMS Sodium cellulose glycolate; Na CMC; cellulose gum; sodium CMC; INS No. 466

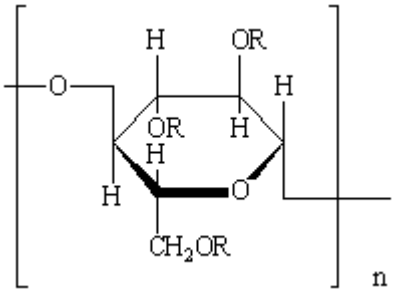
DEFINITION Prepared from cellulose by treatment with alkali and monochloro-acetic acid or its sodium salt. The article of commerce can be specified further by viscosity.

Chemical names: Sodium salt of carboxymethyl ether of cellulose

C.A.S. number: 9004-32-4

Chemical formula: $[C_6H_7O_2(OH)_x(OCH_2COONa)_y]_n$
where
n is the degree of polymerization
x = 1.50 to 2.80
y = 0.2 to 1.50
x + y = 3.0
(y = degree of substitution)

Structural formula



where R = H or CH_2COONa

Formula weight: Structural unit with a degree of substitution of 0.20: 178.14
Structural unit with a degree of substitution of 1.50: 282.18
Macromolecules: greater than about 17,000 (n about 100)

Assay: Not less than 99.5% of sodium carboxymethyl cellulose, calculated on the dried basis

DESCRIPTION White or slightly yellowish, almost odourless hygroscopic granules, powder or fine fibres

FUNCTIONAL USES Thickening agent, stabilizer, suspension agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Yield viscous colloidal solution with water; insoluble in ethanol
Foam test	Vigorously shake a 0.1% solution of the sample. No layer of foam appears. This test distinguishes sodium carboxymethyl cellulose from other cellulose ethers and from alginates and natural gums.
Precipitate formation	To 5 ml of a 0.5% solution of the sample add 5 ml of a 5% solution of copper sulfate or of aluminium sulfate. A precipitate appears. (This test permits the distinction of sodium carboxymethyl cellulose from other cellulose ethers, and from gelatine, carob bean gum and tragacanth gum).
Colour reaction	Add 0.5 g of powdered carboxymethylcellulose sodium to 50 ml of water, while stirring to produce a uniform dispersion. Continue the stirring until a clear solution is produced. To 1 ml of the solution, diluted with an equal volume of water, in a small test tube, add 5 drops of 1-naphthol TS. Incline the test tube, and carefully introduce down the side of the tube 2 ml of sulfuric acid so that it forms a lower layer. A red-purple colour develops at the interface.

PURITY

Loss on drying (Vol. 4)	Not more than 12% after drying (105°, to constant weight)
pH (Vol. 4)	6.0 - 8.5 (1 in 100 solution)
Sodium (Vol. 4)	Not more than 12.4% on the dried basis Determine total sodium content by *Atomic Absorption Spectroscopy* or *Flame Photometry*
Sodium chloride	Not more than 0.5% on the dried basis See description under TESTS
Free glycolate	Not more than 0.4% calculated as sodium glycolate on the dried basis See description under TESTS
Degree of substitution	Not less than 0.20 and not more than 1.50 See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under “General Methods, Metallic Impurities”).

TESTS

PURITY TESTS

Sodium chloride

Heat 5 g of the sample, weighed to the nearest 0.1 mg, in a platinum or porcelain crucible, first with a small flame so that the sample does not ignite and then, when the charring is complete, heat further in an electric oven for 15 min at about 500°. After cooling, pulverize the ashes thus obtained and extract several times with warm water. Filter the extracts into a 500-ml volumetric flask, acidify with nitric acid and dilute to the mark. Determine the NaCl content of 100 ml of this extract by the method of Volhard, using 0.02 N silver nitrate and 0.02 N ammonium thiocyanate. Each ml of 0.02 N silver nitrate is equivalent to 1.169 mg of NaCl. Calculate the sodium chloride content by the formula:

$$\% \text{ NaCl} = \frac{a \times 0.001169 \times 5}{b} \times 100$$

where

a is ml of 0.02 N silver nitrate used; and
b is the dry weight of 5 g of the sample.

Free glycolate

Weigh 0.5 g of the sample to the nearest 0.1 mg, and transfer to a 100-ml beaker. Moisten the sample thoroughly with 5 ml of glacial acetic acid, followed by 5 ml of water, and stir with a glass rod until the solution is complete (usually about 15 min are required). Slowly add 50 ml of acetone while stirring and then approximately 1 g of sodium sulfate. Continue the stirring for several min to ensure complete precipitation of the carboxymethyl cellulose. Filter through a soft, open-texture paper, previously wetted with a small amount of acetone, and collect the filtrate in a 100-ml volumetric flask. Use 30 ml of acetone to facilitate the transfer of the solids and to wash the filter cake. Make up to volume with acetone and mix.

Prepare a blank solution containing 5 ml of water, 5 ml of glacial acetic acid and acetone in another 100-ml volumetric flask. Pipet 2 ml of the sample solution and 2 ml of the blank solution into two 25-ml volumetric flasks. Remove the acetone by heating the uncovered flasks upright in a boiling water bath for exactly 20 min. Cool to room temperature and add 5 ml of naphthalenediol TS, mix thoroughly, then add 15 ml more of the TS and mix. Cover the mouth of the flask with a small piece of aluminium foil and heat upright in the boiling water bath for 20 min. Cool to room temperature and make up to volume with naphthalenediol TS.

Measure the absorbance of sample solution against blank solution at 540 nm using 1-cm cells. Read the corresponding mg of glycolic acid from the calibration curve obtained as follows:

Introduce 0, 1, 2, 3 and 4-ml aliquots of standard glycolic acid solution (1 mg per ml, prepared by weighing accurately 0.100 g of glycolic acid, previously dried in a vacuum desiccator for at least 16 hours, and then dissolving in 100 ml of water; do not keep the

solution longer than 30 days) into a series of five 100-ml volumetric flasks. Add water to each flask to a volume of 5 ml, then add 5 ml of glacial acetic acid and make up with acetone to mark and mix. Pipet 2 ml of each solution (containing, respectively, 0, 1, 2, 3, and 4 mg of glycolic acid per 100 ml) into a series of five 25-ml volumetric flasks and proceed in the same manner as described for the Test Solution. Plot the mg of glycolic acid in the original 100 ml of solution against absorbance to give a calibration curve.

Calculate the sodium glycolate (free glycolate) content by the formula:

$$\% \text{ Sodium glycolate} = \frac{a \times 0.129}{b}$$

where

a is mg of glycolic acid read from the calibration curve; and
b is g of dry-weight of the sample.

Degree of substitution

Sample preparation

Weigh 5 g of the sample to the nearest 0.1 mg, and transfer into a 500-ml conical flask. Add 350 ml of methanol or ethanol (80% by volume). Shake the suspension mechanically for 30 min. Decant through a tared glass filtering crucible under gentle suction. Avoid, at the end of the decanting, suction of air through the crucible. Repeat the treatment with the extraction liquid until the test for chloride ions with a solution of silver nitrate TS is negative. Normally three treatments are sufficient. Transfer the sodium carboxymethyl cellulose into the same crucible. Displace the extraction liquid that adheres to the substance with acetone. Dry the crucible in an oven at 110^{o} until constant in weight. Weigh the first time after 2 h. Cool the crucible every time in a desiccator and pay attention during weighing to the fact that sodium carboxymethyl cellulose is slightly hygroscopic.

Procedure

Weigh 2 g, to the nearest 0.1 mg, of the bone dry substance, obtained with the above-mentioned alcohol-extraction procedure, in a tared porcelain crucible. Initially, char carefully with a small flame and afterwards for 10 min, with a large flame. Cool and then moisten the residue with 3-5 ml of concentrate sulfuric acid. Heat cautiously until the fuming is finished. After some cooling add about 1 g of ammonium carbonate, distributing the powder over the whole contents of the crucible. Heat again, initially with a small flame until the fuming is finished and heat then at a dull red heat for 10 min. Repeat the treatment with sulfuric acid and ammonium carbonate if the residual sodium sulfate still contains some carbon. Cool the crucible in a desiccator and weigh. Instead of adding ammonium carbonate and heating further with a flame, the crucible can be placed for 1 h in an oven at about 600^{o}.

Calculate the sodium content of the alcohol-extracted sample by the formula:

$$\% \text{ Sodium} = \frac{a \times 32.38}{b}$$

where
a is the weight of residual sodium sulfate; and
b is the weight of the alcohol-extracted dry sample.

Calculate the degree of substitution by the formula:

$$\text{Degree of substitution} = \frac{162 \times \% \text{ sodium}}{2300 - (80 \times \% \text{ sodium})}$$

METHOD OF ASSAY

Calculate the percentage of sodium carboxymethyl cellulose in the sample by subtracting from 100% the sum of the percentages of sodium chloride and sodium glycolate (free glycolate), determined separately by the procedures above.

$$\text{Content \%} = 100 - (\%\text{NaCl} + \% \text{ sodium glycolate})$$

SODIUM CARBOXYMETHYL CELLULOSE, ENZYMATICALLY HYDROLYZED

Prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998) superseding tentative specifications prepared at the 49th JECFA (1997), published in FNP 52 Add 5 (1997). This substance was included at the 51st JECFA (1998) in the group ADI "not specified" for modified celluloses, established at the 35th JECFA in 1989.

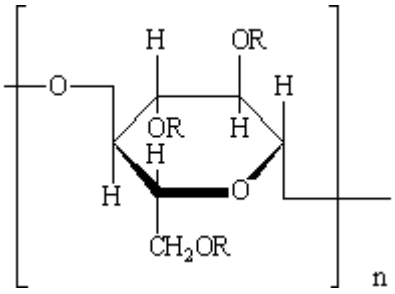
SYNONYMS Enzymatically hydrolyzed carboxymethyl cellulose; CMC-ENZ; INS No. 469

DEFINITION The product is the sodium salt of a carboxymethyl ether of cellulose, which has been partially hydrolyzed by enzymatic treatment with food-grade *Trichoderma reesei* cellulase. The total content of mono- and disaccharides does not exceed about 7.5%.

Chemical names: Carboxymethyl cellulose, sodium, partially enzymatically hydrolyzed

Chemical formula: Sodium salts of polymers containing substituted anhydroglucose units with the general formula:
$[C_6H_7O_2(OH)_x(OCH_2COONa)_y]_n$
where
n is the degree of polymerization
x = 1.50 to 2.80
y = 0.2 to 1.50
x + y = 3.0
(y = degree of substitution)

Structural formula:



where R = H, CH_2COONa or CH_2COOH

Formula weight: 178.14, where y = 0.20
282.18, where y = 1.50
Macromolecules: Not less than 800 (n about 4)

Assay: Not less than 99.5%, including mono- and disaccharides, on the dried basis

DESCRIPTION White or slightly yellowish or greyish, odourless, slightly hygroscopic granular or fibrous powder

FUNCTIONAL USES Carrier, glazing agent, stabilizer, thickener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water; insoluble in ethanol
Foam test	Vigorously shake a 0.1% solution of the sample. No layer of foam appears. This test distinguishes sodium carboxymethyl cellulose, whether hydrolysed or not, from other cellulose ethers and from alginates and natural gums.
Precipitate formation	To 5 ml of a 0.5% solution of the sample add 5 ml of a 5% solution of copper or aluminium sulfate. A precipitate appears. This test distinguishes sodium carboxymethyl cellulose, whether hydrolysed or not, from other cellulose ethers, and from gelatine, carob bean gum and tragacanth gum.
Colour reaction	Add 0.5 g of the powdered sample to 50 ml of water, while stirring to produce a uniform dispersion. Continue the stirring until a clear solution is produced. Dilute 1 ml of the solution with 1 ml of water in a small test tube. Add 5 drops of 1-naphthol TS. Incline the tube, and carefully introduce down the side of the tube 2 ml of sulphuric acid so that it forms a lower layer. A red-purple colour develops at the interface.
Viscosity (60% solids)	Not less than 2500 mPas corresponding to an average molecular weight of 5000 D. This test also distinguishes enzymatically hydrolyzed CMC from non-hydrolyzed CMC since it is not possible to make a 60% solution of ordinary CMC. See description under TESTS

PURITY

Loss on drying (Vol. 4)	Not more than 12% (105° to constant weight)
pH (Vol. 4)	Not less than 6.0 and not more than 8.5 (1 in 100 solution)
Sodium chloride and sodium glycolate	Not more than 0.5%, singly or in combination See descriptions under TESTS
Degree of substitution	Not less than 0.2 and not more than 1.50 carboxymethyl groups (CH_2COOH) per anhydroglucose unit on the dried basis See description under TESTS
Residual enzyme activity	Passes test See description under TESTS
Lead (Vol. 4)	Not more than 3 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

IDENTIFICATION TESTS

Viscosity (60% solids)

To 40 ml of water, add 60 g of the sample, stirring continuously. To ensure the formation of a clear solution without air bubbles, leave the solution to stand in a refrigerator (+4°) for several hours. Using a Bohlin viscometer or an equivalent instrument, measure the viscosity of the solution at 25° using a shear rate of 147sec^{-1}.

PURITY TESTS

Sodium chloride

Heat 5 g of the sample, weighed to the nearest 0.1 mg, in a platinum or porcelain crucible, first with a small flame so that the sample does not ignite and then, when the charring is complete, heat further in an electric oven for 15 min at about 500°. After cooling, pulverize the ashes thus obtained and extract several times with warm water. Filter the extracts into a 500-ml volumetric flask, acidify with nitric acid and dilute to the mark with water. Determine the NaCl content of 100 ml of this extract by the method of Volhard, using 0.2 N silver nitrate and 0.02 N ammonium thiocyanate. Each ml of 0.02 N silver nitrate is equivalent to 1.169 mg of NaCl. Calculate the sodium chloride content by the formula:

$$\% \text{ NaCl} = [(a \times 0.001169 \times 5)/b] \times 100$$

where
a = 0.02 N silver nitrate consumed (ml)
b = dry weight of the sample (g)

Sodium glycolate

Proceed as directed under *Chromatography (High Performance Liquid Chromatography)* using the following conditions and using pure glycolic acid as the reference substance:
Column: Two cation exchange columns in the H^+-form in series, e.g. two Fast Fruit Juice columns, 15 cm x 7.8 mm, Waters, or equivalent
Elution: Isocratic
Mobile phase: Aqueous 0.05% phosphoric acid
Flow: 0.5 ml/min
Detector type: UV or diode array, 205 nm
Sample size: 50 µl of a solution of 200.0 mg of the sample in 20 ml of eluent

Degree of substitution

Weigh accurately about 200 mg of the sample, previously dried at 105° to constant weight, and transfer it into a 250-ml glass-stoppered Erlenmeyer flask. Add 75 ml of glacial acetic acid, and connect the flask with a water-cooled condenser, and reflux gently on a hot plate for 2 h. Cool, transfer the solution to a 250-ml beaker with the aid of 50 ml of glacial acetic acid, and titrate with 0.1 N perchloric acid in dioxane while stirring with a magnetic stirrer. Determine the endpoint potentiometrically with a pH meter equipped with a standard glass electrode and a calomel electrode modified as follows: Discard the aqueous potassium chloride solution, rinse and fill with the supernatant liquid obtained by shaking thoroughly 2 g each of potassium chloride and silver chloride (or silver oxide) with 100 ml of methanol, then add a few crystals of potassium chloride and silver chloride (or silver oxide) to the electrode.

Record the ml of 0.1 N perchloric acid versus mV (0 to 700 mV range), and continue the titration to a few ml beyond the endpoint. Plot the titration curve, and read the volume (A), in ml, of 0.1 N perchloric acid at the

inflection point.

Calculate the degree of substitution (DS) by the formula

(16.2 A/G)/[1.000 - (8.0 A/G)],

where
A = the volume of 0.1 N perchloric acid required (ml)
G = weight of the sample taken (mg)
16.2 = one-tenth of the formula weight of one anhydroglucose unit
8.0 = one-tenth of the formula weight of one sodium carboxymethyl group

Residual enzyme activity

Prepare a 5% solution of sodium carboxymethyl cellulose having a viscosity of 25-50 mPas as a 2% solution. To 20 g of this 5%-solution, add 2 g of a 20% aqueous solution of the sample. Using a Bohlin viscometer or an equivalent instrument, follow the viscosity of the mixture for 10 minutes at 25^{o}, using a shear rate of 147sec^{-1}. No change in viscosity (indicating hydrolysis of the sodium carboxymethyl cellulose), occurs. As a control, measure the viscosity of 2 g of water and 20 g of the same sodium carboxymethyl cellulose solution.

METHOD OF ASSAY

Calculate the percentage of enzyme treated sodium carboxymethyl cellulose by subtracting from 100 the sum of the percentages of sodium chloride and sodium glycolate, determined separately by the procedures above.

SODIUM DIHYDROGEN CITRATE

Prepared at the 30th JECFA (1986), published in FNP 37 (1986) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). A group ADI 'not limited' for citric acid and its calcium, potassium, sodium and ammonium salts was established at the 23rd JECFA (1979)

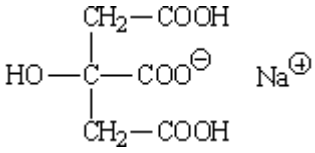
SYNONYMS Monosodium citrate, sodium citrate monobasic; INS No. 331(i)

DEFINITION

Chemical names: Monosodium citrate, monosodium salt of 2-hydroxy-1,2,3-propanetricarboxylic acid

Chemical formula: $C_6H_7NaO_7$

Structural formula:



Formula weight: 214.11

Assay: Not less than 99.0% and not more than 101.0%

DESCRIPTION White, odourless crystals or crystalline powder

FUNCTIONAL USES Buffering agent, sequestrant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water; practically insoluble in ethanol

pH (Vol. 4): 3.4 - 3.8 (1 in 10 soln)

Test for citrate (Vol. 4): Passes test

Test for sodium (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4): Not more than 0.4% (105°, 4 h)

Oxalate (Vol. 4): Passes test

Lead (Vol. 4): Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in

Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh accurately about 180 mg of the dried sample. Dissolve it in 25 ml of water and titrate with 0.1 N sodium hydroxide (potentiometric end-point determination). Each ml of 0.1 N sodium hydroxide is equivalent to 10.706 mg of $C_6H_7NaO_7$.

SODIUM DL-MALATE

Prepared at the 67th JECFA (2006) and published in FAO JECFA Monographs 3 (2006), superseding specifications prepared at the 30th JECFA (1986) and published in FNP 52 (1992) and in the Combined Compendium of Food Additive specifications, FAO JECFA monographs 1 (2005). Metals and arsenic specifications were revised at the 59th JECFA (2002). An ADI 'not specified' was established at the 23rd JECFA (1979).

SYNONYMS Malic acid sodium salt; INS No. 350(ii)

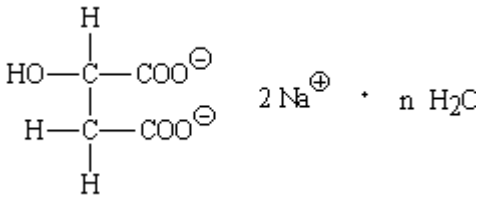
DEFINITION

Chemical names: Disodium DL-malate, hydroxybutanedioic acid disodium salt

C.A.S. number: 676-46-0

Chemical formula: Hemihydrate: $C_4H_4Na_2O_5 \cdot 1/2\ H_2O$
Trihydrate: $C_4H_4Na_2O_5 \cdot 3\ H_2O$

Structural formula:



Formula weight: Hemihydrate: 187.1
Trihydrate: 232.1

Assay: Not less than 98% and not more than 102% on the dried basis

DESCRIPTION Odourless white crystalline powder or lumps

FUNCTIONAL USES Acidity regulator

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water

Test for sodium (Vol. 4): Passes test

Test for malate (Vol. 4): Passes test
Test 5 ml of a 1 in 20 solution of the sample

PURITY

Loss on drying (Vol. 4)	Hemihydrate: Not more than 7% (130°, 4 h) Trihydrate: 20.5% - 23.5% (130°, 4 h)
Alkalinity	Not more than 0.2% as Na_2CO_3 Dissolve 1 g of the sample in 20 ml of freshly boiled and cooled water, and add 2 drops of phenolphthalein TS. If a pink colour is produced, add 0.4 ml of 0.1 N sulfuric acid. The colour of the solution disappears.
Fumaric and maleic acid (Vol. 4)	Not more than 1.0% of fumaric acid and not more than 0.05% of maleic acid
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4.
METHOD OF ASSAY	Dissolve about 0.25 g of the dried sample, accurately weighed, in 50 ml of glacial acetic acid, and titrate with 0.1 N perchloric acid, determining the endpoint potentiometrically. Each ml of 0.1 N perchloric acid is equivalent to 8.903 mg of $C_4H_4Na_2O_5$.

SODIUM FUMARATE

Prepared at the 30th JECFA (1986), published in FNP 37 (1986) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI 'Not specified' for fumaric acid and its salts was established at the 35th JECFA (1989).

SYNONYMS Monosodium fumarate; INS No. 365

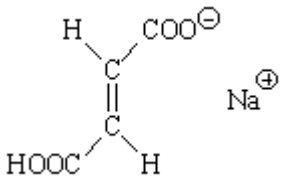
DEFINITION

Chemical names: Monosodium fumarate, monosodium trans-butenedioic acid, monosodium trans-1,2-ethylenedicarboxylate; monosodium trans-1,2-ethylenedicarboxylic acid

C.A.S. number: 7704-73-6

Chemical formula: $C_4H_3NaO_4$

Structural formula:



Formula weight: 138.06

Assay: Not less than 98.0% and not more than 102.0% on the dried basis

DESCRIPTION Odourless, white crystalline powder

FUNCTIONAL USES Buffering agent, acidulant, flavouring enhancer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Soluble in water

pH (Vol. 4): 3 - 4 (1 in 30 solution)

1,2-Dicarboxylic acid: Place 50 mg of the sample in a test tube, add 2 to 3 mg of resorcinol and 1 ml of sulfuric acid, shake, heat at 130° for 5 min. and cool. Dilute with water to 5 ml and add sodium hydroxide solution (2 in 5) dropwise to render the solution alkaline, cool and dilute with water to 10 ml. A greenish blue

	fluorescence is observed under an ultraviolet lamp.
Test for double bond	Add 10 ml of water to 0.5 g of the sample and dissolve by boiling. Add 2 or 3 drops of bromine TS to the hot solution. The colour of bromine TS disappears.
Test for sodium (Vol. 4)	Passes test
PURITY	
Loss on drying (Vol. 4)	Not more than 0.5% (120^{o}, 4 h)
Sulfates (Vol. 4)	Not more than 0.01% Add 30 ml of water to 1 g of the sample, shake, add 1 drop of phenolphthalein TS, and add ammonia TS dropwise until a slight pink colour is produced. Add 1 ml of dilute hydrochloric acid TS. Perform the test for the Limit Test. The solution corresponds to not more than 0.2 ml of 0.01 N sulfuric acid.
Maleic acid (Vol. 4)	Not more than 0.05%
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Weigh accurately about 0.3 g of the dried sample and dissolve in 30 ml of water. Titrate with 0.1 N sodium hydroxide, using 2 drops of phenolphthalein TS as the indicator. Each ml of 0.1 N sodium hydroxide is equivalent to 13.81 mg of $C_4H_3NaO_4$.

SODIUM GLUCONATE

Prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998) superseding specifications prepared at the 23rd JECFA (1979), published in FNP 12 (1979) and republished in FNP 52 (1992). Group ADI "not specified" for glucono-delta-lactone and gluconates, excluding ferrous gluconate, established at the 51st JECFA in 1998.

SYNONYMS INS No. 576

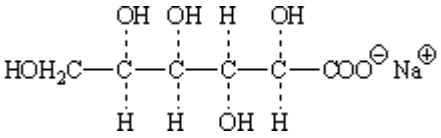
DEFINITION

Chemical names Sodium D-gluconate

C.A.S. number 527-07-1

Chemical formula $C_6H_{11}NaO_7$

Structural formula



Formula weight 218.14

Assay Not less than 98.0%

DESCRIPTION White to tan, granular to fine, crystalline powder

FUNCTIONAL USES Sequestrant, yeast food

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Very soluble in water; sparingly soluble in ethanol

Test for sodium (Vol. 4) Passes test

Test for gluconate (Vol. 4) Passes test

PURITY

Reducing substances (Vol. 4) Not more than 1.0% calculated as D-glucose (Method I)

Lead (Vol. 4) Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in

Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Transfer about 150 mg of the sample, accurately weighed, into a clean, dry 200-ml Erlenmeyer flask, add 75 ml of glacial acetic acid and dissolve by heating on a hot plate. Cool, add quinaldine red TS, and titrate with 0.1 N perchloric acid in glacial acetic acid, using a 10-ml microburet, to a colourless end point. Each ml of 0.1 N perchloric acid is equivalent to 21.81 mg of $C_6H_{11}NaO_7$.

SODIUM HYDROGEN CARBONATE

Prepared at the 19th JECFA (1975), published in NMRS 55B (1976) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI 'not limited' was established at the 9th JECFA (1965).

SYNONYMS Baking soda, bicarbonate of soda, sodium bicarbonate; INS No. 500(ii)

DEFINITION

Chemical names	Sodium hydrogen carbonate, sodium acid carbonate
C.A.S. number	144-55-8
Chemical formula	$NaHCO_3$
Formula weight	84.01
Assay	Not less than 99.0% after drying

DESCRIPTION Colourless, white, crystalline masses or crystalline powder

FUNCTIONAL USES Alkali, leavening agent, buffer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water; insoluble in ethanol
pH (Vol. 4)	8.0 - 8.6 (1 in 100 soln of the sample in cold water without shaking)
Test for sodium (Vol. 4)	Passes test
Test for carbonate (Vol. 4)	Passes test

PURITY

Loss on drying (Vol. 4)	Not more than 0.25% (over silica gel, 4 h)
Water insoluble substances	1 g of the sample dissolves completely in 20 ml of water and gives a clear solution
Ammonium salts	Heat 1 g of the sample in a test tube. No odour of ammonia is detected.
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample

preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh accurately about 3 g of the dried sample, add methyl orange TS and titrate with 1 N sulfuric acid. Each ml of 1 N sulfuric acid is equivalent to 84.01 mg of $NaHCO_3$.

SODIUM HYDROGEN DL-MALATE

Prepared at the 67th JECFA (2006) and published in FAO JECFA Monographs 3 (2006), superseding specifications prepared at the 26th JECFA (1982) and published in FNP 52 (1992) and in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). Heavy metals and arsenic specifications were revised at the 59th JECFA (2002). An ADI 'not specified' was established at the 26th JECFA (1982).

SYNONYMS Malic acid monosodium salt; INS No. 350(i)

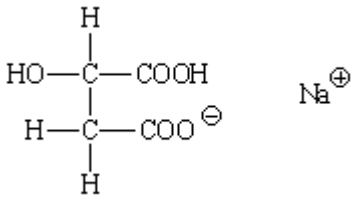
DEFINITION

Chemical names: Monosodium DL-malate, 2-hydroxybutanedioic acid monosodium salt

C.A.S. number: 58214-38-3

Chemical formula: $C_4H_5NaO_5$

Structural formula:



Formula weight: 156.1

Assay: Not less than 99.0% on the dried basis

DESCRIPTION Odourless white powder

FUNCTIONAL USES Buffering agent, humectant

CHARACTERISTICS

IDENTIFICATION

Test for sodium (Vol. 4): Passes test

Test for malate (Vol. 4): Passes test
Test 5 ml of a 1 in 20 solution of the sample

PURITY

Loss on drying (Vol. 4): Not more than 2% (110°, 3 h)

Fumaric and maleic acid (Vol. 4)	Not more than 1.0% of fumaric acid and not more than 0.05% of maleic acid
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4.

METHOD OF ASSAY

Weigh accurately about 1.5 g of the dried sample and transfer into a platinum or porcelain crucible of 20 to 30 mm in diameter. Heat very gently, and gradually raise the temperature. Continue heating for 2 h, and carbonize thoroughly. The heating temperature is between 300° and 400°, at which the crucible shows a dull red colour. If a gas burner is used, the flame should not contact with the carbonized mass. After allowing the carbonized mass to cool, disintegrate with a glass rod, and transfer the mass and crucible into a beaker. Add 50 ml of water and 50 ml of 0.5 N sulfuric acid, cover the beaker with a watch glass, heat the contents on a water bath for 1 h, and filter. If the filter is coloured, weigh the sample again, and carbonize it thoroughly. Wash the beaker, the crucible and the residue on the filter paper with hot water until the washings become neutral to blue litmus paper. Combine the washings to the filtrate. Titrate an excess of sulfuric acid with 0.5 N sodium hydroxide, using 3 drops of methyl red TS as the indicator. Each ml of 0.5 N sulfuric acid is equivalent to 78.04 mg of $C_4H_5NaO_5$.

SODIUM HYDROGEN SULFITE

Prepared at the 53rd JECFA (1999) and published in FNP 52 Add 7 (1999), superseding tentative specifications prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998). Group ADI 0-0.7 mg/kg bw as SO2 for sulfites established at the 51st JECFA in 1998.

SYNONYMS INS No. 222

DEFINITION

Chemical names	Sodium hydrogen sulfite, sodium bisulfite
C.A.S. number	7631-90-5
Chemical formula	$NaHSO_3$
Formula weight	104.06
Assay	Not less than 58.5% and not more than 67.4% of SO_2

DESCRIPTION White crystals or granular powder having an odour of sulfur dioxide

FUNCTIONAL USES Antibrowning agent, antioxidant, preservative

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Freely soluble in water; slightly soluble in ethanol
Test for sodium (Vol. 4)	Passes test
Test for sulfite (Vol. 4)	Passes test

PURITY

Water insolubles	Dissolve 20 g of the sample in 200 ml of water. The solution should be clear with only a trace of suspended matter
pH (Vol. 4)	2.5 - 4.5 (1 in 10 soln)
Iron (Vol. 4)	Not more than 10 mg/kg Proceed as directed in the Limit Test using 0.5 ml of Iron Standard Solution (5 µg Fe) in the control
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
Selenium	Not more than 5 mg/kg

See description under TESTS

TESTS

PURITY TESTS

Selenium

Reagents:
Hydrochloric acid, hydrazinium sulfate, standard selenium solution (100 µg Se/ml)

Procedure
Weigh 2.0 ± 0.1 g of sample and transfer to a 50-ml beaker. Add 10 ml water, 5 ml hydrochloric acid and boil to remove SO_2.

Into a second beaker, weigh 1.0 ± 0.1 g of sample, add 0.05 ml standard selenium solution and proceed as above.

To each beaker add 2 g hydrazinium sulfate and warm to dissolve. Let stand for 5 min. Dilute the contents of each beaker to 50 ml in a Nessler tube and compare the colour of the two solutions. The sample should be less pink than the sample with the added standard.

METHOD OF ASSAY

Weigh 0.2 g of the sample, to the nearest mg, add 50.0 ml of 0.1 N iodine in a glass-stoppered flask, and stopper the flask. Allow to stand for 5 min, add 1 ml of hydrochloric acid, and titrate the excess iodine with 0.1 N sodium thiosulfate, adding starch TS as the indicator. Each ml of 0.1 N iodine is equivalent to 3.203 mg of SO_2.

SODIUM HYDROXIDE

Prepared at the 19th JECFA (1975), published in NMRS 55B (1976) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI not limited' was established at the 9th JECFA (1965)

SYNONYMS Caustic soda, lye, sodium hydrate, INS No. 524

DEFINITION

Chemical names	Sodium hydroxide
C.A.S. number	1310-73-2
Chemical formula	NaOH
Formula weight	40.00
Assay	Not less than 95.0% of total alkali calculated as NaOH

DESCRIPTION White or nearly white pellets, flakes, sticks, fused masses or other forms

FUNCTIONAL USES Alkali

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Very soluble in water; freely soluble in ethanol
Test for alkali	A 1 in 100 solution of the sample is strongly alkaline
Test for sodium (Vol. 4)	Passes test

PURITY

Water insoluble substances	1 g of the sample dissolves completely in 20 ml of water and gives a clear and colourless solution
Carbonate	Not more than 3% as sodium carbonate Each ml of 1 N sulfuric acid required between the phenolphthalein and methyl orange end-points in the Method of assay is equivalent to 106.0 mg of Na_2CO_3
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

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METHOD OF ASSAY Dissolve about 1.5 g of the sample, accurately weighed, in 40 ml of recently boiled and cooled water, cool to 15°, add phenolphthalein TS and titrate with 1 N sulfuric acid. At the discharge of the pink colour, record the volume

of acid required, then add methyl orange TS and continue to titrate to a persistent pink colour. Record the total volume of acid required for the titration. Each ml of 1 N sulfuric acid is equivalent to 40.00 mg of total alkali, calculated as NaOH.

SODIUM LACTATE (SOLUTION)

Prepared at the 18th JECFA (1974), published in NMRS 54B (1975) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003). An ADI 'not limited' for lactic acid and its salts was established at the 23rd JECFA (1979)

SYNONYMS INS No. 325

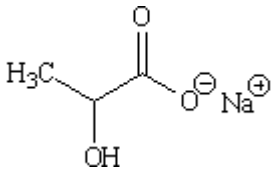
DEFINITION

Chemical names: Sodium lactate, sodium 2-hydroxypropanoate

C.A.S. number: 72-17-3

Chemical formula: $C_3H_5NaO_3$

Structural formula:



Formula weight: 112.06 (anhydrous)

Assay: Not less than 95% and not more than 110% of the labelled amount. This specification is based on a 60% w/w solution of $C_3H_5NaO_3$ in water.

DESCRIPTION Colourless, transparent, liquid. Odourless, or with a slight, characteristic odour

FUNCTIONAL USES Antioxidant synergist, bodying agent, humectant

CHARACTERISTICS

IDENTIFICATION

Ignition: Ignite to an ash. The ash is alkaline, and an effervescence occurs when acid is added

Colour reaction: Overlay 2 ml of the sample on 5 ml of a 1 in 100 solution of catechol in sulfuric acid. A deep red colour is produced at the zone of contact

Test for lactate (Vol. 4): Passes test

Test for sodium (Vol. 4): Passes test

PURITY

Acidity: Neutralization of 1 g of sample shall require not more than 0.5 ml of 0.1 N sodium hydroxide solution, using phenolphthalein TS

pH (Vol. 4)	6.5 - 7.5 (1 in 5 soln)
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh accurately about 0.6 g of the sample into a small beaker, and evaporate to dryness. Add to the residue 60 ml of a 1 in 5 mixture of acetic anhydride in glacial acetic acid, and stir until the residue is completely dissolved. Add crystal violet TS and titrate with 0.1 N perchloric acid to a blue end-point. Perform a blank determination, and make any necessary correction. Each ml of 0.1 N perchloric acid is equivalent to 11.21 mg of $C_3H_5NaO_3$.

SODIUM PROPIONATE

Prepared at the 49th JECFA (1997), published in FNP 52 Add 5 (1997) superseding specifications prepared at the 44th JECFA (1995), published in FNP 52 Add 3 (1995). An ADI not limited' was established at the 17th JECFA (1973)

SYNONYMS Sodium propanoate, INS No. 281

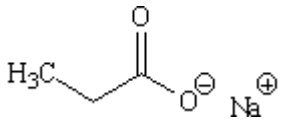
DEFINITION

Chemical name: Sodium propionate

C.A.S. number: 137-40-6

Chemical formula: $C_3H_5NaO_2$

Structural formula:



Formula weight: 96.06

Assay: Not less than 99.0% on the dried basis

DESCRIPTION White or colourless, hygroscopic crystals with not more than a faint characteristic odour

FUNCTIONAL USES Preservative, antimould and antirope agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water, soluble in ethanol

Test for sodium (Vol. 4): Passes test

Test for propionate: Warm the sample with sulfuric acid. The propionic acid evolved may be recognized by its odour.

Test for alkali salt of organic acid: Ignite the sample at a relatively low temperature. The alkaline residue effervesces with acid.

PURITY

Loss on drying (Vol. 4): Not more than 4 % (105°, 2 h)

pH (Vol. 4): 7.5 - 10.5 (1 in 10 soln)

Water-insoluble matter: Not more than 0.1%
Weigh 5 g of the sample to the nearest mg, transfer into a 100-ml beaker and add 50 ml of water. Stir until all the sample appears to be completely

dissolved. Filter through a Gooch crucible, tared to an accuracy of ± 0.2 mg. Rinse the beaker with 20 ml of water. Dry the crucible with its contents in a 60º oven to constant weight. Cool in a desiccator, weigh, and calculate as percentage.

Iron (Vol. 4)

Not more than 50 mg/kg
Test 0.5 g of the sample as described in the Limit Test using 2.5 ml of Iron Standard Solution (25 µg Fe) in the control.

Lead (Vol. 4)

Not more than 5 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh, to the nearest mg, 3 g of the sample previously dried at 105º for 1 h, into a distillation flask and add 200 ml of 50% phosphoric acid. Boil for 2 h and collect the distillate. During distillation keep the volume in the flask at about 200 ml by adding water using a dropping funnel. Titrate the distillate with 1N sodium hydroxide using phenolphthalein TS as indicator. Each ml of 1N sodium hydroxide corresponds to 96.06 mg of $C_3H_5NaO_2$.

SODIUM SESQUICARBONATE

Prepared at the 28th JECFA (1981), published in FNP 31/2 (1984) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI 'not specified' was established at the 25th JECFA (1981)

SYNONYMS Sodium monohydrogendicarbonate; INS No. 500(iii)

DEFINITION

Chemical names	Sodium sesquicarbonate, sodium salt of carbonic acid
C.A.S. number	533-96-0
Chemical formula	$C_2HNa_3O_6 \cdot 2H_2O$ Na_2CO_3-$NaHCO_3 \cdot 2H_2O$
Formula weight	Na_2CO_3-$NaHCO_3 \cdot 2H_2O$: 226.03 Na_2CO_3: 105.99 $NaHCO_3$: 84.00
Assay	Between 35.0 and 38.6% of $NaHCO_3$ Between 46.4 and 50.0% of Na_2CO_3

DESCRIPTION White flakes, crystals or crystalline powder

FUNCTIONAL USES Alkali, buffering agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Freely soluble in water
Test for sodium (Vol. 4)	Passes test
Test for carbonate (Vol. 4)	Passes test

PURITY

Water	Between 13.8 and 16.7% Calculate the percent of water by subtracting from 100 the sum of the percents of sodium bicarbonate, sodium carbonate, and sodium chloride found in the sample.
Sodium chloride	Not more than 0.5% See description under TESTS
Iron (Vol. 4)	Not more than 20 mg/kg See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the

specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Sodium chloride

Dissolve about 10 g of the sample, accurately weighed, in 50 ml of water in a 250-ml beaker, add sufficient nitric acid to make the solution slightly acid, then add 1 ml of ferric ammonium sulfate TS, and 1.0 ml of 0.05 N ammonium thiocyanate, and titrate with 0.05 N silver nitrate, stirring constantly, until the red colour is completely discharged. Finally, back titrate with 0.05 N ammonium thiocyanate, until a faint reddish colour is obtained. Subtract the total volume of 0.05 N ammonium thiocyanate added from the volume of 0.05 N silver nitrate required. Each ml of 0.05 N silver nitrate is equivalent to 2.922 mg of NaCl. Calculate the percent of sodium chloride in the sample taken.

Iron

Dissolve 500 mg of the sample in 10 ml of dilute hydrochloric acid TS, and dilute to 50 ml with water. Add about 40 mg of ammonium persulfate crystals and 10 ml of ammonium persulfate crystals and 10 ml of ammonium thiocyanate TS. Any red or pink colour does not exceed that produced by 1.0 ml of Iron Standard Solution (10 µg Fe) in an equal volume of solution containing 2 ml of hydrochloric acid TS and the quantities of ammonium persulfate and ammonium thiocyanate TS used in the test.

METHOD OF ASSAY

$NaHCO_3$: Weigh accurately about 8.5 g of the sample in a 250-ml flask, and dissolve it in 50 ml of carbon dioxide-free water. Titrate with 1 N sodium hydroxide until a drop of the solution, when added to a drop of a 1-in-10 solution of silver nitrate TS on a spot plate, produces a dark brown colour. Each ml of 1 N sodium hydroxide is equivalent to 84.01 mg of $NaHCO_3$. Na_2CO_3: Weigh accurately about 4.2 g of the sample in a 250 ml flask, and dissolve it by adding 100 ml of water. Add 3 drops of methyl orange TS and titrate with 1 N sulfuric acid, stirring vigorously near the end point to expel carbon dioxide. Each ml of 1 N sulfuric acid is equivalent to 30.99 mg of Na_2O. Calculate the percent of sodium carbonate in the sample by the formula

$$[\%\ Na_2O - (\%\ NaHCO_3 \times 0.3689)] \times 1.7099$$

where % $NaHCO_3$ is the percent of sodium bicarbonate determined in the Assay for sodium bicarbonate; 0.3689 is a factor converting $NaHCO_3$ to Na_2O and 1.7099 is a factor converting Na_2O to Na_2CO_3.

SODIUM SULFATE

Prepared at the 55th JECFA (2000), maintained at 57th JECFA and published in FNP 52 Add 9 (2001). ADI "not specified" was established at the 57th JECFA (2001).

SYNONYMS Glauber's salt (decahydrate from); INS No. 514(i)

DEFINITION

C.A.S. number	7757-82-6 (Anhydrous) 7727-73-3 (Decahydrate)
Chemical formula	$Na_2SO_4 \cdot xH_2O$ (x = 0 or 10)
Formula weight	142.04 (Anhydrous) 322.19 (Decahydrate)
Assay	Not less than 99.0 % on the dry basis

DESCRIPTION

FUNCTIONAL USES Acidity regulator

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Freely soluble in water; practically insoluble in ethanol
Test for sodium (Vol. 4)	Passes Test
Test for sulfate (Vol. 4)	Passes Test

PURITY

Loss on drying (Vol. 4)	Anhydrous: Not more than 1% (105°, 4 h) Decahydrate: Between 51.0% and 57.0% (105°, 4h)
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").
Selenium (Vol. 4)	Not more than 30 mg/kg Test 0.2 g of the sample as directed in the Limit Test (Method II)

METHOD OF ASSAY Weigh accurately about 0.5 g of the dried sample, dissolve in 200 ml of water, add 1 ml of hydrochloric acid and heat to boiling. Gradually add, in small portions and while stirring constantly, an excess of hot

barium chloride TS (about 10 ml), and heat the mixture on a steam bath for 1 h. Collect the precipitate on a filter, wash until free from chloride, dry, ignite and weigh. The weight of the barium sulfate so obtained, multiplied by 0.6086 corresponds to the equivalent amount of Na_2SO_4.

SORBIC ACID

Prepared at the 20th JECFA (1976), published in FNS 1B (1977) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). A group ADI 0-25 mg/kg bw for sorbic acid and its Ca, K, & Na salts was established at the 17th JECFA (1973)

SYNONYMS INS No. 200

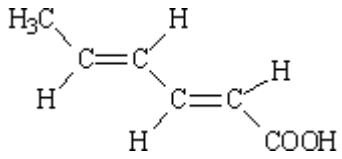
DEFINITION

Chemical names: Sorbic acid, 2,4-hexadienoic acid, 2-propenylacrylic acid

C.A.S. number: 110-44-1

Chemical formula: $C_6H_8O_2$

Structural formula:



Formula weight: 112.12

Assay: Not less than 99.0% calculated on the anhydrous basis

DESCRIPTION Colourless needles or white free flowing powder, having a slight characteristic odour

FUNCTIONAL USES Antimicrobial preservative, fungistatic agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Slightly soluble in water, soluble in ethanol.

Melting range (Vol. 4): Between 132 and 135° (the melting apparatus should be preheated to 125° before introducing the sample).

Spectrophotometry (Vol. 4): A 1 in 400,000 solution in isopropanol solution shows absorbance maximum at 254±2 nm

Test for double bond: Shake about 0.02 g of the sample with 1 ml bromine TS; the colour disappears

PURITY

Water (Vol. 4): Not more than 0.5% (Karl Fischer Method)

Sulfated ash (Vol. 4): Not more than 0.2%.

	Test 2 g of the sample (Method I)
Aldehydes	Not more than 0.1% (as formaldehyde) To 1 ml of a saturated aqueous solution of the sample, add 0.5 ml of Schiff's reagent TS and allow to stand for 10-15 min. Compare the colour with that produced by 1 ml of formaldehyde solution (containing 2 µg) with the same amount of Schiff's reagent under the same conditions. The colour of the test solution should not be more intense than that of the formaldehyde solution
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Dissolve about 0.25 g of the sample, accurately weighed, in 50 ml of anhydrous methanol previously neutralized with 0.1 N sodium hydroxide, add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide to the first pink colour which persists for at least 30 sec. Each ml of 0.1 N sodium hydroxide is equivalent to 11.21 mg of $C_6H_8O_2$

POTASSIUM SORBATE

Prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998) superseding specifications prepared at the 17th JECFA (1973), published in FNP 4 (1978) and republished in FNP 52 (1992). Group ADI 0-25 mg/kg bw for sorbic acid and its calcium, potassium and sodium salts, expressed as sorbic acid, established at the 17th JECFA in 1973.

SYNONYMS

INS No. 202

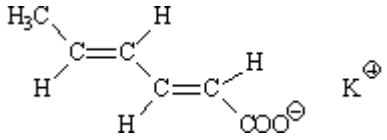
DEFINITION

Chemical names: Potassium sorbate, potassium salt of *trans, trans*-2,4-hexadienoic acid

C.A.S. number: 24634-61-5

Chemical formula: $C_6H_7KO_2$

Structural formula:



Formula weight: 150.22

Assay: Not less than 98% and not more than 102% at the dried basis

DESCRIPTION

White or yellowish-white crystals or crystalline powder or granules

FUNCTIONAL USES

Preservative

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water; soluble in ethanol

Test for potassium (Vol. 4): Passes test

Melting range of sorbic acid derived from the sample: 132- 135°
Acidify a solution of the sample with dilute hydrochloric acid TS. Collect the precipitated sorbic acid on a filter paper, wash free of chloride with water and dry under vacuum over sulfuric acid.

Test for unsaturation: To 2 ml of a 1 in 10 solution of the sample, add a few drops of bromine TS. The colour of the bromine disappears.

PURITY

Loss on drying (Vol. 4): Not more than 1% (105º, 3 h)

Acidity or alkalinity: Not more than about 1% (as sorbic acid or potassium carbonate)
Dissolve 1.1 g of the sample in 20 ml of water and add 3 drops of

phenolphthalein TS. If the solution is colourless, titrate with 0.1 N sodium hydroxide to a pink colour that persists for 15 sec. Not more than 1.1 ml should be required. If the solution is pink in colour titrate with 0.1 N hydrochloric acid. Not more than 0.8 ml should be required to discharge the pink colour.

Aldehydes

Not more than 0.1% as formaldehyde
Prepare a 0.3% solution of the sample, adjust the pH to 4 with 1N HCl and filter. To 5 ml of the filtrate add 2.5 ml of Schiff's reagent TS and allow to stand for 10 - 15 min. Compare the colour with that produced by 5 ml of a control solution containing 15 µg of formaldehyde instead of the sample. The colour of the test solution should not be more intense than that of the control solution.

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh, to the nearest 0.1 mg, 0.25 g of the sample, previously dried at 105º for 3 h. Dissolve in 36 ml of glacial acetic acid and 4 ml acetic anhydride in a 250-ml glass-stoppered flask, warming to effect solution. Cool to room temperature, add 2 drops of crystal violet TS and titrate with 0.1 N perchloric acid in glacial acetic acid to a blue-green end point which persists for at least 30 sec. Perform a blank determination and make any necessary correction. Each ml of 0.1 N perchloric acid is equivalent to 15.02 mg of $C_6H_7KO_2$.

CALCIUM SORBATE

Prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998) superseding specifications prepared at the 17th JECFA (1973), published in FNP 4 (1978) and republished in FNP 52 (1992). Group ADI 0-25 mg/kg bw for sorbic acid and its calcium, potassium and sodium salts, expressed as sorbic acid, established at the 17th JECFA in 1973.

SYNONYMS INS No. 203

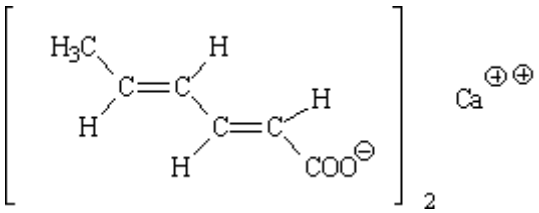
DEFINITION

Chemical names: Calcium sorbate; calcium salt of *trans, trans*-2,4-hexadienoic acid.

C.A.S. number: 7492-55-9

Chemical formula: $C_{12}H_{14}CaO_4$

Structural formula:



Formula weight: 262.32

Assay: Not less than 98% and not more than 102% after drying

DESCRIPTION Fine white crystalline powder not showing any change in colour after heating at 105° for 90 min

FUNCTIONAL USES Preservative

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Soluble in water; practically insoluble in ethanol.

Test for calcium (Vol. 4): Passes test

Melting range of sorbic acid derived from the sample (Vol. 4): 132 - 135°
Acidify a solution of the sample with dilute hydrochloric acid TS. Collect the precipitated sorbic acid on a filter paper, wash free of chloride with water and dry under vacuum over sulfuric acid.

Test for unsaturation: To 2 ml of a 1 in 10 solution of the sample, add a few drops of bromine TS. The colour of the bromine disappears.

PURITY

Loss on drying (Vol. 4) — Not more than 3% (over sulfuric acid in vacuum, 4h)

Fluoride (Vol. 4) — Not more than 10 mg/kg
Weigh 5 g of the sample to the nearest mg and proceed as directed in the Fluoride Limit Test (Method I or III)

Aldehydes — Not more than 0.1% (as formaldehyde)
Prepare a 0.3% solution of the sample, adjust the pH to 4 with 1N HCl and filter. To 5 ml of the filtrate add 2.5 ml of Schiff's reagent TS and allow to stand for 10-15 min. Compare the colour with that produced by 5 ml of a control solution containing 15 µg of formaldehyde instead of the sample. The colour of the test solution should not be more intense than that of the control solution.

Lead (Vol. 4) — Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY — Weigh to the nearest mg, 0.25 g of the dried sample. Dissolve in 35 ml of glacial acetic acid and 4 ml of acetic anhydride in a 250-ml glass-stoppered flask, warming to effect solution. Cool to room temperature, add 2 drops of crystal violet TS and titrate with 0.1 N perchloric acid in glacial acetic acid to a blue-green end point which persists for at least 30 sec. Perform a blank determination and make any necessary correction. Each ml of 0.1 N perchloric acid is equivalent to 13.12 mg of $C_{12}H_{14}CaO_4$.

SORBITOL

Prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 33rd JECFA (1988), published in FNP 38 (1988). Metals and arsenic specifications revised at the 57th JECFA (2001). An ADI 'Not specified' was established at the 26th JECFA (1982).

SYNONYMS D-Glucitol, D-sorbitol, sorbit, sorbol, INS No. 420(i)

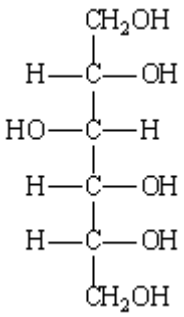
DEFINITION

Chemical names D-Sorbitol

C.A.S. number 50-70-4

Chemical formula $C_6H_{14}O_6$

Structural formula



Formula weight 182.17

Assay Not less than 97.0% of $C_6H_{14}O_6$ of total glycitols and not less than 91.0% of D-sorbitol on the anhydrous basis. The term glycitols refers to compounds with the structural formula CH_2OH-$(CHOH)_n$-CH_2OH, where n is an integer less than or equal to 4.

DESCRIPTION White hygroscopic powder, crystalline powder, flakes or granules

FUNCTIONAL USES Sweetener, humectant, sequestrant, texturizer, stabilizer, bulking agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Very soluble in water, slightly soluble in ethanol

Melting range (Vol. 4) 88 - 102°

Thin layer chromatography (Vol. 4)	Passes test Proceed as directed under *Thin Layer Chromatography of Polyols* Use the following: Standard solution: Dissolve 50 mg of reference standard sorbitol (available from US Pharmacopeial Convention, Inc. 12601 Twinbrook Parkway, Rockville, MD 20852, USA) in 20 ml water Test solution: Dissolve 50 mg of the sample in 20 ml of water
PURITY	
Water (Vol. 4)	Not more than 1% (Karl Fischer Method)
Sulfated ash (Vol. 4)	Not more than 0.1% Test 2 g of sample (Method I)
Chlorides (Vol. 4)	Not more than 50 mg/kg Test 10 g of sample by the Limit Test using 1.5 ml of 0.01N hydrochloric acid in the control
Sulfates (Vol. 4)	Not more than 100 mg/kg Test 10 g of sample by the Limit Test using 2.0 ml of 0.01N sulfuric acid in the control
Nickel (Vol. 4)	Not more than 2 mg/kg Proceed as directed under *Nickel in Polyols*
Reducing sugars	Not more than 0.3% Proceed as directed under *Reducing Substances (as Glucose*), Method II. The weight of cuprous oxide shall not exceed 50 mg
Total sugars	Not more than 1% (as glucose) Weigh 2.1 g of the sample into a 250 ml flask fitted with a ground glass joint, add 40 ml of 0.1N hydrochloric acid, attach a reflux condenser, and reflux for 4 h. Transfer the solution to a 400 ml beaker, rinsing the flask with about 10 ml of water, neutralize with 6N sodium hydroxide and proceed as directed under *Reducing Substances(as Glucose*) Method II. The weight of the cuprous oxide shall not exceed 50 mg.
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Determine the polyol content of the sample using *liquid chromatography* (see Volume 4).

Apparatus
Liquid chromatograph (HPLC)
Detection: differential refractometer maintained at constant temperature
Integrator recorder
Column: AMINEX HPX 87 C (or equivalent resin in calcium form), length 30 cm, internal diameter 9 mm
Eluent: double distilled degassed water (filtered through Millipore membrane filter 0.45 µm)
Chromatographic conditions
Column temperature: 85±0.5^{o}
Eluent flow rate: 0.5 ml/min

Standard preparation
Dissolve an accurately weighed quantity of sorbitol in water to obtain a solution having known concentration of about 10.0 mg of sorbitol per ml.

Sample preparation
Transfer about 1 g of the sample accurately weighed to a 50 ml volumetric flask, dilute with water to volume and mix.

Procedure
Separately inject equal volumes (about 20 µl) of the sample preparation and the standard preparation into the chromatograph. Record the chromatograms and measure the responses of each peak. Calculate separately the quantities, in mg, of sorbitol and other glycitols in the portion of sample taken by the following formula:

$$50 \times C \times \frac{R_U}{R_S}$$

in which C is the concentration, in mg per ml, of sorbitol in the standard preparation; R_U is the peak response of the sample preparation and R_S is the peak response of the standard preparation.

SORBITOL SYRUP

Prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 33rd JECFA (1988), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). No ADI was allocated at the 33rd JECFA (1988)

SYNONYMS D-Glucitol syrup, INS No. 420(ii)

DEFINITION Formed by hydrogenation of glucose syrup; composed of D-sorbitol, D-mannitol and other hydrogenated saccharides
The part of the product which is not D-sorbitol is composed mainly of hydrogenated oligosaccharides formed by the hydrogenation of glucose syrup used as raw material (in which case the syrup is non-crystallizing) or mannitol; minor quantities of hydrogenated di-, tri- and tetrasaccharides may be present

Assay — Not less than 99.0% hydrogenated saccharides and not less than 50.0% of D-sorbitol on the anhydrous basis

DESCRIPTION Clear colourless aqueous solution

FUNCTIONAL USES Sweetener, humectant, sequestrant, texturizer, bulking agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) — Soluble in water, glycerol and propan-1,2-diol

Thin layer chromatography (Vol. 4) — Passes test
Proceed as directed under *Thin Layer Chromatography of Polyols*
Use the following:

Standard solution:
Dissolve 50 mg of reference standard sorbitol (available from US Pharmacopeial Convention, Inc. 12601 Twinbrook Parkway, Rockville, MD 20852, USA) in 20 ml water

Test solution:
Dissolve 50 mg of the sample in 20 ml of water

PURITY

Water (Vol. 4) — Not more than 31% (Karl Fischer Method)

Sulfated ash (Vol. 4) — Not more than 0.1%
Test 3 g of sample (Method I)

Chlorides (Vol. 4)

Not more than 50 mg/kg
Test 10 g of sample by the Limit Test using 1.5 ml of 0.01N hydrochloric acid in the control

Sulfates (Vol. 4)

Not more than 100 mg/kg
Test 10 g of sample by the Limit Test using 2.0 ml of 0.01N sulfuric acid in the control

Nickel (Vol. 4)

Not more than 2 mg/kg
Proceed as directed under *Nickel in Polyols*

Reducing sugars

Not more than 0.3%
Proceed as directed under *Reducing Substances (as Glucose*), Method II. The weight of cuprous oxide shall not exceed 50 mg

Lead (Vol. 4)

Not more than 1 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

METHOD OF ASSAY

Total hydrogenated saccharides (%):

$$\frac{100 - (Water\% + Sulfated\ ash\% + Reducing\ sugars\%)}{100 - Water\%} \times 100$$

Determine the sorbitol content of the sample using *liquid chromatography.*

Apparatus
Liquid chromatograph (HPLC)
Detection: differential refractometer maintained at constant temperature
Integrator recorder
Column: AMINEX HPX 87 C (or equivalent resin in calcium form), length 30 cm, internal diameter 9 mm
Eluent: double distilled degassed water (filtered through Millipore membrane filter 0.45 µm)

Chromatographic conditions
Column temperature: 85±0.5^{o}
Eluent flow rate: 0.5 ml/min

Standard preparation
Dissolve an accurately weighed quantity of sorbitol in water to obtain a solution having known concentration of about 10.0 mg of sorbitol per ml.

Sample preparation
Transfer about 1 g of the sample accurately weighed to a 50 ml volumetric flask, dilute with water to volume and mix.

Procedure
Separately inject equal volumes (about 20 µl) of the sample preparation

and the standard preparation into the chromatograph. Record the chromatograms and measure the responses of each polyol peak. Calculate separately the quantity, in mg, of sorbitol in the portion of sample taken by the following formula:

$$50 \times C \times \frac{R_U}{R_S}$$

where
C = concentration, in mg per ml, of sorbitol in the standard preparation
R_U = the peak response of the sample preparation
R_S = the peak response of the standard preparation.

STANNOUS CHLORIDE

Prepared at the 22nd JECFA (1978), published in FNP 7 (1978) and in FNP 52 (1992). Heavy metals and arsenic specifications revised at the 61st JECFA (2003). A PTWI of 0-14 mg/kg bw for tin was established at the 33rd JECFA (1988).

SYNONYMS Tin dichloride; INS No. 512

DEFINITION

Chemical names	Tin (II) chloride, stannous chloride dihydrate
C.A.S. number	7772-99-8
Chemical formula	$SnCl_2 \cdot 2H_2O$
Formula weight	225.63
Assay	Not less than 98.0% and not more than 102.0%

DESCRIPTION Colourless or white crystals, odourless or having slight odour of hydrochloric acid

FUNCTIONAL USES Reducer or antioxidant in some bottled or lacquered canned vegetables

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water in less than its own weight of water, but it forms an insoluble basic salt with excess water; soluble in ethanol
Test for stannous ion	To a 1 in 20 solution of the sample in dilute hydrochloric acid TS add mercuric chloride TS dropwise. White or greyish precipitate forms
Test for chloride (Vol. 4)	Passes test

PURITY

Hydrochloric acid insoluble matter	Heat 5 g of the sample to 40° in a mixture of 5 ml of water and 5 ml of hydrochloric acid. The sample should dissolve completely, and the solution should be clear.
Sulfates	Not more than 30 mg/kg Dissolve 5 g of the sample in 5 ml of hydrochloric acid, dilute to 50 ml with water, filter if not clear and heat the filtrate or clear solution to boiling. Add 5 ml of barium chloride TS, digest in a covered beaker on a steam bath for

2 h, and allow to stand overnight. No precipitate forms.

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Transfer about 2 g of sample accurately weighed, to a 250 ml volumetric flask, dissolve in 25 ml of hydrochloric acid, dilute to volume with water, and mix well. Transfer 50 ml of this solution to a 500 ml conical flask, and add 5 g of potassium sodium tartrate, and then a cold saturated solution of sodium bicarbonate until de solution is alkaline to litmus paper. Titrate at once with 0.1 N iodine using starch TS as the indicator. Each ml of 0.1N iodine consumed is equivalent to 11.28 mg of $SnCl_2 \cdot 2H_2O$.

(Note: Stannous salts are readily susceptible to oxidation, yet the method of assay does not take account of this. Distilled water contains dissolved oxygen, therefore water used in the method of assay should be "oxygen free"; this may be achieved by purging the water with nitrogen or carbon dioxide or by boiling the air out. In addition to this the iodine solution used in the determination should be free from dissolved oxygen; ideally the iodine solution should be stored in a self-filling apparatus under carbon dioxide.)

SODIUM STEAROYL-2-LACTYLATE

Prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 44th JECFA (1995), published in FNP 52 Add 3 (1995). Metals and arsenic specifications revised at the 55th JECFA (2000). An ADI of 0-20 mg/kg bw was established at the 17th JECFA (1973)

SYNONYMS Sodium stearoyl lactylate, sodium stearoyl lactate; INS No. 481(i)

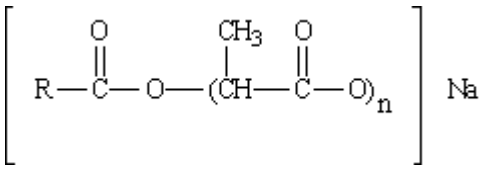
DEFINITION A mixture of sodium salts of stearoyl lactylic acids and minor proportions of other salts of related acids, formed by the esterification of commercial stearic acid with lactic acid and neutralized to the sodium salts; may contain unneutralized palmitoyl and stearoyl lactylic acid, free fatty acids (principally palmitic and stearic), free lactic acid and salts of fatty acid esters of lactic acid and polymerized lactic acid.

Chemical names Sodium di-2-stearoyl lactate, sodium di-(2-stearoyloxy)propionate (major component)

C.A.S. number 25383-99-7

Chemical formula $C_{21}H_{39}O_4Na$; $C_{19}H_{35}O_4Na$ (major components)

Structural formula



where R is $C_{17}H_{35}$ or $C_{15}H_{31}$ and the mean value of n is normally 2

DESCRIPTION White or slightly yellowish powder or brittle solid with a characteristic odour

FUNCTIONAL USES Emulsifier, stabilizer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water; soluble in ethanol

Test for sodium Add 10 ml of dilute hydrochloric acid TS to 2 g of the sample, heat for 5 min in a water bath, filter and neutralize the filtrate with ammonia TS. Retain the residue from the filter for test C. To the filtrate, add uranyl zinc acetate TS; a yellow crystalline precipitate appears within a few min.

Test for fatty acids Take the residue from the filter in the test for sodium, add 30 ml of sodium hydroxide TS, heat for 30 min on a steam bath and filter. Add 20 ml of dilute hydrochloric acid TS to the filtrate after cooling, extract twice with 30 ml of diethyl ether, wash the ether solution with 20 ml of water, dry with anhydrous sodium sulfate and evaporate the ether. The residue melts between 54 and 69º.

Test for lactate (Vol. 4) — Passes test

PURITY

Sodium content — Not less than 2.5% and not more than 5.0%
See description under TESTS

Total lactic acid — Not less than 15% and not more than 40%
See description under TESTS

Acid value — Not less than 60 and not more than 130
See description under TESTS

Ester value — Not less than 90 and not more than 190
See description under TESTS

Lead (Vol. 4) — Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

TESTS

PURITY TESTS

Sodium content

Stock Lanthanum Solution:
Transfer 5.86 g of lanthanum oxide, La_2O_3, to a 100 ml volumetric flask, wet with a few ml of water, slowly add 25 ml of hydrochloric acid, and swirl until the material is completely dissolved. Dilute to volume with water, and mix.

Stock Sodium Solution:
Use a solution containing 1 mg of Na in each ml (1000 mg/l Na). The solution may be obtained commercially or be prepared as follows: transfer 1.271 g of sodium chloride, previously dried at 105° for 2 h and accurately weighed, to a 500 ml volumetric flask, dilute to volume with water, and mix.

Standard Sodium Solutions:
Transfer 10.0 ml of the Stock Lanthanum Solution to each of three 100 ml volumetric flasks. Using a microlitre syringe, transfer 0.20 ml of the Stock Sodium Solution to the first flask, 0.40 ml to the second flask, and 0.50 ml to the third flask. Dilute each flask to volume with water, and mix. The flasks contain 2.0, 4.0 and 5.0 µg of Na per ml, respectively. Prepare these solutions fresh daily.

Sample Preparation:
Transfer about 250 mg of the sample, accurately weighed, to a 30 ml beaker, dissolve with heating in 10 ml of alcohol, and quantitatively transfer the solution into a 25 ml volumetric flask. Wash the beaker with two 5 ml portions of alcohol, adding the washings to the flask, dilute to volume with

alcohol, and mix. Transfer 2.5 ml of the Stock Lanthanum Solution to a second 25 ml volumetric flask. Using a microlitre syringe, transfer 0.25 ml of the alcoholic solution of the sample to the second flask, dilute to volume with water, and mix.

Procedure:
Concomitantly determine the absorbance of each Standard Sodium preparation and of the Sample preparation at 589 nm, with a suitable atomic absorption spectrophotometer, following the operating parameters as recommended by the manufacturer of the instrument. Plot the absorbance of the Standard Sodium preparations vs. concentration of Na, in µg per ml, and from the curve so obtained determine the concentration C, in µg per ml, of Na in the Sample preparation. Calculate the quantity, in mg, of Na in the sample taken by the formula 2.5 C.

Total lactic acid

Standard curve:
Dissolve 1.067 g of lithium lactate in sufficient water to make 1000.0 ml. Transfer 10.0 ml of this solution into a 100 ml volumetric flask, dilute to volume with water, and mix. Transfer 1.0, 2.0, 4.0, 6.0 and 8.0 ml of the diluted standard solution into separate 100 ml volumetric flasks, dilute each flask to volume with water, and mix. These standards represent 1, 2, 4, 6 and 8 µg of lactic acid per ml, respectively. Transfer 1.0 ml of each solution into separate test tubes, and continue as directed in the Procedure, beginning with "Add one drop of cupric sulfate TS ..." After colour development and reading the absorbance values, construct a Standard curve by plotting absorbance versus µg of lactic acid.

Test preparation:
Transfer about 200 mg of the sample, accurately weighed, into a 125 ml Erlenmeyer flask, add 10 ml of 0.5N ethanolic potassium hydroxide and 10 ml of water, attach an air condenser, and reflux gently for 45 min. Wash the sides of the flask and the condenser with about 40 ml of water, and heat on a steam bath until no odour of alcohol remains. Add 6 ml of dilute sulfuric acid (1 in 2), heat until the fatty acids are melted, then cool to about 600, and add 25 ml of petroleum ether. Swirl the mixture gently, and transfer quantitatively to a separatory funnel. Collect the water layer in a 100 ml volumetric flask, and wash the petroleum ether layer with two 20 ml portions of water, adding the washings to the volumetric flask. Dilute to volume with water, and mix. Transfer 1.0 ml of this solution into a second 100 ml volumetric flask, dilute to volume with water, and mix.

Procedure:
Transfer 1.0 ml of the Test preparation into a test tube, and transfer 1.0 ml of water to a second test tube to serve as the blank. Treat each tube as follows: Add one drop of cupric sulfate TS, swirl gently, and then add rapidly from a burette 9.0 ml of sulfuric acid. Loosely stopper the tube, and heat in a water bath at 900 for exactly 5 min. Cool immediately to below 200 in an ice bath for 5 min, add 3 drops of p-phenylphenol TS (on the day of use dissolve 750 mg of p-phenylphenol in 50 ml sodium hydroxide TS), shake immediately and heat in a water bath at 300 for 30 min, shaking the tube twice during this time to disperse the reagent. Heat the tube in a water bath at 90º for exactly 90 sec, and then cool immediately to room temperature in an ice water bath. Determine the absorbance of the solution in a 1 cm cell,

at 570 nm, with a suitable spectrophotometer, using the blank to set the instrument. Obtain the weight, in µg, of lactic acid in the portion of the Test preparation taken from the Procedure by means of the Standard curve.

Acid value

Transfer about 1 g, accurately weighed, to a 100 ml Erlenmeyer flask, add 25 ml of alcohol, previously neutralized to phenolphthalein TS, and heat on a hot plate until the sample is dissolved. Cool, add 5 drops of phenolphthalein TS, and titrate rapidly with 0.1N sodium hydroxide to the first pink colour that persists for at least 30 sec. Calculate the acid value by the formula 56.1 x V x N/W, in which V is the volume, in ml, and N is the normality of the sodium hydroxide solution, and W is the weight, in grams, of the sample taken. Retain the neutralized solution for the determination of Ester value.

Ester value

To the neutralized solution retained in the test for acid value add 10.0 ml of alcoholic potassium hydroxide solution prepared by dissolving 11.2 g of potassium hydroxide in 250 ml of alcohol and diluting with 25 ml of water. Add 5 drops of phenolphthalein TS, connect a suitable condenser, and reflux for 2 hours. Cool, add 5 additional drops of phenolphthalein TS and titrate the excess alkali with 0.1N hydrochloric acid. Perform a blank determination using 10.0 ml of the alcoholic potassium hydroxide solution. Calculate the ester value by the formula 56.1 (B - S) x N/W, in which B - S represents the difference between the volumes of 0.1N hydrochloric acid required for the blank and the sample, respectively, N is the normality of the hydrochloric acid, and W is the weight, in grams, of the sample taken.

CALCIUM STEAROYL-2-LACTYLATE

Prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 44th JECFA (1995), published in FNP 52 Add 3 (1995). Metals and arsenic specifications revised at the 55th JECFA (2000). An ADI of 0-20 mg/kg bw was established at the 17th JECFA (1973).

SYNONYMS Calcium stearoyl lactylate, calcium stearoyl lactate, INS No. 482(i)

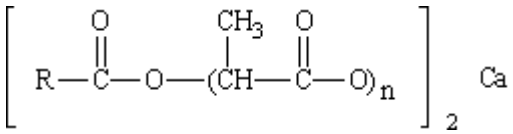
DEFINITION A mixture of calcium salts of stearoyl lactylic acids and minor proportions of other salts of related acids, formed by the esterification of commercial stearic acid with lactic acid and neutralized to the calcium salts; may contain unneutralized palmitoyl and stearoyl lactylic acid, free fatty acids (principally palmitic and stearic acid), free lactic acid and salts of fatty acid esters of lactic and polymerized lactic acid.

Chemical names Calcium di-2-stearoyl lactate, calcium di-(2-stearoyloxy)propionate (major components)

C.A.S. number 5793-94-2

Chemical formula $C_{42}H_{78}O_8Ca$; $C_{38}H_{70}O_8Ca$ (major components)

Structural formula



where R is $C_{17}H_{35}$ or $C_{15}H_{31}$ and the mean value of n dependent on the proportion of lactic acid present

DESCRIPTION White or slightly yellowish powder or brittle solid with a characteristic odour

FUNCTIONAL USES Emulsifier, stabilizer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Slightly soluble in hot water

Test for calcium Add 10 ml of dilute hydrochloric acid TS to 2 g of the sample, heat for 5 min in a water bath, filter and neutralize the filtrate with ammonia TS. Retain the residue from the filter for the test for fatty acids. To the filtrate, add 5 ml of ammonium oxalate TS. A white precipitate is formed, soluble in dilute hydrochloric acid TS, but insoluble in dilute acetic acid TS.

Test for fatty acids Take the residue from the filter in the test for calcium, add 30 ml of sodium hydroxide TS, heat for 30 min on a steam bath and filter. Add 20 ml of dilute hydrochloric acid TS to the filtrate after cooling, extract twice with 30 ml of

diethyl ether, wash the ether solution with 20 ml of water, dry with anhydrous sodium sulfate and evaporate the ether. The residue melts between 54 and 69º.

Test for lactate (Vol. 4) — Passes test

PURITY

Calcium content — Not less than 1.0% and not more than 5.2%
See description under TESTS

Total lactic acid — Not less than 15% and not more than 40%
See description under TESTS

Acid value — Not less than 50 and not more than 130
See description under TESTS

Ester value — Not less than 125 and not more than 190
See description under TESTS

Lead (Vol. 4) — Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Calcium content

Stock Lanthanum Solution:
Transfer 5.86 g of lanthanum oxide, La_2O_3, to a 100 ml volumetric flask, wet with a few ml of water, slowly add 25 ml of hydrochloric acid TS, and swirl until the material is completely dissolved. Dilute to volume with water, and mix.

Stock Calcium Solution:
Use a solution containing 0.5 mg of Ca in each ml (500 mg/l Ca). The solution may be obtained commercially or be prepared as follows: Transfer 124.8 mg of calcium carbonate previously dried at 200º for 4 h, into a 100 ml volumetric flask, carefully dissolve in 2 ml of diluted hydrochloric acid TS, dilute to volume with water and mix.

Standard Calcium Solutions:
Transfer 10.0 ml of the Stock Lanthanum Solution into each of three 50 ml volumetric flasks. Using a microlitre syringe, transfer 0.20 ml of the Stock Calcium Solution to the first flask, 0.40 ml to the second flask and 0.50 ml to the third flask. Dilute each flask to volume with water, and mix. The flasks contain 2.0, 4.0 and 5.0 µg of Ca per ml, respectively. Prepare these solutions fresh daily.

Sample Preparation:
Transfer about 250 mg of the sample, accurately weighed, to a 30 ml beaker, dissolve with heating in 10 ml of alcohol, and quantitatively transfer the

solution into a 25 ml volumetric flask. Wash the beaker with two 5 ml portions of alcohol, adding the washings to the flask, dilute to volume with alcohol, and mix. Transfer 2.5 ml of the Stock Lanthanum Solution to a second 25 ml volumetric flask. Using a microlitre syringe, transfer 0.25 ml of the alcoholic solution of the sample to the second flask, dilute to volume with water, and mix.

Procedure:
Concomitantly determine the absorbance of each Standard Calcium preparation and of the Sample preparation at 422.7 nm with a suitable atomic absorption spectrophotometer, following the operating parameters as recommended by the manufacturer of the instrument. Plot the absorbance of the Standard Calcium preparations vs. concentration of Ca in µg per ml, and from the curve so obtained determine the concentration C, in µg per ml, of Ca in the sample preparation. Calculate the quantity, in mg, of Ca in the sample taken by the formula 2.5 C.

Total lactic acid

Standard curve:
Dissolve 1.067 g of lithium lactate in sufficient water to make 1000.0 ml. Transfer 10.0 ml of this solution into a 100 ml volumetric flask, dilute to volume with water, and mix. Transfer 1.0, 2.0, 4.0, 6.0 and 8.0 ml of the diluted standard solution into separate 100 ml volumetric flasks, dilute each flask to volume with water, and mix. These standards represent 1, 2, 4, 6 and 8 µg of lactic acid per ml, respectively. Transfer 1.0 ml of each solution into separate test tubes, and continue as directed in the Procedure, beginning with "Add one drop of cupric sulfate TS ..." After colour development and reading the absorbance values, construct a Standard curve by plotting absorbance versus µg of lactic acid.

Test preparation:
Transfer about 200 mg of the sample, accurately weighed, into a 125 ml Erlenmeyer flask, add 10 ml of 0.5N ethanolic potassium hydroxide and 10 ml of water, attach an air condenser, and reflux gently for 45 min. Wash the sides of the flask and the condenser with about 40 ml of water, and heat on a steam bath until no odour of alcohol remains. Add 6 ml of dilute sulfuric acid (1 in 2), heat until the fatty acids are melted, then cool to about 600, and add 25 ml of petroleum ether. Swirl the mixture gently, and transfer quantitatively to a separatory funnel. Collect the water layer in a 100 ml volumetric flask, and wash the petroleum ether layer with two 20 ml portions of water, adding the washings to the volumetric flask. Dilute to volume with water, and mix. Transfer 1.0 ml of this solution into a second 100 ml volumetric flask, dilute to volume with water, and mix.

Procedure:
Transfer 1.0 ml of the Test preparation into a test tube, and transfer 1.0 ml of water to a second test tube to serve as the blank. Treat each tube as follows: Add one drop of cupric sulfate TS, swirl gently, and then add rapidly from a burette 9.0 ml of sulfuric acid. Loosely stopper the tube, and heat in a water bath at 900 for exactly 5 min. Cool immediately to below 200 in an ice bath for 5 min, add 3 drops of p-phenylphenol TS (on the day of use dissolve 750 mg of p-phenylphenol in 50 ml sodium hydroxide TS), shake immediately and heat in a water bath at 300 for 30 min, shaking the tube twice during this time to disperse the reagent. Heat the tube in a water bath at 90º for exactly 90 sec,

and then cool immediately to room temperature in an ice water bath. Determine the absorbance of the solution in a 1 cm cell, at 570 nm, with a suitable spectrophotometer, using the blank to set the instrument. Obtain the weight, in µg, of lactic acid in the portion of the Test preparation taken from the Procedure by means of the Standard curve.

Acid value

Transfer about 1 g, accurately weighed, to a 100 ml Erlenmeyer flask, add 25 ml of alcohol, previously neutralized to phenolphthalein TS, and heat on a hot plate until the sample is dissolved. Cool, add 5 drops of phenolphthalein TS, and titrate rapidly with 0.1N sodium hydroxide to the first pink colour that persists for at least 30 sec. Calculate the acid value by the formula 56.1 x V x N/W, in which V is the volume, in ml, and N is the normality of the sodium hydroxide solution, and W is the weight, in grams, of the sample taken. Retain the neutralized solution for the determination of Ester value.

Ester value

To the neutralized solution retained in the test for acid value add 10.0 ml of alcoholic potassium hydroxide solution prepared by dissolving 11.2 g of potassium hydroxide in 250 ml of alcohol and diluting with 25 ml of water. Add 5 drops of phenolphthalein TS, connect a suitable condenser, and reflux for 2 hours. Cool, add 5 additional drops of phenolphthalein TS and titrate the excess alkali with 0.1N hydrochloric acid. Perform a blank determination using 10.0 ml of the alcoholic potassium hydroxide solution. Calculate the ester value by the formula 56.1 (B - S) x N/W, in which B - S represents the difference between the volumes of 0.1N hydrochloric acid required for the blank and the sample, respectively, N is the normality of the hydrochloric acid, and W is the weight, in grams, of the sample taken.

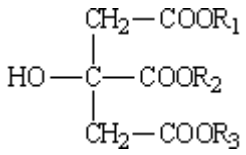
STEARYL CITRATE

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 55th JECFA (2000). An ADI of 0-50 mg/kg bw was established at the 17th JECFA (1973)

SYNONYMS INS No. 484

DEFINITION Formed by esterifying citric acid with commercial stearyl alcohol, which consists essentially of n-octadecanol and up to 50% of n-hexadecanol and conforms to the following specifications.
The article of commerce can be further specified by saponification value, total content and composition of stearyl alcohol, iodine value, acid value, and citric acid content.

Structural formula (approximate composition)



where
R_1, R_2 and R_3 each may be $C_{18}H_{37}$ (stearyl), $C_{16}H_{33}$ (palmityl) or H

DESCRIPTION Cream-coloured unctuous substance

FUNCTIONAL USES Emulsifier, sequestrant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water and in cold ethanol. Soluble in hot ethanol

Test for stearyl alcohol Hydrolyse approximately 2 g of the sample by heating with 50 ml sodium hydroxide TS under reflux for 1 h. Cool and extract the aqueous solution with petroleum ether, evaporate the petroleum ether in an evaporating dish. The residue has a melting range of 43° to 58°.

Test for citrate To 5 ml of the aqueous solution obtained in the Test for stearyl alcohol, add 1 ml of calcium chloride TS and 3 drops of bromothymol blue TS and slightly acidify with dilute hydrochloric acid TS. Add sodium hydroxide TS until the colour changes to a clear blue, then boil the solution for 3 min, agitating it gently during the heating period: a white crystalline precipitate appears which is insoluble in sodium hydroxide TS but is soluble in acetic acid TS.

To 10 ml of the aqueous solution obtained in the Test for stearyl alcohol, add 1 ml of mercuric sulfate TS. Heat the mixture to boiling, and add a few

drops of potassium permanganate TS: a white precipitate of the acetone dicarboxylic acid salt of mercury is formed.

PURITY

Other acids and alcohols	Acids other than citric and alcohols other than those present in commercial stearyl alcohol must not be present
Chloroform insoluble matter	Not more than 0.5% Dissolve about 50.0 g of sample in 400 ml chloroform. Filter the solution through a sintered glass filter of porosity 3 previously weighed to the nearest 0.001 g. Keep the filter warm and wash the residue in the filter with chloroform, then dry at 100°.
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

STEVIOL GLYCOSIDES

Prepared at the 73rd JECFA (2010) and published in FAO JECFA Monographs 10 (2010), superseding specifications prepared at the 69th JECFA (2008) and published in FAO JECFA Monographs 5 (2008). An ADI of 0 - 4 mg/kg bw (expressed as steviol) was established at the 69th JECFA (2008).

SYNONYMS INS no. 960

DEFINITION The product is obtained from the leaves of *Stevia rebaudiana* Bertoni. The leaves are extracted with hot water and the aqueous extract is passed through an adsorption resin to trap and concentrate the component steviol glycosides. The resin is washed with a solvent alcohol to release the glycosides and the product is recrystallized from methanol or aqueous ethanol. Ion exchange resins may be used in the purification process. The final product may be spray-dried.

Stevioside and rebaudioside A are the component glycosides of principal interest for their sweetening property. Associated glycosides include rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside and steviolbioside which are generally present in preparations of steviol glycosides at levels lower than stevioside or rebaudioside A.

Chemical name

Stevioside: 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester

Rebaudioside A: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester

C.A.S. number

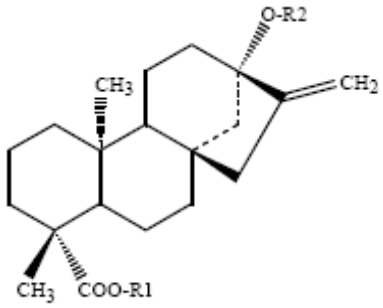
Stevioside: 57817-89-7
Rebaudioside A: 58543-16-1

Chemical formula

Stevioside: $C_{38}H_{60}O_{18}$
Rebaudioside A: $C_{44}H_{70}O_{23}$

Structural Formula

The nine named steviol glycosides:



Compound name	*R1*	*R2*
Stevioside	*β*-Glc	*β*-Glc-*β*-Glc(2→1)
Rebaudioside A	*β*-Glc	*β*-Glc-*β*-Glc(2→1) | *β*-Glc(3→1)
Rebaudioside B	H	*β*-Glc-*β*-Glc(2→1) | *β*-Glc(3→1)
Rebaudioside C	*β*-Glc	*β*-Glc-*α*-Rha(2→1) | *β*-Glc(3→1)
Rebaudioside D	*β*-Glc-*β*-Glc(2→1)	*β*-Glc-*β*-Glc(2→1) | *β*-Glc(3→1)
Rebaudioside F	*β*-Glc	*β*-Glc-*β*-Xyl(2→1) | *β*-Glc(3→1)
Dulcoside A	*β*-Glc	*β*-Glc-*α*-Rha(2→1)
Rubusoside	*β*-Glc	*β*-Glc
Steviolbioside	H	*β*-Glc-*β*-Glc(2→1)

Steviol (R1 = R2 = H) is the aglycone of the steviol glycosides. Glc, Rha and Xyl represent, respectively, glucose, rhamnose and xylose sugar moieties.

Formula weight

Stevioside: 804.88
Rebaudioside A: 967.03

Assay	Not less than 95% of the total of the nine named steviol glycosides on the dried basis.
DESCRIPTION	White to light yellow powder, odourless or having a slight characteristic odour. About 200 - 300 times sweeter than sucrose.
FUNCTIONAL USES	Sweetener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Freely soluble in water
Stevioside and rebaudioside A	The main peak in the chromatogram obtained by following the procedure in Method of Assay corresponds to either stevioside or rebaudioside A.
pH (Vol. 4)	Between 4.5 and 7.0 (1 in 100 solution)

PURITY

Total ash (Vol. 4)	Not more than 1%
Loss on drying (Vol. 4)	Not more than 6% (105°, 2h)
Residual solvents (Vol. 4)	Not more than 200 mg/kg methanol and not more than 5000 mg/kg ethanol (Method I in Vol. 4, General Methods, Organic Components, Residual Solvents)
Arsenic (Vol. 4)	Not more than 1 mg/kg Determine by the atomic absorption hydride technique (Use Method II to prepare the test (sample) solution)
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Vol. 4 (under “General Methods, Metallic Impurities”).

METHOD OF ASSAY Determine the percentages of the individual steviol glycosides by HPLC (Vol. 4) under the following conditions.

Reagents
Acetonitrile: more than 95% transmittance at 210 nm.

Standards
Stevioside: more than 99.0% purity on the dried basis.
Rebaudioside A: more than 99.0% purity on the dried basis.
Mixture of nine steviol glycosides standard solution: Containing stevioside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside and

steviolbioside. This solution is diluted with water-acetonitrile (7:3) accordingly and is used for the confirmation of retention times. Standards are available from Wako Pure Chemical Industries, Ltd. Japan and ChromaDex, USA.

Standard solution
Accurately weigh 50 mg of stevioside and rebaudioside A standard into each of two 50-ml volumetric flasks. Dissolve and make up to volume with water-acetonitrile (7:3).

Sample solution
Accurately weigh 50-100 mg of sample into a 50-ml volumetric flask. Dissolve and make up to volume with water-acetonitrile (7:3).

Procedure
Inject 5 µl of sample solution under the following conditions.
Column: Capcell pak C_{18} MG II (Shiseido Co.Ltd) or Luna 5µ C18(2) 100A (Phenomenex) or equivalent (length: 250 mm; inner diameter: 4.6 mm, particle size: 5µm)
Mobile phase: 32:68 mixture of acetonitrile and 10 mmol/L sodium phosphate buffer (pH 2.6)
Flow rate: 1.0 ml/min
Detector: UV at 210 nm
Column temperature: 40°
Record the chromatogram for about 30 min.

Identification of the peaks and Calculation
Identify the peaks from the sample solution by comparing the retention time with the peaks from the mixture of nine steviol glycosides standard solution (see under figure). Measure the peak areas for the nine steviol glycosides from the sample solution. Measure the peak area for stevioside and rebaudioside A from their standard solutions.
Calculate the percentage of each of the eight steviol glycosides except rebaudioside A in the sample from the formula:

$$\%X = [W_S/W] \times [f_X A_X/A_S] \times 100$$

Calculate the percentage of rebaudioside A in the sample from the formula:

$$\%\textit{Rebaudioside A} = [W_R/W] \times [A_X/A_R] \times 100$$

where
X is each steviol glycoside;
W_S is the amount (mg) calculated on the dried basis of stevioside in the standard solution;
W_R is the amount (mg) calculated on the dried basis of rebaudioside A in the standard solution;
W is the amount (mg) calculated on the dried basis of sample in the sample solution;
A_S is the peak area for stevioside from the standard solution;
A_R is the peak area for rebaudioside from the standard solution;

A_X is the peak area of *X* for the sample solution; and
f_X is the ratio of the formula weight of *X* to the formula weight of stevioside: 1.00 (stevioside), 1.20 (rebaudioside A), 1.00 (rebaudioside B), 1.18 (rebaudioside C), 1.40 (rebaudioside D), 1.16 (rebaudioside F), 0.98 (dulcoside A), 0.80 (rubusoside) and 0.80 (steviolbioside).

Calculate the percentage of total steviol glycosides (sum the nine percentages).

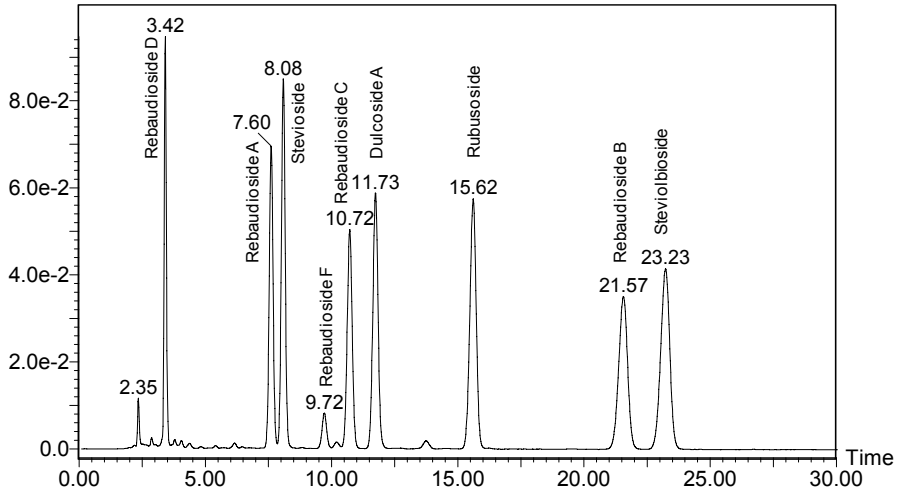


Figure. Chromatogram of mixture of nine steviol glycosides standard solution
Column: Capcell pak C_{18} MG II
Concentration: 0.5 mg/ml each except rebaudioside F (about 0.1 mg/ml)

SUCRALOSE

Prepared at the 41st JECFA (1993), published in FNP 52 Add 2 (1993). Metals and arsenic specifications revised at the 63rd JECFA (2004). An ADI of 0-15 mg/kg bw was established at the 37th JECFA (1990)

SYNONYMS 4,1',6'-trichlorogalactosucrose; INS No. 955

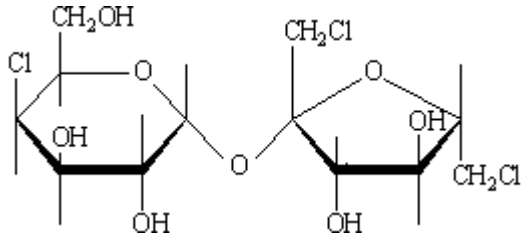
DEFINITION

Chemical names 1,6-Dichloro-1,6-dideoxy-ß-D-fructofuranosyl-4-chloro-4-deoxy-alpha-D-galactopyranoside

C.A.S. number 56038-13-2

Chemical formula $C_{12}H_{19}Cl_3O_8$

Structural formula



Formula weight 397.64

Assay Not less than 98% and not more than 102% calculated on an anhydrous basis

DESCRIPTION White to off-white, practically odourless crystalline powder

FUNCTIONAL USES Sweetener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Freely soluble in water, methanol and ethanol; slightly soluble in ethyl acetate

Infrared absorption The infrared spectrum of a potassium bromide dispersion of the sample corresponds with the reference infrared spectrum in the Appendix

Thin layer chromatography (Vol. 4) The main spot in the test solution has the same R_f value as that of the main spot of Standard Solution A obtained in the test for Other chlorinated disaccharides.

PURITY

Water (Vol. 4) Not more than 2.0% (Karl Fischer Method)

Specific rotation (Vol. 4)	[alpha] 20, D: Between +84.0 and +87.5° (10% w/v solution)
Sulfated ash (Vol. 4)	Not more than 0.7%
Other chlorinated disaccharides	Passes test See description under TESTS
Chlorinated monosaccharides	Passes test See description under TESTS
Triphenylphosphine oxide	Not more than 150 mg/kg See description under TESTS
Methanol	Not more than 0.1% See description under TESTS
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Other chlorinated disaccharides

TLC Plates:
Use reverse phase Thin-layer chromatography plates coated with 0.20 mm layer of silica gel absorbent (e.g., Whatman LKC_{18}).

Mobile Phase:
Mix 7 volumes of a 5.0% w/v aqueous solution of sodium chloride and 3 volumes of acetonitrile.

Spray Reagent:
Use 15% v/v solution of concentrated sulfuric acid in methanol.

Standard Solutions:
Dissolve 1.0 g of sucralose Reference Standard (available e.g. from Tate & Lyle Speciality Sweeteners, PO Box 68 Whiteknights, Reading, RG6 2 BX, United Kingdom) in 10 ml of methanol (Solution A). Dilute 0.5 ml of Solution A with methanol to 100 ml (Solution B).

Test Solution:
Dissolve 1.0 g of sample in 10 ml of methanol

Procedure
Apply 5 µl each of Solution A, Solution B and Test Solution to the bottom of the chromatographic plate. Place the plate in a suitable chromatography chamber containing freshly prepared Mobile Phase and allow the solvent front to ascend 15 cm. Remove the plate from the chamber, allow it to dry and spray with Spray Reagent. Heat the plate in an oven at 125° for 10 min. The main spot in the Test Solution has the same R_f value as the main

spot in Solution A and no other spot in the Test Solution is more intense than the 0.5% spot in Solution B.

Chlorinated monosaccharides

TLC plates:
Use a thin-layer chromatography plate coated with a 0.25 mm thickness of Merck-silica gel 60 or equivalent.

Spray Reagent:
Dissolve 1.23 g p-anisidine and 1.66 g phthalic acid in 100 ml methanol. Store the solution in darkness and refrigerate to prevent it becoming decolourised. Discard if solution becomes discoloured. (Note: p-anisidine is toxic by skin absorption and inhalation and should be used with due caution).

Standard Solution A:
Dissolve 10.0 g of mannitol, weighed to 0.001 g, in water in a 100 ml volumetric flask, and dilute to volume with water.

Standard Solution B:
Dissolve 10 g of mannitol and 40 mg of fructose (Analar grade) in 25 ml of water in a 100 ml volumetric flask and make up to volume with water.

Sample Solution:
Dissolve 2.5 g of sample in 5 ml of methanol in a 10 ml volumetric flask and make up to volume with methanol.

Procedure:
Spot 5 µl of each of Standard Solutions A and B onto the TLC plate applying the solution slowly in 1 µl aliquots and allowing the plate to dry between applications. Spot 5 µl of the Sample Solution onto the plate in a similar manner. The three spots should be of similar size. Spray the plate with Spray Reagent and heat at 100 ± 2^{o} for 15 min. Immediately after heating, view the plate against a dark background. The spot from the Sample Solution is not more coloured than the spot from Solution B (equivalent to a limit of 0.1% maximum total chlorinated monosaccharides). (Darkening of the mannitol spot from Standard Solution A indicates that the plate has been held too long in the oven, and a second plate should be prepared.)

Triphenylphosphine oxide

Chromatographic system:
Typically a high pressure liquid chromatograph, operated at room temperature, is fitted with a radial compression module containing a 5 µm C_{18} Rad Pak reverse phase column (10 cm x 8 mm). The mobile phase is maintained at a pressure and flow rate (typically 1.5 ml/min) capable of giving the required elution time. The chromatograph is equipped with a UV detector (220 nm).

Mobile Phase:
Add 67 volumes of acetonitrile (HPLC grade, far UV, filtered through a 0.45 µm Millipore filter or equivalent) to 33 volumes of water (glass distilled, filtered through a 0.45 µm Millipore filter or equivalent). Mix and de-gas thoroughly.

Standard Solution:
Weigh accurately 100 mg of triphenylphosphine oxide into a 10 ml volumetric flask. Dissolve and make up to volume using the Mobile Phase. Take 1.0 ml of the resulting solution and make up to 100 ml with Mobile Phase. From this solution prepare a further 100-fold dilution with Mobile Phase and use this as the Standard Solution. Filter through a 0.45 µm Millipore filter or equivalent.

Test Solution:
Weigh accurately about 100 mg of sample into a 10 ml volumetric flask. Dissolve and make up to volume with Mobile Phase. Filter through a 0.45 µm Millipore filter or equivalent. Record the weight of sample as W_t mg.

Procedure:
Inject duplicate 25 µl portions of the Standard and Test Solutions into the chromatograph. Under the conditions stated above the retention time for triphenylphosphine oxide is 6 min. Record the mean peak areas for the Standard and Test Solutions as A_s and A_t respectively. Calculate the concentration of triphenylphosphine oxide (TPPO) in the sample from the following formula:

$$TPPO\ mg/kg = \frac{A_t}{A_s} x \frac{10000}{W_t}$$

Methanol

Apparatus:
Use a suitable gas chromatograph equipped with a hydrogen flame ionization detector containing a 2.1 m x 4.0 mm (id) glass column packed with Porapak PS 80-100 mesh or equivalent materials.

Operating conditions:
The operating conditions may vary depending upon the particular instrument used but a suitable chromatogram may be obtained by using the following conditions:
- Column temperature: 150^o (isothermal)
- Inlet temperature: 200^o
- Detector temperature: 250^o
- Carrier gas nitrogen:20 ml/min

Standard Solution:
Using a 2.0-ml class A volumetric pipet, pipet 2.0 ml of methanol into a 100-ml volumetric flask, dilute to volume with pyridine, and mix. Transfer 1.0 ml of this solution to a 100-ml volumetric flask, dilute to volume with pyridine, and mix.

Sample Solution:
Weigh accurately about 2 g of the sample into a 10-ml volumetric flask, dilute to volume with pyridine, and mix.

Procedure:
Inject a 1-µl portion of the Standard Solution onto a gas chromatography column, obtain the chromatogram, and measure the area of the peak produced. The relative standard deviation for replicate injections is not more than 2.0%. Calculate the mean peak areas for the Standard Solution.

Similarly, inject a 1-µl portion of the Sample Solution into the gas chromatograph, and measure the areas of the peaks produced by methanol. Calculate the mean peak areas, and determine the methanol concentration using the following formula:

$$\%\ methanol\ =\ \frac{S_A\ x\ C_S\ x\ V_S}{A_S\ x\ W_S}$$

where
S_A = the sample area
C_S = the concentration of methanol in the standard in percent (volume of methanol X dilution factor X density of solvent equals 2 X 10^{-4} X 0.79 X 100)
V_S = the volume of the Sample Solution
A_S = the standard area
W_S = the weight of the sample

METHOD OF ASSAY

Chromatographic system:
Fit a high pressure liquid chromatograph, operated at room temperature, with a radial compression module containing a 10 cm 5 µm C_{18} reverse phase column. The mobile phase is maintained at a pressure and flow rate (typically 1.5 ml/min) capable of giving the required elution time (see System Suitability Test). An ultraviolet detector that monitors absorption at 190 nm, or a refractive index detector, is used.

Mobile Phase:
Add 150 ml of acetonitrile (HPLC grade, far UV, filtered through a 0.45 µm Millipore filter or equivalent) to 850 ml of water (glass distilled, filtered through a 0.45 µm Millipore filter or equivalent). Mix and de-gas thoroughly.

Standard Solution:
Weigh accurately about 250 mg of sucralose Reference Standard into a 25 ml volumetric flask. Dissolve and make up to volume using the Mobile Phase. Filter the solution through a 0.45 µm Millipore filter or equivalent. Record the weight of Reference Standard as W_s.

Test solution:
Weigh accurately about 250 mg of sample into a 25 ml volumetric flask. Dissolve and make up to volume using the mobile phase. Filter the solution through a 0.45 µm Millipore filter or equivalent. Record the weight of sample as W_t.

System Suitability Test:
Inject duplicate 20 µl portions of Standard Solution into the chromatograph. The retention time of the sucralose should be approximately 9 min. (NOTE: The retention time quoted is appropriate for a 10 cm 5 µm Rad-Pak C_{18} column. If a column of a different make or length is used it may be necessary to adjust the proportion of acetonitrile in the eluent to obtain the required retention time). The co-efficient of variation (100 x standard deviation divided by mean peak area) for the peak areas should not exceed 2%.

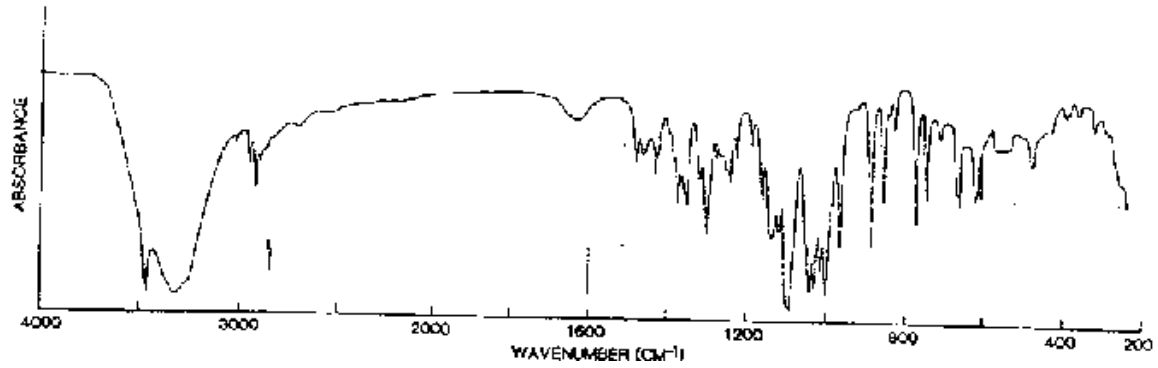
Procedure:

Analyse the Test Solution under the conditions described above, making duplicate 20 µl injections, and calculate the mean peak area. Calculate the percentage purity from the relative peak areas of the Test (A_t) and Standard (A_s) Solutions according to the following formula:

$$\% \, Purity = \frac{A_t \times W_s}{A_s \times W_t} \times 100$$

Calculate the percentage purity on a water-free and methanol-free basis using the values obtained in the tests for water and methanol.

Infrared spectrum — Sucralose



SUCROGLYCERIDES

Prepared at the 49th JECFA (1997) and published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A group ADI of 0 - 30 mg/kg bw for this substance and sucrose esters of fatty acids, sucrose oligoesters type I and type II and sucrose monoesters of lauric, palmitic or stearic acid was established at the 73rd JECFA (2010).

SYNONYMS INS No. 474

DEFINITION Sucroglycerides are obtained by reacting sucrose with an edible fat or oil with or without the presence of a solvent. They consist of a mixture of mono- and di-esters of sucrose and fatty acids together with mono-, di- and triglycerides from the fat or oil. Only the following solvents may be used in the production: dimethyl formamide, cyclohexane, isobutanol, isopropanol and ethyl acetate.

Assay Not less than 40% and not more than 60% of sucrose esters

DESCRIPTION Odourless, soft, solid masses, white to off-white powders, or stiff gels

FUNCTIONAL USES Emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in cold water; soluble in ethanol

Test for fatty acids Add 1 ml of ethanol to 0.1 g of the sample, dissolve by warming, add 5 ml of dilute sulfuric acid TS, heat in a water bath for 30 min and cool. A yellowish white solid or oil is formed, which is soluble in 3 ml of ether.

Test for sugar To 2 ml of the solution separated from the solid or oil in the Test for fatty acids add 1 ml of anthrone TS carefully down the inside of the test tube. The boundary surface of the two layers turns to blue or green.

PURITY

Sulfated ash (Vol. 4) Not more than 2%
Test 2 g of the sample (Method I)

Acid value (Vol. 4) Not more than 6

Free sucrose Not more than 5%
See description under TESTS

Dimethyl formamide Not more than 1 mg/kg
See description under TESTS

Cyclohexane and isobutanol	Not more than 10 mg/kg, singly or in combination See description under TESTS
Ethyl acetate and isopropanol	Not more than 350 mg/kg, singly or in combination
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").

TESTS

PURITY TESTS

Free sucrose

Determine by *gas liquid chromatography* (see Volume 4) using the following conditions:

Reagents

- Internal Standard: 5 mg/ml cholesterol in chloroform or 10 mg/ml tetracosane in chloroform
- Pyridine (dried over molecular sieve)
- N,O-Bis-(Trimethylsilyl)-acetamide (BSA)
- Trimethylchlorosilane (TMCS)

Procedure

Weigh accurately 20-50 mg of the sample into a silylation vial, add 1 ml internal standard solution, 1 ml pyridine, and 0.5 ml each of BSA and TMCS. Seal vial, and heat at 70° for 30 min. Inject 1 µl into the gas liquid chromatograph.

Conditions

Column:

- length: 0.3 m
- diameter: 4 mm (i.d.)
- material: glass
- packing: Dexil

Carrier gas: Nitrogen
Flow rate: 40 ml/min
Detector: FID

Temperature programme: Hold for 1 min at 160°, then 160-375° at 15°/min.
Measure peak areas for sucrose and internal standard. The response factor (RF) is calculated from a number of gas liquid chromatography runs with standard solutions of sucrose containing internal standard.

Calculation

$$RF = \frac{\text{mg of internal standard} \times \text{area sucrose}}{\text{area internal standard} \times \text{mg sucrose}}$$

and

$$\% \text{ free sucrose} = \frac{\text{mg internal standard} \times \text{area sucrose} \times 100}{\text{RF} \times \text{area internal standard} \times \text{mg sample}}$$

Dimethyl formamide

Determine by hydrolysis to dimethylamine and analysis by *gas liquid chromatography* (see Volume 4) using the following conditions:

Reagents

- Dimethyl formamide
- Dimethylamine hydrochloride
- Methanol
- Ethanol
- Hydrochloric acid
- Sodium hydroxide

Standard solutions

Prepare 4.47 mg/ml (equivalent to 4.0 mg/ml of dimethyl formamide) stock solution of dimethylamine hydrochloride in ethanol, and prepare standard solutions equivalent to 4, 2 and 1 µg/ml of dimethyl formamide, respectively, by dilution of the stock solution with 0.1% sodium hydroxide solution in ethanol.

Sample preparation

The apparatus for the hydrolysis is shown in the Appendix. Weigh accurately about 40 g of the sample into a 1000-ml round-bottomed flask. Add 500 ml of 5% methanolic solution of sodium hydroxide, and attach the flask to the apparatus. Set an Erlenmeyer flask containing 10 ml of 1% methanolic solution of hydrochloric acid to the apparatus. Heat the round-bottomed flask and let the content reflux for 1 hour, then distil to collect about 50 ml of the distillate while cooling water of the reflux condenser is stopped. Evaporate the distillate to almost dryness on a boiling water bath. Dissolve the residue with a small amount of ethanol, add 2.5 ml of 5% ethanolic solution of sodium hydroxide, and dilute to 25 ml with ethanol to prepare a sample solution.

Procedure

Inject 2 µl of the sample solution into the gas liquid chromatograph under the conditions below.

Calibration curve

Prepare a calibration curve by injecting each 2 µl of the standard solutions into the gas chromatograph.

Conditions

Column:

- length: 2 m
- diameter: 2 mm (i.d.)
- material: glass
- packing: 10% amine 220 and 10% KOH on 80/100 weak acid washed

Chromosorb W

- conditioning: Heat to 130° overnight with 5 ml/min of nitrogen flow rate

Carrier gas: Nitrogen
Flow rate: 17 ml/min
Detector: FID

Temperatures
- injection port: 198±5°
- column: 60°

Calculation

$$C_{DFA}\ (mg/kg) = \frac{C\ (\mu g/ml) \times 25\ (ml)}{W\ (g)}$$

where
C_{DFA} is the Concentration of dimethyl formamide;
C is the Concentration of dimethyl formamide detected; and
W is the weight of sample taken.

Cyclohexane and isobutanol

Determine by *gas liquid chromatography* (see Volume 4) using the following conditions:

Reagents
- Dimethylformamide (GLC purity grade)
- Cyclohexane (UV spectrophotometric grade)
- Isobutanol (analytical grade)

Standard solutions
Prepare a 0.1% stock solution of cyclohexane and isobutanol in dimethylformamide by pipetting 130 µl of cyclohexane and 125 µl of isobutanol into dimethylformamide and making up the volume to 10 ml.
Prepare by dilution a range of solutions containing 5, 10 and 20 mg/kg of cyclohexane and isobutanol. Prepare a response curve by injecting 5 µl of these diluted standard solutions into the gas chromatograph under the conditions below.

Sample preparation
Weigh 5 g of sample to the nearest 10 mg into a flask with a ground glass stopper, add 5 g of dimethylformamide and warm to dissolve. Cool and inject 5 µl into the gas chromatograph under the conditions below.

Column
- length: 3 m
- diameter: 4.5 mm
- material: stainless steel
- packing: 20% Carbowax 20 M on Chromosorb G 60/80

Carrier gas: Helium (1.6 bar)
Detector: Flame ionization
Temperatures
- injection port: 130°
- column: 130°
- detector: 200°

Determine the concentration of cyclohexane and isobutanol in the sample solution (50%) by comparison with the standard solutions and multiply the concentration by two to convert the results to correspond to the original sucroglycerides.

Isopropanol and ethyl acetate

Determine by *gas chromatography* (see Volume 4) with a head space sampler using the following conditions:

Reagents

- Isopropanol
- Ethyl acetate

Standard solutions

Take each 1 g of isopropanol and ethyl acetate in a volumetric flask and add water to total volume of 100 ml, and prepare 0.02-0.4 g/100 ml solutions by dilution of this solution.
If necessary, prepare standard solutions containing up to 7 g/100 ml of isopropanol and ethyl acetate.

Procedure

Place 1 g (1.0 ± 0.1 g) of powdered sample in a sample vial. Add 5 µl of water to the sample vial and seal it quickly with a septum. Set the sample vial in a pre-conditioned gas chromatograph and start the analysis under the below-mentioned conditions.

Calibration curve

Take 1 g of powdered sucrose esters of fatty acids, solvent free or known residual solvent contents, in a sample vial, add 5 µl of the standard solution and seal it quickly with a septum. Set the sample vial in a pre-conditioned gas chromatograph and start the analysis under the following conditions and obtain calibration curves for each solvent.

Column:

- length: 30 m
- diameter: 0.53 mm (i.d.)
- material: Silica capillary
- film: 100% methyl polysiloxane
- conditioning: Heat to 60° for 2-3 h with approximately 10 ml/min of nitrogen

Carrier gas: Nitrogen
Flow rate: 5 ml/min
Detector: Flame ionization
Temperatures:

- injection port: 110°
- column: 40°
- detector: 110°

Head space sampler:

- Sample volume: 1.0 g ± 0.1 g + 5 µl
- Sample heating temp.: 80°
- Sample heating time: 40 min
- Syringe temperature: 85°
- Sample gas injection: 0.4 ml

Calculation

$C_i = A_i \times Cf_i \times 1000$

where

C_i is the Concentration of solvent i (mg/kg);
A_i is the Peak area of solvent i (µv.sec.); and
Cf_i is the Conversion coefficient for solvent i (slope of the calibration curve) (µg/µv.sec).

METHOD OF ASSAY Determine by *high pressure liquid chromatography* (see Volume 4) using the following conditions:

Sample preparation
Add about 250 mg of the sample, accurately weighed to a 50 ml volumetric flask. Dilute to volume with tetrahydrofuran, and mix. Filter through a 0.5-µm membrane filter.

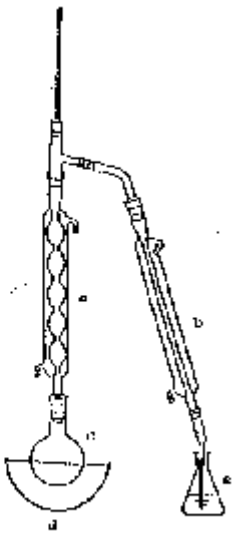
Procedure
Inject 100 µl of the sample into the pre-stabilized high pressure liquid chromatograph.

Conditions
Column: Styrene-divinylbenzene copolymer for gel permeation chromatography (TSK-GEL G2000 (Supelco) or equivalent)
Mobile phase: HPLC-grade degassed tetrahydrofuran
Flow rate: 0.7 ml/min
Detector: Refractive index detector
Temperatures:
Column: 38°
Detector: 38°
Record the chromatogram for about 90 min. Calculate the percentage of sucrose ester content in the sample taken by the formula:

$$100\ A/T$$

where
A is the the sum of peak areas for the three main components, the mono-, di- and triesters, eluting at about 65, 68 and 73 min, respectively; and
T is the sum of all peak areas eluting within 90 min.

Appendix



Apparatus for hydrolysis

a: Reflux condenser
b: Condenser
c: Round bottomed flask
d: Water bath
e: Erlenmeyer flask

SUCROSE ACETATE ISOBUTYRATE

Prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 41st JECFA (1993), published in FNP 52 Add 2 (1993). Metals and arsenic specifications revised at the 61st JECFA (2003. An ADI of 0-20 mg/kg bw was established at the 46th JECFA (1996))

SYNONYMS SAIB, INS No. 444

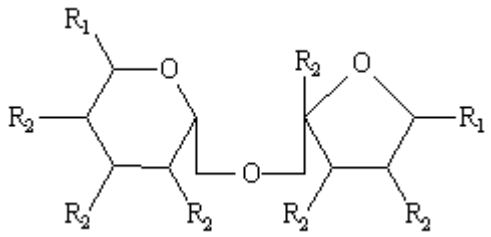
DEFINITION A mixture of the reaction products formed by the esterification of food grade sucrose with acetic anhydride and isobutyric anhydride, followed by distillation. The mixture contains all possible combinations of esters in which the molar ratio of acetate to isobutyrate is about 2:6

Chemical names: Sucrose diacetate hexaisobutyrate (approximate)

C.A.S. number: 137204-24-1; 27216-37-1; 126-13-6

Chemical formula: $C_{40}H_{62}O_{19}$ for sucrose diacetate hexaisobutyrate

Structural formula



where
R_1 = $-CH_2OCOCH_3$, and
R_2 = $-CH_2OCOCH(CH_3)_2$, or $-OCOCH(CH_3)_2$

Formula weight: 832 - 856 (approximate), $C_{40}H_{62}O_{19}$ = 846.9

Assay: Not less than 98.8% and not more than 101.9% of $C_{40}H_{62}O_{19}$

DESCRIPTION Pale straw coloured liquid, clear and free of sediment and having a bland odour

FUNCTIONAL USES Density adjusting agent, cloud-producing agent in non-alcoholic beverages

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Insoluble in water, soluble in most organic solvents

Refractive index (Vol. 4): n (40, D): 1.4492 - 1.4504

Specific gravity (Vol. 4)	d (25, 25): 1.141 - 1.151
Infrared absorption	The infrared spectrum of a potassium bromide dispersion of the sample corresponds with the reference infrared spectrum in the Appendix

PURITY

Acid value (Vol. 4)	Not more than 0.2 Proceed as directed under *Acid Value*, using 50 g of the sample and a microburette
Saponification value (Vol. 4)	Between 524 and 540 Use 1 g of the sample
Triacetin	Not more than 0.1% See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

TESTS

PURITY TESTS

Triacetin

Test by the following *gas chromatographic* procedure

Apparatus:

Gas chromatograph equipped with a flame ionization detector
Column: Stainless steel, 1.5 m, 3.2 mm i.d.

Preparation of sample:

Dilute the sample by adding an equal volume of carbon disulfide.

Conditions

Stationary phase: SE-30, 3%
Solid phase: Chromosorb AW-DMCS, 80-100 mesh
Carrier gas: Helium
Flow rate: 20 ml/min
Temperatures
- Column: Programmed at 10° per min from 100° to 300° immediately after injection of the sample
- Injector: 300°

Injected volume: 1 µl

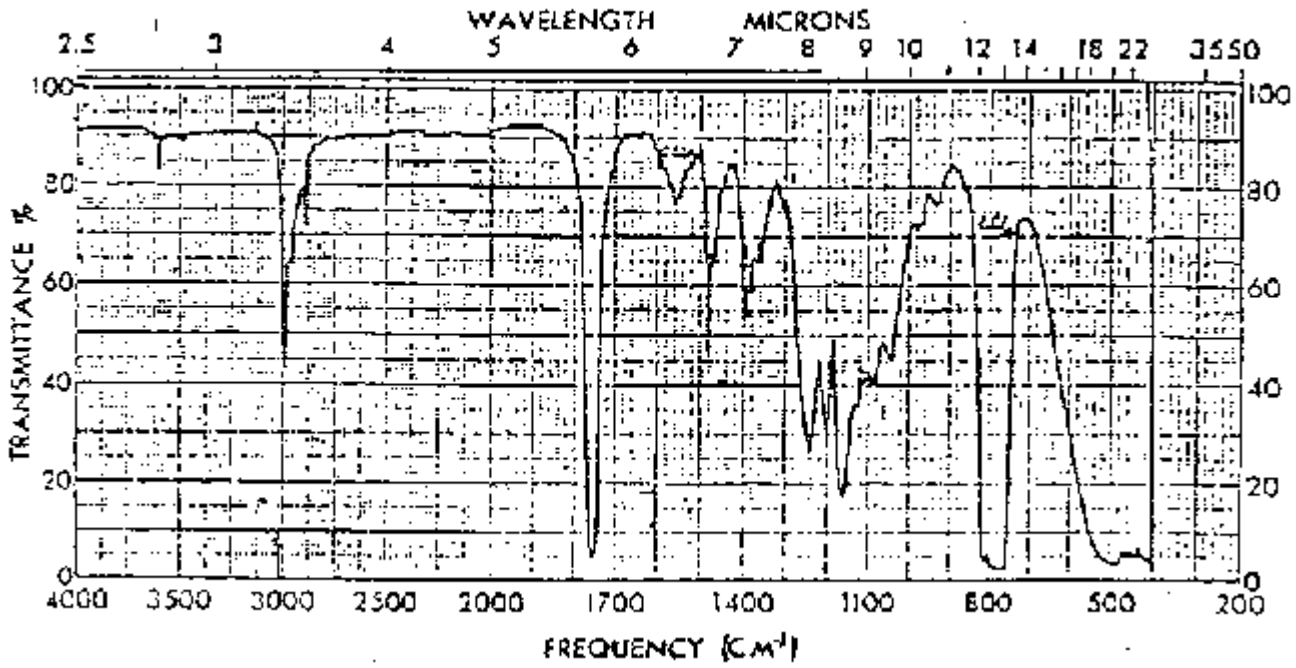
METHOD OF ASSAY

Using the saponification value, calculate the percentage of $C_{40}H_{62}O_{19}$ by the formula:

$$\frac{SV \times 0.10586}{56.1} \times 100$$

where
SV = saponification value

Infrared spectrum Sucrose acetate isobutyrate



SULFUR DIOXIDE

Prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998) superseding specifications prepared at the 49th JECFA (1997), published in FNP 52 Add 5 (1997). Group ADI 0-0.7 mg/kg bw as SO2 for sulfites, established at the 51st JECFA in 1998.

SYNONYMS

INS No. 220

DEFINITION

C.A.S. number: 7446-09-5

Chemical formula: SO_2

Formula weight: 64.07

Assay: Not less than 99.9 % SO_2 by weight

DESCRIPTION

Colourless, non-flammable gas, with strong, pungent, suffocating odour. Its vapour density is 2.26 times that of air at atmospheric pressure and 0^o. The specific gravity of the liquid is about 1.436 at $0^o/4^o$. At 20^o the solubility is about 10 g of SO_2 per 100 g of solution. It is normally supplied under pressure in containers in which it is present in both liquid and gaseous phases.

Caution: Sulfur dioxide gas is intensely irritating to the eyes, throat, and upper respiratory system. Liquid sulfur dioxide may cause skin burns, which result from the freezing effect of the liquid on tissue. Safety precautions to be observed in handling of the material are specified in technical brochures from liquid sulfur dioxide manufacturers, suppliers or organizations of gas manufacturers or suppliers (For example, "Pamphlet G-3" published by the Compressed Gas Association, Suite 1004, 1725 Jefferson Davis Highway, Arlington, VA 22202, USA).

FUNCTIONAL USES

Preservative, antibrowning agent, antioxidant,

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Soluble in water (36 v in 1 v) and ethanol (114 v in 1 v).

Test for sulfurous substances: The sample blackens filter paper moistened with mercurous nitrate TS.

Oxidizing activity: Expose a filter paper, treated with potassium iodate and starch TS, to the sample. A blue colour is developed that fades on continued exposure.

PURITY

Water (Vol. 4): Not more than 0.05%

Transfer about 50 ml of liquid sulfur dioxide into a Karl Fischer titration jar, determine the weight of the sample taken, and determine the water content by Karl Fischer Method

Non-volatile residue

Not more than 0.05%
Measure out 200 ml of sulfur dioxide (288 g) into a 250-ml Erlenmeyer flask, and determine the weight of sample taken by the loss in weight of the sample bomb. Evaporate to dryness on a steam bath, and displace the residual vapours with dry air. Wipe the flask dry, cool in a desiccator, and weigh.

Selenium (Vol. 4)

Not more than 20 mg/kg
A 2.0-ml portion of the Sample Solution meets the requirements of the Selenium Limit Test, Method II. For sampling and sample preparation, see TESTS

Lead (Vol. 4)

Not more than 5 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

Sampling:

Samples of sulfur dioxide may be safely withdrawn from a tank or transfer lines, either of which should be equipped with a 1-cm nozzle and valve. Samples should be taken in bombs constructed of 316 stainless steel, designed to withstand 7 MPa (1000 psig) and equipped with 316 stainless steel needle valves on both ends. To draw a sample, the bomb is first flushed with dry air to remove any sulfur dioxide, remaining from previous sample drawings, and then attached to the tank or transfer lines with a solid pipe connection. A hose is connected to the other end of the bomb and submerged in either a weak caustic solution or water. Any gas in the bomb is discharged into the caustic or water by first opening the valve at the pipe end, followed by slowly opening at the valve at the hose end. When all of the gas is dispelled and liquid sulfur dioxide begins to emerge into the solution, the valve at the hose end is blocked off. The other valves are then tightly closed, and the bomb is detached from the pipe connecting it to the tank or transfer line. Approximately 15% of the liquid sulfur dioxide in the bomb is then discharged into the water or caustic solution. The bomb is then capped at its end and transferred to the laboratory for analysis.

Caution: The bomb should never be stored with more than 85% of the total water capacity of the bomb.

Sample Solution for the Determination of Lead, and Selenium:
Measure out 100 ml of sulfur dioxide (144 g) into a 125-ml Erlenmeyer flask, and determine the weight of sample taken by the loss in weight of the sample bomb. Evaporate to dryness on a steam bath, add 3 ml of nitric acid and 10 ml of water to the dry flask, and warm gently on a hot plate for 15 min. Transfer the contents of the flask to a 100-ml volumetric flask, dilute to volume with water, and mix. Transfer a 10.0-ml aliquot into a second 100-ml volumetric flask, dilute to volume with water, and mix.

Note: The tests in which this solution is to be used will be accurate assuming a 144 g sample has been taken; if not, the weight of sample actually taken must be considered in the calculations.

METHOD OF ASSAY

Subtract from 100 the percentages of non-volatile residue and of water, as determined herein, to obtain the percentage of SO_2.

SODIUM SULFITE

Prepared at the 53rd JECFA (1999) and published in FNP 52 Add 7 (1999), superseding tentative specifications prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998). Group ADI 0-0.7 mg/kg bw as SO2 for sulfites established at the 51st JECFA in 1998.

SYNONYMS Disodium sulfite, INS No. 221

DEFINITION

Chemical names	Sodium sulfite
C.A.S. number	7757-83-7
Chemical formula	Na_2SO_3
Formula weight	126.04
Assay	Not less than 95.0%

DESCRIPTION White powder with not more than a faint odour of sulfur dioxide

FUNCTIONAL USES Antibrowning agent, antioxidant, preservative

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Freely soluble in water; sparingly soluble in ethanol
Test for sodium (Vol. 4)	Passes test
Test for sulfite (Vol. 4)	Passes test

PURITY

pH (Vol. 4)	8.5 - 10.0 (1 in 10 soln)
Thiosulfate	Not more than 0.1% A 10% solution of the sample should remain clear on acidification with sulfuric or hydrochloric acid.
Iron (Vol. 4)	Not more than 10 mg/kg Proceed as directed in the Limit Test using 0.5 ml of Iron Standard Solution (5 µg Fe) in the control
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

Selenium — Not more than 5 mg/kg
See description under TESTS

TESTS

PURITY TESTS

Selenium

Reagents:
Hydrochloric acid, hydrazinium sulfate, standard selenium solution (100 µg Se/ml)

Procedure
Weigh 2.0 ± 0.1 g of sample and transfer to a 50-ml beaker. Add 10 ml water, 5 ml hydrochloric acid and boil to remove SO_2.
Into a second beaker, weigh 1.0 ± 0.1 g of sample, add 0.05 ml standard selenium solution and proceed as above.

To each beaker add 2 g hydrazinium sulfate and warm to dissolve. Let stand for 5 min. Dilute the contents of each beaker to 50 ml in a Nessler tube and compare the colour of the two solutions. The sample should be less pink than the sample with the added standard.

METHOD OF ASSAY

Weigh 250 mg of the sample, add to 50.0 ml of 0.1 N iodine in a glass-stoppered flask, and stopper the flask. Allow to stand for 5 min, add 1 ml of hydrochloric acid and titrate the excess iodine with 0.1 N sodium thiosulfate, adding starch TS as the indicator. Each ml of 0.1 N iodine is equivalent to 6.302 mg of Na_2SO_3.

SODIUM HYDROGEN SULFITE

Prepared at the 53rd JECFA (1999) and published in FNP 52 Add 7 (1999), superseding tentative specifications prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998). Group ADI 0-0.7 mg/kg bw as SO2 for sulfites established at the 51st JECFA in 1998.

SYNONYMS INS No. 222

DEFINITION

Chemical names	Sodium hydrogen sulfite, sodium bisulfite
C.A.S. number	7631-90-5
Chemical formula	$NaHSO_3$
Formula weight	104.06
Assay	Not less than 58.5% and not more than 67.4% of SO_2

DESCRIPTION White crystals or granular powder having an odour of sulfur dioxide

FUNCTIONAL USES Antibrowning agent, antioxidant, preservative

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Freely soluble in water; slightly soluble in ethanol
Test for sodium (Vol. 4)	Passes test
Test for sulfite (Vol. 4)	Passes test

PURITY

Water insolubles	Dissolve 20 g of the sample in 200 ml of water. The solution should be clear with only a trace of suspended matter
pH (Vol. 4)	2.5 - 4.5 (1 in 10 soln)
Iron (Vol. 4)	Not more than 10 mg/kg Proceed as directed in the Limit Test using 0.5 ml of Iron Standard Solution (5 µg Fe) in the control
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
Selenium	Not more than 5 mg/kg

See description under TESTS

TESTS

PURITY TESTS

Selenium

Reagents:
Hydrochloric acid, hydrazinium sulfate, standard selenium solution (100 µg Se/ml)

Procedure
Weigh 2.0 ± 0.1 g of sample and transfer to a 50-ml beaker. Add 10 ml water, 5 ml hydrochloric acid and boil to remove SO_2.

Into a second beaker, weigh 1.0 ± 0.1 g of sample, add 0.05 ml standard selenium solution and proceed as above.

To each beaker add 2 g hydrazinium sulfate and warm to dissolve. Let stand for 5 min. Dilute the contents of each beaker to 50 ml in a Nessler tube and compare the colour of the two solutions. The sample should be less pink than the sample with the added standard.

METHOD OF ASSAY

Weigh 0.2 g of the sample, to the nearest mg, add 50.0 ml of 0.1 N iodine in a glass-stoppered flask, and stopper the flask. Allow to stand for 5 min, add 1 ml of hydrochloric acid, and titrate the excess iodine with 0.1 N sodium thiosulfate, adding starch TS as the indicator. Each ml of 0.1 N iodine is equivalent to 3.203 mg of SO_2.

SODIUM METABISULFITE

Prepared at the 53rd JECFA (1999) and published in FNP 52 Add 7 (1999), superseding tentative specifications prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998). Group ADI 0-0.7 mg/kg bw as SO2 for sulfite established at the 51st JECFA in 1998.

SYNONYMS INS No. 223

DEFINITION

Chemical names	Sodium disulfite, disodium pentaoxodisulfate, disodium pyrosulfite
C.A.S. number	7681-57-4
Chemical formula	$Na_2S_2O_5$
Formula weight	190.11
Assay	Not less than 90.0%

DESCRIPTION White crystals or crystalline powder having an odour of sulfur dioxide

FUNCTIONAL USES Antibrowning agent, antioxidant, flour treatment agent, preservative

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Freely soluble in water; slightly soluble in ethanol
Test for sodium (Vol. 4)	Passes test
Test for sulfite (Vol. 4)	Passes test

PURITY

Water insolubles	Dissolve 20 g of the sample in 200 ml of water. The solution should be clear with only a trace of suspended matter
pH (Vol. 4)	4.0 - 4.5 (1 in 10 soln)
Thiosulfate	Not more than 0.1% A 10% solution of the sample should remain clear on acidification with sulfuric or hydrochloric acid.
Iron (Vol. 4)	Not more than 10 mg/kg Proceed as directed in the Limit Test using 0.5 ml of Iron Standard Solution (5 µg Fe) in the control
Lead (Vol. 4)	Not more than 2 mg/kg

Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

Selenium

Not more than 5 mg/kg
See description under TESTS

TESTS

PURITY TESTS

Selenium

Reagents:
Hydrochloric acid, hydrazinium sulfate, standard selenium solution (100 µg Se/ml)

Procedure
Weigh 2.0 ± 0.1 g of sample and transfer to a 50-ml beaker. Add 10 ml water, 5 ml hydrochloric acid and boil to remove SO_2.
Into a second beaker, weigh 1.0 ± 0.1 g of sample, add 0.05 ml standard selenium solution and proceed as above.

To each beaker add 2 g hydrazinium sulfate and warm to dissolve. Let stand for 5 min. Dilute the contents of each beaker to 50 ml in a Nessler tube and compare the colour of the two solutions. The sample should be less pink than the sample with the added standard.

METHOD OF ASSAY

Weigh 0.2 g of the sample to the nearest mg, add to 50.0 ml of 0.1 N iodine in a glass-stoppered flask, and stopper the flask. Allow to stand for 5 min, add 1 ml of hydrochloric acid, and titrate the excess iodine with 0.1 N sodium thiosulfate, adding starch TS as the indicator. Each ml of 0.1 N iodine is equivalent to 4.753 mg of $Na_2S_2O_5$.

POTASSIUM METABISULFITE

Prepared at the 53rd JECFA (1999) and published in FNP 52 Add 7 (1999), superseding tentative specifications prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998). Group ADI 0-0.7 mg/kg bw as SO2 for sulfite established at the 51st JECFA in 1998.

SYNONYMS INS No. 224

DEFINITION

Chemical names	Potassium disulfite, potassium pentaoxodisulfate, potassium pyrosulfite
C.A.S. number	16731-55-8
Chemical formula	$K_2S_2O_5$
Formula weight	222.33
Assay	Not less than 90%

DESCRIPTION Colourless free-flowing crystals, crystalline powder, or granules, usually having an odour of sulfur dioxide

FUNCTIONAL USES Antibrowning agent, antioxidant, preservative

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water; insoluble in ethanol
Test for potassium (Vol. 4)	Passes test
Test for sulfite (Vol. 4)	Passes test

PURITY

Water insolubles	Dissolve 20 g of the sample in 200 ml of water. The solution should be clear with only a trace of suspended matter.
Thiosulfate	Not more than 0.1% A 10% solution of the sample should remain clear on acidification with sulfuric or hydrochloric acid
Iron (Vol. 4)	Not more than 10 mg/kg Determine as directed in the Limit Test using 0.5 ml of Iron Standard Solution (5 µg Fe) in the control
Lead (Vol. 4)	Not more than 2 mg/kg

Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

Selenium

Not more than 5 mg/kg
See description under TESTS

TESTS

PURITY TESTS

Selenium

Reagents:
Hydrochloric acid, hydrazinium sulfate, standard selenium solution (100 µg Se/ml)

Procedure
Weigh 2.0 ± 0.1 g of sample and transfer to a 50-ml beaker. Add 10 ml water, 5 ml hydrochloric acid and boil to remove SO_2.
Into a second beaker, weigh 1.0 ± 0.1 g of sample, add 0.05 ml standard selenium solution and proceed as above.

To each beaker add 2 g hydrazinium sulfate and warm to dissolve. Let stand for 5 min. Dilute the contents of each beaker to 50 ml in a Nessler tube and compare the colour of the two solutions. The sample should be less pink than the sample with the added standard.

METHOD OF ASSAY

Weigh 250 mg of the sample, add to 50.0 ml of 0.1 N iodine in a glass stoppered flask, and stopper the flask. Allow to stand for 5 min, add 1 ml of dilute hydrochloric acid TS and titrate the excess iodine with 0.1 N sodium thiosulfate, using starch TS as the indicator. Each ml of 0.1 N iodine is equivalent to 5.558 mg of $K_2S_2O_5$.

POTASSIUM SULFITE

Prepared at the 53rd JECFA (1999) and published in FNP 52 Add 7 (1999), superseding tentative specifications prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998). Group ADI 0-0.7 mg/kg bw as SO2 for sulfites established at the 51st JECFA in 1998.

SYNONYMS INS No. 225

DEFINITION

Chemical names: Potassium sulfite

C.A.S. number: 10117-38-1

Chemical formula: K_2SO_3

Formula weight: 158.25

Assay: Not less than 90.0%

DESCRIPTION White, odourless, granular powder

FUNCTIONAL USES Antibrowning agent, antioxidant, preservative

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water; slightly soluble in ethanol

Test for potassium (Vol. 4): Passes test

Test for sulfite (Vol. 4): Passes test

PURITY

Alkalinity: Between 0.25 and 0.45% as K_2CO_3
Dissolve 1 g of the sample in 20 ml of water and add 25 ml of 3% hydrogen peroxide, previously neutralized to methyl red TS. Mix thoroughly, cool to room temperature, and titrate with 0.02 N hydrochloric acid. Perform a blank determination using 25 ml of neutralized hydrogen peroxide solution. Each ml of 0.02 N hydrochloric acid is equivalent to 1.38 mg of K_2CO_3.

Iron (Vol. 4): Not more than 10 mg/kg
Proceed as directed in the Limit Test using 0.5 ml of Iron Standard Solution (5 µg Fe) in the control

Lead (Vol. 4): Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in

Volume 4, “Instrumental Methods.”

Selenium

Not more than 5 mg/kg
See description under TESTS

TESTS

PURITY TESTS

Selenium

Reagents:
Hydrochloric acid, hydrazinium sulfate, standard selenium solution (100 µg Se/ml)

Procedure
Weigh 2.0 ± 0.1 g of sample and transfer to a 50-ml beaker. Add 10 ml water, 5 ml hydrochloric acid and boil to remove SO_2.

Into a second beaker, weigh 1.0 ± 0.1 g of sample, add 0.05 ml standard selenium solution and proceed as above.

To each beaker add 2 g hydrazinium sulfate and warm to dissolve. Let stand for 5 min. Dilute the contents of each beaker to 50 ml in a Nessler tube and compare the colour of the two solutions. The sample should be less pink than the sample with the added standard.

METHOD OF ASSAY

Weigh accurately about 0.75 g of the sample and dissolve in a mixture of 100 ml of 0.1 N iodine and 5 ml of dilute hydrochloric acid TS. Titrate the excess iodine with 0.1 N sodium thiosulfate, adding starch TS as the indicator. Each ml of 0.1 N iodine is equivalent to 7.912 mg of K_2SO_3.

SODIUM THIOSULFATE

Prepared at the 53rd JECFA (1999) and published in FNP 52 Add 7 (1999), superseding tentative specifications prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998). Group ADI 0-0.7 mg/kg bw as SO2 for sulfites established at the 51st JECFA in 1998.

SYNONYMS Sodium hyposulfite; INS No. 539

DEFINITION

Chemical names	Sodium thiosulfate
C.A.S. number	7772-98-7
Chemical formula	$Na_2S_2O_3 \cdot 5H_2O$
Formula weight	248.17
Assay	Not less than 99.0% on the dried basis

DESCRIPTION Colourless crystals or coarse crystalline powder; deliquesces in moist air and effloresces in dry air above 33°

FUNCTIONAL USES Antibrowning agent, antioxidant, sequestrant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Very soluble in water; insoluble in ethanol
Reducing activity	To a 1 in 10 solution of the sample add a few drops of iodine TS; the colour is discharged
Test for sodium (Vol. 4)	Passes test
Test for thiosulfate (Vol. 4)	Passes test

PURITY

Loss on drying (Vol. 4)	32-37% (40-45°, 16 h, under vacuum)
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
Iron (Vol. 4)	Not more than 10 mg/kg Proceed as directed in the Limit Test using 0.5 ml of Iron Standard Solution (5 µg Fe) in the control

Selenium — Not more than 5 mg/kg
See description under TESTS

TESTS

PURITY TESTS

Selenium

Reagents:
Hydrochloric acid, hydrazinium sulfate, standard selenium solution (100 µg Se/ml)

Procedure
Weigh 2.0 ± 0.1 g of sample and transfer to a 50-ml beaker. Add 10 ml water, 5 ml hydrochloric acid and boil to remove SO_2.
Into a second beaker, weigh 1.0 ± 0.1 g of sample, add 0.05 ml standard selenium solution and proceed as above.

To each beaker add 2 g hydrazinium sulfate and warm to dissolve. Let stand for 5 min. Dilute the contents of each beaker to 50 ml in a Nessler tube and compare the colour of the two solutions. The sample should be less pink than the sample with the added standard.

METHOD OF ASSAY

Dissolve about 0.5 g of the dried sample, accurately weighed, in 30 ml of water and titrate with 0.1 N iodine solution using starch TS as the indicator. Each ml of 0.1 N iodine is equivalent to 15.81 mg of $Na_2S_2O_3$.

SUNSET YELLOW FCF

Prepared at the 69th JECFA (2008) and published in FAO JECFA Monographs 5 (2008), superseding specifications prepared at the 28th JECFA (1984), published in combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI of 0-4 mg/kg bw was established at the 74th JECFA (2011).

SYNONYMS CI Food Yellow 3; Orange Yellow S; CI (1975) No. 15985; INS No. 110

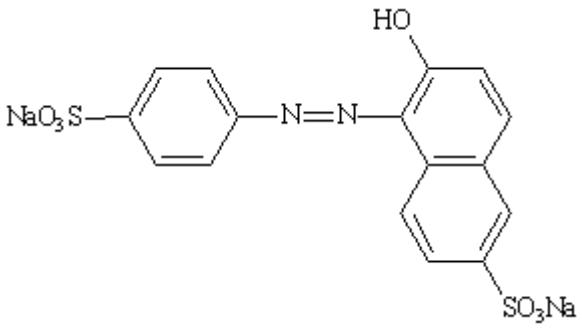
DEFINITION Sunset Yellow FCF consists principally of the disodium salt of 6-hydroxy-5-[(4-sulfophenyl)azo]-2-naphthalenesulfonic acid and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.
(NOTE: The colour may be converted to the corresponding aluminium lake, in which case only the *General Specifications for Aluminium Lakes of Colouring Matters* apply.)

Chemical names: Principal component:
Disodium 6-hydroxy-5-(4-sulfonatophenylazo)-2-naphthalene-sulfonate

C.A.S. number: 2783-94-0

Chemical formula: $C_{16}H_{10}N_2Na_2O_7S_2$ (Principal component)

Structural formula



(Principal component)

Formula weight: 452.38 (Principal component)

Assay: Not less than 85% total colouring matters

DESCRIPTION Orange-red powder or granules

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water; sparingly soluble in ethanol
Colour test	In water, neutral or acidic solutions of Sunset Yellow FCF are yellow-orange, whereas basic solutions are red-brown. When dissolved in concentrated sulfuric acid, the additive yields an orange solution that turns yellow when diluted with water.
Colouring matters, identification (Vol. 4)	Passes test

PURITY

Water content (Loss on drying) (Vol. 4)	Not more than 15% together with chloride and sulfate calculated as sodium salts
Water-insoluble matter (Vol. 4)	Not more than 0.2%
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under “General Methods, Metallic Impurities”).
Subsidiary colouring matter content (Vol. 4)	Not more than 5% Not more than 2% shall be colours other than trisodium 2-hydroxy-1-(4-sulfonatophenylazo)naphthalene-3,6-disulfonate Use the following conditions: Chromatography solvent: 2-Butanone:acetone:water:ammonia (s.g. 0.880) (700:300:300:2) Height of ascent of solvent front: approximately 17 cm
Sudan I (1-(Phenylazo)-2-naphthalenol)	Not more than 1 mg/kg See description under TESTS
Organic compounds other than colouring matters (Vol. 4)	Not more than 0.5%, sum of the: monosodium salt of 4-aminobenzenesulfonic acid, disodium salt of 3-hydroxy-2,7-naphthalenedisulfonic acid, monosodium salt of 6-hydroxy-2-naphthalenesulfonic acid, disodium salt of 7-hydroxy-1,3-naphthalenedisulfonic acid, disodium salt of 4,4'-diazoaminobis-benzenesulfonic acid, and disodium salt of 6,6'-oxybis-2-naphthalenesulfonic acid Proceed as directed under *Determination by High Performance Liquid Chromatography* using an elution gradient of 2 to 100% at 4% per min (linear) followed by elution at 100%.
Unsulfonated primary aromatic amines (Vol. 4)	Not more than 0.01%, calculated as aniline

Ether-extractable matter (Vol. 4)

Not more than 0.2%

TESTS

PURITY TESTS

Sudan I (1-(Phenylazo)-2-naphthalenol)

Principle
The additive is dissolved in water and methanol and filtered solutions are analysed by Reverse-Phase Liquid Chromatography (Volume 4 under "Analytical Techniques, Chromatography"), without extraction or concentration. (Based on *J.AOAC Intl* 90, 1373-1378 (2007).)

Mobile phase
Eluant A: Ammonium acetate (LC grade), 20 mM aqueous
Eluant B: Methanol (LC grade)

Sample solution
Accurately weigh 200 mg of Sunset yellow FCF and transfer it into a 10-ml volumetric flask. Dissolve the sample in 4 ml water via swirling or sonication. Add 5 ml of methanol and swirl. Allow the solution to cool for 5 min and adjust the volume to the mark with water. Filter a part of the solution for analysis through a 13 mm syringe filter with a 0.2 µm pore size PTFE membrane by using a 5 ml polypropylene/polyethylene syringe. (NOTE: Do not substitute a PVDF filter for the PTFE filter, as a PVDF filter adsorbs Sudan I.)

Standard
Sudan I (>97%, Sigma Aldrich, or equivalent), recrystallized from absolute ethanol (5g/150 ml)

Standard stock solution
Accurately weigh a sufficient quantity of the *Standard* to prepare a solution in methanol of 0.0100 mg/ml.

Standard solutions
Transfer 0, 20, 50, 100, 150, 200, and 250 µl aliquots of the *Standard stock solution* to seven 10-ml volumetric flasks. To each flask, add 5 ml of methanol, swirl to mix, and add 4 ml of water. Dilute to volume with water, mix, and filter each solution through a PTFE membrane syringe filter (see *Sample solution*, above) into LC vials for analysis. (NOTE: These solutions nominally contain 0, 0.02, 0.05, 0.10, 0.15, 0.20, and 0.25 µg of Sudan I/ml.)

Chromatographic system
Detector: Photodiode Array (485 nm)
Columns: 150 mm x 2.1 mm id, packed with 5 µm reversed-phase C18, or equivalent, with a guard column (10 mm x 2.1 mm i.d.) – Waters Corp., or equivalent
Column temperature: 25°
Flow rate: 0.25 ml/min
Injection volume: 50 µl
Elution: 50% *Eluant A*/50% *Eluant B* for 5 min; 50 to 100% *Eluant B* in 10 min; 100% *Eluant B* for 10 min. (NOTE: The column

should be requilibrated with 50% *Eluant A*/50% *Eluant B* for 10 min.)

System suitability: Inject three replicates of the *Standard solutions* with concentrations of 0.05 and 0.25 µg of Sudan I/ml. The responses for each set of three injections show relative standard deviations of not more than 2%.

Procedure

Separately inject the seven *Standard solutions* and the *Sample solution* into the chromatograph and measure the peak areas for Sudan I. From the chromatograms for the *Standard solutions*, prepare a standard curve of the concentration of Sudan I vs. the peak areas. (NOTE: The retention time for Sudan I is 19.0 min. Other peaks appearing at earlier retention times in the sample chromatograph are likely attributed to sulfonated subsidiary colours.) Determine the concentration of Sudan I in the *Sample solution* and convert it to mg/kg in the sample of Sunset Yellow FCF.

(NOTE: The limit of determination is 0.4 mg/kg.)

METHOD OF ASSAY

Proceed as directed under *Colouring Matters Content by Titration with Titanous Chloride* (Volume 4, under "Food Colours, Colouring Matters"), using the following:

Weight of sample: 0.5-0.6 g

Buffer: 10 g sodium citrate

Weight (*D*) of colouring matters equivalent to 1.00 ml of 0.1 N $TiCl_3$: 11.31 mg

TALC

Prepared at the 61st JECFA (2003) and published in FNP 52 Add 11 (2003), superseding specifications prepared at the 55th JECFA (2000) and published in FNP 52 Add 8 (2000). An ADI "not specified" was established at the 30th JECFA (1986).

SYNONYMS Talcum; INS No. 553(iii)

DEFINITION Powdered, natural, hydrated magnesium silicate containing varying proportions of such associated materials as alpha-quartz, calcite, chlorite, dolomite, magnesite and phlogopite.

C.A.S. number 14807-96-6

DESCRIPTION Odourless, very fine, white or greyish white crystalline powder; unctuous, adheres readily to the skin, free from grittiness.

FUNCTIONAL USES Anticaking agent, filtering aid, coating agent, surface-finishing agent, texturizing agent, component of chewing gum base.

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water and ethanol

Infrared absorption The infrared absorption spectrum of a potassium bromide dispersion of the sample exhibits major peaks as follows:
3677 ± 2 cm^{-1} OH group
1018 ± 2 cm^{-1} Si-O-Si group
669 ± 2 cm^{-1} Mg-O-Si group

X-Ray diffraction The X-ray diffraction pattern of a random powder sample exhibits reflections at d values of 9.34 Å, 4.66 Å and 3.12 Å

PURITY

Loss on drying (Vol. 4) Not more than 0.5 % (105 ^{o}, 1 h)

Loss on ignition (Vol. 4) Not more than 9 %

Water-soluble substances Not more than 0.2 %
Boil 20 g sample with 200 ml of water in a 250 ml beaker for 15 min, stir to avoid spurting. Cool to room temperature, transfer the contents to a 250 ml volumetric flask, rinse beaker with 25 ml water, add rinsings to the volumetric flask and make up to volume. Allow the mixture to stand for 15 min and filter (use filtrate for the determination of water-soluble iron, below). Evaporate 100 ml of this solution, representing 8 g of talc, in a tared platinum dish on a steam bath to dryness and ignite gently to constant weight. The weight of the residue does not exceed 16 mg.

Water-soluble iron

Using hydrochloric acid TS, dilute, slightly acidify the remaining part of the filtrate obtained in the test for water-soluble substances (above) and add 1 ml of potassium ferrocyanide TS. The solution does not turn blue.

Acid-soluble substances

Not more than 2.5 %
Accurately weigh 2 g sample into a beaker and add 35 ml of 3N hydrochloric acid. Digest the sample at 50o for 15 min. Cool, transfer the contents into a 50 ml volumetric flask, rinse the beaker with water, add rinsings to the volumetric flask and make up to volume. Mix the contents and filter. To 20 ml of the filtrate in a tared platinum dish, add 2 ml of sulfuric acid TS, dilute, evaporate to dryness and ignite to constant weight. The residue does not exceed 20 mg.

Asbestos

Free from asbestos as demonstrated by the test for amphiboles and serpentines
See description under TESTS

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4, “Instrumental Methods”.

TESTS

PURITY TESTS

Asbestos

The presence of amphiboles and of serpentines is revealed by infrared absorption or by X-ray diffraction (see A and B). If detected, the presence of asbestos is confirmed by optical microscopy.

A. An absorption band at 758 ± 1 cm^{-1} in the infrared spectrum of a potassium bromide dispersion of the material remaining after ignition of the substance at 850^{o} for at least 30 min, indicates the presence of tremolite, an amphibole. Absorption bands or shoulders between 600 and 650 cm^{-1} may indicate serpentines.
B. An X-ray powder diffraction pattern exhibiting diffraction peaks at 10.5 ± 0.1o 2θ indicates amphiboles. Peaks at 24.3 ± 0.1o 2θ and 12.1 ± 0.1o 2θ indicate serpentines.

If amphiboles or serpentines are indicated, examine the sample using optical microscopy to confirm the presence of asbestos. Asbestos is confirmed if the following criteria are met:

- a range of length to width ratios of 20:1 to 100:1, or higher for fibres longer than 5 m,
- capability of splitting into very thin fibrils,
- and if 2 or more of the following 4 criteria are met:
- parallel fibres occurring in bundles,
- fibre bundles displaying frayed ends,
- fibres in the form of thin needles,
- matted masses of individual fibres and/or fibres showing curvature

TARA GUM

Prepared at the 30th JECFA (1986), published in FNP 37 (1986) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). An ADI 'not specified' was established at the 30th JECFA (1986)

SYNONYMS Peruvian carob; INS No. 417

DEFINITION Obtained by grinding the endosperm of the seeds of *Caesalpinia spinosa* (Fam. *Leguminosae*); consists chiefly of polysaccharides of high molecular weight composed mainly of galactomannans. The principal component consists of a linear chain of (1,4)-beta-D-mannopyranose units with alpha-D-galacto- pyranose units attached by (1 6) linkages; the ratio of mannose to galactose in tara gum is 3:1. (In carob bean gum this ratio is 4:1 and in guar gum 2:1.) The article of commerce may be further specified as to viscosity and loss on drying.

DESCRIPTION White to white-yellow, nearly odourless powder

FUNCTIONAL USES Thickening agent, stabilizer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in water; insoluble in ethanol

Gel test To an aqueous solution of the sample add small amounts of sodium borate; a gel is formed

Viscosity Transfer 2 g of the sample into a 400-ml beaker and moisten it thoroughly with about 4 ml of isopropanol. Add, with vigorous stirring, 200 ml of water and continue stirring until the gum is completely and uniformly dispersed. An opalescent, moderately viscous solution is formed. (This solution is less viscous than a guar gum solution, but more viscous than a carob bean gum solution when prepared and tested as indicated in the above described test). Transfer 100 ml of this solution into another 400-ml beaker, heat the mixture in a boiling water-bath for about 10 min and cool to room temperature. The solution shows a marked increase in viscosity.

Gum constituents (Vol. 4) Proceed as directed under G*um Constituents Identification*, using galactose and mannose as standards. Galactose and mannose should be present

Microscopic examination Place some ground sample in an aqueous solution containing 0.5% iodine and 1% potassium iodide on a glass slide and examine under a microscope. Tara gum contains groups of round to pear-shaped cells; their contents are yellow to brown.

(Guar gum cells are similar in form but markedly larger in size. Carob bean gum shows long, stretched tubiform cells, separate or slightly interspaced and can be easily distinguished from tara gum.)

PURITY

Loss on drying (Vol. 4)	Not more than 15%
Ash (Vol. 4)	Not more than 1.5%
Acid insoluble matter (Vol. 4)	Not more than 2%
Protein	Not more than 3.5% Proceed as directed under *Nitrogen Determination (Kjeldahl method)* (see Volume 4). The percentage of nitrogen determined multiplied by 5.7 gives the percentage of protein in the sample.
Starch	Not detectable To a 1 in 10 solution of the sample, add a few drops of iodine TS. No blue colour is produced.
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

L(+)-TARTARIC ACID

Prepared at the 53rd JECFA (1999) and published in FNP 52 Add 7 (1999), superseding specifications prepared at the 21st JECFA (1977), published in NMRS 57 (1977) and in FNP 52 (1992). An ADI of 0-30 mg/kg bw was established at the 17th JECFA (1973) and reconfirmed at the 21st JECFA (1977)

SYNONYMS INS No. 334

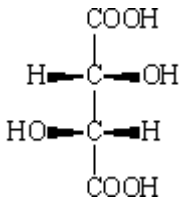
DEFINITION

Chemical names L-Tartaric acid, L-2,3-dihydroxybutanedioic acid, L-2,3-dihydroxysuccinic acid

C.A.S. number 87-69-4

Chemical formula $C_4H_6O_6$

Structural formula



Formula weight 150.09

Assay Not less than 99.5% on the dried basis

DESCRIPTION Colourless or translucent crystals, or white, fine to granular, crystalline powder; odourless

FUNCTIONAL USES Synergist for antioxidants, acid, sequestrant, flavouring agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Very soluble in water; freely soluble in ethanol

Specific rotation (Vol. 4 A 1 in 10 solution is dextrorotatory

Test for tartrate (Vol. 4) Passes test

PURITY

Loss on drying (Vol. 4) Not more than 0.5% (over P_2O_5, 3 h)

Specific rotation (Vol. 4) [alpha] 20, D: Between +11.5° and +13.5°

Sulfated ash (Vol. 4) Not more than 0.1%
Test 2 g of the sample (Method I)

Sulfates (Vol. 4)	Not more than 0.05% 0.4 g of the sample meets the requirements of the Limit Test using 0.2 mg of sulfate ion (SO_4) in the control
Oxalate	Nearly neutralize 10 ml of a 1 in 10 solution of the sample with ammonia TS, and add 10 ml of calcium sulfate TS. No turbidity is produced
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Weigh accurately about 2 g of the dried sample, dissolve in 40 ml of water, add phenolphthalein TS, and titrate with 1 N sodium hydroxide. Each ml of 1 N sodium hydroxide is equivalent to 75.04 mg of $C_4H_6O_6$.

SODIUM L(+)-TARTRATE

Prepared at the 7th JECFA (1963), published in NMRS 35 (1964) and in FNP 52 (1992). Metal and arsenic specifications revised at the 63rd JECFA (2004) (identical to those listed for potassium sodium L(+)-tartrate). An ADI of 0-30 mg/kg bw was established at the 17th JECFA (1973) and reconfirmed at the 21st JECFA (1977).

SYNONYMS Sodium dextro-tartrate; INS No. 335(ii)

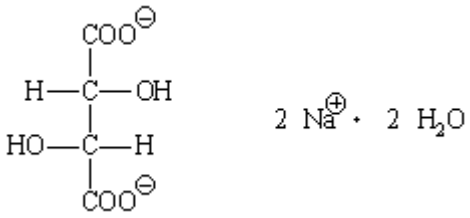
DEFINITION

Chemical names: Disodium L-tartrate, disodium (+)-tartrate, disodium (+)-2,3-dihydroxybutanedioic acid

C.A.S. number: 868-18-8

Chemical formula: $C_4H_4Na_2O_6 \cdot 2H_2O$

Structural formula:



Formula weight: 230.8

Assay: Not less than 99% after drying

DESCRIPTION Transparent, colourless and odourless crystals

FUNCTIONAL USES Sequestrant and stabilizer in meat products and sausage casings

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): One gram is soluble in 3 ml of water; insoluble to ethanol

Test for tartrate (Vol. 4): Passes test

Test for sodium (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4): Not more than 17% and not less than 14% (150°, 3 h)

pH (Vol. 4): 7.0 - 7.5 (1 in 10 solution)

Oxalate: Add 5 drops of dilute acetic acid TS and 2 ml of calcium chloride TS to 10 ml of a 10% solution of sodium tartrate. No turbidity is

produced within 1 h.

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

METHOD OF ASSAY

Weigh 1.500 g of the dried sample into a tared porcelain crucible, ignite gently at first, until the salt is thoroughly carbonized, protecting the carbonized salt from contact with the flame at all times. Cool the crucible, place in a glass beaker and break up the carbonized mass with a glass rod. Without removing the glass rod or the crucible, add 50 ml of water, 50 ml of 0.5 N sulfuric acid, cover the beaker, and boil the solution for 30 min. Filter, and wash with hot water until the last washing is neutral to litmus. Cool the combined filtrate and washings, add methyl orange TS, and titrate the excess acid with 0.5 N sodium hydroxide. Each ml of 0.5 N sulfuric acid is equivalent to 0.0485 g of $C_4H_4Na_2O_6$.

Alternative method of assay:

Weigh 0.4500 g of the dried sample and transfer to a 250-ml beaker. Add 100 ml of glacial acetic acid, and stir the solution (e.g. with a magnetic stirrer) until the sample is dissolved. Titrate the solution with 0.1 N perchloric acid in glacial acetic acid, adding the titrant in 0.2-ml increments as the end-point is neared, and determine the end-point by the potentiometric method. Each ml of 0.1 N perchloric acid is equivalent to 0.0097 g of $C_4H_4Na_2O_6$.

POTASSIUM SODIUM L(+)-TARTRATE

Prepared at the 7th JECFA (1963), published in NMRS 35 (1964) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). An ADI of 0-30 mg/kg bw was established at the 17th JECFA (1973)

SYNONYMS Rochelle salt, Seignette salt, Potassium sodium dextro-tartrate; INS No. 337

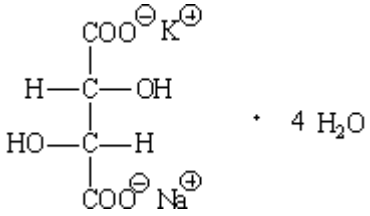
DEFINITION

Chemical names: Potassium sodium L-tartrate, potassium sodium (+)-tartrate, potassium sodium (+)-2,3-dihydroxybutanedioic acid

C.A.S. number: 304-59-6

Chemical formula: $C_4H_4KNaO_6 \cdot 4H_2O$

Structural formula:



Formula weight: 282.23

Assay: Not less than 99% after drying

DESCRIPTION Colourless crystals, or as a white, crystalline powder

FUNCTIONAL USES Sequestrant, stabilizer in cheese products, minced meat, and sausage casings

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): One gram is soluble in 1 ml of water; insoluble to ethanol

Test for tartrate (Vol. 4): Passes test

Test for sodium (Vol. 4): Passes test

Test for potassium (Vol. 4): Passes test

PURITY

Loss on drying (Vol. 4): Not more than 26.0% and not less than 21.0% (150°, 3 h)

pH (Vol. 4): 6.5 - 7.5 (1 in 10 soln)

Oxalate

Add 3 drops of dilute acetic acid TS and 2 ml of calcium chloride TS to 10 ml of a 10% solution of potassium sodium tartrate. No turbidity is produced within 1 h.

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh 1.500 g of the dried sample into a tared porcelain crucible and ignite. Heat gently at first, until the salt is thoroughly carbonized, protecting the carbonized salt from contact with the flame at all times. The final temperature must not be above that of a dull read heat. Cool the crucible, place in a glass beaker, and break up the carbonized mass with a glass rod. Without removing the glass rod or the crucible, add 50 ml of water, 50 ml of 0.5 N sulfuric acid, cover the beaker, and boil the solution for 30 min. Filter, and wash with hot water until the last washing is neutral to litmus. Cool the combined filtrate and washings, add methyl orange TS, and titrate the excess acid with 0.5 N sodium hydroxide. Each ml of 0.5 N sulfuric acid is equivalent to 0.05254 g of $C_4H_4KNaO_6$.

TERTIARY BUTYLHYDROQUINONE

Prepared at the 49th JECFA (1997) , published in FNP 52 Add 5 (1997) superseding specifications prepared at the 44th JECFA (1995), published in FNP 52 Add 3 (1995). Metals and arsenic specifications revised at the 61st JECFA (2003). An ADI of 0-0.7 mg/kg bw was established at the 49th JECFA (1997).

SYNONYMS TBHQ, INS No 319

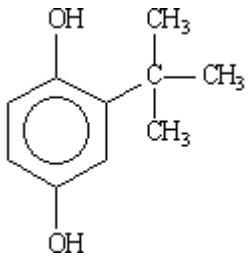
DEFINITION

Chemical names: Mono-tert-butylhydroquinone, *t*-butylhydroquinone, 2-(1,1-dimethylethyl)-1,4-benzenediol

C.A.S. number: 1948-33-0

Chemical formula: $C_{10}H_{14}O_2$

Structural formula:



Formula weight: 166.22

Assay: Not less than 99.0% of $C_{10}H_{14}O_2$

DESCRIPTION White, crystalline solid having a characteristic odour.

FUNCTIONAL USES Antioxidant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Practically insoluble in water; soluble in ethanol

Melting point (Vol. 4): Not less than 126.5°

Phenolics: Dissolve about 5 mg of the sample in 10 ml of methanol, and add 10.5 ml of dimethylamine solution (1 in 4). A red to pink colour is produced.

PURITY

t-Butyl-p-benzoquinone: Not more than 0.2%
See description under TESTS

2,5-Di-t-butyl hydroquinone — Not more than 0.2%
See description under TESTS

Hydroxyquinone — Not more than 0.1%
See description under TESTS

Toluene — Not more than 25 mg/kg
See description under TESTS

Lead (Vol. 4) — Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

t-Butyl-p-benzoquinone

Apparatus:
Use a suitable double-beam infrared spectrophotometer and matched 0.4 mm liquid sample cells with calcium fluoride windows.

Reagents and Solutions:
Standard preparation: Transfer about 10 mg of mono-tertiary-butyl-p-benzoquinone Reference Standard (available from US Pharmacopeial Convention, Inc., 12601 Twinbrook Parkway, Rockville, MD 20852, USA), accurately weighed, into a 10-ml volumetric flask, dissolve in chloroform, dilute to volume with the same solvent and mix.
Sample preparation: Transfer about 1 g of the sample, previously ground to a fine powder in a high-speed blender and accurately weighed, into a 10-ml volumetric flask, dissolve in chloroform, dilute to volume with the same solvent, and mix. Filter through a Millipore filter (UHWPO1300), or equivalent, before use in the Procedure below.

Procedure:
Fill the reference cell with chloroform and the sample cell with the Standard preparation. Place the cells in the respective reference and sample beam of the spectrophotometer, and record the infrared spectrum from 1600 to 1775 cm^{-1}. On the spectrum draw a background line from 1612 to 1750 cm^{-1}, and determine the net absorbence (A_S) of the Standard preparation at 1659 cm^{-1}.
Similarly, obtain the spectrum of the sample preparation, and determine its net absorbence (A_U) at 1659 cm^{-1}.

Calculation:
Calculate the percent of t-butyl-p-benzoquinone in the sample by the formula:

$$100 \times A_U/A_S \times W_S/W_U$$

where
W_S = the exact weight, in mg, of the mono-tertiary-butyl-p-benzoquinone Reference Standard taken
W_U = the exact weight, in mg, of the sample taken.

2,5-Di-t-butylhydroquinone and hydroquinone

Apparatus:
Use a suitable gas chromatograph equipped with a thermal conductivity detector (F and M Model 810 or equivalent), containing a 0.61-m (2 ft) × 6.35-mm (outside diameter) stainless steel column packed with 20% Silicone SE-30, by weight and 80% Diatoport S (60/80-mesh), or equivalent materials.

Operating conditions:
The operating parameters may vary, depending upon the particular instrument used, but a suitable chromatogram may be obtained using the following conditions:
- Column temperature: programmed from 100 to 270°, at 15° per min
- Injection port temperature: 300°
- Carrier gas: helium, flowing at a rate of 100 ml per min
- Bridge current: 140 mA
- Sensitivity: 1 × for integrator (Infotronics CRS 100), 2 × for recorder

Reagents and Solutions:
Stock solution: Weigh accurately about 50 mg each of hydroquinone (HQ), 2,5-di-t-butylhydroquinone (DTBHQ), and methyl benzoate (internal standard), transfer into separate 50-ml volumetric flasks, dilute to volume with pyridine, and mix.
Calibration standards: Into separate 10-ml volumetric flasks add 0.50, 1.0, 2.0 and 3.0 ml of the HQ stock solution, then to each flask add 2 ml of the methyl benzoate (internal standard) stock solution, dilute each to volume with pyridine, and mix. In the same manner prepare four DTBHQ calibrating solutions. Prepare the trimethylsilyl derivative of each solution as follows. Add 9 drops of calibration solution to a 2-ml gas syringe, add 250 µl of N,O-bistrimethylsilylacetamide, and heat at about 80° for 10 min. Chromatograph 10-µl portions of each standard in duplicate, and plot the concentration ratio of HQ to internal standard (X-axis) against the response ratio of HQ to internal standard (Y-axis). Plot the same relationships between DTBHQ and the internal standard.

Procedure:
Transfer about 1 g of the sample, accurately weighed, into a 10-ml volumetric flask, add 2 ml of the methyl benzoate internal standard stock solution, dilute to volume with pyridine, and mix. Prepare the trimethylsilyl derivative as described above under Calibration standards, and then chromatograph duplicate 10-µl portions to obtain the chromatogram. The approximate peak times, in minutes, are: methyl benzoate, 2.5; TMS derivative of HQ, 5.5; TMS derivative of tert-butylhydroquinone, 7.3; TMS derivative of DTBHQ, 8.4.

Calculation:
Determine the peak areas (response) of interest by automatic integration or manual triangulation. Calculate the response ratio of HQ and DTBHQ

to internal standard. From the calibration curves determine the concentration ratio of HQ and DTBHQ to internal standard, and calculate the % HQ and % DTBHQ in the sample by the formula:

$A = Y \times I \times 10/S$

where
A = the % HQ or % DTBHQ in the sample
Y = the concentration ratio (X-axis on calibration curve)
I = the percentage (w/v) of internal standard in the Sample preparation
S = the weight of sample taken, in g.

Toluene

Apparatus:
Use a suitable gas chromatograph equipped with a flame ionization detector (F and M Model 810 or equivalent), containing a 3.66-m (12-ft) × 3.18-mm (outside diameter) stainless steel column packed with 10% Silicone SE-30, by weight, and 90% Diatoport S (60/80 mesh), or equivalent materials.

Operating conditions:
The operating parameters may vary, depending upon the particular instrument used, but a suitable chromatogram may be obtained using the following conditions:
- Column temperature: programmed from 70 to 280° at 15° per minute and held
- Injection port temperature: 275°
- Cell temperature: 300°
- H_2 and O_2 (or air) settings: 1.4 atm (20 psi) each

Reagents and solutions:
Standard solution: Prepare a solution of toluene in octanol containing approximately 50 µg per ml, and calculate the exact concentration (C_R) in percent (w/v).
Sample solution: Transfer about 2 g of the sample, accurately weighed, into a 10-ml volumetric flask, dissolve in octanol, dilute to volume with the same solvent, and mix. Calculate the exact concentration of the solution (C_S) in percent (w/v).

Procedure:
Inject a 5-µl portion of the Standard solution into the chromatograph, and measure the height of the toluene peak (H_R) on the chromatogram. The toluene retention time is 3.3 min; other peaks are of no interest in this analysis. Similarly, obtain the chromatogram on a 5-µl portion of the Sample solution and of a blank consisting of octanol, and measure the height of the toluene peak (H_S).

Calculation:
Calculate the mg/kg of toluene in the sample by the formula:

$H_S / C_R \times C_R/C_S \times 10^6$

METHOD OF Transfer about 170 mg of the sample, previously ground to a fine powder

ASSAY

and accurately weighed, into a 250-ml wide-mouth conical flask, and dissolve in 10 ml of methanol. Add 150 ml of water, 1 ml of N sulfuric acid, and 4 drops of diphenylamine indicator (3 mg of p-diphenylaminesulfonic acid, sodium salt, per ml of 0.1 N sulfuric acid), and titrate with 0.1 N ceric sulfate to the first complete colour change from yellow to red-violet. Record the volume, in ml, of 0.1 N ceric sulfate required as V.

Calculate the percent of $C_{10}H_{14}O_2$ in the sample, uncorrected for hydroquinone (HQ) and 2,5-di-tert-butylhydro- quinone (DTBHQ), by the formula:

$$(V - 0.1\ ml) \times N \times 8.311/W$$

where
0.1 ml = the volume of ceric sulfate consumed by the primary oxidation products of tert-butylhydroquinone ordinarily present in the sample
N = the normality of the standard ceric sulfate solution
W = the weight of the sample taken, in g.

Record the uncorrected percentage thus calculated as A. If HQ and DTBHQ are present in the sample, they will be included in the titration.

Calculate the corrected percentage of $C_{10}H_{14}O_2$ in the sample by the formula:

$$A - (\%HQ \times 1.51) - (\%DTBHQ \times 0.75)$$

using the respective values for % HQ and % DTBHQ as determined by the gas chromatographic procedures given above.

THAUMATIN

Prepared at the 53rd JECFA (1999) and published in FNP 52 Add 7 (1999), superseding tentative specifications prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998). ADI "not specified", established at the 29th JECFA in 1985.

SYNONYMS INS No. 957

DEFINITION Obtained by aqueous extraction (pH 2.5-4.0) of the arils of the fruit of *Thaumatococcus daniellii* (Benth); consists essentially of the proteins Thaumatin I and Thaumatin II together with minor amounts of plant constituents derived from the source material.

C.A.S. number 53850-34-3

Formula weight Thaumatin I: 22,209
Thaumatin II: 22,293

Assay Not less than 15.1% nitrogen on the dried basis equivalent to not less than 93% protein (N x 6.2)

DESCRIPTION Odourless, cream-coloured powder

FUNCTIONAL USES Sweetener, flavour enhancer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Very soluble in water; insoluble in acetone

Ninhydrin test To 5 ml of a 1 in 1000 ml solution of the sample add 1 ml of freshly prepared triketohydrine hydrate (ninhydrin) solution (dissolve 200mg of triketohydrine hydrate in water and dilute to 100 ml). A bluish colour is produced.

Infrared absorption The infrared spectrum of a potassium bromide dispersion of the sample (1-2 mg of sample ground in a mortar with 100-200 mg potassium bromide) corresponds to the infrared spectrum below. Characteristic maxima of absorption are shown at the following wavenumbers: 3300, 2960, 1650, 1529, 1452, 1395, 1237, 1103 and 612 cm^{-1}

PURITY

Loss on drying (Vol. 4) Not more than 9.0% (105° to constant weight)

Spectrophotometry (Vol. 4) The specific absorption, $A^{1\%}_{1\ cm}$ at the wavelength of maximum absorption (about 279 nm) shall be not less than 11.5 and not more than 13.0 determined on the dried basis and using a 1 in 100 w/v solution of the sample in water at pH 2.7.

Sulfated ash (Vol. 4) Not more than 2.0% on the dried basis

Carbohydrates	Not more than 3.0% on the dried basis See description under TESTS
Microbiological criteria (Vol. 4)	Total aerobic plate count: Not more than 1000 cfu/g *E. coli*: Negative in 1 g
Aluminium	Not more than 100 mg/kg Determine by atomic absorption spectroscopy (Vol. 4)
Lead (Vol. 4)	Not more than 3 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Carbohydrates

Reagent:
cysteine-sulfuric acid
Mix immediately before use 0.5 ml of 3% w/v aqueous solution of L-cysteine hydrochloride monohydrate and 25 ml of 86% v/v sulfuric acid. Cool in ice. Do not store for reuse.

Procedure
Dissolve 0.2 g of sample, accurately weighed, in water and make up to 100 ml. Place a 0.2-ml portion in a very clean dust-free glass tube and cool in an ice-bath. Add 1.2 ml of ice-cold cysteine-sulfuric reagent, cover with a glass ball, and mix thoroughly. After 2 min in ice, remove to room temperature for 3 min, then plunge into a boiling water bath for 3 min. Immediately cool in ice for 5 min, before reading the absorbance in a 1-cm cell at 412 nm.

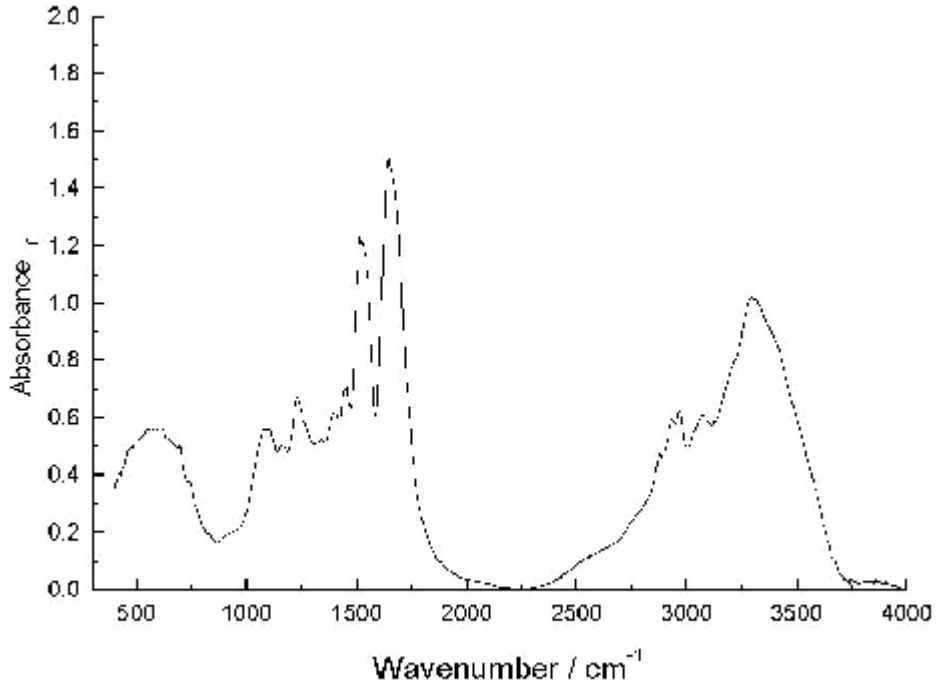
Standard curve
Prepare standard glucose solutions ranging in concentration from 10 -100 µg/ml and construct a standard curve from the absorbance of these solutions following treatment of 0.2 ml samples according to the above procedure.

Determine the carbohydrate concentration (as glucose) in the test sample by reference to the standard curve.

METHOD OF ASSAY

Proceed as directed under Nitrogen Determination (Kjeldahl Method; Volume 4), Method II.

Infrared spectrum — Thaumatin


Absorbance
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
500
1000
1500
2000
2500
3000
3500
4000
Wavenumber / cm-1

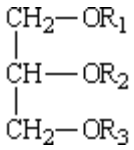
THERMALLY OXIDIZED SOYA BEAN OIL interacted with MONO- and DIGLYCERIDES of FATTY ACIDS

Prepared at the 39th JECFA (1992), published in FNP 52 Add 1 (1992). Metals and arsenic specifications revised at the 55th JECFA (2000) An ADI of 0-30 mg/kg bw was established at the 39th JECFA (1992)

SYNONYMS INS no. 479, TOSOM

DEFINITION A complex mixture of esters of glycerol and fatty acids found in edible fat and fatty acids from thermally oxidized soya bean oil; produced by interaction and desodourization under vacuum at 130° of 10 % w/w of thermally oxidized soya bean oil (thermally oxidized soya bean oil is obtained by oxidation of refined soya bean oil with air at 190 - 200°) and 90 % w/w of mono- and diglycerides of food fatty acids.

Structural formula (principal component)



where R_1, R_2 and R_3 variously may be a:
- normal fatty acid
- oxidized fatty acid (e.g. hydroxyl and/or carbonyl compound of fatty acid)
- hydrogen
- short chain fatty acid
- di- and polymer of oxidized fatty acids

The product may contain small quantities of free fatty acids and free glycerol.

DESCRIPTION Pale yellow to light brown with a waxy or solid consistency.

FUNCTIONAL USES Emulsifier, antispattering agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water; soluble in hot fats and oils

PURITY

Melting range (Vol. 4) 55 ° - 65°

Free fatty acids (Vol. 4) Not more than 1.5 % w/w calculated as oleic acid
Proceed as directed under *Free Fatty Acids* using the equivalence factor e = 28.2.

Free glycerol (Vol. 4) Not more than 2 % w/w

Total fatty acids 83 - 90 % w/w

	See description under TESTS
Total glycerol	16-22% w/w See description under TESTS
Fatty acids, insoluble in petroleum ether	Not more than 2 % w/w of total fatty acids See description under TESTS
Fatty acid methyl esters, not forming adduct with urea	Not more than 9.0 % w/w of total fatty acid methyl esters See description under TESTS
Peroxide value	Not more than 3 See description under TESTS
Epoxides	Not more than 0.03 % w/w oxiran oxygen See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Total fatty acids

Weigh accurately about 12 g of the sample into a 500-ml conical flask, add 100 ml of 2 N alcoholic potassium hydroxide solution and reflux for 1 hour on a sand bath. It is necessary to swirl the flask every 5 to 10 min during the reflux period.

Transfer quantitatively the hot content of the saponification flask to a 500-ml separating funnel, using 100 ml of water and set aside to cool. Extract the aqueous solution by vigorously shaking for 1 min with 3 100-ml portions of petroleum ether (40-60^{o}). Transfer the aqueous solution to a clean 1000-ml round bottomed flask and discard the organic extracts. Reduce the volume of the aqueous solution by evaporation till about 200 ml, using a rotatory evaporator under vacuum at 70^{o} (the smell of ethanol has disappeared). Add cautiously 75 ml of 4 N hydrochloric acid and shake vigorously. Transfer the content of the flask to a 500-ml separating funnel, using 2 25-ml portions of water. Set aside to cool to 30-35^{o} and then add 2 50-ml portions of ether. When the mixture has cooled to room temperature, shake vigorously for about 1 min. Set aside for separation of layers.

Transfer the aqueous layer to a 500 ml separation funnel and extract with further 2 100-ml portions of ether. Transfer the aqueous solution to the 1000 ml round bottomed flask and combine all 3 ether extracts in a separating funnel. Wash the combined extracts with 3 100-ml portions of water and combine the washings with the aqueous solution in the round bottomed flask. This solution is used for the test for Total glycerol. Transfer the ether fraction to a dry and previously tared 500-ml round

bottomed flask. Evaporate to dryness using a rotatory evaporator at vacuum and slowly increasing the temperature from 40° to 70°. Add 100 ml of acetone and evaporate to dryness. Empty the receiver and continue to evaporate at full vacuum at 100° for further 20 min.
Place the flask in an oven at 110±5° for 1 hour. Cool in a desiccator and weigh the flask, now containing the isolated fatty acids.

Calculation:

$$Total\ fatty\ acids\ (\%) = \frac{(B - A) \times 100}{W}$$

where
A = weight of empty flask (g)
B = weight of flask and fatty acids (g)
W = weight of sample (g)

Total glycerol

Use the aqueous solution obtained by the test for Total fatty acids. Transfer quantitatively the aqueous solution to a 1000-ml volumetric flask. Dilute to mark with water. Pipet 50 ml of periodic acid into a 400 ml beaker and then 15 ml of the sample solution. Shake gentle to affect thorough mixing. Cover with a watch glass and allow to stand for 30 min. Add 20 ml of potassium iodide solution (150 g/l), mix by gentle shaking and allow to stand 1 min (never more than 5 min) protected from light. Add water to approximately 200 ml and titrate with 0.1 N sodium thiosulfate TS solution using Starch solution TS as indicator. Carry out a blank using 15 ml of water instead of sample solution.

Calculation:

$$Total\ glycerol\ (\%) = \frac{(B - S) \times 2.302 \times 1000}{W \times 15}$$

where,
B = ml of sodium thiosulfate used for the blank
S = ml of sodium thiosulfate used for the sample
N = normality of sodium thiosulfate
W = weight of sample (g)

Fatty acids, insoluble in petroleum ether

Weigh accurately about 5 g of the isolated fatty acids, obtained by the test for Total fatty acids, into a 250-ml round bottomed flask (flask I). Add 100 ml of petroleum ether (40-60°) and reflux for 30 min. at 55° on a water bath. Cool, close the flask with a glass stopper and leave over-night.

Heat the flask under reflux to 55° and decant and discard the organic solution. Wash the flask and its content with 2 25-ml portions and 1 10-ml portion of petroleum ether. Discard the washings.
Add 30 ml of 96 % v/v solution of ethanol to flask I and dissolve the content at low heat. Filter the solution into a dry and previously weighed 100-ml round bottomed flask (flask II). Wash flask I and the filter thoroughly with 3 10 ml-portions of 96 % v/v solution of ethanol.

Evaporate the content of flask II to dryness using a rotatory evaporator under vacuum at 70°. Add 50 ml of petroleum ether. Heat to 55° at a water bath under reflux for 30 min. Cool and decant and discard the petroleum ether solution. Wash the content of flask II with further 2 25-ml portions of petroleum ether. Discard the washings.

Evaporate the content of flask II to dryness using a rotatory evaporator under vacuum at 70° at a water bath. Continue to evaporate for further 15 min at full vacuum. Leave the flask in an oven at 105±5° for 1 h. Cool in an desiccator and weigh the flask.

Calculate the Fatty acids, not soluble in petroleum ether (% w/w of total fatty acids, from:

$$\frac{(B - A) \times 100}{W}$$

where
A = weight of empty flask II (g)
B = weight of flask II with content (g)
W = weight of sample of fatty acids (g)

Fatty acid methyl esters, not forming adduct with urea

Weigh accurately about 5 g of the isolated fatty acids, obtained by the test for Total fatty acids, into a dry previously weighed 250-ml round bottomed flask. Add 10.0 ml of methanol, 1.0 ml of conc. hydrochloric acid and 25 ml of dimethoxypropane (mix after each addition). Close the flask using a glass stopper, swirl if necessary to dissolve and leave for reaction at room temperature for 1 hour.

Add 50 ml of toluene and evaporate to dryness under vacuum at 60° at water bath using a rotatory evaporator. Dissolve the residue in 50 ml of petroleum ether and evaporate to dryness under the same conditions as before. Continue to evaporate for further 15 min under full vacuum at 100°.

Place the flask now containing the fatty acid methyl esters in an oven at 105±5° for 1 hour. Cool in a desiccator.

Introduce in small portions 250 g of urea into a 30 x 2 cm glass column with a fritted glass disk at bottom tapping the column to assure optimal packing. Connect a separatory funnel to the top of the column through a stopper. Add to the separator y funnel, 150 ml of methanol, previously saturated with urea at room temperature. Introduce the methanol through the stopcock of the separatory funnel at a flow rate of approximately 10 ml/min.

Weigh accurately about 5 g of the fatty acid methyl esters into a 250-ml conical flask and dissolve in 100 ml of methanol. Transfer quantitatively the solution to the separatory funnel using 2 25-ml portions of methanol, previously saturated with urea at room temperature. Elute the solution through the stopcock of the separatory funnel at a flow rate of approximately 10 ml/min. Collect the eluate in a 500-ml roundbottomed

flask. Add to the separatory funnel when empty, 200 ml of methanol, previously saturated with urea at room temperature and continue elution until the flow from the column stops.

Evaporate the eluate, using a rotatory evaporator under vacuum at 60°, until crystals accurately appear in the liquid. Add 200 ml of water to the flask and diluted hydrochloric acid till pH less than 3.

Transfer quantitatively the solution to a 1000-ml separatory funnel using 2 25-ml portions of water and 1 50-ml portion of ether. Shake vigorously and set aside to separate. Repeat the extraction with 3 50-ml portions of ether further, collecting the ether fractions in a 500-ml separatory funnel. Discard the water fraction. Wash the combined ether fractions with 2 50-ml portions of water. Discard the washings.

Transfer quantitatively the ether solution to a dry previously weighed 500-ml round bottomed flask using a small quantity of acetone. Evaporate to dryness using a rotatory evaporator under vacuum at 40-50°. Add 50 ml of acetone and dissolve the residue. Evaporate to dryness under the same conditions. Add further 50 ml of acetone, dissolve and evaporate to dryness. Continue evaporation under full vacuum at 100° for 45 min. Place the flask in an oven at 105±5° for 1 hour, cool in a desiccator and weigh the flask with content.

Calculate Fatty acid esters not forming adduct with urea (% of total fatty acid esters) from

$$\frac{(B - A) \times 100}{W}$$

where,
A = weight of empty flask (g)
B = weight of flask with content (g)
W = weight of fatty acid esters (g)

Peroxide value

Weigh accurately about 5 g of the sample into a 200-ml conical flask. Add 30 ml of a 2:3 solution of chloroform and acetic acid TS and close the flask with a stopper. Heat with warm water and swirl to dissolve the sample. Cool to room temperature and add 0,5 ml of saturated potassium iodide solution. Close the flask with the stopper and shake vigorously for 60±5 sec.

Add 30 ml of acetic acid TS and titrate immediately with 0.01 N Sodium thiosulfate using Starch TS as indicator.

Carry out a blank determination without sample.
Calculation:

$$Peroxide\ value = \frac{(a - b) \times N \times 1000}{W}$$

where

a = amount of sodium thiosulfate used for the sample (ml)
b = amount of sodium thiosulfate used for the blank (ml)
N = normality of the sodium thiosulfate
W = weight of sample (g)

Epoxides

Accurately weigh about 3 g of the sample into 250-ml round bottomed flask, add 10 ml of monochlorobenzene and dissolve the sample. Dilute with 40 ml of 2-propanol, add 10 ml of 0.1 N 2,4,6-trimethylpyridin hydrochloride solution and reflux for 1 hour at a warm sand bath. Let cool to room temperature and add 25 ml of water. Measure the temperature of the solution and determine the excess of 2,4,6-trimethylpyridin hydrochloride by potentiometric titration with 0.1 N sodium methylate solution. Carry out a blank without sample.

Calculation:

$$\%\ of\ oxiran\ oxygen = \frac{(a - b) \times N \times 16}{10 \times W}$$

where
a = amount of sodium methanolate solution used for the sample (ml)
b = amount of sodium methanolate solution used for the blank (ml)
N = normality of sodium methanolate solution
W = weight of sample (g)

THIODIPROPIONIC ACID

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003). An ADI of 0-3 mg/kg bw was established at the 17th JECFA (1973)

SYNONYMS INS No. 388

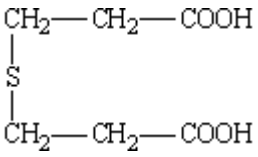
DEFINITION

Chemical names 3,3'-Thiodipropionic acid, diethyl sulfide 2,2'-dicarboxylic acid, thiodihydracrylic acid, ß,ß'-thiodipropionic acid

C.A.S. number 111-17-1

Chemical formula $C_6H_{10}O_4S$

Structural formula



Formula weight 178.21

Assay Not less than 98.5%

DESCRIPTION White crystalline solid having a slight characteristic odour

FUNCTIONAL USES Antioxidant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in water; freely soluble in ethanol

Melting range (Vol. 4) 130 - 134°

Sulfur Between 17.5% and 18.5%
See description under TESTS

PURITY

Sulfated ash (Vol. 4) Not more than 0.2%

Selenium (Vol. 4) Not more than 30 mg/kg

Lead (Vol. 4) Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

IDENTIFICATION TESTS

Sulfur

Weigh 0.700 g of the sample and add 100 ml of acetic acid and 50 ml of ethanol and heat the mixture gently until the sample dissolves completely. Add 3 ml of hydrochloric acid and add 4 drops of p-ethoxychrysoidin TS and immediately titrate with 0.1 N bromide-bromate TS. As the end point is approached (pink colour) add 4 more drops of the indicator solution and continue the titration dropwise, to a colour change from red to pale yellow. Perform a blank determination and make any necessary correction. Each ml of 0.1 N bromide-bromine TS is equivalent to 1.603 mg of S.

METHOD OF ASSAY

Dissolve 0.350 g of the sample in 40 ml of water, add phenolphthalein TS and titrate with 0.1 N sodium hydroxide to the first appearance of a faint pink colour that persists for at least 30 sec. Each ml of 0.1 N sodium hydroxide is equivalent to 8.910 mg of $C_6H_{10}O_4S$.

DILAURYL THIODIPROPIONATE

Prepared at the 17th JECFA (1973), published in FNP 4 (1978) and FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003). An ADI of 0-3 mg/kg bw was established at the 17thJECFA (1973)

SYNONYMS INS No. 389

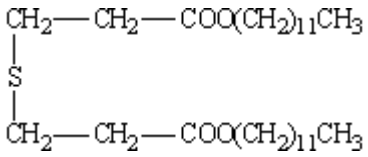
DEFINITION Ester of thiodipropionic acid and a food grade lauryl alcohol

Chemical names Didodecyl 3,3'-thiodipropionic acid, dilauryl ester of ß,ß'-thiodi-propionic acid

C.A.S. number 123-28-4

Chemical formula $C_{30}H_{58}O_4S$

Structural formula



Formula weight 514.86

Assay Not less than 99%

DESCRIPTION White crystalline flakes having a characteristic sweetish ester-like odour

FUNCTIONAL USES Antioxidant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water, soluble in ethanol and ether

Solidification point (Vol. 4) Not below 40°

Saponification value (Vol. 4) 205 - 215

PURITY

Acidity Not more than 0.2% (as thiopropionic acid)
To 50 ml of a mixture of 1 part of methanol and 3 parts of benzene, add 5 drops of phenolphthalein TS and neutralize with ethanolic potassium hydroxide. Add 2 g, accurately weighed, of the sample, swirl to dissolve and titrate with 0.1 N ethanolic potassium hydroxide. Each ml of 0.1 N ethanolic potassium hydroxide is equivalent to 8.91 mg $C_6H_{10}O_4S$.

Lead (Vol. 4) Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the

specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Weigh out 0.700 g of the sample, transfer to a 250-ml Erlenmeyer flask, add 100 ml of acetic acid and 50 ml of ethanol, and heat the mixture gently until the sample dissolves completely. Add 3 ml of hydrochloric acid and 4 drops of p-ethoxy-chrysoidin TS and immediately titrate with 0.1 N bromide-bromate TS. As the end-point is approached (pink colour), add 4 more drops of the indicator solution and continue the titration, dropwise, to a colour change from red to pale yellow. Perform a blank determination and make any necessary correction. Each ml of 0.1 N bromide-bromate TS is equivalent to 25.74 mg of $C_{30}H_{58}O_4S$. Convert to percentage and subtract thiodipropionic acid content determined in the Acidity test to obtain percentage of $C_{30}H_{58}O_4S$.

TITANIUM DIOXIDE

Prepared at the 73rd JECFA (2010) and published in FAO JECFA Monographs 10 (2010), superseding specifications prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs 7 (2009). An ADI "not limited" was established at the 13th JECFA (1969).

SYNONYMS Titania; CI Pigment white 6; CI (1975) No. 77891; INS No. 171

DEFINITION Titanium dioxide is produced by either the sulfate or the chloride process. Processing conditions determine the form (anatase or rutile structure) of the final product.

In the sulfate process, sulfuric acid is used to digest ilmenite ($FeTiO_3$) or ilmenite and titanium slag. After a series of purification steps, the isolated titanium dioxide is finally washed with water, calcined, and micronized.

In the chloride process, chlorine gas is reacted with a titanium-containing mineral under reducing conditions to form anhydrous titanium tetrachloride, which is subsequently purified and converted to titanium dioxide either by direct thermal oxidation or by reaction with steam in the vapour phase. Alternatively, concentrated hydrochloric acid can be reacted with the titanium-containing mineral to form a solution of titanium tetrachloride, which is then further purified and converted to titanium dioxide by hydrolysis. The titanium dioxide is filtered, washed, and calcined.

Commercial titanium dioxide may be coated with small amounts of alumina and/or silica to improve the technological properties of the product.

C.A.S. number 13463-67-7

Chemical formula TiO_2

Formula weight 79.88

Assay Not less than 99.0% on the dried basis (on an aluminium oxide and silicon dioxide-free basis)

DESCRIPTION White to slightly coloured powder

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water, hydrochloric acid, dilute sulfuric acid, and organic solvents. Dissolves slowly in hydrofluoric acid and hot concentrated sulfuric acid.

Colour reaction Add 5 ml sulfuric acid to 0.5 g of the sample, heat gently until fumes of sulfuric acid appear, then cool. Cautiously dilute to about 100 ml with water and filter. To 5 ml of this clear filtrate, add a few drops of

hydrogen peroxide; an orange-red colour appears immediately.

PURITY

Loss on drying (Vol. 4)	Not more than 0.5% (105°, 3 h)
Loss on ignition (Vol. 4)	Not more than 1.0% (800°) on the dried basis
Aluminium oxide and/or silicon dioxide	Not more than 2%, either singly or combined See descriptions under TESTS
Acid-soluble substances	Not more than 0.5%; Not more than 1.5% for products containing alumina or silica. Suspend 5 g of the sample in 100 ml 0.5 N hydrochloric acid and place on a steam bath for 30 min with occasional stirring. Filter through a Gooch crucible fitted with a glass fibre filter paper. Wash with three 10-ml portions of 0.5 N hydrochloric acid, evaporate the combined filtrate and washings to dryness, and ignite at a dull red heat to constant weight.
Water-soluble matter (Vol. 4)	Not more than 0.5% Proceed as directed under acid-soluble substances (above), using water in place of 0.5 N hydrochloric acid.
Impurities soluble in 0.5 N hydrochloric acid	
Antimony	Not more than 2 mg/kg See description under TESTS
Arsenic	Not more than 1 mg/kg See description under TESTS
Cadmium	Not more than 1 mg/kg See description under TESTS
Lead	Not more than 10 mg/kg See description under TESTS
Mercury (Vol. 4)	Not more than 1 mg/kg Determine using the cold vapour atomic absorption technique. Select a sample size appropriate to the specified level

TESTS

PURITY TESTS

Impurities soluble in 0.5 N hydrochloric acid	
Antimony, arsenic, cadmium and lead (Vol.4)	Transfer 10.0 g of sample into a 250-ml beaker, add 50 ml of 0.5 *N* hydrochloric acid, cover with a watch glass, and heat to boiling on a hot plate. Boil gently for 15 min, pour the slurry into a 100- to 150-ml centrifuge bottle, and centrifuge for 10 to 15 min, or until undissolved material settles. Decant the supernatant through Whatman No. 4 filter paper, or equivalent, collecting the filtrate in a 100-ml volumetric flask and retaining as much as possible of the undissolved material in the centrifuge bottle. Add 10 ml of hot water to the original beaker,

washing off the watch glass with the water, and pour the contents into the centrifuge bottle. Form a slurry, using a glass stirring rod, and centrifuge. Decant through the same filter paper, and collect the washings in the volumetric flask containing the initial extract. Repeat the entire washing process two more times. Finally, wash the filter paper with 10 to 15 ml of hot water. Cool the contents of the flask to room temperature, dilute to volume with water, and mix.

Determine antimony, cadmium, and lead using an AAS/ICP-AES technique appropriate to the specified level. Determine arsenic using atomic absorption hydride technique.

Aluminium oxide

Reagents and sample solutions

Ammonium acetate buffer solution
In a 1000-ml volumetric flask, dissolve 77 g of ammonium acetate in about 500 ml of water, add 10 ml of glacial acetic acid and dilute to volume with water.

Diammonium hydrogen phosphate solution
In a 1000-ml volumetric flask, dissolve 150 g of diammonium hydrogen phosphate in about 700 ml of water, adjust pH to 5.5 using a 1 in 2 solution of hydrochloric acid, then dilute to volume with water.

Zinc Sulfate solution (0.01 N)
Dissolve 2.9 g of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) in sufficient water and make up to 1000 ml in a volumetric flask. Standardize the solution as follows: Dissolve 500 mg of high-purity (99.9%) aluminium wire, accurately weighed, in 20 ml of concentrated hydrochloric acid, heating gently to effect solution, then transfer the solution into a 1000-ml volumetric flask, dilute to volume with water, and mix. Transfer a 10 ml aliquot of this solution into a 500 ml Erlenmeyer flask containing 90 ml of water and 3 ml of concentrated hydrochloric acid, add 1 drop of methyl orange TS and 25 ml of 0.02 M disodium ethylenediaminetetraacetate (EDTA) Add, dropwise, ammonia solution (1 in 5) until the colour is just completely changed from red to orange-yellow. Then, add 10 ml of ammonium acetate buffer solution and 10 ml of diammonium hydrogen phosphate solution. Boil the solution for 5 min, cool it quickly to room temperature in a stream of running water, add 3 drops of xylenol orange TS, and mix.

Using zinc sulfate solution as titrant, titrate the solution to the first yellow-brown or pink end-point colour that persists for 5-10 sec. (NOTE: This titration should be performed quickly near the end-point by adding rapidly 0.2 ml increments of the titrant until the first colour change occurs; although the colour will fade in 5-10 sec, it is the true end-point. Failure to observe the first colour change will result in an incorrect titration. The fading end-point does not occur at the second end-point)

Add 2 g of sodium fluoride, boil the mixture for 2-5 min, and cool in a stream of running water. Titrate this solution, using the zinc sulfate solution as titrant, to the same fugitive yellow-brown or pink end-point as described above.

Calculate mass (mg) of Al_2O_3 per ml of zinc sulfate solution (T) from the formula

$$T = 18.896\ W/V$$

where

W is the mass (g) of aluminium wire;
V is the ml of the zinc sulfate solution consumed in the second titration;
18.896 = (R × 1000 mg/g × 10 ml/2)/1000 ml; and
R is the ratio of the formula weight of aluminium oxide to that of elemental aluminium.

Sample Solution A

Accurately weigh 1.0 g of the sample and transfer to a 250-ml high-silica glass Erlenmeyer flask. Add 10 g of sodium bisulfate ($NaHSO_4 \cdot H_2O$). (*Note*: Do not use more sodium bisulfate than specified, as an excess concentration of salt will interfere with the EDTA titration later on in the procedure.) Begin heating the flask at low heat on a hot plate, and then gradually raise the temperature until full heat is reached. (*Caution:* perform this procedure in a well ventilated area) When spattering has stopped and light fumes of SO_3 appear, heat in the full flame of a Meeker burner, with the flask tilted so that the fusion of the sample and sodium bisulfate is concentrated at one end of the flask. Swirl constantly until the melt is clear (except for silica content), but guard against prolonged heating to avoid precipitation of titanium dioxide. Cool, add 25 ml sulfuric acid solution (1 in 2), and heat until the mass has dissolved and a clear solution results. Cool, and dilute to 120 ml with water. Introduce a magnetic stir bar into the flask.

Sample Solution B

Prepare 200 ml of an approximately 6.25 M solution of sodium hydroxide. Add 65 ml of this solution to Sample Solution A, while stirring with the magnetic stirrer; pour the remaining 135 ml of the alkali solution into a 500-ml volumetric flask.

Slowly, with constant stirring, add the sample mixture to the alkali solution in the 500-ml volumetric flask; dilute to volume with water, and mix. (*Note*: If the procedure is delayed at this point for more than 2 hours, store the contents of the volumetric flask in a polyethylene bottle.) Allow most of the precipitate to settle (or centrifuge for 5 min), then filter the supernatant liquid through a very fine filter paper. Label the filtrate Sample Solution B.

Sample Solution C

Transfer 100 ml of the Sample Solution B into a 500-ml Erlenmeyer flask, add 1 drop of methyl orange TS, acidify with hydrochloric acid solution (1 in 2), and then add about 3 ml in excess. Add 25 ml of 0.02 M disodium EDTA, and mix. [*Note*: If the approximate Al_2O_3 content is known, calculate the optimum volume of EDTA solution to be added by the formula: (4 x % Al_2O_3) + 5 ml]

Add, dropwise, ammonia solution (1 in 5) until the colour is just completely changed from red to orange-yellow. Then add10 ml each of ammonium acetate and diammonium hydrogen phosphate solution and boil for 5 min. Cool quickly to room temperature in a stream of running water, add 3 drops of xylenol orange TS, and mix. If the solution is purple, yellow-brown, or pink, bring the pH to 5.3 - 5.7 by the addition of acetic acid. At the desired pH, a pink colour indicates that not enough of the EDTA solution has been added, in which case,

discard the solution and repeat this procedure with another 100 ml of Sample Solution B, using 50 ml, rather than 25 ml, of 0.02 M disodium EDTA.

Procedure

Using the standardized zinc sulfate solution as titrant, titrate Sample Solution C to the first yellow-brown or pink end-point that persists for 5-10 sec. (*Important:* See Note under "*0.01 Zinc sulfate*"). This first titration should require more than 8 ml of titrant, but for more accurate work a titration of 10-15 ml is desirable.

Add 2 g of sodium fluoride to the titration flask, boil the mixture for 2-5 min, and cool in a stream of running water. Titrate this solution, using the standardized zinc sulfate solution as titrant, to the same fugitive yellow-brown or pink end-point as described above.

Calculation

Calculate the percentage of aluminium oxide (Al_2O_3) in the sample taken by the formula:

$$\% Al_2O_3 = 100 \times (0.005VT)/S$$

where

V is the number of ml of 0.01 N zinc sulfate consumed in the second titration;
T is the mass of Al_2O_3 per ml of zinc sulfate solution;
S is the mass (g) of the sample taken; and
0.005 = 500 ml / (1000mg/g × 100 ml).

Silicon dioxide

Accurately weigh 1 g of the sample and transfer to a 250-ml high-silica glass Erlenmeyer flask. Add 10 g of sodium bisulfate ($NaHSO_4 \cdot H_2O$). Heat gently over a Meeker burner, while swirling the flask, until decomposition and fusion are complete and the melt is clear, except for the silica content, and then cool. (*Caution:* Do not overheat the contents of the flask at the beginning, and heat cautiously during fusion to avoid spattering.)

To the cooled melt add 25 ml of sulfuric acid solution (1 in 2) and heat carefully and slowly until the melt is dissolved. Cool, and carefully add 150 ml of water by pouring very small portions down the sides of the flask, with frequent swirling to avoid over-heating and spattering. Allow the contents of the flask to cool, and filter through fine ashless filter paper, using a 60 degree gravity funnel. Rinse out all the silica from the flask onto the filter paper with sulfuric acid solution (1 in 10). Transfer the filter paper and its contents into a platinum crucible, dry in an oven at 120°, and heat the partly covered crucible over a Bunsen burner. To prevent flaming of the filter paper, first heat the cover from above, and then the crucible from below.

When the filter paper is consumed, transfer the crucible to a muffle furnace and ignite at 1000° for 30 min. Cool in a desiccator, and weigh. Add 2 drops of sulfuric acid (1 in 2) and 5 ml of concentrated hydrofluoric acid (sp.gr. 1.15), and carefully evaporate to dryness, first on a low-heat hot plate (to remove the HF) and then over a Bunsen burner (to remove the H_2SO_4). Take precautions to avoid spattering, especially after removal of the HF. Ignite at 1000° for 10 min, cool in a desiccator, and weigh again. Record the difference

between the two weights as the content of SiO_2 in the sample.

METHOD OF ASSAY Accurately weigh about 150 mg of the sample, previously dried at 105° for 3 hours, and transfer into a 500-ml conical flask. Add 5 ml of water and shake until a homogeneous, milky suspension is obtained. Add 30 ml of sulfuric acid and 12 g of ammonium sulfate, and mix. Initially heat gently, then heat strongly until a clear solution is obtained. Cool, then cautiously dilute with 120 ml of water and 40 ml of hydrochloric acid, and stir. Add 3 g of aluminium metal, and immediately insert a rubber stopper fitted with a U-shaped glass tube while immersing the other end of the U-tube into a saturated solution of sodium bicarbonate contained in a 500-ml wide-mouth bottle, and generate hydrogen. Allow to stand for a few minutes after the aluminium metal has dissolved completely to produce a transparent purple solution. Cool to below 50° in running water, and remove the rubber stopper carrying the U-tube. Add 3 ml of a saturated potassium thiocyanate solution as an indicator, and immediately titrate with 0.1 N ferric ammonium sulfate until a faint brown colour that persists for 30 seconds is obtained. Perform a blank determination and make any necessary correction. Each ml of 0.1 N ferric ammonium sulfate is equivalent to 7.990 mg of TiO_2.

d- α -TOCOPHEROL, CONCENTRATE

Prepared at the 55th JECFA (2000) and published in FNP 52 Add 8 (2000), superseding tentative specifications prepared at the 30th JECFA (1986) and published in FNP 37 (1986) and in FNP 52 (1992). A group ADI of 0.15-2 mg/kg bw for dl-α-tocopherol and d-α-tocopherol, concentrate, singly or in combination, was established at the 30th JECFA (1986).

SYNONYMS Vitamin E, RRR-alpha -tocopherol, 5,7,8-trimethyltocol, (+)-alpha-Tocopherol; INS No. 307a

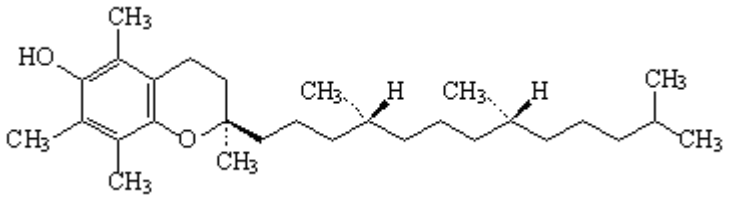
DEFINITION d-alpha-Tocopherol, concentrate is a form of Vitamin E obtained by the vacuum steam distillation of edible vegetable oil products, comprising a concentrated form of d-alpha-tocopherol. It may contain an edible vegetable oil added to adjust the required amount of total tocopherols, and the content of d-alpha-tocopherol may be adjusted by suitable physical and chemical means.

Chemical names (2R,4'R,8'R)-2,5,7,8-tetramethyl-2-(4',8',12'-trimethyltridecyl)-chroman-6-ol

C.A.S. number No. 59-02-9 (Vitamin E)

Chemical formula $C_{29}H_{50}O_2$

Structural formula



Formula weight 430.71

Assay Not less than 40% of total tocopherols, of which not less than 95% consists of d-alpha-tocopherol

DESCRIPTION Brownish red to light yellow, nearly odourless, clear viscous oil, which oxidizes and darkens slowly in air and on exposure to light

FUNCTIONAL USES Antioxidant, nutrient

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water; soluble in ethanol; miscible with ether

Chromatography The retention time of the major peak in the chromatogram of the sample solution is the same as that of the standard solution, both relative to the

internal standard, as obtained in the Assay.

Colour reaction — Dissolve about 0.05 g of the sample in 10 ml of absolute ethanol. Add, with swirling, 2 ml of nitric acid and heat at about 75° for 15 min. A bright red to orange colour develops

PURITY

Specific rotation — alpha (25, D): Not less than +24°
See description under TESTS

Acidity — Dissolve 1 g of the sample in 25 ml of a mixture of equal volumes of ethanol and ether that has been neutralized to phenolphthalein TS with 0.1 N sodium hydroxide, add 0.5 ml of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide until the solution remains faintly pink after shaking for 30 sec. Not more than 1.0 ml of 0.1 N sodium hydroxide is required.

Lead (Vol. 4) — Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental methods”.

TESTS

PURITY TESTS

Specific rotation — Transfer an accurately weighed sample, equivalent to about 100 mg of total tocopherols, to a separator, and dissolve it in 50 ml of ether. To the separator add 20 ml of a 10% solution of potassium ferricyanide in 0.8% sodium hydroxide solution, and shake for 3 min. Wash the ether solution with four 50-ml portions of water, discard the washings, and dry over anhydrous sodium sulfate.

Evaporate the dried ether solution on a water bath under reduced pressure or in an atmosphere of nitrogen until about 7 or 8 ml remain, and then complete the evaporation, removing the last traces of ether without the application of heat. Immediately dissolve the residue in 5 ml of isooctane, and determine the optical rotation. Calculate the specific rotation, using as c the concentration expressed as the number of g of total tocopherols, determined in the Assay, in 100 ml of the solution.

METHOD OF ASSAY

Gas Liquid Chromatographic Method (Volume 4)

Reagents and solutions
Internal Standard Solution: Transfer about 600 mg of hexadecyl hexadecanoate, accurately weighed, to a 200-ml volumetric flask, dissolve in a solution containing 2 parts of pyridine and 1 part of propionic anhydride, dilute to volume with the solution, and mix.

Standard solution
Transfer 12-, 25-, 37-, and 50-mg portions of USP Alpha Tocopherol Reference Standard, accurately weighed, to separate 50-ml Erlenmeyer flasks having 19/38 standard-taper ground-glass necks. Pipet 25.0 ml of the Internal Standard Solution into each flask, mix, and reflux for 10 min. under water-cooled condensers.

Sample solution
Transfer about 60 mg of the sample, accurately weighed, to another 50-ml Erlenmeyer flask, pipet 10.0 ml of the Internal Standard Solution into the flask, mix, and reflux for 10 min. under a water-cooled condenser.

Chromatographic System
Use a gas chromatograph equipped with a flame-ionization detector and a glass-lined sample-introduction system or on-column injection. Under typical conditions, the instrument contains a 2-m x 4-mm borosilicate glass column packed with 2% to 5% methylpolysiloxane on 80- to 100-mesh acid-base washed siliconized chromatographic diatomaceous earth. The column is maintained isothermally between 240^{o} and 260^{o}, the injection port at about 290^{o}, and the detector block at about 300^{o}. The flow rate of dry carrier gas is adjusted to obtain a hexadecyl-hexadecanoate peak approximately 18 to 20 min after sample introduction when a 2% stationary phase is used, or 30 to 32 min when a 5% stationary phase is used. (Note: Cure and condition the column as necessary).

System Suitability
Chromatograph a suitable number of injections of the sample solution, as directed under Calibration, to assure that the resolution factor R, between the major peaks occurring at retention times of approximately 0.50 (delta-tocopherol propionate) and 0.63 (beta- and gamma-tocopheryl propionates), relative of hexadecyl hexadecanoate at 1.00, is not less than 2.5.

Calibration
Chromatograph successive 2- to 5-µl portions of each standard solution until the relative response factor F, for each is constant (i.e. within a range of approximately 2%) for three consecutive injections. If graphic integration is used, adjust the instrument to obtain at least 70% maximum recorder response for the hexadecyl hexadecanoate peak. Measure the areas under the first (alpha-tocopheryl propionate) and second (hexadecyl hexadecanoate) major peaks, and record the values as A_s and A_1, respectively. Calculate the factor F, for each concentration of $(A_s/A_1) \times (C_1/C_s)$, in which C_1 and C_s are the exact concentrations, in mg per ml, of hexadecyl hexadecanoate and of USP Alpha Tocopherol Reference Standard in the Standard Preparation, respectively. Prepare a relative response factor curve by plotting area of alpha-tocopheryl propionate versus relative response factor.

Procedure
Inject a suitable portion (2 to 5 µl) of the Assay Preparation into the chromatograph, and record the chromatogram. Measure the areas under the four major peaks occurring at relative retention times of 0.50, 0.63, 0.76, and 1.00, and record the values as A_δ, $A_{\beta+\gamma}$, A_α and A_1, corresponding

to delta-tocopherol propionate, beta- and gamma-tocopheryl propionates, alpha-tocopheryl propionate, and hexadecyl hexadecanoate, respectively.

Calculate the weight, in mg, of each tocopherol form in the sample by the following formulas.

delta-tocopherol = $(10C_1/F) \times (A_\delta/A_1)$
beta- and gamma-tocopherols = $(10C_1/F) \times (A_{\beta+\gamma}/A_1)$
alpha-tocopherol = $(10C_1/F) \times (A_\alpha/A_1)$

where
F is obtained from the relative response factor curve (see Calibration) for each of the corresponding areas under the delta-, beta- and gamma-, and alpha-tocopheryl propionate peaks produced by the Assay Preparation.

(NOTE: The relative response factors for delta-tocopheryl propionate and for beta- and gamma-tocopheryl propionates have been determined empirically to be the same as for alpha-tocopheryl propionate).

TOCOPHEROL CONCENTRATE, MIXED

Prepared at the 30th JECFA (1986), published in FNP 37 (1986) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003). A group ADI of 0.15-2 mg/kg bw for dl-α-tocopherol and d-α-tocopherol, concentrate, singly or in combination, was established at the 30th JECFA (1986).

SYNONYMS Vitamin E, INS No 307b

DEFINITION A form of Vitamin E obtained by the vacuum steam distillation of edible vegetable oil products, comprising concentrated tocopherols.
It may contain an edible vegetable oil added to adjust the required amount of total tocopherols, and the tocopherol forms may be adjusted by suitable physical and chemical means.

Chemical names Mixed Tocopherol Concentrate contains tocopherols such as d-alpha-, d-ß-, d-gamma-, d-delta-tocopherols

C.A.S. number No single definite C.A.S. number is for this substance. No. 59-02-9 is for vitamin E, 1406-18-4 is for alpha-tocopherol, 2074-53-5 is for all-rac-alpha-tocopherol, and 10191-40-0 is for racemic-alpha-tocopherol synthesized from natural phytol or its derivative.

Assay Not less than 34% of total tocopherols

DESCRIPTION Brownish red to red, clear, viscous oil having a mild, characteristic odour; may show a slight separation of waxlike constituents in microcrystalline form

It oxidizes and darkens slowly in air and on exposure to light, particularly when in alkaline media.

FUNCTIONAL USES Antioxidant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water; soluble in ethanol; miscible in ether

Chromatography The retention time of the third major peak (i.e. the peak occurring just before that of the internal standard) in the chromatogram of the Assay Preparation is the same as that of the Standard Preparation, both relative to the internal standard, as obtained in the Assay.

Colour reaction Dissolve about 0.05 g of the sample in 10 ml of absolute ethanol. Add, with swirling, 2 ml of nitric acid and heat at about 75^{o} for 15 min. A bright red to orange colour develops

PURITY

Specific rotation (Vol. 4) — [alpha] 25, D: Not less than +20°
See description under TESTS

Sulfated ash (Vol. 4) — Not more than 0.1%
Test 1 g of the sample (Method II)

Acidity — Dissolve 1 g of the sample in 25 ml of a mixture of equal volumes of ethanol and ether that has been neutralized to phenolphthalein TS with 0.1 N sodium hydroxide, add 0.5 ml of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide until the solution remains faintly pink after shaking for 30 sec. Not more than 1.0 ml of 0.1 N sodium hydroxide is required.

Lead (Vol. 4) — Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TESTS

PURITY TESTS

Specific rotation — Transfer an accurately weighed amount of sample, equivalent to about 100 mg of total tocopherols, to a separator, and dissolve it in 50 ml of ether. To the separator add 20 ml of a 10% solution of potassium ferricyanide in sodium hydroxide solution (1 in 125), and shake for 3 min. Wash the ether solution with four 50-ml portions of water, discard the washings, and dry over anhydrous sodium sulfate. Evaporate the dried ether solution on a water bath under reduced pressure or in an atmosphere of nitrogen until about 7 or 8 ml remain, and then complete the evaporation, removing the last traces of ether without the application of heat. Immediately dissolve the residue in 5 ml of isooctane, and determine the optical rotation. Calculate the specific rotation, using as c the concentration expressed as the number of g of total tocopherols, determined in the Assay, in 100 ml of the solution.

METHOD OF ASSAY

Gas Liquid Chromatographic Method

Reagents and solutions:
Internal Standard Solution: Transfer about 600 mg of hexadecyl hexadecanoate, accurately weighed, to a 200-ml volumetric flask, dissolve in a solution containing 2 parts of pyridine and 1 part of propionic anhydride, dilute to volume with the solution, and mix.
Standard Preparations: Transfer 12-, 25-, 37-, and 50-mg portions of USP Alpha Tocopherol Reference Standard, accurately weighed, to separate 50-ml Erlenmeyer flasks having 19/38 standard-taper ground-glass necks. Pipet 25.0 ml of the Internal Standard Solution into each flask, mix, and reflux for 10 min. under water-cooled condensers.
Assay Preparation: Transfer about 60 mg of the sample, accurately weighed, to another 50-ml Erlenmeyer flask, pipet 10.0 ml of the Internal

Standard Solution into the flask, mix, and reflux for 10 min. under a water-cooled condenser.

Chromatographic System

Use a gas chromatograph equipped with a flame-ionization detector and a glass-lined sample-introduction system or on-column injection. Under typical conditions, the instrument contains a 2-m x 4-mm borosilicate glass column packed with 2% to 5% methylpolysiloxane gum on 80- to 100-mesh acid-base washed silinized chromatographic diatomaceous earth. The column is maintained isothermally between 240^o and 260^o, the injection port at about 290^o, and the detector block at about 300^o. The flow rate of dry carrier gas is adjusted to obtain a hexadecyl-hexadecanoate peak approximately 18 to 20 min. after sample introduction when a 2% column is used, or 30 to 32 min. when a 5% column is used. (NOTE: Cure and condition the column as necessary).

System Suitability

Chromatograph a suitable number of injections of the Assay Preparation, as directed under Calibration, to assure that the resolution factor, R, between the major peaks occurring at retention times of approximately 0.50 (delta-tocopherol propionate) and 0.63 (ß-plus gamma-tocopheryl propionates), relative of hexadecyl hexadecanoate at 1.00, is not less than 2.5.

Calibration

Chromatograph successive 2- to 5-µl portions of each Standard Preparation until the relative response factor, F, for each is constant (i.e. within a range of approximately 2%) for three consecutive injections. If graphic integration is used, adjust the instrument to obtain at least 70% maximum recorder response for the hexadecyl hexadecanoate peak. Measure the areas under the first (l-tocopheryl propionate) and second (hexadecyl hexadecanoate) major peaks (excluding the solvent peak), and record the values as A_s and A_1, respectively. Calculate the factor, F, for each concentration of $(A_s/A_1) \times C_1/C_s)$, in which C_1 and C_s are the exact concentrations, in mg per ml, of hexadecyl hexadecanoate and of USP Alpha Tocopherol Reference Standard in the Standard Preparation, respectively. Prepare a relative response factor curve by plotting area of alpha-tocopheryl propionate versus relative response factor.

Procedure

Inject a suitable portion (2 to 5 µl) of the Assay Preparation into the chromatograph, and record the chromatogram. Measure the areas under the four major peaks occurring at relative retention times of 0.50, 0.63, 0.76, and 1.00, and record the values as a_{delta}, $a_{beta+gamma}$, a_{alpha} and a_1, corresponding to delta-tocopheryl propionate, ß-plus gamma-tocopheryl propionates, alpha-tocopheryl propionate, and hexadecyl hexadecanoate, respectively.

Calculate the weight, in mg, of each tocopherol form in the sample by the following formulas:

delta-tocopherol = $(10C_1/F) \times (a_{delta}/a_1)$;
ß-plus gamma-tocopherols = $(10C_1/F) \times (a_{beta+gamma}/a_1)$;

alpha-tocopherol = $(10C_1/F) \times (a_{alpha}/a_1)$,

where
F is obtained from the relative response factor curve (see Calibration) for each of the corresponding areas under the delta-, ß- plus gamma-, and alpha-tocopheryl propionate peaks produced by the Assay Preparation. (NOTE: The relative response factor for delta-tocopheryl propionate and for ß- plus gamma-tocopheryl propionates has been determined empirically to be the same as for alpha-tocopheryl propionate).

dl- α -TOCOPHEROL

Prepared at the 30th JECFA (1986), published in FNP 37 (1986) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003). A group ADI of 0.15-2 mg/kg bw for dl-α-tocopherol and d-α-tocopherol, concentrate, singly or in combination, was established at the 30th JECFA (1986).

SYNONYMS Vitamin E; INS No. 307c

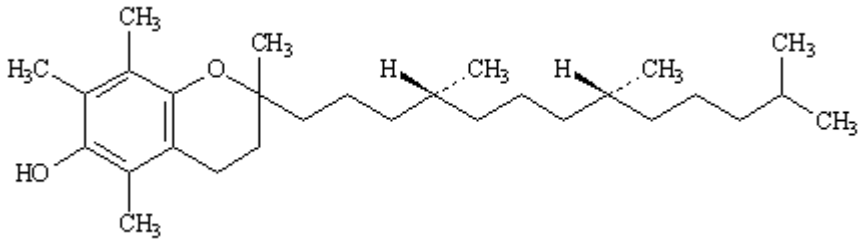
DEFINITION

Chemical names: dl-5,7,8-Trimethyltocol, dl-2,5,7,8-tetramethyl-2-(4',8',12'- trimethyltridecyl)-6-chromanol

C.A.S. number: No single definite C.A.S. number is for this substance. No. 59-02-9 is for vitamin E, 1406-18-4 is for alpha-tocopherol, 2074-53-5 is for all-rac-alpha-tocopherol, and 10191-40-0 is for racemic-alpha-tocopherol synthesized from natural phytol or its derivative.

Chemical formula: $C_{29}H_{50}O_2$

Structural formula:



Formula weight: 430.71

Assay: Not less than 96% and not more than 102%

DESCRIPTION Slightly yellow to amber, nearly odourless, clear, viscous oil (It oxidizes and darkens in air and on exposure to light.)

FUNCTIONAL USES Antioxidant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Insoluble in water, freely soluble in ethanol, miscible with ether

Specific rotation (Vol. 4): [alpha] 20, D: 0±0.05° (1 in 10 soln in chloroform)

Spectrophotometry (Vol. 4): In absolute ethanol the maximum absorption is about 292 nm

Colour reaction — Dissolve about 0.01 g of the sample in 10 ml of absolute ethanol. Add, with swirling, 2 ml of nitric acid and heat at about 75° for 15 min. A bright red to orange colour develops

PURITY

Refractive index — n (20, D): 1.503 - 1.507

Spectrophotometry (Vol. 4) — E (1%, 1 cm) (292 nm): 71-76 (0.01 g in 200 ml of absolute ethanol)

Sulfated ash (Vol. 4) — Not more than 0.1%
Test 1 g of the sample (Method II)

Acidity — Dissolve 1 g of the sample in 25 ml of a mixture of equal volumes of ethanol and ether that has been neutralized to phenolphthalein TS with 0.1 N sodium hydroxide, add 0.5 ml of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide until the solution remains faintly pink after shaking for 30 sec. Not more than 1.0 ml of 0.1 N sodium hydroxide is required.

Lead (Vol. 4) — Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

METHOD OF ASSAY

Gas Liquid Chromatographic Method

Reagents and solutions
Internal Standard Solution: Prepare a solution in n-hexane containing a 3 mg of hexadecyl hexadecanoate, accurately weighed, in each ml.
Standard Preparation: dissolve about 30 mg of USP Alpha Tocopherol Reference Standard, accurately weighed, in 10.0 ml of the Internal Standard Solution.

Assay Preparation: dissolve about 30 g of the sample accurately weighed, in 10.0 ml of the International Standard Solution.

Chromatographic System
Use a gas chromatograph equipped with a flame-ionization detector and a glass-lined sample- introduction system or on-column injection. Under typical conditions, the instrument contains a 2-m x 4-mm boro-silicate glass column packed with 2% methylpolysiloxane gum on 80- to 100-mesh acid-base washed silinized chromatographic diatomaceous earth. The column is maintained isothermally between 240° and 260°, the injection port at about 290°, and the detector block at about 300°. The flow rate of dry carrier gas is adjusted to obtain a hexadecyl hexadecanoate peak approximately 18 to 20 min after sample introduction when a 2% column is used, or 30 to 32 min when a 5% column is used. (NOTE: Cure and condition the column as necessary).

System Suitability

Chromatograph a sufficient number of injections of a mixture in n-hexane of 1 mg per ml each of USP Alpha Tocopherol Reference Standard and USP Alpha Tocopheryl Acetate Reference Standard, as directed under Calibration, to assure that the resolution factor, R, is not less than 1.0.

Calibration

Chromatograph successive 2- to 5-µl portions of the Standard Preparation until the relative response factor, F, is constant (i.e., within a range of approximately 2%) for three consecutive injections. If graphic integration is used, adjust the instrument to obtain at least 70% maximum recorder response for the hexadecyl hexadecanoate peak. Measure the areas under the major peaks occurring at relative retention times of approximately 0.51 (alpha-tocopherol) and 1.00 (hexadecyl hexadecanoate), and record the values as A_s and A_1, respectively. Calculate the relative response factor, F, by the formula $(A_s/A_1) \times (C_1/C_s)$, in which C_1 and C_s are the exact concentrations, in mg per ml, of hexadecyl hexadecanoate and of USP Alpha Tocopherol Reference Standard in the Standard Preparation, respectively.

Procedure

Inject a suitable portion (2 to 5 µl) of the Assay Preparation into the chromatograph, and record the chromatogram. Measure the areas under the major peaks occurring at relative retention times of approximately 0.51 (alpha-tocopherol) and 1.00 (hexadecyl hexadecanoate), and record the values as a_u and a_1, respectively.

Calculate the weight, in mg, of dl-alpha-tocopherol in the sample by the formula $(10C_1/F) \times (a_u/a_1)$.

TRAGACANTH GUM

Prepared at the 29th JECFA (1985), published in FNP 34 (1986) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). An ADI 'not specified' was established at the 29th JECFA (1985)

SYNONYMS INS No. 413

DEFINITION A dried exudation obtained from the stems and branches of *Astragalus gummifer* Labillardiere and other Asiatic species of *Astragalus* (Fam. *Leguminosae*); consists mainly of high molecular-weight polysaccharides (galactoarabans and acidic polysaccharides) which, on hydrolysis, yield galacturonic acid, galactose, arabinose, xylose and fucose; small amounts of rhamnose and of glucose (derived from traces of starch and/or cellulose) may also be present.

C.A.S. number 9000-65-1

DESCRIPTION The unground gum occurs as flattened, lamellated, straight or curved fragments or as spirally twisted pieces 0.5 - 2.5 mm thick and up to 3 cm in length; white to pale yellow, but some pieces may have a red tinge; the pieces are horny in texture, with a short fracture; odourless. The powdered gum is white to pale yellow or pinkish brown (pale tan).
Items of commerce may contain extraneous materials such as pieces of bark which must be removed before use in food.
Unground samples should be powdered to pass a No. 45 sieve (355 M) and mixed well before performing any one of the following tests.

FUNCTIONAL USES Emulsifier, stabilizer, thickening agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) 1 g of the sample in 50 ml of water swells to form a smooth, stiff, opalescent mucilage; insoluble in ethanol and does not swell in 60% (w/v) aqueous ethanol.

Microscopy Examine microscopically a suspension of the sample in water. Numerous angular fragments with circular or irregular lamellae, starch grains up to 15 µm in diameter, and stratified cellular membranes, which turn violet in colour on the addition of iodinated zinc chloride solution, are visible.

Precipitate formation The samples gives a precipitation reaction with a saturated aqueous solution of copper (II) acetate.

Gum constituents Identify arabinose, xylose, fucose, galactose and galacturonic acid as follows: Proceed as directed under *Gum Constituents Identification* using the following reference standards: arabinose, mannose, galactose, xylose, fucose, galacturonic acid and glucuronic acid. Arabinose, xylose, fucose, galactose and galacturonic acid should be present; mannose and glucuronic acid should be absent.

PURITY

Loss on drying (Vol. 4)	Not more than 16% (105°, 5 h)
Sulfated ash (Vol. 4)	Not more than 4%
Acid insoluble ash	Not more than 0.5% Boil the ash obtained as directed under Sulfated ash above, with 25 ml of 3 M hydrochloric acid for 5 min., collect the insoluble matter on a tared crucible or ashless filter paper, wash with hot water, ignite, and weigh. Calculate the percentage of Acid-insoluble ash from the weight of the sample.
Acid insoluble matter	Not more than 2% In a 250 ml round-bottomed flask, place 2.0 g of tragacanth and add 95 ml of methanol. Moisten the powder by swirling and add 80 ml of hydrochloric acid. Add a few glass beads of about 4 mm in diameter and heat under reflux in a water-bath for 3 h. shaking occasionally. Eliminate the glass beads and filter by suction the suspension while hot through a previously tared sintered-glass filter. Rinse the flask with a small quantity of water and pass the rinsings through the filter. Wash the residue on the filter with about 40 ml of methanol and dry at 110° to constant weight. Allow to cool in a desiccator and weigh. Calculate as percentage.
Acacia and other soluble gums	To 20 ml of a 0.25% (w/v) suspension of the sample in freshly boiled and cooled water add 10 ml of lead (II) acetate solution. A flocculent precipitate is produced. Filter, and to the filtrate add 10 ml of lead sub-acetate solution. The solution may become slightly cloudy but no precipitate is formed.
Agar	To 4 ml of a dispersion [0.5% w/v] of the sample in water, add 0.5 ml of hydrochloric acid and heat on a boiling water bath for 30 min. Add a few drops of barium chloride solution [3.65%, w/v]. No precipitate is formed.
Dextrin	Mount the sample in aqueous glycerol and examine under the microscope. The addition of 1% aqueous iodine solution does not reveal yellow-brown or purplish-red particles.
Karaya gum	(a) Boil 1 g of the sample with 20 ml of water until a mucilage is formed. Add 5 ml of hydrochloric acid and again boil for 5 min. No permanent pink or red colour develops. (b) Shake 0.2 g with 10 ml of ethanol (60%) in a 10 ml stoppered cylinder, graduated in 0.1 ml intervals. Any gel formed occupies not more than 1.5 ml. (c) Shake 1.0 g with 99 ml of water. Titrate the mucilage so formed with 0.01 M sodium hydroxide, using methyl red solution as indicator. Not more than 5.0 ml of 0.01 M sodium hydroxide is required to change the colour of the solution.
Microbiological criteria	*Salmonella* spp.: Negative in 1 g

(Vol. 4)	*E. coli*: Negative in 1 g
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

TRIACETIN

Prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 19th JECFA (1975), published in NMRS 55B (1976) and in FNP 52 (1992). Metals and arsenic specifications revised at the 63rd JECFA (2004). An ADI 'not specified' was established at the 19th JECFA (1975)

SYNONYMS Glyceryl triacetate, INS No. 1518

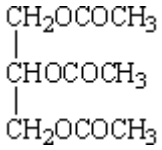
DEFINITION

Chemical names: Glyceryl triacetate

C.A.S. number: 102-76-1

Chemical formula: $C_9H_{14}O_6$

Structural formula:



Formula weight: 218.21

Assay: Not less than 98.5% on the anhydrous basis

DESCRIPTION Colourless, somewhat oily liquid having a slight, fatty odour

FUNCTIONAL USES Humectant, solvent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Sparingly soluble in water, soluble in ethanol

Test for glycerol: Heat a few drops in a test tube with about 0.5 g of potassium bisulfate. Pungent vapours of acrolein are evolved

Test for acetate (Vol. 4): Passes test
To be performed on the solution resulting from the assay

PURITY

Water (Vol. 4): Not more than 1.0% (Karl Fischer Method)

Refractive index (Vol. 4): 1.429 - 1.431 at 25°

Specific gravity (Vol. 4): d (25, 25): 1.154 - 1.158

Distillation range (Vol. 4): 258 - 270°

Sulfated ash (Vol. 4)
Not more than 0.02%
Test 5 g of the sample (Method II)

Acidity
Accurately weigh a sample of 25 g, dilute with 50 ml of neutralized ethanol, and add 5 drops of phenolphthalein TS. Not more than 1 ml of 0.02N sodium hydroxide is required to produce a pink colour.

Unsaturated compounds
To 10 ml of the sample in a glass-stoppered tube add dropwise a 1 in 100 solution of bromine in carbon tetrachloride until a permanent yellow colour is produced. No turbidity or precipitate appears after 18 h in the dark.

Lead (Vol. 4)
Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY
Transfer about 1 g of the sample, accurately weighed, into a suitable pressure bottle, add 25 ml of 1N potassium hydroxide and 15 ml of isopropanol, stopper the bottle, and wrap securely in a canvas bag. Heat in a water bath maintained at 98±2^{o} for 1 h, allowing the water in the bath to just cover the liquid in the bottle. Remove the bottle from the bath, cool in air to room temperature, then loosen the bag, uncap the bottle to release any pressure, and remove the bag. Add 6 to 8 drops of phenolphthalein TS, and titrate the excess alkali with 0.5N sulfuric acid just to the disappearance of the pink colour. Perform a blank determination. Each ml of 0.5N sulfuric acid is equivalent to 36.37 mg of $C_9H_{14}O_6$.

TRIAMMONIUM CITRATE

Prepared at the 27th JECFA (1983), published in FNP 28 (1983) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). A group ADI 'not limited' for citric acid and its calcium, potassium, sodium and ammonium salts was established at the 23rd JECFA (1979)

SYNONYMS Citric acid triammonium salt; ammonium citrate tribasic; INS No. 380

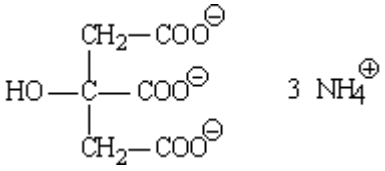
DEFINITION

Chemical names: Triammonium citrate, triammonium salt of 2-hydroxypropan-1,2,3-tricarboxylicacid

C.A.S. number: 3458-72-8

Chemical formula: $C_6H_{17}N_3O_7$

Structural formula:



Formula weight: 243.22

Assay: Not less than 97.0%

DESCRIPTION White crystals or crystalline powder

FUNCTIONAL USES Buffering agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water

Test for citrate (Vol. 4): Passes test

Test for ammonium (Vol. 4): Passes test

PURITY

Oxalate (Vol. 4): Not more than 0.04%

Lead (Vol. 4): Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Dissolve about 3.5 g of the sample, accurately weighed, in 50 ml of water, add 50 ml of 1 N sodium hydroxide, boil for 15 min or until ammonia ceases to be evolved, add sufficient 1 N sulfuric acid to make the solution acid to phenolphthalein TS, boil for 5 min, cool, and titrate with 1 N sodium hydroxide, using phenolphthalein TS as an indicator. Each ml of 1 N sodium hydroxide is equivalent to 81.07 mg of $C_6H_{17}N_3O_7$.

CALCIUM CITRATE

Prepared at the 19th JECFA (1975), published in NMRS 55B (1976) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI 'not limited' was established at the 17th JECFA (1973)

SYNONYMS INS No. 333(iii)

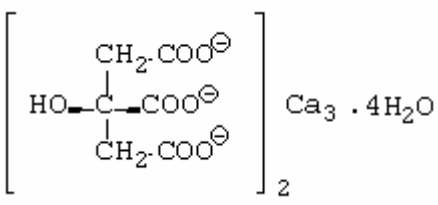
DEFINITION

Chemical names: Tricalcium citrate, tricalcium salt of 2-hydroxy-1,2,3- propanetricarboxylic acid, tricalcium salt of ß-hydroxy-tricarballylic acid

C.A.S. number: 813-94-5

Chemical formula: $C_{12}H_{10}Ca_3O_{14} \cdot 4H_2O$

Structural formula:



Formula weight: 570.51

Assay: Not less than 97.5% after drying

DESCRIPTION Odourless, fine white powder

FUNCTIONAL USES Sequestrant, buffer, firming agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Very slightly soluble in water. Insoluble in ethanol.

Test for citrate (Vol. 4): Passes test

Test for calcium (Vol. 4): Passes test
Test a solution obtained by igniting 0.5 g of the sample at as low a temperature as possible, cooling and dissolving the residue in 10 ml of water and adding 1 ml of glacial acetic acid.

PURITY

Loss on drying (Vol. 4): Not less than 10% and not more than 14% (150°, 4 h)

Fluorides (Vol. 4)	Not more than 30 mg/kg (Method I or III)
Free acid and alkali	Passes test To 1 g of the sample, add 5 ml of water, shake well for 1 min, and add 2 drops of phenolphthalein TS. No pink colour is produced. Add 0.5 ml of 0.1 N sodium hydroxide. A pink colour is produced.
Oxalate	Dissolve 1 g of the sample in 5 ml of warm dilute hydrochloric acid TS and filter the solution if necessary. Add 2 g of sodium acetate and dilute to 10 ml with water. No turbidity is produced within 1 h.
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Weigh accurately about 350 mg of the sample, previously dried at 150^{o} for 4 h, dissolve in a mixture of 10 ml of water and 2 ml of dilute hydrochloric acid TS, and dilute to about 100 ml with water. While stirring (preferably with a magnetic stirrer) add about 30 ml of 0.05 M disodium ethylenediaminetetra-acetate from a 50-ml buret, then add 15 ml of sodium hydroxide TS and 300 mg of hydroxynaphthol blue indicator, and continue the titration to a blue endpoint. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 8.303 mg of $C_{12}H_{10}Ca_3O_{14}$.

TRIETHYL CITRATE

Prepared at the 28th JECFA (1984), published in FNP 31/2 (1984) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003). An ADI of 0-20 mg/kg bw was established at the 28th JECFA (1984).

SYNONYMS Ethyl citrate; INS No. 1505

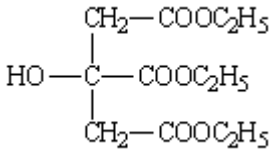
DEFINITION

Chemical names — Triethyl 2-hydroxypropan-1,2,3-tricarboxylate

C.A.S. number — 77-93-0

Chemical formula — $C_{12}H_{20}O_7$

Structural formula



Formula weight — 276.29

Assay — Not less than 99% w/w

DESCRIPTION Odourless, practically colourless, oily liquid

FUNCTIONAL USES Carrier solvent, sequestrant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) — Slightly soluble in water; miscible with ethanol and ether

Refractive index (Vol. 4) — n (20, D):1.439 - 1.441

Specific gravity (Vol. 4) — d (25, 25): 1.135 - 1.139

PURITY

Water (Vol. 4) — Not more than 0.25% w/w (Karl Fischer Method)

Acidity — Not more than 0.02% w/w (as citric acid)
Dissolve 32 g of the sample, accurately weighted, in 30 ml of neutralized ethanol, add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide. Not more than 1.0 ml is required.

Lead (Vol. 4) — Not more than 2 mg/kg
Determine using an AAS/ICP-AES technique appropriate to the

specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

METHOD OF ASSAY

Weigh accurately about 1.5 g of the sample into a 500-ml flask equipped with a standard taper ground joint, and add 25 ml of isopropanol and 25 ml of water. Pipet 50 ml of 0.5 N sodium hydroxide into the mixture, add a few boiling chips, and attach a suitable water-cooled condenser. Reflux for 1.5 h, then cool, wash down the condenser with about 20 ml of water, add 5 drops of bromothymol blue TS, and titrate the excess alkali with 0.5 N sulfuric acid. Perform a blank determination. Each ml of 0.5 N sulfuric acid is equivalent to 46.05 mg of $C_{12}H_{20}O_7$.

TRIPOTASSIUM CITRATE

Prepared at the 19th JECFA (1975), published in NMRS 55B (1976) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI not limited' was established at the 17th JECFA (1973)

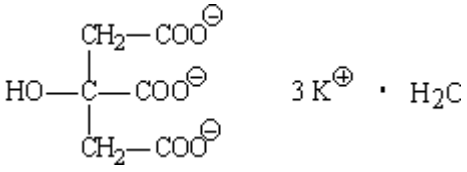
SYNONYMS Potassium citrate; INS No. 332(ii)

DEFINITION

Chemical names: Tripotassium citrate, tripotassium salt of 2-hydroxy-1,2,3-propanetricarboxylic acid, tripotassium salt of ß-hydroxy-tricarballylic acid

C.A.S. number: 866-84-2

Chemical formula:



Structural formula: $C_6H_5K_3O_7 \cdot H_2O$

Formula weight: 324.42

Assay: Not less than 99.0% after drying

DESCRIPTION Deliquescent, odourless, transparent crystals or white, granular powder

FUNCTIONAL USES Sequestrant, stabilizer, buffer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Very soluble in water, insoluble in ethanol

Test for citrate: To 5 ml of a 1 in 10 solution of the sample add 1 ml of calcium chloride TS and 3 drops of bromothymol blue TS, and slightly acidify with dilute hydrochloric acid TS. Add sodium hydroxide TS until the colour changes to a clear blue, then boil the solution for 3 min, agitating gently during the heating period. A white, crystalline precipitate appears which is insoluble in sodium hydroxide TS but dissolves in acetic acid TS.

To 10 ml of a 1 in 10 solution of the sample add 1 ml of mercuric sulfate TS. Heat the mixture to boiling and add a few drops of potassium permanganate TS. A white precipitate is formed.

Test for potassium: When hydrochloric acid is present, a solution of the sample gives with platinum chloride TS a yellow crystalline precipitate (which on ignition leaves a residue of potassium chloride and platinum).

PURITY

Loss on drying (Vol. 4)	Not more than 6% (180^{o}, 4 h)
Alkalinity	A 1 in 20 solution of the sample is alkaline to litmus. After the addition of 0.2 ml of 0.1 N sulfuric acid and 1 drop of phenolphthalein TS to 10 ml of the solution no pink colour is produced.
Oxalate	To 10 ml of a 1 in 10 solution of the sample add 5 drops of dilute acetic acid TS and 2 ml of calcium chloride TS. No turbidity develops within 1 h.
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
METHOD OF ASSAY	Weigh accurately about 250 mg of the dried sample. Dissolve in 40 ml of glacial acetic acid, warming slightly to effect solution. Cool the solution to room temperature, add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid. Perform a blank determination and make any necessary correction. Each ml of 0.1 N perchloric acid is equivalent to 10.21 mg of $C_6H_5K_3O_7$.

TRISODIUM CITRATE

Prepared at the 19th JECFA (1975), published in NMRS 55B (1976) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI not limited' was established at the 17th JECFA (1973)

SYNONYMS Sodium citrate; INS No. 331(iii)

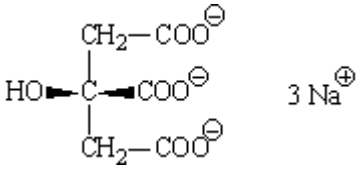
DEFINITION

Chemical names: Trisodium citrate, trisodium salt of 2-hydroxy-1,2,3- propanetricarboxylic acid, trisodium salt of ß-hydroxy-tricarballylic acid

C.A.S. number: 68-04-2

Chemical formula: Anhydrous: $C_6H_5Na_3O_7$
Hydrated: $C_6H_5Na_3O_7 \cdot xH_2O$

Structural formula:



Formula weight: 258.07 (anhydrous)

Assay: Not less than 99.0% calculated on the dried basis

DESCRIPTION Colourless, odourless crystals, or white, crystalline powder; hydrated forms available include the dihydrate and the pentahemihydrate

FUNCTIONAL USES Buffer, sequestrant, emulsion stabilizer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water, insoluble in ethanol

Test for citrate: To 5 ml of a 1 in 10 solution of the sample add 1 ml of calcium chloride TS and 3 drops of bromothymol blue TS, and slightly acidify with dilute hydrochloric acid TS. Add sodium hydroxide TS until the colour changes to a clear blue, then boil the solution for 3 min, agitating gently during the heating period. A white, crystalline precipitate appears which is insoluble in sodium hydroxide TS but dissolves in acetic acid TS.

To 10 ml of a 1 in 10 solution of the sample add 1 ml of mercuric sulfate TS. Heat the mixture to boiling and add a few drops of potassium permanganate TS. A white precipitate is formed.

Test for sodium — To 5 ml of a 1 in 20 solution of the sample, acidified with acetic acid TS add 1 ml of uranyl zinc acetate TS. A yellow crystalline precipitate is formed within a few min.

PURITY

Loss on drying (Vol. 4) — Anhydrous: not more than 1% (180° to constant weight)
Dihydrate: not more than 13% (180° to constant weight)
Pentahemihydrate: not more than 30% (180° to constant weight)

Alkalinity — A 1 in 20 solution of the sample is alkaline to litmus. After the addition of 0.2 ml of 0.1 N sulfuric acid and 1 drop of phenolphthalein TS to 10 ml of the solution no pink colour is produced.

Oxalate — To 10 ml of a 1 in 10 solution of the sample add 5 drops of dilute acetic acid TS and 2 ml of calcium chloride TS. No turbidity develops within 1 h.

Lead (Vol. 4) — Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY — Transfer about 350 mg of the sample, accurately weighed, to a 250-ml beaker. Add 100 ml of glacial acetic acid, stir until completely dissolved, and titrate with 0.1 N perchloric acid, using crystal violet TS as indicator. Perform a blank determination and make any necessary correction. Each ml of 0.1 N perchloric acid is equivalent to 8.602 mg of $C_6H_5Na_3O_7$.

XANTHAN GUM

Prepared at the 53rd JECFA (1999) and published in FNP Add 7 (1999), superseding specifications prepared at the 51st JECFA (1998), published in FNP 52 Add 6 (1998). ADI "not specified", established at the 30th JECFA in 1986.

SYNONYMS INS No. 415

DEFINITION A high molecular weight polysaccharide gum produced by a pure-culture fermentation of a carbohydrate with *Xanthomonas campestris*, purified by recovery with ethanol or isopropanol, dried and milled; contains D-glucose and D-mannose as the dominant hexose units, along with D-glucuronic acid and pyruvic acid, and is prepared as the sodium, potassium or calcium salt; its solutions are neutral.

C.A.S. number 11138-66-2

Assay Yields, on the dried basis, not less than 4.2% and not more than 5.4% of carbon dioxide (CO_2), corresponding to between 91.0% and 117.0% respectively of xanthan gum.

DESCRIPTION Cream-coloured powder

FUNCTIONAL USES Thickener, stabiliser, emulsifier, foaming agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in water; insoluble in ethanol

Gel formation To 300 ml of water, previously heated to 80° and stirred rapidly with a mechanical stirrer in a 400-ml beaker, add, at the point of maximum agitation, a dry blend of 1.5 g of the sample and 1.5 g of carob bean gum. Stir until the mixture goes into solution, and then continue stirring for 30 min longer. Do not allow the water temperature to drop below 60° during stirring. Discontinue stirring, and allow the mixture to cool at room temperature for at least 2 h. A firm rubbery gel forms after the temperature drops below 40°, but no such gel forms in a 1% control solution of the sample prepared in the same manner but omitting the carob bean gum.

PURITY

Loss on drying (Vol. 4) Not more than 15% (105°, 2.5 h)

Ash (total) (Vol. 4) Not more than 16% after drying

Pyruvic acid Not less than 1.5%
See description under TESTS

Nitrogen (Vol. 4) Not more than 1.5%

Proceed according to the Kjeldahl method

Ethanol and isopropanol — Not more than 500 mg/kg, singly or in combination
See description under TESTS

Lead (Vol. 4) — Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

Microbiological criteria (Vol. 4) — Total plate count: Not more than 5,000 cfu/g
E. coli: Negative by test
Salmonella: Negative by test
Yeasts and moulds: Not more than 500 cfu/g
See also description under TESTS

TESTS

PURITY TESTS

Pyruvic acid

Sample preparation
Weigh 600 mg of the sample to the nearest 0.1 mg and dissolve in sufficient water to make 100 ml. Transfer 10.0 ml of the solution into a 50-ml glass-stoppered flask. Pipette 20 ml of N hydrochloric acid into the flask, weigh the flask, and reflux for 3 h, taking precautions to prevent loss of vapours. Cool to room temperature, and add water to make up for any weight loss during refluxing. Pipette 1.0 ml of a 1 in 200 solution of 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid into a 30-ml separatory funnel, then add 2.0 ml of the sample solution, mix, and allow to stand at room temperature for 5 min. Extract the mixture with 5 ml of ethyl acetate, and discard the aqueous layer. Extract the hydrazone from the ethyl acetate with three 5-ml portions of sodium carbonate TS, collecting the extracts in a 50-ml volumetric flask. Dilute to volume with sodium carbonate TS and mix.

Standard preparation
Weigh 45 mg of pyruvic acid, to the nearest 0.1 mg, and transfer into a 500-ml volumetric flask. Dilute to volume with water, and mix. Transfer 10.0 ml of this solution into a 50-ml glass-stoppered flask, and continue as directed under "Sample preparation", beginning with "Pipette 20 ml of N hydrochloric acid into the flask".

Procedure
Determine the absorbance of each solution with a suitable spectrophotometer in 1-cm cells at the maximum of about 375 nm, using sodium carbonate TS as the blank. The absorbance of the "Sample preparation" is equal to or greater than that of the "Standard preparation".

Ethanol and isopropanol

Principle
The alcohols are converted to the corresponding nitrite esters and determined by headspace gas chromatography (see Volume 4).

Sample preparation
Dissolve 100 mg of sample in 10 ml of water using sodium chloride as a

dispersing agent if necessary.

Internal standard solution
Prepare an aqueous solution containing 50 mg/l of n-propanol.

Standard alcohol solution
Prepare an aqueous solution containing 50 mg/l each of ethanol and isopropanol.

Procedure
Weigh 200 mg of urea into a 25-ml "dark vial" (Reacti-flasks, Pierce, Rockford, IL, USA, or equivalent). Purge with nitrogen for 5 min and then add 1 ml of saturated oxalic acid solution, close with a rubber stopper and swirl. Add 1 ml of sample solution, 1 ml of internal standard solution, and simultaneously start a stopwatch (T=0). Swirl the vial and recap with an open screw cap fitted with a silicone rubber septum. Swirl until T=30 sec. At T=45 sec inject through the septum 0.5 ml of an aqueous solution of sodium nitrite (250 g/l). Swirl until T=70 sec and at T=150 sec withdraw through the septum 1 ml of the headspace using a pressure lock syringe (Precision Sampling Corp., Baton Rouge, Louisiana, USA, or equivalent).

Gas chromatography
Insert syringe needle in the injection port; precompress the sample, then open the syringe and inject the sample.
Use the following conditions:

- Column: glass (4mm i.d., 90 cm)
- Packing: first 15 cm packed with chrompack (or equivalent) and the remainder with Porapak R 120-150 mesh (or equivalent)
- Carrier gas: nitrogen (flow rate: 80 ml/min)
- Detector: flame ionization
- Temperatures: injection port: 250º; column: 150º isothermal

Calculation
Quantify the ethanol and isopropanol present in the sample by comparing the peak areas with the corresponding peaks obtained by chromatographing the headspace produced by substituting in the procedure 1 ml of Standard alcohol solution for 1 ml of Sample solution.

Microbiological criteria (Vol. 4)

Total plate count: Using aseptic technique, disperse 1 g of sample into 99 ml of phosphate buffer and use a Stomacher, shaker or stirrer to fully dissolve. Limit dissolving time to about 10 min and then pipette 1 ml of the solution into separate, duplicate, appropriately marked petri dishes. Pour over the aliquot of sample in each petri dish 12-15 ml of Plate Count Agar previously tempered to 44-46°. Mix well by alternate rotation and back and forth motion of the plates, allow the agar to solidify. Invert the plates and incubate for 48±2 h at 35±1°.
After incubation count the growing colonies visible on each plate and record the number of colonies. Take the average of both plates, and multiply by the sample dilution factor, 100. Where no colonies are visible, express the result as less than 100 cfu/g.

E. coli determination

: Using aseptic technique, disperse 1 g of sample in 99 ml of Lactose broth using either a Stomacher, shaker or stirrer to fully dissolve the sample. Limit the dissolving time to about 15 min and then lightly seal the container and incubate the broth for 18-24 h at 35±1°. Using a sterile pipette, inoculate 1 ml of the incubate into a tube containing 10 ml GN broth. Incubate for 18-24 h and then streak any GN broths showing positive growth or gas production onto duplicate plates of Levine EMB agar. Incubate the plates for 24±2 h at 35±1° and then examine for colonies typical of E. coli i.e. showing strong purple growth with dark centre and a green metallic sheen sometimes spreading onto the agar. Record any typical E. coli colonies as presumptive positive, otherwise negative. Streak any well isolated suspect colonies onto a plate of PCA and incubate for 18-24 h at 35±1°. Perform a Gram stain on any growth to confirm it is Gram negative. If so, disperse any colony growth into a small volume of 0.85% saline and perform chemical tests to confirm the identity of the bacterial growth. This can most conveniently be done by using API 20E or Micro ID strips or equivalent systems.

After completion of the tests, identify the organism from the Identification manual of the system used and record the final result.

Media
GN Broth (Gram Negative Broth)
Peptone 20.0 g
Dextrose 1.0 g
Mannitol 2.0 g
Sodium citrate 5.0 g
Sodium deoxycholate 0.5 g
Potassium phosphate (dibasic) 4.0 g
Potassium phosphate (monobasic) 1.5 g
Sodium chloride 5.0 g
Make up to 1 litre with distilled or de-ionised water, pH 7.0±0.2 at 25°

Salmonella determination
: Using aseptic technique, disperse 5 g of sample into 200 ml of sterile lactose broth using either a Stomacher, shaker or stirrer to maximise dissolution over a 15 min period. Loosely seal the container and incubate at 35±1° for 24±2 h.

Tighten lid and gently shake incubated sample mixture; transfer 1 ml mixture to 10 ml selenite cystine broth and another 1 ml mixture to 10 ml tetrathionate broth. Incubate 24±2 h at 35°. Mix (vortex, if tube) and streak 3-mm loopful incubated selenite cystine broth on bismuth sulfite (BS) agar, xylose lysine desoxycholate (XLD) agar, and Hektoen enteric (HE) agar. (Prepare BS plates the day before streaking and store in dark at room temperature until streaked.) Repeat with 3-mm loopful of tetrathionate broth. Incubate plates 24±2 h at 35°. Continue as indicated on pages 221-226 of the Guide to Specifications, FAO Food and Nutrition Paper 5 Revision 2, Rome 1991, “Examine plates for presence of colonies”.

Yeasts and moulds
: Using aseptic technique, disperse 1 g of sample into 99 ml of phosphate buffer and use a Stomacher, shaker or stirrer to fully dissolve. Limit

dissolving time to about 10 min and then pipette 1 ml of the solution into separate, duplicate, appropriately marked petri dishes. Pour over the aliquot of sample in each petri dish 15-20 ml of Potato dextrose agar (either acidified or containing antibiotic) previously tempered to 44-46°. Mix well by alternate rotation and back and forth motion of the plates, and allow the agar to solidify. Invert the plates and incubate for 5 days at 20-25°. After incubation, count the growing colonies visible on each plate using a colony counter and record the number of colonies. Separate the yeasts from the moulds according to their morphology and count them separately. Take the average of both plates and multiply by the sample dilution factor, 100. Where no colonies are visible, express the result as less than 100 cfu/g.

METHOD OF ASSAY

Proceed as directed in the test for Carbon Dioxide Determination by Decarboxylation (Volume 4) using 1.2 g of the sample accurately weighed.

XYLITOL

Prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 33rd JECFA (1988), published in FNP 38 (1988). Metals and arsenic specifications revised at the 57th JECFA (2001). An ADI 'not specified' was established at the 27th JECFA (1983)

SYNONYMS INS No. 967

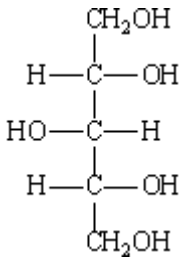
DEFINITION

Chemical names Xylitol

C.A.S. number 87-99-0

Chemical formula $C_5H_{12}O_5$

Structural formula



Formula weight 152.15

Assay Not less than 98.5% and not more than 101.0% on the anhydrous basis

DESCRIPTION White, crystalline powder, practically odourless

FUNCTIONAL USES Sweetener, humectant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Very soluble in water, sparingly soluble in ethanol

Melting range (Vol. 4) 92 - 96°

Infrared absorption The infrared spectrum of a potassium bromide dispersion of the sample corresponds with the reference infrared spectrum below

PURITY

Water (Vol. 4) Not more than 0.5% (Karl Fischer Method)

Sulfated ash (Vol. 4) Not more than 0.1%
Test 2 g of sample (Method I)

Nickel (Vol. 4) Not more than 2 mg/kg
Proceed as directed under *Nickel in Polyols*

Reducing sugars (Vol. 4) Not more than 0.2%
Dissolve about 500 mg of the sample, accurately weighed, in 2 ml of water in a 10 ml conical flask. To a second conical flask add 2 ml of a dextrose solution containing 0.5 mg per ml. Add 2 ml of cupric tartrate, alkaline TS to each flask, heat to boiling, and cool. The sample solution is less turbid than the dextrose solution, in which a reddish brown precipitate is formed.

Other polyols Not more than 1.0%
Proceed as described under Method of Assay and calculate the percentage of each polyol (L-arabinitol, galactitol, mannitol, and sorbitol) by the formula therein given, in which W_S refers to the weight, in mg, of the respective polyol taken for the standards solution; R_S is the peak response ratio of the corresponding polyol obtained from the Standard solution; and R_U is the peak response ratio of the corresponding polyol obtained from the Sample preparation. Sum the four individual polyol percentages to obtain the total.

Lead (Vol. 4) Not more than 1 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

METHOD OF ASSAY

Internal standard solution:
Transfer about 500 mg of erythritol, accurately weighed, into a 25 ml volumetric flask, dilute to volume with water, and mix.

Standard solution:
Transfer about 25 mg each of L-arabinitol, galactitol, mannitol, and sorbitol, accurately weighed, to a 100-ml volumetric flask, dilute to volume with water, and mix. To an accurately measured volume of this solution, add an accurately weighed amount of Reference Standard Xylitol (available from US Pharmacopeial Convention, Inc. 12601 Twinbrook Parkway, Rockville, MD 20852, USA) to obtain a solution with a known concentration of about 49 mg/ml.

Sample preparation:
Transfer about 5 g of the sample, accurately weighed, into a 100-ml volumetric flask, dilute to volume with water, and mix.

Chromatography
Use a gas chromatograph equipped with a flame-ionization detector and a 2-m x 2-mm glass column packed with 3% liquid phase of 25% phenyl-25% cyanopropylmethylsilicone (OV-225 or equivalent) on silanized siliceous earth support (Chromosorb W-HP or equivalent). The carrier gas is nitrogen flowing at about 30 ml/min. The injector port temperature is 250°, the column temperature 200° and the detector temperature 250°. Chromatograph the derivatized Standards Solution prepared as directed under Procedure, and record the peak responses. The relative retention times corresponding to erythritol, L-arabinitol, xylitol, galactitol, mannitol, and sorbitol are usually about 1.0, 2.77, 3.90, 6.96, 7.63 and 8.43,

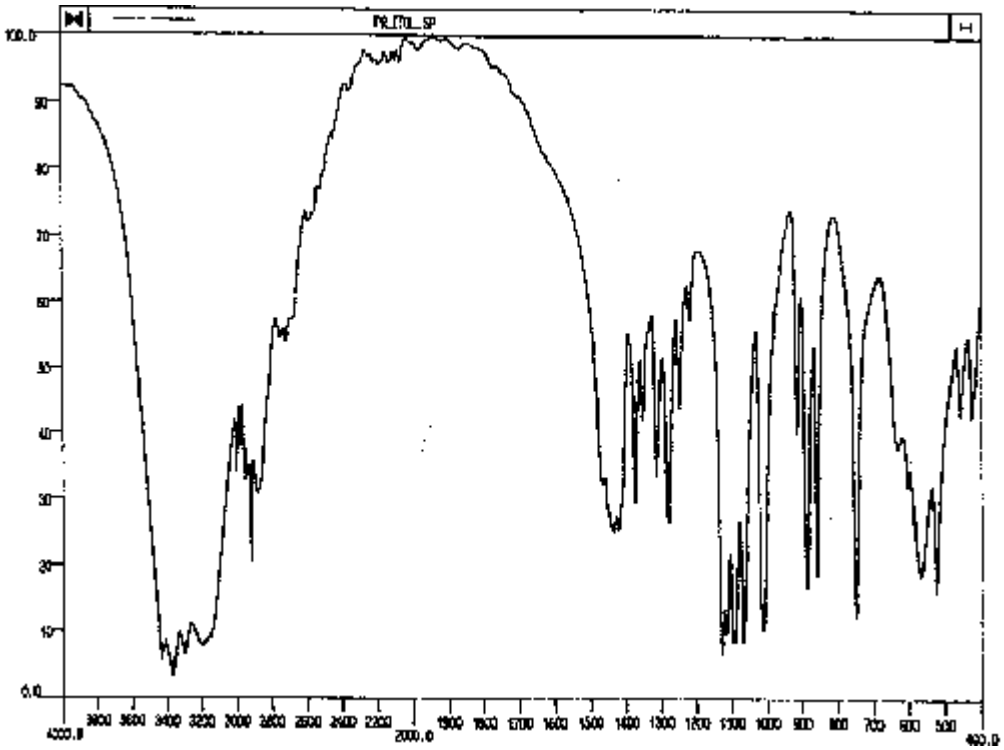
respectively. The relative standard deviation of the response ratios of the derivatized Xylitol to the derivatized erythritol from three replicate injections does not exceed 2.0%.

Procedure:
Pipet 1 ml portions of the standards solution and the sample preparation into separate 100-ml, round-bottom boiling flasks. To each flask, add 1.0 ml of internal standard solution, and evaporate the respective mixtures to dryness on a water bath at 60^{o} with the aid of a rotary evaporator. Dissolve each dry residue in 1 ml of pyridine, and add 1 ml of acetic anhydride to each flask. Boil each solution under reflux for 1 h to complete the acetylation. Separately inject 1-µl portions of the derivatized solutions from the sample preparation and the standard solution into the gas chromatograph and measure the peak responses. Calculate the percentage of xylitol, on the as-is basis, by the formula:

$$100 \times \frac{W_S}{W_U} \times \frac{R_U}{R_S}$$

where
W_S = the weight, in mg, of Reference Standard Xylitol used for the Standard solution
W_U = the weight, in mg, of the sample taken for the Assay preparation
R_U and R_S = the ratios of peak responses of the derivatized analyte to the derivatized erythritol from the Internal Standard solution obtained from the Sample Preparation and the Standard Solution, respectively. Using the value obtained in the water determination, correct the percentage to the anhydrous basis.

Infrared spectrum — Xylitol


PR_1701_SP
100.0
90
80
70
60
50
40
30
20
10
0.0
4000.0
3800
3600
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3000
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1600
1500
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1300
1200
1100
1000
900
800
700
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500
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ที่ปรึกษา

1. นายแพทย์พิพัฒน์ ยิ่งเสรี	เลขาธิการคณะกรรมการอาหารและยา
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3. นางสาวทิพย์วรรณ ปริญญาศิริ	ผู้อำนวยการสำนักอาหาร
4. นายชนินทร์ เจริญพงศ์	ที่ปรึกษาสำนักอาหาร

คณะผู้จัดทำ (สำนักอาหาร สำนักงานคณะกรรมการอาหารและยา)

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