Key to interpreting proposals

Proposed revisions are shown with language proposed for deletion or replacement crossed off. New text (if any) follows, and is enclosed in symbols as shown in the examples below:

▲new text▲*FCC 6*

if slated for FCC 6; and

■new text■ *1S (FCC 6)*

if slated for a Supplement to FCC. The same symbols not set off by an extra paragraph break and enclosing text with no increase in type size indicate recent revisions that are already official. Where the symbols appear together with no enclosed text, such as ■ ■ or ▲▲, it means that text has been deleted and no new text was proposed to replace it. In all revisions, the closing symbol is accompanied by an identifier that indicates the particular Supplement or indicates the FCC as the publication where the revision will appear if approved. For example, ■ *1S (FCC 6)* indicates that the proposed revision is slated for the First Supplement to FCC 6, and ▲*FCC 6* indicates that the revisions are proposed for FCC 6.

BRIEFING

5′-Adenylic Acid. Because there is no existing *FCC* monograph for this food ingredient, a new monograph is proposed on the basis of data received. See the Briefing under *Disodium 5′-Uridylate*.
(FIEC: J. Moore) C82096

Add the following:

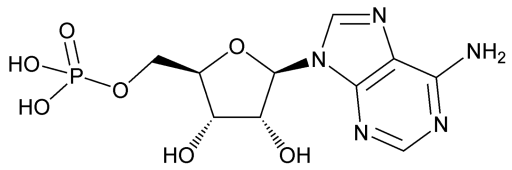
■ ***5′-Adenylic Acid***

Adenosine 5′-monophosphate

Adenylic acid

AMP

Adenosine 5′-phosphoric acid



$C_{10}H_{14}N_5O_7P$

Formula wt 347.23
CAS: [61-19-8]

DESCRIPTION

5′-Adenylic Acid occurs as colorless or white crystals, or as a white, crystalline powder. It is very slightly soluble in water, and practically insoluble in alcohol. It is produced by enzymatic cleavage of natural source yeast RNA with a 5-phosphodiesterase followed by heat treatment, further purification steps, and washing of crystals with ethanol.

Function: Source of 5′-Adenylic Acid

Packaging and Storage: Store in tight containers protected from light and moisture.

IDENTIFICATION

- **A. Infrared Absorption,** *Spectrophotometric Identification Tests,* Appendix IIIC
  Reference standard: USP 5'-Adenylic Acid RS
  Sample and standard preparation: *A*
  Acceptance criteria: The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard*.
- **B. Procedure**
  Acceptance criteria: The retention time of the major peak (excluding the solvent peak) in the chromatogram of the *Sample solution* corresponds to that of the *Standard solution* in the *Assay*.

ASSAY

- **Procedure**

Mobile phase: 0.1 M potassium dihydrogen phosphate (KH_2PO_4) in degassed water, adjusted to pH 5.6 with 0.1 M dipotassium hydrogen phosphate (K_2HP0_4)

Standard solution: 0.02 mg/mL of USP 5'-Adenylic Acid RS in *Mobile phase.* [Note—Ultra-sonication may be necessary to aid in complete dissolution.]

Sample solution: 0.02 mg/mL in *Mobile phase.* [Note—Ultra-sonication may be necessary to aid in complete dissolution.]

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography

Detector: UV 254 nm

Column: 25 cm × 4.6-mm; packed with 5-µm reversed phase C18 silica gel[1]

Column temperature: Ambient

Flow rate: About 1.0 mL/min

Injection size: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Suitability requirement 1: The relative standard deviation of the 5'-adenylic acid area responses from replicate injections is NMT 2.0%.

Suitability requirement 2: The resolution, *R*, between the 5′-adenylic acid peak and all other peaks is NLT 2.0.

Analysis: Separately inject equal volumes of the *Standard solution* and *Sample solution* into the chromatograph, and measure the responses for the major peaks on the resulting chromatograms. [Note—The approximate retention time for 5′-adenylic acid is 27.5 min.] Calculate the percentage of 5′-adenylic acid, $C_{10}H_{14}N_5O_7P$, in the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area response for 5′-adenylic acid in the *Sample solution*

r_S = peak area response for 5′-adenylic acid in the *Standard solution*

C_S = concentration of 5′-adenylic acid in the *Standard solution* (mg/mL)

C_U = concentration of sample in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–103.0%, calculated on the anhydrous basis

IMPURITIES

Inorganic Impurities

- **ARSENIC**

[Note—When water is specified as a diluent, use deionized ultra-filtered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of arsenic as practical.]

Diluent: 4% nitric acid in water

Standard stock solution: 100 µg/mL of arsenic prepared by diluting a commercially available 1000 mg/kg arsenic ICP standard solution

Standard solutions: 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL of arsenic: from *Standard stock solution* diluted with *Diluent*

Sample: 5 g

Sample solution: Dissolve the *Sample* in 40 mL of 10% nitric acid in a 100-mL volumetric flask, and dilute with water to volume.

Spectrophotometric system*, Plasma Spectrochemistry,* Appendix IIC

Mode: Inductively coupled plasma–optical emission spectroscopy (ICP–OES)

Setup: Use a suitable ICP–OES configured in a radial optical alignment. [Note—This method was developed using a Varian Vista MPX ICP–OES unit.]

The instrument parameters are as follows: set the ultra-violet detector to scan arsenic at 188.980 nm. Set the sample read time to 20 s. Set the forward power from the RF generator to 1150 watts. Use an argon plasma feed gas flow of 13.5 L/min with the auxiliary gas set to flow at 2.25 L/min. The sample is delivered to the spray chamber by a multi-channel peristaltic pump set to deliver sample at a rate of 20 rpm. Samples are flushed through the system for 20 s prior to analysis. A 40-s read delay is also programmed into the sampling routine to allow for fluid flow equilibration after the high-speed flush, prior to the first analytical read of the sample. Between samples, the pumping system is washed by flushing the *Diluent* for 20 s.

Analysis: Generate a standard curve using *Diluent* as a blank and the *Standard solutions.* [Note—The correlation coefficient for the best-fit line should not be less than 0.999.]

Similarly, analyze the *Sample solution* on the ICP. Calculate the concentration (mg/kg) of arsenic in the *Sample* taken:

$$\text{Result} = (C/W) \times F$$

C = concentration of arsenic in the *Sample solution* determined from the standard curve (µg/mL)

W = weight of *Sample* taken (g)

F = *Sample solution* final volume, 100 mL

Acceptance criteria: NMT 2 mg/kg

- **CADMIUM**

[Note—When water is specified as a diluent, use deionized ultra-filtered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of cadmium as practical.]

Diluent: 4% nitric acid in water

Standard stock solution: 100 µg/mL of cadmium prepared by diluting a commercially available 1000 mg/kg cadmium ICP standard solution

Standard solutions: 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL of cadmium: from *Standard stock solution* diluted with *Diluent*

Sample: 5 g

Sample solution: Dissolve the *Sample* in 40 mL of 10% nitric acid in a 100-mL volumetric flask, and dilute with water to volume.

Spectrophotometric system*, Plasma Spectrochemistry,* Appendix IIC

Mode: ICP–OES

Setup: Same as that described in the test for *Arsenic*, but set to scan for cadmium at 228.802 nm

Analysis: Generate a standard curve using *Diluent* as a blank and the *Standard solutions.* [Note—The correlation coefficient for the best-fit line should not be less than 0.999.]

Similarly, analyze the *Sample solution* on the ICP. Calculate the concentration (mg/kg) of cadmium in the *Sample* taken:

$$\text{Result} = (C/W) \times F$$

C = concentration of cadmium in the *Sample solution* determined from the standard curve (µg/mL)

W = weight of *Sample* taken (g)

F = *Sample solution* final volume, 100 mL

Acceptance criteria: NMT 0.1 mg/kg

- **LEAD**

[Note—When water is specified as a diluent, use deionized ultra-filtered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of lead as practical.]

Diluent: 4% nitric acid in water

Standard stock solution: 100 µg/mL of lead prepared by diluting a commercially available 1000 mg/kg lead ICP standard solution

Standard solutions: 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL of lead: from *Standard stock solution* diluted with *Diluent*

Sample: 5 g

Sample solution: Dissolve the *Sample* in 40 mL of 10% nitric acid in a 100-mL volumetric flask, and dilute with water to volume.

Spectrophotometric system*, Plasma Spectrochemistry,* Appendix IIC

Mode: ICP–OES

Setup: Same as that described in the test for *Arsenic*, but set to scan for lead at 220.353 nm

Analysis: Generate a standard curve using *Diluent* as a blank and the *Standard solutions.* [Note—The correlation coefficient for the best-fit line should not be less than 0.999.]

Similarly, analyze the *Sample solution* on the ICP. Calculate the concentration (mg/kg) of lead in the *Sample* taken:

$$\text{Result} = (C/W) \times F$$

C = concentration of lead in the *Sample solution* determined from the standard curve (µg/mL)

W = weight of *Sample* taken (g)

F = *Sample solution* final volume, 100 mL

Acceptance criteria: NMT 1 mg/kg

- **MERCURY**

[Note—When water is specified as a diluent, use deionized ultra-filtered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of mercury as practical.]

Diluent: 4% nitric acid in water

Standard stock solution: 100 µg/mL of mercury prepared by diluting a commercially available 1000 mg/kg mercury ICP standard solution

Standard solutions: 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL of mercury: from *Standard stock solution* diluted with *Diluent*

Sample: 5 g

Sample solution: Dissolve the *Sample* in 40 mL of 10% nitric acid in a 100-mL volumetric flask, and dilute with water to volume

Spectrophotometric system*, Plasma Spectrochemistry,* Appendix IIC

Mode: ICP–OES

Setup: Same as that described in the test for *Arsenic*, but set to scan for mercury at 194.164 nm

Analysis: Generate a standard curve using *Diluent* as a blank and the *Standard solutions.* [Note—The correlation coefficient for the best-fit line should not be less than 0.999.]

Similarly, analyze the *Sample solution* on the ICP. Calculate the concentration (mg/kg) of mercury in the *Sample* taken:

$$\text{Result} = (C/W) \times F$$

C = concentration of mercury in the *Sample solution* determined from the standard curve (µg/mL)
W = weight of *Sample* taken (g)
F = *Sample solution* final volume, 100 mL

Acceptance criteria: NMT 0.5 mg/kg

Organic Impurities

- **ETHANOL**

Standard solution: 10 mg/kg of ethanol in 1 N sodium hydroxide. Add 10 mL of this solution to a 20-mL headspace vial, and cap tightly.

Sample solution: 100 mg/g in 1 N sodium hydroxide. Add 10 mL of this solution to a 20-mL headspace vial, and cap tightly.

Chromatographic system, Appendix IIA

Mode: Gas chromatography equipped with pressure-loop headspace autosampler

Detector: Flame ionization

Column: 30-m × 0.53-mm (id) capillary column with a 6% cyanopropylphenyl–94% dimethylpolysiloxane stationary phase and a 3.00-µm film thickness[2]

Column temperature: 20 min at 40°; increase to 240° at 10°/min; maintain at 240° for 10 min

Injection port temperature: 140°

Detector temperature: 250°

Carrier gas: Nitrogen

Flow rate: 2.5 mL/min

Headspace unit: 2.5 mL/min

Equilibration temperature: 80°

Equilibration time: 60 min

Loop temperature: 85°

Transfer temperature: 90°

Pressurization time: 0.5 min

Loop fill time: 0.1 min

Injection time: 1 min

Injection size: 1 mL of headspace

System suitability

Sample: *Standard solution*

Suitability requirement: The relative standard deviation of the ethanol peak area responses from replicate injections is NMT 5.0%.

Analysis: Separately inject equal volumes of the *Standard solution* and *Sample solution* into the chromatograph, record the chromatograms, and measure the peak responses. [Note—The approximate retention time for ethanol is 11 min.]

Acceptance criteria: The peak area from the *Sample solution* does not exceed that from the *Standard solution* (NMT 100 mg/kg).

- **OTHER RIBONUCLEOTIDES**

Mobile phase and **Chromatographic system:** Prepare as directed in the *Assay*.

Sample solution: 1.0 mg/mL. [Note—Ultra-sonication may be necessary to aid in complete dissolution.]

Standard solution: Mixture of USP Disodium 5′-Uridylate RS, USP 5′-Adenylic Acid RS, USP 5′-Cytidylic Acid RS, USP Disodium Guanylate RS, and USP Disodium Inosinate RS, each at 0.02 mg/mL in *Mobile phase*

Suitability requirements

Sample: *Standard solution*

Suitability requirement 1: The relative standard deviation of the 5'-adenylic acid peak area responses from replicate injections is NMT 2.0%.

Suitability requirement 2: The resolution, *R*, between the 5'-adenylic acid peak and all other nucleotide peaks is NLT 2.0.

Analysis: Separately inject equal volumes of the *Standard solution* and *Sample solution* into the chromatograph, and measure the responses for all nucleotide peaks on the resulting chromatograms, except the peak from 5'-adenylic acid. [Note—The approximate retention times are 4.6 min (5′-cytidylic acid), 6.2 min (5′-uridylic acid), 10.3 min (5′-guanylic acid), 11.5 min (5′-inosinic acid), and 27.5 min (5′-adenylic acid).] Separately calculate the percentage of each analyte (5′-cytidylic acid, 5′-guanylic acid, 5′-inosinic acid, and 5′-uridylic acid) in the sample taken:

$$\text{Result} = (C_S/C_U) \times (r_U/r_S) \times 100$$

C_S = concentration of analyte in the *Standard solution* (mg/mL)

C_U = concentration of analyte in the *Sample solution* (mg/mL)

r_U = peak area of the analyte from the *Sample solution*

r_S = peak area of the analyte from the *Standard solution*

Acceptance criteria: The sum of the percentages for all nucleotide impurities is NMT 0.5%, calculated on the anhydrous basis.

SPECIFIC TESTS

- **pH**, *pH Determination,* Appendix IIB
  Sample solution: 10 mg/mL
  Acceptance criteria: 2.5–3.5 for the saturated aqueous solution
- **Water**, *Water Determination, Method I,* Appendix IIB
  Acceptance criteria: NMT 6.0%
- **Bile-Tolerant Gram-Negative Bacteria**, Appendix XIIC
  Sample preparation: Proceed as directed using a 10-g sample and incubating at 30–35° for 18–24 h.
  Acceptance criteria: Negative in 10 g
- **Enterobacter sakazakii** *(Cronobacter Spp.),* Appendix XIIC
  Sample preparation: Proceed as directed using a 10-g sample and incubating at 30–35° for 18–24 h.
  Acceptance criteria: Negative in 10 g
- **Salmonella spp.**, Appendix XIIC
  Sample preparation: Dissolve 25 g of sample at a sample/broth ratio of 1/8, and proceed as directed.
  Acceptance criteria: Negative in 25 g
- **Total Aerobic Microbial Count**, *Method I (Plate Count Method),* Appendix XIIB
  Acceptance criteria: NMT 1,000 CFU/g
- **Total Yeasts and Molds Count**, *Method I (Plate Count Method),* Appendix XIIB
  Acceptance criteria: NMT 100 CFU/g

■ 1S (*FCC7*)

[1] YMC-Pack ODS-AQ (YMC Europe GmbH, Dinslaken, Germany), or equivalent.

[2] CP-Select 624 CB (Varian-Chrompack, Palo Alto, CA), or equivalent.

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

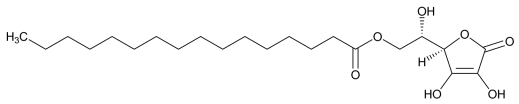
Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(FI07) Food Ingredients Expert Committee

BRIEFING

Ascorbyl Palmitate, *FCC* 6 page 67. On the basis of efforts to modernize *Identification* test procedures in *FCC* and on data received, a new infrared method is proposed under *Identification*.
(FIEC: J. Moore) C83889

Ascorbyl Palmitate

Palmitoyl l-Ascorbic Acid



$C_{22}H_{38}O_7$

Formula wt 414.54

INS: 304

CAS: [137-66-6]

DESCRIPTION

Ascorbyl Palmitate occurs as a white or yellow-white powder. It is very slightly soluble in water and in vegetable oils. One gram dissolves in about 4.5 mL of alcohol.

Function: Antioxidant

Packaging and Storage: Store in tightly closed containers, preferably in a cool, dry place.

IDENTIFICATION

Add the following:

- ■**A. Infrared Absorption,** *Spectrophotometric Identification Tests,* Appendix IIIC
  Reference standard: USP Ascorbyl Palmitate RS
  Sample and standard preparation: *K*
  Acceptance criteria: The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard.*■ 1S (*FCC7*)
- ■**B.**■ 1S (*FCC7*) **Procedure**
  Sample solution: 100 mg/mL in alcohol
  Acceptance criteria: The *Sample solution* decolorizes dichlorophenol-indophenol TS.

ASSAY

- **Procedure**
  Sample: 300 mg
  Analysis: Dissolve the *Sample* in 50 mL of alcohol in a 250-mL Erlenmeyer flask. Add 30 mL of water and immediately titrate with 0.1 N iodine to a yellow color that persists for at least 30 s. Each mL of 0.1 N iodine is equivalent to 20.73 mg of $C_{22}H_{38}O_7$.
  Acceptance criteria: NLT 95.0% of $C_{22}H_{38}O_7$, calculated on the dried basis

IMPURITIES

Inorganic Impurities

- **Lead**, *Lead Limit Test, Flame Atomic Absorption Spectrophotometric Method,* Appendix IIIB
  Sample: 10 g
  Acceptance criteria: NMT 2 mg/kg

SPECIFIC TESTS

- **Loss on Drying**, Appendix IIC**:** Vacuum oven at 56° to 60° for 1 h
  Acceptance criteria: NMT 2%
- **Melting Range or Temperature**, Appendix IIB
  Analysis: Determine as directed in *Procedure for Class Ia*
  Acceptance criteria: Between 107° and 117°
- **Optical (Specific) Rotation**, Appendix IIB
  Sample solution: 1 g in 10 mL of methanol
  Acceptance criteria: $[\alpha]_D^{25}$ Between +21° and +24°, calculated on the dried basis
- **Residue on Ignition (Sulfated Ash)**, *Method I,* Appendix IIC
  Sample: 2 g
  Acceptance criteria: NMT 0.1%

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 67

BRIEFING

Calcium Lignosulfonate (40-65). Because there is no existing *FCC* monograph for this food ingredient, a new monograph is proposed based on the Calcium Lignosulfonate (40-65) monograph prepared at the 69th Session (2008) of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and on the basis of data supplied.

(FIEC: J. Moore) C76501

Add the following:

■ **Calcium Lignosulfonate (40-65)**

Lignosulfonic Acid, Calcium Salt (40-65)

INS: 1522

DESCRIPTION

Calcium Lignosulfonate (40-65) occurs as a light yellow-brown to brown powder. It is an amorphous material obtained from the sulfite pulping of softwood. The lignin framework is a sulfonated random polymer of three aromatic alcohols: coniferyl alcohol, *p*-coumaryl alcohol, and sinapyl alcohol, of which coniferyl alcohol is the principal unit. After completion of the pulping, the water-soluble calcium lignosulfonate is separated from the cellulose, purified (ultrafiltration), and acidified. The recovered material is evaporated and spray dried. It is distinguished from Calcium Lignosulfonate by its characteristic weight average molecular weight, its low degree of sulfonation, and its low level of reducing sugars. It is soluble in water and practically insoluble in organic solvents.

Function: Carrier, encapsulating agent

Packaging and Storage: Store in well-closed containers.

IDENTIFICATION

- **Degree of Sulfonation:** [Note—The degree of sulfonation is determined as the content ratio of organic sulfur to methoxyl. The organic sulfur content is determined indirectly as the difference between the total sulfur content (determined by elemental analysis) and inorganic sulfur content (determined by ion exchange chromatography).]

  Total sulfur determination

  Calibration standards: Add approximately 0.2 mg of vanadium pentoxide into each of four tin capsules. Accurately weigh 0.5, 1.0, 1.5, and 2.0 mg of BBOT (2,5-(Bis(5-*tert*-butyl-2-benzo-oxazol-2-yl) thiophene)) into the four capsules.

  System suitability standard: Add approximately 0.2 mg of vanadium pentoxide and 0.5–2.0 mg of BBOT into a tin capsule.

  Samples: Add approximately 0.2 mg of vanadium pentoxide into each of two tin capsules. Accurately weigh 1–2 mg of sample, previously dried, into each capsule.

  Equipment: Elemental analyzer capable of analyzing for sulfur[1]

  Equipment parameters

  Carrier gas: Helium, 120 mL/min

  Combustion furnace temperature: 1000°

  Oven temperture: 70°

Helium pressure: 150 kPa
Oxygen pressure: 150 kPa
Oxygen loop: 5 mL
Run time: 300 s

System suitability

[Note—A system suitability check should be performed after every fourth sample.]
Sample: *System suitability standard*
Suitability requirement 1: The chromatogram contains a sulfur peak.
Suitability requirement 2: The standard deviation for triplicate analyses is NMT 0.20.

Analysis: Run the four calibration standards and construct a calibration curve. [Note—The correlation coefficient should be at least 0.999.] Run the *Samples*. Obtain the weight (mg) of total sulfur in the *Sample* using the calibration curve. Calculate the percent total sulfur in the portion of the sample taken:

$$\text{Result} = W_U/W_{SMP} \times 100$$

W_U = weight of total sulfur calculated from the standard curve (mg)

W_{SMP} = weight of the sample taken, on the dried basis (mg)

Inorganic sulfur determination

Mobile phase: 0.1 N sodium hydroxide and water (10:90)

Standard stock solution: 1 mg/mL of sulfate, prepared by dissolving 0.1479 g of sodium sulfate in 100 mL of water

Standard solutions: 2.0, 5.0, 20.0, and 40.0 mg/L of sulfate, prepared by pipetting 0.1, 0.25, 1.0, and 2.0 mL of *Standard stock solution* in separate 50-mL volumetric flasks, adding 1 mL of 3% H_2O_2 to each, and diluting with water to volume.

Sample solution: Transfer 30 mg of previously dried sample into a 50-mL volumetric flask, and dissolve in 10 mL of 10 mg/mL NaOH. Add 5 mL of 3% H_2O_2, and allow to stand overnight, then dilute with water to volume.

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography[2]

Detector: Ion detector with anion self-regenerating conductivity suppressor[3]

Column: 25-cm × 4-mm, anion-exchange analytical column[4] , and 5-cm × 4-mm anion-exchange guard column[5]

Flow rate: 0.7 mL/min

Injection size: 10 µL

System suitability

Sample: *Sample solution*
Relative standard deviation: NMT 3.0%

Analysis: Separately inject equal volumes of the *Standard solutions* and *Sample solution* (previously filtered using a 0.2-µm syringe filter) into the chromatograph, and measure the responses for the major peaks on the resulting chromatograms. [Note—The approximate retention time for sulfate is 7 min.] Prepare a standard curve for sulfate by plotting sulfate peak areas versus concentrations in mg/L. From the standard curve, calculate the concentration (C_U) of sulfate in the *Sample solution* in mg/L. Calculate the percent inorganic sulfur in the portion of the sample taken:

$$\text{Result} = C_U/C_{SMP} \times F_1/F_2 \times 100$$

C_U = concentration of sulfate in the *Sample solution* determined from the standard curve (mg/L)

C_{SMP} = concentration of the sample, on the dried basis, in the *Sample solution* (mg/L)

F_1 = formula weight of sulfur, 32

F_2 = formula weight of sulfate, 96

Methoxyl (−OCH3) determination

Sample: 15–20 mg, previously dried and weighed onto a small piece of aluminum foil

0.025 N sodium thiosulfate: Dilute 0.1 N sodium thiosulfate VS with water (1:3).

Analysis

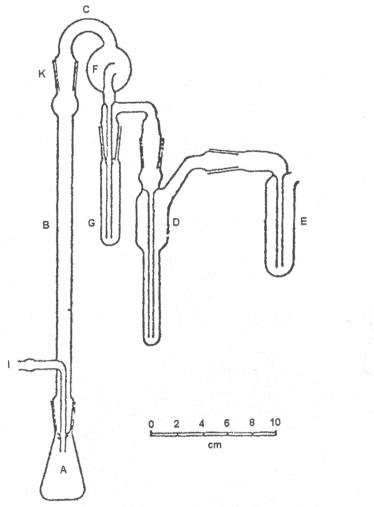


Figure 1.

See *Figure 1* for apparatus setup. Wrap the foil around the *Sample* and put it into the reaction flask (A) to which 5 mL of hydroiodic acid (min. 57%), approximately 2 g of phenol, and a few glass beads have been added. Add 5 mL of 50 mg/mL cadmium sulfate solution containing about 0.3 mg of red phosphorus into the washer (G). Add 10 mL of glacial acetic acid (saturated with sodium acetate) and 10 droplets of bromine to the receiver (D). Finally, fill the U-trap (E) with sodium hydroxide or other suitable absorbant that will prevent bromine from leaving the system. Pass nitrogen gas through a 30 mg/mL Na_2CO_3 solution and into the system through the side arm (I) of the air condensor (B). Heat the reaction flask (A) to 140–145Å for 1 h in a glycerin bath. Wash the contents of the receiver (D) into a 250-mL Erlenmeyer flask containing 10 mL of acetic acid (saturated with sodium acetate). Rotate the flask and add formic acid dropwise until the color disappears. Add 5 mL of 10% potassium iodide solution, and mix. Then add 10 mL of 1 M sulfuric acid and let the flask stand for 3 min. Titrate the solution with *0.025 N sodium thiosulfate* until the color changes from yellowish to colorless. Calculate the percent methoxyl:

$$\text{Result} = V \times F_1 \times F_2/(W \times F_3 \times F_4) \times 100$$

V = volume of *0.025 N sodium thiosulfate* used in the titration (mL)

F_1 = concentration of the *0.025 N sodium thiosulfate*, 0.025

F_2 = formula weight of methoxyl, 31

W = weight of the sample taken on the dried basis (mg)

F_3 = stoichiometric conversion factor from titrant to methoxyl moiety, 6

F_4 = mL-to-L conversion factor for the *0.025 N sodium thiosulfate*, 1000

Degree of sulfonation calculation: Calculate the degree of sulfonation:

$$\text{Result} = (\%OS)/(\%M)$$

%OS = % organic sulfur, as (% total sulfur) − (% inorganic sulfur), determined above in the *Total sulfur determination* and *Inorganic sulfur determination* test procedures

%M = % methoxyl, determined above in the *Methoxyl (−OCH3) determination* test procedure

Acceptance criteria: 0.3–0.7, on the dried basis

- **Infrared Absorption**, *Spectrophotometric Identification Tests,* Appendix IIIC

  Reference standard: USP Calcium Lignosulfonate (40-65) RS

  Sample and standard preparation: *K*

  Acceptance criteria: The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard.*

- **Ultraviolet Absorption**

  Sample stock solution: 500 µg/mL

  Sample solution: 50 µg/mL made from *Sample stock solution*, and adjusted to a pH of 2.0–2.2 with 5 M hydrochloric acid.

  Acceptance criteria: The *Sample solution* exhibits an absorption maximum at 280 nm.

- **Weight-Average Molecular Weight**

  Mobile phase: Combine 1600 g of water with 161.8 g of DMSO in a 2-L flask. Add 21.44 g of dibasic sodium phosphate heptahydrate, and adjust the pH to 10.5 with NaOH. Add 1.6 g of sodium dodecylsulfate and pass through a 0.22-µm filter.

  Standard solutions: Prepare two lignosulfonate calibration standard[6] solutions, each at 2 mg/mL in *Mobile phase*. One should be prepared using a lignosulfonate standard with a weight-average molecular weight from 30,000 to 60,000 g/mol, and the other using a lignosulfonate standard with a weight-average molecular weight from 5,000 to 10,000 g/mol. Filter each solution into a vial using a 0.2-µm syringe filter.

  Sample solution: 2 mg/mL using a previously dried sample. Filter into vial using a 0.2-µm syringe filter.

  Chromatographic system, Appendix IIA

  Mode: High-performance liquid chromatography

  Detector: UV 280 nm

  Column: 50-cm × 10-mm glucose divinylbenzene (DVB), 5-µm, 10^4 Å analytical column[7] , and 4-cm × 6-mm, 7-µm, 300 Å guard column[8]

  Oven temperature: 60°

  Flow rate: 1.0 mL/min

  Injection size: 200 µL

  System suitability

Sample: The *Standard solution* with the highest weight-average molecular weight.

Suitability requirement: The relative standard deviation of the lignosulfonate peak retention time for three injections is NMT 0.5%.

Analysis: Run *Mobile phase* through the system for NLT 2 hr. [Note—Pressure should not exceed 1000 psi.] Inject the *Standards solutions*, then the *Sample solution* followed by another set of *Standard solutions*. Generate a calibration curve using the *Standard solutions*. Calculate the weight-average molecular weight from the chromatogram of the *Sample solution* using suitable software.[9]

Acceptance criteria: Between 40,000 and 65,000 with >90% of the sample ranging from 1,000 to 250,000

IMPURITIES

Inorganic Impurities

- **Arsenic**, *Arsenic Limit Test,* Appendix IIIB**:** Prepare as directed for organic compounds.

  [Note—Alternatively, the arsenic content may be determined by the following graphite furnace atomic absorption spectrophotometric method.]

  Standard solutions: 0–15 ng/mL of arsenic; prepared from a commercially available 1000 mg/kg arsenic standard solution. [Note—Store this solution in polyethylene bottles due to instability in glass.]

  Sample solution [**CAUTION—** Wear proper eye protection, protective clothing, and gloves during sample preparation. Closely follow the manufacturer's safety instructions for use of the microwave digestion apparatus.] Transfer 200 mg of sample into a Teflon digestion vessel liner. Add 3 mL of 65% nitric acid, 2 mL of 30% hydrogen peroxide, and cover. Heat for 20 min in a microwave oven, and allow the vessel to cool to room temperature (a cool water bath may be used to speed the cooling process), and carefully open in a ventilation hood. Dilute the cooled digest with water to 12 mL.

  Reagent blank: Use the same quantities of reagents as used to prepare the *Sample solution*, but omitting the sample.

  Analysis: Use any suitable graphite furnace atomic absorption spectrophotometer. Optimize the instrument according to the manufacturer's instructions. Determine the absorbance of each of the *Standard solutions*, of the *Sample solution*, and of the *Reagent blank* at 193.7 nm. Determine the corrected absorbance values by subtracting the *Reagent blank* absorbance from each of the *Standard solutions* and from the *Sample solution* absorbances. Prepare a standard curve by plotting the corrected absorbance of the *Standard solutions* versus the concentration of arsenic (ng/mL). Calculate the concentration (mg/kg) of arsenic in the sample taken:

$$\text{Result} = C/W \times F_1$$

  C = concentration of arsenic in the *Sample solution* determined from the standard curve (ng/mL)

  W = weight of the sample taken to prepare the *Sample solution* (g)

  F_1 = sample dilution factor, 12 mL

  Acceptance criteria: NMT 1 mg/kg

- **Lead**, *Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace Method, Method II,* Appendix IIIB

  Acceptance criteria: NMT 2 mg/kg

- **Sulfite**

  Mobile phase: 0.1 M sodium hydroxide and water (10:90)

  Diluent: Dilute 0.5 mL of 37% formaldehyde with water to 1000 mL.

  Standard stock solution: 1 mg/mL of sulfite in *Diluent*, prepared by dissolving 0.1574 g of sodium sulfite in and diluting to 100 mL with *Diluent*

Standard solutions: 2.0, 5.0, 10.0, and 20.0 mg/L of sulfite in *Diluent*, made from *Standard stock solution*
Sample solution: 3.0 mg/mL in *Diluent*
Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography[2]

Detector: Ion detector with anion self-regenerating conductivity suppressor[3]

Column: 25-cm × 4-mm, anion-exchange analytical column[4], and 5-cm × 4-mm anion-exchange guard column[5]

Flow rate: 0.7 mL/min
Injection size: 10 µL
System suitability

Sample: *Sample solution*
Relative standard deviation: NMT 3.0% for the sulfite peak area

Analysis: Separately inject equal volumes of the *Standard solutions* and *Sample solution* (previously filtered using a 0.2-µm syringe filter) into the chromatograph, and measure the responses for the major peaks on the resulting chromatograms. [Note—The approximate retention time for sulfite is 6 min.] Prepare a standard curve for sulfite by plotting sulfite peak areas versus concentrations in mg/L. From the standard curve, calculate the concentration (C_U) of sulfite in the *Sample solution* in mg/L. Calculate the percent sulfite in the portion of the sample taken:

$$\text{Result} = C_U/(C_{SMP} \times F_1) \times 100$$

C_U = concentration of sulfite in the *Sample solution* determined from the standard curve (mg/L)
C_{SMP} = concentration of the sample, on the dried basis, in the *Sample solution* (mg/mL)
F_1 = mL-to-L conversion factor, 1000

Acceptance criteria: NMT 0.5%, on the dried basis

Organic Impurities

- **Reducing Sugars**

Standard solutions: 0.10, 1.0, and 2.0 mg/mL of glucose
Sample solution: 10 mg/mL

Equipment: Flow injection analyzer[10] with flow set to "low" position on both pumps, and heater set at 90°. [Note—The signal should be less than ± 1000 micro-absorbance units before starting analysis.]

Analysis: Introduce 100 µL each of the *Sample solution* and the *Standard solutions* into the analyzer. For each analysis, air is introduced followed by addition of 2 mg/mL of Brij-35 (Polyoxyethyleneclycol dodecyl ether[11]), at a continuous flow of 0.287 mL/min. The solutions are then dialyzed through a cellulose membrane[12] . After dialysis add 1 M NaOH at 0.385 mL/min, and $CaCl_2$ and PHBH (*p*-hydroxylbenzoichydrazide), both at 0.074 mL/min, into the mixing chamber of the analyzer. The mixture then enters the heater (previously set at 90°) where bubbles are eliminated, after which it reaches the detector (set at 410 nm). Run duplicate injections of every *Sample solution*. Construct a calibration curve from the *Standard solutions*. Calculate the percent reducing sugars in the portion of the sample taken:

$$\text{Result} = C_U/C_{SMP} \times 100$$

C_U = concentration of reducing sugars, as glucose, in the *Sample solution* determined from the standard curve (mg/mL)

C_{SMP} = concentration of the sample, on the dried basis, in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 5.0% (as glucose), on the dried basis

SPECIFIC TESTS

- **Ash (Total)**, Appendix IIC

  Sample: 0.5–1 g, previously dried

  Analysis: Proceed as directed, but igniting at 550° for 1 h and then 900° for 10 min until all dark particles have disappeared and the ash is white.

  Acceptance criteria: NMT 14%, on the dried basis

- **Calcium**

  Standard stock solution: 3.00 µg/mL of calcium, prepared from a certified Calcium Standard Solution[13]. [Note—Store in polyethylene bottles because of its instability in glass.]

  Standard solutions: 0.750, 1.50, 2.25, and 3.00 µg/mL of calcium, made from *Standard stock solution*. [Note—Store in polyethylene bottles because of its instability in glass.]

  Sample stock solution: 4000 µg/mL prepared as follows. Transfer 0.2 g of a previously dried sample into a graduated flask. Add 5 mL of 65% nitric acid and 2 mL of 30% hydrogen peroxide. Boil for 1 h in a microwave oven. Dilute with water to 50 mL.

  Sample solution: 40 µg/mL from *Sample stock solution*

  Analysis: Using a suitably calibrated atomic absorption spectrophotometer, determine the absorbance of the *Standard solutions* and *Sample solution* at 422.7 nm. Prepare a standard curve for calcium by plotting calcium peak areas versus concentrations in mg/L. From the standard curve, determine the concentration (C_U) of calcium in the *Sample solution* in µg/mL. Calculate the percent calcium in the portion of the sample taken:

$$\text{Result} = C_U/C_{SMP} \times 100$$

  C_U = concentration of calcium in the *Sample solution* determined from the standard curve(µg/mL)

  C_{SMP} = concentration of the sample in the *Sample solution* (µg/mL)

  Acceptance criteria: NMT 5.0%, on the dried basis

- **Loss on Drying**, Appendix IIC: 105° for 24 h

  Acceptance criteria: NMT 8.0%

■1S (*FCC7*)

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1 Thermo Fisher Scientific, or equivalent.

2 Dionex Corporation (Sunnyvale, CA) ion exchange chromatograph, or equivalent.

3 ASRS-300 4mm (Dionex Corporation, Sunnyvale, CA), or equivalent.

4 IonPac AS11 (Dionex Corporation, Sunnyvale, CA), or equivalent.

5 IonPac AG11 (Dionex Corporation, Sunnyvale, CA), or equivalent.

6 Lignosulfonate calibration standards available from Borregaard Industries Limited, Borregaard LignoTech Research and Development, P.O. Box 162, NO-1701 Sarpsborg, Norway. Phone no: +47 69118000; e-mail: borregaard@borregaard.com.

7 Jordi Gel DVB Glucose (Jordi Labs, Bellingham, MA), or equivalent.

[8] TSK-Gel PWXL (TOSOH Bioscience, Montgomeryville, PA), or equivalent.

[9] Empower (Waters, Milford, MA), or equivalent.

[10] O.I. Analytical (College Station, TX), or equivalent.

[11] Ultra grade, O.I. Analytical (College Station, TX), or equivalent.

[12] Type C 25 MM (Astoria-Pacific, Inc, Clackamas, OR), or equivalent.

[13] NIST or equivalent.

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(FI07) Food Ingredients Expert Committee

BRIEFING

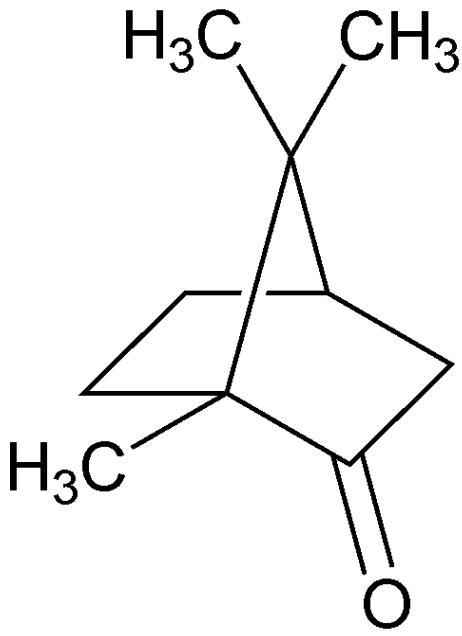
***d*-Camphor,** *FCC 6* page 154. On the basis of comments received, it is proposed to change the title of this monograph to reflect a less confusing and more widely accepted method of distinguishing optically active isomers. Also proposed is the addition of the existing name as a synonym.
(FIEC: K. Bowman) C79653

Change to read:

~~***d*-Camphor**~~■ **(+)-Camphor**■ 1S (*FCC7*)
■

d-Camphor

■ 1S (*FCC7*)



$C_{10}H_{16}O$

Formula wt 152.24
FEMA: 2230

DESCRIPTION

Change to read:

~~*d*-Camphor~~■ (+)-Camphor■ 1S (*FCC7*) occurs as a white to gray translucent crystalline or fused mass.

Odor: Minty, ethereal
Solubility: Soluble in alcohol; insoluble or practically insoluble in most fixed oils, propylene glycol, water
Boiling Point: ~204°
Solubility in Alcohol, Appendix VI**:** One mL dissolves in 1 mL of 95% alcohol.
Function: Flavoring agent

IDENTIFICATION

Change to read:

- ▲**Infrared Absorption**, *Spectrophotometric Identification Tests,* Appendix IIIC
  Reference standard: USP Camphor RS
  Sample and standard preparation: *M*
  Acceptance criteria: The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard.*▲*FCC6*

OTHER REQUIREMENTS

- **Melting Range or Temperature**, Appendix IIB
  Acceptance criteria: Between 174° and 179°
- **Angular Rotation**, *Optical (Specific) Rotation,* Appendix IIB
  Acceptance criteria: Between +41° and +43°

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 154

BRIEFING

Carbon, Activated, *FCC 6* page 165. On the basis of comments received, a revision to reinstate the previous test for *Heavy Metals (as Pb)* and limit to supplement the existing tests for arsenic and lead is proposed. The proposal maintains the quality of this food-grade ingredient and excludes metal-impregnated activated carbon products from this standard.
(FIEC: K. Bowman) C83327

Carbon, Activated

DESCRIPTION

Carbon, Activated occurs as a black substance, varying in particle size from coarse granules to a fine powder. It is a solid, porous, carbonaceous material prepared by carbonizing and activating organic substances. The raw materials, which include sawdust, peat, lignite, coal, cellulose residues, coconut shells, and petroleum coke, may be carbonized and activated at a high temperature with or without the addition of inorganic salts in a stream of activating gases such as steam or carbon dioxide. Alternatively, carbonaceous matter may be treated with a chemical activating agent such as phosphoric acid or zinc chloride, and the mixture carbonized at an elevated temperature, followed by removal of the chemical activating agent by water washing. Activated Carbon is insoluble in water and in organic solvents.

Function: Decolorizing agent; taste- and odor-removing agent; purification agent in food processing
Packaging and Storage: Store in well-closed containers.

IDENTIFICATION

- **A. Procedure**
  Sample: 3 g, powdered
  Control: Dilute 10 mL of iodine TS with water to 50 mL.
  Analysis: Place the *Sample* in a glass-stoppered Erlenmeyer flask containing 10 mL of dilute hydrochloric acid (5%), boil for 30 s, and cool to room temperature. Add 100 mL of iodine TS, stopper, and shake vigorously for 30 s. Filter through Whatman No. 2 filter paper, or equivalent, discarding the first portion of filtrate. Compare 50 mL of the subsequent filtrate with the *Control.*
  Acceptance criteria: The color of the carbon-treated iodine solution is no darker than that of the *Control,* indicating the adsorptivity of the sample.
- **B. Procedure**
  Analysis: Ignite a sample in air.
  Acceptance criteria: Carbon monoxide and carbon dioxide are produced, and an ash remains.

IMPURITIES

Change to read:
Inorganic Impurities

- **Arsenic,** *Arsenic Limit Test,* Appendix IIIB
  Sample solution: Use a 20-mL portion of the filtrate obtained in the test for *Water Extractables*, diluted to 35 mL.
  Acceptance criteria: NMT 3 mg/kg
- **Lead,** *Lead Limit Test,* Appendix IIIB
  Sample solution: Use a 20-mL portion of the filtrate obtained in the test for *Water Extractables*.
  Control: 10 µg Pb (10 mL of *Diluted Standard Lead Solution*)

Acceptance criteria: NMT 10 mg/kg

- **Heavy Metals (as Pb)**

Method I

[Note—This test is designed to limit the content of common metallic impurities colored by sulfide ion (Ag, As, Bi, Cd, Cu, Hg, Pb, Sb, Sn) by comparing the color with a standard containing lead (Pb) ion under the specified test conditions. It demonstrates that the test substance is not grossly contaminated by such heavy metals, and within the precision of the test, that it does not exceed the *Heavy Metals* limit given as determined by concomitant visual comparison with a control solution. In the specified pH range, the optimum concentration of lead (Pb) ion for matching purposes by this method is 20 µg in 50 mL of solution.

The most common limitation of the *Heavy Metals* test is that the color the sulfide ion produces in the *Sample solution* depends on the metals present and may not match the color in the dilution of the *Standard lead solution* used for matching purposes. Lead sulfide is brown, as are Ag, Bi, Cu, Hg, and Sn sulfides. While it is possible that ions not mentioned here may also yield nonmatching colors, among the nine common metallic impurities listed above, the sulfides with different colors are those of As and Cd, which are yellow, and that of Sb, which is orange. If these criteria are met, Cd may be a contributor to the yellow color, so the Cd content should be determined. If an orange color is observed, the Sb content should be determined. These additional tests are in accord with the section on *Trace Impurities* in the *General Provisions*, as follows: "if other possible impurities may be present, additional tests may be required, and should be applied, as necessary, by the manufacturer, vendor, or user to demonstrate that the substance is suitable for its intended application.

Determine the amount of heavy metals by *Method I* for substances that yield clear, colorless solutions before adding sulfide ion. Use *Method II* for those substances that do not yield clear, colorless solutions under the test conditions specified for *Method I* or for substances that by virtue of their complex nature, interfere with the precipitation of metals by sulfide ion.]

[Note—In the following tests, failure to accurately adjust the pH of the solution within the specified limits may result in a significant loss of test sensitivity.]

Lead nitrate stock solution: Dissolve 159.8 mg of Reagent-Grade ACS Lead Nitrate [$Pb(NO_3)_2$] in 100 mL of water containing 1 mL of nitric acid, dilute to 1000.0 mL, and mix. [Note—Prepare and store this solution in glass containers that are free from lead salts.]

Standard lead solution: Dilute 10.0 mL of *Lead nitrate stock solution* with water to 100.0 mL. Each mL is equivalent to 10 µg of lead (Pb) ion. [Note—Prepare on the day of use.]

Sample solution: Use the filtrate obtained in the test for *Water Extractables*.

Solution A: Pipet 2.0 mL of *Standard lead solution* (20 µg of Pb) into a 50-mL color-comparison tube, and add water to make 25 mL. Adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper) by adding 1 N acetic acid or 6 N ammonia, dilute with water to 40 mL, and mix.

Solution B: Transfer 10 mL of the *Sample solution* into a 50-mL color-comparison tube that matches the one used for *Solution A*, adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper) by adding 1 N acetic acid or 6 N ammonia, dilute with water to 40 mL, and mix.

Solution C: Transfer 10 mL of the *Sample solution* into a third color-comparison tube that matches those used for *Solutions A* and *B*, and add 2.0 mL of *Standard lead solution*. Adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper) by adding 1 N acetic acid or 6 N ammonia, dilute with water to 40 mL, and mix.

Analysis: Add 10 mL of freshly prepared hydrogen sulfide TS to each tube, mix, allow to stand for 5 min, and view downward over a white surface. [Note—If the color of *Solution C* is lighter than that of *Solution A*, the sample is interfering with the test procedure and *Method II* must be used.]

Acceptance criteria: The color of *Solution B* is not darker than that of *Solution A*, and the intensity of the

color of *Solution C* is equal to or greater than that of *Solution A* (NMT 0.004%).

Method II

Solution A: Prepare as directed in *Method I*.

Solution B: Place 0.5 g of sample into 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 sample is thoroughly carbonized, add 2 mL of nitric acid and 5 drops of sulfuric acid, cautiously heat until white fumes no longer evolve, then ignite, preferably in a muffle furnace, at 500° to 600° until all of the carbon is burned off. Cool, add 4 mL of 1:2 hydrochloric acid, cover, and digest on a steam bath for 10 to 15 min. Uncover, and slowly evaporate on a steam bath to dryness. Moisten the residue with 1 drop of hydrochloric acid, add 10 mL of hot water, and digest for 2 min. Add 6 N ammonia dropwise until the solution is just alkaline to litmus paper, dilute with water to 25 mL, and adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper) by adding 1 N acetic acid. Filter if necessary, rinse the crucible and the filter with 10 mL of water, transfer the solution and rinsings into a 50-mL color-comparison tube, dilute with water to 40 mL, and mix.

Analysis: Add 10 mL of freshly prepared hydrogen sulfide TS to each tube, mix, allow to stand for 5 min, and view downward over a white surface.

Acceptance criteria: The color of *Solution B* is not darker than that of *Solution A* (NMT 0.004%).

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Organic Impurities

- **Cyanogen Compounds**

Sample: 5 g

Analysis: Mix the *Sample* with 50 mL of water and 2 g of tartaric acid and distill the mixture, collecting 25 mL of distillate below the surface of a mixture of 2 mL of 1 N sodium hydroxide and 10 mL of water contained in a small flask placed in an ice bath. Dilute the distillate with water to 50 mL and mix. Add 12 drops of ferrous sulfate TS to 25 mL of the diluted distillate, heat almost to boiling, cool, and add 1 mL of hydrochloric acid.

Acceptance criteria: No blue color is produced.

- **Higher Aromatic Hydrocarbons**

Sample solution: Extract 1 g of sample with 12 mL of cyclohexane in a continuous-extraction apparatus for 2 h. Place the extract in a Nessler tube.

Control solution: Dissolve 100 µg of quinine sulfate in 1000 mL of 0.1 N sulfuric, and transfer into a matching Nessler tube.

Acceptance criteria: The *Sample solution* shows no more color or fluorescence than the *Control solution* when observed under ultraviolet light.

SPECIFIC TESTS

- **Iodine Number**[1]

Hydrochloric acid solution: (5% by weight): Add 70 mL of concentrated hydrochloric acid to 550 mL of water, and mix well.

Potassium iodate solution: (0.1000 N): Dry 4 or more g of primary standard-grade potassium iodate (KIO_3) at 110 ± 5° for 2 h, and cool to room temperature in a desiccator. Dissolve 3.5667 ± 0.1 mg of the dry potassium iodate in about 100 mL of water. Quantitatively transfer to a 1-L volumetric flask, dilute with water to volume, and mix thoroughly. Store in a glass-stoppered bottle.

Starch solution: Mix 1.0 ± 0.5 g of starch with 5 to 10 mL of cold water to make a paste. Continue to stir while adding an additional 25 ± 5 mL of water to the starch paste. Pour the mixture, while stirring, into 1 L of boiling water, and boil for 4 to 5 min. Make this solution fresh daily.

Sodium thiosulfate solution: (0.100 N): Dissolve 24.820 g of sodium thiosulfate in approximately 75 ± 25

mL of freshly boiled water, and add 0.10 ± 0.01 g of sodium carbonate. Quantitatively transfer the mixture to a 1-L volumetric flask, and dilute with water to volume. Allow the solution to stand for a minimum of 4 days before standardizing. Store the solution in an amber bottle. To standardize the solution, perform the following in triplicate: pipet 25.0 mL of 0.1000 N *Potassium iodate solution* into a wide-mouthed Erlenmeyer flask. Add 2.00 ± 0.01 g of potassium iodide, and shake the flask to dissolve the potassium iodide crystals. Pipet 5.0 mL of concentrated hydrochloric acid into the flask, and titrate the free iodine with *Sodium thiosulfate solution* to a light yellow color. Add a few drops of *Starch solution*, and continue the titration until 1 drop produces a colorless solution. Determine the *Sodium thiosulfate solution* normality:

$$\text{Result} = (P \times R)/S$$

P = volume of 0.1000 N *Potassium iodate solution* (mL)
R = normality of the 0.1000 N *Potassium iodate solution*
S = volume of *Sodium thiosulfate solution* (mL)

Average the three normality results. Repeat the test if the range of values exceeds 0.003 N.

Standard iodine solution: (0.100 ± 0.001 N): Transfer 12.700 g of iodine and 19.100 g of potassium iodide (KI), accurately weighed, into a beaker, and mix. Add 2 to 5 mL of water, and stir well. While stirring, continue to add small increments, approximately 5 mL each, of water until the total volume is 50 to 60 mL. Allow the solution to stand a minimum of 4 h to ensure crystal dissolution, stirring occasionally. Quantitatively transfer the solution to a 1-L volumetric flask, and dilute with water to volume. The iodide-to-iodine weight ratio must be 1.5:1. Store the solution in an amber bottle.

[Note—Standardize this solution just before use.]

To standardize this solution, perform the following in triplicate. Pipet 25.0 mL into a 250-mL wide-mouthed Erlenmeyer flask. Titrate with the standardized *Sodium thiosulfate solution* until a light yellow color develops. Add a few drops of *Starch solution*, and continue the titration until 1 drop produces a colorless solution. Determine the *Iodine solution* normality:

$$\text{Result} = (S \times N_1)/I$$

S = volume of the standardized *Sodium thiosulfate solution* (mL)
N_1 = normality of the standardized *Sodium thiosulfate solution,* determined above
I = volume of *Iodine solution* (mL)

Average the three normality results. Repeat the test if the range of values exceeds 0.003 N. The standardized *Iodine solution* concentration must be 0.100 ± 0.001 N.

Sample preparation: Grind a representative sample until 60 wt % (or more) passes through a 325-mesh screen and 95 wt % (or more) passes through a 100-mesh screen. Dry the ground sample, and cool to room temperature in a desiccator.

Analysis: Three dosages of *Sample preparation* must be estimated to determine the iodine number. Weigh the three dosages (M) of dry carbon to the nearest mg. Transfer each to one of three clean, dry 250-mL Erlenmeyer flasks equipped with ground glass stoppers. Pipet 10.0 mL of *Hydrochloric acid solution* into each flask, stopper each flask, and swirl gently until the carbon is completely wetted. Loosen the stoppers to vent the flasks, place on a hot plate in a fume hood, and bring the contents to a boil. Allow to boil gently for 30 ± 2 s to remove any sulfur (which may interfere with the test results). Remove the flasks from the hot plate and cool to room temperature. Standardize and then pipet 100.0 mL of *Iodine solution* into each flask. [Note—Stagger the addition of standardized *Iodine solution* to the three flasks so that no delays are encountered in handling.]

Immediately stopper the flasks, and shake the contents vigorously for 30 ± 1 s. Quickly filter each mixture by gravity through one sheet of folded filter paper (Whatman No. 2V, or equivalent) into one of three beakers. [Note—Prepare the filtration equipment in advance to avoid delays in filtering the samples.]

For each filtrate, use the first 20 to 30 mL to rinse a pipet, and discard the rinse portions. Use clean beakers to collect the remaining filtrates. Mix each filtrate by swirling the beaker, and pipet 50.0 mL of each filtrate into one of three clean 250-mL Erlenmeyer flasks. Titrate each filtrate with standardized *Sodium thiosulfate solution* until a pale yellow color develops. Add 2 mL of *Starch solution*, and continue the titration with standardized *Sodium thiosulfate solution* until 1 drop produces a colorless solution. Record the volume (S) of standardized *Sodium thiosulfate solution* used.

Calculations: The capacity of a carbon for any adsorbate depends on the concentration of the adsorbate. The concentrations of the standard iodine solution and filtrate must be known to determine an appropriate carbon weight to produce final concentrations agreeing with the definition of iodine number. The amount of sample to be used in the determination is governed by the activity of the sample. If filtrate normalities (C) are not within the range of 0.008 N to 0.040 N, repeat the procedure using different sample weights. Once filtrate normalities are set within the specified range, perform the following calculations for each carbon dosage:

$$A = N_2 \times 12693.0$$

N_2 = normality of the standardized *Iodine solution*

$$B = N_1 \times 126.93$$

N_1 = normality of the standardized *Sodium thiosulfate solution*

Calculate the dilution factor (DF):

$$DF = (I + H)/F$$

I = volume of *Iodine solution* used in the standardization procedure (mL)
H = volume of *Hydrochloric acid solution* used (mL)
F = volume of filtrate (mL)

Calculate the weight, in mg, of iodine adsorbed per g of sample (X/M):

$$X/M = [A - (DF) \times (B) \times (S)]/M$$

S = volume of standardized *Sodium thiosulfate solution* (mL)
M = weight of the *Sample* taken (g)

Calculate the normality of the residual filtrate (C):

$$C = (N_1 \times S)/F$$

Using logarithmic paper, plot X/M (as the ordinate) versus C (as the abscissa) for each of the three carbon dosages. Calculate the least squares fit for the three points, and plot. The iodine number is the X/M value at a residual iodine concentration (C) of 0.02 N. The regression coefficient for the least squares fit should be greater than 0.995. Carbon dosages may be estimated initially by using three values of C (usually 0.01, 0.02, and 0.03):

$$M = [A - (DF) \times (C) \times (126.93) \times (50)]/E$$

M = weight of the carbon dosage (g)
E = nominal iodine number of the *Sample*

If new carbon dosages have been determined, repeat the *Analysis* and *Calculations*.

Acceptance criteria: NLT 400

- **Loss on Drying,** Appendix IIC (120° for 4 h)
  Acceptance criteria: Results conform to the representations of the vendor.
- **Residue on Ignition (Sulfated Ash),** Appendix IIC
  Sample: 500 g
  Acceptance criteria: Results conform to the representations of the vendor.
- **Water Extractables**
  Sample: 5 g
  Analysis: Transfer the *Sample* into a 250-mL flask provided with a reflux condenser and a Bunsen valve. Add 100 mL of water and several glass beads, and reflux for 1 h. Cool slightly, and filter through Whatman No. 2, or equivalent, filter paper, discarding the first 10 mL of filtrate. Cool the subsequent filtrate to room temperature, and pipet 25.0 mL into a tared crystallization dish. [Note—Retain the remainder of the filtrate for the tests for *Arsenic* and *Lead*.] Evaporate the filtrate in the dish to incipient dryness on a hot plate, never allowing the solution to boil. Dry for 1 h at 100° in a vacuum oven, cool, and weigh.
  Acceptance criteria: NMT 4.0%

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[1] Portions of this test are adapted from "ASTM D 4607-94(1999)—Standard Test Method for Determination of Iodine Number of Activated Carbon". The original ASTM method is available in its entirety from ASTM, 100 Barr Harbor Drive, West Conshohocken, PA 19428; phone: 610-832-9585; fax: 610-832-9555; e-mail: service@astm.org; website: www.astm.org.

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 165

BRIEFING

***l*-Carveol,** *FCC 6* page 176. On the basis of comments received, a revision to change the title of this monograph to reflect a less confusing and more widely accepted method of distinguishing optically active isomers is proposed. Also proposed is the addition of the existing name as a synonym.
(FIEC: K. Bowman) C79714

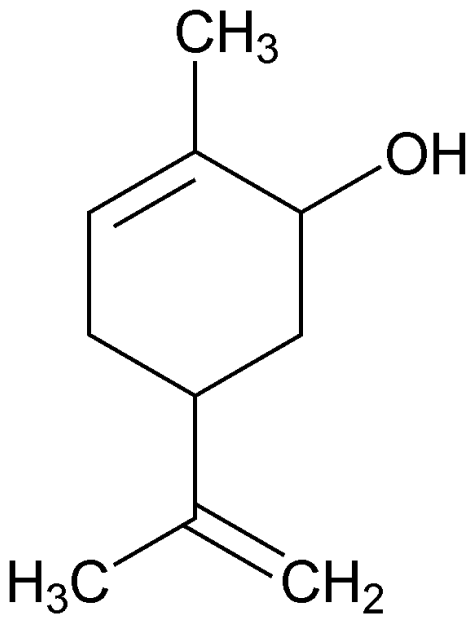
Change to read:
~~***l*-Carveol**~~■ **(–)-Carveol**■ 1S (*FCC7*)

Change to read:
■

l-Carveol

■ 1S (*FCC7*)

p-Mentha-6,8-dien-2-ol



$C_{10}H_{16}O$

Formula wt 152.24
FEMA: 2247

DESCRIPTION

Change to read:
~~*l*-Carveol~~■ (–)-Carveol■ 1S (*FCC7*) occurs as a colorless to pale yellow liquid.

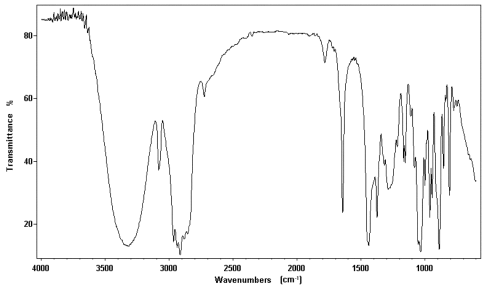
Odor: Spearminty
Solubility: Soluble in propylene glycol, vegetable oils; insoluble or practically insoluble in water

Boiling Point: ~226° to 227° (751 mm Hg)
Solubility in Alcohol, Appendix VI**:** One mL dissolves in 1 mL of 95% alcohol.
Function: Flavoring agent

IDENTIFICATION

Change to read:

- **Infrared Spectra**, *Spectrophotometric Identification Tests,* Appendix IIIC
  Acceptance criteria: The spectrum of the sample exhibits relative maxima at the same wavelengths as those of the spectrum below.



~~*l*-Carveol~~ (–)-Carveol 1S (*FCC7*)

ASSAY

- **Procedure:** Proceed as directed under *M-1b,* Appendix XI.
  Acceptance criteria: NLT 96.0% of $C_{10}H_{16}O$

SPECIFIC TESTS

- **Refractive Index**, Appendix II (at 20°)
  Acceptance criteria: Between 1.493 and 1.497
- **Specific Gravity:** Determine at 25° by any reliable method (see *General Provisions*).
  Acceptance criteria: Between 0.947 and 0.953

OTHER REQUIREMENTS

- **Angular Rotation**, *Optical (Specific) Rotation,* Appendix IIB
  Acceptance criteria: Between −117° and −130°

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

BRIEFING

***d*-Carvone,** *FCC 6* page 177. On the basis of comments received, it is proposed to change the title of this monograph to reflect a less confusing and more widely accepted method of distinguishing optically active isomers. Also proposed is the addition of the existing name as a synonym.
(FIEC: K. Bowman) C79715

Change to read:

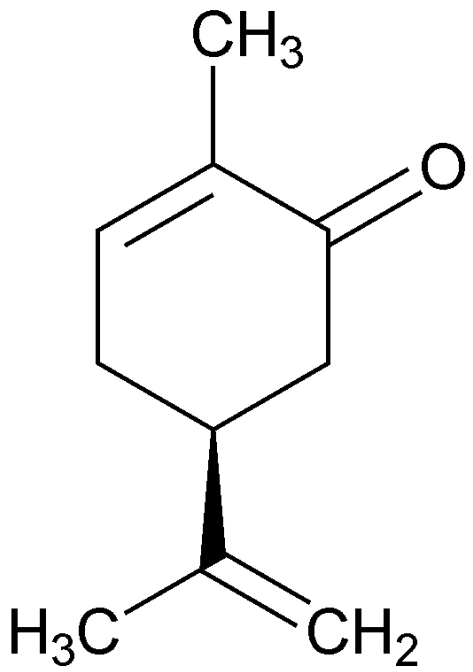
~~***d*-Carvone**~~■ **(+)-Carvone**■ 1S (*FCC7*)

Change to read:

■ *d*-Carvone■ 1S (*FCC7*)

dextro-Carvone

d-1-Methyl-4-isopropenyl-6-cyclohexen-2-one



$C_{10}H_{14}O$

Formula wt 150.22
FEMA: 2249

DESCRIPTION

Change to read:

~~*d*-Carvone~~■ (+)-Carvone■ 1S (*FCC7*) occurs as a colorless to light yellow liquid.

Odor: Caraway

Solubility: Soluble in propylene glycol, most fixed oils; miscible in alcohol; insoluble or practically insoluble in glycerin

Boiling Point: ~230°

Solubility in Alcohol, Appendix VI**:** One mL dissolves in 5 mL of 60% alcohol.

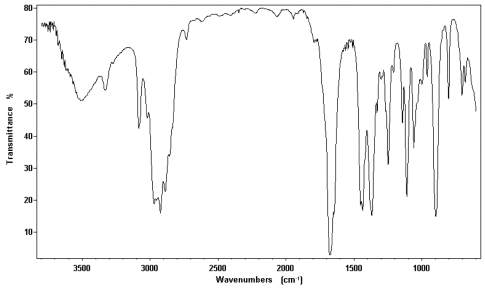
Function: Flavoring agent

IDENTIFICATION

Change to read:

- **Infrared Spectra,** *Spectrophotometric Identification Tests,* Appendix IIIC

  Acceptance criteria: The spectrum of the sample exhibits relative maxima at the same wavelengths as those of the spectrum below.



~~*d*-Carvone~~■ (+)-Carvone■ 1S (*FCC7*)

ASSAY

- **Procedure:** Proceed as directed under *M-1b,* Appendix XI.

  Acceptance criteria: NLT 95.0% of $C_{10}H_{14}O$

SPECIFIC TESTS

- **Refractive Index,** Appendix II (at 20°)

  Acceptance criteria: Between 1.496 and 1.499

- **Specific Gravity:** Determine at 25° by any reliable method (see *General Provisions*).

  Acceptance criteria: Between 0.955 and 0.960

OTHER REQUIREMENTS

- **Angular Rotation,** *Optical (Specific) Rotation,* Appendix IIB (Use a 100-mm tube.)

  Acceptance criteria: Between +50° and +60°

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 177

BRIEFING

***l*-Carvone,** *FCC 6* page 178. On the basis of comments received, a revision to change the title of this monograph to reflect a less confusing and more widely accepted method of distinguishing optically active isomers is proposed. Also proposed is the addition of the existing name as a synonym.
(FIEC: K. Bowman) C79716

Change to read:

~~***l*-Carvone**~~■ **(–)-Carvone**■ 1S (*FCC7*)

Change to read:

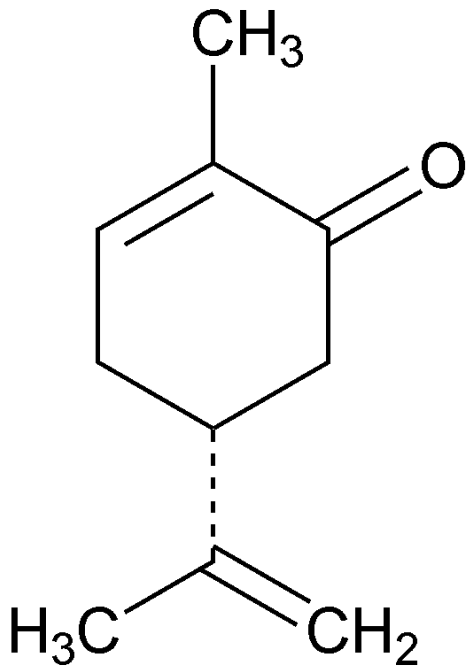
■

l-Carvone

■ 1S (*FCC7*)

levo-Carvone

1-1-Methyl-4-isopropenyl-6-cyclohexen-2-one



$C_{10}H_{14}O$

Formula wt 150.22
FEMA: 2249

DESCRIPTION

Change to read:

~~*l*-Carvone~~■ (–)-Carvone■ 1S (*FCC7*) occurs as a colorless to pale strawberry colored liquid.

Odor: Spearminty

Solubility: Soluble in propylene glycol, most fixed oils; miscible in alcohol; insoluble or practically insoluble in glycerin

Boiling Point: ~231°

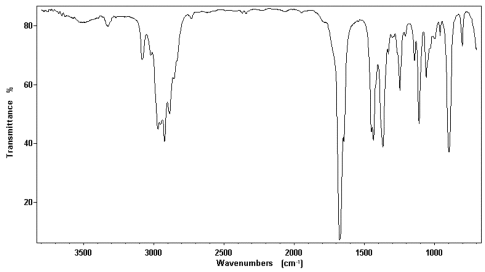
Solubility in Alcohol, Appendix VI**:** One mL dissolves in 2 mL of 70% alcohol.

Function: Flavoring agent

IDENTIFICATION

Change to read:

- **Infrared Spectra**, *Spectrophotometric Identification Tests,* Appendix IIIC
  Acceptance criteria: The spectrum of the sample exhibits relative maxima at the same wavelengths as those of the spectrum below.



~~*l*-Carvone~~■ (–)-Carvone■ 1S (*FCC7*)

ASSAY

- **Procedure:** Proceed as directed under *M-1b,* Appendix XI.
  Acceptance criteria: NLT 97.0% of $C_{10}H_{14}O$

SPECIFIC TESTS

- **Refractive Index**, Appendix II (at 20°)
  Acceptance criteria: Between 1.495 and 1.502
- **Specific Gravity:** Determine at 25° by any reliable method (see *General Provisions*).
  Acceptance criteria: Between 0.956 and 0.960

OTHER REQUIREMENTS

- **Angular Rotation**, *Optical (Specific) Rotation,* Appendix IIB**:** Use a 100-mm tube.
  Acceptance criteria: Between −57° and −62°

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 178

BRIEFING

***l*-Carvyl Acetate,** *FCC 6* page 179. On the basis of comments received, it is proposed to change the title of this monograph to reflect a less confusing and more widely accepted method of distinguishing optically active isomers. Also proposed is the addition of the existing name as a synonym.
(FIEC: K. Bowman) C79717

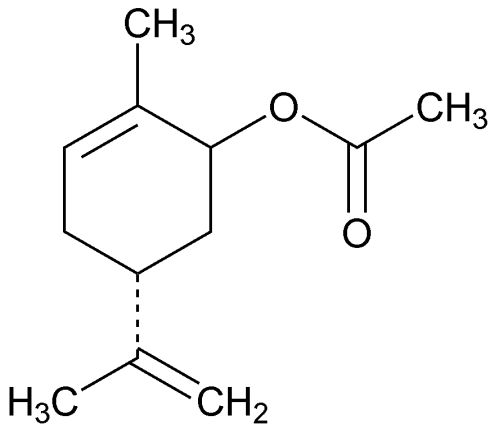
Change to read:
~~***l*-Carvyl Acetate**~~■ **(–)-Carvyl Acetate**■ 1S (*FCC7*)

Change to read:
■

l-Carvyl Acetate

■ 1S (*FCC7*)

p-Mentha-6,8-dien-2-yl Acetate



$C_{12}H_{18}O_2$

Formula wt 194.27
FEMA: 2250

DESCRIPTION

Change to read:
~~*l*-Carvyl Acetate~~■ (–)-Carvyl Acetate■ 1S (*FCC7*) occurs as a colorless to pale yellow liquid.

Odor: Spearminty
Solubility: Soluble in alcohol

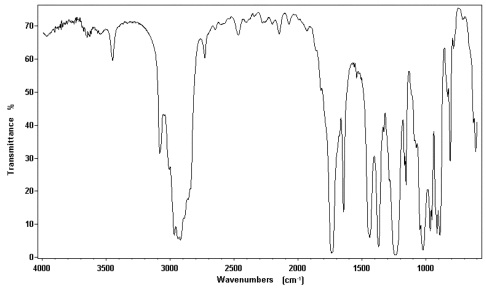
Boiling Point: ~77° to 79° (0.1 mm Hg)
Function: Flavoring agent

IDENTIFICATION

Change to read:

- **INFRARED SPECTRA**, *Spectrophotometric Identification Tests,* Appendix IIIC

  Acceptance criteria: The spectrum of the sample exhibits relative maxima at the same wavelengths as those of the spectrum below.



~~*l*-Carvyl Acetate~~■(–)-Carvyl Acetate■1S (*FCC7*)

ASSAY

- **PROCEDURE:** Proceed as directed under *M-1b,* Appendix XI.

  Acceptance criteria: NLT 98.0% of $C_{12}H_{18}O_2$

SPECIFIC TESTS

- **ACID VALUE**, *M-15,* Appendix XI

  Acceptance criteria: NMT 1.0

- **REFRACTIVE INDEX**, Appendix II (at 20°)

  Acceptance criteria: Between 1.473 and 1.479

- **SPECIFIC GRAVITY:** Determine at 25° by any reliable method (see *General Provisions*).

  Acceptance criteria: Between 0.964 and 0.970

OTHER REQUIREMENTS

- **ANGULAR ROTATION**, *Optical (Specific) Rotation,* Appendix IIB

  Acceptance criteria: Between −90° and −120°

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 179

BRIEFING

5′-Cytidylic Acid. Because there is no existing *FCC* monograph for this food ingredient, a new monograph is proposed on the basis of data received. See the Briefing under *Disodium 5′-Uridylate*.
(FIEC: J. Moore) C82096

Add the following:

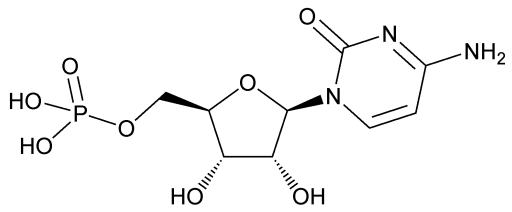
■ ***5′-Cytidylic Acid***

Cytidine 5′-monophosphate

Cytidylic acid

CMP

Cytidine 5′-phosphoric acid



$C_9H_{14}N_3O_8P$

Formula wt 323.20
CAS: [63-37-6]

DESCRIPTION

5′-Cytidylic Acid occurs as colorless or white crystals, or as a white, crystalline powder. It is very slightly soluble in water, and practically insoluble in alcohol. It is produced by enzymatic cleavage of natural source yeast RNA with a 5-phosphodiesterase followed by heat treatment, further purification steps, and washing of crystals with ethanol.

Function: Source of 5′-Cytidylic Acid

Packaging and Storage: Store in tight containers protected from light and moisture.

IDENTIFICATION

- **A. Infrared Absorption,** *Spectrophotometric Identification Tests,* Appendix IIIC
  Reference standard: USP 5′-Cytidylic Acid RS
  Sample and standard preparation: *A*
  Acceptance criteria: The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard.*
- **B. Procedure**
  Acceptance criteria: The retention time of the major peak (excluding the solvent peak) in the chromatogram of the *Sample solution* corresponds to that of the *Standard solution* in the *Assay*.

ASSAY

- **PROCEDURE**

Mobile phase: 0.1 M potassium dihydrogen phosphate (KH_2PO_4) in degassed water, adjusted to pH 5.6 with 0.1 M dipotassium hydrogen phosphate (K_2HP0_4)

Standard solution: 0.02 mg/mL of USP 5′-Cytidylic Acid RS in *Mobile phase.* [Note—Ultra-sonication may be necessary to aid in complete dissolution.]

Sample solution: 0.02 mg/mL in *Mobile phase.* [Note—Ultra-sonication may be necessary to aid in complete dissolution.]

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography

Detector: UV 254 nm

Column: 25-cm × 4.6-mm; packed with 5-µm reversed phase C18 silica gel[1]

Column temperature: Ambient

Flow rate: About 1.0 mL/min

Injection size: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Suitability requirement 1: The relative standard deviation of the 5′-cytidylic acid peak area responses from replicate injections is NMT 2.0%.

Suitability requirement 2: The resolution, *R*, between the 5′-cytidylic acid peak and all other peaks is NLT 2.0.

Analysis: Separately inject equal volumes of the *Standard solution* and *Sample solution* into the chromatograph, and measure the responses for the major peaks on the resulting chromatograms. [Note—The approximate retention time for 5′-cytidylic acid is 4.6 min.] Calculate the percent disodium 5′-cytidylic acid, $C_9H_{14}N_3O_8P$, in the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area response for 5′-cytidylic acid in the *Sample solution*

r_S = peak area response for 5′-cytidylic acid in the *Standard solution*

C_S = concentration of 5′-cytidylic acid in the *Standard solution* (mg/mL)

C_U = concentration of sample in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–103.0%, calculated on the anhydrous basis

IMPURITIES

Inorganic Impurities

- **ARSENIC**

[Note—When water is specified as a diluent, use deionized ultra-filtered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of arsenic as practical.]

Diluent: 4% nitric acid in water

Standard stock solution: 100 µg/mL of arsenic prepared by diluting a commercially available 1000 mg/kg arsenic ICP standard solution

Standard solutions: 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL of arsenic: from *Standard stock solution* diluted with *Diluent*

Sample: 5 g

Sample solution: Dissolve the *Sample* in 40 mL of 10% nitric acid in a 100-mL volumetric flask, and dilute with water to volume.

Spectrophotometric system, *Plasma Spectrochemistry,* Appendix IIC

Mode: Inductively coupled plasma–optical emission spectroscopy (ICP–OES)

Setup: Use a suitable ICP–OES configured in a radial optical alignment. [Note—This method was developed using a Varian Vista MPX ICP OES unit.] The instrument parameters are as follows: set the ultra-violet detector to scan arsenic at 188.980 nm. Set the sample read time to 20 s. Set the forward power from the RF generator to 1150 watts. Use an argon plasma feed gas flow of 13.5 L/min with the auxiliary gas set to flow at 2.25 L/min. The sample is delivered to the spray chamber by a multi-channel peristaltic pump set to deliver sample at a rate of 20 rpm. Samples are flushed through the system for 20 s prior to analysis. A 40-s read delay is also programmed into the sampling routine to allow for fluid flow equilibration after the high-speed flush, prior to the first analytical read of the sample. Between samples, the pumping system is washed by flushing the *Diluent* for 20 s.

Analysis: Generate a standard curve using *Diluent* as a *Blank* and the *Standard solutions.* [Note—The correlation coefficient for the best-fit line should not be less than 0.999.]

Similarly, analyze the *Sample solution* on the ICP. Calculate the concentration (mg/kg) of arsenic in the *Sample* taken:

$$\text{Result} = (C/W) \times F$$

C = concentration of arsenic in the *Sample solution* determined from the standard curve (µg/mL)

W = weight of *Sample* taken (g)

F = *Sample solution* final volume, 100 mL

Acceptance criteria: NMT 2 mg/kg

- **CADMIUM**

[Note—When water is specified as a diluent, use deionized ultra-filtered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of cadmium as practical.]

Diluent: 4% nitric acid in water

Standard stock solution: 100 µg/mL of cadmium prepared by diluting a commercially available 1000 mg/kg cadmium ICP standard solution

Standard solutions: 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL of cadmium: from *Standard stock solution* diluted with *Diluent*

Sample: 5 g

Sample solution: Dissolve the *Sample* in 40 mL of 10% nitric acid in a 100-mL volumetric flask, and dilute with water to volume.

Spectrophotometric system, *Plasma Spectrochemistry,* Appendix IIC

Mode: Inductively coupled plasma–optical emission spectroscopy (ICP–OES)

Setup: Same as that described in the test for *Arsenic,* but set to scan for cadmium at 228.802 nm

Analysis: Generate a standard curve using *Diluent* as a *Blank* and the *Standard solutions.* [Note—The correlation coefficient for the best-fit line should not be less than 0.999.]

Similarly, analyze the *Sample solution* on the ICP. Calculate the concentration (mg/kg) of cadmium in the *Sample* taken:

$$\text{Result} = (C/W) \times F$$

C = concentration of cadmium in the *Sample solution* determined from the standard curve (µg/mL)

W = weight of *Sample* taken (g)

F = *Sample solution* final volume, 100 mL

Acceptance criteria: NMT 0.1 mg/kg

- **LEAD**

[Note—When water is specified as a diluent, use deionized ultra-filtered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of lead as practical.]

Diluent: 4% nitric acid in water

Standard stock solution: 100 µg/mL of lead prepared by diluting a commercially available 1000 mg/kg lead ICP standard solution

Standard solutions: 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL of lead: from *Standard stock solution* diluted with *Diluent*

Sample: 5 g

Sample solution: Dissolve the *Sample* in 40 mL of 10% nitric acid in a 100-mL volumetric flask, and dilute with water to volume.

Spectrophotometric system, *Plasma Spectrochemistry,* Appendix IIC

Mode: Inductively coupled plasma–optical emission spectroscopy (ICP–OES)

Setup: Same as that described in the test for *Arsenic,* but set to scan for lead at 220.353 nm

Analysis: Generate a standard curve using *Diluent* as a *Blank* and the *Standard solutions.* [Note—The correlation coefficient for the best-fit line should not be less than 0.999.]

Similarly, analyze the *Sample solution* on the ICP. Calculate the concentration (mg/kg) of lead in the *Sample* taken:

$$\text{Result} = (C/W) \times F$$

C = concentration of lead in the *Sample solution* determined from the standard curve (µg/mL)

W = weight of *Sample* taken (g)

F = *Sample solution* final volume, 100 mL

Acceptance criteria: NMT 1 mg/kg

- **MERCURY**

[Note—When water is specified as a diluent, use deionized ultra-filtered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of mercury as practical.]

Diluent: 4% nitric acid in water

Standard stock solution: 100 µg/mL of mercury prepared by diluting a commercially available 1000 mg/kg mercury ICP standard solution

Standard solutions: 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL of mercury: from *Standard stock solution* diluted with *Diluent*

Sample: 5 g

Sample solution: Dissolve the *Sample* in 40 mL of 10% nitric acid in a 100-mL volumetric flask, and dilute with water to volume.

Spectrophotometric system, *Plasma Spectrochemistry,* Appendix IIC

Mode: Inductively coupled plasma–optical emission spectroscopy (ICP–OES)

Setup: Same as that described in the test for *Arsenic,* but set to scan for mercury at 194.164 nm

Analysis: Generate a standard curve using *Diluent* as a *Blank* and the *Standard solutions.* [Note—The correlation coefficient for the best-fit line should not be less than 0.999.]

Similarly, analyze the *Sample solution* on the ICP. Calculate the concentration (mg/kg) of mercury in the *Sample* taken:

$$\text{Result} = (C/W) \times F$$

C = concentration of mercury in the *Sample solution* determined from the standard curve (µg/mL)
W = weight of *Sample* taken (g)
F = *Sample solution* final volume, 100 mL

Acceptance criteria: NMT 0.5 mg/kg

Organic Impurities

- **ETHANOL**

Standard solution: 20 mg/kg of ethanol in 1 N sodium hydroxide. Add 10 mL of this solution to a 20-mL headspace vial, and cap tightly.

Sample solution: 100 mg/g in 1 N sodium hydroxide. Add 10 mL of this solution to a 20-mL headspace vial, and cap tightly.

Chromatographic system, Appendix IIA

Mode: Gas chromatography equipped with pressure-loop headspace autosampler

Detector: Flame ionization

Column: 30-m × 0.53-mm (id) capillary column with a 6% cyanopropylphenyl–94% dimethylpolysiloxane stationary phase and a 3.00-µm film thickness[2]

Column temperature: 20 min at 40°; increase to 240° at 10°/min; maintain at 240° for 10 min

Injection port temperature: 140°

Detector temperature: 250°

Carrier gas: Nitrogen

Flow rate: 2.5 mL/min

Headspace unit: 2.5 mL/min

Equilibration temperature: 80°

Equilibration time: 60 min

Loop temperature: 85°

Transfer temperature: 90°

Pressurization time: 0.5 min

Loop fill time: 0.1 min

Injection time: 1 min

Injection size: 1 mL of headspace

System suitability

Sample: *Standard solution*

Suitability requirement: The relative standard deviation of the ethanol peak area responses from replicate injections is NMT 5.0%.

Analysis: Separately inject equal volumes of the *Standard solution* and *Sample solution* into the chromatograph, record the chromatograms, and measure the peak responses. [Note—The approximate retention time for ethanol is 11 min.]

Acceptance criteria: The peak area from the *Sample solution* does not exceed that from the *Standard solution* (NMT 200 mg/kg).

- **OTHER RIBONUCLEOTIDES**

Mobile phase and **Chromatographic system:** Prepare as directed in the *Assay*.

Standard solution: Mixture of USP Disodium 5′-Uridylate RS, USP 5′-Adenylic Acid RS, USP 5′-Cytidylic Acid RS, USP Disodium Guanylate RS, and USP Disodium Inosinate RS each at 0.02 mg/mL in *Mobile phase*

Sample solution: 1.0 mg/mL. [Note—Ultra-sonication may be necessary to aid in complete dissolution.]

Suitability requirements

Sample: *Standard solution*

Suitability requirement 1: The relative standard deviation of the 5′-cytidylic acid peak area responses from replicate injections is NMT 2.0%.

Suitability requirement 2: The resolution, *R*, between the 5′-cytidylic acid peak and all other nucleotide peaks is NLT 2.0.

Analysis: Separately inject equal volumes of the *Standard solution* and *Sample solution* into the chromatograph, and measure the responses for all nucleotide peaks on the resulting chromatograms, except the peak from 5′-cytidylic acid. [Note—The approximate retention times are 4.6 min (5′-cytidylic acid), 6.2 min (5′-uridylic acid), 10.3 min (5′-guanylic acid), 11.5 min (5′-inosinic acid), and 27.5 min (5′-adenylic acid).] Separately calculate the percentage of each analyte (disodium 5′-uridylate, 5′-guanylic acid, 5′-inosinic acid, and 5′-adenylic acid) in the sample taken:

$$\text{Result} = (C_S/C_U) \times (r_U/r_S) \times 100$$

C_S = concentration of analyte in the *Standard solution* (mg/mL)

C_U = concentration of analyte in the *Sample solution* (mg/mL)

r_U = peak area of the analyte from the *Sample solution*

r_S = peak area of the analyte from the *Standard solution*

Acceptance criteria: The sum of the percentages for all nucleotide impurities is NMT 0.5%, calculated on the anhydrous basis.

SPECIFIC TESTS

- **pH**, *pH Determination,* Appendix IIB
  Sample solution: 10 mg/mL
  Acceptance criteria: 2.5–3.5
- **Water**, *Water Determination, Method I,* Appendix IIB
  Acceptance criteria: NMT 6.0%
- **Bile-Tolerant Gram-Negative Bacteria,** Appendix XIIC
  Sample preparation: Proceed as directed using a 10-g sample and incubating at 30–35° for 18–24 h.
  Acceptance criteria: Negative in 10 g
- **Enterobacter sakazakii** *(Cronobacter spp.),* Appendix XIIC
  Sample preparation: Proceed as directed using a 10-g sample and incubating at 30–35° for 18–24 h.
  Acceptance criteria: Negative in 10 g
- **Salmonella spp.**, Appendix XIIC
  Sample preparation: Dissolve 25 g of sample at a sample/broth ratio of 1/8, and proceed as directed.
  Acceptance criteria: Negative in 25 g
- **Total Aerobic Microbial Count**, *Method I (Plate Count Method),* Appendix XIIB
  Acceptance criteria: NMT 1,000 CFU/g
- **Total Yeasts and Molds Count**, *Method I (Plate Count Method),* Appendix XIIB
  Acceptance criteria: NMT 100 CFU/g

[1] YMC-Pack ODS-AQ (YMC Europe GmbH, Dinslaken, Germany), or equivalent.

[2] CP-Select 624 CB (Varian-Chrompack, Palo Alto, CA), or equivalent.

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(FI07) Food Ingredients Expert Committee

BRIEFING

DHA Algal Oil, Crypthecodinium Type. Because there is no existing *FCC* monograph for this food ingredient, a new monograph is proposed based on a new proposed monograph for *Crypthecodinium Cohnii Oil* in *Pharmacopeial Forum* 35(4).

1. The proposed methods for *Arsenic*, *Lead*, and *Mercury* are taken from the methods appearing in the *FCC* monograph for *Menhaden Oil, Hydrogenated*. The proposed limits are consistent with the proposal for *Crypthecodinium Cohnii Oil* in *Pharmacopeial Forum* 35(4).
2. The *Description* section and regulatory approval documents for this ingredient indicate that the ingredient may be manufactured with solvent extraction, but no method or limit appears in this proposed monograph for residual solvents. Comments, methods of analysis, and data regarding development of a residual solvent limit for this ingredient are encouraged.
3. The *Identification* and *Assay* sections reference a new proposed method for *Fatty Acid Composition (Saturated,* cis*-Monounsaturated, and* cis*-Polyunsaturated) in Oils Containing Long Chain Polyunsaturated Fatty Acids,* Appendix VII. See briefing under *Fats and Related Substances,* Appendix VII.
4. The *Specific Tests* section references a new proposed method for *Anisidine Value*. See briefing under *Fats and Related Substances,* Appendix VII.
5. A *Labeling* requirement has been included such that the presence of oil(s) added to standardize DHA content should be indicated. Comments are requested regarding whether or not the labeling should indicate the specific oil(s) added for standardization.

(FIEC: K. Bowman) C74208

Add the following:

■ **DHA Algal Oil, Crypthecodinium Type**

Crypthecodinium cohnii Oil

DESCRIPTION

DHA Algal Oil, Crypthecodinium Type occurs as a yellow to orange colored oil providing a source of docosahexaenoic acid (DHA, $C_{22}H_{32}O_2$) (C22:6 n-3), an omega-3 long-chain polyunsaturated fatty acid. It is obtained from fermentation of the species of microalgae *Crypthecodinium cohnii*, usually by solvent extraction. The oil may be winterized, bleached, and deodorized to substantially remove free fatty acids, phospholipids, odor and flavor components, and other material. Docosahexaenoic acid is the only significant polyunsaturated fatty acid present; DHA content may be standardized with other oils. Suitable antioxidants may be added.

Function: Nutrient; source of DHA

Packaging and Storage: Store in tight, light-resistant containers. Avoid exposure to excessive heat.

IDENTIFICATION

- **Fatty Acid Composition,** *Fatty Acid Composition (Saturated,* cis*-Monounsaturated, and* cis*-Polyunsaturated) in Oils Containing Long Chain Polyunsaturated Fatty Acids,* Appendix VII

  Acceptance criteria: The retention time of the peak of the docosahexaenoic acid methyl ester from the *Sample Preparation* corresponds to that from the *Standard Solution*. The area percent for the methyl esters of the fatty acids from the *Sample Preparation* meet the requirements for each fatty acid indicated

in the table below.

Fatty Acid	Shorthand Notation	Lower Limit (area %)	Upper Limit (area %)
Linoleic acid	18:2 n-6	0	1.0
Dihomo-gamma-linolenic acid	20:3 n-6	0	0.1
Eicosapentanoic acid	20:5 n-3	0	0.1
Docosapentaenoic acid	22:5 n-6	0	0.1
Docosahexaenoic acid	22:6 n-3	40.0	47.0

ASSAY

- **DHA,** *Fatty Acid Composition (Saturated,* cis-*Monounsaturated, and* cis-*Polyunsaturated) in Oils Containing Long Chain Polyunsaturated Fatty Acids,* Appendix VII

Acceptance criteria: NLT 40.0% docosahexaenoic acid (DHA)

IMPURITIES

Inorganic Impurities

- **ARSENIC**

Apparatus

Sample digestion: Use a microwave oven (CEM Model MDS-2100, or equivalent) equipped with advanced composite vessels with 100-mL Teflon liners. Use rupture membranes to vent vessels should the pressure exceed 125 psi. The vessels fit into a turntable, and each vessel can be vented into an overflow container. Equip the microwave oven with an exhaust tube to ventilate fumes.

Sample analysis: Use a suitable graphite furnace atomic absorption spectrophotometer (GFAAS) equipped with an autosampler, pyrolytically coated graphite tubes, solid pyrolytic graphite platforms, and an adequate means of background correction. This method was developed using a Perkin-Elmer Model 5100, HGA-600 furnace, and an AS-60 autosampler with Zeeman effect background correction. An electrodeless discharge lamp serves as the source, argon as the purge gas, and air as the alternate gas. Set up the instrument according to manufacturer's specifications, with consideration of current good GFAAS practices. The instrument parameters are as follows:

Wavelength: 193.7 nm

Lamp current: 300 (EDL) modulated

Pyrolysis: 1000°

Atomization: 2400°

Slit: 0.7

Characteristic mass: 15 pg

Glassware: Acid wash all glass, Teflon, and plastic vessels by soaking them in a nitric acid bath containing a 4:1 solution of water:nitric acid. [**CAUTION—** Wear a full face shield and protective clothing and gloves at all times when working with acid baths.] After acid soaking, rinse acid-washed items in deionized water, dry them, and store them in clean, covered cabinets.

Calibration standard stock solution: 100 µg/L

Prepare from a suitable standard, which may be purchased [accuracy certified against National Institute of Standards and Technology (NIST) spectrometric standard solutions].

Calibration standard solutions: 2.0, 5.0, 10.0, 25.0, and 50.0 µg/L in 2% nitric acid, from the *Calibration standard stock solution*

1% Palladium stock solution: Mix 1 g of ultrapure palladium metal, with 20 mL of water and 10 mL of nitric acid in a Teflon beaker, and warm the solution on a hot plate to dissolve the palladium. Allow the solution

to cool to room temperature, transfer it into a 100-mL volumetric flask, and dilute with deionized water to volume.

1% Magnesium nitrate stock solution: Mix 1 g of ultrapure magnesium nitrate, with 40 mL of water and 1 mL of nitric acid in a Teflon beaker, and warm the solution on a hot plate to dissolve. Allow the solution to cool to room temperature, transfer it into a 100-mL volumetric flask, and dilute with deionized water to volume.

[Note—Because of the difficulty in preparing matrix modifier stock solutions with the required purity, purchasing modifier stock solutions and using them to prepare working modifier solutions is recommended. A palladium (0.3%) and magnesium nitrate (0.2%) solution may be purchased from High Purity Standards, or equivalent.]

Modifier working solution: Transfer 3 mL of *1% Palladium stock solution* and 2 mL of *1% Magnesium nitrate stock solution* to a 10-mL volumetric flask and dilute with 2% nitric acid to volume. A volume of 5 µL provides 0.015 mg of palladium and 0.01 mg of magnesium nitrate.

Sample solution: [**CAUTION—** Wear proper eye protection and protective clothing and gloves during sample preparation. Closely follow the manufacturer's safety instructions for use of the microwave digestion apparatus.]

Transfer 500 mg of sample into a Teflon digestion vessel liner. Prepare samples in duplicate. Add 15 mL of nitric acid, and swirl gently. Cover the vessels with lids, leaving the vent fitting off. Predigest overnight under a hood. Place the rupture membrane in the vent fitting, and tighten the lid. Place all vessels on the microwave oven turntable. Connect the vent tubes to the vent trap, and connect the pressure-sensing line to the appropriate vessel. Initiate a two-stage digestion procedure by heating the microwave at 15% power for 15 min followed by 25% power for 45 min. Remove the turntable of vessels from the oven, and allow the vessels to cool to room temperature (a cool water bath may be used to speed the cooling process). Vent the vessels when they reach room temperature. Remove the lids, and slowly add 2 mL of 30% hydrogen peroxide to each. Allow the reactions to subside, and seal the vessels. Return the vessels on the turntable to the microwave oven and heat for an additional 15 min at 30% power. Remove the vessels from the oven, and allow them to cool to room temperature. Transfer the cooled digests into 25-mL volumetric flasks, and dilute with deionized water to volume.

Analysis: The graphite furnace program is as follows:

1. Dry at 115° using a 1-s ramp, a 65-s hold, and a 300-mL/min argon flow.
2. Char the sample at 1000° using a 1-s ramp, a 20-s hold, and a 300-mL/min air flow.
3. Cool down and purge the air from the furnace for 10 s using a 20° set temperature and a 300-mL/min argon flow.
4. Atomize at 2400° using a 0-s ramp and a 5-s hold with the argon flow stopped.
5. Clean out at 2600° with a 1-s ramp and a 5-s hold.

Use the autosampler to inject 20-µL aliquots of blanks, *Calibration standard solutions*, and *Sample solutions* and 5 µL of *Modifier working solution*. Inject each solution in duplicate, and average the results. Use peak area measurement for all quantitations. After ensuring that the furnace is clean by running a 5% nitric acid blank, check the instrument's sensitivity by running a 20-µL aliquot of the 25-µg *Calibration standard solution*. Compare the results obtained with the expected results for the equipment used, and take the necessary steps to correct any problems.

Calculate the characteristic mass. Record and track the integrated absorbance and characteristic mass for reference and quality assurance.

Inject each *Calibration standard solution* in duplicate. Use the algorithms provided in the instrument

software to establish calibration curves. Recheck calibration periodically, and recalibrate if the recheck differs from the original calibration by more than 10%.

Inject the *Sample solution* in duplicate, and record the integrated absorbance. If the instrument response exceeds that of the calibration curve, dilute with 5% nitric acid to bring the sample's response into the working range, and note the dilution factor (DF). All sample analyses should be blank corrected using a sample solution blank.

If a computer-based instrument is used, the data output is reported as µg/L. Calculate the concentration of arsenic, in µg/g (equivalent to mg/kg), in the original sample taken by the formula:

$$\text{Result} = (C \times DF \times V)/W$$

C = concentration of arsenic in the sample aliquot injected (µg/L)
DF = dilution factor of the *Sample solution*
V = final volume of the *Sample solution* (L)
W = weight of the sample taken to prepare the *Sample solution* (g)

[Note—To monitor recovery and ensure analytical accuracy for proper quality assurance, analyze blanks, spiked blanks, and a spiked oil with each digestion set.]

Acceptance criteria: NMT 0.1 mg/kg

- **LEAD**

Apparatus

Sample digestion: Use a microwave oven (CEM Model MDS-2100, or equivalent) equipped with advanced composite vessels with 100-mL Teflon liners. Use rupture membranes to vent vessels should the pressure exceed 125 psi. The vessels fit into a turntable, and each vessel can be vented into an overflow container. Equip the microwave oven with an exhaust tube to ventilate fumes.

Sample analysis: See *Apparatus* in *Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace Method, Method I,* Appendix IIIB

Calibration standard stock solution: 100 µg/L

Prepare from a suitable standard, which may be purchased [accuracy certified against National Institute of Standards and Technology (NIST) spectrometric standard solutions].

Calibration standard solutions: 2.0, 5.0, 10.0, 25.0, and 50.0 µg/L in 2% nitric acid, from the *Calibration standard stock solution*

10% Ammonium dihydrogen phosphate stock solution: Mix 10 g of ultrapure ammonium dihydrogen phosphate, with 40 mL of water and 1 mL of nitric acid to dissolve the phosphate. Dilute with deionized water to 100 mL.

1% Magnesium nitrate stock solution: Mix 1 g of ultrapure magnesium nitrate, with 40 mL of water and 1 mL of nitric acid in a Teflon beaker, and warm on a hot plate to dissolve the solids. Allow the solution to cool to room temperature, transfer it into a 100-mL volumetric flask, and dilute with deionized water to volume.

[Note—Because of the difficulty in preparing matrix modifier stock solutions with the required purity, purchasing modifier stock solutions and using them to prepare working solutions is recommended. An ammonium dihydrogen phosphate (4%) and magnesium nitrate (0.2%) solution may be purchased from High Purity Standards, or equivalent.]

Modifier working solution: Transfer 4 mL of *10% Ammonium dihydrogen phosphate stock solution* and 2 mL of *1% Magnesium nitrate stock solution* to a 10-mL volumetric flask and dilute with 2% nitric acid to volume. A volume of 5 µL provides 0.2 mg of phosphate plus 0.01 mg of magnesium nitrate.

Sample solution: Prepare as directed for *Sample solution* in the *Arsenic* test (above).

[**CAUTION—** Wear proper eye protection and protective clothing and gloves during sample preparation.

Closely follow the manufacturer's safety instructions for use of the microwave digestion apparatus.]

Analysis: The graphite furnace program is as follows:

1. Dry at 120° using a 1-s ramp, a 55-s hold, and a 300-mL/min argon flow.
2. Char the sample at 850° using a 1-s ramp, a 30-s hold, and a 300-mL/min air flow.
3. Cool down and purge the air from the furnace for 10 s using a 20° set temperature and a 300-mL/min argon flow.
4. Atomize at 2100° using a 0-s ramp and a 5-s hold with the argon flow stopped.
5. Clean out at 2600° with a 1-s ramp and a 5-s hold.

Use the autosampler to inject 20-µL aliquots of blanks, *Calibration standard solutions*, *Sample solutions*, and 5 µL of *Modifier working solution*. Inject each solution in duplicate, and average the results. Use peak-area measurement for all quantitation. After ensuring that the furnace is clean by running a 5% nitric acid blank, check instrument sensitivity by running an aliquot of the 25-µg *Calibration standard solution*. Compare the results obtained with the expected results for the equipment used, and take the necessary steps to correct any problems.

Calculate the characteristic mass, and record and track the integrated absorbance and characteristic mass for reference and quality assurance.

Inject each *Calibration standard solution* in duplicate. Use the algorithms provided in the instrument software to establish calibration curves. Recheck the calibration periodically and recalibrate if recheck differs from the original calibration by more than 10%.

Inject the *Sample solution* in duplicate, and record the integrated absorbance. If the instrument response exceeds that of the calibration curve, dilute with 5% nitric acid to bring the sample response into the working range, and note the dilution factor (DF). All sample analyses should be blank corrected using a sample solution blank.

If a computer-based instrument is used, the data output is reported as micrograms per liter. Calculate the concentration, in µg/g (equivalent to mg/kg), of lead in the original sample by the following formula:

$$\text{Result} = (C \times DF \times V)/W$$

C = concentration of lead in the sample aliquot injected (µg/L)
DF = dilution factor of the *Sample solution*
V = final volume of the *Sample solution* (L)
W = weight of the sample taken to prepare the *Sample solution* (g)

[Note—To monitor recovery and ensure analytical accuracy for proper quality assurance, analyze blanks, spiked blanks, and a spiked oil with each digestion set.]

Acceptance criteria: NMT 0.1 mg/kg

- **MERCURY**

Apparatus

Sample digestion: Use a microwave oven (CEM Model MDS-2100, or equivalent) equipped with advanced composite vessels with 100-mL Teflon liners. Use rupture membranes to vent vessels should the pressure exceed 125 psi. The vessels fit into a turntable, and each vessel can be vented into an overflow container. Equip the microwave oven with an exhaust tube to ventilate fumes.

Sample analysis: Use a suitable atomic absorption spectrophotometer equipped with an atomic vapor assembly. This method was developed using a Perkin-Elmer Model 5100 and IL 440 Thermo Jarrell Ash atomic vapor assembly. An electrodeless discharge lamp serves as the source, with an inert gas such

as argon or nitrogen as the purge gas. Set up the instrument according to manufacturer specifications. Instrument parameters are as follows:

Wavelength: 253.6 nm
Slit: 0.7
Reagent setting: 5
Gas flow: 5 to 6 L/min
Reaction time: 0.5 min

Glassware: Acid wash all glass, Teflon, and plastic vessels by soaking them in a nitric acid bath containing a 4:1 solution of water:nitric acid. [**CAUTION—** Wear a full face shield and protective clothing and gloves at all times when working with acid baths.] After acid soaking, rinse acid-washed items in deionized water, dry, and store them in clean, covered cabinets.

Calibration standard stock solution: 200 ng/g of mercury. Prepare from a suitable standard, which may be purchased [accuracy certified against National Institute of Standards and Technology (NIST) spectrometric standard solutions].

Calibration standard solutions: 20, 60, 100, 200, and 400 ng of mercury in 1 N hydrochloric acid from the *Calibration standard stock solution*

Reducing reagent: 5% Stannous chloride in 25% hydrochloric acid (trace-metal grade). [Note—Prepare daily.]

Sample solution: Prepare as directed for the *Sample solution* in the *Arsenic* test (above).

[**CAUTION—** Wear proper eye protection and protective clothing and gloves during sample preparation. Closely follow the manufacturer's safety instructions for use of the microwave digestion apparatus.]

Analysis: Optimize the instrument settings for the spectrophotometer as described in the instrument manual. The instrument parameters for cold vapor generation are as follows:

Wavelength: 253.6 nm
Slit: 0.70 nm
Reagent setting: 5
Gas flow: 5 to 6 L/min
Reaction time: 0.5 min

Use a peak height integration method with a 40-s integration time and a 20-s read delay in an unheated absorption cell. Zero the instrument as follows: Place a Fleaker containing 50 mL of 1 N hydrochloric acid in the sample well of the hydride generator. Press “start” on the vapor generator and “read” on the atomic absorption spectrophotometer. The instrument will automatically flush the sample container with nitrogen, dispense the designated amount of reagent, stir the sample for a designated reaction time, and purge the head volume again with nitrogen, sweeping any vapor into the quartz cell for determination of absorption. The atomic absorption spectrophotometer will automatically zero on this sample when “autozero” is selected from the calibration menu.

Generate a standard curve of concentration versus absorption by analyzing the five *Calibration standard solutions* prepared as described for daily standards in *Calibration standard solutions*. Analyze each solution in duplicate, generate the calibration curve, and store, using procedures specific for the instrumentation.

Transfer an appropriate aliquot of *Sample solution* (usually 2 mL) in a Fleaker containing 50 mL of 1 N hydrochloric acid. Analyze solutions in duplicate using the procedure specified in the instrument manual. Using the calibration algorithm provided in the instrument software, calculate and report the mercury concentration in nanograms of mercury in the aliquot analyzed.

Calculate the level of mercury as µg/g (equivalent to mg/kg), in the original sample by the formula:

$$\text{Result} = (A \times DF)/(W \times 1000)$$

A = amount of mercury in the aliquot analyzed (ng)
DF = dilution factor (final volume of *Sample solution*/volume taken for analysis)
W = weight of the sample taken to prepare the *Sample solution* (g)

[Note—To monitor recovery and ensure analytical accuracy for proper quality assurance, analyze blanks, spiked blanks, and a spiked oil with each digestion set.]

Acceptance criteria: NMT 0.1 mg/kg

SPECIFIC TESTS

- **Anisidine Value,** Appendix VII
  Acceptance criteria: NMT 20.0
- **Free Fatty Acids (as Oleic Acid),** Appendix VII
  Analysis: Use 28.2 for the equivalence factor (*e*) in the formula given in the procedure.
  Acceptance criteria: NMT 0.4%
- **Peroxide Value,** Appendix VII
  Acceptance criteria: NMT 5.0 mEq/kg
- **Total Oxidation Value**
  Analysis: Calculate by the formula:

$$\text{Result} = (2 \times PV) + AV$$

PV = peroxide value, determined above
AV = anisidine value, determined above

Acceptance criteria: NMT 26

- **Unsaponifiable Matter,** Appendix VII
  Acceptance criteria: NMT 3.5%

OTHER REQUIREMENTS

- **Labeling** Label to indicate the content of docosahexaenoic acid in mg/g. Indicate the name and concentration of any added antioxidant and the presence of any other oil(s) used to standardize the docosahexaenoic acid content.

■ 1S (*FCC7*)

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

BRIEFING

DHA Algal Oil, Schizochytrium Type. Because there is no existing *FCC* monograph for this food ingredient, a new monograph is proposed based on a new proposed monograph for *Schizochytrium Oil* in *Pharmacopeial Forum* 35(4).

1. The proposed methods for *Arsenic*, *Lead*, and *Mercury* are taken from the methods appearing in the *FCC* monograph for *Menhaden Oil, Hydrogenated*. The proposed limits are consistent with the proposal for *Schizochytrium Oil* in *Pharmacopeial Forum* 35(4).
2. The *Description* section and regulatory approval documents for this ingredient indicate that the ingredient may be manufactured with solvent extraction, but no method or limit appears in this proposed monograph for residual solvents. Comments, methods of analysis, and data regarding development of a residual solvent limit for this ingredient are encouraged.
3. The *Identification* and *Assay* sections reference a new proposed method for *Fatty Acid Composition (Saturated,* cis*-Monounsaturated, and* cis*-Polyunsaturated) in Oils Containing Long Chain Polyunsaturated Fatty Acids,* Appendix VII. See briefing under *Fats and Related Substances,* Appendix VII.
4. The *Specific Tests* section references a new proposed method for *Anisidine Value,* Appendix VII. See briefing under *Fats and Related Substances,* Appendix VII.
5. A *Labeling* requirement has been included such that the presence of oil(s) added to standardize DHA content should be indicated. Comments are requested regarding whether or not the labeling should indicate the specific oil(s) added for standardization.

(FIEC: K. Bowman) C74211

Add the following:

■ **DHA Algal Oil, Schizochytrium Type**

Schizochytrium Oil

DESCRIPTION

DHA Algal Oil, Schizochytrium Type occurs as a yellow to orange colored oil providing a source of docosahexaenoic acid (DHA, $C_{22}H_{32}O_2$) (C22:6 n-3), an omega-3 long-chain polyunsaturated fatty acid. It is obtained from fermentation of the species of microalgae *Schizochytrium* sp., usually by solvent extraction. The oil may be winterized, bleached, and deodorized to substantially remove free fatty acids, phospholipids, odor and flavor components, and other material. Docosahexaenoic acid is the main polyunsaturated fatty acid present; DHA content may be standardized with other oils. Suitable antioxidants may be added.

Function: Nutrient; source of DHA

Packaging and Storage: Store in tight, light-resistant containers. Avoid exposure to excessive heat.

IDENTIFICATION

- **Fatty Acid Composition,** *Fatty Acid Composition (Saturated,* cis*-Monounsaturated, and* cis*-Polyunsaturated) in Oils Containing Long Chain Polyunsaturated Fatty Acids,* Appendix VII

  Acceptance criteria: The retention times of the peaks of the docosahexaenoic acid methyl ester and eicosapentanoic acid methyl ester from the *Sample Preparation* correspond to those from the *Standard Solution*. The area percent for the methyl esters of the fatty acids from the *Sample Preparation* meet the requirements for each fatty acid indicated in the table below.

Fatty Acid	Shorthand Notation	Lower Limit (area %)	Upper Limit (area %)
Dihomo-gamma-linolenic acid	20:3 n-6	1.7	2.8
Arachidonic acid	20:4 n-6	0.6	1.3
Eicosapentanoic acid	20:5 n-3	1.3	3.9
Docosapentaenoic acid	22:5 n-6	10.5	16.5
Docosahexaenoic acid	22:6 n-3	30.0	40.0

ASSAY

- **DHA,** *Fatty Acid Composition (Saturated,* cis*-Monounsaturated, and* cis*-Polyunsaturated) in Oils Containing Long Chain Polyunsaturated Fatty Acids,* Appendix VII

  Acceptance criteria: NLT 30.0% docosahexaenoic acid (DHA)

IMPURITIES

Inorganic Impurities

- **ARSENIC**

  Apparatus

  Sample digestion: Use a microwave oven (CEM Model MDS-2100, or equivalent) equipped with advanced composite vessels with 100-mL Teflon liners. Use rupture membranes to vent vessels should the pressure exceed 125 psi. The vessels fit into a turntable, and each vessel can be vented into an overflow container. Equip the microwave oven with an exhaust tube to ventilate fumes.

  Sample analysis: Use a suitable graphite furnace atomic absorption spectrophotometer (GFAAS) equipped with an autosampler, pyrolytically coated graphite tubes, solid pyrolytic graphite platforms, and an adequate means of background correction. This method was developed using a Perkin-Elmer Model 5100, HGA-600 furnace, and an AS-60 autosampler with Zeeman effect background correction. An electrodeless discharge lamp serves as the source, argon as the purge gas, and air as the alternate gas. Set up the instrument according to manufacturer's specifications, with consideration of current good GFAAS practices. The instrument parameters are as follows:

  Wavelength: 193.7 nm

  Lamp current: 300 (EDL) modulated

  Pyrolysis: 1000°

  Atomization: 2400°

  Slit: 0.7

  Characteristic mass: 15 pg

  Glassware: Acid wash all glass, Teflon, and plastic vessels by soaking them in a nitric acid bath containing a 4:1 solution of water:nitric acid. [**CAUTION—** Wear a full face shield and protective clothing and gloves at all times when working with acid baths.] After acid soaking, rinse acid-washed items in deionized water, dry them, and store them in clean, covered cabinets.

  Calibration standard stock solution: 100 µg/L

  Prepare from a suitable standard, which may be purchased [accuracy certified against National Institute of Standards and Technology (NIST) spectrometric standard solutions].

  Calibration standard solutions: 2.0, 5.0, 10.0, 25.0, and 50.0 µg/L in 2% nitric acid, from the *Calibration standard stock solution*

  1% Palladium stock solution: Mix 1 g of ultrapure palladium metal, with 20 mL of water and 10 mL of nitric acid in a Teflon beaker, and warm the solution on a hot plate to dissolve the palladium. Allow the solution to cool to room temperature, transfer it into a 100-mL volumetric flask, and dilute with deionized water to

volume.

1% Magnesium nitrate stock solution: Mix 1 g of ultrapure magnesium nitrate, with 40 mL of water and 1 mL of nitric acid in a Teflon beaker, and warm the solution on a hot plate to dissolve. Allow the solution to cool to room temperature, transfer it into a 100-mL volumetric flask, and dilute with deionized water to volume.

[Note—Because of the difficulty in preparing matrix modifier stock solutions with the required purity, purchasing modifier stock solutions and using them to prepare working modifier solutions is recommended. A palladium (0.3%) and magnesium nitrate (0.2%) solution may be purchased from High Purity Standards, or equivalent.]

Modifier working solution: Transfer 3 mL of *1% Palladium stock solution* and 2 mL of *1% Magnesium nitrate stock solution* to a 10-mL volumetric flask and dilute with 2% nitric acid to volume. A volume of 5 µL provides 0.015 mg of palladium and 0.01 mg of magnesium nitrate.

Sample solution: [**CAUTION—** Wear proper eye protection and protective clothing and gloves during sample preparation. Closely follow the manufacturer's safety instructions for use of the microwave digestion apparatus.]

Transfer 500 mg of sample into a Teflon digestion vessel liner. Prepare samples in duplicate. Add 15 mL of nitric acid, and swirl gently. Cover the vessels with lids, leaving the vent fitting off. Predigest overnight under a hood. Place the rupture membrane in the vent fitting, and tighten the lid. Place all vessels on the microwave oven turntable. Connect the vent tubes to the vent trap, and connect the pressure-sensing line to the appropriate vessel. Initiate a two-stage digestion procedure by heating the microwave at 15% power for 15 min followed by 25% power for 45 min. Remove the turntable of vessels from the oven, and allow the vessels to cool to room temperature (a cool water bath may be used to speed the cooling process). Vent the vessels when they reach room temperature. Remove the lids, and slowly add 2 mL of 30% hydrogen peroxide to each. Allow the reactions to subside, and seal the vessels. Return the vessels on the turntable to the microwave oven and heat for an additional 15 min at 30% power. Remove the vessels from the oven, and allow them to cool to room temperature. Transfer the cooled digests into 25-mL volumetric flasks, and dilute with deionized water to volume.

Analysis: The graphite furnace program is as follows:

1. Dry at 115° using a 1-s ramp, a 65-s hold, and a 300-mL/min argon flow.
2. Char the sample at 1000° using a 1-s ramp, a 20-s hold, and a 300-mL/min air flow.
3. Cool down and purge the air from the furnace for 10 s using a 20° set temperature and a 300-mL/min argon flow.
4. Atomize at 2400° using a 0-s ramp and a 5-s hold with the argon flow stopped.
5. Clean out at 2600° with a 1-s ramp and a 5-s hold.

Use the autosampler to inject 20-µL aliquots of blanks, *Calibration standard solutions*, and *Sample solutions* and 5 µL of *Modifier working solution*. Inject each solution in duplicate, and average the results. Use peak area measurement for all quantitations. After ensuring that the furnace is clean by running a 5% nitric acid blank, check the instrument's sensitivity by running a 20-µL aliquot of the 25-µg *Calibration standard solution*. Compare the results obtained with the expected results for the equipment used, and take the necessary steps to correct any problems.

Calculate the characteristic mass. Record and track the integrated absorbance and characteristic mass for reference and quality assurance.

Inject each *Calibration standard solution* in duplicate. Use the algorithms provided in the instrument software to establish calibration curves. Recheck calibration periodically, and recalibrate if the recheck

differs from the original calibration by more than 10%.

Inject the *Sample solution* in duplicate, and record the integrated absorbance. If the instrument response exceeds that of the calibration curve, dilute with 5% nitric acid to bring the sample's response into the working range, and note the dilution factor (DF). All sample analyses should be blank corrected using a sample solution blank.

If a computer-based instrument is used, the data output is reported as µg/L. Calculate the concentration of arsenic, in µg/g (equivalent to mg/kg), in the original sample taken by the formula:

$$\text{Result} = (C \times DF \times V)/W$$

C = concentration of arsenic in the sample aliquot injected (µg/L)
DF = dilution factor of the *Sample solution*
V = final volume of the *Sample solution* (L)
W = weight of the sample taken to prepare the *Sample solution* (g)

[Note—To monitor recovery and ensure analytical accuracy for proper quality assurance, analyze blanks, spiked blanks, and a spiked oil with each digestion set.]

Acceptance criteria: NMT 0.1 mg/kg

- **LEAD**

Apparatus

Sample digestion: Use a microwave oven (CEM Model MDS-2100, or equivalent) equipped with advanced composite vessels with 100-mL Teflon liners. Use rupture membranes to vent vessels should the pressure exceed 125 psi. The vessels fit into a turntable, and each vessel can be vented into an overflow container. Equip the microwave oven with an exhaust tube to ventilate fumes.

Sample analysis: See *Apparatus* in *Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace Method, Method I,* Appendix IIIB

Calibration standard stock solution: 100 µg/L

Prepare from a suitable standard, which may be purchased [accuracy certified against National Institute of Standards and Technology (NIST) spectrometric standard solutions].

Calibration standard solutions: 2.0, 5.0, 10.0, 25.0, and 50.0 µg/L in 2% nitric acid, from the *Calibration standard stock solution*

10% Ammonium dihydrogen phosphate stock solution: Mix 10 g of ultrapure ammonium dihydrogen phosphate, with 40 of mL water and 1 mL of nitric acid to dissolve the phosphate. Dilute with deionized water to 100 mL.

1% Magnesium nitrate stock solution: Mix 1 g of ultrapure magnesium nitrate, with 40 mL of water and 1 mL of nitric acid in a Teflon beaker, and warm on a hot plate to dissolve the solids. Allow the solution to cool to room temperature, transfer it into a 100-mL volumetric flask, and dilute with deionized water to volume.

[Note—Because of the difficulty in preparing matrix modifier stock solutions with the required purity, purchasing modifier stock solutions and using them to prepare working solutions is recommended. An ammonium dihydrogen phosphate (4%) and magnesium nitrate (0.2%) solution may be purchased from High Purity Standards, or equivalent.]

Modifier working solution: Transfer 4 mL of *10% Ammonium dihydrogen phosphate stock solution* and 2 mL of *1% Magnesium nitrate stock solution* to a 10-mL volumetric flask and dilute with 2% nitric acid to volume. A volume of 5 µL provides 0.2 mg of phosphate plus 0.01 mg of magnesium nitrate.

Sample solution: Prepare as directed for *Sample solution* in the *Arsenic* test (above).

[**CAUTION—** Wear proper eye protection and protective clothing and gloves during sample preparation. Closely follow the manufacturer's safety instructions for use of the microwave digestion apparatus.]

Analysis: The graphite furnace program is as follows:

1. Dry at 120° using a 1-s ramp, a 55-s hold, and a 300-mL/min argon flow.
2. Char the sample at 850° using a 1-s ramp, a 30-s hold, and a 300-mL/min air flow.
3. Cool down and purge the air from the furnace for 10 s using a 20° set temperature and a 300-mL/min argon flow.
4. Atomize at 2100° using a 0-s ramp and a 5-s hold with the argon flow stopped.
5. Clean out at 2600° with a 1-s ramp and a 5-s hold.

Use the autosampler to inject 20-µL aliquots of blanks, *Calibration standard solutions*, *Sample solutions*, and 5 µL of *Modifier working solution*. Inject each solution in duplicate, and average the results. Use peak-area measurement for all quantitation. After ensuring that the furnace is clean by running a 5% nitric acid blank, check instrument sensitivity by running an aliquot of the 25-µg *Calibration standard solution*. Compare the results obtained with the expected results for the equipment used, and take the necessary steps to correct any problems.

Calculate the characteristic mass, and record and track the integrated absorbance and characteristic mass for reference and quality assurance.

Inject each *Calibration standard solution* in duplicate. Use the algorithms provided in the instrument software to establish calibration curves. Recheck the calibration periodically and recalibrate if recheck differs from the original calibration by more than 10%.

Inject the *Sample solution* in duplicate, and record the integrated absorbance. If the instrument response exceeds that of the calibration curve, dilute with 5% nitric acid to bring the sample response into the working range, and note the dilution factor (DF). All sample analyses should be blank corrected using a sample solution blank.

If a computer-based instrument is used, the data output is reported as micrograms per liter. Calculate the concentration, in µg/g (equivalent to mg/kg), of lead in the original sample by the following formula:

$$\text{Result} = (C \times DF \times V)/W$$

- C = concentration of lead in the sample aliquot injected (µg/L)
- DF = dilution factor of the *Sample solution*
- V = final volume of the *Sample solution* (L)
- W = weight of the sample taken to prepare the *Sample solution* (g)

[Note—To monitor recovery and ensure analytical accuracy for proper quality assurance, analyze blanks, spiked blanks, and a spiked oil with each digestion set.]

Acceptance criteria: NMT 0.1 mg/kg

- **MERCURY**

Apparatus

Sample digestion: Use a microwave oven (CEM Model MDS-2100, or equivalent) equipped with advanced composite vessels with 100-mL Teflon liners. Use rupture membranes to vent vessels should the pressure exceed 125 psi. The vessels fit into a turntable, and each vessel can be vented into an overflow container. Equip the microwave oven with an exhaust tube to ventilate fumes.

Sample analysis: Use a suitable atomic absorption spectrophotometer equipped with an atomic vapor assembly. This method was developed using a Perkin-Elmer Model 5100 and IL 440 Thermo Jarrell Ash atomic vapor assembly. An electrodeless discharge lamp serves as the source, with an inert gas such as argon or nitrogen as the purge gas. Set up the instrument according to manufacturer specifications.

Instrument parameters are as follows:
Wavelength: 253.6 nm
Slit: 0.7
Reagent setting: 5
Gas flow: 5 to 6 L/min
Reaction time: 0.5 min

Glassware: Acid wash all glass, Teflon, and plastic vessels by soaking them in a nitric acid bath containing a 4:1 solution of water:nitric acid. [**CAUTION—** Wear a full face shield and protective clothing and gloves at all times when working with acid baths.] After acid soaking, rinse acid-washed items in deionized water, dry, and store them in clean, covered cabinets.

Calibration standard stock solution: 200 ng/g of mercury. Prepare from a suitable standard, which may be purchased [accuracy certified against National Institute of Standards and Technology (NIST) spectrometric standard solutions].

Calibration standard solutions: 20, 60, 100, 200, and 400 ng of mercury in 1 N hydrochloric acid from the *Calibration standard stock solution*

Reducing reagent: 5% Stannous chloride in 25% hydrochloric acid (trace-metal grade) [Note—Prepare daily.]

Sample solution: Prepare as directed for the *Sample solution* in the *Arsenic* test (above).

[**CAUTION—** Wear proper eye protection and protective clothing and gloves during sample preparation. Closely follow the manufacturer's safety instructions for use of the microwave digestion apparatus.]

Analysis: Optimize the instrument settings for the spectrophotometer as described in the instrument manual. The instrument parameters for cold vapor generation are as follows:
Wavelength: 253.6 nm
Slit: 0.70 nm
Reagent setting: 5
Gas flow: 5 to 6 L/min
Reaction time: 0.5 min

Use a peak height integration method with a 40-s integration time and a 20-s read delay in an unheated absorption cell. Zero the instrument as follows: Place a Fleaker containing 50 mL of 1 N hydrochloric acid in the sample well of the hydride generator. Press “start” on the vapor generator and “read” on the atomic absorption spectrophotometer. The instrument will automatically flush the sample container with nitrogen, dispense the designated amount of reagent, stir the sample for a designated reaction time, and purge the head volume again with nitrogen, sweeping any vapor into the quartz cell for determination of absorption. The atomic absorption spectrophotometer will automatically zero on this sample when “autozero” is selected from the calibration menu.

Generate a standard curve of concentration versus absorption by analyzing the five *Calibration standard solutions* prepared as described for daily standards under *Calibration standard solutions*. Analyze each solution in duplicate, generate the calibration curve, and store, using procedures specific for the instrumentation.

Transfer an appropriate aliquot of *Sample solution* (usually 2 mL) in a Fleaker containing 50 mL of 1 N hydrochloric acid. Analyze solutions in duplicate using the procedure specified in the instrument manual. Using the calibration algorithm provided in the instrument software, calculate and report the mercury concentration in nanograms of mercury in the aliquot analyzed.

Calculate the level of mercury as µg/g (equivalent to mg/kg), in the original sample by the formula:

$$\text{Result} = (A \times DF)/(W \times 1000)$$

A = amount of mercury in the aliquot analyzed (ng)

DF = dilution factor (final volume of *Sample solution*/volume taken for analysis)

W = weight of the sample taken to prepare the *Sample solution* (g)

[Note—To monitor recovery and ensure analytical accuracy for proper quality assurance, analyze blanks, spiked blanks, and a spiked oil with each digestion set.]

Acceptance criteria: NMT 0.1 mg/kg

SPECIFIC TESTS

- **Anisidine Value,** Appendix VII
  Acceptance criteria: NMT 20.0
- **Free Fatty Acids (as Oleic Acid),** Appendix VII
  Analysis: Use 28.2 for the equivalence factor (*e*) in the formula given in the procedure.
  Acceptance criteria: NMT 0.4%
- **Peroxide Value,** Appendix VII
  Acceptance criteria: NMT 5.0 mEq/kg
- **Total Oxidation Value**
  Analysis: Calculate by the formula:

$$\text{Result} = (2 \times \text{PV}) + \text{AV}$$

PV = peroxide value, determined above

AV = anisidine value, determined above

Acceptance criteria: NMT 26

- **Unsaponifiable Matter,** Appendix VII
  Acceptance criteria: NMT 4.5%

OTHER REQUIREMENTS

- **Labeling** Label to indicate the content of docosahexaenoic acid in mg/g. Indicate the name and concentration of any added antioxidant and the presence of any other oil(s) used to standardize the docosahexaenoic acid content.

■ 1S (*FCC7*)

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

BRIEFING

Diacetyl Tartaric Acid Esters of Mono- and Diglycerides, *FCC 6* page 256. On the basis of comments received, a revision to the *Residue on Ignition (Sulfated Ash)* acceptance criteria is proposed. The revision aligns this specification with that in the *Diacetyltartaric and Fatty Acid Esters of Glycerol* monograph prepared at the 71st session of the Joint FAO/WHO Expert Committee on Food Additives (2009) and the purity criteria for this food additive set in Commission Directive 98/86/EC (Official Journal of the European Communities L334, 9.12.98, page 48).
(FIEC: J. Moore) C84110

Diacetyl Tartaric Acid Esters of Mono- and Diglycerides

DATEM

INS: 472e

CAS: [91052-83-4]
CAS: [100085-39-0]

DESCRIPTION

Diacetyl Tartaric Acid Esters of Mono- and Diglycerides occur over a range in appearance from sticky, viscous liquids through a fatlike consistency to a waxy solid, depending on the iodine value of the oils or fats used in their manufacture. They are the reaction product of partial glycerides of edible oils, fats, or fat-forming fatty acids with diacetyl tartaric anhydride. The diacetyl tartaroyl esters are miscible in all proportions with oils and fats. They are soluble in most common fat solvents, in methanol, in acetone, and in ethyl acetate, but are insoluble in other alcohols, in acetic acid, and in water. They are dispersible in water and resistant to hydrolysis for moderate periods of time. The pH of a 3% dispersion in water is between 2 and 3.

Function: Emulsifier
Packaging and Storage: Store in well-closed containers.

IDENTIFICATION

- **Procedure**

  Sample solution: 500 mg in 10 mL of methanol
  Analysis: Add, dropwise, lead acetate TS to the *Sample solution*.
  Acceptance criteria: A white, flocculent, practically insoluble precipitate forms.

ASSAY

- **Tartaric Acid**

  Solution A: Transfer 4 g of sample into a 250-mL Erlenmeyer flask, and add 80 mL of 0.5 N potassium hydroxide and 0.5 mL of phenolphthalein TS. Connect an air condenser at least 65 cm long to the flask, and heat the mixture on a hot plate for about 2.5 h. Remove the air condenser and add approximately 10% phosphoric acid to the hot mixture until it is definitely acid to congo red test paper. Reconnect the air condenser, and heat until the fatty acids are liquefied and clear. Cool, and transfer the mixture into a 250-mL separatory funnel with the aid of small portions of water and hexane. Extract the liberated fatty acids with three successive 25-mL portions of hexane, and collect the extracts in a second separatory funnel. Wash the combined hexane extracts with two 25-mL portions of water, and add the washings to the first separatory funnel containing the water layer. Retain the combined hexane extracts for the determination of *Fatty Acids (Total)*. Transfer the contents of the first separatory funnel to a 250-mL beaker, heat on a

steam bath to remove traces of hexane, filter through acid-washed, fine-texture filter paper into a 500-mL volumetric flask, and finally dilute with water to volume.

Solution B: Pipet 25.0 mL of *Solution A* into a 100-mL volumetric flask, and dilute with water to volume (*Solution B*). [Note—Retain the rest of *Solution A* for the determination of *Glycerin*.]

Standard stock solution: 1 mg/mL of tartaric acid (reagent-grade)

Standard solutions: Transfer 3.0-, 4.0-, 5.0-, and 6.0-mL aliquots of the *Standard stock solution* into separate 19- × 150-mm matched cuvettes, and add sufficient water to make 10.0 mL. Add 4.0 mL of a freshly prepared 50 mg/mL solution of sodium metavanadate and 1.0 mL of glacial acetic acid to each cuvette. [Note—Use these solutions within 10 min after color development.]

Blank solution: Prepare in the same manner as the *Standard solutions*, using 10.0 mL of water in the cuvette in place of the tartaric acid solutions.

Sample solution: Prepare in the same manner as the *Standard solutions*, using 10.0 mL of *Solution B* in the cuvette in place of the tartaric acid solutions.

Analysis: Using a suitable spectrophotometer or photoelectric colorimeter equipped with a 520-nm filter that has been set at zero with the *Blank solution*, determine the absorbance of each of the *Standard solutions* and the *Sample solution.* Prepare a standard curve from the data obtained for the *Standard solutions* by plotting the absorbances on the ordinate against the corresponding quantities, in mg, of tartaric acid in each solution on the abscissa. From the curve, determine the weight, in mg, of tartaric acid in the final dilution (W_T). Calculate the percentage of tartaric acid:

$$\text{Result} = (W_T/W_S) \times 20$$

W_T = weight of tartaric acid in the final dilution (mg)

W_S = weight of sample taken (mg)

Acceptance criteria: Between 17.0 and 20.0 g of tartaric acid ($C_4H_6O_6$) per 100 g of sample after saponification

IMPURITIES

Inorganic Impurities

- **Lead**, *Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace Method, Method II,* Appendix IIIB

  Sample: 10 g

  Acceptance criteria: NMT 2 mg/kg

SPECIFIC TESTS

- **Acetic Acid**, *Volatile Acidity,* Appendix VII

  Sample: 4 g

  Analysis: Use 30.03 as the equivalence factor (*e*)

  Acceptance criteria: Between 14.0 and 17.0 g of acetic acid (CH_3COOH) per 100 g of sample after hydrolysis

- **Acid Value**

  Solvent mixture: Prepare a 20% solution of hexane in methanol (v/v) and add phenol red TS. Neutralize the solution if necessary.

  Analysis: Transfer 1 g of sample into a 125-mL Erlenmeyer flask and dissolve in 25 mL of *Solvent mixture*. Titrate with 0.1 N methanolic potassium hydroxide to a light red endpoint. Perform a blank determination (see *General Provisions*) using a 25-mL portion of the *Solvent mixture*, and make any necessary

correction. Calculate the acid value:

$$\text{Result} = 56.1 \times V \times N/W$$

V = volume of the methanolic potassium hydroxide used in the titration (mL)
N = normality of the methanolic potassium hydroxide used
W = weight of the sample taken (g)

Acceptance criteria: Between 62 and 76

- **FATTY ACIDS (TOTAL)**

Sample: The combined hexane extracts of fatty acids obtained in the test for *Tartaric Acid*

Analysis: Dry the *Sample* by shaking with a few grams of anhydrous sodium sulfate. Filter the solution into a tared, 250-mL beaker, evaporate the hexane on a steam bath, cool, and weigh.

Acceptance criteria: NLT 56.0 g of total fatty acids per 100 g of sample after hydrolysis

- **GLYCERIN**

Periodic acid solution: Dissolve 2.7 g of periodic acid (H_5IO_6) in 50 mL of water, add 950 mL of glacial acetic acid, and mix thoroughly. [Note—Protect this solution from light.]

Analysis: Transfer 5.0 mL of *Solution A*, prepared in the test for *Tartaric Acid*, into a 250-mL glass-stoppered Erlenmeyer or iodine flask. Add 15 mL of glacial acetic acid and 25.0 mL of *Periodic acid solution* to the flask, shake the mixture for 1 or 2 min, allow it to stand for 15 min, add 15 mL of a 150 mg/mL potassium iodide solution and 15 mL of water, swirl, and let it stand for 1 min. Titrate the liberated iodine with 0.1 N sodium thiosulfate, using starch TS as the indicator. Perform *Residual Blank Titration* (see *General Provisions*) using water in place of sample, and make any necessary correction. The corrected volume is the number of mL of 0.1 N sodium thiosulfate required for the glycerin and the tartaric acid in the sample represented by the 5 mL of *Solution A*. From the percentage determined in the test for *Tartaric Acid*, calculate the volume of 0.1 N sodium thiosulfate required for the tartaric acid in the titration. The difference between the corrected volume and the calculated volume required for the tartaric acid is the number of mL of 0.1 N sodium thiosulfate consumed because of the glycerin in the sample. One mL of 0.1 N sodium thiosulfate is equivalent to 2.303 mg of glycerin and to 7.505 mg of tartaric acid.

Acceptance criteria: NLT 12.0 g of glycerin ($C_3H_8O_3$) per 100 g of sample after hydrolysis

Change to read:

- **RESIDUE ON IGNITION (SULFATED ASH)**, Appendix IIC

Sample: 10 g

Acceptance criteria: NMT ~~0.01%~~■ 0.5%■ 1S (*FCC7*)

- **SAPONIFICATION VALUE**, Appendix VII

Sample: 2 g

[Note—Add 5 to 10 mL of water to samples and blanks before saponification; otherwise, sufficient salts precipitate during saponification to cause serious bumping and spattering.]

Acceptance criteria: Between 380 and 425

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(FI07) Food Ingredients Expert Committee

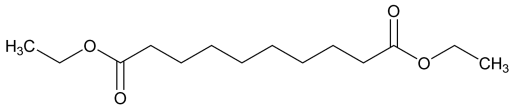
FCC Sixth Edition Page 256

BRIEFING

Diethyl Sebacate, *FCC 6* page 262. On the basis of efforts to modernize *Identification* test methods in *FCC*, it is proposed to change the existing infrared method in the *Identification* section to one that utilizes a USP Reference Standard instead of comparison to a printed spectrum.
(FIEC: K. Bowman) C77400

Diethyl Sebacate

Ethyl Sebacate



$C_{14}H_{26}O_4$

Formula wt 258.36
FEMA: 2376

DESCRIPTION

Diethyl Sebacate occurs as a colorless to slightly yellow liquid.

Odor: Faint, winy, fruity

Solubility: Miscible in alcohol, ether, other organic solvents, most fixed oils; insoluble or practically insoluble in water

Boiling Point: ~302°

Function: Flavoring agent

IDENTIFICATION

Change to read:

- ~~**Infrared Spectra,**~~ ■ **Infrared Absorption,** ■ 1S (*FCC7*) *Spectrophotometric Identification Tests,* Appendix IIIC

  ~~**Acceptance criterion:** The spectrum of the sample exhibits relative maxima at the same wavelengths as those of the spectrum below.~~

  Click to View Image

  ~~Diethyl Sebacate~~

  ■ **Reference standard:** USP Diethyl Sebacate RS

  Sample and standard preparation: *F*

  Acceptance criteria: The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard.* ■ 1S (*FCC7*)

ASSAY

- **Procedure:** Proceed as directed under *M-1b,* Appendix XI.

  Acceptance criteria: NLT 98.0% of $C_{14}H_{26}O_4$

SPECIFIC TESTS

- **Acid Value,** *M-15,* Appendix XI

Acceptance criteria: NMT 1.0

- **Refractive Index,** Appendix II (at 20°)
  Acceptance criteria: Between 1.435 and 1.438
- **Specific Gravity:** Determine at 25° by any reliable method (see *General Provisions*).
  Acceptance criteria: Between 0.960 and 0.965

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 262

BRIEFING

***d*-Dihydrocarvone,** *FCC 6* page 264. On the basis of comments received, it is proposed to change the title of this monograph to reflect a less confusing and more widely accepted method of distinguishing optically active isomers. Also proposed is the addition of the existing name as a synonym.
(FIEC: K. Bowman) C79718

Change to read:

~~***d*-Dihydrocarvone**~~■ **(+)-Dihydrocarvone**■ 1S (*FCC7*)

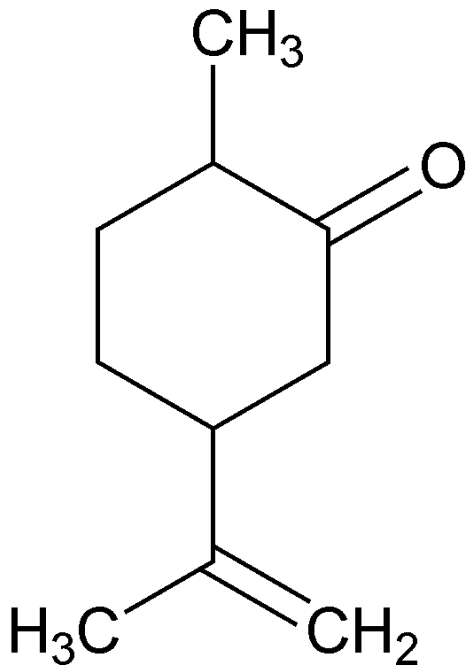
Change to read:

■

d-Dihydrocarvone

■ 1S (*FCC7*)

d-2-Methyl-5-(1-methylethenyl)-cyclohexanone



$C_{10}H_{16}O$

Formula wt 154.24
FEMA: 3565

DESCRIPTION

Change to read:

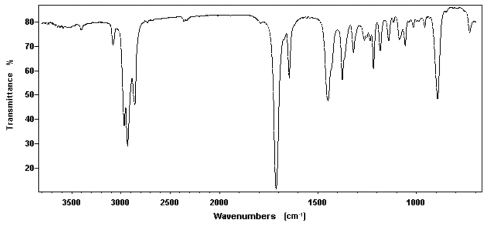
~~*d*-Dihydrocarvone~~■ (+)-Dihydrocarvone■ 1S (*FCC7*) occurs as an almost colorless liquid.

Odor: Herbaceous, spearmint
Solubility: Soluble in alcohol, most fixed oils; insoluble or practically insoluble in water
Boiling Point: ~222°
Solubility in Alcohol, Appendix VI**:** One mL dissolves in 1 mL of 95% alcohol.
Function: Flavoring agent

IDENTIFICATION

Change to read:

- **Infrared Spectra,** *Spectrophotometric Identification Tests,* Appendix IIIC
  Acceptance criteria: The spectrum of the sample exhibits relative maxima at the same wavelengths as those of the spectrum below.



~~*d*-Dihydrocarvone~~ ■ (+)-Dihydrocarvone ■ 1S (*FCC7*)

ASSAY

- **Procedure:** Proceed as directed under *M-1a,* Appendix XI
  Acceptance criteria: NLT 92.0% of $C_{10}H_{16}O$ (sum of two isomers)

SPECIFIC TESTS

- **Refractive Index,** Appendix II (at 20°)
  Acceptance criteria: Between 1.470 and 1.474
- **Specific Gravity:** Determine at 25° by any reliable method (see *General Provisions*).
  Acceptance criteria: Between 0.923 and 0.928

OTHER REQUIREMENTS

- **Angular Rotation,** *Optical (Specific) Rotation,* Appendix IIB
  Acceptance criteria: Between +14.0° and +22°

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 264

BRIEFING

Disodium 5′-Uridylate. Because there is no existing *FCC* monograph for this food ingredient, a new monograph is proposed on the basis of data received. The test procedures for Enterobacteriaceae (*Bile-Tolerant Gram-Negative Bacteria*), *Enterobacter sakazakii (Cronobacter* spp.), *Salmonella* spp., *Total Yeasts and Molds Count*, and *Total Aerobic Microbial Count* are new proposed General Tests and Assays procedures (see the Briefing under Appendix XII). The FCC Policy on Microbial Attributes (*FCC 5*, page 3) indicates that the *FCC* does not list specific microbial criteria...other than those for which scientifically valid data are available to [The Food Ingredients Expert Committee]...that support the need for such criteria. Considering the *Codex Alimentarius* principles for establishing microbial criteria outlined in this FCC policy, data were received to support the proposal of five microbial acceptance criteria on the following basis:

1. These ingredients are currently used predominately in foods for infants and young children, a sub-population recognized internationally as being particlarly vulnerable.
2. For powdered infant formulas manufactured using the dry-mix and combined wet-mix/dry-mix processes, ingredients added, such as nucleotides, may not receive a microbiological heat treatment step. The microbial safety of these finished products is therefore depedent on the microbiological quality of the ingredients used. This indicates that control at the ingredient point in the supply chain for these food products may be an effective way to protect the consumer from microbial contamination. This notion is supported by the 2008 Codex Alimentarius Code of Hygienic Practice For Powdered Formulae for Infants and Young Children (CAC/RCP 66, 2008).
3. The Codex Alimentarius Code of Hygienic Practice For Powdered Formulae for Infants and Young Children (CAC/RCP 66, 2008) recommends criteria for the pathogenic microorganisms *Salmonella* spp. and *Enterobacer sakazakii*, and hygiene standards for aerobic bacteria (Total Aerobic Microbial Count) and Enterobacteriaceae (Bile-Tolerant Gram-Negative Bacteria)
4. Current standards for these three nucleotides in Australia/New Zealand (FSANZ-1.3.4) include specifications for aerobic bacteria, coliforms, yeasts and molds, and *Salmonella*.
5. Current standards for 5′-Adenylic Acid and 5′-Cytidylic Acid (for use in infant formula) in the Korean Food Additives Code include specifications for “General Fungi” and *Salmonella*.

(FIEC: J. Moore) C82096

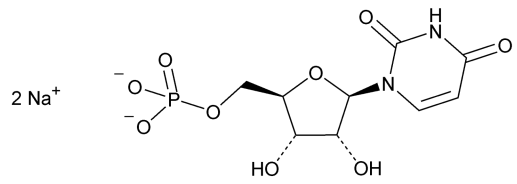
Add the following:

■ **Disodium 5′-Uridylate**

Uridine 5′-monophosphate disodium salt

Disodium uridine 5′-monophosphate

UMP disodium salt



$C_9H_{11}N_2Na_2O_9P \cdot xH_2O$

Formula wt 368.15
CAS: [3387-36-8]

DESCRIPTION

Disodium 5′-Uridylate occurs as colorless or white crystals. It contains approximately seven molecules of water of crystallization. It is soluble in water, sparingly soluble in alcohol, and practically insoluble in ether. It is produced by enzymatic cleavage of natural source yeast RNA with a 5-phosphodiesterase followed by heat treatment, further purification steps, and washing of crystals with ethanol.

Function: Source of Disodium 5'-Uridylate

Packaging and Storage: Store in tight containers, protected from light and moisture.

IDENTIFICATION

- **A. Infrared Absorption,** *Spectrophotometric Identification Tests,* Appendix IIIC
  Reference standard: USP Disodium 5'-Uridylate RS
  Sample and standard preparation: *A*
  Acceptance criteria: The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard*.
- **B. Procedure**
  Acceptance criteria: The retention time of the major peak (excluding the solvent peak) in the chromatogram of the *Sample solution* corresponds to that of the *Standard solution* in the *Assay*.

ASSAY

- **Procedure**
  Mobile phase: 0.1 M potassium dihydrogen phosphate (KH_2PO_4) in degassed water, adjusted to pH 5.6 with 0.1 M dipotassium hydrogen phosphate (K_2HP0_4)
  Standard solution: 0.02 mg/mL of USP Disodium 5'-Uridylate RS in *Mobile phase.* [Note—Ultra-sonication may be necessary to aid in complete dissolution.]
  Sample solution: 0.02 mg/mL in *Mobile phase.* [Note—Ultra-sonication may be necessary to aid in complete dissolution.]
  Chromatographic system, Appendix IIA
  Mode: High-performance liquid chromatography
  Detector: UV 254 nm
  Column: 25 cm × 4.6-mm; packed with 5-µm reversed phase C18 silica gel[1]
  Column temperature: Ambient
  Flow rate: About 1.0 mL/min
  Injection size: 50 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Suitability requirement 1: The relative standard deviation of the disodium 5′-uridylate peak area responses from replicate injections is NMT 2.0%.
  Suitability requirement 2: The resolution, *R*, between the disodium 5′-uridylate peak and all other peaks is NLT 2.0.
  Analysis: Separately inject equal volumes of the *Standard solution* and *Sample solution* into the chromatograph, and measure the responses for the major peaks on the resulting chromatograms. [Note—The approximate retention time for disodium 5′-uridylate is 6.2 min.] Calculate the percentage of

disodium 5′-uridylate, $C_9H_{11}N_2Na_2O_9P$, in the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area response for disodium 5′-uridylate in the *Sample solution*

r_S = peak area response for disodium 5′-uridylate in the *Standard solution*

C_S = concentration of disodium 5′-uridylate in the *Standard solution* (mg/mL)

C_U = concentration of sample in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–103.0%, calculated on the anhydrous basis

IMPURITIES

Inorganic Impurities

- **ARSENIC**

[Note—When water is specified as a diluent, use deionized ultra-filtered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of arsenic as practical.]

Diluent: 4% nitric acid in water

Standard stock solution: 100 µg/mL of arsenic prepared by diluting a commercially available 1000 mg/kg arsenic ICP standard solution

Standard solutions: 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL of arsenic: from *Standard stock solution* diluted with *Diluent*

Sample: 5 g

Sample solution: Dissolve the *Sample* in 40 mL of 10% nitric acid in a 100-mL volumetric flask, and dilute with water to volume.

Spectrophotometric system*, Plasma Spectrochemistry,* Appendix IIC

Mode: Inductively coupled plasma–optical emission spectroscopy (ICP–OES)

Setup: Use a suitable ICP–OES configured in a radial optical alignment. [Note—This method was developed using a Varian Vista MPX ICP–OES unit.] The instrument parameters are as follows: set the ultra-violet detector to scan arsenic at 188.980 nm. Set the sample read time to 20 s. Set the forward power from the RF generator to 1150 watts. Use an argon plasma feed gas flow of 13.5 L/min with the auxiliary gas set to flow at 2.25 L/min. The sample is delivered to the spray chamber by a multi-channel peristaltic pump set to deliver sample at a rate of 20 rpm. Samples are flushed through the system for 20 s prior to analysis. A 40-s read delay is also programmed into the sampling routine to allow for fluid flow equilibration after the high-speed flush, prior to the first analytical read of the sample. Between samples, the pumping system is washed by flushing the *Diluent* for 20 s.

Analysis: Generate a standard curve using *Diluent* as a blank and the *Standard solutions.* [Note—The correlation coefficient for the best-fit line should not be less than 0.999.]

Similarly, analyze the *Sample solution* on the ICP. Calculate the concentration (mg/kg) of arsenic in the *Sample* taken:

$$\text{Result} = (C/W) \times F$$

C = concentration of arsenic in the *Sample solution* determined from the standard curve (µg/mL)

W = weight of *Sample* taken (g)

F = *Sample solution* final volume, 100 mL

Acceptance criteria: NMT 2 mg/kg

- **Cadmium**

[Note—When water is specified as a diluent, use deionized ultra-filtered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of cadmium as practical.]

Diluent: 4% nitric acid in water

Standard stock solution: 100 µg/mL of cadmium prepared by diluting a commercially available 1000 mg/kg cadmium ICP standard solution

Standard solutions: 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL of cadmium: from *Standard stock solution* diluted with *Diluent*

Sample: 5 g

Sample solution: Dissolve the *Sample* in 40 mL of 10% nitric acid in a 100-mL volumetric flask, and dilute with water to volume.

Spectrophotometric system*, Plasma Spectrochemistry,* Appendix IIC

Mode: ICP–OES

Setup: Same as that described in the test for *Arsenic*, but set to scan for cadmium at 228.802 nm

Analysis: Generate a standard curve using *Diluent* as a blank and the *Standard solutions.* [Note—The correlation coefficient for the best-fit line should not be less than 0.999.]

Similarly, analyze the *Sample solution* on the ICP. Calculate the concentration (mg/kg) of cadmium in the *Sample* taken:

$$\text{Result} = (C/W) \times F$$

C = concentration of cadmium in the *Sample solution* determined from the standard curve (µg/mL)

W = weight of *Sample* taken (g)

F = *Sample solution* final volume, 100 mL

Acceptance criteria: NMT 0.1 mg/kg

- **Lead**

[Note—When water is specified as a diluent, use deionized ultra-filtered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of lead as practical.]

Diluent: 4% nitric acid in water

Standard stock solution: 100 µg/mL of lead prepared by diluting a commercially available 1000 mg/kg lead ICP standard solution

Standard solutions: 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL of lead: from *Standard stock solution* diluted with *Diluent*

Sample: 5 g

Sample solution: Dissolve the *Sample* in 40 mL of 10% nitric acid in a 100-mL volumetric flask, and dilute with water to volume.

Spectrophotometric system*, Plasma Spectrochemistry,* Appendix IIC

Mode: ICP–OES

Setup: Same as that described in the test for *Arsenic*, but set to scan for lead at 220.353 nm

Analysis: Generate a standard curve using *Diluent* as a blank and the *Standard solutions.* [Note—The correlation coefficient for the best-fit line should not be less than 0.999.]

Similarly, analyze the *Sample solution* on the ICP. Calculate the concentration (mg/kg) of lead in the *Sample* taken:

$$\text{Result} = (C/W) \times F$$

C = concentration of lead in the *Sample solution* determined from the standard curve (µg/mL)

W = weight of *Sample* taken (g)

F = *Sample solution* final volume, 100 mL

Acceptance criteria: NMT 1 mg/kg

- **MERCURY**

[Note—When water is specified as a diluent, use deionized ultra-filtered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of mercury as practical.]

Diluent: 4% nitric acid in water

Standard stock solution: 100 µg/mL of mercury prepared by diluting a commercially available 1000 mg/kg mercury ICP standard solution

Standard solutions: 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL of mercury: from *Standard stock solution* diluted with *Diluent*

Sample: 5 g

Sample solution: Dissolve the *Sample* in 40 mL of 10% nitric acid in a 100-mL volumetric flask, and dilute with water to volume.

Spectrophotometric system*, Plasma Spectrochemistry,* Appendix IIC

Mode: ICP–OES

Setup: Same as that described in the test for *Arsenic*, but set to scan for mercury at 194.164 nm

Analysis: Generate a standard curve using *Diluent* as a blank and the *Standard solutions.* [Note—The correlation coefficient for the best-fit line should not be less than 0.999.]

Similarly, analyze the *Sample solution* on the ICP. Calculate the concentration (mg/kg) of mercury in the *Sample* taken:

$$\text{Result} = (C/W) \times F$$

C = concentration of mercury in the *Sample solution* determined from the standard curve (µg/mL)

W = weight of *Sample* taken (g)

F = *Sample solution* final volume, 100 mL

Acceptance criteria: NMT 0.5 mg/kg

Organic Impurities

- **ETHANOL**

Standard solution: 100 mg/kg of ethanol in 1 N sodium hydroxide. Add 10 mL of this solution to a 20-mL headspace vial, and cap tightly.

Sample solution: 100 mg/g in 1 N sodium hydroxide. Add 10 mL of this solution to a 20-mL headspace vial, and cap tightly.

Chromatographic system*,* Appendix IIA

Mode: Gas chromatography equipped with pressure-loop headspace autosampler

Detector: Flame ionization

Column: 30-m × 0.53-mm (id) capillary column with a 6% cyanopropylphenyl–94% dimethylpolysiloxane stationary phase and a 3.00-µm film thickness[2]

Column temperature: 20 min at 40°; increase to 240° at 10°/min; maintain at 240° for 10 min

Injection port temperature: 140°

Detector temperature: 250°

Carrier gas: Nitrogen

Flow rate: 2.5 mL/min
Headspace unit: 2.5 mL/min

Equilibration temperature: 80°
Equilibration time: 60 min

Loop temperature: 85°

Transfer temperature: 90°
Pressurization time: 0.5 min
Loop fill time: 0.1 min
Injection time: 1 min
Injection size: 1 mL of headspace

System suitability

Sample: *Standard solution*

Suitability requirement: The relative standard deviation of the ethanol peak area responses from replicate injections is NMT 5.0%.

Analysis: Separately inject equal volumes of the *Standard solution* and *Sample solution* into the chromatograph, record the chromatograms, and measure the peak responses. [Note—The approximate retention time for ethanol is 11 min.]

Acceptance criteria: The peak area from the *Sample solution* does not exceed that from the *Standard solution* (NMT 1000 mg/kg).

- **Other Ribonucleotides**

Mobile phase and **Chromatographic system:** Prepare as directed in the *Assay*.

Sample solution: 1.0 mg/mL. [Note—Ultra-sonication may be necessary to aid in complete dissolution.]

Standard solution: Mixture of USP Disodium 5′-Uridylate RS, USP 5′-Adenylic Acid RS, USP 5′-Cytidylic Acid RS, USP Disodium Guanylate RS, and USP Disodium Inosinate RS each at 0.02 mg/mL in *Mobile phase*

Suitability requirements

Sample: *Standard solution*

Suitability requirement 1: The relative standard deviation of the disodium 5'-uridylate peak area responses from replicate injections is NMT 2.0%.

Suitability requirement 2: The resolution, *R*, between the disodium 5'-uridylate peak and all other nucleotide peaks is NLT 2.0.

Analysis: Separately inject equal volumes of the *Standard solution* and *Sample solution* into the chromatograph, and measure the responses for all nucleotide peaks on the resulting chromatograms, except the peak from disodium 5′-uridylate. [Note—The approximate retention times are 4.6 min (5′-cytidylic acid), 6.2 min (5′-uridylic acid), 10.3 min (5′-guanylic acid), 11.5 min (5′-inosinic acid), and 27.5 min (5′-adenylic acid).] Separately calculate the percentage of each analyte (5′-cytidylic acid, 5′-guanylic acid, 5′-inosinic acid, and 5′-adenylic acid) in the sample taken:

$$\text{Result} = (C_S/C_U) \times (r_U/r_S) \times 100$$

C_S = concentration of analyte in the *Standard solution* (mg/mL)

C_U = concentration of analyte in the *Sample solution* (mg/mL)

r_U = peak area of the analyte from the *Sample solution*

r_S = peak area of the analyte from the *Standard solution*

Acceptance criteria: The sum of the percentages for all nucleotide impurities is NMT 1%, calculated on the anhydrous basis.

SPECIFIC TESTS

- **pH**, *pH Determination,* Appendix IIB
  Sample solution: 50 mg/mL
  Acceptance criteria: 7.0–8.5
- **Water**, *Water Determination, Method I,* Appendix IIB
  Acceptance criteria: NMT 26.0%
- **Bile-Tolerant Gram-Negative Bacteria**, Appendix XIC
  Sample preparation: Proceed as directed using a 10-g sample and incubating at 30–35° for 18–24 h.
  Acceptance criteria: Negative in 10 g
- **Enterobacter sakazakii** *(Cronobacter spp.),* Appendix XIC
  Sample preparation: Proceed as directed using a 10-g sample and incubating at 30–35° for 18–24 h.
  Acceptance criteria: Negative in 10 g
- **Salmonella spp.**, Appendix XIC
  Sample preparation: Dissolve 25 g of sample at a sample/broth ratio of 1/8, and proceed as directed.
  Acceptance criteria: Negative in 25 g
- **Total Aerobic Microbial Count**, *Method I (Plate Count Method),* Appendix XIB
  Acceptance criteria: NMT 1,000 cfu/g
- **Total Yeasts and Molds Count**, *Method I (Plate Count Method),* Appendix XIB
  Acceptance criteria: NMT 100 cfu/g

■ 1S (*FCC7*)

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[1] YMC-Pack ODS-AQ (YMC Europe GmbH, Dinslaken, Germany), or equivalent.

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[2] CP-Select 624 CB (Varian-Chrompack, Palo Alto, CA), or equivalent.

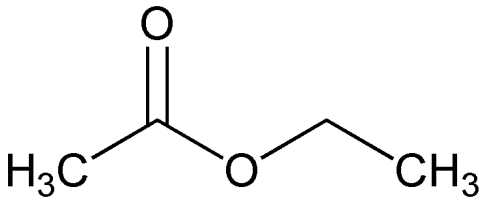
Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(FI07) Food Ingredients Expert Committee

BRIEFING

Ethyl Acetate, *FCC 6* page 301. On the basis of comments received, a revision to the *Boiling Point* is proposed. The revision aligns this value with the monograph for *Ethyl Acetate* prepared at the 46th Session of the Joint FAO/WHO Expert Committee on Food Additives (1996).
(FIEC: K. Bowman) C84051

Ethyl Acetate



$C_4H_8O_2$

Formula wt 88.11
FEMA: 2414

DESCRIPTION
Ethyl Acetate occurs as a colorless liquid; volatile at low temperatures; flammable.

Change to read:
Odor: ▲*FCC6* Acetous, ethereal

Solubility: Miscible in alcohol, ether, glycerin, most fixed oils, volatile oils; 1 mL dissolves in 10 mL water

Change to read:

Boiling Point: ~~~54°~~ ■ ~77° ■ 1S (*FCC7*)

Function: Flavoring agent

IDENTIFICATION

Change to read:
- ▲**Infrared Absorption**, *Spectrophotometric Identification Tests,* Appendix IIIC
  Reference standard: USP Ethyl Acetate RS
  Sample and standard preparation: *F*
  Acceptance criteria: The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard.*▲*FCC6*

ASSAY
- **Procedure:** Proceed as directed under *M-1b,* Appendix XI
  Acceptance criteria: NLT 99.0% of $C_4H_8O_2$

SPECIFIC TESTS
- **Acid Value**, *M-15,* Appendix XI (Use bromocresol purple TS as the indicator.)
  Acceptance criteria: NMT 5.0

- **Refractive Index**, Appendix II (at 20°)
  Acceptance criteria: Between 1.370 and 1.375
- **Specific Gravity:** Determine at 25° by any reliable method (see *General Provisions*).
  Acceptance criteria: Between 0.894 and 0.898

OTHER REQUIREMENTS

- **Distillation Range**, Appendix IIB
  Acceptance criteria: Between 76° and 77.5°
- **Methyl Compounds**, *M-10*, Appendix XI
  Acceptance criteria: Passes test
- **Readily Carbonizable Substances**, *M-12*, Appendix XI
  Acceptance criteria: Passes test
- **Residue on Evaporation**, *M-16*, Appendix XI (105°)
  Sample: 10 g
  Acceptance criteria: NMT 0.02%

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

BRIEFING

Ethyl Lauroyl Arginate. Because there is no existing *FCC* monograph for this food ingredient, a new monograph is proposed based on the Ethyl Lauroyl Arginate monograph from the 71st session (2009) of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and based on data received. While the *Lead* limit was based on the JECFA monograph, it is proposed for the testing procedure to use the FCC Atomic Absorption Spectrophotometric Graphite Furnace Method instead of the AAS/ICP-AES testing procedure described in the JECFA monograph. Interested parties are encourage to submit comments to Jeff Moore, Ph.D., email jm@usp.org.

(FIEC: J. Moore) C69184

Add the following:

■ **Ethyl Lauroyl Arginate**

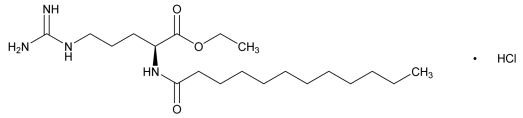
Ethyl-N^{α}-Lauroyl-l-Arginate · HCl

Ethyl-N^{α}-Dodecanoyl-l-Arginate · HCl

Lauric Arginate Ethyl Ester

Lauramide Arginine Ethyl Ester

LAE



$C_{20}H_{41}N_4O_3Cl$

Formula wt 421.02

INS: 243

CAS: [60372-77-2]

DESCRIPTION

Ethyl Lauroyl Arginate occurs as a white powder. It is synthesized by esterifying arginine with ethanol, followed by reacting the ester with lauroyl chloride. The resultant ethyl lauroyl arginate is recovered as hydrochloride salt which is filtered off and dried. It is freely soluble in water, ethanol, propylene glycol, and glycerol.

Function: Preservative

Packaging and Storage: Store in tight containers in a dry place.

IDENTIFICATION

- **Procedure**

  Acceptance criteria: The retention time of the major peak (excluding the solvent peak) in the chromatogram of the *Sample solution* corresponds to that of the *Standard solution* in the *Assay* (below).

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile and water (50:50, v/v) containing 0.1% trifluoroacetic acid

Standard stock solution: 3000 µg/mL USP Ethyl Lauroyl Arginate Hydrochloride RS[1] and 100 µg/mL USP Lauroyl Arginine RS[2] in *Mobile phase*

Standard solutions: Separately dilute 2-, 4-, 6-, 8-, and 10-mL aliquots of *Standard stock solution* with *Mobile phase* to 25 mL.

Sample solution: 1000 µg/mL in *Mobile phase*

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography

Detector: UV 215 nm

Column: 15-cm × 3.9-mm, packed with octadecylsilane chemically bonded to porous silica or ceramic micro-particles (5-µm particle diameter)[3]

Column temperature: Ambient

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solutions*

Suitability requirement: The relative standard deviation is NMT 2.0% for the slope of the ethyl-N^{α}-lauroyl-l-arginate · HCl standard curve.

Analysis: [Note—Equilibrate the column by pumping *Mobile phase* through it for 30 min. All injections should be done in triplicate.] Separately inject equal volumes of the *Standard solutions* and *Sample solution* into the chromatograph and measure the responses for the major peaks on the resulting chromatograms. [Note—The retention times for ethyl-N^{α}-lauroyl-l-arginate · HCl and N^{α}-lauroyl-l-arginine are approximately 4.3 and 2.2 min, respectively.]

Prepare a standard curve for ethyl-N^{α}-lauroyl-l-arginate · HCl by plotting peak areas versus concentrations in µg/mL of the *Standard solutions*. Calculate the percentage of ethyl-N^{α}-lauroyl-l-arginate · HCl in the portion of the sample taken:

$$\text{Result} = C_U / C_{SMP} \times 100$$

C_U = concentration of ethyl-N^{α}-lauroyl-l-arginate · HCl in the *Sample solution* determined from the standard curve (µg/mL)

C_{SMP} = concentration of sample in the *Sample solution* (µg/mL)

Acceptance criteria: 85%–95%

IMPURITIES

Inorganic Impurities

- **LEAD,** *Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace Method, Method I,* Appendix IIIB

Acceptance criteria: NMT 1 mg/kg

Organic Impurities

- **L-ARGININE HYDROCHLORIDE**

Mobile phase: Methanol, 15 mM sodium heptanesulfonate, 27 mM phosphoric acid solution, and 3 mM sodium di-hydrogen phosphate solution (1.5:1:1:1) (v/v/v/v)

Standard stock solution: 400 µg/mL USP Arginine Hydrochloride RS[4]

Standard solutions: 20, 40, and 60 µg/mL of USP Arginine Hydrochloride RS, made from *Standard stock solution*

Sample solution: 4000 µg/mL

Derivatizing solution: Mix 1 L of 0.2 M borate buffer solution (pH 9.4) with 0.8 g of *o*-phthaldialdehyde dissolved in 5 mL of methanol and 2 mL of 2-mercaptoethanol. [Note—This solution is stable for 48 h at room temperature and without additional preventative measure but it is advisable to keep the solution under nitrogen and to prepare it freshly every 24–48 h.]

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography (equipped with post-column derivatization)

Detector: UV 340 nm

Column: 30 cm × 3.9-mm, packed with octadecylsilane chemically bonded to porous silica or ceramic micro-particles (10-µm particle diameter)[5]

Column temperature: 65°

Flow rate: 0.8 mL/min

Injection size: 10 µL

Derivatization: Post-column derivatization is produced employing the *Derivatizing solution* at 65° in a teflon tubular reactor (650–800 × 0.3 mm)

System suitability

Sample: *Standard solutions*

Suitability requirement: The relative standard deviation is NMT 5.0% for the slope of the l-arginine · HCl standard curve.

Analysis: [Note—Equilibrate the column by pumping *Mobile phase* through it for 30 min. All injections should be done in triplicate.] Separately inject equal volumes of the *Standard solutions* and *Sample solution* into the chromatograph and measure the responses for the major peaks on the resulting chromatograms. [Note—The retention time for l-arginine · HCl is approximately 5.03 min.]

Prepare standard curves for l-arginine · HCl by plotting peak areas versus concentrations in µg/mL of the *Standard solutions*. Calculate the percentage of l-arginine · HCl in the portion of the sample taken:

$$\text{Result} = C_U/C_{SMP} \times 100$$

C_U = concentration of l-arginine · HCl in the *Sample solution* determined from the standard curve (µg/mL)

C_{SMP} = concentration of sample in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 1%

- **L-ARGININE ETHYL ESTER DIHYDROCHLORIDE**

Mobile phase, Derivatizing solution, and **Chromatographic system:** Prepare as directed in the test procedure for *L-Arginine Hydrochloride* (above).

Standard stock solution: 8000 µg/mL USP Arginine Ethyl Ester Dihydrochloride RS[6]

Standard solutions: 400, 800, and 1200 µg/mL of USP Arginine Ethyl Ester Dihydrochloride RS, made from *Standard stock solution*

Sample solution: 80 mg/mL

System suitability

Sample: *Standard solutions*

Suitability requirement: The relative standard deviation is NMT 5.0% for the slope of the l-arginine ethyl ester · 2HCl standard curve.

Analysis: [Note—Equilibrate the column by pumping *Mobile phase* through it for 30 min. All injections should be done in triplicate.] Separately inject equal volumes of the *Standard solutions* and *Sample solution* into the chromatograph and measure the responses for the major peaks on the resulting chromatograms. [Note—The retention time for l-arginine ethyl ester · 2HCl is approximately 6.70 min.]

Prepare a standard curve for l-arginine ethyl ester · 2HCl by plotting peak areas versus concentrations in µg/mL of the *Standard solutions*. Calculate the percentage of l-arginine ethyl ester · 2HCl in the portion of the sample taken:

$$\text{Result} = C_U/C_{SMP} \times 100$$

C_U = concentration of analyte in the *Sample solution* determined from the standard curve(µg/mL)

C_{SMP} = concentration of sample in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 1%

- **Ethyl Laurate and Lauric Acid**

Mobile phase: Acetonitrile and water (85:15) (v/v), containing 0.1% trifluoroacetic acid

Standard stock solution: 2500 µg/mL of USP Lauric Acid RS and 1500 µg/mL of USP Ethyl Laurate RS in *Mobile phase*

Standard solutions: Separately dilute 5-, 10-, and 15-mL aliquots of *Standard stock solution* with *Mobile phase* to 50 mL.

Sample solution: 10 mg/mL

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography

Detector: UV 212 nm

Column: 15 cm × 3.9-mm, packed with octadecylsilane chemically bonded to porous silica or ceramic micro-particles (5-µm particle diameter)[7]

Column temperature: Ambient

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solutions*

Suitability requirement: The relative standard deviation is NMT 5.0% for the slope of the lauric acid and ethyl laurate standard curves.

Analysis: [Note—Equilibrate the column by pumping *Mobile phase* through it for 30 min. All injections should be done in triplicate.] Separately inject equal volumes of the *Standard solutions* and *Sample solution* into the chromatograph and measure the responses for the major peaks on the resulting chromatograms. [Note—The retention times for lauric acid and ethyl laurate are approximately 3.65 and 11.2 min, respectively.]

Prepare standard curves for ethyl laurate and lauric acid by plotting their peak areas versus concentrations in µg/mL of the *Standards solutions*, corrected for purity. Separately calculate the percentages of ethyl laurate and lauric acid in the portion of the sample taken:

$$\text{Result} = C_U/C_{SMP} \times 100$$

C_U = concentration of analyte in the *Sample solution* determined from the standard curve (µg/mL)

C_{SMP} = concentration of sample in the *Sample solution* (µg/mL)

Acceptance criteria

Ethyl laurate: NMT 3%

Lauric acid: NMT 5%

- **LAUROYL ARGININE**

Mobile phase, Standard stock solution, Standard solutions, Sample solution, and **Chromatographic system:** Proceed as directed under *Assay.*

System suitability

Sample: *Standard solutions*

Suitability requirement: The relative standard deviation is NMT 5.0% for the slope of the N^{α}-lauroyl-l-arginine standard curve.

Analysis: Proceed as directed under *Assay.* Prepare a standard curve for lauroyl arginine by plotting N^{α}-lauroyl-l-arginine peak areas versus concentrations in µg/mL of the *Standards solutions*. Calculate the percentage of N^{α}-lauroyl-l-arginine in the portion of the sample taken:

$$\text{Result} = C_U/C_{SMP} \times 100$$

C_U = concentration of N^{α}-lauroyl-l-arginine in the *Sample solution* determined from the standard curve (µg/mL)

C_{SMP} = concentration of sample in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 3%

SPECIFIC TESTS

- *Ash (Total),* Appendix IIC

Analysis: Proceed as directed, but igniting at 700° instead of 550°.

Acceptance criteria: NMT 2%

- **pH,** *pH Determination,* Appendix IIB

Sample: 10 mg/mL

Acceptance criteria: Between 3.0 and 5.0

- **WATER,** *Water Determination, Method I,* Appendix IIB

Acceptance criteria: NMT 5%

■ 1S (*FCC7*)

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1 Ethyl-N^{α}-lauroyl-l-arginate · HCl.

2 N^{α}-Lauroyl-l-arginine.

3 Symmetry C18 (Waters Corporation, Milford, MA, USA), or equivalent.

4 l-Arginine · HCl.

5 µBondapack C18 (Waters Corporation, Milford, MA, USA), or equivalent.

6 l-Arginine ethyl ester dihydrochloride.

7 Symmetry C18 (Waters Corporation, Milford, MA, USA), or equivalent.

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(FI07) Food Ingredients Expert Committee

BRIEFING

***d*-Fenchone,** *FCC 6* page 352. On the basis of comments received, it is proposed to change the title of this monograph to reflect a less confusing and more widely accepted method of distinguishing optically active isomers. Also proposed is the addition of the existing name as a synonym.
(FIEC: K. Bowman) C79746

Change to read:

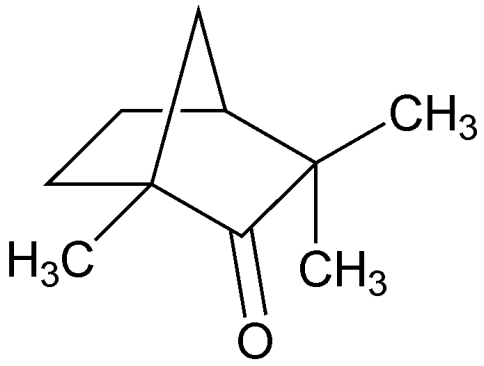
~~***d*-Fenchone**~~■ **(+)-Fenchone**■ 1S (*FCC7*)

Change to read:

■

d-Fenchone

■ 1S (*FCC7*)



$C_{10}H_{16}O$

Formula wt 152.24
FEMA: 2479

DESCRIPTION

Change to read:

~~*d*-Fenchone~~■ (+)-Fenchone■ 1S (*FCC7*) occurs as a colorless to pale yellow liquid.

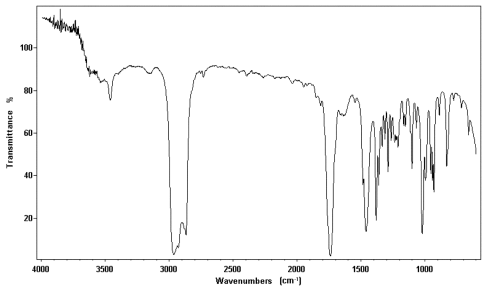
Odor: Camphoraceous
Solubility: Soluble in propylene glycol, vegetable oils; insoluble or practically insoluble in water
Boiling Point: ~192°
Solubility in Alcohol, Appendix VI**:** One mL dissolves in 1 mL of 95% ethanol.
Function: Flavoring agent

IDENTIFICATION

Change to read:

- **Infrared Spectra**, *Spectrophotometric Identification Tests,* Appendix IIIC

Acceptance criteria: The spectrum of the sample exhibits relative maxima at the same wavelengths as those of the spectrum below.



~~*d*-Fenchone~~■ (+)-Fenchone■ 1S (*FCC7*)

ASSAY

- **PROCEDURE:** Proceed as directed under *M-1b,* Appendix XI.
  Acceptance criteria: NLT 97.0% of $C_{10}H_{16}O$

SPECIFIC TESTS

- **REFRACTIVE INDEX,** Appendix II (at 20°)
  Acceptance criteria: Between 1.460 and 1.467
- **SPECIFIC GRAVITY:** Determine at 25° by any reliable method (see *General Provisions*).
  Acceptance criteria: Between 0.940 and 0.948

OTHER REQUIREMENTS

- **ANGULAR ROTATION,** *Optical (Specific) Rotation,* Appendix IIB
  Acceptance criteria: Between +46° and −68°

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 352

BRIEFING

Ferric Phosphate, *FCC 6* page 358. On the basis of information submitted, it is proposed to revise the physicochemical characteristics of this ingredient in the *Description* section to reflect those of materials currently in commerce. It should be noted that according to the *FCC General Provisions*, "Characteristics described and statements made in the *Description* section of a monograph are not requirements, but are provided as information that may assist with the overall evaluation of a food ingredient." (*FCC 6*, page 2).
(FIEC: J. Moore) C84018

Ferric Phosphate

Change to read:

Iron Phosphate

Ferric Orthophosphate

$FePO_4 \cdot xH_2O$

Formula wt, anhydrous 150.82
CAS: ■anhydrous■ 1S (*FCC7*) [10045-86-0]
■CAS: dihydrate (strengite) [13824-49-2]
CAS: dihydrate (phosphosiderite) [14567-75-0]
CAS: trihydrate (koninckite) [14567-93-2]■ 1S (*FCC7*)

DESCRIPTION

Change to read:

Ferric Phosphate occurs as a ~~yellow-white to buff colored powder~~■ crystalline or amorphous material in shades of yellow, white, purple, or pink.■ 1S (*FCC7*) It contains from one to four molecules of water of hydration. It is insoluble in water and in glacial acetic acid, but is soluble in ~~mineral acids~~■ hydrochloric and sulfuric acids. ■ 1S (*FCC7*)

Function: Nutrient
Packaging and Storage: Store in well-closed containers.

IDENTIFICATION

- **PROCEDURE**

  Sample: 1 g

  Analysis: Dissolve the *Sample* in 5 mL of 1:2 hydrochloric acid, and add an excess of 1 N sodium hydroxide. A red-brown precipitate forms. Boil the mixture, filter to remove the iron, and strongly acidify a portion of the filtrate with hydrochloric acid. Cool, mix with an equal volume of magnesia mixture TS, and treat with a slight excess of 6 N ammonium oxide. An abundant white precipitate forms. Wash the precipitate and add a few drops of silver nitrate TS.

  Acceptance criteria: The white precipitate turns green-yellow when treated with the silver nitrate TS.

ASSAY

- **PROCEDURE**

Sample: 3.5 g

Analysis: Dissolve the *Sample* in 75 mL of 1:2 hydrochloric acid, heat to boiling, and boil for about 5 min. Cool, transfer into a 100-mL volumetric flask, dilute with the dilute hydrochloric acid to volume, and mix. Add 100 mL of the dilute hydrochloric acid to 25.0 mL of this solution, boil again for 5 min, and add, dropwise and while stirring, stannous chloride TS to the boiling solution until the iron is just reduced as indicated by the disappearance of the yellow color. Add 2 drops in excess (but no more) of the stannous chloride TS, dilute with about 50 mL of water, and cool to room temperature. While stirring vigorously, add 15 mL of a saturated solution of mercuric chloride, and then allow to stand for 5 min. Add 15 mL of a sulfuric acid–phosphoric acid mixture, prepared by slowly adding 75 mL of sulfuric acid to 300 mL of water, cooling, adding 75 mL of phosphoric acid, and then diluting with water to 500 mL. Mix, add 0.5 mL of barium diphenylamine sulfonate TS, and titrate with 0.1 N potassium dichromate to a red-violet endpoint. Each mL of 0.1 N potassium dichromate is equivalent to 5.585 mg of Fe.

Acceptance criteria: 26.0%–32.0% of Fe

IMPURITIES

Inorganic Impurities

- **Arsenic,** *Arsenic Limit Test,* Appendix IIIB

[Note—Assemble the special distillation apparatus as shown in Fig. 13.]

Sample solution: Transfer 2 g of sample, 50 mL of hydrochloric acid, and add 5 g of cuprous chloride into the distilling flask (B). Reassemble the distillation apparatus and apply gentle suction to flask F to produce a continuous stream of bubbles. Heat the solution in flask B to boiling and distill until between 30 and 35 mL of distillate has been collected in flask D. Quantitatively transfer the distillate to a 100-mL volumetric flask with the aid of water, dilute with water to volume, and mix.

Standard solution: Prepare this solution in the same manner as the *Sample solution*, but use 6.0 mL of *Standard Arsenic Solution* in place of the sample.

Blank solution: Prepare this solution in the same manner as the *Sample solution,* but use 6.0 mL of water in place of the sample.

Analysis: Transfer 50.0 mL of the *Sample solution* into the generator flask, add 2 mL of a 150 mg/mL solution of potassium iodide, and continue as directed in the *Procedure* beginning with "[add] 0.5 mL of *Stannous Chloride Solution*, and mix. …" Modify the *Procedure* by using 5.0 g of Devarda's metal in place of the 3.0 g of 20-mesh granular zinc, and maintain the temperature of the reaction mixture in the generator flask between 25° and 27°. Treat 50.0 mL each of the *Standard solution* and of the *Blank solution* in the same manner and under the same conditions. Determine the absorbance at 525 nm produced by each solution as directed under *Procedure.* Calculate the arsenic content (in mg/kg) of the sample:

$$\text{Result} = 3 \times (A_U - A_B)/(A_S - A_B)$$

A_U = absorbance produced by the *Sample solution*

A_B = absorbance produced by the *Blank solution*

A_S = absorbance produced by the *Standard solution*

[Note—If A_B exceeds 0.300, different samples of reagent-grade cuprous chloride and Devarda's metal should be tested for arsenic content by the procedure described herein, and lots of these reagents should be selected that will give blank readings that do not exceed 0.300.]

Acceptance criteria: NMT 3 mg/kg

- **Fluoride,** *Fluoride Limit Test,* Appendix IIIB

  Sample: 1.0 g

  Acceptance criteria: NMT 0.005%

- **Lead**

  [Note—When preparing all aqueous solutions and rinsing glassware before use, employ water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin before use. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Clean glassware before use by soaking in warm 8 N nitric acid for 30 min and by rinsing with deionized water.]

  Ascorbic acid–sodium iodide solution: 100 mg/mL of ascorbic acid and 192.5 mg/mL of sodium iodide

  Trioctylphosphine oxide solution: 50 mg/mL of trioctylphosphine oxide in 4-methyl-2-pentanone. [**CAUTION—** This solution causes irritation. Avoid contact with eyes, skin, and clothing. Take special precautions in disposing of unused portions of solutions to which this reagent is added.]

  Standard stock solution: Transfer 159.8 mg of reagent-grade lead nitrate to a 1000-mL volumetric flask, dissolve it in 100 mL of water containing 1 mL of nitric acid, and dilute with water to volume. This solution contains 100 µg/mL of lead.

  Standard preparation: Transfer 5.0 mL of *Standard stock solution* into a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 2.0 mL of this resulting solution into a 50-mL volumetric flask. Add 10 mL of 9 N hydrochloric acid and about 10 mL of water to the volumetric flask. Add 20 mL of *Ascorbic acid–sodium iodide solution* and 5.0 mL of *Trioctylphosphine oxide solution* to the flask. Shake for 30 s and allow the layers to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow the layers to separate. The organic solvent layer is the *Standard preparation* and contains 2.0 µg/mL of lead.

  Blank preparation: Transfer 10 mL of 9 N hydrochloric acid and about 10 mL of water to a 50-mL volumetric flask. Add 20 mL of *Ascorbic acid–sodium iodide solution* and 5.0 mL of *Trioctylphosphine oxide solution* to the flask. Shake for 30 s and allow the layers to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow the layers to separate. The organic solvent layer remaining is the *Blank preparation* and contains 0.0 µg/mL of lead.

  Sample preparation: Add 2.5 g of sample, 10 mL of 9 N hydrochloric acid, about 10 mL of water, 20 mL of *Ascorbic acid–sodium iodide solution*, and 5.0 mL of *Trioctylphosphine oxide solution* to a 50-mL volumetric flask, shake for 30 s, and allow the layers to separate. Add water to bring the organic solvent layer into the neck of the flask, shake again, and allow the layers to separate. The organic solvent layer is the *Sample preparation.*

  Analysis: Using a suitable atomic absorption spectrophotometer equipped with a lead hollow-cathode lamp and an air–acetylene flame set at the lead emission line of 283.3 nm, with 4-methyl-2-pentanone used to set the instrument to zero, concomitantly determine the absorbance of the *Blank preparation,* the *Standard preparation*, and the *Sample preparation.* [Note—In a suitable analysis, the absorbance of the *Blank preparation* is NMT 20% of the difference between the absorbance of the *Standard preparation* and the absorbance of the *Blank preparation*.]

  Acceptance criteria: The absorbance of the *Sample preparation* does not exceed that of the *Standard preparation.* (NMT 4 mg/kg)

- **Mercury**

  10% Stannous chloride solution: Dissolve 20 g of stannous chloride ($SnCl_2 \cdot 2H_2O$) in 40 mL of warm hydrochloric acid and dilute with 160 mL of water.

  Sample solution: Transfer 5.00 g of sample into a 150-mL beaker, add 25 mL of aqua regia, cover with a watch glass, and allow to stand at room temperature for about 5 min. Heat just to boiling, allow to simmer

for about 5 min, and cool. Transfer the solution into a 250-mL volumetric flask, dilute with water to volume, and mix. [Note—Disregard any undissolved material that may be present.] Transfer a 50.0-mL aliquot of this solution into a 150-mL beaker, and add 1.0 mL of 1:5 sulfuric acid and 1.0 mL of a filtered solution of 40 mg/mL potassium permanganate. Heat the solution just to boiling, simmer for about 5 min, and cool. Prepare a *Reagent blank* in the same manner.

Standard stock solution: Dissolve 338.5 mg of mercuric chloride, in 200 mL of water in a 250-mL volumetric flask, add 14 mL of 1:2 sulfuric acid, dilute with water to volume, and mix. Pipet 10.0 mL of this solution into a 1000-mL volumetric flask containing about 800 mL of water and 56 mL of 1:2 sulfuric acid, dilute with water to volume, and mix. Pipet 10.0 mL of the second solution into a second 1000-mL volumetric flask containing 800 mL of water and 56 mL of 50% sulfuric acid, dilute with water to volume, and mix. Each mL of this diluted stock solution contains 0.1 µg of mercury.

Standard solutions: Pipet 1.25, 2.50, 5.00, 7.50, and 10.00 mL of the *Standard stock solution* (equivalent to 0.125, 0.250, 0.500, 0.750, and 1.00 µg of mercury, respectively) into five separate 150-mL beakers. Add 25 mL of aqua regia to each beaker, cover with watch glasses, heat just to boiling, simmer for about 5 min, and cool to room temperature. Transfer the solutions into separate 250-mL volumetric flasks, dilute with water to volume, and mix. Transfer a 50.0-mL aliquot from each solution into five separate 150-mL beakers, and add 1.0 mL of 1:5 sulfuric acid and 1.0 mL of a filtered solution of 40 mg/mL potassium permanganate solution to each. Heat the solutions just to boiling, simmer for about 5 min, and cool.

Analysis: Use a Mercury Detection Instrument as described and an Aeration Apparatus as shown in Fig. 16 under *Mercury Limit Test,* Appendix IIIB. For the purposes of the test described in this monograph, the Techtron AA-1000 atomic absorption spectrophotometer, equipped with a 10-cm silica absorption cell (Beckman Part No. 75144, or equivalent) and coupled with a strip chart recorder (Varian Series A-25, or equivalent), is satisfactory.

Assemble the Aeration Apparatus as shown in Fig. 16 under *Mercury Limit Test,* Appendix IIIB. Use magnesium perchlorate as the absorbent in the absorption cell (e), fill gas-washing bottle (c) with 60 mL of water, and place stopcock (b) in the bypass position. Connect the assembly to the 10-cm absorption cell (analogous to f in the figure) of the spectrophotometer, and adjust the air or nitrogen flow rate so that, in the following procedure, maximum absorption and reproducibility are obtained without excessive foaming in the test solution. Obtain a baseline reading at 253.7 nm by following the equipment manufacturer's operating instructions.

Using the Techtron AA-1000 spectrophotometer, the following conditions are suitable: slit width: 2 Å; lamp current: 3 mA; and scale expansion: × 1. With the strip chart recorder, set the chart speed at 25 in./h and the span at 2 mV. Precondition the apparatus by an appropriate modification of the procedures described below for treatment of the test solutions.

[Note—The fritted bubbler in gas-washing bottle (c) should be kept immersed in water between determinations. After each determination, wash the bubbler with a stream of water.]

Treat the *Reagent blank*, each of the *Standard solutions*, and the *Sample solution* as follows: transfer the solution to be tested into a 125-mL gas-washing bottle (c), using a few drops of 100 mg/mL hydroxylamine hydrochloride solution to remove any manganese hydroxide from the beaker. Dilute with water to about 55 mL, and add a magnetic stirring bar. Discharge the permanganate color by adding, dropwise, the hydroxylamine hydrochloride solution, swirling after each drop is added. Add 15.0 mL of *10% Stannous chloride solution,* and immediately connect gas-washing bottle c to the aeration apparatus. Switch on the magnetic stirrer, turn stopcock b from the bypass to the aerating position, and obtain the absorbance reading. Disconnect bottle c from the aeration apparatus, discard the solution just tested, wash bottle c and the fritted bubbler with water, and repeat the procedure with the remaining solutions. Correct the sample readings for the *Reagent blank*, and determine the mercury concentration of the *Sample solution* from a standard curve prepared by plotting the readings obtained with the

Standard solutions against mercury concentration, in mg/kg, with suitable adjustments being made for dilution factors.

Acceptance criteria: NMT 3 mg/kg

SPECIFIC TESTS

- **Loss on Ignition**

Analysis: Ignite a sample at 800° for 1 h.
Acceptance criteria: NMT 32.5%

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 358

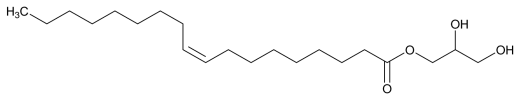
BRIEFING

Glyceryl Monooleate, *FCC 6* page 417. On the basis of comments and supporting data received, it is proposed to replace the existing outdated packed column GC method used in both the *Assay* and test for *Free Glycerin* with a more modern HPLC method currently used in the *USP32–NF27 Glyceryl Monooleate* monograph. The proposed HPLC method eliminates the use of chloroform as a solvent and the use of a derivitization step. For use in the *Assay*, a new quantitative glyceryl monooleate USP Reference Standard is also proposed which allows more accurate quantitation compared to the currently used USP RS which does not contain glyceryl monooleate but rather a mixture of glyceryl palmitate and glyceryl stearate.

(FIEC: J. Moore) C83867

Glyceryl Monooleate

Monoolein



$C_{21}H_{40}O_4$

Formula wt 356.54

INS: 471

CAS: [25496-72-4]
FEMA: 2526

DESCRIPTION

Glyceryl Monooleate occurs as a clear liquid at room temperature. It has a mild, fatty taste. It is prepared by esterifying glycerin with food-grade oleic acid in the presence of a suitable catalyst such as aluminum oxide. It also occurs in many animal and vegetable fats such as tallow and cocoa butter. It is soluble in hot alcohol and in chloroform; very slightly soluble in cold alcohol, in ether, and in petroleum ether; and insoluble in water. It melts at around 15°. It may also contain tri- and diesters.

Function: Emulsifier; flavoring agent

Packaging and Storage: Store in tight, light-resistant containers.

IDENTIFICATION

- **Fatty Acid Composition,** Appendix VII

  Acceptance criteria: A sample exhibits the following composition profile of fatty acids:

Fatty Acid	Weight % (Range)
≤12	0
12:0	0
14:0	<4
16:0	1–5
16:1	<9
18:0	<3.0
18:1	≥82
18:2	3–7
≥20	<1.5

ASSAY

Change to read:

- **PROCEDURE**

Propionating reagent: Mix 10 mL of pyridine with 20 mL of propionic anhydride.

Internal standard solution: 4 mg/mL hexadecyl hexadecanoate in chloroform

Standard preparation: Transfer 50 mg of USP Monoglycerides RS into a 25-mL flask, add 5 mL of *Internal standard solution* by pipet, and mix. When dissolution is complete, immerse the flask in a water bath maintained at a temperature between 45° and 50°, and volatilize the chloroform with the aid of a stream of nitrogen. Add 3.0 mL of *Propionating reagent*, and heat on a hot plate at 75° for 30 min. Evaporate the reagents with the aid of a stream of nitrogen and gentle steam heat. Add 15 mL of chloroform, and swirl to dissolve the residue.

Sample preparation: Transfer 50 mg of sample into a 25-mL conical flask, and add 5 mL of *Internal standard solution* by pipet, and mix. When dissolution is complete, immerse the flask in a water bath maintained at a temperature between 45° and 50°, and volatilize the chloroform with the aid of a stream of nitrogen. Add 3.0 mL of *Propionating reagent*, and heat on a hot plate at 75° for 30 min. Evaporate the reagents with the aid of a stream of nitrogen and gentle steam heat. Add 15 mL of chloroform, and swirl to dissolve the residue.

Chromatographic system, Appendix IIA

Mode: Gas chromatography

Detector: Flame-ionization detector

Column: 2.4-m × 4-mm (id) borosilicate glass column, or equivalent, packed with 2% liquid phase, 5% phenyl methyl silicone on 80- to 100-mesh support (Supelcoport, or equivalent)

Temperature

Column: Between 270° and 280°, isothermal

Injection port : About 310°

Detector block: About 310°

Carrier gas: Helium

Flow rate: About 70 mL/min

System suitability

Sample: *Standard preparation* (6 to 10 replicate injections)

~~**Suitability requirement 1:** The resolution, *R*, between the peaks for the derivatized glyceryl hexadecanoate and glyceryl octadecanoate is NLT 2.0.~~

~~**Suitability requirement 2:** The relative standard deviation of the ratio of the peak area of the derivatized glyceryl *cis*-9-octadecanoate to that of the hexadecyl hexadecanoate is NMT 2.0%.~~

~~**Analysis:** Inject a suitable portion of the *Standard solution* and record the resulting chromatogram. Calculate the response factor, *F*, taken by the equation:~~

$$F = (A_S/A_D)(W_D/W_S)$$

~~A_S = Sum of areas under the derivatized monoglyceride peaks~~

~~A_D = Area under the hexadecyl hexadecanoate peak~~

~~W_D = Weight (mg) of hexadecyl hexadecanoate in the *Standard preparation*~~

~~W_S = Weight (mg) of USP Monoglycerides RS, in the *Standard preparation*~~

~~Similarly inject a suitable portion of the *Sample preparation* and record the resulting chromatogram. Calculate the quantity, in mg, of monoglycerides in the amount of sample taken by the formula:~~

$$(W_D/F)(a_U/a_D)$$

~~W_D = Weight (mg) of hexadecyl hexadecanoate in the *Standard preparation*~~

~~F = Response factor, determined above~~

~~a_U = Sum of areas under the derivatized monoglyceride peaks~~

~~a_D = Area under the hexadecyl hexadecanoate peak~~

■ **Mobile phase:** Tetrahydrofuran

Standard solution: 20 mg/mL of USP Glyceryl Monooleate 90% RS in *Mobile phase*

Sample solution: 40 mg/mL in *Mobile phase*

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography

Detector: Refractive index

Column: Two 7.5-mm × 30-cm columns in tandem containing spherical styrene-divinylbenzene copolymer packing (5- to 10-µm particle diameter)[1]. [Note—Alternatively, one 7.5-mm × 60-cm column with an equivalent packing can be used provided that system suitability criteria are met.]

Column temperature: 40°. [Note—Column temperature may be lowered to ambient, but working at 40° provides stable separation conditions and ensures better sample solubility.]

Flow rate: 1 mL/min

Injection size: 40 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Suitabilty requirement 1: The resolution between the diglycerides and monoglycerides peaks is NLT 1.0.

Suitability requirement 2: The relative standard deviation for the monoglycerides peak area is NMT 2.0%.

Analysis: Separately inject equal volumes of the *Standard solution* and *Sample solution* into the chromatograph, and measure the responses for the major peaks on the resulting chromatograms. [Note—The approximate relative retention times for triglycerides, diglycerides, monoglycerides, and glycerin are 0.76, 0.79, 0.85, and 1.0, respectively.]

Calculate the percent of monoglycerides in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area response for monoglycerides from the *Sample solution*

r_S = peak area response for monoglycerides from the *Standard solution*

C_S = concentration of monoglycerides in the *Standard solution* corrected for purity based on the USP RS label claim (mg/mL)

C_U = concentration of sample in the *Sample solution* (mg/mL)

■1S (*FCC7*)

Acceptance criteria: NLT 35.0% monoglycerides, calculated on the anhydrous basis

IMPURITIES

Inorganic Impurities

- **LEAD**, *Lead Limit Test, Flame Atomic Absorption Spectrophotometric Method,* Appendix IIIB
  Sample: 10 g
  Acceptance criteria: NMT 1 mg/kg
- **WATER**, *Water Determination,* Appendix IIB
  Sample: 0.5 g
  Analysis: Proceed as directed using 20 mL of a 1:1 methanol:chloroform mixture
  Acceptance criteria: NMT 1.0%

SPECIFIC TESTS

- **ACID VALUE**, Appendix VII
  Acceptance criteria: NMT 6

Change to read:

- **FREE GLYCERIN**

  ~~**Propionating reagent:** 10 mL of pyridine and 20 mL of propionic anhydride~~

  ~~**Internal standard solution:** 0.2 mg/mL tributyrin in chloroform~~

  ~~**Standard stock preparation:** Transfer 15 mg of glycerin and 50 mg of tributyrin into a 25-mL glass-stoppered conical flask. Add 3.0 mL of *Propionating reagent*, and heat on a hot plate at 75° for 30 min. Volatilize the reagents with the aid of a stream of nitrogen at room temperature, add about 12 mL of chloroform, and mix.~~

  ~~**Standard preparation:** 1 mL of *Standard stock preparation* diluted to 20 mL with chloroform~~

  ~~**Sample preparation:** Transfer 50 mg of sample into a 25-mL glass-stoppered conical flask, and add 5 mL of *Internal standard solution* by pipet, and mix to dissolve. Immerse the flask in a water bath maintained at a temperature between 45° and 50°, and volatilize the chloroform with the aid of a stream of nitrogen.~~

Add 3.0 mL of *Propionating reagent*, and heat on a hot plate at 75° for 30 min. Evaporate the reagents with the aid of a stream of nitrogen at room temperature. Add 5 mL of chloroform, and mix.

Chromatographic system, Appendix IIA

Mode: Gas chromatography

Detector: Flame-ionization detector

Column: 2.4-m × 4-mm (id) borosilicate glass column, or equivalent, packed with 2% liquid phase consisting of a high-molecular-weight compound of polyethylene glycol and a diepoxide (Carbowax 20 M, or equivalent) on an 80- to 100-mesh siliceous earth support (Chromosorb W AW DMCS, or equivalent)

Temperature

Column: Between 190° and 200°, isothermal

Injection port : About 300°

Detector block: About 310°

Carrier gas: Helium

Flow rate: About 70 mL/min

System suitability

Sample: *Standard preparation* (6 to 10 replicate injections)

Suitability requirement 1: The resolution factor, *R*, between the peaks for the derivatized glycerin and tributyrin is NLT 4.0.

Suitability requirement 2: The relative standard deviation of the ratio of the peak areas of the derivatized glycerin and tributyrin is NMT 2.0%.

Analysis: Inject a suitable portion of the *Standard preparation* and record the resulting chromatogram. Calculate the response factor, *F*, taken by the formula:

$$F = (A_D/A_S)(W_S/W_D)$$

A_S = Area under the tripropionin peak

A_D = Area under the tributyrin peak

W_D = Weight (mg) of tributyrin in the *Standard preparation*

W_S = Weight (mg) of glycerin, in the *Standard preparation*

Similarly inject a suitable portion of the *Sample preparation* and record the resulting chromatogram. Calculate the percentage of glycerin by the formula:

$$100F(a_U/a_D)(w_D/w_U)$$

F = Response factor, determined above

a_U = Area under the tripripionin peak

a_D = Area under the tributyrin peak

w_D = Weight of tributyrin (mg) in 5 mL of *Internal standard solution*

w_U = Weight of sample (mg) in the *Sample preparation*

■ **Glycerin standard solutions:** 0.4, 1.0, 2.0, and 4.0 mg/mL of USP Glycerin RS in tetrahydrofuran
Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay.*
System suitabilty solution: Use the *Standard solution* prepared in the *Assay*.
System suitabilty: Proceed as directed in the *Assay* using the *System suitability solution*.
Analysis: Separately inject equal volumes of the *Glycerin standard solutions* and *Sample solution* into the chromatograph, and measure the responses for the major peaks on the resulting chromatograms.
[Note—The approximate relative retention times for triglycerides, diglycerides, monoglycerides, and glycerin are 0.76, 0.79, 0.85, and 1.0, respectively.]
Prepare a standard curve for glycerin by plotting glycerin peak areas (obtained from the chromatograms of the *Glycerin standard solutions*) versus concentrations in mg/mL. From the standard curve, calculate the concentration (C_U) of glycerin in the *Sample solution* in mg/mL. Calculate the percentage of glycerin in the portion of the sample taken:

$$\text{Result} = C_U/C_{SMP} \times 100$$

C_U = concentration of glycerin in the *Sample solution* determined from the standard curve (mg/mL)
C_{SMP} = concentration of sample in the *Sample solution* (mg/mL)

■ 1S (*FCC7*)

Acceptance criteria: NMT 6.0%

- **Hydroxyl Value**, *Method II,* Appendix VII
  Acceptance criteria: Between 300 and 330
- **Iodine Value**, Appendix VII
  Acceptance criteria: Between 58 and 80
- **Saponification Value**, Appendix VII
  Acceptance criteria: Between 160 and 176
- **Residue on Ignition (Sulfated Ash)**, Appendix IIC
  Sample: 5 g
  Acceptance criteria: NMT 0.1%

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[1] PLgel (Phenomenex, Torrance, CA), or equivalent.

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(FI07) Food Ingredients Expert Committee

BRIEFING

4-(*p*-Hydroxyphenyl)-2-butanone, *FCC 6* page 469. On the basis of comments received, a revision to add two chemical names (synonyms) is proposed. On the basis of efforts to modernize *Identification* test methods in *FCC*, it is proposed to change the existing infrared method in the *Identification* section to one that utilizes a USP Reference Standard instead of comparison to a printed spectrum.
(FIEC: K. Bowman) C84054

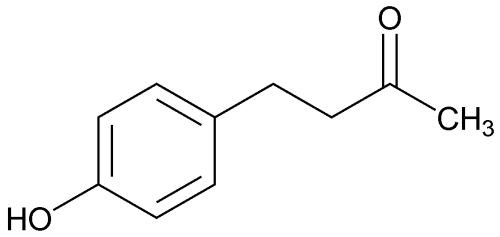
4-(*p*-Hydroxyphenyl)-2-butanone

Change to read:
■

4-(4-Hydroxyphenyl)-2-butanone

Raspberry Ketone

■1S (*FCC7*)



$C_{10}H_{12}O_2$

Formula wt 164.20
FEMA: 2588

DESCRIPTION

4-(*p*-Hydroxyphenyl)-2-butanone occurs as a white solid.
Odor: Raspberry
Solubility: Insoluble or practically insoluble in propylene glycol, vegetable oils, water
Solubility in Alcohol, Appendix VI**:** One g dissolves in 2 mL of 95% alcohol.
Function: Flavoring agent

IDENTIFICATION

Change to read:

- ~~**Infrared Spectra,** ***Spectrophotometric Identification Tests,*** Appendix IIIC~~■ **Infrared Absorption,** *Spectrophotometric Identification Tests,* Appendix IIIC■1S (*FCC7*)

  ~~**Acceptance criterion:** The spectrum of the sample exhibits relative maxima at the same wavelengths as those of the spectrum below.~~

  Click to View Image
  ~~4-(*p*-Hydroxyphenyl)-2-butanone~~

  ■**Reference standard:** USP Raspberry Ketone RS

Sample and standard preparation: *K*

Acceptance criteria: The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard.* ■1S (*FCC7*)

ASSAY

- **Procedure:** Proceed as directed under *M-1b,* Appendix XI.

  Acceptance criteria: NLT 98.0% of $C_{10}H_{12}O_2$

OTHER REQUIREMENTS

- **Melting Range or Temperature,** Appendix IIB

  Acceptance criteria: Between 82° and 84°

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 469

BRIEFING

Isopropyl Alcohol, *FCC 6* page 511. The following revisions are proposed.

1Ω On the basis of efforts to modernize the methods given in *FCC* and on the basis of data received, a new *Assay* is proposed to replace the existing packed-column gas chromatography method with a capillary column gas chromatography method. The new procedure is based on analysis performed with a Restek Rtx®-1301 column and includes system suitability requirements based on the use of a USP Reference Standard containing isopropyl alcohol and 0.1% each of ethyl ether, acetone, diisopropyl ether, 1-propanol, and 2-butanol.

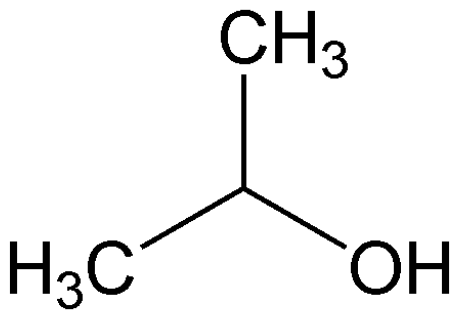
2Ω On the basis of comments received, a revision to the *Acceptance criteria* for the *Refractive Index* test under *Identification* is proposed. The new proposed limit is consistent with the monograph for *Propan-2-ol* prepared at the 51st Session of the Joint Expert Committee on Food Additives (1998).

(FIEC: K. Bowman) C79201; C79289

Isopropyl Alcohol

2-Propanol

Isopropanol



C_3H_8O

Formula wt 60.10
67-63-0

DESCRIPTION

Isopropyl Alcohol occurs as a clear, colorless, flammable liquid. It is miscible with water, with ethyl alcohol, with ether, and with many other organic solvents. Its refractive index at 20° is about 1.377.

Function: Extraction solvent

Packaging and Storage: Store in tight containers, remote from fire.

IDENTIFICATION

Change to read:

- **Refractive Index,** Appendix IIB

  [Note—Use an Abbé or other refractometer of equal or greater accuracy.]

~~**Acceptance criterion:** About 1.377 at 20°~~

■ **Acceptance criteria:** Between 1.377 and 1.380 at 20° ■1S (*FCC7*)

ASSAY

Change to read:

- **PROCEDURE**

~~**Chromatographic system,** Appendix IIA~~

~~**Mode:** Gas chromatography~~

~~**Detector:** Flame ionization detector~~

~~**Column:** 1.8-m × 6-mm (id) steel column, or equivalent, packed with 10% P.E.G. 400 on 60- to 80-mesh Chromosorb W (or equivalent)~~

~~**Temperature**~~

~~**Column:** 90°~~

~~**Injection port:** 150°~~

~~**Detector:** 150°~~

~~**Carrier gas:** Helium~~

~~**Flow rate:** 45 mL/min~~

~~**Injection size:** Between 1 µL and 5 µL~~

~~**Analysis:** Inject samples into the chromatogram and record the resulting chromatograms. Determine the content of propan-2-ol and volatile impurities by the method of area normalization.~~

~~**Acceptance criterion:** NLT 99.7% C_3H_8O, by weight~~

■ **System suitability solution:** USP 2-Propanol System Suitability RS

Chromatographic system, Appendix IIA

Mode: Gas chromatography

Detector: Flame ionization detector

Column: 60-m × 0.25-mm fused silica column with 1.4-µm film thickness of 6% cyanopropylphenyl/94% dimethylpolysiloxane stationary phase[1] with a 4-mm straight liner

Temperature

Injector: 150°

Detector: 200°

Column: Hold at 35° for 5 min; ramp to 45° at 1°/min; ramp to 100° at 10°/min; hold at 100° for 1 min

Carrier gas: Helium

Linear velocity: 35 cm/s

Injection size: 1 µL

Split ratio: 50:1

System suitability

Sample: *System suitability solution*

[Note—Approximate relative retention times for ethyl ether, acetone, isopropyl alcohol, diisopropyl ether, 1-propanol, and 2-butanol are 0.7, 0.9, 1.0, 1.4, 1.5, and 2.0, respectively.]

Suitability requirement 1: The relative standard deviation for each peak area is NMT 1.0%.

Suitability requirement 2: The resolution for the ethyl ether and isopropyl alcohol peaks is NLT 2.0.

Suitability requirement 3: The signal-to-noise ratio is NLT 500 for any of the following peaks: ethyl ether, acetone, diisopropyl ether, 1-propanol, and 2-butanol.

Analysis: Inject the sample into the chromatograph and record the resulting chromatogram. Determine the percentage of C_3H_8O present in the sample through peak area normalization:

$$\text{Result} = 100 \times (R_i/R_T)$$

R_i = peak area for isopropyl alcohol

R_T = sum of all of the peak areas

Acceptance criteria: NLT 99.7% C_3H_8O ■ 1S (*FCC7*)

IMPURITIES

Inorganic Impurities

- **Lead**, *Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace Method, Method I,* Appendix IIIB

  Acceptance criteria: NMT 1 mg/kg

SPECIFIC TESTS

- **Acidity (as Acetic Acid)**

  Sample: 50 mL (about 39 g)

  Analysis: Add 2 drops of phenolphthalein TS to 100 mL of water, then add 0.01 N sodium hydroxide to the first pink color that persists for at least 30 s. Add the *Sample* to this solution, and mix. Continue addition of 0.01 N sodium hydroxide until the pink color is restored.

  Acceptance criteria: NMT 0.7 mL of sodium hydroxide is required to restore the pink color (NMT 10 mg/kg).
- **Distillation Range**, Appendix IIB

  Acceptance criteria: Within a range of 1°, including 82.3°
- **Nonvolatile Residue**

  Sample: 125 mL (about 100 g)

  Analysis: Evaporate the *Sample* to dryness in a tared dish on a steam bath, dry the residue at 105° for 30 min, cool, and weigh.

  Acceptance criteria: NMT 10 mg/kg
- **Solubility in Water**

  Sample: 10 mL

  Analysis: Mix the *Sample* with 40 mL of water.

  Acceptance criteria: After 1 h, the solution is as clear as an equal volume of water.
- **Specific Gravity:** Determine by any reliable method (see *General Provisions*).

  Acceptance criteria: NMT 0.7840 at 25°/25° (equivalent to 0.7870 at 20°/20°)
- **Substances Reducing Permanganate**

  Sample: 50 mL

  Analysis: Transfer the *Sample* into a 50-mL glass-stoppered cylinder, add 0.25 mL of 0.1 N potassium permanganate, mix, and allow to stand for 10 min.

  Acceptance criteria: The pink color is not entirely discharged.
- **Water**, *Water Determination,* Appendix IIB

  Acceptance criteria: NMT 0.2%

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[1] Restek Rtx®-1301, or equivalent. Available at www.restek.com

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 511

BRIEFING

Alpha-Lactalbumin. Because there is no existing *FCC* monograph for this food ingredient, a new monograph is being proposed, based on the proposed *NF* monograph for *Alpha-Lactalbumin* appearing in *Pharmacopeial Forum* 34(3) [May–June 2008]. The retention time of alpha-lactalbumin, as determined in the *Assay*, is approximately 15 min. The generic *Heavy Metals* test and limit proposed in *PF* have been replaced by a specific test and limit for lead consistent with the *FCC* monograph for *Whey Protein Isolate*.
(FIEC: K. Bowman) C79529

Add the following:

■ **Alpha-Lactalbumin**
$C_{626}H_{958}N_{162}O_{196}S_9$

Formula wt 14,178
CAS: [9051-29-0]

DESCRIPTION

Alpha-Lactalbumin occurs as a homogenous, free-flowing, semi-hygroscopic, light cream-colored powder. It is a lyophilized or spray-dried powder of compact globular metalloprotein that may contain a single bound calcium ion and is capable of binding zinc and other metals. Alpha-Lactalbumin is isolated either from bovine milk or from whey, both of which should be from edible sources suitable for human use. It may contain suitable stabilizers.

Function: Nutrient; source of tryptophan; source of alpha-lactalbumin
Packaging and Storage: Store in tight containers.

IDENTIFICATION

- **A. Procedure**

  Acceptance criteria: The retention time of the major peak for alpha-lactalbumin in the chromatogram of the *Sample solution* corresponds to that in the chromatogram of the *Standard solution* as obtained in the *Assay*.

- **B. SDS-Polyacrylamide Gel Electrophoresis**

  Alcoholic solution: Specially denatured alcohol 3A and isopropyl alcohol (95:5)[1]
  Gel fixing solution: *Alcoholic solution*, glacial acetic acid, and water (400:100:500)
  Gel staining solution: 0.25 g/L of Coomassie Blue G-250 in 10% (v/v) acetic acid solution.[2] [Note—Store at room temperature.]
  Destaining solution: 10.0% acetic acid in water (v/v). [Note—This solution may be stored at room temperature for up to 6 months.]
  Sample buffer: Prepare a solution containing 200 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), 2% (w/v) sodium dodecyl sulfate (SDS), 40% (v/v) glycerol, and 0.04% (w/v) Coomassie Blue G-250. If necessary, adjust with hydrochloric acid or sodium hydroxide to a pH of 6.8.[3]
  Running buffer: Prepare a solution containing 100 mM tris(hydroxymethyl)aminomethane, 100 mM *N*-tris(hydroxymethyl)methylglycine (tricine), and 0.1%(w/v) SDS in water. If necessary, adjust with hydrochloric acid or sodium hydroxide to a pH of 8.3.[4] In a 400-mL beaker, thoroughly mix 35 mL of this solution with 315 mL of water.
  Molecular weight marker: Use a suitable molecular weight marker containing protein bands between 3.5 and 27 kD.

Molecular weight standard solution: Transfer 16 µL of the *Sample buffer* into a 0.5-mL microcentrifuge tube. Pipet 4 µL of the *Molecular weight marker* into the microcentrifuge tube. Incubate the mixture in the closed microcentrifuge tube for 5 min at 95°, then allow the tube to stand at room temperature for 5 min. Centrifuge at 5000 rpm for 1 min.

Alpha-lactalbumin standard stock solution: 1.0% (w/v) USP Alpha-Lactalbumin RS in water. [Note—Prepare in a 2-mL centrifuge tube.]

Alpha-lactalbumin standard solution: Pipet 21 µL of the *Sample buffer* and 3 µL of the *Alpha-lactalbumin standard stock solution* into a 0.5-mL microcentrifuge tube. Incubate the mixture in the closed microcentrifuge tube for 5 min at 95°, then allow the tube to stand at room temperature for 5 min. Centrifuge at 5000 rpm for 1 min.

Sample stock solution: 1.0% (w/v) sample in water. [Note—Prepare in a 2-mL centrifuge tube.]

Sample solution: Pipet 21 µL of the *Sample buffer* and 3 µL of the *Sample stock solution* into a 0.5-mL microcentrifuge tube. Incubate the mixture in the closed microcentrifuge tube for 5 min at 95°, then allow the tube to stand at room temperature for 5 min. Centrifuge at 5000 rpm for 1 min.

SDS-PAGE gel and apparatus: Following the manufacturer's instructions, assemble and fill a 16.5% Tris-Tricine Ready Gel[5] or equivalent, in the Mini-Protean III Electrophoresis Module[6] or in an equivalent module. Add *Running buffer* appropriately to the apparatus.

Gel loading: Load 10 µL of the *Molecular weight standard solution*, 2.5 µL of the *Alpha-lactalbumin standard solution*, and 2.5 µL of the *Sample solution*, respectively, into the 16.5% Tris-Tricine SDS-PAGE gel. [Note—The loaded samples contain approximately 3 µg of protein, based on the sample weight.]

Running the gel: Set the voltage to 100 V and run at a constant voltage until the tracking dye front is approximately 10 mm from the bottom of the gel (approximately 80–90 min).

Gel fixing: Remove the gel, transfer to a plastic container, and soak in the *Gel fixing solution* for 30 min on a shaking rack. Decant the *Gel fixing solution*, rinse with water, and decant the water.

Gel staining: Pour approximately 100 mL of *Gel staining solution* into the staining container. Place the gel into the staining container, and allow the stain to completely cover the gel. Place the staining container on an appropriate shaker and stain the gel for 60–90 min with gentle shaking.

Destaining: Drain the *Gel staining solution* into an appropriate waste container, and add 100 mL of *Destaining solution* to the container to cover the gel. Place the container on an appropriate shaker and shake with gentle agitation for 30 min. Discard the used *Destaining solution* and repeat destaining as necessary. Repeat rinsing with *Destaining* solution three to four times at 30-min intervals or until the gel is destained to the desired clarity.

Acceptance criteria: The *Sample solution* gives one major band at 14 kD, a minor band at 16 kD, and has a molecular weight that is similar to that of the *Alpha-lactalbumin standard solution*.

ASSAY

- **Procedure**

Mobile phase: Prepare a solution containing 0.02 M Tris-HCl, 0.5% SDS, and 0.1 N sodium chloride. Adjust the pH of the solution to 5.95 ± 0.05. Pass the solution through a filter having a 0.5-µm or finer porosity, and degas.

Standard solution: 1.0 mg/mL of USP Alpha-Lactalbumin RS in *Mobile phase*, calculated on the dried basis. [Note—Prepare immediately before use.]

Sample solution: 1.0 mg/mL in *Mobile phase*

System suitability solution: 0.5 mg/mL each of USP Alpha-Lactalbumin RS and beta-lactoglobulin in *Mobile phase*

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography

Detector: UV 280 nm

Column: 7.8-mm × 30-cm size exclusion chromatography column containing a hydrophilic bonded silica packing of 5-µm particles (pore size 290Å)[7]

Flow rate: 0.6 mL/min

Injection size: 20 µL

System suitability

[Note—Before performing the *System suitability* and *Analysis*, equilibrate the column with *Mobile phase* at 0.6 mL/min for approximately 90 min, or until a stable baseline is achieved.]

Sample: *System suitability solution*

[Note—The relative retention times are approximately 1.00 for alpha-lactalbumin and 0.91 for beta-lactoglobulin.]

Suitability requirement 1: The resolution between beta-lactoglobulin and alpha-lactalbumin is NLT 1.65.

Suitability requirement 2: The tailing factor for the alpha-lactalbumin peak is NMT 1.1.

Analysis: Separately inject the *Standard solution* and the *Sample solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks.

Calculate the alpha-lactalbumin content of the sample as a percentage of total protein:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (100/P)$$

r_U = peak response for alpha-lactalbumin obtained from the chromatogram of the *Sample solution*

r_S = peak response for alpha-lactalbumin obtained from the chromatogram of the *Standard solution*

C_S = concentration of alpha-lactalbumin in the *Standard solution* (mg/mL)

C_U = concentration of alpha-lactalbumin in the *Sample solution* (mg/mL)

P = total protein percentage (labeled)

Acceptance criteria: NLT 90.0% of the labeled total protein content

IMPURITIES

Inorganic Impurities

- **Lead,** *Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace Method, Method II,* Appendix IIIB

  Acceptance criteria: NMT 0.5 mg/kg, on the dried basis

- **Phosphorus**

  Solution A: Dilute 250 mL of hydrochloric acid with water to 1000 mL.

  Solution B: Dissolve 20 g of ammonium molybdate in 200 mL of water with heat, then allow the solution to cool. Dissolve 1.0 g of ammonium vanadate in 125 mL of water with heat, cool, and add 160 mL of hydrochloric acid. Gradually add, with stirring, the molybdate solution to the vanadate solution, then dilute with water to 1000 mL.

  Standard stock solution 1: 2 mg/mL of phosphorus from monobasic potassium phosphate (KH_2PO_4), previously dried for 2 h at 105°. [Note—Store this solution in a refrigerator.]

  Standard stock solution 2: 0.1 mg/mL of phosphorus, from *Standard stock solution 1.* [Note—Prepare this solution immediately before use. Store in a refrigerator.]

  Standard solutions: Transfer 5.0, 8.0, 10.0, and 15.0 mL of *Standard stock solution 2* to four separate 100-

mL volumetric flasks.

Sample solution: Transfer 4.0 g of the sample to an ashing dish. Dry the sample on a hot plate or steam bath. Ignite in a muffle furnace at a maximum temperature of 600° until free of carbon. Cool, add 40 mL of *Solution A* and several drops of nitric acid, and bring to a boil on a hot plate. Cool, transfer to a 100-mL volumetric flask, and dilute with water to volume. Pipet 20.0 mL of this solution into a 100-mL volumetric flask.

Analysis: To each of the flasks containing the *Standard solutions* and the *Sample solution*, add 20.0 mL of *Solution B*, dilute with water to volume, and allow to stand for exactly 10 min for maximum color development. To an empty 100-mL volumetric flask, add 20.0 mL of *Solution B*, dilute with water to volume, and allow to stand for exactly 10 min for maximum color development (this solution will be used as the blank). Concomitantly determine the absorbance of each solution in 1-cm cells with a suitable spectrophotometer at a wavelength of 400 nm, using the blank to zero the instrument. Plot the absorbances of the *Standard solutions* versus concentration, in µg/mL, of phosphorus, and draw the straight line best fitting the plotted points. From the graph so obtained, determine the concentration, C, in µg/mL, of phosphorus in the *Sample solution*.

Calculate the quantity, in µg/g, of phosphorus in the sample taken:

$$\text{Result} = (F \times C/W) \times 100$$

F = dilution factor, 5

C = concentration of phosphorus in the *Sample solution*, determined from the standard curve (µg/mL)

W = weight of the sample used to prepare the *Sample solution* (g)

Acceptance criteria: NMT 700 µg/g

Organic Impurities

- **BETA-LACTOGLOBULIN**

Mobile phase, Sample solution, System suitability solution, Chromatographic system and **System suitability:** Proceed as directed in the *Assay*.

Standard solution: 1.0 mg/mL of beta-lactoglobulin in *Mobile phase*, calculated on the dried basis. [Note—Prepare immediately before use.]

Analysis: Separately inject the *Standard solution* and the *Sample solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks.

Calculate the percentage of beta-lactoglobulin in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (100/P)$$

r_U = peak response for beta-lactoglobulin obtained from the chromatogram of the *Sample solution*

r_S = peak response for beta-lactoglobulin obtained from the chromatogram of the *Standard solution*

C_S = concentration of beta-lactoglobulin in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

P = percentage of *Total Protein* as determined below

Acceptance criteria: NMT 6.5%, calculated on total protein basis

- **LACTOSE**

Solution A: 36.0 mg/mL of potassium ferrocyanide trihydrate ($K_4Fe(CN)_6 \cdot 3H_2O$)

Solution B: 72.0 mg/mL of zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$)

Test reagent 1: 600 mg of lyophilisate consisting of a mixture of citrate buffer (pH 6.6), nicotinamide adenine dinucleotide (NAD) (35 mg), anhydrous magnesium sulfate, and stabilizers (added if necessary). Dissolve lyophilisate in 7.0 mL of water before use.

Test reagent 2: 1.7 mL of a suspension of β-galactosidase (approximately 100 Units)

Test reagent 3: 34 mL of a solution consisting of 0.51 M potassium diphosphate buffer (pH 8.6), and stabilizers (added if necessary)

Test reagent 4: 1.7 mL of a suspension of galactose dehydrogenase (approximately 40 Units).

[Note—*Test reagents 1–4* are commercially available in a test kit.[8]]

Sample solution: Transfer 1.0 g of the sample to a 100-mL volumetric flask and add 60 mL of water. Add 5 mL of *Solution A*, mix, then add 5 mL of *Solution B*. Add 10 mL of 0.1 N sodium hydroxide solution and mix vigorously. Dilute with water to volume and pass through a filter paper. Use the clear filtrate. [Note—This procedure breaks emulsions, absorbs some colors, and precipitates proteins.]

Analysis: Label one glass or disposable 1-cm plastic cuvette as "blank" and a second, equivalent, cuvette as "test." To each cuvette, pipet 0.20 mL of *Test reagent 1* and 0.05 mL of *Test reagent 2*. Pipet 0.10 mL of the *Sample solution* into the cuvette that is labeled "test." Mix both cuvettes with stirrers and incubate at 20°–25° for 20 min. Pipet 1.00 mL of *Test reagent 3* into each cuvette. Pipet 2.00 mL of water into the "blank" cuvette and 1.90 mL of water into the cuvette containing the *Sample solution*. Incubate at 20°–25° for 2 min. Determine the absorbances, A_{S1} and A_{B1}, at 340 nm for the *Sample solution* and the blank, respectively. Next, add 0.05 mL of *Test reagent 4* to each cuvette. Incubate at 20°–25° until the reaction has stopped (about 10–15 min). Again determine the absorbances, A_{S2} and A_{B2}, at 340 nm for the *Sample solution* and the blank, respectively. If the reaction has not stopped after 15 min, continue to read the absorbances at 2-min intervals until the absorbance for the *Sample solution* remains constant for two successive measurements.

Calculate the percentage of lactose in the portion of the sample taken:

$$\text{Result} = 100 \times V_T \times M_{r1} \times [(A_{S2} - A_{S1}) - (A_{B2} - A_{B1})]/(E \times V_U \times C_U)$$

V_T = total volume in the cuvette, 3.30 mL

M_{r1} = molecular weight of lactose monohydrate, 360.32

E = absorption coefficient of nicotinamide adenine dinucleotide reduced form (NADH) at 340 nm, 6300 $L \cdot mol^{-1} \cdot cm^{-1}$

V_U = volume of *Sample solution* in the cuvette, 0.10 mL

C_U = concentration of the *Sample solution* (mg/L)

Acceptance criteria: NMT 1.0%

- **Lipid (Fat)**

Weighing dish preparation: Predry the clean dishes under the same conditions that will be used for final drying after fat extraction. Ensure that all surfaces where weighing dishes will be placed are clean and free of particulates. At the end of the oven drying, place the weighing dishes in a desiccator and cool to room temperature. Immediately before use, weigh the dishes to the nearest 0.1 mg and record the weights. Check the balance zero after weighing each dish. Protect the weighed dishes from contamination with extraneous matter.

Sample: 0.5 g

Analysis: Transfer the *Sample* to a Mojonnier-style ether extraction flask that has the capacity to hold a volume of 21–23 mL in the lower bulb plus neck at the bottom of the flask. The flask should have a smooth,

round opening at the top that can be sealed when closed with a cork. Add 1.5 mL of ammonium hydroxide to the flask. Add 3 drops of phenolphthalein TS to help sharpen the visual appearance of the interface between the ether and the aqueous layers during extraction. Add 10 mL of alcohol, close the flask with a cork stopper that has been water-soaked, and shake the flask for 15 s.

For the first extraction, add 25 mL of ether, replace the cork stopper, and shake the flask very vigorously for 1 min, releasing built-up pressure by loosening the stopper as necessary. Add 25 mL of petroleum ether, replace the cork stopper, and repeat vigorous shaking for 1 min. Centrifuge the flask at 600 rpm for NLT 30 s to obtain a clean separation of the aqueous (bright pink) and ether phases. Decant the ether solution into a suitable weighing dish prepared as directed in *Weighing dish preparation*. When the ether solution is decanted into the dish, be careful not to pour any suspended solids or aqueous phase into the weighing dish. Ether can be evaporated from the dish at NMT 100° while conducting the second extraction.

For the second extraction, add 5 mL of alcohol to the original flask, close with the cork stopper, and shake vigorously for 15 s. Add 15 mL of ether, replace the cork, and shake vigorously for 1 min. Add 15 mL of petroleum ether, replace the cork stopper, and repeat vigorous shaking for 1 min. Centrifuge the flask at 600 rpm for NLT 30 s to obtain a clean separation of the aqueous (bright pink) and ether phases. If the interface is below the neck of the flask, add water to bring the level about halfway up to the neck. Add water slowly down the inside surface of the flask so that there is minimum disturbance of the interface. Decant the ether solution for the second extraction into the same weighing dish used for the first extraction.

For the third extraction, omit addition of the alcohol and repeat the procedure used for the second extraction. Completely evaporate the solvents in a hood on a hot plate at NMT 100°, avoiding spattering. Dry the extracted fat and the weighing dish to constant weight in a forced air oven at 100 ± 1° for NLT 30 min or in a vacuum oven at 70°–75° and NMT 50.8 cm (20 inches) of vacuum for NLT 7 min. Remove the weighing dish from the oven and place in a desiccator to cool to room temperature. Record the weight of the weighing dish containing the fat.

Run a blank determination using water and record the weight of any dry residue collected. The reagent blank should be less than 2.0 mg of residue. [Note—A negative number is not acceptable.]

Calculate the weight percent of lipid (fat) in the portion of the sample taken:

$$\text{Result} = 100 \times (W_2 - W_1 - W_3)/W$$

W_2 = weight of the weighing dish containing fat

W_1 = weight of the empty weighing dish

W_3 = weight of the reagent blank residue

W = weight of the *Sample*

Acceptance criteria: NMT 1.0% lipid (fat); the difference between duplicate runs is NMT 0.03% lipid (fat)

SPECIFIC TESTS

- **Ash (Total),** Appendix IIC

  Sample: 1 g

  Acceptance criteria: NMT 3.5%

- **Calcium Content**

  Standard stock solution: Dissolve 1.249 g of calcium carbonate in 270 mL of 3 N hydrochloric acid in a 1000-mL volumetric flask. Dilute with water to volume. Dilute 50 mL of the solution so obtained to 1000 mL. (Contains 25 µg/mL of calcium.)

Solution A: Weigh 11.7 g (±100 mg) of lanthanum oxide and transfer to a 1000-mL volumetric flask. Add enough water to wet the powder, then slowly add 50 mL of hydrochloric acid. [**CAUTION—** Exothermic reaction.] Let the powder dissolve, then dilute with water to volume. This solution contains 1% (w/v) of lanthanum and is stable for up to 6 months when stored at room temperature.

Standard solutions: To five identical 25-mL volumetric flasks, add 0, 5, 10, 15, and 20 mL, respectively, of *Standard stock solution*. Add 2.5 mL of *Solution A* to each flask then dilute with water to volume. The solutions contain 0, 5, 10, 15, and 20 µg/mL of calcium, respectively, and each contains 0.1% (w/v) of lanthanum.

Sample solution: Transfer 1.0 g of the sample to a 100-mL volumetric flask, add 10 mL of *Solution A*, and dilute with water to volume.

Blank: Dilute *Solution A* 10-fold.

Analysis: Concomitantly determine the absorbances of the *Standard solutions* and the *Sample solution* against the *Blank* at the calcium emission line of 422.7 nm with a suitable atomic absorption spectrophotometer equipped with a calcium hollow-cathode lamp and a reduced air–acetylene flame. [Note—Optimize flame parameters in accordance with the instrument manufacturer's instructions.] Plot the absorbances of the *Standard solutions* versus the concentration, in µg/mL of calcium, and draw the straight line best fitting the plotted points. From the graph so obtained, determine the concentration, C, in µg/mL, of calcium in the *Sample solution*.

Calculate the quantity of calcium, in mg, in each g of Alpha-Lactalbumin taken:

$$\text{Result} = (F \times V \times C)/W$$

F = factor converting µg to mg, 0.001
V = final volume of the *Sample solution*, 100 mL
C = concentration of calcium in the *Sample solution*, determined from the standard curve (µg/mL)
W = weight of Alpha-Lactalbum used to prepare the *Sample solution* (g)

Acceptance criteria: NMT 1 mg/g

- **Denaturation Temperature**

Sample preparation: Prepare a protein dough by mixing 3 g of the sample with 2 g of water. Place the dough into a well-sealed sample container.

Analysis: Perform two measurements on the *Sample preparation* using a differential scanning calorimeter. Heat to 140° and scan. Cool rapidly to below room temperature, and rescan. Apply a scan rate of 10°/min. Weigh pans before and after scanning to verify that no moisture loss occurs during the scanning process. Measure and record the denaturation temperatures as peak temperatures. The formation of two peaks indicates the presence of both the holo form and the apo form of alpha-lactalbumin.

Acceptance criteria

Apo form: 50°–52°

Holo form: 58°–61°

- **Loss on Drying,** Appendix IIC (vacuum oven at 100° and 660 mm Hg with continuous dry air feed for 5 h)

Sample: 1.0–1.5 g

Acceptance criteria: NMT 6.5%

- **pH,** Appendix IIB

Sample solution: 100 mg/mL

Acceptance criteria: NMT 7.5

- **Total Protein**

Sample: 250 mg

Analysis: Combust the *Sample* in the presence of pure oxygen (99.9%) in an airtight oven at 950° with a suitable nitrogen analyzer. The components such as carbon dioxide, sulfur dioxide, and moisture are absorbed by various in-line chemical filters. All nitrogenous matter is converted into nitrogen in the presence of catalytic converters. The weight percent of nitrogen is measured by a thermal conductivity detector. Blank the system by analyzing a suitable nitrogen blank material, such as powdered cellulose, and obtaining a zero reading. Calibrate and qualify the system using EDTA. The relative standard deviation for replicate runs is NMT 0.5%.

Calculate the weight percent of total protein in the sample by multiplying the percentage of nitrogen found by 6.23.

Acceptance criteria: NLT 95.0%, calculated on the dried basis

OTHER REQUIREMENTS

- **Labeling:** Label to state protein content, expressed as total protein percentage on the dried basis. Indicate the type of source material, expressed as bovine milk, whey, or both, used to manufacture the final product.

■ 1S (*FCC7*)

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[1] A suitable grade of this solution is available, for example, as Reagent Alcohol, catalog number R8382, from www.sigma-aldrich.com.

[2] Suitable gel staining solutions are commercially available, for example, from Bio-Rad. Use Coomassie Brilliant Blue G-250 from Bio-Rad (catalog number 161-0406, www.bio-rad.com), or equivalent.

[3] A suitable sample buffer is commercially available, for example, as Tricine sample buffer from Bio-Rad, catalog number 161-0739, www.bio-rad.com.

[4] A suitable undiluted running buffer is available, for example, as 10x Tris/Tricine/SDS buffer from Bio-Rad, catalog number 161-0744, www.bio-rad.com.

[5] Available from Bio-Rad, catalog number 161-1107 or 161-1179, www.bio-rad.com.

[6] Available from Bio-Rad, catalog number 165-3302, www.bio-rad.com.

[7] Phenomenex BioSep 3000 SEC, or equivalent, www.phenomenex.com.

[8] Available, for example, from Boehringer-Mannheim (R-Biopharm, Inc., 7950 Old US 27S, Marshall, MI 49068 USA; Tel: 1-877-789-3033 or 1-269-789-3033; Fax: 1-269-789-3070; www.r-biopharm.com).

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

BRIEFING

***d*-Limonene,** *FCC 6* page 545 and page 1293 of the *First Supplement.* On the basis of comments received, a revision to change the title of this monograph to reflect a less confusing and more widely accepted method of distinguishing optically active isomers is proposed. Also proposed is the addition of the existing name as a synonym.

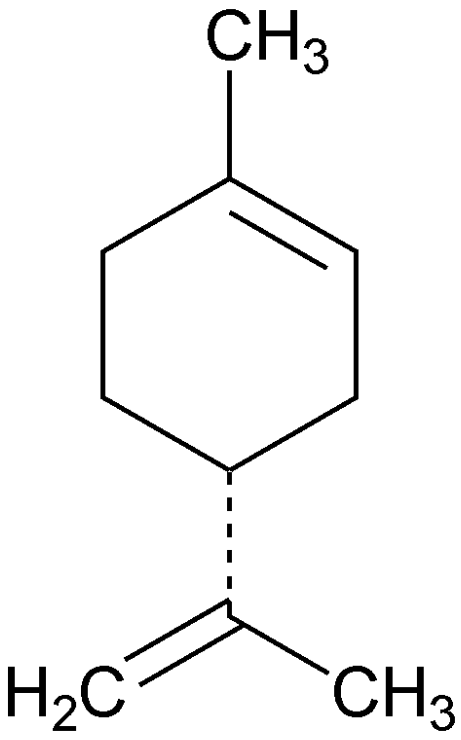
(FIEC: K. Bowman) C79751

~~***d*-Limonene**~~ ■ **(+)-Limonene** ■ 1S (*FCC7*)

■ *d*-Limonene ■ 1S (*FCC7*)

d-*p*-Mentha-1,8-diene

Cinene



$C_{10}H_{16}$

Formula wt 136.24
FEMA: 2633

DESCRIPTION

Change to read:

~~*d*-Limonene~~ ■ (+)-Limonene ■ 1S (*FCC7*) occurs as a colorless liquid. ■ It may contain a suitable antioxidant.

■ 1S (*FCC6*)

Odor: Mildly citrus, free from camphoraceous and terpene notes

Solubility: Slightly soluble in glycerin; miscible in alcohol, most fixed oils; insoluble or practically insoluble in propylene glycol, water

Boiling Point: ~177°

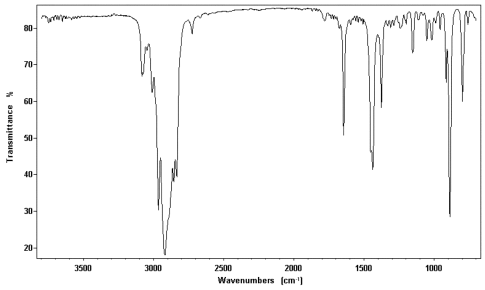
Function: Flavoring agent

IDENTIFICATION

Change to read:

- **Infrared Spectra**, *Spectrophotometric Identification Tests,* Appendix IIIC

  Acceptance criteria: The spectrum of the sample exhibits relative maxima at the same wavelengths as those of the spectrum below.



~~*d*-Limonene~~ (+)-Limonene 1S (*FCC7*)

ASSAY

- **Procedure:** Proceed as directed under *M-1a,* Appendix XI.

  Acceptance criteria: NLT 93.0% of $C_{10}H_{16}$

SPECIFIC TESTS

- **Refractive Index**, Appendix II (at 20°)

  Acceptance criteria: Between 1.471 and 1.474

- **Specific Gravity:** Determine at 25° by any reliable method (see *General Provisions*).

  Acceptance criteria: Between 0.838 and 0.843

OTHER REQUIREMENTS

- **Angular Rotation**, *Optical (Specific) Rotation,* Appendix IIB (Use a 100-mm tube.)

  Acceptance criteria: Between +96° and +104°

- **Peroxide Value**, *M-11,* Appendix XI

  Acceptance criteria: NMT 5.0

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 545

FCC Sixth Edition Supplement 1 Page 1293

BRIEFING

***l*-Limonene,** *FCC 6* page 546 and page 1294 of the *First Supplement.* On the basis of comments received, a revision to change the title of this monograph to reflect a less confusing and more widely accepted method of distinguishing optically active isomers is proposed. Also proposed is the addition of the existing name as a synonym.

(FIEC: K. Bowman) C79757

Change to read:

~~***l*-Limonene**~~ ■ **(–)-Limonene** ■ 1S (*FCC7*)

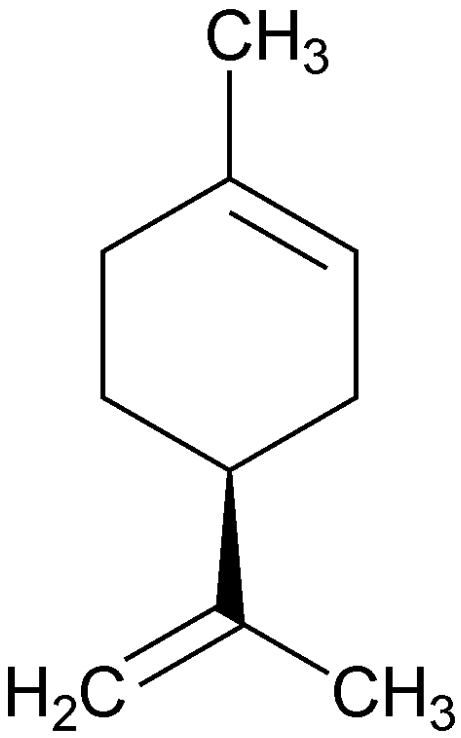
Change to read:

■

l-Limonene

■ 1S (*FCC7*)

l-p-Mentha-1,8-diene



$C_{10}H_{16}$

Formula wt 136.24

DESCRIPTION

Change to read:

~~l-Limonene~~■ (–)-Limonene■ 1S (*FCC7*) occurs as a colorless liquid. ■ It may contain a suitable antioxidant.

■ 1S (*FCC6*)

Odor: Refreshing, light, clean

Solubility: Miscible in alcohol, most fixed oils; insoluble or practically insoluble in water

Boiling Point: ~177°

Function: Flavoring agent

ASSAY

- **Procedure:** Proceed as directed under *M-1a,* Appendix XI.
  Acceptance criteria: NLT 95.0% of $C_{10}H_{16}$

SPECIFIC TESTS

- **Refractive Index,** Appendix II (at 20°)
  Acceptance criteria: Between 1.469 and 1.473
- **Specific Gravity:** Determine at 25° by any reliable method (see *General Provisions*).
  Acceptance criteria: Between 0.837 and 0.841

OTHER REQUIREMENTS

- **Angular Rotation,** *Optical (Specific) Rotation,* Appendix IIB**:** Use a 100-mm tube.
  Acceptance criteria: Between −90° and −61°
- **Peroxide Value,** *M-11,* Appendix XI
  Acceptance criteria: NMT 5.0

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 546

FCC Sixth Edition Supplement 1 Page 1294

BRIEFING

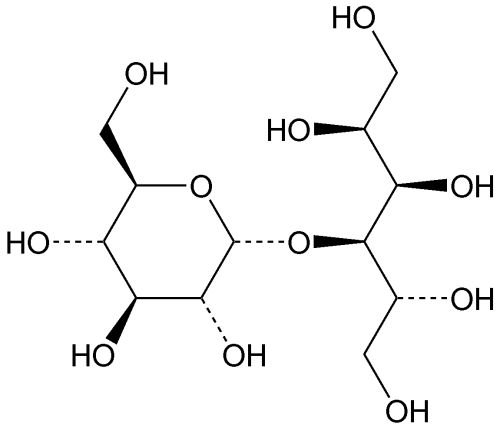
Maltitol, *FCC 6* page 574 and page 1294 of the *First Supplement.* On the basis of comments and data received, it is proposed to replace the use of the test for *Water* (Karl Fischer Titrimetric Method), with a *Loss on Drying* procedure. Data received indicate that the proposed test for *Loss on Drying* has improved precision and accuracy compared to the Karl Fischer test for *Water* for determining the moisture content of this food ingredient. Interested parties are encouraged to submit comments.
(FIEC: J. Moore) C70573

Maltitol

d-Maltitol

Hydrogenated Maltose

α-d-Glucopyranosyl-1,4-d-glucitol



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

Formula wt 344.31

INS: 965

CAS: [585-88-6]

DESCRIPTION

Maltitol occurs as a white, crystalline powder containing small amounts of sorbitol and related polyhydric alcohols. It is very soluble in water and slightly soluble in ethanol.

Function: Sweetener; humectant; stabilizer

Packaging and Storage: Store in well-closed containers.

IDENTIFICATION

- **Thin-Layer Chromatography,** Appendix IIA
  Adsorbent: 0.25-mm layer of chromatographic silica gel
  Standard solution: 2.5 mg/mL of USP Maltitol RS
  Sample solution: 2.5 mg/mL, on the anhydrous basis
  Application volume: 2 µL
  Developing solvent system: *n*-Propyl alcohol, ethyl acetate, and water (70:20:10)

Spray reagent A: 2 mg/mL of sodium metaperiodate

Spray reagent B: 20 mg/mL of 4,4′-tetramethyl-diaminodiphenylmethane in 4:1 acetone–glacial acetic acid

Analysis: Following development, spray the plate with *Spray reagent A*, air-dry for 15 min, and spray with *Spray reagent B*.

Acceptance criteria: The principal spot obtained from the *Sample solution* corresponds in R_F value and color to that obtained from the *Standard solution*.

ASSAY

- **PROCEDURE**

Mobile phase: Degassed water

Standard solution: 10.0 mg/mL of USP Maltitol RS

Sample solution: Transfer 0.7 g of sample into a 50-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography

Detector: Refractive index detector

Column: 9-mm × 30-cm column packed with a strong cation-exchange resin, about 9 µm in diameter, or equivalent, consisting of sulfonated cross-linked styrene–divinylbenzene copolymer in the calcium form (Aminex HPX-87c, or equivalent)

Column temperature: 85 ± 0.5°

Flow rate: About 0.5 mL/min

Injection volume: About 20 µL

System suitability

Sample: *Standard solution*

Suitability requirement: The relative standard deviation for replicate injections is NMT 2.0%.

Analysis: Separately inject the *Standard solution* and the *Sample solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. [Note—The elution pattern includes the higher-molecular-weight hydrogenated polysaccharides, followed by three individual peaks representing maltotriitol, maltitol, and sorbitol. The principal peak is maltitol, which elutes at about twice the retention time of the void volume, and the retention time for sorbitol is about 1.7 relative to maltitol.]

Calculate the quantity, in mg, of maltitol in the portion of the sample taken:

$$\text{Result} = 50 \times C \times (r_U/r_S)$$

C = concentration of maltitol in the *Standard solution* (mg/mL)

r_U = peak response of maltitol obtained from the *Sample solution*

r_S = peak response of maltitol obtained from the *Standard solution*

Acceptance criteria: 92.0%–100.5% of maltitol as $C_{12}H_{24}O_{11}$, calculated on the dried basis

IMPURITIES

Inorganic Impurities

- **LEAD**, *Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace Method, Method I,* Appendix IIIB

Acceptance criteria: NMT 1 mg/kg

- **NICKEL**, *Nickel Limit Test,* Appendix IIIB

Sample: 20.0 g

Acceptance criteria: NMT 1 mg/kg

SPECIFIC TESTS

- **Other Hydrogenated Saccharides**

Analysis: Proceed as directed in the *Assay*, but use the following calculation.

Calculate the quantity, in mg, of maltitol and other hydrogenated saccharides in the *Sample solution*:

$$\text{Result} = 50 \times C \times (r_U/r_S)$$

C = concentration of maltitol in the *Standard solution* (mg/mL)

r_U = peak response of hydrogenated saccharide obtained from the *Sample solution*

r_S = peak response of maltitol obtained from the *Standard solution*

Add the percentages of higher-molecular-weight hydrogenated polysaccharides, maltotriitol, and sorbitol to obtain the total.

Acceptance criteria: NMT 7.0%

Change to read:

- **Reducing Sugars (as Glucose)**

0.05 N iodine VS: Dilute 0.1 N iodine VS with water (1:1).

0.05 N sodium thiosulfate VS: Dilute 0.1 N sodium thiosulfate VS with water (1:1).■1S (*FCC6*)

Sample: ■3.3 g■1S (*FCC6*)

Analysis: Dissolve the *Sample* ■in 3 mL of water with the aid of gentle heat. Cool, and add 20.0 mL of alkaline cupric citrate TS and a few glass beads. Heat so that boiling begins after 4 min, and maintain the boiling for 3 min. Cool rapidly, and add 40 mL of diluted acetic acid TS, 60 mL of water, and 20.0 mL of *0.05 N iodine VS*. With continuous shaking, add 25 mL of a mixture of 6 mL of hydrochloric acid and 94 mL of water. When the precipitate has dissolved, titrate the excess iodine with *0.05 N sodium thiosulfate VS*. Use 2 mL of starch TS, added toward the end of the titration, as an indicator.■1S (*FCC6*)

Acceptance criteria: ■NLT 12.8 mL of *0.05 N sodium thiosulfate VS* is required. (NMT 0.3% reducing sugars, as glucose)■1S (*FCC6*)

- **Residue on Ignition (Sulfated Ash)**, *Method I (for solids),* Appendix IIC

Sample: 2 g

Acceptance criteria: NMT 0.1%

Change to read:

- ~~**Water**, *Water Determination,* Appendix IIB~~■**Loss on Drying**, Appendix IIC

Sample: 1.5 g

Analysis: 80° for 3 hr in a vacuum of NMT 10 mm Hg■1S (*FCC7*)

Acceptance criteria: NMT 1.5%

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Supplement 1 Page 1294

BRIEFING

***l*-Menthone,** *FCC 6* page 598. On the basis of comments received, it is proposed to change the title of this monograph to reflect a less confusing and more widely accepted method of distinguishing optically active isomers. Also proposed is the addition of the existing name as a synonym.
(FIEC: K. Bowman) C79758

Change to read:
~~***l*-Menthone**~~■ **(–)-Menthone**■ 1S (*FCC7*)

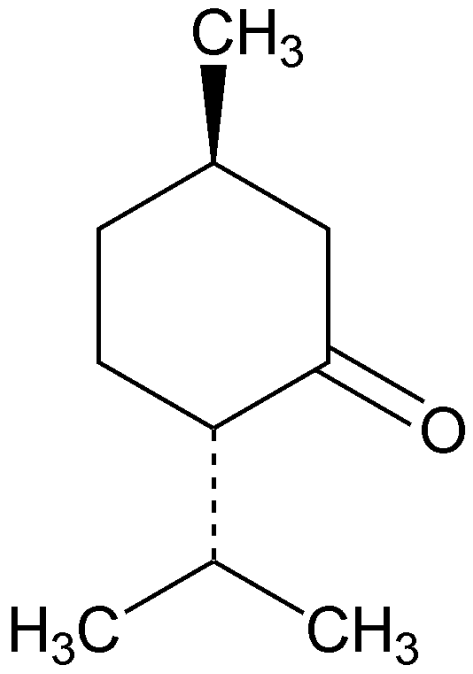
Change to read:

l-p-Menthan-3-one

■

l-Menthone

■ 1S (*FCC7*)



$C_{10}H_{18}O$

Formula wt 154.25
FEMA: 2667

DESCRIPTION

Change to read:
~~*l*-Menthone~~■ (–)-Menthone■ 1S (*FCC7*) occurs as an almost colorless liquid.

Odor: Mint
Solubility: Soluble in alcohol, most fixed oils; very slightly soluble in water
Boiling Point: ~207°
Solubility in Alcohol, Appendix VI**:** One mL dissolves in 1 mL of 95% ethanol.
Function: Flavoring agent

ASSAY

- **Procedure:** Proceed as directed under *M-1b,* Appendix XI.
  Acceptance criteria: NLT 96.0% of $C_{10}H_{18}O$ (sum of two isomers)

SPECIFIC TESTS

- **Acid Value,** *M-15,* Appendix XI
  Acceptance criteria: NMT 1.0
- **Refractive Index,** Appendix II (at 20°)
  Acceptance criteria: Between 1.448 and 1.453
- **Specific Gravity:** Determine at 25° by any reliable method (see *General Provisions*).
  Acceptance criteria: Between 0.888 and 0.895

OTHER REQUIREMENTS

- **Angular Rotation,** *Optical (Specific) Rotation,* Appendix IIB (Use a 100-mm tube.)
  Acceptance criteria: Between 0° and −25°

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 598

BRIEFING

***dl*-Menthyl Acetate,** *FCC 6* page 598. On the basis of comments received, it is proposed to change the title of this monograph to remove confusing nomenclature previously used for optically active isomers. Also proposed is the addition of the existing name as a synonym.
(FIEC: K. Bowman) C81953

Change to read:

~~***dl*-Menthyl Acetate**~~■ **Menthyl Acetate, Racemic**■ 1S (*FCC7*)

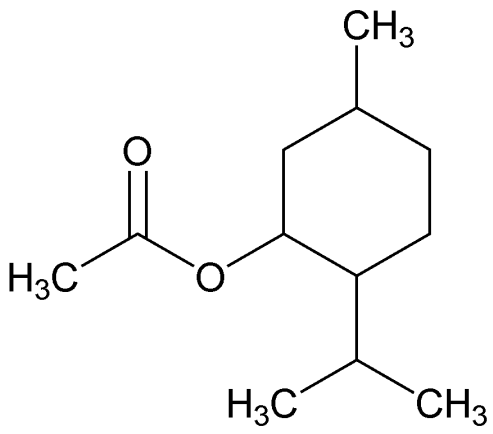
Change to read:

dl-p-Menthan-3-yl Acetate

■

dl-Menthyl Acetate

■ 1S (*FCC7*)



$C_{12}H_{22}O_2$

Formula wt 198.31
FEMA: 2668

DESCRIPTION

Change to read:

~~*dl*-Menthyl Acetate~~■ Menthyl Acetate, Racemic■ 1S (*FCC7*) occurs as a colorless liquid.

Odor: Mild, minty
Solubility: Soluble in alcohol, most fixed oils, propylene glycol; slightly soluble in glycerin, water
Boiling Point: ~228° to 229°
Solubility in Alcohol, Appendix VI**:** One mL dissolves in 1 mL of 95% ethanol.
Function: Flavoring agent

ASSAY

- **Procedure:** Proceed as directed under *M-1b,* Appendix XI.
  Acceptance criteria: NLT 97.0% of $C_{12}H_{22}O_2$ (sum of two isomers)

SPECIFIC TESTS

- **Acid Value**, *M-15,* Appendix XI
  Acceptance criteria: NMT 2.0
- **Refractive Index**, Appendix II (at 20°)
  Acceptance criteria: Between 1.443 and 1.450
- **Specific Gravity:** Determine at 25° by any reliable method (see *General Provisions*).
  Acceptance criteria: Between 0.921 and 0.926

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 598

BRIEFING

***l*-Menthyl Acetate,** *FCC 6* page 599. On the basis of comments received, it is proposed to change the title of this monograph to reflect a less confusing and more widely accepted method of distinguishing optically active isomers. Also proposed is the addition of the existing name as a synonym.
(FIEC: K. Bowman) C81924

Change to read:

~~***l*-Menthyl Acetate**~~■ **(–)-Menthyl Acetate**■ 1S (*FCC7*)

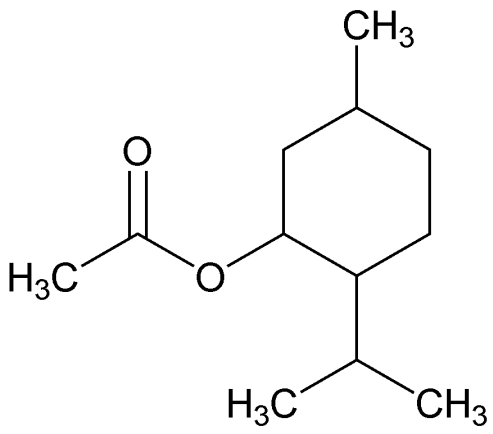
Change to read:

l-p-Menthan-3-yl Acetate

■

l-Menthyl Acetate

■ 1S (*FCC7*)



$C_{12}H_{22}O_2$

Formula wt 198.31
FEMA: 2668

DESCRIPTION

Change to read:

~~*l*-Menthyl Acetate~~■ (–)-Menthyl Acetate■ 1S (*FCC7*) occurs as a colorless liquid.

Odor: Mild, minty
Solubility: Soluble in alcohol, propylene glycol, most fixed oils; slightly soluble in water
Boiling Point: ~229° to 230°
Function: Flavoring agent

ASSAY

- **Procedure:** Proceed as directed under *M-1b,* Appendix XI.
  Acceptance criteria: NLT 98.0% of $C_{12}H_{22}O_2$

SPECIFIC TESTS

- **Acid Value,** *M-15,* Appendix XI
  Acceptance criteria: NMT 2.0
- **Refractive Index,** Appendix II (at 20°)
  Acceptance criteria: Between 1.443 and 1.447
- **Specific Gravity:** Determine at 25° by any reliable method (see *General Provisions*).
  Acceptance criteria: Between 0.921 and 0.926

OTHER REQUIREMENTS

- **Angular Rotation,** *Optical (Specific) Rotation,* Appendix IIB (Use a 100-mm tube.)
  Acceptance criteria: Between −70° and −69°

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 599

BRIEFING

Nisin Preparation, *FCC 6* page 675. On the basis of comments received, it is proposed to harmonize the test for *Sodium Chloride Content* with changes made at the 71st Session of the Joint FAO/WHO Expert Committee on Food Additives (2009).

(FIEC: K. Bowman) C84538

Change to read:

~~**Nisin Preparation**~~▲**Nisin A Preparation**▲*FCC7*

Change to read:

(contains 34 amino acids and has an approximate empirical formula $C_{143}H_{230}O_{37}N_{42}S_7$)

Formula wt ~3348

INS: 234

CAS:▲Nisin A▲*FCC7* [1414-45-5]

DESCRIPTION

Change to read:

Nisin ▲A▲*FCC7* Preparation occurs as a white, free-flowing powder. It is a mixture of closely related polypeptides produced by strains of the *Lactococcus lactis* subsp. *lactis* ~~Lancefield Group N~~▲▲*FCC7* in an appropriate growth medium. Nisin ▲A▲*FCC7* in the fermentation broth can be recovered by various methods, such as injecting sterile, compressed air (froth concentration), acidification, salting out, and spray-drying. The product comprises Nisin ▲A▲*FCC7* and sodium chloride that is adjusted to an activity level of not less than 900 IU/mg by the addition of sodium chloride ~~and nonfat milk solids~~▲▲*FCC7*.

Function: Antimicrobial agent

Packaging and Storage: Store in well-closed containers at temperatures not exceeding 22°.

ASSAY

Change to read:

- **PROCEDURE**

  ~~**Assay medium:** Dissolve 10 g of bacteriological peptone, 3 g of beef extract, 3 g of sodium chloride, 1.5 g of autolyzed yeast, 1 g of brown sugar, and 15 g of agar in distilled water to a final volume of 1000 mL. Sterilize in an autoclave at 121° for 15 min. The medium can be stored in a covered container at room temperature until use. At the time of use, melt the medium, and cool to approximately 50°. Add 2% of a 1:1 mixture of Tween 20 (polyoxyethylene sorbitan monolaurate) and distilled water, previously held for 20 to 30 min at 48°.~~

  ~~**Assay organism:** Maintain *Micrococcus luteus* (ATCC 10240,[1] NCIMB 8166[2]) by subculturing on agar slants of the *Assay medium* and incubating at 30° for 48 h. Prepared slants may be stored for a~~

~~maximum of 14 days at 4° until required. When required for use, the growth on the slant cultures is suspended in 7 mL of sterilized normal saline solution.~~

~~**Standard stock solution:** 1000 IU/mL of nisin prepared as follows: Suspend 100 mg of Nisin International Reference Preparation[3] (1000 IU/mg of nisin) in 80 mL of 0.02 N hydrochloric acid. Set aside at room temperature for 2 h. Dilute the suspension to a final volume of 100.0 mL with 0.02 N hydrochloric acid.~~

~~**Standard solutions:** 0.5, 1.0, 2.5, 5.0 and 10.0 IU/mL of nisin in 0.02 N hydrochloric acid; from *Standard stock solution* [Note:Store the *Standard stock solution* for up to 7 days at 4°, or prepare a fresh solution each day.]~~

~~**Sample stock preparation:** Suspend 100 mg of sample in 80 mL of 0.02 *N* hydrochloric acid in a 100-mL volumetric flask, and set aside at room temperature for 2 h. Dilute the solution to volume by adding 0.02 *N* hydrochloric acid.~~

~~**Sample preparation:** 1 mL of *Sample stock preparation* diluted to 200 mL with 0.02 *N* hydrochloric acid~~

~~**Analysis:** Using normal saline solution, dilute the suspension of the *Assay organism* to 1:10 and, mixing thoroughly, add 2 mL of this dilution to each 100 mL of melted *Assay medium* held at 48°. Pour the inoculated medium to a depth of 3 to 4 mm (approximately 15 mL) into 5 sterile, flat-bottomed Petri dishes, and allow to solidify. Invert the plates, and store at 4° for 1 h. Bore four 8-mm holes on 30-mm centers in each plate of the agar medium with the aid of a sterile, hollow-steel borer, 8 to 9 mm in diameter, and discard the agar discs. Transfer, in quadruplicate, 0.20-mL aliquots of each of the *Standard solutions* into the holes, (using one concentration of nisin standard to a plate). Cover the plates, and incubate them overnight at 30°. Repeat the preceding plating method using the *Sample preparation* in place of the *Standard solutions*.~~

~~After incubation, measure the zones of inhibition to the nearest 0.1 mm by means of calipers or other appropriate devices. Prepare a standard curve by plotting the log of the nisin concentration (from the *Standard solutions*) in the critical range against the zone diameters, and drawing the best straight line through the plotted points.~~

~~From the standard curve, determine the nisin concentration in the sample, and average the results.~~

~~**Acceptance criteria:** NLT 900 IU of nisin/mg of sample~~

▲**Assay organism:** *Lactococcus lactis* sbsp. *cremoris* (ATCC 14365[1] , NCDO 495[2]) is subcultured daily in sterile separated milk by transferring one loopful to a McCartney bottle of litmus milk and incubating at 30° .

Inoculated milk: Inoculate a suitable quantity of sterile skim milk with 2% of a 24-h culture of the *Assay organism*, and place it in a water bath at 30° for 90 min. [Note—Use immediately after preparation.]

Standard stock solution: Dissolve 1 g of standard nisin A in 1 L of 0.02N hydrochloric acid (1000 units/mL). [Note—Nisin A preparation containing 2.5% nisin A, minimum potency of 10^6 IU/g, obtainable from Sigma, St. Lous, MO or Fluka Buchs, Switzerland, may be used for the *Standard stock solution*. A similar preparation is available from Danisco, Copenhagen, Denmark.]

Standard solution: Dilute a portion of the *Standard stock solution* in 0.02 N hydrochloric acid to 50 units/mL. [Note—Prepare immediately before use.]

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 A content in the sample and standard is the same). Dilute in 0.02 N hydrochloric acid to 50 units/mL of nisin A.

Resazurin solution: 0.0125% solution of resazurin in water. [Note—Prepare immediately before use.]

Analysis: Separately pipet graded volumes (0.60, 0.55, 0.50, 0.45, 0.41, 0.38, 0.34, 0.31, 0.28, and 0.26 mL) of the *Standard solution* and the *Sample solution* into two rows of 10 dry 6-in × 5/8-in bacteriological test tubes. Add 4.6 mL of *Inoculated milk* to each tube by means of an automatic pipetting device. The

addition of *Inoculated milk* should be 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 of *Resazurin solution* to each using an automatic pipetting device and in the same order used for the addition of the *Inoculated milk*. 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 matte-finish cabinet. The tube containing the highest concentration of the *Sample solution* which shows the first clear difference in color (i.e. has changed from blue to mauve) is compared with tubes containing the *Standard solution* to find the nearest in color. Make further matches of tubes containing the next two lower concentrations of the *Sample solution* and the *Standard solution*. Interpolation of matches may be made at half dilution steps. Calculate the concentration of nisin A in the *Sample solution* using the known amounts of nisin A in the *Standard solution*. Obtain three readings of the solution and average them. Calculate the activity in terms of IU/g of preparation.

Acceptance criteria: NLT 900 IU of nisin A/mg of sample▲*FCC7*

IMPURITIES

Inorganic Impurities

- **Lead,** *Lead Limit Test, Flame Atomic Absorption Spectrophotometric Method,* Appendix IIIB

  Sample: 10 g

  Acceptance criteria: NMT 2 mg/kg

SPECIFIC TESTS

Change to read:

- **Differentiation of Nisin ▲A▲*FCC7* from Other Antimicrobial Substances, Stability to Acid, ▲Non-Stability to Alkali▲*FCC7***

  Sample ▲stock▲*FCC7* preparation: ~~Suspend 100 mg of sample in 80 mL of 0.02 *N* hydrochloric acid in a 100-mL volumetric flask, and set aside at room temperature for 2 h. Dilute the solution to volume by adding 0.02 *N* hydrochloric acid.~~▲Dissolve 1 g of sample in 1 L of 0.02 N hydrochloric acid to give a solution containing 1000 units/mL.

  Sample preparation: Dilute the *Sample stock preparation* further with 0.02 N hydrochloric acid to a concentration of 50 units/mL. [Note—Prepare immediately before use.] ▲*FCC7*

  Analysis 1: Boil the *Sample preparation* for 5 min. Determine the nisin ▲A▲*FCC7* concentration of the boiled *Sample preparation* as directed in the *Assay*. [Note—Save the unused portion of the boiled *Sample preparation* for use in *Analysis 2*.]

  Analysis 2: Adjust the pH of the solution obtained in *Analysis 1* 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. Determine the nisin ▲A▲*FCC7* concentration of the resulting solution as directed in the *Assay*.

  Acceptance criteria

  Analysis 1: The calculated nisin ▲A▲*FCC7* concentration of the boiled sample is 100% ± 5% of the *Assay* value (indicating no significant loss of activity due to heat treatment).

  Analysis 2: Complete loss of the antimicrobial activity is observed following the treatment described.

Change to read:

- **Differentiation of Nisin ▲A▲*FCC7* from Other Antimicrobial Substances, Tolerance of Lactococcus lactis to High Concentrations of Nisin ▲A▲*FCC7***

  Assay medium: Prepare one or more flasks containing 100 mL of litmus milk, and sterilize at 121° for 15 min.

  Assay organism: Prepare cultures of *Lactococcus lactis* (ATCC 11454, NCIMB 8586) in sterile skim milk by incubating for 18 h at 30°.

  Sample: 0.1 g

  Analysis: Suspend the *Sample* in the *Assay medium* and allow to stand at room temperature for 2 h. Add 0.1 mL of the *Assay organism*, and incubate at 30° for 24 h.

  Acceptance criteria: The *Assay organism* (*L. lactis*) grows in this concentration of the *Sample* (about 1000 IU/mL), but it will not grow in similar concentrations of other antimicrobial substances. [Note—This test will not differentiate nisin ▲A▲*FCC7* from subtilin.]

- **Loss on Drying,** Appendix IIC (105° for 2 h, dry to constant weight)

  Sample: 2 g

  Acceptance criteria: NMT 3.0%

- **Microbial Limits**

  [Note—Current methods for the following tests may be found by accessing the Food and Drug Administration's Bacteriological Analytical Manual (BAM) online at www.cfsan.fda.gov.]

  Acceptance criteria

  Aerobic plate count: NMT 10 CFU/g

  E. coli: Negative in 25 g

  Salmonella: Negative in 25 g

Change to read:

- **Sodium Chloride Content**

  ~~**Sample:** 20 mg~~

  ~~**Analysis:** Dissolve the *Sample* in 50 mL of water contained in a glass-stoppered flask. While agitating, add 3 mL of nitric acid, 5 mL of nitrobenzene, 50.0 mL of 0.1 N silver nitrate, and 2 mL of ferric ammonium sulfate TS. Shake well, and titrate the excess silver nitrate with 0.2 N ammonium thiocyanate. The titration endpoint is indicated by the appearance of a red color. Perform a blank determination (see *General Provisions*). Calculate the content of sodium chloride in the *Sample* taken by the formula:~~

  ~~$$(2 \times 5.844)(A - B)$$~~

  ~~2 = Equivalence factor~~

  ~~5.844 = Equivalence factor~~

  ~~*A* = Volume of 0.2 N ammonium thiocyanate consumed by the blank (mL)~~

  ~~*B* = Volume of 0.2 N ammonium thiocyanate consumed by the *Sample* (mL)~~

  ■ **Sample:** 200 mg

  Analysis: Dissolve the *Sample* in 50 mL of water in a glass-stoppered flask. While agitating, add 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 well, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate. The titration endpoint is indicated by the appearance of a red color. Perform a blank determination (see *General Provisions*), and subtract the volume required for the blank titration from the volume required for

the *Sample* titration; the difference in these volumes is the amount of ammonium thiocyanate consumed by the *Sample*, V. Each mL of reacted 0.1 N silver nitrate is equivalent to 5.844 mg of NaCl. Calculate the percentage (w/w) of sodium chloride in the *Sample*:

$$\text{Result} = F \times [(50 \times A) - (V \times B)]/W \times 100$$

F = formula weight of sodium chloride, 58.44
A = concentration of the silver nitrate solution (mol/L)
V = volume of the ammonium thiocyanate consumed by the *Sample* (mL)
B = concentration of the ammonium thiocyanate solution (mol/L)
W = weight of the *Sample* (mg)

■ 1S (*FCC7*)

Acceptance criteria: NLT 50.0%

~~1ATCC is the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110.~~

~~2NCIMB Ltd. is located at 23 St. Machar Drive, Aberdeen, Scotland AB24 3RY.~~

~~3The Nisin International Reference Preparation is available from the WHO International Laboratory for Biological Standards, Ministry of Agriculture, Fisheries & Food, Central Veterinary Laboratory, New Haw, Weybridge, Surrey, England.~~

[1] ATCC is the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110.

[2] NCDO is the National Collection of Dairy Organisms. Contact NCDO c/o NCIMB, 23 St. Marchar Drive, Aberdeen, Scotland AB24 3RY.

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

BRIEFING

Phosphoric Acid, *FCC 6* page 761 and page 1316 of the *First Supplement.* On the basis of comments and data received, a revision to the test for *Fluoride* is proposed to provide a more accurate method that includes a heating step for the *Sample preparation* necessary for converting fluoride in the sample to free F ion.
(FIEC: K. Bowman) C83924

Phosphoric Acid

Orthophosphoric Acid

H_3PO_4

Formula wt 98.00

INS: 338

CAS: [7664-38-2]

DESCRIPTION

Phosphoric Acid occurs as a colorless, aqueous solution, usually available in concentrations ranging from 75.0% to 85.0%. It is miscible with water and with alcohol.

Function: Acidifier; sequestrant

Packaging and Storage: Store in tight containers.

IDENTIFICATION

- **Phosphate,** Appendix IIIA
  Sample solution: 100 mg/mL
  Acceptance criteria: Passes tests

ASSAY

- **Procedure**
  Sample: 1.5 g
  Analysis: Transfer the *Sample* into a tared glass-stoppered flask and dilute with water to 120 mL. Add 0.5 mL of thymolphthalein TS, mix, and titrate with 1 N sodium hydroxide to the first appearance of a blue color. Each mL of 1 N sodium hydroxide is equivalent to 49.00 mg of H_3PO_4.
  Acceptance criteria: NLT the minimum or within the range of percentage claimed by the vendor

IMPURITIES

Change to read:

Inorganic Impurities

- **Arsenic,** *Arsenic Limit Test,* Appendix IIIB
  Sample solution: 1 g dissolves in 35 mL of water.
  Acceptance criteria: NMT 3 mg/kg
- **Cadmium,** *Cadmium Limit Test,* Appendix IIIB
  [Note—Alternately, use the Inductively Coupled Plasma Emission Method below to determine the cadmium content.]
  Apparatus: Inductively Coupled Plasma Emission Spectrophotometer set to 226.502 nm for cadmium and to 371.029 for yttrium (internal standard) with an axial view mode. [Note—This method was developed using

a Perkin-Elmer Model 3300 DV equipped with a sapphire injector, low-flow GemCone nebulizer, cyclonic spray chamber, and yttrium internal standard.] Use acid-rinsed plastic volumetric flasks and other labware.

Standard stock solution: Use commercially available certified stock standard solutions of 10-, 100-, or 1000-µg/mL cadmium in 2% to 5% nitric acid. Use higher purity nitric acid for standards and samples. Where possible, match the sample matrix by adding a material of known high purity to the standards.

Internal standard solution: 10-µg/mL yttrium in 2% nitric acid, from a certified stock solution

Standard solutions: 0.250-, 0.050-, and 0-µg/mL cadmium containing 5% nitric acid; 0.100-µg/mL yttrium; and 5% high-purity sample matrix matching reagent (if available): made from *Standard stock solution* and *Internal standard solution.* [Note—Prepare monthly.]

Sample solution: Dissolve 2.5 g of sample in water, and add 2.5 mL of nitric acid and 500 µL of 10-µg/mL yttrium. Dilute to 50 mL.

Analysis: Set up the instrumental method to measure the area of the 0-µg/mL *Standard solution* (blank) peaks and then the net intensities of the 0.050- and 0.250-µg/mL *Standard solutions* with the yttrium *Internal standard solution*. The calibration curve should be linear. Examine the spectra of the cadmium and yttrium, and make any necessary adjustments to the exact peak locations and baselines to ensure proper integration of the areas under the respective peaks.

Analyze the *Sample solution* and calculate the concentration, in µg/mL, of the cadmium in the *Sample solution*. Calculate the quantity, in mg/kg, of cadmium in the sample by multiplying this value by 20.

[Note—Some sample types may naturally contain significant levels of yttrium. In these cases, choose a suitable alternative internal standard, or run the test without an internal standard. Use of the internal standard is not required, but it is helpful when there are variations in the viscosity among sample types. Samples may be prepared in higher or lower concentrations as needed. Standard concentrations may be adjusted as needed. Alternative procedures should be validated before use.]

Acceptance criteria: NMT 3 mg/kg

- ~~**FLUORIDE**, *Fluoride Limit Test, Method IV,* Appendix IIIB~~

~~**Sample:** 1 g~~

~~**Analysis:** Proceed as directed using *Buffer Solution B.*~~ 1S (*FCC6*)

~~**Acceptance criteria:** NMT 10 mg/kg~~

- **FLUORIDE**

Fluoride standard solution: 100 µg/mL of fluoride ion; prepare by dissolving 22.2 mg of sodium fluoride, previously dried at 200° for 4 h, in sufficient water to make 100.0 mL.

Electrode calibration: Determine the electrode slope, S, according to the manufacturer's instructions or according to the method described under *Fluoride Limit Test, Method IV,* Appendix IIIB.

Sample preparation: Weigh 5 g of sample into a 100-mL volumetric flask, add 30 mL water, and place the uncapped flask on a hot plate capable of maintaining a temperature of 80°–90° for 10 min without boiling. (Alternatively, suspend the uncapped flask in a boiling water bath for 10 min.) Allow the contents to cool to room temperature, and dilute with water to volume.

Analysis: Transfer the *Sample preparation* to a 150-mL plastic beaker with a magnetic stir bar. Place in the solution the fluoride ion and reference electrodes (or a combination fluoride electrode) of a suitable ion-selectrive apparatus with a magnetic stirrer. Begin stirring slowly and allow the instrument to equilibrate. Obtain the initial mV reading from the instrument, E1. Add 1 mL of the *Fluoride standard solution* to the beaker, allow the electrode to equilibrate with continued stirring, and take the final mV reading, E2. [Note—The ion-selective electrode responds more slowly than does a pH electrode, and a stable reading may not be obtained for 2–3 min. The mV displayed should be stable for 30 s before taking readings.]

Calculate the amount of fluoride, in mg/kg, in the sample:

$$\text{Result} = 20/[10^{(E2 - E1)/S} - 1]$$

Acceptance criteria: NMT 10 mg/kg

■ 1S (*FCC7*)

- **LEAD**, *Lead Limit Test, APDC Extraction Method,* Appendix IIIB

  ■ ■ 1S (*FCC6*)

  Acceptance criteria: NMT 3 mg/kg

OTHER REQUIREMENTS

- **LABELING:** Indicate the percent or the percent range of phosphoric acid (H_3PO_4).

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 1316

FCC Sixth Edition Supplement 1 Page 1316

BRIEFING

Polyvinylpolypyrrolidone, *FCC 6* page 794 and page 1318 of the *First Supplement.* On the basis of efforts to modernize *Identification* tests in *FCC*, a new infrared method is proposed as *Identification* test *A*. The existing *Procedure* under *Identification* is now *Identification* test *B*.
(FIEC: K. Bowman) C79489

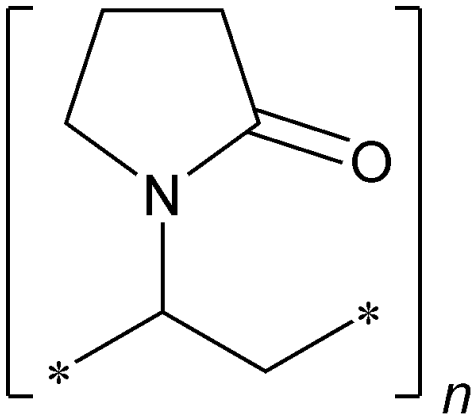
Polyvinylpolypyrrolidone

(Current title—not to change until February 2010)
(Monograph title change—to become effective February 2010)
See *Crospovidone*

Crospovidone

PVPP

1-Vinyl-2-pyrrolidone Crosslinked Insoluble Polymer



INS: 1202

DESCRIPTION

Polyvinylpolypyrrolidone occurs as a white to off-white, hygroscopic, free-flowing powder. It is a crosslinked homopolymer of purified vinylpyrrolidone, produced catalytically. It is insoluble in water and in other common solvents.

Function: Clarifying agent; stabilizer
Packaging and Storage: Store in tight containers.

IDENTIFICATION

Add the following:

- ■**A. Infrared Absorption,** *Spectrophotometric Identification Tests,* Appendix IIIC
  Reference standard: USP Crospovidone RS

  Standard and sample preparation: *K*; previously dried in vacuum at 105° for 1 h

Acceptance criteria: The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard*.

■1S (*FCC7*)

Change to read:

- ■**B.**■1S (*FCC7*) **Procedure**

Sample: 1 g

Analysis: Add 0.1 mL of iodine TS to a suspension of the *Sample* in 10 mL of water, and shake the mixture for 30 s. [Note—The reagent is discolored, a distinction from polyvinylpyrrolidone, which produces a red color.] Add 1 mL of starch TS, and shake the mixture.

Acceptance criteria: No blue color appears.

ASSAY

- **Nitrogen Determination**, *Method II,* Appendix IIIC

Sample: 100 mg

Analysis: Determine as directed, except in the wet-digestion step, repeat the addition of hydrogen peroxide (usually three to six times) until a clear, light green solution is obtained, then heat for an additional 4 h, and continue as directed, beginning with "Cautiously add 20 mL of water".

Acceptance criteria: 11.0%–12.8% of nitrogen (N)

IMPURITIES

Inorganic Impurities

- **Lead**, *Lead Limit Test, Flame Atomic Absorption Spectrophotometric Method,* Appendix IIIB

Sample: 10 g

Acceptance criteria: NMT 2 mg/kg

SPECIFIC TESTS

- **Acid-Alcohol Soluble Substances**

Sample: 1 g

Solubility solution: 15 g of glacial acetic acid in 50 mL of ethanol and sufficient water to make 500 mL of solution

Analysis: Place the *Sample* into a flask containing 500 mL of the *Solubility solution*. Allow the contents of the flask to rest for 24 h. Filter on a filter screen with a porosity of 2.5 µm, then on a filter screen with a porosity of 0.8 µm. Concentrate the filtrate over a water bath. Finish evaporation over the water bath in a 70-mm diameter tared silica capsule.

Acceptance criteria: The dry residue remaining after evaporation must be less than 10 mg, taking into account any residue left by the evaporation of 500 mL of the acetic acid–ethanol mixture. (NMT 1.0%.)

- **pH**, *pH Determination,* Appendix IIB

Sample suspension: 1 g in 100 mL of water

Acceptance criteria: Between 5.0 and 11.0

- **Residue on Ignition (Sulfated Ash)**, Appendix IIC

Sample: 2 g

Acceptance criteria: NMT 0.4%

Change to read:

- **Unsaturation (as Vinylpyrrolidone)**

■**Mobile phase:** Acetonitrile and water (10:90)

Resolution solution: Transfer 10 mg of vinylpyrrolidone and 500 mg of vinyl acetate to a 100-mL volumetric flask, and dissolve in and dilute with methanol to volume. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Standard solution: Transfer 50 mg of vinylpyrrolidone to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Sample solution: Suspend 1.250 g in 50.0 mL of methanol, and shake for 60 min. Leave the bulk to settle, and filter through a 0.2-µm filter.

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography

Detector: UV 235 nm

Column: Stainless steel column about 4-mm × 250-mm, packed with octadecylsilanized silica gel (5 µm in particle diameter), with a guard column about 4-mm × 25-mm with the same packing

Column temperature: 40°

Flow rate: Adjust so that the retention time of vinylpyrrolidone is about 10 min.

Injection volume: About 50 µL

System suitability

Samples: *Resolution solution* and *Standard solution*

Suitability requirement 1: The resolution, *R*, between vinylpyrrolidone and vinyl acetate for the *Resolution solution* is NLT 2.0.

Suitability requirement 2: The relative standard deviation for replicate injections of the *Standard solution* is NMT 2.0%.

Analysis: Separately inject the *Standard solution* and the *Sample solution* into the chromatograph, record the chromatograms, and measure the responses for the vinylpyrrolidone peak area. [Note—If necessary, after each injection of the *Sample solution*, wash the polymeric material from the guard column by passing the *Mobile phase* through the column backwards for about 30 min at the same flow rate.]

Calculate the concentration (mg/mL) of vinylpyrrolidinone in the sample:

$$\text{Result} = 1000 \times (C/W) \times (r_U/r_S)$$

C = concentration of vinylpyrrolidinone in the *Standard solution* (mg/mL)

W = weight of sample taken to prepare the *Sample solution* (mg)

r_U = peak area response for vinylpyrrolidinone obtained from the *Sample solution*

r_S = peak area response for vinylpyrrolidinone obtained from the *Standard solution*

■1S (*FCC6*)

Acceptance criteria: ■NMT 0.001%■ 1S (*FCC6*)

- **WATER**, *Water Determination,* Appendix IIB

  Acceptance criteria: NMT 6.0%

Change to read:

- **WATER SOLUBLE SUBSTANCES**

  Sample: 10 g

  Analysis: Place the *Sample* into a 200-mL flask containing 100 mL of water. Shake the flask, and allow the contents to rest for 24 h. ■Filter through a membrane filter having a 0.45-µm porosity, protected against

clogging by superimposing a membrane filter having a 3-µm porosity.■1S (*FCC6*)Evaporate the filtrate over a water bath until dry.

Acceptance criteria: The residue left by evaporating the filtrate is less than ■150■1S (*FCC6*)mg. (NMT ■1.5%■1S (*FCC6*))

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 794
FCC Sixth Edition Supplement 1 Page 1318

BRIEFING

Potassium Phosphate, Dibasic, *FCC 6* page 814. On the basis of comments received, it is proposed to revise the *Assay* to clarify instructions for determining the amount of hydrochloric acid consumed by the sample. (FIEC: K. Bowman) C83932

Potassium Phosphate, Dibasic

Dipotassium Monophosphate

Dipotassium Phosphate

K_2HPO_4

Formula wt 174.18

INS: 340(ii)

CAS: [7758-11-4]

DESCRIPTION

Potassium Phosphate, Dibasic, occurs as a colorless or white, granular salt that is deliquescent when exposed to moist air. One g is soluble in about 3 mL of water. It is insoluble in alcohol. The pH of a 1% solution is about 9.

Function: Buffer; sequestrant; yeast food

Packaging and Storage: Store in tight containers.

IDENTIFICATION

- **Phosphate,** Appendix IIIA
  Sample solution: 50 mg/mL
  Acceptance criteria: Passes tests
- **Potassium,** Appendix IIIA
  Sample solution: 50 mg/mL
  Acceptance criteria: Passes tests

ASSAY

Change to read:

- **Procedure**
  Sample: 6.5 g, previously dried
  Analysis: Transfer the *Sample* into a 250-mL beaker. Add 50.0 mL of 1 N hydrochloric acid and 50.0 mL of water, and stir until the sample is completely dissolved. Place the electrodes of a suitable pH meter in the solution and, stirring constantly, slowly titrate the excess acid 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:

$$\blacksquare A = 50 - x$$

  x = volume of 1 N sodium hydroxide used in the titration (mL)

■ 1S (*FCC7*)

  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 to pH 8.8). When A is equal to or less than B, each mL of the volume A of 1 N hydrochloric acid is equivalent to 174.2 mg of K_2HPO_4. When A is greater than B, each mL of the volume 2B – A of 1 N sodium hydroxide is equivalent to 174.2 mg of K_2HPO_4.

Acceptance criteria: NLT 98.0% of K_2HPO_4, on the dried basis

IMPURITIES

Inorganic Impurities

- **Arsenic**, *Arsenic Limit Test,* Appendix IIIB
  Sample solution: 1 g in 10 mL
  Acceptance criteria: NMT 3 mg/kg
- **Fluoride**, *Fluoride Limit Test, Method IV,* Appendix IIIB
  Sample: 2 g
  Acceptance criteria: NMT 10 mg/kg
- **Lead**, *Lead Limit Test, APDC Extraction Method,* Appendix IIIB
  Acceptance criteria: NMT 2 mg/kg

SPECIFIC TESTS

- **Insoluble Substances**
  Sample: 10 g
  Analysis: Dissolve the *Sample* in 100 mL of hot water and filter through a tared filtering crucible. Wash the insoluble residue with hot water, dry it at 105° for 2 h, cool, and weigh.
  Acceptance criteria: NMT 0.2%
- **Loss on Drying**, Appendix IIC (105° for 4 h)
  Acceptance criteria: NMT 2.0%

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 814

BRIEFING

Potassium Phosphate, Monobasic, *FCC 6* page 815. On the basis of comments received, it is proposed to revise the *Assay* to clarify instructions for determining the amount of hydrochloric acid consumed by the sample.
(FIEC: K. Bowman) C83931

Potassium Phosphate, Monobasic

Potassium Biphosphate

Potassium Dihydrogen Phosphate

Monopotassium Phosphate

KH_2PO_4

Formula wt 136.09

INS: 340(i)

CAS: [7778-77-0]

DESCRIPTION

Potassium Phosphate, Monobasic, occurs as colorless crystals or as a white, granular or crystalline powder. It is stable in air. It is freely soluble in water, but is insoluble in alcohol. The pH of a 1:100 aqueous solution is between 4.2 and 4.7.

Function: Buffer; sequestrant; yeast food

Packaging and Storage: Store in tight containers.

IDENTIFICATION

- **Phosphate,** Appendix IIIA
  Sample solution: 50 mg/mL
  Acceptance criteria: Passes tests
- **Potassium,** Appendix IIIA
  Sample solution: 50 mg/mL
  Acceptance criteria: Passes tests

ASSAY

Change to read:

- **Procedure**
  Sample: 5 g, previously dried
  Analysis: Transfer the *Sample* into a 250-mL beaker. Add 5.0 mL of 1 N hydrochloric acid and 100 mL of water, and stir until the sample is completely dissolved. Place the electrodes of a suitable pH meter in the solution, and, stirring constantly, slowly titrate the excess acid 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:

$$A = 5 - x$$

x = volume of 1 N sodium hydroxide used in the titration (mL)

■ 1S (*FCC7*)

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 to 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.

Acceptance criteria: NLT 98.0% of KH_2PO_4, on the dried basis

IMPURITIES

Inorganic Impurities

- **Arsenic**, *Arsenic Limit Test,* Appendix IIIB
  Sample solution: 1 g in 10 mL
  Acceptance criteria: NMT 3 mg/kg
- **Fluoride**, *Fluoride Limit Test, Method IV,* Appendix IIIB
  Sample: 2 g
  Acceptance criteria: NMT 10 mg/kg
- **Lead**, *Lead Limit Test, APDC Extraction Method,* Appendix IIIB
  Acceptance criteria: NMT 2 mg/kg

SPECIFIC TESTS

- **Insoluble Substances**
  Sample: 10 g
  Analysis: Dissolve the *Sample* in 100 mL of hot water, and filter through a tared filtering crucible. Wash the insoluble residue with hot water, dry it at 105° for 2 h, cool, and weigh.
  Acceptance criteria: NMT 0.2%
- **Loss on Drying**, Appendix IIC (105° for 4 h)
  Acceptance criteria: NMT 1.0%

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 815

BRIEFING

Potassium Phosphate, Tribasic, *FCC 6* page 815. On the basis of comments received, it is proposed to revise the *Assay* to clarify instructions for determining the amount of hydrochloric acid consumed by the sample. (FIEC: K. Bowman) C83934

Potassium Phosphate, Tribasic

Tripotassium Phosphate

K_3PO_4

Formula wt 212.27

INS: 340(iii)

CAS: [7778-53-2]

DESCRIPTION

Potassium Phosphate, Tribasic occurs as white, hygroscopic crystals or granules. It is anhydrous or may contain one molecule of water of hydration. It is freely soluble in water, but is insoluble in alcohol. The pH of a 1:100 aqueous solution is about 11.5.

Function: Emulsifier

Packaging and Storage: Store in tight containers.

IDENTIFICATION

- **Phosphate,** Appendix IIIA
  Sample solution: 50 mg/mL
  Acceptance criteria: Passes test
- **Potassium,** Appendix IIIA
  Sample solution: 50 mg/mL
  Acceptance criteria: Passes test

ASSAY

Change to read:

- **Procedure**

  Sample: Quantity equivalent to 8 g of anhydrous K_3PO_4

  Analysis: Dissolve the *Sample* in 40 mL of water in a 400-mL beaker, and add 100.0 mL of 1 N hydrochloric acid. Pass a stream of fine bubbles of carbon dioxide-free air through the solution for 30 min to expel carbon dioxide, covering the beaker loosely to prevent any 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. Protect the solution from absorbing carbon dioxide. Titrate the solution with 1 N sodium hydroxide until the inflection point occurs at about pH 4, and then calculate the volume (A) of 1 N hydrochloric acid consumed:

$$\blacksquare A = 100 - x$$

  x = volume of 1 N sodium hydroxide used in the titration (mL)

  ■1S (*FCC7*)

  Continue the titration with 1 N sodium hydroxide until the inflection point occurs at about pH 8.8. Calculate

the volume (B) of 1 N sodium hydroxide consumed in this titration. When A is equal to or greater than 2B, 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 2B, each mL of the volume A – B of 1 N sodium hydroxide is equivalent to 212.3 mg of K_3PO_4.

Acceptance criteria: NLT 97.0% of K_3PO_4, calculated on the ignited basis

IMPURITIES

Inorganic Impurities

- **Arsenic**, *Arsenic Limit Test,* Appendix IIIB
  Sample solution: 1 g in 10 mL of water
  Acceptance criteria: NMT 3 mg/kg
- **Fluoride**, *Fluoride Limit Test, Method IV,* Appendix IIIB
  Sample: 2 g
  Acceptance criteria: NMT 10 mg/kg
- **Lead**, *Lead Limit Test, APDC Extraction Method,* Appendix IIIB
  Sample: 10 g
  Acceptance criteria: NMT 2 mg/kg

SPECIFIC TESTS

- **Insoluble Substances**
  Sample: 10 g
  Analysis: Dissolve 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.
  Acceptance criteria: NMT 0.2%
- **Loss on Ignition**
  Analysis: Ignite a sample at 800° for 30 min using a platinum, quartz, or porcelain dish instead of the weighing bottle.
  Acceptance criteria
  Anhydrous: NMT 5.0%
  Monohydrate: Between 8% and 20.0%

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 815

BRIEFING

Propylene Oxide. Because there is no existing *FCC* monograph for this food chemical, a new monograph based on comments received and based partially on the specifications set forth in the Propylene Oxide monograph prepared at the 3rd Session of the Joint FAO/WHO Expert Committee on Food Additives (JEFCA) is proposed. Interested parties are encouraged to submit comments.

1. The first *Function* given indicates the use of this ingredient as a chemical reactant used in the production of food ingredients. This is a new category of *Function* for *FCC*.
2. The *Assay* procedure given is a gas chromatographic procedure utilizing hydrogen as the carrier gas. It is our understanding that methods which utilize helium as a carrier gas are also used in industry. Methods and data supporting the use of an alternate carrier gas are encouraged. Also regarding the *Assay*, comments are requested concerning the limit requirement of NLT 99.90% (as opposed to 99.9%).
3. The JECFA monograph referenced above includes specifications for Aldehydes and Distillation Range. We have not included these specifications because of comments received indicating that JECFA's limit for Aldehydes (0.1%) would actually indicate significant contamination, that propylene oxide commercially available would be well below the JECFA limit, and it is not clear that the test referenced would test to an appropriate limit. Comments received also indicated that the Distillation Range specification given by JECFA would be more likely to indicate only the identification of the material and not the quality of the material. Because two appropriate *Identification* tests are already included in this monograph, it was determined that the Distillation Range test was not necessary.
4. The limits for *Nonvolatile Residue*, *Acidity (as Acetic Acid)*, and *Total Chlorides* given are lower than current JECFA limits, but we do recognize the age of the original JECFA specifications and comments received indicated that the lower limits are reasonable limits for modern commercially available material.
5. The method included for *Total Chlorides* is adapted from ASTM method D4929-07 (Method B) based on comments received. Additional data and comments are encouraged.

(FIEC: K. Bowman) C73611

Add the following:

■ **Propylene Oxide**

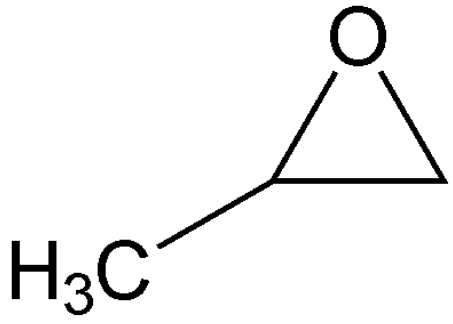
Epoxypropane

Methyl Ethylene Oxide

Methyl Oxirane

Propene Oxide

1,2-Propylene Oxide



C_3H_6O

Formula wt 58.08
CAS: [75-56-9]

DESCRIPTION

Propylene Oxide occurs as a colorless volatile liquid with an ether-like odor. It is usually produced by peroxide-based epoxidation of propylene or dehydrochlorination of propylene chlorohydrin. Propylene Oxide is freely soluble in water and miscible in ethanol and ether.

Function: Reactive chemical intermediate used in the manufacture of food ingredients (polyols, propylene glycol, modified food starch, etc.); preservative; antimicrobial agent

Packaging and Storage: Store in tight containers in a cool place, remote from fire. [**CAUTION—** Propylene Oxide is highly volatile and flammable and is classified as a suspect human carcinogen. Keep away from sources of ignition. The material should be handled with appropriate ventilation and personal protective equipment.]

IDENTIFICATION

- **A. Infrared Absorption,** *Spectrophotometric Identification Tests,* Appendix IIIC
  Reference standard: USP Propylene Oxide RS
  Sample and standard preparation: *F*
  Acceptance criteria: The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard*.
- **B. Procedure**
  Acceptance criteria: The major peak in the chromatogram of the sample is at the same retention time as the major peak in the chromatogram of the *Standard*, as determined from the *Assay* below.
- **C. Specific Gravity:** Determine by any reliable method (see *General Provisions*).
  Acceptance criteria: 0.829–0.831 at $20^{\circ}/20^{\circ}$

ASSAY

- **Procedure**
  Standard: USP Propylene Oxide RS
  Chromatographic system, Appendix IIA
  Mode: Gas chromatography
  Detector: Flame ionization detector
  Column: 60-m × 0.32-mm (i.d.) fused silica capillary column with a 1.5-µm trifluoropropylmethylpolysiloxane stationary phase[1]
  Temperature

Injector: 65°

Detector: 175°

Column: See table below.

Time (min)	Temperature (°)
0	33
7.5	33
19.7	94
22	94
24.8	150
28	150

Carrier gas: Hydrogen

Detector gas flows

Hydrogen carrier: 28 mL/min

Make-up (helium): 30 mL/min

Air: 400 mL/min

Head pressure: 8.3 psi

Linear velocity: 40 cm/s

Flow rate: 1.97 mL/min

Injection size: 1 µL

Split ratio: 25:1

Septum purge: 2.0 mL/min

Analysis: Separately inject a sample of propylene oxide and the *Standard* into the chromatograph and record the chromatograms.

Identify the impurities present and calculate their concentrations using pre-determined response factors. [Note—Potential organic impurities include acetone, methanol, methyl formate, and propionaldehyde.] Calculate the percent propylene oxide in the sample as the difference between 100.0 and the sum of all impurities detected from the chromatogram of the sample.

Acceptance criteria: The percent propylene oxide in the sample is NLT 99.90%.

IMPURITIES

Inorganic Impurities

- **Total Chlorides**[2]

Apparatus

Combustion furnace: Electric furnace capable of maintaining a temperature of 800°

Combustion tube: Quartz; constructed such that the sample, vaporized in the inlet section, is swept into the oxidation zone by an inert gas (argon, helium, nitrogen, or carbon dioxide of high purity grade) where it mixes with oxygen and is burned. The inlet end of the tube contains a septum and has side arms for the introduction of oxygen and the inert gas. The center section of the tube should be of sufficient volume to allow complete oxidation of the sample.

Titration cell: The cell should contain a sensor-reference pair of electrodes (to detect changes in silver ion concentration), a generator anode-cathode pair of electrodes (to maintain constant silver ion concentration), and an inlet for the gaseous sample from the pyrolysis tube. The sensor, reference, and anode electrodes should be silver electrodes and the cathode electrode should be a platinum wire. The reference electrode should reside in a saturated silver acetate half-cell and the electrolyte should contain

70% acetic acid in water.

Microcoulometer: Use a microcoulometer having variable gain and bias control, capable of measuring the potential of the sensing-reference electrode pair and of comparing this potential with a bias potential, and of applying the amplified difference to the working-auxiliary electrode pair so as to generate a titrant. The output of the microcoulometer signal should be proportional to the generating current. Use an apparatus with a digital meter and circuitry to convert the output signal directly to mass (in ng or µg) of chloride.

Sampling syringe: A µL sampling syringe capable of delivering 5- to 50-µL samples into the pyrolysis tube should be used. The recommended needle length is 3- or 6-in in order to reach the inlet zone.

Pump: A constant-rate syringe pump or manual dispensing adapter may be used to regulate flow of the sample into the combustion tube. Flow rate should not exceed 0.5 µL/s.

Operating conditions

[Note—Set up the analyzer as directed by the manufacturer. Typical operating conditions are given below.]

Reactant gas flow (O_2)	160 mL/min
Carrier gas flow (Argon, Helium, Nitrogen, or Carbon Dioxide)	40 mL/min
Furnace temperature:	
Inlet zone	700°
Center/outlet zones	800°
Coulometer:	
Bias voltage	240–265 mV
Gain	~1200

Sample: Propylene oxide

Standard solution: Dissolve 3.174 mg/mL of chlorobenzene in 2,2,4-trimethyl pentane and dilute with the same solvent to 500 mL (1000 mg/L of chloride). Dilute 1.0 mL of this solution with the same solvent to 100 mL (10 mg/L of chloride).

Blank: 2,2,4-trimethyl pentane

Injection size: 40 µL

Analysis: Separately inject the *Standard solution*, *Sample*, and *Blank* into the pyrolysis tube at a rate of NMT 0.5 µL/s. Use the readouts obtained from the microcoulometer to calculate the chloride content, in µg/g, in the sample taken:

$$\text{Result} = [R_U/(V_U \times D_U \times RF)] - [R_B/(V_B \times D_B \times RF)]$$

R_U = microcoulometer readout obtained from the *Sample* (ng)

V_U = injection size for the *Sample* (µL)

D_U = density of the *Sample* (g/mL)

RF = recovery factor; calculate as directed below

R_B = microcoulometer readout obtained from the *Blank* (ng)

V_B = injection size for the *Blank* (µL)

D_B = density of the *Blank* (g/mL)

$$\text{Recovery Factor, RF} = [R_S/(V_S \times D_S \times C_S)] - [R_B/(V_B \times D_B)]$$

R_S = microcoulometer readout obtained from the *Standard solution* (ng)

V_S = injection size for the *Standard solution* (µL)

D_S = density of the *Standard solution* (g/mL)

C_S = concentration of chloride in the *Standard solution* (mg/kg)

R_B = microcoulometer readout obtained from the *Blank*

V_B = injection size for the *Blank* (µL)

D_B = density of the *Blank* (g/mL)

Acceptance criteria: NMT 10 µg/g

Organic Impurities

- **Acidity (as Acetic Acid)**

Solution A: Dissolve 0.2 g of phenolphthalein in 60 mL of 90% ethanol. Dilute with water to 100 mL.

Sample: 50 mL (measure in a chilled graduated cylinder)

Analysis: Transfer the *Sample* to a chilled 250-mL Erlenmeyer flask, add 1 mL of *Solution A*, and titrate with 0.01 N sodium hydroxide to a faint pink endpoint which persists for NLT 15 s.

Calculate the percentage of acetic acid present:

$$\text{Result} = V_T \times 0.060 \times (1/V_{PO}) \times (1/S) \times 100$$

V_T = volume of 0.01 N sodium hydroxide required for the titration (mL)

V_{PO} = volume of the propylene oxide *Sample* taken (mL)

S = specific gravity of the *Sample*

Acceptance criteria: NMT 0.002%

SPECIFIC TESTS

- **Nonvolatile Residue**

[Note—Perform analysis in a fume hood to avoid exposure to propylene oxide vapors.]

Sample: 100 mL

Analysis: Evaporate the *Sample* to dryness in a tared platinum dish (previously heated at 105° to constant weight) on a steam bath, heat at 105° for 30 min or to constant weight, cool in a desiccator, and weigh.

Acceptance criteria: NMT 0.002 g/100 mL

- **Water,** *Water Determination,* Appendix IIB

Acceptance criteria: NMT 0.01%

■ 1S (*FCC7*)

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[1] Restek Chromatography Products RTX-200, or equivalent. Available at www.restek.com.

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[2] Adapted from ASTM D4929-07 Standard Test Methods for Determination of Organic Chloride Content in Crude Oil, Method B. The original ASTM method is available in its entirety from ASTM International, 100 Barr Harbor Drive, West Conshocken, PA 19428. Phone: 610-832-9585, Fax: 610-832-9555, Email: service@astm.org, Website: www.astm.org.

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

BRIEFING

Rebaudioside A, page 1438 of the *Second Supplement* to *FCC 6*. On the basis of comments and data received, it is proposed to revise the *Assay* method used to quantify the purity and steviol glycoside impurites of this food ingredient. The proposed method uses a Cosmosil Sugar-D column with better stationary phase stability compared to the current Zorbax NH_2 column resulting in longer column life, more reproducible retention times, and lower LOD and LOQ. It also eliminates the long column equilibration step.
(FIEC: J. Moore) C83480

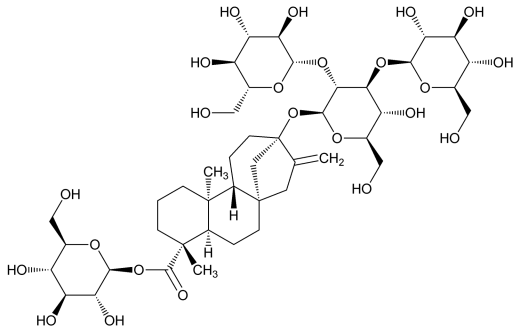
Rebaudioside A

Reb A

Rebiana

Kaur-16-en-18-oic acid, 13-[(*O*-β-d-glucopyranosyl-(1→2)-*O*-[β-d-glucopyranosyl-(1→3)]-β-d-glucopyranosyl)oxy]-, β-d-glucopyranosyl ester, (4α)

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



$C_{44}H_{70}O_{23}$

Formula wt 967.01
CAS: [58543-16-1]

DESCRIPTION

Rebaudioside A is a white to off-white, hygroscopic fine crystal, granule, or powder having a sweet taste. It is freely soluble in ethanol:water 50/50 (v/v), sparingly soluble in water, and sparingly soluble in ethanol. It is obtained from the leaves of the *Stevia rebaudiana* (Bertoni) plant in a multi-step separation and purification process. Principle steps include extraction of steviol glycosides from the leaves using an aqueous or aqueous alcoholic (ethanol or methanol) solvent, and purification of rebaudioside A from the resulting mixture of steviol glycosides by resin absorption followed by recrystallization from an aqueous or aqueous alcoholic (ethanol or methanol) solvent. It is composed predominantly of rebaudioside A, a glycoside of the *ent*-kaurenoid diterpenoid aglycone known as steviol.

Function: Nonnutritive sweetener; sugar substitute

Change to read:

Packaging and Storage: Keep dry, and store in tight containers at ambient temperature.

~~[Note—Rebaudioside A is hygroscopic and should be stored in a desicator prior to weighing for appropriate test procedures.]~~

■ ■ 1S (*FCC7*)

IDENTIFICATION

- **A. Spectrophotometric Identification Tests,** *Infrared Absorption,* Appendix IIIC

  Reference standard: USP Rebaudioside A RS

  Sample and standard preparation: *A*

  Acceptance criteria: The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard*.

- **B. Procedure**

  Acceptance criteria: The retention time of the major peak (excluding the solvent peak) in the chromatogram of the *Sample solution* is the same as that of the 4750 mg/L *Rebaudioside A standard solution* in the *Assay*.

ASSAY

Change to read:

- **Procedure** ■

  [Note—Rebaudioside A is hygroscopic, and accurate quantitative analysis requires moisture equilibration before analysis. Equilibrate sample and rebaudioside A standard specimens in lab no less than 24 h before weighing. Intermittent stirring will ensure uniform moisture absorption. *Water* content used in calculation should be determined at time of weighing and after equilibration.]

■ 1S (*FCC7*)

Acetate buffer: Dissolve 0.125 g of ammonium acetate in 900 mL of water, adjust pH to 4.3 with glacial acetic acid solution, and dilute to 1 L. [Note—It may be necessary to adjust the ratio of ammonium acetate to acetic acid. Changing the pH adjusts the retention time of rebaudioside A and related glycosides. Decreasing the pH of the buffer will decrease the retention time of rebaudioside A.]

Mobile phase: ~~20%~~ ■ 13% ■ 1S (*FCC7*) (v/v) *Acetate buffer* in acetonitrile ~~[Note—Equilibrate the column by flushing with 75 mL of isopropyl alcohol and then 50 mL of acetonitrile. After that, flush the column with *Mobile phase* until the retention time of rebaudioside A is less than 8 min (may take up to one day of flushing).]~~ ■ ■ 1S (*FCC7*)

Diluent: 25% (v/v) *Acetate buffer* in acetonitrile. [Note—Allow *Diluent* to come to room temperature before use.]

Rebaudioside A standard solutions: ~~4500, 4750, 5250, and 5500~~ ■ 250, 1000, 2500, and 5000 ■ 1S (*FCC7*) mg/L of USP Rebaudioside A RS in *Diluent*

Stevioside standard stock solution: 250 mg/L of USP Stevioside RS in *Diluent*

Stevioside standard solutions: ~~2.5, 5.0, 10, 25, and 50~~ ■ 0.5, 5.0, 25, and 250 ■ 1S (*FCC7*) mg/L of USP Stevioside RS in *Diluent*: from *Stevioside standard stock solution*

Sample solution: 5000 mg/L in *Diluent*

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography

Detector: UV 210 nm

Column: 15-cm × 4.6-mm, packed with a propyl-amino silane phase bonded to silica gel (5-µm particle diameter)[1]

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection size: ~~12~~ 15 (*FCC7*) µL

System suitability

Samples: ~~5250~~ 5000 (*FCC7*) mg/L *Rebaudioside A standard solution*, ~~2.5~~ 0.5 (*FCC7*) mg/L *Stevioside standard solution*~~, and *Sample solution*~~ (*FCC7*)

Suitability requirements

Detector response: Peak-to-noise ratio (peak height/baseline noise) is NLT 3 for the stevioside peak from the ~~2.5~~ 0.5 (*FCC7*) mg/L *Stevioside standard solution*, where peak height is expressed in mAU, and baseline noise is the maximum deflection of the baseline (mAU) in a blank at the retention time of stevioside over the same baseline peak width in min.

Relative standard deviation: NMT 2.0% for rebaudioside A peak area and retention time from the ~~5250~~ 5000 (*FCC7*) mg/L *Rebaudioside A standard solution*

~~**Column efficiency:** NLT 5000 theoretical plates count, *N*, using the rebaudioside B peak from the *Sample solution*~~

(*FCC7*)

Retention time: The retention time for the rebaudioside A peak from the ~~5250~~ 5000 (*FCC7*) mg/L *Rebaudioside A standard solution* is less than ~~8.0~~ 15.0 (*FCC7*) min.

Tailing factor: NMT 2.0 for the rebaudioside ~~B~~ A (*FCC7*) peak from the ~~*Sample solution*~~ 5000 mg/L *Rebaudioside A standard solution* (*FCC7*)

Analysis: Separately inject equal volumes of the *Rebaudioside A standard solutions, Stevioside standard solutions*, and *Sample solution* into the chromatograph, and measure the responses for the major peaks on the resulting chromatograms. [Note—The approximate retention times for rebaudioside A and its related steviol glycosides are listed in *Chromatographic Profile Table 1*. If the retention time for rebaudioside A is below ~~6~~ 11 (*FCC7*) min, adjust the ratio of ammonium acetate to acetic acid.]

~~**Chromatographic Profile Table 1**~~

~~Compound~~	~~Approx. Retention Time (min)~~	~~Molecular Weight (g/mol)~~
~~Rubusoside~~	~~3.0~~	~~642.73~~
~~Dulcoside A~~	~~3.9~~	~~788.87~~
~~Stevioside~~	~~4.5~~	~~804.88~~
~~Rebaudioside C~~	~~5.2~~	~~951.01~~
~~Rebaudioside F~~	~~5.6~~	~~936.99~~
~~Rebaudioside A~~	~~7.1~~	~~967.01~~
~~Rebaudioside D~~	~~16~~	~~1129.15~~
~~Steviolbioside~~	~~18~~	~~642.73~~
~~Rebaudioside B~~	~~36~~	~~804.88~~

Chromatographic Profile Table 1

Compound	Approx. Retention Time (min)	Molecular Weight (g/mol)
Rubusoside	2.6	642.73
Dulcoside A	4.3	788.87
Stevioside	6.6	804.88
Rebaudioside C	8.5	951.01
Rebaudioside F	9.6	936.99
Rebaudioside A	14	967.01
Steviolbioside	29	642.73
Rebaudioside D	41	1129.15
Rebaudioside B	66	804.88

■ 1S (*FCC7*)

Prepare a standard curve for rebaudioside A by plotting rebaudioside A peak areas versus concentrations in mg/L, corrected for purity, based on the USP Reference Standard label claim. [Note—Peak responses for all other steviol glycosides besides rebaudioside A are used in the *Related Steviol Glycosides* impurities test procedure.] From the standard curve, calculate the concentration (C_U) of rebaudioside A in the *Sample solution* in mg/L. Calculate the percentage of rebaudioside A in the portion of the sample taken:

$$\text{Result} = C_U/C_{SMP} \times 100\%$$

C_U = concentration of rebaudioside A in the *Sample solution* determined from the standard curve (mg/L)

C_{SMP} = concentration of the sample in the *Sample solution* (mg/L)

Acceptance criteria: NLT 95.0%, calculated on the anhydrous and solvent-free basis

IMPURITIES

Inorganic Impurities

• **Arsenic,** *Arsenic Limit Test,* Appendix IIIB

[Note—Alternatively, the arsenic content may be determined by the following method.]

[Note—When water is specified as a diluent, use deionized ultra-filtered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of arsenic as practical.]

Dilute nitric acid: Dilute 2.0 mL of nitric acid with water to 100 mL.

Yttrium internal standard solution: Use a commercially available 1000 µg/kg yttrium ICP standard solution. [Note—The internal standard should be 20 µg/kg in all blanks, standards, and samples.]

Standard stock solution: Dilute a 1000 mg/kg commercially available arsenic ICP standard solution with *Dilute nitric acid* to 1000 µg/kg, transfer 10.0 mL of this solution to a 100-mL volumetric flask, add 2.0 mL of *Dilute nitric acid*, and dilute with water to volume (100 µg/kg). [Note—Prepare this solution fresh every 2 weeks.]

Standard solution: 10 µg/kg of arsenic prepared as follows: transfer 5.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, add 3.0 mL of *Dilute nitric acid*, add 1.0 mL of *Yttrium internal standard solution*, and dilute with water to volume (10 µg/kg). [Note—Prepare this solution fresh weekly.]

Standard blank solution: Transfer 1.0 mL of the *Yttrium internal standard solution* to a 50-mL volumetric flask, add 3.0 mL of *Dilute nitric acid*, and dilute with water to volume.

Sample solution [**CAUTION—** Wear proper eye protection, protective clothing, and gloves during sample

preparation. Closely follow the manufacturer's safety instructions for use of the microwave digestion apparatus.] Transfer 500 mg of sample into a Teflon digestion vessel liner. Prepare samples in duplicate. Add 10 mL of nitric acid, and swirl gently. Cover the vessels with lids, leaving the vent fitting off. Predigest for at least 1 h under a hood. Place the rupture membrane in the vent fitting, and tighten the lid. Place all vessels on the turntable of a microwave oven with a magnetron frequency of about 2455 MHz and a selectable output power of 0 to 950 watts in 1% increments, equipped with advanced composite vessels with 100-mL polytef liners[2]. Connect the vent tubes to the vent trap, and connect the pressure-sensing line to the appropriate vessel. Initiate a two-stage digestion procedure by heating the microwave at 15% power for 10 min, followed by 25% power for 10 min. Remove the turntable of vessels from the oven, and allow the vessels to cool to room temperature (a cool water bath may be used to speed the cooling process). Vent the vessels when they reach room temperature. Transfer the cooled digests into 50-mL volumetric flasks, add 1.0 mL of the *Yttrium internal standard solution*, and dilute with deionized water to volume.

Spectrophotometric system*, Plasma Spectrochemistry,* Appendix IIC

Mode: Inductively coupled plasma-mass spectrometer (ICP-MS)

ICP-MS: Use a system equipped with a quadrupole mass spectrometer and an ion detector maintained under vacuum; the system may include a suppression system to mitigate interference from the $^{40}Ar^{35}Cl^{+}$ ion. If not, correction for this interference must be determined by a suitable method, such as that recommended by the instrument manufacturer. The isotope ratio of $^{40}Ar^{35}Cl^{+}/^{40}Ar^{37}Cl^{+}$ in the *Standard blank solution* may be used to correct this interference.

Analysis: [Note—Instrument performance must be verified to conform to the manufacturer's specifications for resolution and sensitivity. Before analyzing samples, the instrument must pass a suitable performance check.] Aspirate the *Standard blank solution, Standard solution*, and *Sample solution*, at least in duplicate. The *Standard blank solution* should not yield a significant intensity for arsenic. Calculate the internal standard ratios for the *Sample solution* and *Standard solution* as ratio of the arsenic to the yttrium intensities. Calculate the concentration (mg/kg) of arsenic in the sample taken:

$$\text{Result} = (R_U/R_S) \times C_S \times (50/S)$$

R_U = internal standard ratio (arsenic response/yttrium response) from the *Sample solution*

R_S = internal standard ratio (arsenic response/yttrium response) from the *Standard solution*

C_S = concentration of arsenic in the *Standard solution* (µg/kg)

50 = sample dilution factor

S = weight of sample used to prepare the *Sample solution* (mg)

Acceptance criteria: NMT 1 mg/kg, calculated on the anhydrous basis

- **Lead,** *Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace Method, Method I,* Appendix IIIB

[Note—Alternatively, the lead content may be determined by the following method.]

[Note—When water is specified as a diluent, use deionized ultra-filtered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of lead as practical.]

Dilute nitric acid: Dilute 2.0 mL of nitric acid with water to 100 mL.

Thallium internal standard solution: Commercially available 1000 µg/kg thallium ICP standard solution [Note—The internal standard should be 20 µg/kg in all blanks, standards, and samples.]

Standard stock solution: Dilute a 1000 mg/kg commercially available lead ICP standard solution with *Dilute nitric acid* to 1000 µg/kg, transfer 10.0 mL of this solution to a 100-mL volumetric flask, add 2.0 mL of *Dilute nitric acid*, and dilute with water to volume (100 µg/kg). [Note—Prepare this solution fresh every 2

weeks.]

Standard solution: 10 µg/kg of lead prepared as follows: transfer 5.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, add 3.0 mL of *Dilute nitric acid*, add 1.0 mL of *Thallium internal standard solution*, and dilute with water to volume (10 µg/kg). [Note—Prepare this solution fresh weekly.]

Standard blank solution: Transfer 1.0 mL of the *Thallium internal standard solution* to a 50-mL volumetric flask, add 3.0 mL of *Dilute nitric acid*, and dilute with water to volume.

Sample solution [**CAUTION—** Wear proper eye protection, protective clothing, and gloves during sample preparation. Closely follow the manufacturer's safety instructions for use of the microwave digestion apparatus.] Transfer 500 mg of sample into a Teflon digestion vessel liner. Prepare samples in duplicate. Add 10 mL of nitric acid, and swirl gently. Cover the vessels with lids, leaving the vent fitting off. Predigest for at least 1 h under a hood. Place the rupture membrane in the vent fitting, and tighten the lid. Place all vessels on the turntable of a microwave oven with a magnetron frequency of about 2455 MHz and a selectable output power of 0 to 950 watts in 1% increments, equipped with advanced composite vessels with 100-mL polytef liners[2]. Connect the vent tubes to the vent trap, and connect the pressure-sensing line to the appropriate vessel. Initiate a two-stage digestion procedure by heating the microwave at 15% power for 10 min, followed by 25% power for 10 min. Remove the turntable of vessels from the oven, and allow the vessels to cool to room temperature (a cool water bath may be used to speed the cooling process). Vent the vessels when they reach room temperature. Transfer the cooled digests into 50-mL volumetric flasks, add 1.0 mL of the *Thallium internal standard solution*, and dilute with deionized water to volume.

Spectrophotometric system*, Plasma Spectrochemistry,* Appendix IIC

Mode: Inductively coupled plasma-mass spectrometer (ICP-MS)

ICP-MS: Use a system equipped with a quadrupole mass spectrometer and an ion detector maintained under vacuum: the instrument should read all isotopes for lead (206, 207, and 208 amu) and the thallium internal standard (205 amu), and should report the total lead content using the most naturally abundant isotope at 208 amu.

Analysis: [Note—Instrument performance must be verified to conform to the manufacturer's specifications for resolution and sensitivity. Before analyzing samples, the instrument must pass a suitable performance check.] Aspirate the *Standard blank solution, Standard solution*, and *Sample solution*, at least in duplicate. The *Standard blank solution* should not yield a significant intensity for lead. Calculate the internal standard ratios for the *Sample solution* and *Standard solution* as ratio of the lead to the thallium intensities. Calculate the concentration (mg/kg) of lead in the sample taken:

$$\text{Result} = (R_U/R_S) \times C_S \times (50/S)$$

R_U = internal standard ratio (lead response/thallium response) from the *Sample solution*

R_S = internal standard ratio (lead response/thallium response) from the *Standard solution*

C_S = concentration of lead in the *Standard solution* (µg/kg)

50 = sample dilution factor

S = weight of sample used to prepare the *Sample solution* (mg)

Acceptance criteria: NMT 1 mg/kg, calculated on the anhydrous basis

Organic Impurities

- **Ethanol and Methanol**

Internal standard solution: 10 µg/mL of 1-butanol

Standard stock solution: 12.5 mg/mL of ethanol and 12.5 mg/mL of methanol. [Note—Use water free of organics. Prepare fresh daily.]

Standard solutions: By serial dilution of the *Standard stock solution*, prepare solutions with ethanol and

methanol concentrations of 1250, 625, 125, 62.5, 12.5, and 1.25 µg/mL. Separately add 4.0 mL each of these ethanol–methanol solutions and 1.0 mL of *Internal standard solution* to headspace vials, and cap tightly.

Sample: 100 mg

Sample solution: Transfer the *Sample* into a headspace vial, add 4.0 mL of water, add 1 mL of *Internal standard solution*, and cap tightly.

Blank: Transfer 4.0 mL of water into a headspace vial, and cap tightly.

Chromatographic system, Appendix IIA

Mode: Gas chromatography equipped with a headspace analyzer

Detector: Flame ionization

Column: 30-m × 0.32-mm (id) high polarity capillary column with a crosslinked and bonded poly(ethylene glycol) stationary phase and a 1-µm film thickness[3]

Column temperature: 3 min at 35°; increase to 180° at 10°/min; maintain at 180° for 1 min

Injection port temperature: 250°

Detector temperature: 250°

Carrier gas: Helium

Flow rate: 35 cm/s linear velocity

Incubation: 80° for 20 min

Injection syringe: Heated, gas-tight, 85°

Injection size: 1 mL of headspace

System suitability

Sample: *Standard solution*

Suitability requirement 1: The resolution, *R*, between any two components is NLT 3.

Suitability requirement 2: The relative standard deviation of the individual peak responses from replicate injections is NMT 15%.

Analysis: Separately inject equal volumes of the *Standard solutions* and *Sample solution* into the chromatograph, record the chromatograms, and measure the peak responses. [Note—The approximate retention times for ethanol and methanol are 8.1 and 7.4 min, respectively.]

Prepare standard curves for ethanol and methanol by plotting on the y-axis the ratios of analyte peak area to internal standard peak area and on the x-axis the concentration of analyte (µg/mL). [Note—The coefficent of determination for each standard curve should be NLT 0.995.]

Determine the concentration (C_U), in µg/mL, of each analyte in the *Sample solution* using the appropriate standard curve and the ratio of each analyte peak area to the internal standard peak area from the *Sample solution* chromatogram. Determine the percent of each analyte (ethanol and methanol) in the portion of the *Sample* taken:

$$\text{Result} = C_U \times 4/S \times 0.1$$

C_U = concentration of analyte in the *Sample solution* determined from the standard curve (µg/mL)

4 = total volume of water used dissolve the *Sample* for the *Sample solution* (mL)

S = *Sample* weight (mg)

0.1 = correction factor, taking into account unit conversion from µg/mg to µg/µg and conversion to percent

Acceptance criteria

Ethanol: NMT 0.50%

Methanol: NMT 0.020%

- **Related Steviol Glycosides**

Acetate buffer, Mobile phase, Diluent, Rebaudioside A standard solutions, Stevioside standard stock solution, Stevioside standard solutions, Sample solution, Chromatographic system, and **System suitability:** Prepare as directed in the *Assay*.

Analysis: Proceed as directed in the *Assay*, but with the following modifications for the standard curve and calculations.

Using the peak area responses from the *Stevioside standard solutions*, prepare a standard curve for stevioside by plotting stevioside peak areas versus concentrations, in mg/L, corrected for purity, based on the USP Reference Standard label claim. From this standard curve, determine the concentration (mg/L) of stevioside in the *Sample solution*. Calculate the percent of stevioside in the sample taken:

$$\text{Result} = C_U/C_{SMP} \times 100\%$$

C_U = concentration of stevioside in the *Sample solution* determined from the standard curve (mg/L)

C_{SMP} = concentration of the sample in the *Sample solution* (mg/L)

For the seven other steviol glycoside impurities (rubusoside, dulcoside A, rebaudioside C, rebaudioside F, rebaudioside D, steviolbioside, and rebaudioside B) use the stevioside standard curve prepared above to calculate the mg/L stevioside equivalents for each. Separately calculate the percentage of each analyte (rubusoside, dulcoside A, rebaudioside C, rebaudioside F, rebaudioside D, steviolbioside, and rebaudioside B) in the sample taken using the following formula, which takes into account the differences in molecular weights between the analytes and stevioside:

$$\text{Result} = (C_U \times M_{r1}/M_{r2})/C_{SMP} \times 100\%$$

C_U = concentration of stevioside equivalents in the *Sample solution* determined from the standard curve (mg/L)

M_{r1} = molecular weight of the analyte (see *Chromatographic Profile Table 1* in the *Assay*)

M_{r2} = molecular weight of stevioside, 804.88

C_{SMP} = concentration of the sample in the *Sample solution* (mg/L)

Acceptance criteria: The sum of the percentages for all eight steviol glycoside impurities is NMT 5%, calculated on the anhydrous and solvent-free basis.

SPECIFIC TESTS

- **pH**, *pH Determination,* Appendix IIB

Sample: 10 mg/mL

Acceptance criteria: Between 4.5 and 7.0

- **Residue on Ignition (Sulfated Ash)**, Appendix IIC

Sample: 1 g

Acceptance criteria: NMT 1%, calculated on the anhydrous basis

- **Water**, *Water Determination, Method I,* Appendix IIB

Acceptance criteria: NMT 6%

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[1] ~~Zorbax NH2 (Agilent Technologies)~~ ■ Cosmosil Sugar-D (Nacalai Tesque) ■ 1S (*FCC7*), or equivalent.

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[2] MDS 2100 (CEM Corporation, Matthews, NC, USA) or equivalent.

[3] DB-WAXetr (Agilent Technologies), or equivalent.

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 1438

FCC Sixth Edition Supplement 2 Page 1438

BRIEFING

Sodium Phosphate, Monobasic, page 1332 of the *First Supplement* to *FCC 6*. On the basis of comments received, a revision to the *Assay* to clarify instructions for determining the amount of hydrochloric acid consumed by the sample is proposed. A second revision to the volume of hydrochloric acid used in the *Analysis* under the *Assay* is proposed. The large volume of hydrochloric acid used in the existing method represents an unnecessary excess because very little alkalinity is present in the sample.
(FIEC: K. Bowman) C83936

Sodium Phosphate, Monobasic

Monosodium Phosphate

Sodium Biphosphate

Monosodium Dihydrogen Phosphate

NaH_2PO_4

Formula wt, anhydrous 119.98

$NaH_2PO_4 \cdot H_2O$

Formula wt, monohydrate 137.99

INS: 339(i)

CAS: anhydrous [7558-80-7]
CAS: monohydrate [10049-21-5]

DESCRIPTION

Sodium Phosphate, Monobasic is anhydrous or contains one or two molecules of water of hydration and is slightly hygroscopic. The anhydrous form occurs as a white, crystalline powder or granules. The hydrated forms occur as white or transparent crystals or granules. All forms are freely soluble in water, but are insoluble in alcohol. The pH of a 1:100 solution is between 4.1 and 4.7.

Function: Buffer; emulsifier; nutrient
Packaging and Storage: Store in tightly closed containers.

IDENTIFICATION

- **Phosphate,** Appendix IIIA
  Sample solution: 50 mg/mL
  Acceptance criteria: Passes tests
- **Sodium,** Appendix IIIA
  Sample solution: 50 mg/mL
  Acceptance criteria: Passes tests

ASSAY

Change to read:

- **Procedure**

  Sample: 5 g, previously dried at 105° for 4 h

Analysis: Transfer the *Sample* into a 250-mL beaker and add 100 mL of water and ~~50.0~~■ 5.0 ■1S (*FCC7*) mL of 1 N hydrochloric acid. 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 ■ using the equation:

$$A = 5 - x$$

x = volume of 1 N sodium hydroxide used in the titration (mL)

■1S (*FCC7*)

Continue the titration with 1 N sodium hydroxide to the inflection point occurring at about pH 8.8. 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 120.0 mg of NaH_2PO_4.

Acceptance criteria: NLT 98.0% and NMT 103.0% NaH_2PO_4, on the dried basis

IMPURITIES

Change to read:

Inorganic Impurities

- **Arsenic,** *Arsenic Limit Test,* Appendix IIIB
  Sample solution: 1 g in 35 mL of water
  Acceptance criteria: NMT 3 mg/kg
- **Fluoride,** *Fluoride Limit Test, Method IV,* Appendix IIIB
  Sample: 2 g
  Acceptance criteria: NMT 0.005%
- **Lead,** *Lead Limit Test, APDC Extraction Method,* Appendix IIIB
  ■ ■1S (*FCC6*)
  Acceptance criteria: NMT 4 mg/kg

SPECIFIC TESTS

- **Insoluble Substances**
  Sample solution: 10 g
  Analysis: Dissolve the *Sample* in 100 mL of hot water, and pass the solution through a tared filtering crucible (not glass). Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.
  Acceptance criteria: NMT 0.2%
- **Loss on Drying,** Appendix IIC: 60° for 1 h, then 105° for 4 h
  Acceptance criteria
  Anhydrous: NMT 2.0%
  Monohydrate: Between 10.0% and 15.0%
  Dihydrate: Between 20.0% and 25.0%

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 1332

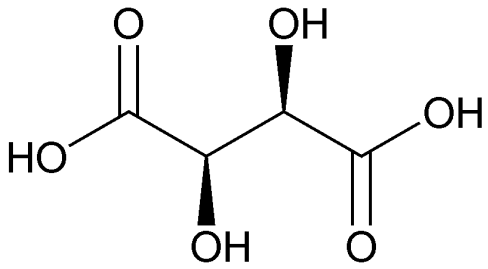
FCC Sixth Edition Supplement 1 Page 1332

BRIEFING

Tartaric Acid, *FCC 6* page 948. On the basis of efforts to modernize *Identification* test procedures in *FCC* and data received, a new infrared method is proposed under *Identification.*
(FIEC: J. Moore) C83899

Tartaric Acid

l(+)-Tartaric Acid



$C_4H_6O_6$

Formula wt 150.09

INS: 334

CAS: [87-69-4]
FEMA: 3044

DESCRIPTION

Tartaric Acid occurs as colorless or translucent crystals or as a white, fine to granular, crystalline powder. It is stable in air. One g dissolves in 0.8 mL of water at 25°, in about 0.5 mL of boiling water, and in about 3 mL of alcohol. Its solutions are dextrorotatory.

Function: Acidifier; sequestrant; flavoring agent

Packaging and Storage: Store in well-closed containers.

IDENTIFICATION

Add the following:

- ■**Infrared Absorption,** *Spectrophotometric Identification Tests,* Appendix IIIC
  Reference standard: USP Tartaric Acid RS
  Sample and standard preparation: *K*
  Acceptance criteria: The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard.*■ 1S (*FCC7*)
- **Tartrate,** Appendix IIIA
  Acceptance criteria: Passes test

ASSAY

- **Procedure**
  Sample: 2 g, previously dried
  Analysis: Dissolve the *Sample* 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 $C_4H_6O_6$.

Acceptance criteria: 99.7%–100.5% of $C_4H_6O_6$, on the dried basis

IMPURITIES

Inorganic Impurities

- **Lead**, *Lead Limit Test, Flame Atomic Absorption Spectrophotometric Method,* Appendix IIIB
  Sample: 5.0 g
  Acceptance criteria: NMT 2 mg/kg
- **Sulfate**
  Sample solution: 10 mg/mL
  Analysis: Add 3 drops of hydrochloric acid and 1 mL of barium chloride TS to 10 mL of the *Sample solution*.
  Acceptance criteria: No turbidity forms.

Organic Impurities

- **Oxalate**
  Sample solution: 100 mg/mL
  Analysis: Nearly neutralize 10 mL of the *Sample solution* with 6 N ammonium hydroxide and add 10 mL of calcium sulfate TS.
  Acceptance criteria: No turbidity forms.

SPECIFIC TESTS

- **Loss on Drying**, Appendix IIC (Dry over phosphorus pentoxide for 3 h.)
  Acceptance criteria: NMT 0.5%
- **Optical (Specific) Rotation**, Appendix IIB
  Sample solution: 200 mg/mL
  Acceptance criteria: $[\alpha]_D^{25}$ Between +12.0° and +13.0°
- **Residue on Ignition (Sulfated Ash)**, Appendix IIC
  Sample: 4 g
  Acceptance criteria: NMT 0.05%

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

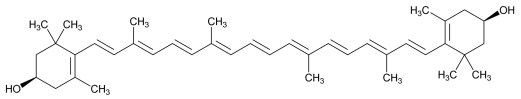
FCC Sixth Edition Page 948

BRIEFING

Zeaxanthin. Because there is no existing *FCC* monograph for this food ingredient, a new monograph is being proposed on the basis of data and comments received.
(FIEC: K. Bowman) C77847

Add the following:

■ **Zeaxanthin**



$C_{40}H_{56}O_2$

Formula wt 568.88
CAS: [144-68-3]

DESCRIPTION

Zeaxanthin occurs as a free-flowing, orange to pale-yellow powder. It is the purified fraction obtained from isomerization of lutein from *Tagetes erecta L.* It is sparingly soluble in chloroform and practically insoluble in water and ethanol.

Function: Source of zeaxanthin; color

Packaging and Storage: Store in tight, light-resistant containers in a cool place.

IDENTIFICATION

- **A. Ultraviolet Absorption**

  Acceptance criteria: The *Sample solution* from the test for *Total Carotenoids* shows an absorption maximum at about 453 nm.

- **B. Procedure**

  Acceptance criteria: The retention time of the major peak in the chromatogram of the *Sample solution* corresponds to that in the chromatogram of the *Standard solution* as obtained in the *Assay* for *Zeaxanthin* (below).

ASSAY

- **Zeaxanthin**

  [Note—Use low-actinic glassware.]

  Mobile phase: Hexane and ethyl acetate (75:25); filtered and degassed. Make adjustments if necessary.

  Standard solution: 150 µg/mL of USP Zeaxanthin RS prepared as follows: dissolve 15.0 mg of USP Zeaxanthin RS in 10 mL of chloroform, swirling briefly, and dilute with *Mobile phase* to 100 mL.

  Sample solution: Transfer 15.0 mg of sample to a 100-mL volumetric flask, add 10 mL of chloroform, and place the flask in an ultrasonic bath at 30° for 2–5 min to obtain a clear solution. Dilute with *Mobile phase* to volume.

  Chromatographic system, Appendix IIA

  Mode: High-performance liquid chromatography

  Detector: 453 nm

  Column: 4.6-mm × 25-cm column that contains 5- to 10-µm porous silica packing[1]

  Flow rate: 1.5 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

[Note—The approximate relative retention times are about 1.0 for zeaxanthin and 0.95 for lutein.]

Suitability requirement 1: The resolution between zeaxanthin and lutein is NLT 1.0.

Suitability requirement 2: The tailing factor is NMT 2.

Suitability requirement 3: The relative standard deviation for replicate injections is NMT 2.0%.

Analysis: Inject the *Sample solution* into the chromatograph, record the chromatogram, and measure the peak area responses. [Note—The peak area of zeaxanthin is NLT 90.0% of the total detected area of peaks in the chromatogram.]

Calculate the percentage of Zeaxanthin in the sample taken:

$$\text{Result} = T \times (r_U/r_T)$$

T = percentage of *Total Carotenoids* determined below

r_U = peak response of zeaxanthin

r_T = sum of the responses of all of the peaks

Acceptance criteria: NLT 74.0%

- **Total Carotenoids**

[Note—Use low-actinic glassware.]

Sample stock solution: Transfer 25.0 mg of sample to a 100-mL volumetric flask, add 20 mL of chloroform, and place the flask in an ultrasonic bath at 30° for 2–5 min to obtain a clear solution. Dilute with cyclohexane to volume to obtain a solution containing 250 µg/mL.

Sample solution: 2.5 µg/mL in cyclohexane; from the *Sample stock solution*

Blank: Cyclohexane

Analysis: Determine the absorbance of the *Sample solution* against that of the *Blank* at the wavelength of maximum absorbance at about 453 nm, with a suitable spectrophotometer.

Calculate the percentage of total carotenoids as Zeaxanthin ($C_{40}H_{56}O_2$):

$$\text{Result} = A/(C \times F)$$

A = absorbance of the *Sample solution*

C = concentration of the *Sample solution* (g/mL)

F = absorptivity of zeaxanthin in cyclohexane (2540 $mL \cdot g^{-1} \cdot cm^{-1}$)

Acceptance criteria: NLT 80.0%

IMPURITIES

Inorganic Impurities

- **Lead,** *Lead Limit Test, Flame Atomic Absorption Spectrophotometric Method,* Appendix IIIB

Sample: 10 g

Acceptance criteria: NMT 1 mg/kg

Organic Impurities

- **Lutein and Other Related Compounds**

[Note—Use low-actinic glassware.]

Mobile phase: Hexane and ethyl acetate (75:25); filtered and degassed. Make adjustments if necessary.

Standard solution: 150 µg/mL of USP Zeaxanthin RS prepared as follows: dissolve 15.0 mg of USP

Zeaxanthin RS in 10 mL of chloroform, swirling briefly, and dilute with *Mobile phase* to 100 mL.

Sample solution: Transfer 15.0 mg of sample to a 100-mL volumetric flask, add 10 mL of chloroform, and place the flask in an ultrasonic bath at 30° for 2–5 min to obtain a clear solution. Dilute with *Mobile phase* to volume.

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography

Detector: 453 nm

Column: 4.6-mm × 25-cm column that contains 5- to 10-µm porous silica packing[1]

Flow rate: 1.5 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

[Note—The approximate relative retention times are about 1.0 for zeaxanthin and 0.95 for lutein.]

Suitability requirement 1: The resolution between zeaxanthin and lutein is NLT 1.0.

Suitability requirement 2: The tailing factor is NMT 2.

Suitability requirement 3: The relative standard deviation for replicate injections is NMT 2.0%.

Analysis: Inject the *Sample solution* into the chromatograph, record the chromatogram, and measure the peak area responses. [Note—The peak area of lutein is NMT 9.0% of the total detected area of peaks in the chromatogram of the *Sample solution*.]

Calculate the percentage of lutein in the portion of the sample taken:

$$\text{Result} = T \times (r_U/r_T)$$

T = percentage of *Total Carotenoids* determined above

r_U = peak response of lutein

r_T = sum of the responses of all of the peaks

Calculate the percentage of other related compounds in the sample taken:

$$\text{Result} = 100 \times (r_O/r_T)$$

r_O = individual peak response of any other peak in the chromatogram, excluding zeaxanthin and lutein

r_T = sum of the responses of all of the peaks

Acceptance criteria

Lutein: NMT 8.5%

Other related compounds: NMT 1.0% of any other single related compound

SPECIFIC TESTS

- **Residue on Ignition (Sulfated Ash),** Appendix IIC

Analysis: Proceed as directed, but igniting at 600° ± 50°.

Acceptance criteria: NMT 1.0%

- **Water,** *Water Determination,* Appendix IIB

Acceptance criteria: NMT 1.0%

■1S (*FCC7*)

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[1] Agilent Zorbax Rx-SIL, or equivalent.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Bowman Scientific Liaison 1-301-816-8345	(FI07) Food Ingredients Expert Committee

BRIEFING

Appendix III: Chemical Tests and Determinations, page 1487 of the *Second Supplement* to *FCC 6* and *FCC Forum* (June 2009].

1. On the basis of comments received, a revision to *Fluoride Limit Test, Method III* to allow the use of a graphing calculator or spreadsheet for creation of the *Calibration Curve* is proposed. Addition of the formula for calculating the fluoride content of the sample in percentage is also proposed.
2. On the basis of comments received, a revision to *Fluoride Limit Test, Method IV* to remove the target ranges from the *Electrode Calibration* section is proposed. The existing ranges may not be appropriate for all fluoride electrodes and may conflict with directions from manufacturers, therefore, it is proposed that analysts use manufacturers' instructions to evaluate the calibration of the electrode.

(FIEC: K. Bowman) C81956; C81964

APPENDIX III: CHEMICAL TESTS AND DETERMINATIONS

A. IDENTIFICATION TESTS

The identification tests described in *Section A* of this Appendix are frequently referred to in the *Food Chemicals Codex* for the presumptive identification of FCC-grade chemicals taken from labeled containers. These tests are not intended to be applied to mixtures unless so specified.

Acetate

Acetic acid or acetates, when warmed with sulfuric acid and alcohol, form ethyl acetate, recognizable by its characteristic odor. With neutral solutions of acetates, ferric chloride TS produces a deep red color that is destroyed by the addition of a mineral acid.

Aluminum

Solutions of aluminum salts yield with 6 *N* ammonia a white, gelatinous precipitate that is insoluble in an excess of the 6 *N* ammonia. The same precipitate is produced by 1 *N* sodium hydroxide, but it dissolves in an excess of this reagent.

Ammonium

Ammonium salts are decomposed by 1 *N* sodium hydroxide with the evolution of ammonia, recognizable by its alkaline effect on moistened red litmus paper. The decomposition is accelerated by warming.

Benzoate

Neutral solutions of benzoates yield a salmon colored precipitate with ferric chloride TS. From moderately

concentrated solutions of benzoate, 2 *N* sulfuric acid precipitates free benzoic acid, which is readily soluble in ether.

Bicarbonate

See Carbonate.

Bisulfite

See Sulfite.

Bromide

Free bromine is liberated from solutions of bromides upon the dropwise addition of chlorine TS. When shaken with chloroform, the bromine dissolves, coloring the chloroform red to red-brown. A yellow-white precipitate, which is insoluble in nitric acid and slightly soluble in 6 *N* ammonia, is produced when solutions of bromides are treated with silver nitrate TS.

Calcium

Insoluble oxalate salts are formed when solutions of calcium salts are treated in the following manner: Using 2 drops of methyl red TS as the indicator, neutralize a 1:20 solution of a calcium salt with 6 *N* ammonia, then add 2.7 *N* hydrochloric acid, dropwise, until the solution is acid. A white precipitate of calcium oxalate forms upon the addition of ammonium oxalate TS. This precipitate is insoluble in acetic acid but dissolves in hydrochloric acid.

Calcium salts moistened with hydrochloric acid impart a transient yellow-red color to a nonluminous flame.

Carbonate

Carbonates and bicarbonates effervesce with acids, yielding a colorless gas that produces a white precipitate immediately when passed into calcium hydroxide TS. Cold solutions of soluble carbonates are colored red by phenolphthalein TS, whereas solutions of bicarbonates remain unchanged or are slightly changed.

Chloride

Solutions of chlorides yield with silver nitrate TS a white, curdy precipitate that is insoluble in nitric acid but soluble in a slight excess of 6 *N* ammonia.

Citrate

To 15 mL of pyridine add a few milligrams of a citrate salt, dissolved or suspended in 1 mL of water, and shake. Add 5 mL of acetic anhydride to this mixture, and shake. A light red color appears.

Cobalt

Solutions of cobalt salts (1:20) in 2.7 *N* hydrochloric acid yield a red precipitate when heated on a steam bath with an equal volume of a hot, freshly prepared 1:10 solution of 1-nitroso-2-naphthol in 9 *N* acetic acid. Solutions of cobalt salts yield a yellow precipitate when saturated with potassium chloride and treated with potassium nitrite and acetic acid.

Copper

When solutions of cupric compounds are acidified with hydrochloric acid, a red film of metallic copper is deposited on a bright untarnished surface of metallic iron. An excess of 6 *N* ammonia, added to a solution of a cupric salt, produces first a blue precipitate and then a deep blue colored solution. Solutions of cupric salts yield with potassium ferrocyanide TS a red-brown precipitate, insoluble in diluted acids.

Hypophosphite

Hypophosphites evolve spontaneously flammable phosphine when strongly heated. Solutions of hypophosphites yield a white precipitate with mercuric chloride TS. This precipitate becomes gray when an excess of hypophosphite is present. Hypophosphite solutions, acidified with sulfuric acid and warmed with copper sulfate TS, yield a red precipitate.

Iodide

Solutions of iodides, upon the dropwise addition of chlorine TS, liberate iodine, which colors the solution yellow to red. Chloroform is colored violet when shaken with this solution. The iodine thus liberated gives a blue color with starch TS. In solutions of iodides, silver nitrate TS produces a yellow, curdy precipitate that is insoluble in nitric acid and in 6 *N* ammonia.

Iron

Solutions of ferrous and ferric compounds yield a black precipitate with ammonium sulfide TS. This precipitate is dissolved by cold 2.7 *N* hydrochloric acid with the evolution of hydrogen sulfide.

Ferric Salts: Potassium ferrocyanide TS (10%) produces a dark blue precipitate in acid solutions of ferric salts. With an excess of 1 *N* sodium hydroxide, a red-brown precipitate is formed. Solutions of ferric salts produce with ammonium thiocyanate TS (1.0 *N*) a deep red color that is not destroyed by diluted mineral acids.

Ferrous Salts: Potassium ferricyanide TS (10%) produces a dark blue precipitate in solutions of ferrous salts. This precipitate, which is insoluble in dilute hydrochloric acid, is decomposed by 1 *N* sodium hydroxide. Solutions of ferrous salts yield with 1 *N* sodium hydroxide a green-white precipitate, the color rapidly changing to green and then to brown when shaken.

Lactate

When solutions of lactates are acidified with sulfuric acid, potassium permanganate TS (0.1 *N*) is added, and the mixture is heated, acetaldehyde is evolved. This can be detected by allowing the vapor to come into contact with a filter paper that has been moistened with a freshly prepared mixture of equal volumes of 20% aqueous morpholine and sodium nitroferricyanide TS. A blue color is produced.

Magnesium

Solutions of magnesium salts in the presence of ammonium chloride yield no precipitate with ammonium carbonate TS, but a white crystalline precipitate, which is insoluble in 6 *N* ammonium hydroxide, is formed on the subsequent addition of sodium phosphate TS (6%).

Manganese

Solutions of manganous salts yield with ammonium sulfide TS a salmon colored precipitate that dissolves in acetic acid.

Nitrate

When a solution of a nitrate is mixed with an equal volume of sulfuric acid, the mixture cooled, and a solution of ferrous sulfate superimposed, a brown color is produced at the junction of the two liquids. Brown-red fumes are evolved when a nitrate is heated with sulfuric acid and metallic copper. Nitrates do not decolorize acidified potassium permanganate TS (0.1 *N*) (distinction from nitrites).

Nitrite

Nitrites yield brown-red fumes when treated with diluted mineral acids or acetic acid. A few drops of potassium iodide TS (15%) and a few drops of 2 *N* sulfuric acid added to a solution of nitrite liberate iodine, which colors starch TS blue.

Peroxide

Solutions of peroxides slightly acidified with sulfuric acid yield a deep blue color on the addition of potassium dichromate TS. On shaking the mixture with an equal volume of diethyl ether and allowing the liquids to separate, the blue color is transferred to the ether layer.

Phosphate

Neutral solutions of orthophosphates yield with silver nitrate TS (0.1 *N*) a yellow precipitate, which is soluble in 1.7 *N* nitric acid or in 6 *N* ammonium hydroxide. With ammonium molybdate TS, a yellow precipitate, which is soluble in 6 *N* ammonium hydroxide, is formed.

Potassium

Potassium compounds impart a violet color to a nonluminous flame if not masked by the presence of small quantities of sodium. In neutral, concentrated or moderately concentrated solutions of potassium salts, sodium bitartrate TS (10%) slowly produces a white, crystalline precipitate that is soluble in 6 *N* ammonium hydroxide and in solutions of alkali hydroxides or carbonates. The precipitation may be accelerated by stirring or rubbing the inside of the test tube with a glass rod or by the addition of a small amount of glacial acetic acid or alcohol.

Sodium

Dissolve 0.1 g of the sodium compound in 2 mL of water. Add 2 mL of 15% potassium carbonate, and heat to boiling. No precipitate is formed. Add 4 mL of potassium pyroantimonate TS, and heat to boiling. Allow to cool in ice water, and if necessary, rub the inside of the test tube with a glass rod. A dense precipitate is formed. Sodium compounds impart an intense yellow color to a nonluminous flame.

Change to read:

Sulfate

Solutions of sulfates yield with barium chloride TS ~~(10%)~~▲ ▲*FCC7* a white precipitate that is insoluble in

hydrochloric and nitric acids. Sulfates yield with lead acetate TS ~~(8%)~~ ▲*FCC7* a white precipitate that is soluble in ammonium acetate solution. Hydrochloric acid produces no precipitate when added to solutions of sulfates (distinction from thiosulfates).

Sulfite

When treated with 2.7 *N* hydrochloric acid, sulfites and bisulfites yield sulfur dioxide, recognizable by its characteristic odor. This gas blackens filter paper moistened with mercurous nitrate TS.

Tartrate

When a few milligrams of a tartrate are added to a mixture of 15 mL of pyridine and 5 mL of acetic anhydride, an emerald green color is produced.

Thiosulfate

With hydrochloric acid, solutions of thiosulfates yield a white precipitate that soon turns yellow, liberating sulfur dioxide, recognizable by its odor. The addition of ferric chloride TS to solutions of thiosulfates produces a dark violet color that quickly disappears.

Zinc

Zinc salts, in the presence of sodium acetate, yield a white precipitate with hydrogen sulfide. This precipitate, which is insoluble in acetic acid, is dissolved by 2.7 *N* hydrochloric acid. A similar precipitate is produced by ammonium sulfide TS in neutral or alkaline solutions. Solutions of zinc salts yield with potassium ferrocyanide TS (10%) a white precipitate that is insoluble in 2.7 *N* hydrochloric acid.

B. LIMIT TESTS

Add the following:

▪ALUMINUM LIMIT TEST

[Note—The *Standard Solutions* and *Sample Solution* may be modified, if necessary, to obtain solutions of suitable concentrations adaptable to the linear or working range of the instrument.]

Nitric Acid Diluent: Dilute 40 mL of nitric acid with water to 1000 mL.

Standard Aluminum Solutions: Treat a quantity of aluminum wire with 6 N hydrochloric acid at 80° for a few minutes. Dissolve 100 mg of the treated wire in a mixture consisting of 10 mL of hydrochloric acid and 2 mL of nitric acid, by heating at 80° for about 30 min. Continue heating until the volume is reduced to about 4 mL. Cool to room temperature, and add 4 mL of water. Evaporate to about 2 mL by heating. Cool, and transfer this solution, with the aid of water, to a 100-mL volumetric flask, and dilute with water to volume (1 mg/mL aluminum). Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, and dilute with water to volume (100 µg/mL aluminum). Transfer 1.0 mL of this solution to a third 100-mL volumetric flask, and dilute with water to volume (1 µg/mL aluminum). [Note—If more diluted *Standard Aluminum Solutions* are required,

transfer 1.0-, 2.0-, and 4.0-mL portions of the 1 µg/mL *Standard Aluminum Solution* to separate 100-mL volumetric flasks, dilute with *Nitric Acid Diluent* to volume, and mix. These solutions contain 0.01, 0.02, and 0.04 µg/mL of aluminum, respectively.]

Sample Solution: Transfer the amount of sample specified in the monograph to a plastic 100-mL volumetric flask. Add 50 mL of water, and sonicate for 30 min. Add 4 mL of nitric acid, and dilute with water to volume.

Procedure: Determine the absorbances of the *Standard Aluminum Solutions* and the *Sample Solution* at the aluminum emission line at 309.3 nm with a suitable atomic absorption spectrophotometer equipped with an aluminum hollow-cathode lamp and a flameless electrically heated furnace, using the *Nitric Acid Diluent* as the blank. Plot the absorbances of the *Standard Solutions* versus the concentration of aluminum, in µg/mL, drawing a straight line best fitting the three points. From the graph so obtained, determine the concentration, in µg/mL, of aluminum in the *Sample Solution*.

Calculate the amount of aluminum in the sample taken, in µg/g, using the following formula

$$\text{Result} = C_A/C_S$$

in which C_A is the concentration of aluminum in the *Sample Solution*, in µg/mL, obtained from the standard curve; and C_S is the concentration of the *Sample Solution*, in g/mL.

■2S (*FCC6*)

ARSENIC LIMIT TEST

Silver Diethyldithiocarbamate Colorimetric Method

[Note—All reagents used in this test should be very low in arsenic content.]

Apparatus: Use the general apparatus shown in *Fig. 11* unless otherwise specified in an individual monograph. It consists of a 125-mL arsine generator flask (*a*) fitted with a scrubber unit (*c*) and an absorber tube (*e*), with a 24/40 standard-taper joint (*b*) and a ball-and-socket joint (*d*), secured with a No. 12 clamp, connecting the units. The tubing between *d* and *e* and between *d* and *c* is a capillary having an id of 2 mm and an od of 8 mm. Alternatively, an apparatus embodying the principle of the general assembly described and illustrated may be used.

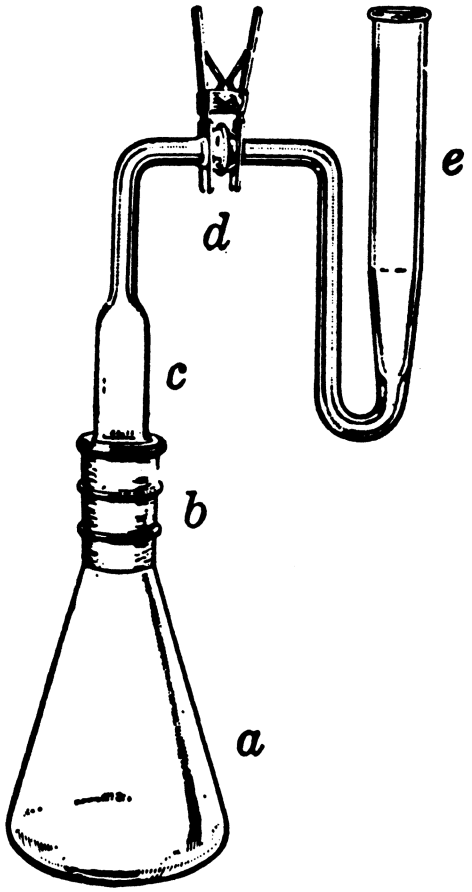

FIGURE 11 General Apparatus for Arsenic Limit Test. (Courtesy of the Fisher Scientific Co., Pittsburgh, PA.)

[Note— The special assemblies shown in *Figs. 12*, *13*, and *14* are to be used only when specified in certain monographs.]

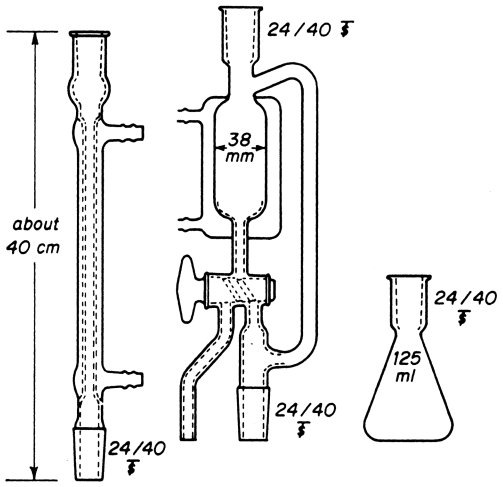

FIGURE 12 Modified Bethge Apparatus for the Distillation of Arsenic Tribromide.

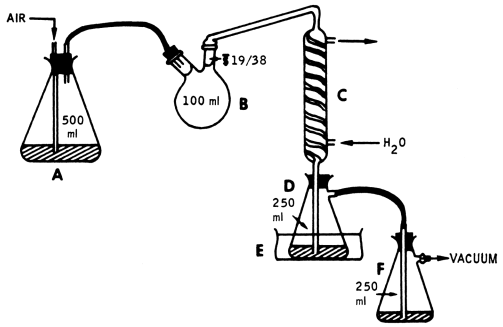

FIGURE 13 Special Apparatus for the Distillation of Arsenic Trichloride. (Flask *A* contains 150 mL of hydrochloric acid; flasks *D* and *F* contain 20 mL of water. Flask *D* is placed in an ice water bath, *E*.)

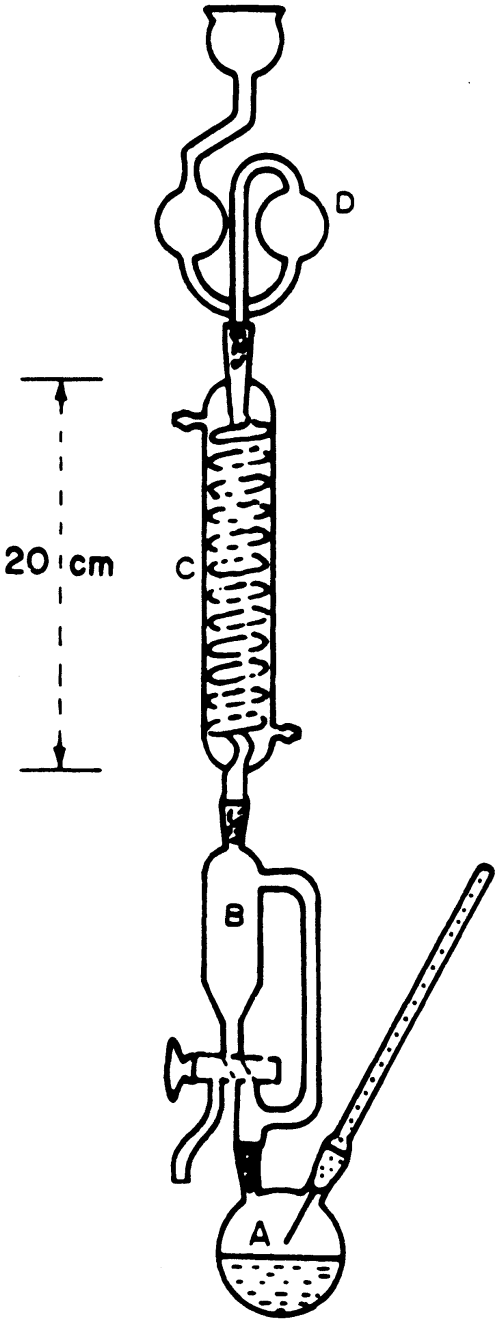

FIGURE 14 Special Apparatus for the Determination of Inorganic Arsenic. (*A*, 250-mL distillation flask; *B*,

receiver chamber, approximately 50-mL capacity; *C*, reflux condenser; *D*, splash head.)

Standard Arsenic Solution: Accurately weigh 132.0 mg of arsenic trioxide that has been previously dried at 105° for 1 h, and dissolve it in 5 mL of a 1:5 sodium hydroxide solution. Neutralize the solution with 2 *N* sulfuric acid, add 10 mL in excess, and dilute with recently boiled water to 1000.0 mL. Transfer 10.0 mL of this solution into a 1000-mL volumetric flask, add 10 mL of 2 *N* sulfuric acid, dilute with recently boiled water to volume, and mix. Use this final solution, which contains 1 µg of arsenic in each milliliter, within 3 days.

Silver Diethyldithiocarbamate Solution: Dissolve 1 g of ACS reagent-grade silver diethyldithiocarbamate in 200 mL of recently distilled pyridine. Store this solution in a light-resistant container and use within 1 month.

Stannous Chloride Solution: Dissolve 40 g of stannous chloride dihydrate ($SnCl_2 \cdot 2H_2O$) in 100 mL of hydrochloric acid. Store the solution in glass containers and use within 3 months.

Lead Acetate-Impregnated Cotton: Soak cotton in a saturated solution of lead acetate trihydrate, squeeze out the excess solution, and dry in a vacuum at room temperature.

Sample Solution: Use directly as the *Sample Solution* in the *Procedure* the solution obtained by treating the sample as directed in an individual monograph. Prepare sample solutions of organic compounds in the generator flask (*a*), unless otherwise directed, according to the following general procedure:

[**CAUTION—** Some substances may react unexpectedly with explosive violence when digested with hydrogen peroxide. Use appropriate safety precautions at all times.]

[Note—If halogen-containing compounds are present, use a lower temperature while heating the sample with sulfuric acid; do not boil the mixture; and add the peroxide, with caution, before charring begins to prevent loss of trivalent arsenic.]

Transfer 1.0 g of sample into the generator flask, add 5 mL of sulfuric acid and a few glass beads, and digest at a temperature not exceeding 120° until charring begins, preferably using a hot plate in a fume hood. (Additional sulfuric acid may be necessary to completely wet some samples, but the total volume added should not exceed about 10 mL.) After the acid has initially decomposed the sample, cautiously add, dropwise, hydrogen peroxide (30%), allowing the reaction to subside and reheating the sample between drops. Add the first few drops very slowly with sufficient mixing to prevent a rapid reaction, and discontinue heating if foaming becomes excessive. Swirl the solution in the flask to prevent unreacted substance from caking on the walls or bottom of the flask during digestion.

[Note—Maintain oxidizing conditions at all times during the digestion by adding small quantities of the peroxide whenever the mixture turns brown or darkens.]

Continue the digestion until the organic matter is destroyed, gradually raising the temperature of the hot plate to 250° to 300° until fumes of sulfur trioxide are copiously evolved and the solution becomes colorless or retains only a light straw color. Cool, cautiously add 10 mL of water, heat again to strong fuming, and cool. Cautiously add 10 mL of water, mix, wash the sides of the flask with a few milliliters of water, and dilute to 35 mL.

Procedure: If the *Sample Solution* was not prepared in the generator flask, transfer to the flask a volume of the solution, prepared as directed, equivalent to 1.0 g of the substance being tested, and add water to make 35 mL. Add 20 mL of 1:5 sulfuric acid, 2 mL of potassium iodide TS, 0.5 mL of *Stannous Chloride Solution*, and 1 mL of isopropyl alcohol, and mix. Allow the mixture to stand for 30 min at room temperature. Pack the scrubber unit (*c*) with two plugs of *Lead Acetate-Impregnated Cotton*, leaving a small air space between the two plugs, lubricate joints *b* and *d* with stopcock grease, if necessary, and connect the scrubber unit with the absorber tube (*e*). Transfer 3.0 mL of *Silver Diethyldithiocarbamate Solution* to the absorber tube, add 3.0 g of granular zinc (20-mesh) to the mixture in the flask, and immediately insert the standard-taper joint (*b*) into the

flask. Allow the evolution of hydrogen and color development to proceed at room temperature (25° ± 3°) for 45 min, swirling the flask gently at 10-min intervals. Disconnect the absorber tube from the generator and scrubber units, and transfer the *Silver Diethyldithiocarbamate Solution* to a 1-cm absorption cell. Determine the absorbance at the wavelength of maximum absorption between 535 nm and 540 nm, with a suitable spectrophotometer or colorimeter, using *Silver Diethyldithiocarbamate Solution* as the blank. The absorbance due to any red color from the solution of the sample does not exceed that produced by 3.0 mL of *Standard Arsenic Solution* (3 µg As) when treated in the same manner and under the same conditions as the sample.

The room temperature during the generation of arsine from the standard should be held to within ±2° of that observed during the determination of the sample.

Interferences: Metals or salts of metals such as chromium, cobalt, copper, mercury, molybdenum, nickel, palladium, and silver may interfere with the evolution of arsine. Antimony, which forms stibine, is the only metal likely to produce a positive interference in the color development with the silver diethyldithiocarbamate. Stibine forms a red color with silver diethyldithiocarbamate that has a maximum absorbance at 510 nm, but at 535 to 540 nm, the absorbance of the antimony complex is so diminished that the results of the determination would not be altered significantly.

CADMIUM LIMIT TEST

Spectrophotometer: Use any suitable atomic absorption spectrophotometer equipped with a Boling-type burner, an air–acetylene flame, and a hollow-cathode cadmium lamp. The instrument should be capable of operating within the sensitivity necessary for the determination.

Standard Solution: Transfer 100 mg of cadmium chloride crystals ($CdCl_2 \cdot 2½H_2O$), accurately weighed, into a 1000-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Pipet 25 mL of this solution into a 100-mL volumetric flask, add 1 mL of hydrochloric acid, dilute with water to volume, and mix. Each milliliter contains 12.5 µg of cadmium.

Sample Solution: Transfer 10 g of sample, accurately weighed, into a 50-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Test Solutions: Transfer 5.0 mL of *Sample Solution* into each of five separate 25-mL volumetric flasks. Dilute the contents of *Flask 1* with water to volume, and mix. Add 1.00, 2.00, 3.00, and 4.00 mL of *Standard Solution*, to *Flasks 2*, *3*, *4*, and *5*, respectively, then dilute each flask with water to volume, and mix. The *Test Solutions* contain, respectively, 0, 0.5, 1.0, 1.5, and 2.0 µg/mL of cadmium.

Procedure: Determine the absorbance of each *Test Solution* at 228.8 nm, setting the instrument to previously established optimum conditions, using water as a blank. Plot the absorbance of the *Test Solutions* versus their contents of cadmium, in micrograms per milliliter. Draw the straight line best fitting the five points, and extrapolate the line until it intercepts the concentration axis. From the intercept, determine the amount, in micrograms, of cadmium in each milliliter of the *Test Solution* containing 0 mL of the *Standard Preparation.* Calculate the quantity, in milligrams per kilogram, of cadmium in the sample by multiplying this value by 25.

CHLORIDE AND SULFATE LIMIT TESTS

Where limits for chloride and sulfate are specified in the individual monograph, compare the *Sample Solution* and control in appropriate glass cylinders of the same dimensions and matched as closely as practicable with

respect to their optical characteristics.

If the solution is not perfectly clear after acidification, pass it through filter paper that has been washed free of chloride and sulfate. Add identical quantities of the precipitant (silver nitrate TS or barium chloride TS) in rapid succession to both the *Sample Solution* and the control solution.

Experience has shown that visual turbidimetric comparisons are best made between solutions containing from 10 to 20 µg of chloride (Cl) ion or from 200 to 400 µg of sulfate (SO_4) ion in 50 mL. Weights of samples are specified on this basis in the individual monographs in which these limits are included.

Chloride Limit Test

Standard Chloride Solution: Dissolve 165 mg of sodium chloride in water and dilute to 100.0 mL. Transfer 10.0 mL of this solution into a 1000-mL volumetric flask, dilute with water to volume, and mix. Each milliliter of the final solution contains 10 µg of chloride (Cl) ion.

Procedure: Unless otherwise directed, dissolve the specified amount of the test substance in 30 to 40 mL of water; neutralize to litmus external indicator with nitric acid, if necessary; and add 1 mL in excess. Add 1 mL of silver nitrate TS to the clear solution or filtrate, dilute with water to 50 mL, mix, and allow to stand for 5 min protected from direct sunlight. Compare the turbidity, if any, with that produced similarly in a control solution containing the required volume of *Standard Chloride Solution* and the quantities of the reagents used for the sample.

Sulfate Limit Test

Standard Sulfate Solution: Dissolve 148 mg of anhydrous sodium sulfate in water, and dilute to 100.0 mL. Transfer 10.0 mL of this solution to a 1000-mL volumetric flask, dilute with water to volume, and mix. Each milliliter of the final solution contains 10 µg of sulfate (SO_4).

Procedure: Unless otherwise directed, dissolve the specified amount of the test substance in 30 to 40 mL of water; neutralize to litmus external indicator with hydrochloric acid, if necessary; then add 1 mL of 2.7 *N* hydrochloric acid. Add 3 mL of barium chloride TS to the clear solution or filtrate, dilute with water to 50 mL, and mix. After 10 min compare the turbidity, if any, with that produced in a solution containing the required volume of *Standard Sulfate Solution* and the quantities of the reagents used for the sample.

1,4-DIOXANE LIMIT TEST

Vacuum Distillation Apparatus: Assemble a closed-system vacuum distillation apparatus employing glass vacuum stopcocks (*A*, *B*, and *C*), as shown in *Fig. 15.*

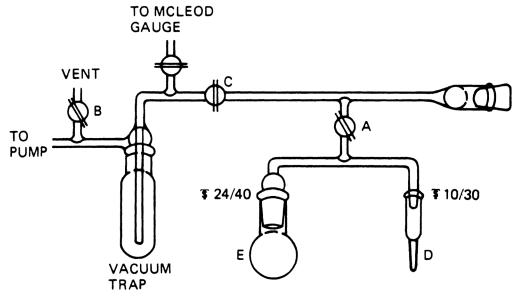

FIGURE 15 Closed-System Vacuum Distillation Apparatus for 1,4-Dioxane.

The concentrator tube (*D*) is made of borosilicate or quartz (not flint) glass, graduated precisely enough to measure the 0.9 mL or more of distillate and marked so that the analyst can accurately dilute to 2.0 mL (available as Chromaflex concentrator tube, Kontes Glass Co., Vineland, NJ, Catalog No. K42560-0000).

Standard Preparation: Prepare a solution of 1,4-dioxane in water containing 100 µg/mL. Keep the solution refrigerated, and prepare fresh weekly.

Sample Preparation: Transfer 20 g of the sample, accurately weighed, into a 50-mL round-bottom flask (*E*) having a 24/40 ground-glass neck. Semisolid or waxy samples should be liquefied by heating on a steam bath before making the transfer. Add 2.0 mL of water to the flask for crystalline samples, and 1.0 mL for liquid, semisolid, or waxy samples. Place a small Teflon-covered stirring bar in the flask, stopper, and stir to mix. Immerse the flask in an ice bath, and chill for about 1 min.

Wrap heating tape around the tube connecting the Chromaflex tube (*D*) and the round-bottom flask (*E*), and apply about 10 V to the tape. Apply a light coating of high-vacuum silicone grease to the ground-glass joints, and connect the Chromaflex tube to the 10/30 joint and the round-bottom flask to the 24/40 joint. Immerse the vacuum trap in a Dewar flask filled with liquid nitrogen, close stopcocks *A* and *B*, open stopcock *C*, and begin evacuating the system with a vacuum pump. Prepare a slush bath from powdered dry ice and methanol, and raise the bath to the neck of the round-bottom flask. After freezing the contents of the flask for about 10 min, and when the vacuum system is operating at 0.05 mm pressure or lower, open stopcock *A* for 20 s, and then close it. Remove the slush bath, and allow the flask to warm in air for about 1 min. Immerse the flask in a water bath at 20° to 25°, and after about 5 min warm the water in the bath to 35° to 40° (sufficient to liquefy most samples) while stirring slowly but constantly with the magnetic bar. Cool the water in the bath by adding ice, and chill for about 2 min. Replace the water bath with the slush bath, freeze the contents of the flask for about 10 min, then open stopcock *A* for 20 s, and close it. Remove the slush bath, and repeat the heating steps as before, this time reaching a final temperature of 45° to 50° or a temperature necessary to melt the sample completely. If there is any condensation in the tube connecting the round-bottom flask to the Chromaflex tube, slowly increase the voltage to the heating tape and heat until condensation disappears.

Stir with the magnetic stirrer throughout the following steps: Very slowly immerse the Chromaflex tube in the Dewar flask containing liquid nitrogen.

[**CAUTION—** When there is liquid distillate in the Chromaflex tube, the tube must be immersed in the nitrogen very slowly, or the tube will break.]

Water will begin to distill into the tube. As ice forms in the tube, raise the Dewar flask to keep the liquid nitrogen level only slightly below the level of ice in the tube. When water begins to freeze in the neck of the 10/30 joint, or when liquid nitrogen reaches the 2.0-mL graduation mark on the Chromaflex tube, remove the Dewar flask and let the ice melt without heating. After the ice has melted, check the volume of water that has distilled, and repeat the sequence of chilling and thawing until at least 0.9 mL of water has been collected. Freeze the tube once again for about 2 min, and release the vacuum first by opening stopcock *B*, followed by stopcock *A*. Remove the Chromaflex tube from the apparatus, close it with a greased stopper, and let the ice melt without heating. Mix the contents of the tube by swirling, note the volume of distillate, and dilute with water to 2.0 mL, if necessary. Use this *Sample Preparation* as directed under *Chromatography* (below).

Chromatography: (*See Chromatography, Appendix IIA*.) Use a gas chromatograph equipped with a flame-ionization detector. Under typical conditions, the instrument contains a 4-mm (id) × 6-ft glass column, or equivalent, packed with 80-/100- or 100-/120-mesh Chromosorb 104, or equivalent. The column is maintained isothermally at about 140°, the injection port at 200°, and the detector at 250°. Nitrogen is the carrier gas,

flowing at a rate of about 35 mL/min. Install an oxygen scrubber between the carrier gas line and the column. The column should be conditioned for about 72 h at 250° with 30 to 40 mL/min carrier flow.

[Note—Chromosorb 104 is oxygen sensitive. Both new and used columns should be flushed with carrier gas for 30 to 60 min before heating each time they are installed in the gas chromatograph.]

Inject a volume of the *Standard Preparation*, accurately measured, to give about 20% of maximum recorder response. Where possible, keep the injection volume in the range of 2 to 4 µL, and use the solvent-flush technique to minimize errors associated with injection volumes. In the same manner, inject an identical volume of the *Sample Preparation*. The height of the peak produced by the *Sample Preparation* does not exceed that produced by the *Standard Preparation*.[1]

FLUORIDE LIMIT TEST

Method I (Thorium Nitrate Colorimetric Method)

Use this method unless otherwise directed in the individual monograph.

[**CAUTION—** When applying this test to organic compounds, rigidly control at all times the temperature at which the distillation is conducted to the recommended range of 135° to 140° to avoid the possibility of explosion.]

[Note—To minimize the distillation blank resulting from fluoride leached from the glassware, treat the distillation apparatus as follows: Treat the glassware with hot 10% sodium hydroxide solution, followed by flushing with tap water and rinsing with distilled water. At least once daily, treat in addition by boiling down 15 to 20 mL of 1:2 sulfuric acid until the still is filled with fumes; cool, pour off the acid, treat again with 10% sodium hydroxide solution, and rinse thoroughly. For further details, see AOAC method 944.08.]

Unless otherwise directed, place a 5.0-g sample and 30 mL of water in a 125-mL Pyrex distillation flask having a side arm and trap. The flask is connected with a condenser and carries a thermometer and a capillary tube, both of which must extend into the liquid. Slowly add, with continuous stirring, 10 mL of 70% perchloric acid, and then add 2 or 3 drops of a 1:2 solution of silver nitrate and a few glass beads. Connect a small dropping funnel or a steam generator to the capillary tube. Support the flask on a flame-resistant mat or shielding board, with a hole that exposes about one-third of the flask to the low, “clean” flame of a Bunsen burner.

[Note—The shielding is essential to prevent the walls of the flask from overheating above the level of its liquid contents.]

Distill until the temperature reaches 135°. Add water from the funnel or introduce steam through the capillary, maintaining the temperature between 135° and 140° at all times. Continue the distillation until 100 mL of distillate has been collected. After the 100-mL portion (*Distillate A*) is collected, collect an additional 50-mL portion of distillate (*Distillate B*) to ensure that all of the fluorine has been volatilized.

Place 50 mL of *Distillate A* in a 50-mL Nessler tube. In another, similar Nessler tube, place 50 mL of water distilled through the apparatus as a control. Add to each tube 0.1 mL of a filtered 1:1000 solution of sodium alizarinsulfonate and 1 mL of a freshly prepared 1:4000 solution of hydroxylamine hydrochloride, and mix well. Add, dropwise and with stirring, either 1 *N* or 0.05 *N* sodium hydroxide, depending on the expected volume of volatile acid distilling over, to the tube containing the distillate until its color just matches that of the control, which is faintly pink. Then add to each tube 1.0 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 of a 1:4000 solution of thorium nitrate so that, after mixing, the color of the liquid just changes to a faint pink. Note the volume of the solution added, then add exactly the same volume to the control, and mix. Now add to the control solution sodium fluoride TS (10 µg F per milliliter) from a buret to make the colors of the two tubes match after dilution to the same volume. Mix well, and allow all air bubbles to escape before making the final color comparison. Check the endpoint by adding 1 or 2 drops of sodium fluoride TS to the control. A distinct change in color should take place. Note the volume of sodium fluoride TS added.

Dilute *Distillate B* to 100 mL, and mix well. Place 50 mL of this solution in a 50-mL Nessler tube, and follow the procedure used for *Distillate A*. The total volume of sodium fluoride TS required for the solutions from both *Distillate A* and *Distillate B* should not exceed 2.5 mL.

Method II (Ion-Selective Electrode Method A)

Buffer Solution: Dissolve 36 g of cyclohexylenedinitrilotetraacetic acid (CDTA) in sufficient 1 *N* sodium hydroxide to make 200 mL. Transfer 20 mL of this solution (equivalent to 4 g of disodium CDTA) into a 1000-mL beaker containing 500 mL of water, 57 mL of glacial acetic acid, and 58 g of sodium chloride, and stir to dissolve. Adjust the pH of the solution to between 5.0 and 5.5 by the addition of 5 *N* sodium hydroxide, then cool to room temperature, dilute with water to 1000 mL, and mix.

Procedure: Unless otherwise directed in the individual monograph, transfer 8.0 g of sample and 20 mL of water into a 250-mL distilling flask, cautiously add 20 mL of perchloric acid, and then add 2 or 3 drops of a 1:2 solution of silver nitrate and a few glass beads.
[**CAUTION—** Handle perchloric acid in an appropriate fume hood.]

Following the directions, and observing the *Caution* and *Notes*, as given under *Method I*, distill the solution until 200 mL of distillate has been collected.

Transfer a 25.0-mL aliquot of the distillate into a 250-mL plastic beaker, and dilute with the *Buffer Solution* to 100 mL. Place the fluoride ion and reference electrodes (or a combination fluoride electrode) of a suitable ion-selective electrode apparatus in the solution. Adjust the calibration control until the indicator needle points to the center on the logarithmic concentration scale, allowing sufficient time for equilibration (about 20 min), and stirring constantly during the equilibration period and throughout the remainder of the procedure. Pipet 1.0 mL of a solution containing 100 µg of fluoride (F) ion per milliliter (prepared by dissolving 22.2 mg of sodium fluoride, previously dried at 200° for 4 h, in sufficient water to make 100.0 mL) into the beaker, allow the electrode to come to equilibrium, and record the final reading on the logarithmic concentration scale.

[Note—Follow the instrument manufacturer's instructions regarding precautions and interferences, electrode filling and check, temperature compensation, and calibration.]

Calculations: Calculate the fluoride content, in milligrams per kilogram, of the sample taken

$$\text{Result} = [IA/(R - I)] \times 100 \times (200/25W)$$

in which I is the initial scale reading before the addition of the sodium fluoride solution; A is the concentration, in micrograms per milliliter, of fluoride in the sodium fluoride solution added to the sample solution; R is the final scale reading after addition of the sodium fluoride solution; and W is the original weight, in grams, of the sample.

Change to read:

Method III (Ion-Selective Electrode Method B)

Sodium Fluoride Solution (5 µg F per milliliter)**:** Transfer 2.210 g of sodium fluoride, previously dried at 200° for 4 h and accurately weighed, into a 400-mL plastic beaker, add 200 mL of water, and stir until dissolved. Quantitatively transfer this solution into a 1000-mL volumetric flask with the aid of water, dilute with water to volume, and mix. Store this stock solution in a plastic bottle. On the day of use, transfer 5.0 mL of the stock solution into a 1000-mL volumetric flask, dilute with water to volume, and mix.

Calibration Curve: Transfer 1.0, 2.0, 3.0, 5.0, 10.0, and 15.0 mL of the *Sodium Fluoride Solution* into separate 250-mL plastic beakers; add 50 mL of water, 5 mL of 1 *N* hydrochloric acid, 10 mL of 1 *M* sodium citrate, and 10 mL of 0.2 *M* disodium EDTA to each beaker; and mix. Transfer each solution into separate 100-mL volumetric flasks, dilute with water to volume, and mix. Transfer a 50-mL portion of each solution into separate 125-mL plastic beakers, and measure the potential of each solution with a suitable ion-selective electrode apparatus (such as the Orion Model No. 94-09, with solid-state membrane), using a suitable reference electrode (such as the Orion Model No. 90-01, with single junction). Plot the calibration curve on two-cycle semilogarithmic paper (such as K & E No. 465130) ■or with the use of a suitable graphing calculator or spreadsheet program,■ 1S (*FCC7*) with micrograms of F per 100 mL solution on the logarithmic scale.

Procedure: Transfer 1.00 g of sample into a 150-mL glass beaker, add 10 mL of water, and, while stirring continuously, slowly add 20 mL of 1 *N* hydrochloric acid to dissolve the sample. Boil rapidly for 1 min, then transfer into a 250-mL plastic beaker, and cool rapidly in ice water. Add 15 mL of 1 *M* sodium citrate and 10 mL of 0.2 *M* disodium EDTA, and mix. Adjust the pH to 5.5 ± 0.1 with 1 *N* hydrochloric acid or 1 *N* sodium hydroxide, if necessary; transfer into a 100-mL volumetric flask; dilute with water to volume; and mix. Transfer a 50-mL portion of this solution into a 125-mL plastic beaker, and measure the potential of the solution with the apparatus described under *Calibration Curve*. Determine the fluoride content, in micrograms, of the sample from the *Calibration Curve*. ■Determine the percentage of fluoride in the sample by the formula

$$\text{Result} = (C/W_S) \times 0.000001 \times 100\%$$

in which C is the content of fluoride, in µg, in the sample, determined from the *Calibration Curve*; W_S is the sample weight, in g; and 0.000001 is a factor converting micrograms to grams.■ 1S (*FCC7*)

Change to read:

Method IV (Ion-Selective Electrode Method C)

■

[Note—Unless directed otherwise by the individual monograph, use *Buffer Solution A* for samples with a neutral to higher pH, and use *Buffer Solution B* for samples with a neutral to lower pH.]

■ 1S (*FCC6*)

Buffer Solution ■A■ 1S (*FCC6*)**:** Add 2 volumes of 6 *N* acetic acid to 1 volume of water, and adjust the pH to 5.0 with 50% potassium hydroxide solution.

■**Buffer Solution B:** Dissolve 150 g of sodium citrate dihydrate and 10.3 g of disodium EDTA dihydrate in 800 mL of water, adjust the pH to 8.0 with 50% sodium hydroxide solution, and dilute with water to 1000 mL. ■ 1S (*FCC6*)

Fluoride Standard Solutions

1000 mg/kg Fluoride Standard: Transfer 2.2108 g of sodium fluoride, previously dried at 200° for 4 h, into

a 1000-mL volumetric flask and dissolve in and dilute with water to volume. The resulting solution contains 1000 µg of fluoride per milliliter.

50 mg/kg Fluoride Standard: Pipet 50 mL of the *1000 mg/kg Fluoride Standard* into a 1000-mL volumetric flask. Dilute with water to volume.

10 mg/kg Fluoride Standard: Pipet 100 mL of the *50 mg/kg Fluoride Standard* into a 500-mL volumetric flask. Dilute with water to volume.

Fluoride Limit Solutions (for a 1-g sample)

50 mg/kg Fluoride Limit Solution: (1 mg/kg fluoride standard) Pipet 50 mL of the *10 mg/kg Fluoride Standard* into a 500-mL volumetric flask, and dilute with water to volume.

10 mg/kg Fluoride Limit Solution: (0.2 mg/kg fluoride standard) Pipet 10 mL of the *10 mg/kg Fluoride Standard* into a 500-mL volumetric flask, and dilute with water to volume.

Fluoride Limit Solutions (for a 2-g sample)

50 mg/kg Fluoride Limit Solution: (2 mg/kg fluoride standard) Pipet 100 mL of the *10 mg/kg Fluoride Standard* into a 500-mL volumetric flask, and dilute with water to volume.

10 mg/kg Fluoride Limit Solution: (0.4 mg/kg fluoride standard) Pipet 20 mL of the *10 mg/kg Fluoride Standard* into a 500-mL volumetric flask, and dilute with water to volume.

[Note—Store all standard and limit solutions in plastic containers.]

Sample Preparation: Accurately weigh the amount of sample specified in the monograph, transfer it into a 100-mL volumetric flask, and dissolve it in a minimal amount of water. Add 50.0 mL of the ■appropriate ■1S (*FCC6*) *Buffer Solution,* dilute with water to volume, and mix.

Electrode Calibration: Pipet 50 mL of the ■appropriate■1S (*FCC6*) *Buffer Solution* into a plastic beaker. Place the fluoride ion and reference electrodes (or a combination fluoride electrode) into the plastic beaker and stir. At 5-min intervals, add 100 µL and 1000 µL of the *1000 mg/kg Fluoride Standard* and read the potential, in millivolts, after each addition. The difference between the two readings is the slope of the fluoride electrode and should typically be in the range of ■63 to 70 mV at 25° for *Buffer Solution A* and in the range of■1S (*FCC6*) 54 to 60 mV at 25° ■for *Buffer Solution B.*■1S (*FCC6*) If the difference in potential is not within this range, check, and, if necessary, replace the electrode, instrument, or solutions.

■Alternatively, the electrode calibration should be performed according to the manufacturer's instructions and should comply with the manufacturer's calibration range at 25°. If the difference in potential is not within this range, evaluate the system and equipment as necessary.■1S (*FCC7*)

Procedure: Transfer the entire sample into a plastic beaker. Place the electrode into the beaker, allow the solution to equilibrate for 5 min with stirring, and read the potential, in millivolts. Remove and rinse the electrode(s) with water. In another beaker, using a pipet, add 50 mL of the ■appropriate■1S (*FCC6*) *Buffer Solution* followed by 50 mL of the *Fluoride Limit Solution* that best reflects the fluoride limit of the sample. Place the electrode in the beaker, equilibrate for 3 min, and read the potential in millivolts. If the potential of the *Fluoride Limit Solution* is less than that of the sample, the sample passes the test criteria for maximum acceptable fluoride level limit.

Method V

Lime Suspension: Carefully shake about 56 g of low-fluorine calcium oxide (about 2 mg/kg of F) with 250 mL of water, and while stirring, slowly add 250 mL of 60% perchloric acid. Add a few glass beads, and boil

until copious fumes of perchloric acid evolve, then cool, add 200 mL of water, and boil again. [**CAUTION—** Handle perchloric acid in an appropriate fume hood.]

Repeat the dilution and boiling once more, cool, dilute considerably, and if precipitated silicon dioxide forms, pass through a fritted-glass filter. While stirring, pour the clear solution into 1000 mL of a 1:10 solution of sodium hydroxide, allow the precipitate to settle, and siphon off the supernatant liquid. Remove the sodium salts from the precipitate by washing five times with water in large centrifuge bottles, shaking the mass thoroughly each time. Finally, shake the precipitate into a suspension and dilute with water to 2000 mL. Store in paraffin-lined bottles, and shake well before use.

[Note—100 mL of this suspension should give no appreciable fluoride blank when evaporated, distilled, and titrated as directed under *Method I*.]

Procedure: Assemble the distilling apparatus as described under *Method I*, and add 1.67 g of sample, accurately weighed, and 25 mL of 1:2 sulfuric acid to the distilling flask. Distill until the temperature reaches 160°, then maintain at 160° to 165° by adding water from the funnel, collecting 300 mL of distillate. Oxidize the distillate by cautiously adding 2 or 3 mL of fluorine-free 30% hydrogen peroxide (to remove sulfates), allow to stand for a few minutes, and evaporate in a platinum dish with an excess of *Lime Suspension*. Ignite briefly at 600°, then cool and wet the ash with about 10 mL of water. Cover the dish with a watch glass, and cautiously introduce under the watch glass just sufficient 60% perchloric acid to dissolve the ash. Add the contents of the dish through the dropping funnel of a freshly prepared distilling apparatus (the distilling flask should contain a few glass beads), using a total of 20 mL of the 60% perchloric acid to dissolve the ash and transfer the solution. Add 10 mL of water and a few drops of a 1:2 solution of silver perchlorate through the dropping funnel, and continue as directed under *Method I*, beginning with "Distill until the temperature reaches 135°. . . ."

LEAD LIMIT TEST

[Note—Unless otherwise specified in the monograph, use the *Dithizone Method* to determine lead levels.]

Change to read:

Dithizone Method

Special Reagents: Select reagents having as low a lead content as practicable, and store all solutions in containers of borosilicate glass. Rinse all glassware thoroughly with warm, 1:2 nitric acid followed by water.

Ammonia–Cyanide Solution: Dissolve 2 g of potassium cyanide in 15 mL of ammonium hydroxide, and dilute with water to 100 mL.

Ammonium Citrate Solution: Dissolve 40 g of citric acid in 90 mL of water, add 2 or 3 drops of phenol red TS, then cautiously add ammonium hydroxide until the solution acquires a red color. Extract it with 20-mL portions of *Dithizone Extraction Solution* until the dithizone solution retains its green color or remains unchanged.

Diluted Standard Lead Solution: (1 µg Pb in 1 mL)

Lead Nitrate Stock Solution: Dissolve 159.8 mg of ACS Reagent-Grade Lead Nitrate [$Pb(NO_3)_2$] in 100 mL of water containing 1 mL of nitric acid, dilute with water to 1000.0 mL, and mix. Prepare and store this

solution in glass containers that are free from lead salts.

Standard Lead Solution: On the day of use, dilute 10.0 mL of *Lead Nitrate Stock Solution* with water to 100.0 mL. Each milliliter of *Standard Lead Solution* contains the equivalent of 10 µg of lead (Pb) ion.

Diluted Standard Lead Solution: Immediately before use, transfer 10.0 mL of *Standard Lead Solution* into a 100-mL volumetric flask, dilute to volume with 1:100 nitric acid, and mix.

Dithizone Extraction Solution: Dissolve 30 mg of dithizone in 1000 mL of chloroform, add 5 mL of alcohol, and mix. Store in a refrigerator. Before use, shake a suitable volume of the solution with about half its volume of 1:100 nitric acid, discarding the nitric acid. ■■ 1S (*FCC6*)

Hydroxylamine Hydrochloride Solution: Dissolve 20 g of hydroxylamine hydrochloride in sufficient water to make about 65 mL, transfer the solution into a separator, add a few drops of thymol blue TS, then add ammonium hydroxide until the solution assumes a yellow color. Add 10 mL of a 1:25 solution of sodium diethyldithiocarbamate, mix, and allow to stand for 5 min. Extract the solution with successive 10- to 15-mL portions of chloroform until a 5-mL test portion of the chloroform extract does not assume a yellow color when shaken with cupric sulfate TS. Add 2.7 *N* hydrochloric acid until the extracted solution is pink, adding 1 or 2 drops more of thymol blue TS if necessary, then dilute with water to 100 mL, and mix.

Potassium Cyanide Solution: Dissolve 50 g of potassium cyanide in sufficient water to make 100 mL. Remove the lead from the solution by extraction with successive portions of *Dithizone Extraction Solution* as described under *Ammonium Citrate Solution*, then extract any dithizone remaining in the cyanide solution by shaking with chloroform. Finally, dilute the cyanide solution with sufficient water so that each 100 mL contains 10 g of potassium cyanide.

Standard Dithizone Solution: Dissolve 10 mg of dithizone in 1000 mL of chloroform, keeping the solution in a glass-stoppered, lead-free bottle suitably wrapped to protect it from light and stored in a refrigerator.

Sample Solution: Use the solution obtained by treating the sample as directed in an individual monograph as the *Sample Solution* in the *Procedure*. Sample solutions of organic compounds are prepared, unless otherwise directed, according to the following general method:

[**CAUTION—** Some substances may react unexpectedly with explosive violence when digested with hydrogen peroxide. Use appropriate safety precautions at all times.]

Transfer 1.0 g of sample into a suitable flask, add 5 mL of sulfuric acid and a few glass beads, and digest at a temperature not exceeding 120° until charring begins, using preferably, a hot plate in a fume hood. (Additional sulfuric acid may be necessary to completely wet some samples, but the total volume added should not exceed about 10 mL.) After the sample has initially been decomposed by the acid, add with caution, dropwise, hydrogen peroxide (30%), allowing the reaction to subside and reheating between drops. The first few drops must be added very slowly with sufficient mixing to prevent a rapid reaction, and heating should be discontinued if foaming becomes excessive. Swirl the solution in the flask to prevent unreacted substance from caking on the walls or bottom of the flask during the digestion.

[Note—Add small quantities of the peroxide when the solution begins to darken.]

Continue the digestion until the organic matter is destroyed, gradually raising the temperature of the hot plate to 250° to 300° until fumes of sulfur trioxide are copiously evolved and the solution becomes colorless or retains only a light straw color. Cool, cautiously add 10 mL of water, again evaporate to strong fuming, and cool. Quantitatively transfer the solution into a separator with the aid of small quantities of water.

Procedure: Transfer the *Sample Solution*, prepared as directed in the individual monograph, into a separator,

and unless otherwise directed, add 6 mL of *Ammonium Citrate Solution* and 2 mL of *Hydroxylamine Hydrochloride Solution*. (Use 10 mL of the citrate solution when determining lead in iron salts.) Add 2 drops of phenol red TS to the separator, and make the solution just alkaline (red in color) by the addition of ammonium hydroxide. Cool the solution, if necessary, under a stream of tap water, then add 2 mL of *Potassium Cyanide Solution*. Immediately extract the solution with 5-mL portions of *Dithizone Extraction Solution*, draining each extract into another separator, until the dithizone solution retains its green color. Shake the combined dithizone solutions for 30 s with 20 mL of 1:100 nitric acid; discard the chloroform layer; add 5.0 mL of *Standard Dithizone Solution* and 4 mL of *Ammonia–Cyanide Solution* to the acid solution; and shake for 30 s. The purple hue in the chloroform solution of the sample caused by any lead dithizonate present does not exceed that in a control, containing the volume of *Diluted Standard Lead Solution* equivalent to the amount of lead specified in the monograph, when treated in the same manner as the sample.

Flame Atomic Absorption Spectrophotometric Method

Select reagents having as low a lead content as practicable, and store all solutions in high-density polyethylene containers. Rinse all plastic and glassware thoroughly with warm, 1:2 nitric acid followed by water.

Lead Nitrate Stock Solution (100 µg/mL)**:** Dissolve 159.8 mg of reagent-grade lead nitrate [$Pb(NO_3)_2$] in 100 mL of water containing 1 mL of nitric acid in a 1000-mL volumetric flask, and dilute with water to volume.

Standard Lead Solution (10 µg/mL)**:** On the day of use, transfer 10 mL of *Lead Nitrate Stock Solution* into a 100-mL volumetric flask, and dilute with water to volume.

Diluted Standard Lead Solutions: On the day of use, prepare a set of standard lead solutions that corresponds to the lead limit specified in the monograph:

1 mg/kg Lead Limit: (0.5, 1.0, and 1.5 µg/mL standards) On the day of use, transfer 5.0, 10.0, and 15.0 mL of *Standard Lead Solution* into three separate 100-mL volumetric flasks, add 10 mL of 3 *N* hydrochloric acid to each, and dilute with water to volume.

5 mg/kg Lead Limit: (1.0, 5.0, and 10.0 µg/mL standards) On the day of use, transfer 10.0 and 50.0 mL of *Standard Lead Solution* into two separate 100-mL volumetric flasks, add 10 mL of 3 *N* hydrochloric acid to each, and dilute with water to volume. The final standard, 10.0 µg/mL, is taken directly from the *Standard Lead Solution*.

10 mg/kg Lead Limit: (5.0, 10.0, and 15.0 µg/mL standards) On the day of use, transfer 5.0, 10.0, and 15.0 mL of *Lead Nitrate Stock Solution* into three separate 100-mL volumetric flasks, add 10 mL of 3 *N* hydrochloric acid to each, and dilute with water to volume.

25% Sulfuric Acid Solution (by volume): Cautiously add 100 mL of sulfuric acid to 300 mL of water with constant stirring while cooling in an ice bath.

Sample Preparation: Transfer the sample weight as specified in the monograph, weighed to the nearest 0.1 mg, into an evaporating dish. Add a sufficient amount of *25% Sulfuric Acid Solution*, and distribute the sulfuric acid uniformly through the sample. Within a hood, place the dish on a steam bath to evaporate most of the water. Place the dish on a burner, and slowly pre-ash the sample by expelling most of the sulfuric acid. Place the dish in a muffle furnace that has been set at 525°, and ash the sample until the residue appears free from carbon. Prepare a *Sample Blank* by ashing 5 mL of 25% sulfuric acid. Cool and cautiously wash down the inside of each evaporation dish with water.

Add 5 mL of 1 *N* hydrochloric acid. Place the dish on a steam bath, and evaporate to dryness. Add 1.0 mL of 3 *N* hydrochloric acid and approximately 5 mL of water, and heat briefly on a steam bath to dissolve any residue. Transfer each solution quantitatively to a 10-mL volumetric flask, dilute to volume, and mix.

Procedure: Concomitantly determine the absorbances of the *Sample Blank*, the *Diluted Standard Lead Solutions*, and the *Sample Preparation* at the lead emission line of 283.3 nm, using a slit-width of 0.7 nm. Use a suitable atomic absorption spectrophotometer equipped with a lead electrodeless discharge lamp (EDL), an air–acetylene flame, and a 4-in. burner head. Use water as the blank.

Calculations: Determine the corrected absorbance values by subtracting the *Sample Blank* absorbance from each of the *Diluted Standard Lead Solutions* and from the *Sample Preparation* absorbances. Prepare a standard curve by plotting the corrected *Diluted Standard Lead Solutions* absorbance values versus their corresponding concentrations expressed as micrograms per milliliter. Determine the lead concentration in the *Sample Preparation* by reference to the calibration curve. Calculate the quantity of lead, in milligrams per kilogram, in the sample taken:

$$\text{Result} = 10C/W_S$$

in which C is the concentration, in micrograms per milliliter, of lead from the standard curve; and W_S is the weight, in grams, of the sample taken.

Change to read:

Atomic Absorption Spectrophotometric Graphite Furnace Method

The following methods are primarily intended for the analysis of applicable substances containing less than 1 mg/kg of lead.

Method I

This method is intended for the quantitation of lead in substances that are soluble in water, such as sugars and sugar syrups, at levels as low as 0.03 mg/kg. The method detection limit is approximately 5 ng/kg.

Apparatus: Use a suitable graphite furnace atomic absorption spectrophotometer set at 283.3 nm and equipped with an autosampler, pyrolytically coated graphite tubes, solid pyrolytic graphite platforms, and an adequate means of background correction. Zeeman effect or Smith-Hieftje background correction is preferred, but deuterium arc background correction should be acceptable. (This method was developed on a Perkin-Elmer Model Z5100, 0.7-nm slit, HGA-600 furnace, AS-60 autosampler with Zeeman background correction.) If the instrument does not have a well-defined calibration function, a separate calculator or computer is required for linear least squares, nonlinear, or quadratic calibrations. Use either a hollow cathode lamp or an electrode-less discharge lamp as the source, and use argon as the purge gas and breathing-quality air (for oxygen ashing to avoid residue build up during the char step) as the alternate gas. Set up the instrument according to the manufacturer's specifications with consideration of current good GFAAS practices—addressing such factors as line voltage, cooling water temperature, graphite part specifications, and furnace temperature. If an optical pyrometer or thermocouple is not available to check the furnace controller temperature calibration, dim the room lights, and observe the furnace emission through the sample introduction port while increasing the furnace temperature. A characteristic cherry red glow should begin to appear at 800°. If it glows at a lower temperature, then the furnace is hotter, and temperatures must be adjusted downward accordingly.

Use acid-cleaned [in a mixture of 5% sub-boiling, distilled nitric acid and 5% sub-boiling, distilled hydrochloric acid made up in deionized, distilled water (18 megohm), and thoroughly rinsed with deionized, distilled water (18 megohm)] autosampler cups (PE B008-7600 Teflon, or equivalent) to avoid contamination. Use micropipets with disposable tips free of lead contamination for dilution. Ensure accuracy and precision of

micropipets and tips by dispensing and weighing 5 to 10 replicate portions of water onto a microbalance. Use acid-cleaned volumetric glassware to prepare standards and dilute samples to a final volume. For digestion, use acid-cleaned, high-density polyethylene tubes, polypropylene tubes, Teflon tubes, or quartz tubes. Store final diluted samples in plastic tubes.

Standard Solutions: Prepare all lead solutions in 5% sub-boiling distilled nitric acid. Use a single-element 1000- or 10,000-µg/mL lead stock to prepare (weekly) an intermediate 10-µg/mL standard in 5% nitric acid. Prepare (daily) a *Lead Standard Solution* (1 µg/mL) by diluting the intermediate 10-µg/mL stock solution 1:10. Prepare *Working Calibration Standards* of 100.0, 50.0, 25.0, and 10.0 ng/mL from this, using appropriate dilutions. Store standards in acid-cleaned polyethylene test tubes or bottles. If the GFAAS autosampler is used to automatically dilute standards, ensure calibration accuracy by pipetting volumes of 3 µL or greater.

Modifier Stock Solution: Weigh 20 g of ultrapure magnesium nitrate hexahydrate and dilute to 100 mL. Just before use, prepare a *Modifier Working Solution* by diluting stock solution 1:10. A volume of 5 µL will provide 0.06 mg of magnesium nitrate.

Sample Digestion [**CAUTION—** Perform the procedure in a fume hood, and wear safety glasses.] Obtain a representative subsample to be analyzed. For liquid samples such as sugar syrups, ultrasonicate and/or vortex mix before weighing. For solid samples such as crystalline sucrose, make a sugar solution using equal weights of sample (5-g minimum) and deionized, distilled (18 megohm) water. Mix samples until completely dissolved. Transfer approximately 1.5 g (record to nearest mg) of sample (or 3.0 g of sugar solution), accurately weighed, into a digestion tube. Run a *Sample Preparation Blank* of 1.5 g of deionized, distilled (18 megohm) water through the entire procedure with each batch of samples. Add 0.75 mL of sub-boiling, distilled nitric acid. Heat plastic tubes in a water bath, quartz tubes in a water bath or heating block, warming slowly to between 90° and 95° to avoid spattering. Monitor the temperature by using a "dummy" sample. Heat until all brown vapors have dissipated and any rust-colored tint is gone (20 to 30 min). Cool. Add 0.5 mL of 50% hydrogen peroxide dropwise, heat at 90° to 95° for 5 min, and cool. Add a second 0.5-mL portion of 50% hydrogen peroxide, dropwise, and heat at 90° to 100° for 5 to 10 min until clear. Cool, and dilute to a final volume of 10 mL.

Procedure: The furnace program is as follows: (1) dry at 200°, using a 20-s ramp and a 30-s hold and a 300-mL/min argon flow; (2) char the sample at 750°, using a 40-s ramp and a 40-s hold and a 300-mL/min air flow; (3) cool down, and purge the air from the furnace for 60 s, using a 20° set temperature and a 300-mL/min argon flow; (4) atomize at 1800°, using a 0-s ramp and a 10-s hold with the argon flow stopped; (5) clean out at 2600°, with a 1-s ramp and a 7-s hold; (6) cool down the furnace (if necessary) at 20°, with a 1-s ramp and a 5-s hold with a 300-mL/min argon flow.

Use the autosampler to inject 20 µL of blanks, calibration standards, and sample solutions and 5 µL of *Modifier Working Solution*. Inject each respective solution in triplicate, and average results. Use peak area measurements for all quantitation. After ensuring that the furnace is clean by running a 5% nitric acid blank, check the instrument sensitivity by running the 25-ng/mL calibration standard. If the integrated absorbance is less than 0.14 abs-sec for a standard, 28-mm × 6-mm, end-heated furnace tube, correct the cause of insufficient sensitivity before proceeding. If the integrated absorbance is greater than 0.25 abs-sec, contamination is likely, and the source should be investigated. Calculate the characteristic mass (m_o) (mass of Pb pg necessary to produce an integrated absorbance of 0.0044 abs-sec) as follows:

$$m_o = (0.0044 \text{ abs-sec})(25 \text{ pg/µL})(20 \text{ µL})/ (\text{measured } 25 \text{ pg/µL abs-sec})$$

Record and track the integrated absorbance and m_o for reference and quality assurance.

Standard Curve: Inject each calibration standard in triplicate. Normal instrument linearity extends to 25 ng/mL. If nonlinear calibration capability is not available, limit the working calibration curve to ≤25 ng/mL. Use the calibration algorithms provided in the instrument software. Recheck calibration periodically (≤15 samples) by running a 25- or 50-ng/mL calibration standard interspersed with samples. If recheck differs from calibration by >10%, recalibrate the instrument. The instrumental detection limit (DL) and quantitation limit (QL), in picograms, may be based on 7 to 10 replicates of the *Sample Preparation Blank* and calculated as follows:

DL = (3)(s.d. blank abs-sec)(10 pg/µL)(20 µL)/ (abs-sec 10 ng/mL std)

QL = (10)(s.d. blank abs-sec)(10 pg/µL)(20 µL)/(abs-sec 10 ng/mL std)

During method development, detection limits were typically 10 to 14 pg, corresponding to 0.5 to 0.7 ng/mL for 20 µL. This corresponds to a method detection limit of 3.3 to 4.7 ng/g of sugar.

Sample Analyses: Inject each sample digest in triplicate, and record the integrated absorbance. If instrument response exceeds that of the calibration curve, dilute with 5% nitric acid to bring the sample response into working range, and note the dilution factor (DF). Sample solutions having a final concentration of >25 ng/mL should be diluted 1:10 to facilitate analysis in the linear range for systems not equipped with nonlinear calibration. All sample analyses should be blank corrected using the sample preparation blank. This can typically be done automatically by the software after identifying and running a representative sample preparation blank. Use the calibration algorithm provided in the instrument software to calculate a blank-corrected, digest lead concentration (in nanograms per milliliter).

Calculation of Lead Content: Calculate the lead level in the original sample as follows:

Pb(ng/g) = (blank-corrected Pb ng/mL)(DF)[sample vol (10 mL)][sample wt (approx. 1.5 g)].[2]

Quality Assurance: To ensure analytical accuracy, NIST SRM 1643c acidified water or a similar material should be analyzed before the unknown samples are. The certified content of SRM 1643c is 35.3 ± 0.9 ng/mL. If the concentration determined is not within 10% of the mean reference value (31.8 to 38.8 ng/mL), the reason for inaccuracy should be evaluated, and unknown samples should not be analyzed until acceptable accuracy is achieved. Also prepare an in-house control solution made from uncontaminated table sugar or reagent-grade sucrose (or other appropriate substance with a Pb content <5 ng/g as received) mixed with an equal volume of water. Spike this solution with Pb to produce a concentration of 100 ng/g. Analyze with each batch of samples. Recoveries should be 100% ± 20%, and the precision for complete replicate digestions should be <5% RSD. Periodically, a sample digest should be checked using the method of standard additions to ensure that there are no multiplicative or chemical interferences. Spiking samples and checking recoveries is always a good practice.

Method II

This method is primarily intended for the determination of lead at levels of less than 1 mg/kg in substances immiscible with water, such as edible oils.

Apparatus: Use a suitable atomic absorption spectrophotometer (Perkin-Elmer Model 3100 or equivalent) fitted with a graphite furnace (Perkin-Elmer HGA 600 or equivalent). Use a lead hollow-cathode lamp (Perkin-Elmer or equivalent) with argon as the carrier gas. Follow the manufacturers' directions for setting the appropriate instrument parameters for lead determination.

[Note—For this test, use reagent-grade chemicals with as low a lead content as is practicable, as well as high-purity water and gases. Before use in this analysis, rinse all glassware and plasticware twice with 10% nitric acid and twice with 10% hydrochloric acid, and then rinse them thoroughly with high-purity water, preferably obtained from a mixed-bed strong-acid, strong-base ion-exchange cartridge capable of producing water with an electrical resistivity of 12 to 15 megohms.]

Hydrogen Peroxide–Nitric Acid Solution: Dissolve equal volumes of 10% hydrogen peroxide and 10% nitric acid.
[Note—Use caution.]

Lead Nitrate Stock Solution: Dissolve 159.8 mg of ACS Reagent-Grade Lead Nitrate (alternatively, use NIST Standard Reference Material, containing 10 mg of lead per kilogram, or equivalent) in 100 mL of *Hydrogen Peroxide–Nitric Acid Solution*. Dilute with *Hydrogen Peroxide–Nitric Acid Solution* to 1000.0 mL, and mix. Prepare and store this solution in glass containers that are free from lead salts. Each milliliter of this solution contains the equivalent of 100 µg of lead (Pb) ion.

Standard Lead Solution: On the day of use, dilute 10.0 mL of *Lead Nitrate Stock Solution* with *Hydrogen Peroxide–Nitric Acid Solution* to 100.0 mL, and mix. Each milliliter of *Standard Lead Solution* contains the equivalent of 10 µg of lead (Pb) ion.

Butanol–Nitric Acid Solution: Slowly add 50 mL of nitric acid to approximately 500 mL of butanol contained in a 1000-mL volumetric flask. Dilute with butanol to volume, and mix.

Standard Solutions: Prepare a series of lead standard solutions serially diluted from the *Standard Lead Solution* in *Butanol–Nitric Acid Solution*. Pipet into separate 100-mL volumetric flasks 0.2, 0.5, 1, and 2 mL, respectively, of *Standard Lead Solution*, dilute with *Butanol–Nitric Acid Solution* to volume, and mix. The *Standard Solutions* contain, respectively, 0.02, 0.05, 0.1, and 0.2 µg of lead per milliliter. (For lead limits greater than 1 mg/kg, prepare a series of standard solutions in a range encompassing the expected lead concentration in the sample.)

Sample Solution [**CAUTION—** Perform this procedure in a fume hood, and wear safety glasses.] Transfer 1 g of sample, accurately weighed, into a large test tube. Add 1 mL of nitric acid. Place the test tube in a rack in a boiling water bath. As soon as the rusty tint is gone, add 1 mL of 30% hydrogen peroxide dropwise to avoid a vigorous reaction, and wait for bubbles to form. Stir with an acid-washed plastic spatula if necessary. Remove the test tube from the water bath, and let it cool. Transfer the solution into a 10-mL volumetric flask, and dilute with *Butanol–Nitric Acid Solution* to volume, and mix. Use this solution for analysis.

Procedure

Tungsten Solution: Transfer 0.1 g of tungstic acid (H_2WO_4) and 5 g of sodium hydroxide pellets into a 50-mL plastic bottle. Add 5.0 mL of high-purity water, and mix. Heat the mixture in a hot water bath until complete solution is achieved. Cool, and store at room temperature.

Procedure: Place the graphite tube in the furnace. Inject a 20-µL aliquot of the *Tungsten Solution* into the graphite tube, using a 300-mL/min argon flow and the following sequence of conditions: Dry at 110° for 20 s, char at 700° to 900° for 20 s, and with the argon flow stopped, atomize at 2700° for 10 s; repeat this procedure once more using a second 20-µL aliquot of the *Tungsten Solution*. Clean the quartz windows.

Standard Curve

[Note—The sample injection technique is the most crucial step in controlling the precision of the analysis; the volume of the sample must remain constant. Rinse the µL pipet tip (Eppendorf or equivalent) three times with either the *Standard Solutions* or *Sample Solution* before injection. Use a fresh pipet tip for each

injection, and start the atomization process immediately after injecting the sample. Between injections, flush the graphite tube of any residual lead by purging at a high temperature as recommended by the manufacturer.]

With the hollow cathode lamp properly aligned for maximum absorbance and the wavelength set at 283.3 nm, atomize 20-µL aliquots of the four *Standard Solutions*, using a 300-mL/min argon flow and the following sequence of conditions: Dry at 110° for 30 s, with a 20-s ramp period and a 10-s hold time; then char at 700° for 42 s, with a 20-s ramp period and a 22-s hold time; and then, with the argon flow stopped, atomize at 2300° for 7 s.

Plot a standard curve using the concentration, in micrograms per milliliter, of each *Standard Solution* versus its maximum absorbance value compensated for background correction as directed for the particular instrument, and draw the best straight line.

Atomize 20 µL of the *Sample Solution* under identical conditions, and measure its corrected maximum absorbance. From the *Standard Curve*, determine the concentration, *C*, in micrograms per milliliter, of the *Sample Solution*. Calculate the quantity, in milligrams per kilogram, of lead in the sample by the formula

$$\text{Result} = 10C/W$$

in which W is the weight, in grams, of the sample taken.

APDC Extraction Method

Select reagents having as low a lead content as practicable, and store all solutions in high-density polyethylene containers. Rinse all plastic and glassware thoroughly with warm, 1:2 nitric acid followed by water.

2% APDC Solution: Dissolve 2.0 g of ammonium pyrrolidinedithiocarbamate (APDC) in 100 mL of water. Filter any slight residue of insoluble APDC from the solution before use.

Lead Nitrate Stock Solution (100 µg/mL)**:** Dissolve 159.8 mg of reagent-grade lead nitrate [$Pb(NO_3)_2$] in 100 mL of water containing 1 mL of nitric acid in a 1000-mL volumetric flask, and dilute with water to volume.

■ **Standard Lead Solutions**

2 mg/kg Lead Standard: On the day of use, transfer 2.0 mL of *Lead Nitrate Stock Solution* into a 100-mL volumetric flask, and dilute with water to volume. The resulting solution contains 2 µg of lead per milliliter.

3 mg/kg Lead Standard: On the day of use, transfer 3.0 mL of *Lead Nitrate Stock Solution* into a 100-mL volumetric flask, and dilute with water to volume. The resulting solution contains 3 µg of lead per milliliter.

4 mg/kg Lead Standard: On the day of use, transfer 4.0 mL of *Lead Nitrate Stock Solution* into a 100-mL volumetric flask, and dilute with water to volume. The resulting solution contains 4 µg of lead per milliliter.

10 mg/kg Lead Standard: On the day of use, transfer 10.0 mL of *Lead Nitrate Stock Solution* into a 100-mL volumetric flask, and dilute with water to volume. The resulting solution contains 10 µg of lead per milliliter.

■ 1S (*FCC6*)

Sample Preparation: Transfer a 10.0-g sample to a clean 150-mL beaker, and 10 mL of water to a second 150-mL beaker to serve as the blank. Add to each 30 mL of water and the minimum amount of hydrochloric

acid needed to dissolve the sample, plus an additional 1 mL of hydrochloric acid to ensure the dissolution of any lead present. Heat to boiling, and boil for several minutes. Allow to cool, and dilute with deionized water to about 100 mL. Adjust the pH of the resulting solution to between 1.0 and 1.5 with 25% NaOH. Quantitatively transfer the pH-adjusted solution to a clean 250-mL separatory funnel, and dilute with water to about 200 mL. Add 2 mL of *2% APDC Solution*, and mix. Extract with two 20-mL portions of chloroform, collecting the extracts in a clean 50-mL beaker. Evaporate to dryness on a steam bath. Add 3 mL of nitric acid to the residue, and heat to near dryness. Then add 0.5 mL of nitric acid and 10 mL of deionized water to the beaker, and heat until the volume is reduced to about 3 to 5 mL. Transfer the digested extract to a clean 10-mL volumetric flask, and dilute with water to volume.

Procedure: Concomitantly determine the absorbances of the ■appropriate■ 1S (*FCC6*) *Standard Lead Solution* and the *Sample Preparation* against the blank at the lead emission line of 283.3 nm, using a slit-width of 0.7 nm. Use a suitable atomic absorption spectrophotometer equipped with a lead electrode-less discharge lamp (EDL), or equivalent; an air–acetylene flame; and a 4-in. burner head. Use water as the blank. The absorbance of the *Sample Preparation* is not greater than that of the *Standard Lead Solution*.

MANGANESE LIMIT TEST

Manganese Detection Instrument: Use any suitable atomic absorption spectrophotometer equipped with a fast-response recorder or other readout device and capable of measuring the radiation absorbed by manganese atoms at the manganese resonance line of 279.5 nm.

Standard Preparations: Transfer 1000 mg, accurately weighed, of manganese metal powder into a 1000-mL volumetric flask, dissolve by warming in a mixture of 10 mL of water and 10 mL of 0.5 *N* hydrochloric acid, cool, dilute with water to volume, and mix. Pipet 5.0 mL of this solution into a 50-mL volumetric flask, dilute with water to volume, and mix. Finally, pipet 5.0, 10.0, 15.0, and 25.0 mL of this solution into separate 1000-mL volumetric flasks, dilute each flask with water to volume, and mix. The final solutions contain 0.5, 1.0, 1.5, and 2.5 mg/kg of Mn, respectively.

Sample Preparation: 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 with 0.5 *N* hydrochloric acid to volume, and shake. Centrifuge approximately 100 mL of the sample mixture in a heavy-walled centrifuge tube at 2000 rpm for 5 min, and use the clear supernatant liquid in the following *Procedure*.

Procedure: Aspirate 0.5 *N* hydrochloric acid through the air–acetylene burner for 5 min, and obtain a baseline reading at 279.5 nm, following the manufacturer's instructions for operating the atomic absorption spectrophotometer being used for the analysis. Aspirate a portion of each *Standard Preparation* in the same manner, note the readings, then aspirate a portion of the *Sample Preparation*, and note the reading. Prepare a standard curve by plotting the mg/kg of Mn in each *Standard Preparation* against the respective readings. From the graph determine the mg/kg of Mn in the *Sample Preparation*, and multiply this value by 20 to obtain the mg/kg of Mn in the original sample taken for analysis.

MERCURY LIMIT TEST

Method I

Mercury Detection Instrument: Use any suitable atomic absorption spectrophotometer equipped with a fast-response recorder and capable of measuring the radiation absorbed by mercury vapors at the mercury resonance line of 253.6 nm. A simple mercury vapor meter or detector equipped with a variable span recorder also is satisfactory.

[Note—Wash all glassware associated with the test with nitric acid, and rinse thoroughly with water before use.]

Aeration Apparatus: The apparatus, shown in *Fig. 16*, consists of a flowmeter (*a*), capable of measuring flow rates from 500 to 1000 mL/min, connected via a three-way stopcock (*b*), with a Teflon plug, to 125-mL gas washing bottles (*c* and *d*), followed by a drying tube (*e*), and finally a suitable quartz liquid absorption cell (*f*), terminating with a vent (*g*) to a fume hood.

[Note— The absorption cell will vary in optical pathlength depending on the type of mercury detection instrument used.]

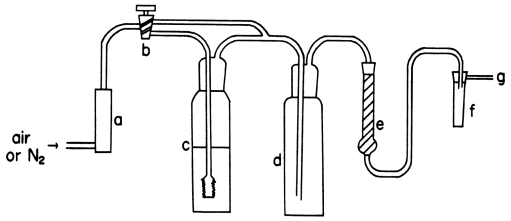

FIGURE 16 Aeration Apparatus for Mercury Limit Test.

Bottle *c* is fitted with an extra-coarse fritted bubbler (Corning 31770 125 EC, or equivalent), and the bottle is marked with a 60-mL calibration line. The drying tube *e* is lightly packed with magnesium perchlorate. Bottle *c* is used for the test solution, and bottle *d*, which remains empty throughout the procedure, is used to collect water droplets.

Alternatively, an apparatus embodying the principle of the assembly described and illustrated may be used. The aerating medium may be either compressed air or compressed nitrogen.

Standard Preparation: Transfer 1.71 g of mercuric nitrate [$Hg(NO_3)\cdot H_2O$] into a 1000-mL volumetric flask, dissolve in a mixture of 100 mL of water and 2 mL of nitric acid, dilute with water to volume, and mix. Discard after 1 month. Transfer 10.0 mL of this solution into a second 1000-mL volumetric flask, acidify with 5 mL of a 1:5 sulfuric acid solution, dilute with water to volume, and mix. Discard after 1 week. On the day of use, transfer 10.0 mL of the second solution into a 100-mL volumetric flask, acidify with 5 mL of 1:5 sulfuric acid, dilute with water to volume, and mix. Each milliliter of this solution contains 1 µg of mercury. Transfer 2.0 mL of this solution (2 µg Hg) into a 50-mL beaker, and add 20 mL of water, 1 mL of a 1:5 sulfuric acid solution, and 1 mL of a 1:25 solution of potassium permanganate. Cover the beaker with a watch glass, boil for a few seconds, and cool.

Sample Preparation: Prepare as directed in the individual monograph.

Procedure: Assemble the aerating apparatus as shown in *Fig. 16*, with bottles *c* and *d* empty and stopcock *b* in the bypass position. Connect the apparatus to the absorption cell (*f*) in the instrument, and adjust the air or nitrogen flow rate so that in the following procedure, maximum absorption and reproducibility are obtained without excessive foaming in the test solution. Obtain a baseline reading at 253.6 nm, following the manufacturer's instructions for operating the instrument.

Treat the *Standard Preparation* as follows: Destroy the excess permanganate by adding a 1:10 solution of hydroxylamine hydrochloride, dropwise, until the solution is colorless. Immediately wash the solution into bottle *c* with water, and dilute with water to the 60-mL mark. Add 2 mL of 10% stannous chloride solution (prepared fresh each week by dissolving 10 g of $SnCl_2\cdot 2H_2O$ in 20 mL of warm hydrochloric acid and diluting

with 80 mL of water), and immediately reconnect bottle *c* to the aerating apparatus. Turn stopcock *b* from the bypass to the aerating position, and continue the aeration until the absorption peak has been passed and the recorder pen has returned to the baseline. Disconnect bottle *c* from the aerating apparatus, discard the *Standard Preparation* mixture, wash bottle *c* with water, and repeat the foregoing procedure using the *Sample Preparation*; any absorbance produced by the *Sample Preparation* does not exceed that produced by the *Standard Preparation*.

Method II

Dithizone Extraction Solution: Dissolve 30 mg of dithizone in 1000 mL of chloroform, add 5 mL of alcohol, and mix. Store in a refrigerator. Before use, shake a suitable volume of the solution with about half its volume of 1:100 nitric acid, discarding the nitric acid. Discard the solution after 1 month.

Diluted Dithizone Extraction Solution: Just before use, dilute 5 mL of *Dithizone Extraction Solution* with 25 mL of chloroform.

Hydroxylamine Hydrochloride Solution: Dissolve 20 g of hydroxylamine hydrochloride in sufficient water to make about 65 mL, transfer the solution into a separator, add a few drops of thymol blue TS, and then add ammonium hydroxide until a yellow color develops. Add 10 mL of a 1:25 solution of sodium diethyldithiocarbamate, mix, and allow to stand for 5 min. Extract the solution with successive 10- to 15-mL portions of chloroform until a 5-mL test portion of the chloroform extract does not develop a yellow color when shaken with a dilute solution of cupric sulfate. Add 2.7 *N* hydrochloric acid until the extracted solution is pink, adding one or two more drops of thymol blue TS, if necessary, then dilute with water to 100 mL, and mix.

Mercury Stock Solution: Transfer 135.4 mg of mercuric chloride, accurately weighed, into a 100-mL volumetric flask, dissolve in and dilute with 1 *N* sulfuric acid to volume, and mix. Dilute 5.0 mL of this solution to 500.0 mL with 1 *N* sulfuric acid. Each milliliter contains the equivalent of 10 µg of mercury.

Diluted Standard Mercury Solution: On the day of use, transfer 10.0 mL of *Mercury Stock Solution* into a 100-mL volumetric flask, dilute with 1 *N* sulfuric acid to volume, and mix. Each milliliter contains the equivalent of 1 µg of mercury.

Sodium Citrate Solution: Dissolve 250 g of sodium citrate dihydrate in 1000 mL of water.

Sample Solution: Dissolve 1 g of sample in 30 mL of 1.7 *N* nitric acid by heating on a steam bath. Cool to room temperature in an ice bath, stir, and pass through S and S No. 589, or equivalent, filter paper that has been previously washed with 1.7 *N* nitric acid, followed by water. Add 20 mL of *Sodium Citrate Solution* and 1 mL of *Hydroxylamine Hydrochloride Solution* to the filtrate.

Procedure [Note—Because mercuric dithizonate is light sensitive, perform this procedure in subdued light.] Prepare a control containing 3.0 mL of *Diluted Standard Mercury Solution* (3 µg Hg), 30 mL of 1.7 *N* nitric acid, 5 mL of *Sodium Citrate Solution*, and 1 mL of *Hydroxylamine Hydrochloride Solution*. Treat the control and the *Sample Solution* as follows: Using a pH meter, adjust the pH of each solution to 1.8 with ammonium hydroxide, and transfer the solutions into different separators. Extract each with two 5-mL portions of *Dithizone Extraction Solution*, and then extract again with 5 mL of chloroform, discarding the aqueous solutions. Transfer the combined extracts from each separator into different separators, add 10 mL of 1:2 hydrochloric acid to each, shake well, and discard the chloroform layers. Extract the acid solutions with about 3 mL of chloroform, shake well, and discard the chloroform layers. Add 0.1 mL of 0.05 *M* disodium EDTA and 2 mL of 6 *N* acetic acid to each separator, mix, and then slowly add 5 mL of ammonium hydroxide. Stopper the separators, and cool under a stream of cold water, and dry the outside of the separators. To avoid loss, carefully pour the solutions through the tops of the separators into separate beakers, and using a pH meter,

adjust the pH of both solutions to 1.8 with 6 *N* ammonium hydroxide. Return the sample and control solutions to their original separators, add 5.0 mL of *Diluted Dithizone Extraction Solution*, and shake vigorously. Any color developed in the *Sample Solution* does not exceed that in the control.

NICKEL LIMIT TEST

[Note—Unless otherwise specified in the individual monograph, use *Method I*.]

Method I

Atomic Absorption System Apparatus: Use a suitable atomic absorption spectrometer equipped with a nickel hollow-cathode lamp and an air–acetylene flame to measure the absorbance of the *Blank Preparation*, the *Standard Preparations,* and the *Test Preparation* as directed under *Procedure* (below).

Test Preparation: Dissolve 20.0 g of sample in strong acetic acid TS, and dilute with the same solvent to 150.0 mL. Add 2.0 mL of a saturated solution of ammonium pyrrolidinedithiocarbamate (about 10 g/L of water), and 10.0 mL of methyl isobutyl ketone, and shake for 30 s. Protect from bright light. Allow the two layers to separate, and use the methyl isobutyl ketone layer.

Blank Preparation: Prepare in the same manner as in the *Test Preparation,* but omit the sample.

Standard Preparations: Prepare three *Standard Preparations* in the same manner as in the *Test Preparation,* but add 0.5 mL, 1.0 mL, and 1.5 mL, respectively, of 10 mg/kg nickel standard solution TS in addition to 20.0 g of sample.

Procedure: Zero the instrument with the *Blank Preparation*. Concomitantly determine the absorbances of each of the *Standard Preparations* and of the *Test Preparation* at least three times each, and record the average of the steady readings for each. Between each measurement, aspirate the *Blank Preparation,* and ascertain that the reading returns to its initial blank value.

Calculation: Calculate the linear equation of the graph using a least-squares fit, and derive from it the concentration of nickel in the *Test Preparation*. Alternatively, plot on a graph the mean of the readings against the added quantity of nickel. Extrapolate the line joining the points on the graph until it meets the concentration axis. The distance between this point and the intersection of the axes represents the concentration of nickel in the *Test Preparation*.

Add the following:

▲Method II

[Note—All glassware used must be soaked in *1% Nitric Acid* for at least 2 h, and then rinsed with water.]

1% Nitric Acid: Cautiously add 10 mL of nitric acid to a 1000-mL volumetric flask containing about 500 mL of water. Mix, and dilute with water to volume.

Blank Solution: Use *1% Nitric Acid*.

Nickel Stock Standard Solution: Immediately before use, dilute appropriate amount of nickel standard[3] with *1% Nitric Acid* to prepare a solution containing the equivalent of 10 µg of nickel per mL.

Standard Solutions: Into three identical 100-mL volumetric flasks, introduce respectively 2.0, 5.0, and 10.0

mL of *Nickel Stock Standard solution*. Dilute with *1% Nitric Acid* to volume and mix. These standards contain 0.2 µg, 0.5 µg, and 1.0 µg of nickel per mL.

Test Solution: Weigh accurately a quantity of test specimen containing about 5 g of solids into a 100-mL volumetric flask. Dissolve in and dilute with *1% Nitric Acid* to volume, and mix.

Procedure: Concomitantly determine the absorbances of the *Standard Solutions* and the *Test Solution* at least three times each, at the wavelength of maximum absorbance at 352.0 nm, with a suitable atomic absorption spectrophotometer equipped with an air–acetylene flame and a nickel hollow-cathode lamp using the *Blank Solution* to zero the instrument. Record the average of the steady readings for each of the *Standard Solutions* and the *Test Solution*. Clear the nebulizer using the *Blank Solution* and aspirate each of the *Standard Solutions* and the *Test Solution* in turn. The standard chosen for reslope should be run every 4 to 5 samples. If there is a significant change in its response, reslope and repeat the previous samples. The standard deviation for the *Standard Solution* of 0.2 µg of nickel per mL must be less than 20%. Plot the absorbances of the *Standard Solutions* versus concentration, in µg/mL, of nickel, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, *C,* in µg/mL, of nickel in the *Test Solution*. Calculate the quantity, in µg, of nickel in each g of test specimen taken:

$$\text{Result} = 100C/W$$

in which W is the weight, in g, of test specimen taken to prepare the *Test Solution*.▲*FCC6*

PHOSPHORUS LIMIT TEST

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 with water to 1000 mL, and mix.

Ammonium Vanadate Solution (0.25%)**:** Dissolve 2.5 g of ammonium metavanadate, NH_4VO_3, in 600 mL of boiling water, cool to 60° to 70°, and add 20 mL of nitric acid. Cool to room temperature, dilute with water to 1000 mL, and mix.

Zinc Acetate Solution (10%)**:** Dissolve 120 g of zinc acetate dihydrate, $Zn(C_2H_3O_2)_2\cdot 2H_2O$, in 880 mL of water, and pass 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, KH_2PO_4, in water in a 1000-mL volumetric flask, dilute with water to volume, 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 with water to volume, 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 to zero. Prepare a standard curve by plotting the absorbance of each solution versus its concentration, in mg of phosphorus (P) per 100 mL.

Treated Sample: Place 20 to 25 g of the starch sample in a 250-mL beaker, add 200 mL of a 7:3 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 Büchner 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 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 h 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 Büchner 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 basis 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 slowly wash 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 with water to volume, 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 50 mL of water to a second flask to serve as a blank. To each 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 with water to volume, 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 equation

$$\text{mg/kg P} = (a \times 200 \times 1000)/(V \times W)$$

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

SELENIUM LIMIT TEST

Reagents and Solutions

2,3-Diaminonaphthalene Solution: On the day of use, dissolve 100 mg of 2,3-diaminonaphthalene ($C_{10}H_{10}N_2$) and 500 mg of hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) in sufficient 0.1 *N* hydrochloric acid to make 100 mL.

Selenium Stock Solution: Transfer 40.0 mg of powdered metallic selenium into a 1000-mL volumetric flask, and dissolve in 100 mL of 1:2 nitric acid, warming gently on a steam bath to effect solution. Cool, dilute with

water to volume, and mix.

*Selenium Standard Solution***:** Pipet 5.0 mL of *Selenium Stock Solution* into a 200-mL volumetric flask, dilute with water to volume, and mix. Each milliliter of this solution contains the equivalent of 1 µg of selenium (Se).

Method I

Standard Preparation: Pipet 6.0 mL of *Selenium Standard Solution* into a 150-mL beaker, add 50 mL of 0.25 *N* nitric acid, and mix.

Sample Preparation: Using a 1000-mL combustion flask and 25 mL of 0.5 *N* nitric acid as the absorbing liquid, proceed as directed under *Oxygen Flask Combustion*, Appendix I, using the amount of sample specified in the individual monograph (and the magnesium oxide or other reagent, where specified).

[Note—If the sample contains water of hydration or more than 1% of moisture, dry it at 140° for 2 h before combustion, unless otherwise directed.]

Upon completion of combustion, place a few milliliters of water in the cup or lip of the combustion flask, loosen the stopper of the flask, and rinse the stopper, sample holder, and sides of the flask with about 10 mL of water. Transfer the solution, with the aid of about 20 mL of water, into a 150-mL beaker, heat gently to boiling, boil for 10 min, and cool.

Procedure: Treat the *Sample Preparation*, the *Standard Preparation*, and 50 mL of 0.25 *N* nitric acid, to serve as the blank, similarly and in parallel as follows: Add a 1:2 solution of ammonium hydroxide to adjust the pH of the solution to 2.0 ± 0.2. Dilute with water to 60.0 mL, and transfer to a low-actinic separator with the aid of 10.0 mL of water, adding the 10.0 mL of rinsings to the separator. Add 200 mg of hydroxylamine hydrochloride, swirl to dissolve, immediately add 5.0 mL of *2,3-Diaminonaphthalene Solution*, insert the stopper, and swirl to mix. Allow the solution to stand at room temperature for 100 min. Add 5.0 mL of cyclohexane, shake vigorously for 2 min, and allow the layers to separate. Discard the aqueous phases, and centrifuge the cyclohexane extracts to remove any traces of water. Determine the absorbance of each extract in a 1-cm cell at the maximum at about 380 nm with a suitable spectrophotometer, using the extract from the blank to set the instrument. The absorbance of the extract from the *Sample Preparation* is not greater than that from the *Standard Preparation* when a 200-mg sample is tested, or not greater than one-half the absorbance of the extract from the *Standard Preparation* when a 100-mg sample is tested.

Method II

Standard Preparation: Pipet 6.0 mL of *Selenium Standard Solution* into a 150-mL beaker, add 50 mL of 2 *N* hydrochloric acid, and mix.

Sample Preparation: Transfer the amount of sample specified in the individual monograph into a 150-mL beaker, dissolve in 25 mL of 4 *N* hydrochloric acid, swirling if necessary to effect solution, heat gently to boiling, and digest on a steam bath for 15 min. Remove from heat, add 25 mL of water, and allow to cool to room temperature.

Procedure: Place the beakers containing the *Standard Preparation* and the *Sample Preparation* in a fume hood, and to a third beaker, add 50 mL of 2 *N* hydrochloric acid to serve as the blank. Cautiously add 5 mL of ammonium hydroxide to each beaker, mix, and allow the solution to cool. Treat each solution, similarly and in parallel, as directed under *Procedure* in *Method I*, beginning with “Add a 1:2 solution of ammonium hydroxide...”

C. OTHERS

ALGINATES ASSAY

In a suitable closed system, liberate the carbon dioxide from the uronic acid groups of about 250 mg of the test sample by heating with hydrochloric acid, and sweep the carbon dioxide, by means of an inert gas, into a titration vessel containing excess standardized sodium hydroxide. Any suitable system may be used as long as it provides precautions against leakage and overheating of the reaction mixture, adequate sweeping time, avoidance of entrainment of hydrochloric acid, and meets the requirements of the *System Suitability Test*. One suitable system, with accompanying procedure, is given below.

Apparatus: The apparatus is shown in *Fig. 17*. It consists essentially of a soda lime column, *A*, a mercury valve, *B*, connected through a side arm, *C*, to a reaction flask, *D*, by means of a rubber connection. Flask *D* is a 100-mL round-bottom, long-neck boiling flask, resting in a suitable heating mantle, *E*.

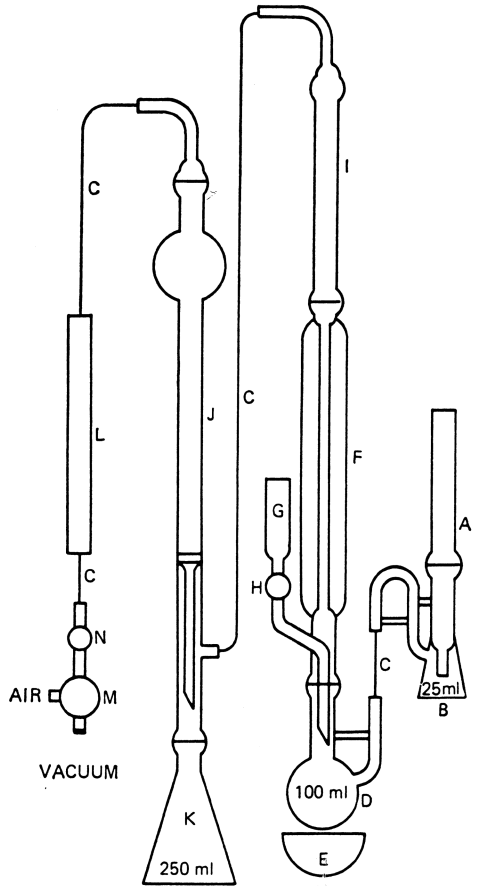

FIGURE 17 Apparatus for Alginates Assay.

The reaction flask is provided with a reflux condenser, *F*, to which is fitted a delivery tube, *G*, of 40-mL capacity, having a stopcock, *H*. The reflux condenser terminates in a trap, *I*, containing 25 g of 20-mesh zinc or tin, which can be connected with an absorption tower, *J*.

The absorption tower consists of a 45-cm tube fitted with a medium-porosity fritted glass disk sealed to the inner part above the side arm and having a delivery tube sealed to it extending down to the end of the tube. A trap, consisting of a bulb of approximately 100-mL capacity, is blown above the fritted disk and the outer portion of a ground spherical joint is sealed on above the bulb. A 250-mL Erlenmeyer flask, *K*, is connected to

the bottom of the absorption tower. The top of the tower is connected to a soda lime tower, *L*, which is connected to a suitable pump to provide vacuum and air supply, the choice of which is made by a three-way stopcock, *M*. The volume of air or vacuum is controlled by a capillary-tube regulator or needle valve, *N*.

All joints are a size 35/25 ground spherical type.

Standard D-Glucurono-6,3-lactone: This chemical ($C_6H_8O_6$) is available as a reference standard with an assay of 100.0 ± 1.0% (24.99 ± 0.25% CO_2) from Aldrich Chemical Co.

System Suitability Test: Transfer about 250.0 mg of *Standard d-Glucurono-6,3-lactone*, accurately weighed, into the reaction flask, *D*, and carry out the *Procedure* described below. The system is considered suitable when the net titration results in a calculation of %CO_2 in a range of 24.73 to 25.26, which is equivalent to a range of 98.95 to 101.06% d-*Glucurono-6,3-lactone*.

Procedure: Transfer about 250 mg of sample, accurately weighed, into the reaction flask, *D*, add 25 mL of 0.1 *N* hydrochloric acid, insert several boiling chips, and connect the flask to the reflux condenser, *F*, using syrupy phosphoric acid as a lubricant.

[Note—Stopcock grease may be used for the other connections.]

Check the system for air leaks by forcing mercury up into the inner tube of the mercury valve, *B*, to a height of about 5 cm. Turn off the pressure using the stopcock, *M*. If the mercury level does not fall appreciably after 1 to 2 min, the apparatus may be considered to be free from leaks. Draw carbon dioxide-free air through the apparatus at a rate of 3000 to 6000 mL/h. Raise the heating mantle, *E*, to the flask, heat the sample to boiling, and boil gently for 2 min. Turn off and lower the mantle, and allow the sample to cool for 15 min. Charge the delivery tube, *G*, with 23 mL of hydrochloric acid. Disconnect the absorption tower, *J*, rapidly transfer 25.0 mL of 0.25 *N* sodium hydroxide into the tower, add 5 drops of *n*-butanol, and reconnect the absorption tower. Draw carbon dioxide-free air through the apparatus at the rate of about 2000 mL/h, add the hydrochloric acid to the reaction flask through the delivery tube, raise the heating mantle, and heat the reaction mixture to boiling. After 2 h, discontinue the current of air and heating. Force the sodium hydroxide solution down into the flask, *K*, using gentle air pressure, and then rinse down the absorption tower with three 15-mL portions of water, forcing each washing into the flask with air pressure. Remove the flask, and add to it 10 mL of a 10% solution of barium chloride ($BaCl_2 \cdot 2H_2O$). Stopper the flask, shake gently for about 2 min, add phenolphthalein TS, and titrate with 0.1 *N* hydrochloric acid. Perform a blank determination (see *General Provisions*). Each milliliter of 0.25 *N* sodium hydroxide consumed is equivalent to 5.5 mg of carbon dioxide (CO_2). Calculate the results on the dried basis.

α-AMINO NITROGEN (AN) DETERMINATION

Transfer 7 to 25 g of sample, accurately weighed, into a 500-mL volumetric flask with the aid of several 50-mL portions of warm, ammonia-free water, dilute with water to volume, and mix. Neutralize 20.0 mL of the solution with 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide, using phenolphthalein TS as the indicator, and add 10 mL of freshly prepared phenolphthalein–formol solution (50 mL of 40% formaldehyde containing 1 mL of 0.05% phenolphthalein in 50% alcohol neutralized exactly to pH 7 with 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide). Titrate with 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide to a distinct red color, add a small, but accurately measured, volume of 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide in excess, and back titrate to neutrality with 0.2 *N* hydrochloric acid. Conduct a blank titration using the same reagents, with 20 mL of water in place of the test solution. Each milliliter of 0.2 *N* barium hydroxide or 0.2 *N* sodium hydroxide is equivalent to 2.8 mg of α-amino nitrogen.

AMMONIA NITROGEN (NH_3-N) DETERMINATION

[**CAUTION—** Provide adequate ventilation.]

[Note—Use nitrogen-free reagents, where available, or reagents very low in nitrogen content.]

Transfer between 700 mg and 2.2 g of sample into a 500- to 800-mL Kjeldahl digestion flask of hard, moderately thick, well-annealed glass. If desired, wrap the sample, if solid or semisolid, in nitrogen-free filter paper to facilitate the transfer.

Add about 200 mL of water, and mix. Add a few granules of zinc to prevent bumping, tilt the flask, and cautiously pour sodium hydroxide pellets, or a 2:5 sodium hydroxide solution, down the inside of the flask so that it forms a layer under the solution, using a sufficient amount (usually about 25 g of solid sodium hydroxide) to make the mixture strongly alkaline. Immediately connect the flask to a distillation apparatus consisting of a Kjeldahl connecting bulb and a condenser that has a delivery tube extending well beneath the surface of a measured excess of 0.5 *N* hydrochloric or sulfuric acid contained in a 500-mL flask. Add 5 to 7 drops of methyl red indicator (1 g of methyl red in 200 mL of alcohol) to the receiver flask. Rotate the Kjeldahl flask to mix its contents thoroughly, and heat until all of the ammonia has distilled, collecting at least 150 mL of distillate. Wash the tip of the delivery tube, collecting the washings in the receiving flask, and titrate the excess acid with 0.5 *N* sodium hydroxide. Perform a blank determination (see *General Provisions*), substituting 2 g of sucrose for the sample, and make any necessary correction. Each milliliter of 0.5 *N* acid consumed is equivalent to 7.003 mg of ammonia nitrogen.

[Note—If it is known that the substance to be determined has a low nitrogen content, 0.1 *N* acid and alkali may be used, in which case each milliliter of 0.1 *N* acid consumed is equivalent to 1.401 mg of nitrogen.]

Calculate the percent ammonia nitrogen by the formula

$$\text{Result} = (NH_3\text{-N}/S) \times 100$$

in which NH_3-N is the weight, in milligrams, of ammonia nitrogen, and S is the weight, in milligrams, of sample.

BENZENE (in Paraffinic Hydrocarbon Solvents)

Apparatus: (See *Chromatography, Appendix IIA*.) Use a suitable gas chromatograph, equipped with a column, or equivalent, that will elute *n*-decane before benzene under the conditions of the *System Suitability Test* (below). Column materials and conditions that have been found suitable for this method are listed in the accompanying tables. See *Fig. 18* for a typical chromatogram obtained with column No. 5.

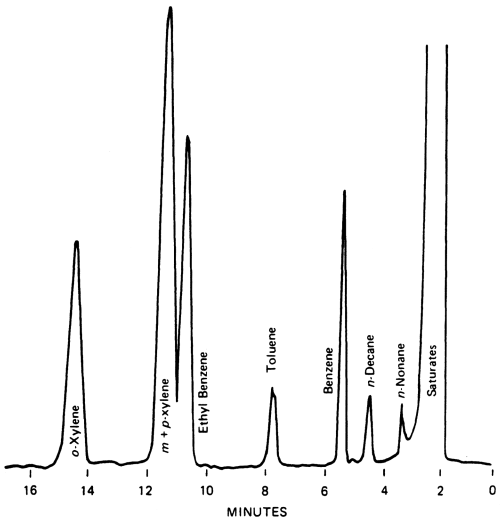

FIGURE 18 Typical Chromatogram for the Determination of Benzene in Hexanes Using Column No. 5.

Reagents

Isooctane: 99 mole percent minimum containing less than 0.05 mole percent aromatic material.

Benzene: 99.5 mole percent minimum.

Internal Standard: *n*-Decane and either *n*-undecane or *n*-dodecane according to the requirement of the *System Suitability Test*.

Reference Solution A: Prepare a standard solution containing 0.5% by weight each of the *Internal Standard* and of benzene in isooctane.

Reference Solution B: Prepare a standard solution containing about 0.5% by weight each of *n*-decane, of *Internal Standard*, and of benzene in isooctane.

Calibration: Select the instrument conditions necessary to give the desired sensitivity. Inject a known volume of *Reference Solution A*, and change the attenuation, if necessary, so that the benzene peak is measured with a chart deflection of not less than 25% or more than 95% of full scale. When choosing the attenuation, consider all unresolved peaks to represent a single compound. There may be tailing of the nonaromatic peak, but do not use any conditions that lead to a depth of the valley ahead of the benzene peak (A) less than 50% of the weight of the benzene peak (B) as depicted in *Fig. 19*.

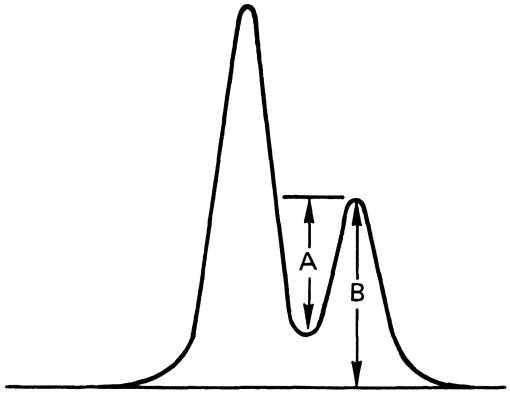

FIGURE 19 Illustration of A/B Ratio.

If there is tailing of the nonaromatic material, construct a baseline by drawing a line from the bottom of the

valley ahead of the benzene peak to the point of tangency after the peak (see *Fig. 20*). Measure the areas of the benzene peak and the internal standard peak by any of the following means: triangulation, planimeter, paper cutout, or mechanical or electronic integrator. Do not use integrators on peaks without a constant baseline, unless the integrator has provision for making baseline corrections with accuracy at least as good as that of manual methods.

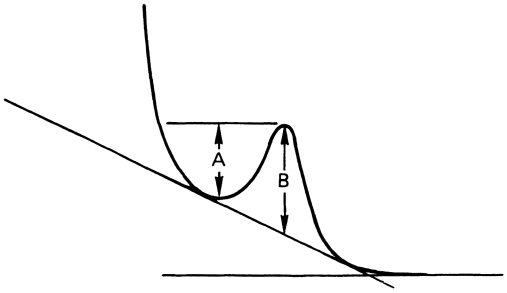

FIGURE 20 Illustration of A/B Ratio for a Small Component Peak on the Tail of a Large Peak.

Calculate a response factor for benzene (R_b) relative to the *Internal Standard* by the formula

$$\text{Result} = A_i/W_i \times W_b/A_b$$

in which A_i is the area of the *Internal Standard* peak in arbitrary units corrected for attenuation; W_i is the weight percent of *Internal Standard* in *Reference Solution A*; A_b is the area of the benzene peak in arbitrary units corrected for attenuation; and W_b is the weight percent of benzene in *Reference Solution A*.

Procedure: Place approximately 0.1 mL of *Internal Standard* into a tared 25-mL volumetric flask, weigh on an analytical balance, dissolve in and dilute with the sample to be analyzed to volume.

Using the exact instrumental conditions that were used in the calibration, inject the same volume of sample containing the *Internal Standard*. Before measuring the area of the *Internal Standard* and benzene peaks, change the attenuation to ensure at least 25% chart deflection.

Measure the area of the *Internal Standard* and benzene peaks in the same manner as was used for the calibration. Calculate the weight percent of benzene in the sample (W_B) by the formula

$$\text{Result} = (A_b \times R_b \times W_i \times 100)/(A_i \times S)$$

in which A_b is the area of the benzene peak corrected for attenuation; R_b is the relative response factor for benzene; W_i is the weight, in grams, of *Internal Standard* added; A_i is the area of the *Internal Standard* peak corrected for attenuation; and S is the weight, in grams of the sample taken.

System Suitability Test: Inject the same volume of *Reference Solution B* as in the *Calibration* and record the chromatogram. *n*-Decane must be eluted before benzene, and the ratio of A to B (*Fig. 19*) must be at least 0.5 where *A* is equal to the depth of the valley between the *n*-decane and benzene peaks and B is equal to the height of the benzene peak.

Column Materials and Conditions for the Determination of Benzene in Hexanes

Column No.	1	2	3	4	5	6	7
Liquid phase	CEF	PEF 200	CEF	DEGS	TCEPE	TCEPE	DEGS
Length, ft	15	6	16	10	15	100	12
m		4.5	2	5	3.1	—	313.7
Diameter, in (mm) Inside	0.07(1.8)	—	0.07	0.18(4.5)	0.06(1.5)	0.01(.254)	
Outside	1/8(3.2)	¼(6.4)	1/8	—	—	—	1/8
Weight, percent	17	30	20	20	10	—	20
Solid support	Chromosorb P	Chromosorb P	Chromosorb P	Chromosorb P	Chromosorb P	Capillary	Chromosorb P
Mesh	60–80	60–80	60–80	80–100	60–80	—	80–100
Treatment	AW	AW	AW	none	AW	none	AW Sil
Inlet, deg	200	210	250	260	250	275	260
Detector, deg	200	155	250	200	175	250	240
Column, deg	115	95	90	100	115	95	65
Carrier gas	N_2	He	He	He	N_2	N_2	He
Flow rate, cm^3/min	30	60	60	60	1	3	52
Detector	FI	TC	FI	FI	FI	FI	FI
Recorder, mV	5	1	1	1	10	1	1
Sample, 1	5	10	1	2	5	0.8	5
Split	9 + 1	—	—	—	100 + 1	100 – 1	—
Area	Tri	EI	DI	Tri Plan	EI	EI	Tri

Abbreviations Used in Table

AW—Acid washed; CEF—*N,N*-Bis(2-cyanoethyl)formamide; DEGS—Diethylene Glycol Succinate; DI—Disk integrator; EI—Electronic integrator; FI—Flame ionization; Sil—Silanized; TC—Thermal conductivity; TCEPE—Tetracyanoethylated Pentaerythritrol; Tri—Triangulation.

Retention Times in Minutes for Selected Hydrocarbons Under the Conditions for the Determination of Benzene in Hexanes

Column No.	1	2	3	4	5	6	7
Benzene	3.4	2.0	6.5	6.7	5.4	6.1	6.7
Toluene	4.4	3.2	9.0	10.3	7.8	7.0	10.3
Ethylbenzene	5.4	5.2	11.5	14.8	10.8	8.0	14.8
p-m-Xylenes	5.8	—	12.5	—	11.4	8.5	—
o-Xylene	7.5	6.8	17.0	16.1	14.5	10.0	—
n-Undecane	3.0	2.8	3.5	—	—	—	—
n-Dodecane	—	—	—	12.8	8.5	6.5	—

COLORS[4]

Chromium

Standards

Standard Chromium Solution (1000 mg/kg)*:* Transfer 2.829 g of $K_2Cr_2O_7$, accurately weighed (National Institute of Standards and Technology No. 136) into a 1-L volumetric flask; dissolve in and dilute with water to volume.

Standard Colorant Solution: Transfer 62.5 g of colorant previously shown to be free of chromium to a 1-L volumetric flask; dissolve in and dilute with water to volume.

Apparatus: Use any suitable atomic absorption spectrophotometer equipped with a fast response recorder and capable of measuring the radiation absorbed at 357.9 nm.

Instrument Parameters: *Wavelength setting*: 357.9 nm; *optical passes*: 5; *lamp current*: 8 mA; *lamp voltage*: 500 v; *fuel*: hydrogen; *oxidant*: air; *recorder*: l mv with a scale expansion of 5 or 10. Alternatively, follow the instructions supplied with the instrument.

Procedure: Set the instrument at the optimum conditions for measuring chromium as directed by the manufacturer's instructions. Prepare a series of seven standard chromium solutions containing Cr at approximately 5, 10, 15, 20, 40, 50, and 60 mg/kg by appropriate dilutions of the *Standard Chromium Solution* into 100-mL volumetric flasks; add 80 mL of the *Standard Colorant Solution*, and dilute each flask with water to volume.

Transfer 5 g of the colorant to be analyzed to a 100-mL volumetric flask; dissolve in and dilute with water to volume. Prepare a calibration curve using the series of standards, and using this curve, determine the chromium content of the colorant samples.

Ether Extracts

[**CAUTION—** Isopropyl ether forms explosive peroxides. To ensure the absence of peroxides, perform the following test: Prepare a colorless solution of ferrous thiocyanate by mixing equal volumes of 0.1 *N* ferrous sulfate and 0.1 *N* ammonium thiocyanate. Using titanous chloride, carefully discharge any red coloration due to ferric ions. Add 10 mL of ether to 50 mL of the solution, and shake vigorously for 2 to 3 min. A red color indicates the presence of peroxides. If redistillation is necessary, the usual precautions against peroxide detonation should be observed. Immediately before use, pass the ether through a 30-cm column of chromatography-grade aluminum oxide to remove peroxides and inhibitors.]

Apparatus: Use an upward displacement-type liquid–liquid extractor, as shown in *Fig. 21*, with a sintered-glass diffuser and a working capacity of 200 mL. Suspend a piece of bright copper wire through the condenser, and place a small coil of copper wire (about 0.5 g) in the distillation flask.

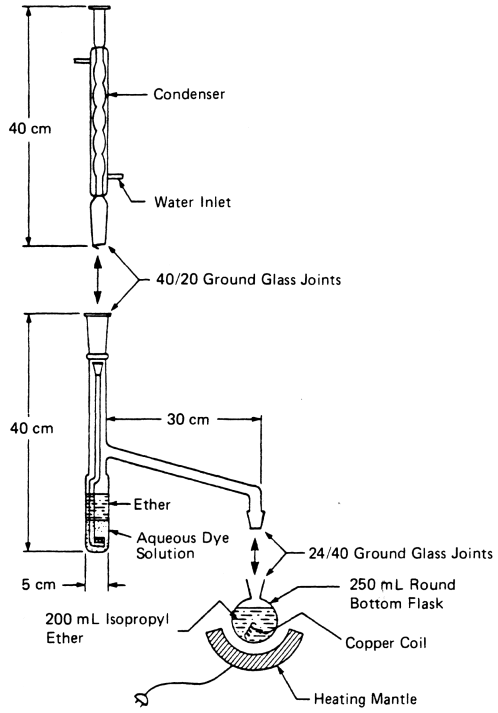

FIGURE 21 Upward Displacement-Type Liquid–Liquid Extractor with Sintered-Glass Diffuser.

Alkaline Ether Extract: Transfer 5 g of the colorant to a beaker, and dissolve in 150 mL of water. Add 2 mL of 2.5 *N* NaOH solution, transfer the solution into the extractor; and dilute with water to approximately 200 mL. Add 200 mL of ether to the distillation flask, and extract for 2 h with a reflux rate of about 15 mL/min. Set the extracted colorant solution aside. Transfer the ether extract into a separatory funnel, and wash with two 25-mL portions of 0.1 *N* NaOH followed by two 25-mL portions of water. Reduce the volume of the ether extract to about 5 mL by distillation (in portions) from a tared flask containing a small piece of clean copper coil.

Acid Ether Extract: Add 5 mL of 3 *N* hydrochloric acid to the extracted colorant solution set aside in the alkaline ether extract procedure above, mix, and extract with ether as directed above. Wash the ether extract with two 25-mL portions of 0.1 *N* hydrochloric acid and water. Transfer the washed ether in portions to the flask containing the evaporated alkaline extract, and carefully remove all the ether by distillation. Dry the residue in an oven at 85° for 20 min. Then allow the flask to cool in a desiccator for 30 min, and weigh. Repeat drying and cooling until a constant weight is obtained. The increase in weight of the tared flask, expressed as a percentage of the sample weight, is the combined ether extract.

Leuco Base

Reagents and Solutions

Cupric Chloride Solution: Transfer 10.0 g of $CuCl_2 \cdot 2H_2O$ to a 1-L volumetric flask; dissolve in and dilute with dimethylformamide (DMF) to volume.

Sample Solution: Prepare as directed in the individual monograph.

Procedure

Solution 1: Pipet 50 mL of DMF into a 250-mL volumetric flask, cover, and place in the dark.

Solution 2: Pipet 10 mL of the *Sample Solution* into a 250-mL volumetric flask, add 50 mL of DMF, and place in the dark.

Solution 3: Pipet 50 mL of *Cupric Chloride Solution* into a 250-mL volumetric flask, and gently bubble air through the solution for 30 min.

Solutions 4a and 4b: Pipet 10 mL of the *Sample Solution* into each of two 250-mL volumetric flasks, add 50 mL of *Cupric Chloride Solution* to each, and bubble air gently through the solutions for 30 min.

Dilute all of the solutions with water nearly to volume; incubate for 5 to 10 min, but no longer, in a water bath cooled with tap water; and dilute to volume. Record the spectrum for each solution between 500 nm and 700 nm using an absorbance range of 0 to 1 and a 1-cm pathlength cell; record all spectra on the same spectrogram.

Curve No.	Solution in Sample Cell	Solution in Reference Cell
I	1	1
II	1	2
III	3	3
IVa	3	4a
IVb	3	4b

Calculation

Click to View Image

in which the Roman numerals I through IV represent the absorbance readings for solutions of the corresponding Arabic numerals (above) at the wavelength maximum; a is the absorptivity (for Fast Green, a = 0.156 at 625 nm; for Brillant Blue, a = 0.164 at 630 nm); W is the weight, in grams, of the sample taken; and r is the ratio of the molecular weights of colorant and leuco base (for Fast Green, r = 0.9712; for Brillant Blue, r = 0.9706).

Mercury

Apparatus: The apparatus used for the direct microdetermination of mercury is shown in *Fig. 22*. It consists of a quartz combustion tube designed to hold a porcelain combustion boat (60 × 10 × 8 mm) and a small piece of copper oxide wire. The combustion tube is placed in a heavy-duty hinged combustion tube furnace (Lindburg Type 70T, or equivalent), and it is connected by clamped ball-joints at one end to a source of nitrogen and connected to a series of three traps at the other. The traps are constructed of a linear array of 18- × 2-mm Pyrex tubes connected by clamped ball-joints and extend from the connection at the combustion tube. Trap I contains anhydrous calcium sulfate packed between quartz-wool plugs, trap II contains Ascarite packed between cotton plugs, and trap III contains aluminum oxide packed between cotton plugs. The nitrogen flow forces the mercury through the combustion tube, the three traps, and a section of Tygon tube to a mercury vapor meter (Beckman model K-23, or equivalent). The mercury released from a sample during combustion is quantitated by comparing the recorder response with that given by a series of mercury standards.

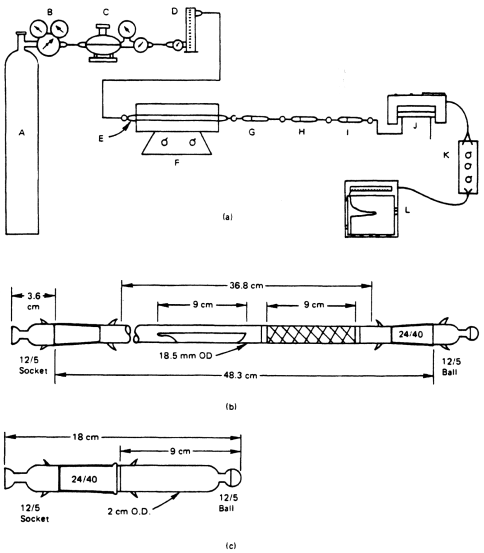

FIGURE 22 (a) Schematic Diagram of Apparatus for Photometric Mercury Vapor M Method:

A. Tank of nitrogen	G. Dehydrite trap
B. Two-stage pressure regulator	H. Ascarite trap
C. Low-pressure regulator	I. Aluminum oxide trap
D. Flowmeter	J. Mercury vapor meter
E. Combustion tube	K. Atenuator
F. Combustion-tube furnace	L. Recorder
(b) Quartz Combustion Tube with Boat and Copper Oxide Packing; (c) Schematic Diagram of Trap Used to Contain Ascarite, Dehydrite, and Aluminum Oxide.	

Reagents and Equipment

Absorbent Cotton

Aluminum Oxide: Anhydrous.

Calcium Sulfate: Anhydrous, dehydrate, or equivalent

Asbestos Pads, (1 × 0.5 × 1 cm)*:* Preheated at 800° for 1 h.

Ascarite: 20- to 30-mesh.

Copper Oxide Wire: Preheated at 850° for 2 h.

Nitrogen: Purified grade.

Quartz Wool

Sodium Carbonate: Anhydrous, fine granular.

Standard Solution: Transfer approximately 1.35 g of reagent-grade mercurous chloride, accurately weighed, into a 1-L volumetric flask. Dissolve in and dilute with water to volume. When diluted 100-fold, the solution contains 0.01 µg Hg per microliter (*Diluted Standard Solution*).

Procedure: Preheat the furnace to 650°, and adjust the nitrogen flow to 1 L/min.

Blank Analysis: Place a square piece of preheated asbestos pad in the combustion boat, and cover it with sodium carbonate. Stop the nitrogen flow, disconnect the ball-joint, quickly insert the boat into the combustion tube with large forceps, and reconnect the joint. Note the time, allow the boat to sit in the tube with no nitrogen flow for exactly 1 min, and then restart the flow of nitrogen. Mercury elutes almost immediately with the reinstated nitrogen flow; note the recorder response. Allow about 30 s between runs.

Calibration: Determine the recorder response after the application to the asbestos pad of 1, 2, and 3 µL of the *Diluted Standard Solution*.

Sample Analysis: Transfer 25 mg of colorant, accurately weighed, to the combustion boat, and cover the sample completely with sodium carbonate. Follow the procedure used for the *Blank Analysis* above, and calculate the mercury content using the standard curve.

Trap Problems: (1) Some colorants (e.g., Brillant Blue and Fast Green) may give a response that is symmetrically dissimilar to the Hg peak. If such a response "carries over" to the next sample, then the aluminum oxide trap may need to be changed. (2) If the recorder response is of inadequate sensitivity (peak height induced by 0.01 µg less than 0.5 cm), then the traps are packed too tightly. Remove or redistribute packing first in the aluminum oxide trap, then try the other traps. (3) The traps will need changing periodically as indicated by a change in the physical appearance of the trap material or by chart responses of different retention times or different symmetry from that of mercury standards. (4) If two or more standards are run in succession, a later sample might give an erroneous mercury response. Run blanks and then repeat the sample analysis to confirm the validity of the response.

Sodium Chloride

Dissolve approximately 2 g of colorant, accurately weighed, in 100 mL of water, and add 10 g of activated carbon that is free of chloride and sulfate. Boil gently for 2 to 3 min. Cool to room temperature, add 1 mL of 6 *N* nitric acid, and stir. Dilute with water to volume in a 200-mL volumetric flask, and then filter through dry paper. Repeat the treatment with 2-g portions of carbon until no color is adsorbed onto filter paper dipped into the filtrate.

Transfer 50 mL of filtrate to a 250-mL flask. Add 2 mL of 6 *N* nitric acid, 5 mL of nitrobenzene, and 10 mL of standardized 0.1 *N* silver nitrate solution. Shake the flask until the silver chloride coagulates. Prepare a saturated solution of ferric ammonium sulfate, and add just enough concentrated nitric acid to discharge the red color; add 1 mL of this solution to the 250-mL flask to serve as the indicator. Titrate with 0.1 *N* ammonium thiocyanate solution that has been standardized against the silver nitrate solution until the color persists after shaking for 1 min. Calculate the weight percent of sodium chloride, P, by the equation

$$P = [(V \times N)/W] \times 22.79$$

in which V is the net volume, in milliliters, of silver nitrate solution required; N is the normality of the silver nitrate solution; and W is the weight, in grams, of the sample taken. The factor 22.79 incorporates a total volume of 195 mL because 10 g of activated carbon occupies 5 mL.

Sodium Sulfate

Place 25 mL of the decolorized filtrate obtained from the *Sodium Chloride* test (above) into a 125-mL Erlenmeyer flask, and add 1 drop of a 0.5% phenolphthalein solution in 50% ethanol. Add 0.05 *N* sodium hydroxide, dropwise, until the solution is alkaline to pH paper, and then add 0.002 *N* hydrochloric acid until the indicator is decolorized. Add 25 mL of ethanol and about 0.2 g of tetrahydroquinone sulfate indicator. Titrate with 0.03 *N* barium chloride solution to a red endpoint. Make a blank determination.

Calculate the weight percent, P, of sodium sulfate by the equation

$$P = [(V - B) \times N/W] \times 55.4$$

in which V is the volume, in milliliters, of barium chloride solution required to titrate the sample; B is the volume, in milliliters, of barium chloride solution required for the blank; N is the normality of the barium chloride solution; and W is the weight, in grams, of the sample taken. The factor 55.4 incorporates a total volume of

195 mL because 10 g of activated carbon occupies 5 mL.

Total Color

Method I (Spectrophotometric)

Pipet 10.0 mL of the dissolved colorant into a 250-mL Erlenmeyer flask containing 90 mL of 0.04 *N* ammonium acetate, and mix well. Determine the net absorbance of the solution relative to water at the wavelength maximum given for each color. Calculate the percentage of colorant present using the following equation, which presumes a 1-cm pathlength cell:

$$\% \text{ total color} = (A \times 100)/(a \times W)$$

in which A is the absorbance; a is the absorptivity; and W is the weight, in grams, of the sample taken.

Method II (Titration with Titanium Chloride)

Apparatus: The apparatus for determining total color by titration with titanium chloride ($TiCl_3$) is shown in *Fig. 23*. It consists of a storage bottle, *A*, of 0.1 *N* titanium chloride titrant maintained under hydrogen produced by a Kipp generator; an Erlenmeyer flask, *B*, equipped with a source of CO_2 or N_2 to maintain an inert atmosphere in which the reaction takes place; a stirrer; and the buret, *C*.

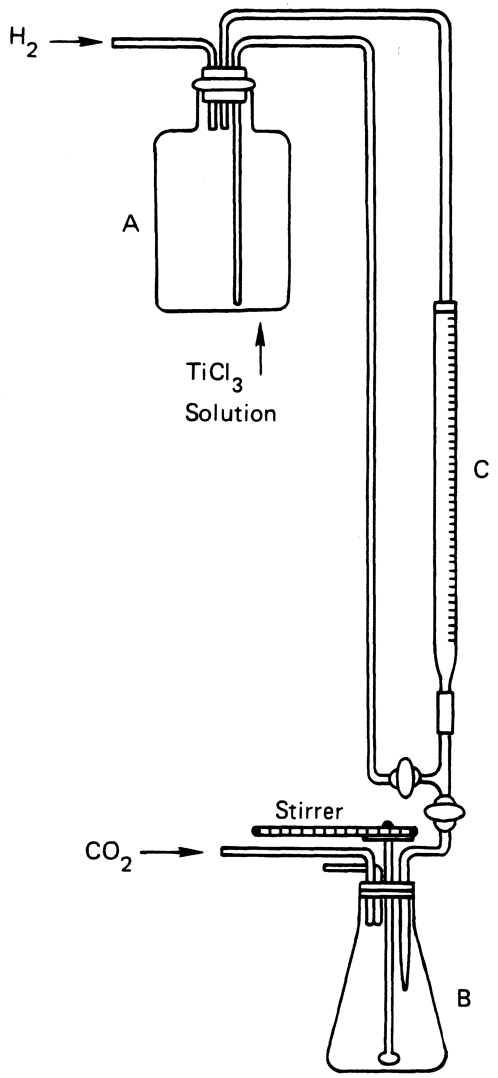

FIGURE 23 Titanous Chloride Titration Apparatus.

Reagents and Solutions

Titanium Chloride Solution (0.1 *N*)*:* Transfer 73 mL of commercially prepared 20% $TiCl_3$ solution into a storage bottle, and carefully add 82 mL of concentrated HCl per L of final solution. Mix well, and bubble CO_2 or N_2 through the solution for 1 h. Before standardizing, maintain the solution under a hydrogen atmosphere for at least 16 h using a Kipp generator.

Potassium Dichromate Solution (0.1 *N*, primary standard)*:* Transfer 4.9032 g of $K_2Cr_2O_7$ (National Institute of Standards and Technology No. 136) to a 1-L volumetric flask; dissolve in and dilute with water to volume.

Ammonium Thiocyanate (50%)*:* Transfer 500 g of NH_4SCN, ACS certified, to a 1-L volumetric flask; dissolve in about 600 mL of water, warming if necessary; and dilute to volume.

Ferrous Ammonium Sulfate: $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$, ACS certified.

Sodium Bitartrate

Standardization of the Titanium Chloride Solution: Drain any standing titanium chloride ($TiCl_3$) from the feed lines and buret, and refill with fresh solution. Add 3.0 g of *Ferrous Ammonium Sulfate* to a wide-mouth Erlenmeyer flask followed by 200 mL of water, 25 mL of 50% sulfuric acid, 25 mL of *0.1* N *Potassium*

Dichromate Solution (by pipet), and 2 or 3 boiling chips. Boil the solution vigorously on a hot plate for 30 s to remove dissolved air, then quickly transfer the flask to the titration apparatus, securely connect the stopper assembly, and start the carbon dioxide flow and stirrer. Pass carbon dioxide over the solution for 1 min before beginning the titration.

Add the *0.1* N *Titanium Chloride Solution* at a fast, steady drip to within 1 mL of the estimated endpoint (about 20 mL). Reduce the carbon dioxide flow, remove the solid-glass rod from the stopper assembly, pipet 10 mL of *Ammonium Thiocyanate* (50%) into the flask, insert the glass rod, and increase the carbon dioxide flow. Continue titrating slowly until the endpoint: A color change from brown-red to light green is observed. Perform a blank determination using the same reagents and quantities, and calculate the normality, N, of the *0.1* N *Titanium Chloride Solution* on the basis of three titrations by the equation

$$N = (V_r \times N_r/V_t - V_b)$$

in which V_r is the volume, in milliliters, of *0.1* N *Potassium Dichromate* used; N_r is the normality of the *0.1* N *Potassium Dichromate*; V_t is the volume, in milliliters, of *0.1* N *Titanium Chloride Solution* used; and V_b is the volume, in milliliters, of titanium dichloride used in the blank titration.

Procedure: Transfer the quantity of colorant prescribed in the individual monograph into a 500-mL wide-mouth Erlenmeyer flask and add 21 to 22 g of *Sodium Bitartrate* (sodium citrate for Sunset Yellow), 275 mL of water, and two or three boiling chips. Boil the solution vigorously on a hot plate for 30 s to remove dissolved air, then quickly transfer the flask to the titration apparatus, securely connect the stopper assembly, and start the carbon dioxide flow and stirrer. Pass carbon dioxide over the solution for 1 min before beginning the titration.

Titrate the sample until the color lightens, wait 20 s, and then continue the addition with about 2 s between drops. When the color is almost completely bleached, wait 20 s, and then continue the addition with 5 s between drops. A complete color change indicates the endpoint. Perform a blank determination using the same reagents and quantities, and calculate the total color, T, in percent and on the basis of three titrations, by the equation

$$T = [(V_t - V_b)/(W \times F_s)] \times 100 \times N$$

in which V_t is the volume of titrant used; V_b is the volume of titrant required to produce the endpoint in a blank; N is the normality of the titrant; W is the weight, in grams, of the sample taken, and F_s is a factor derived from the stoichiometry of the reaction characteristics of each colorant and is given in the individual monograph.

Method III (Gravimetric)

Transfer approximately 0.5 g of colorant, accurately weighed, to a 400-mL beaker, add 100 mL of water, and heat to boiling. Add 25 mL of 1:50 hydrochloric acid, and bring to a boil. Wash down the sides of the beaker with water, cover, and keep on a steam bath for several hours or overnight. Cool to room temperature, and quantitatively transfer the precipitate into a tared filtering crucible with 1:100 hydrochloric acid. Wash the precipitate with two 15-mL portions of water, and dry the crucible for 3 h at 135°. Cool in a desiccator, and weigh. Calculate the total color, *P*, in weight percent, by the equation

$$P = [(W_p \times F)/W_s] \times 100$$

in which W_p is the weight, in grams, of the precipitate; F is the gravimetric conversion factor given in the individual monograph; and W_s is the original weight, in grams, of the sample taken.

Uncombined Intermediates and Products of Side Reactions

Method I

Sample Solution: Transfer approximately 2 g of colorant to a 100-mL volumetric flask; dissolve in and dilute with water to volume.

Apparatus: Pack a 2.5- × 45-cm glass column with approximately 20 g of cellulose (Whatman CF-11 grade, or equivalent) that has been slurried in the eluant and from which the fines have been removed by decantation. Equilibrate the column thoroughly with the eluant, 35% ammonium sulfate.

Procedure: Pipet 5 mL of *Sample Solution* into a beaker containing 5 g of cellulose that has been slurried in eluant and from which the fines have been removed by decantation. Stir the mixture thoroughly, add 10 g of ammonium sulfate, and stir until uniformly mixed. Mix the slurry with 15 mL of eluant, and apply it to the column. Allow the fluid to enter the column, and wash the beaker with eluant until the sample is quantitatively transferred. Elute the column with approximately 500 mL of 35% ammonium sulfate, and collect a total of eight 60-mL fractions. Divide each collected fraction in half and add 0.5 mL of NH_4OH to one half and 0.5 mL of HCl to the other.

Calculation: After identifying each intermediate and side product by comparing spectra of the fractions with commercial standards, calculate the concentration, C, of each using the equation

$$C = A/(a \times b)$$

in which A is the absorbance at the wavelength of maximal absorption; b is the cell pathlength, in centimeters; and a is the absorptivity given in the individual monograph.

Method II

Apparatus: Use a suitable high-performance liquid chromatography system (see *Chromatography, Appendix IIA*) equipped with a dual wavelength detector system such that the effluent can be monitored serially at 254 nm and 325 to 385 nm (wide-band pass). Use a 1-m × 2.1-mm (id) column, or equivalent, packed with a strong anion-exchange resin (Dupont No. 830950405, or equivalent).

Operating Conditions: The operating conditions required may vary depending on the system used. The following conditions have been shown to give suitable results for Allura Red, Tartrazine, and Sunset Yellow.

Allura Red

Primary Eluant: 0.01 *M* aqueous $Na_2B_4O_7$.

Secondary Eluant: 0.20 *M* $NaClO_4$ in aqueous 0.01 *M* $Na_2B_4O_7$.

Sample Size: 20 µL of a 0.25% solution.

Flow Rate: 0.60 mL/min.

Gradient: Linear, in two phases: 0% to 18% in 40 min, 18% to 62% in 8 min more, then hold for 18 min more at 62%.

Temperature: 50°.

Pressure: 1000 psi.

Order of Elution: (1) Cresidinesulfonic acid (CSA); (2) unknown; (3) Schaeffer's salt (SS); (4) unknown; (5) 4,4′-diazoaminobis(5-methoxy-2-methylbenzenesulfonic acid) (DMMA); (6) unknown; (7) Allura Red; (8) 6,6′-oxybis(2-naphthalenesulfonic acid) (DONS).

Tartrazine

Primary Eluant: 0.01 *M* aqueous $Na_2B_4O_7$.

Secondary Eluant: 0.10 *M* $NaClO_4$ in aqueous 0.01 *M* $Na_2B_4O_7$.

Sample Size: 50 µL of a 0.15% solution, prepared within 13 min of injection.
Flow Rate: 1.00 mL/min.
Gradient: Exponential at 4%/min: 0.95%.

Temperature: 50°.
Pressure: 1000 psi.
Order of Elution: (1) Phenylhydrazine-*p*-sulfonic acid (PHSA); (2) sulfanilic acid (SA); (3) 1-(4-sulfophenyl)-3-ethylcarboxy-5-hydroxypyrazolone (PY-T); (4) 1-(4-sulfophenyl)-3-carboxy-5-hydroxypyrazolone (EEPT); (5) 4,4′-(diazoamino)-dibenzenesulfonic acid (DAADBSA).

Sunset Yellow

Primary Eluant: 0.01 *M* aqueous $Na_2B_4O_7$.

Secondary Eluant: 0.20 *M* $NaClO_4$ in aqueous 0.01 *M* $Na_2B_4O_7$.

Sample Size: 5 µL of a 1% solution.
Flow Rate: 0.50 mL/min.
Gradient: Linear in four phases: 0% to 11% in 10 min; hold 25 min; 11% to 38% in 10 min; 38% to 42% in 10 min; 42% to 98% in 20 min; hold 20 min.

Temperature: 50°.
Pressure: 1000 psi.
Order of Elution: (1) Sulfanilic acid (SA); (2) Schaeffer's salt (SS); (3) 4,4′-(diazoamino)-dibenzenesulfonic acid (DAADBSA); (4) *R*-salt dye; (5) Sunset Yellow; (6) 6,6′-oxybis(2-naphthalenesulfonic acid) (DONS).

Standard Solutions

Allura Red: Prepare a solution containing 0.25 g of colorant, 0.5 mg of CSA, 0.75 mg of SS, 0.25 mg of DMMA, and 1.25 mg of DONS in a 100-mL volumetric flask. Dissolve in and dilute with 0.1 *M* $Na_2B_4O_7$ to volume.

Tartrazine: Prepare a solution containing 0.15 g of colorant and 0.3 mg each of PHSA, SA, PY-T, EEPT, and DAADBSA in a 100-mL volumetric flask. Dissolve in and dilute with 0.1 *M* $Na_2B_4O_7$ to volume.

Sunset Yellow: Prepare a solution containing 0.25 g of colorant, 0.5 mg of SA, 0.75 mg of SS, 0.25 mg of DAADBSA, and 1.25 mg of DONS in a 100-mL volumetric flask. Dissolve in and dilute with 0.1 *M* $Na_2B_4O_7$ to volume.

Test Solutions: Prepare at least four test solutions, each containing the colorant, and one impurity, accurately weighed, dissolved in 0.1 *M* $Na_2B_4O_7$, and diluted to volume in a 100-mL volumetric flask. The solutions should encompass the range of concentrations, evenly spaced, given below for each constituent:

Allura Red (250 mg)*:* CSA (0.05 to 0.5 mg); SS (0.05 to 0.75 mg); DONS (0.5 to 2.5 mg); DMMA (0.025 to 0.25 mg). Inject 20 µL of each solution.
Tartrazine (150 mg)*:* SA (7.5 to 300 µg); PY-T (7.5 to 300 µg); EEPT (7.5 to 300 µg); DAADBSA (7.5 to 300 µg). Inject 50 µL of each solution.
Sunset Yellow (250 mg)*:* SA (0.05 to 0.5 mg); SS (0.05 to 0.75 mg); DONS (0.5 to 2.5 mg); DAADBSA (0.05 to 0.25 mg). Inject 20 µL of each solution.

System Suitability

Resolution: Elute the column, or equivalent, with the gradient specified under *Operating Conditions* until a

smooth baseline is obtained. Inject an aliquot of the *Standard Solution*. The resolution of the eluted components matches or exceeds that shown for the corresponding colorant (see *Figs. 24*, *25*, and *26*). After determining that the column, or equivalent, will give the required resolution, allow it to rest for 2 weeks before use.

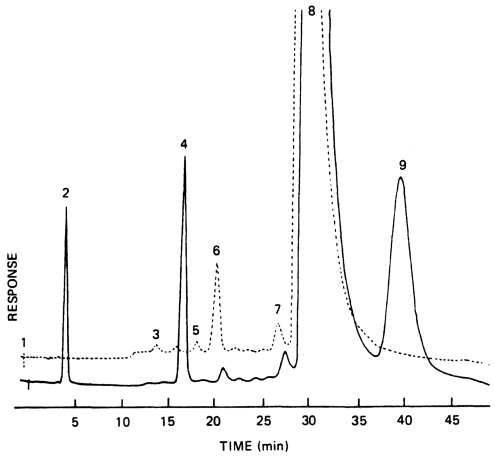

FIGURE 24 Allura Red–Top Trace: Eluant Monitored at 254 nm; Bottom Trace: Eluant Monitored at 375 to 385 nm.

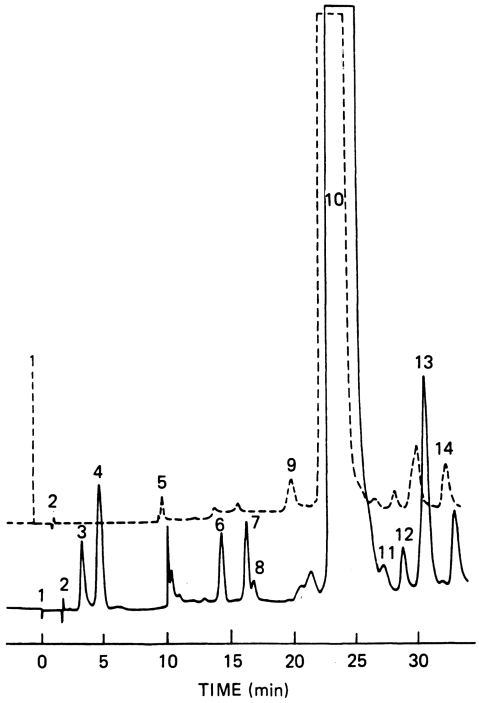

FIGURE 25 Tartrazine–Top Trace: Eluant Monitored at 254 nm; Bottom Trace: Eluant Monitored at 375 to 385 nm.

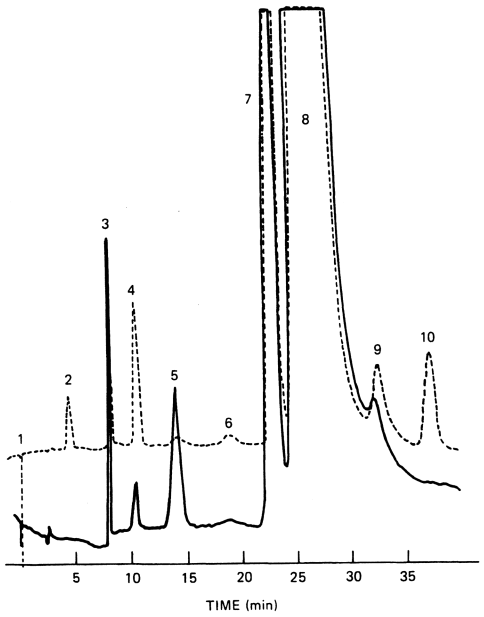

FIGURE 26 Sunset Yellow–Top Trace: Eluant Monitored at 254 nm; Bottom Trace: Eluant Monitored at 375 to 385 nm.

Calibration: Inject the designated volume of each *Test Solution* onto a conditioned column, and prepare a standard curve corresponding to each unreacted intermediate and side reaction product. Determine the area, *A*, for each peak from the integrator if an automated system is used or by multiplying the peak height by the width at one-half the height. The peak height alone may be used for EEPT, PY-T, and DAADBSA. Calculate the concentration, C_i, of each intermediate or side product using the equation

$$C_i = mA_i + b$$

in which A_i is the area of its corresponding chromatographic peak. Calculate the slope, m, and intercept, b, using the following linear regression equations:

$$m = [N\Sigma C_iA_i - \Sigma C_i\Sigma A_i]/[N\Sigma A_i^2 - [(\Sigma A_i)^2],$$

$$b = [\overline{A}]_i - m[\overline{C}]_i$$

in which $\overline{C}$ and $\overline{A}$ are the calculated averages of the concentrations and peak areas, respectively, used to construct the standard curve for one intermediate or side reaction product. Calculate the correlation coefficient, r, from the following equation:

$$r = [\Sigma(C_i - \overline{C})(A_i - A)]/[\Sigma(C_i - \overline{C}^2) \times \Sigma(A_i - \overline{A})^2].$$

Each time the system is calibrated, add the new data to those accumulated from previous analyses. The correlation coefficient must be between 0.95 and 1.00 for any single experiment or from accumulated data.

Recalibrate the system after every ten determinations or 2 days, whichever occurs first.

Sample Preparation: Prepare as directed in the individual monograph.

Procedure: Inject the volume of *Sample Preparation* as designated in the monograph into the column.

Determine the concentration of intermediates and side reaction products from the peak areas using the slope, m, and intercept, b, calculated under *Calibration* by the equation

$$C_S = mA_S + b$$

in which C_S is the concentration of the unknown in the *Sample Preparation* and A_S its corresponding peak area.

Loss on Drying (Volatile Matter)

Transfer 1.5 to 2.5 g of colorant, accurately weighed, to a tared crucible. Heat in a vacuum oven at 135° for 12 to 15 h. Lower the pressure in the oven to −125 mm Hg, and continue heating for an additional 2 h. Cover the crucible, and allow to cool in a desiccator. Reweigh the crucible when cool. The loss of weight is defined as the volatile matter.

Water-Insoluble Matter

Transfer about 1 g of colorant, accurately weighed, to a 250-mL beaker, and add 200 mL of boiling water. Stir to facilitate dissolution of the color.

Tare a filtering crucible equipped with a glass fiber filter (Reeve Angel, No. 5270, or equivalent). Filter the solution with the aid of suction when it has cooled to ambient temperature. Rinse the beaker three times, pouring the rinsings through the crucible. Wash the filter with water until the filtrate is colorless.

Dry the crucible and filter in an oven at 135° for at least 3 h, cool them in a desiccator and reweigh to the nearest 0.1 mg. Calculate the percent water-insoluble matter, I, by the equation

$$I = (W_C/W_S) \times 100$$

in which W_C is the difference in crucible weight and W_S is the sample weight.

GLUTAMIC ACID

Apparatus: Use an ion-exchange amino acid analyzer, equipped with sulfonated polystyrene columns, in which the effluent from the sample is mixed with ninhydrin reagent and the absorbance of the resultant color is measured continuously and automatically at 570 and 440 nm by a recording photometer.

Standard Solution: Transfer 1250 ± 2 mg of reagent-grade glutamic acid, accurately weighed, into a 500-mL volumetric flask. Fill the flask half-full with water, add 5 mL of hydrochloric acid to help dissolve the amino acid, dilute with water to volume, and mix. Prepare the standard for analysis by diluting 1 mL of this solution with 4 mL of 0.2 *N* sodium citrate, pH 2.2, buffer. This *Standard Solution* contains 0.5 mg of glutamic acid per milliliter (C_S).

Sample Preparation: Dilute 5 mg of sample, accurately weighed, to exactly 5 mL with 0.2 *N* sodium citrate, pH 2.2, buffer. Remove any insoluble material by centrifugation or filtration.

Procedure: Using 2-mL aliquots of the *Standard Solution* and *Sample Preparation*, proceed according to the apparatus manufacturer's instructions. From the chromatograms thus obtained, match the retention times produced by the *Standard Preparation* with those produced by the *Sample Solution*, and identify the peak

produced by glutamic acid. Record the area of the glutamic acid peak from the sample as A_U, and that from the standards as A_S.

Calculations: Calculate the concentration, C_A, in milligrams per milliliter, of glutamic acid in the *Sample Preparation* by the formula

$$A_U \times C_S/A_S$$

in which C_S is the concentration, in milligrams per milliliter, of glutamic acid in the *Standard Solution*.

Calculate the percent glutamic acid, on the basis of total protein, by the formula

$$(100 \times C_A)/(6.25 \times N_T)$$

in which N_T is the percent total nitrogen determined in the monograph *Assay*, and 6.25 is the conversion factor for protein and amino acids.

Calculate the percent glutamic acid in the sample by the formula

$$100 \times C_A/S_W$$

in which S_W is the weight, in milligrams, of the sample taken.

HYDROXYPROPOXYL DETERMINATION

Apparatus The apparatus for hydroxypropoxyl group determination is shown in *Fig. 27*.

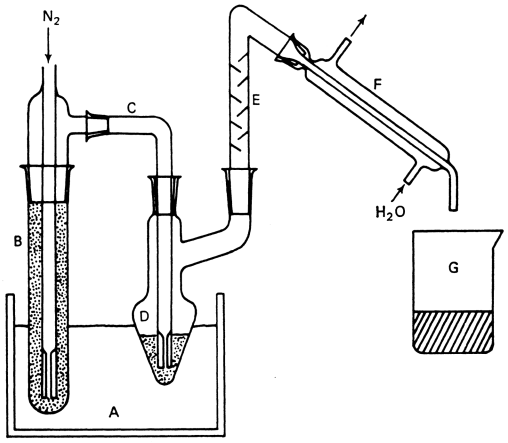

FIGURE 27 Apparatus for Hydroxypropoxyl Determination.

The boiling flask, *D*, is fitted with an aluminum foil-covered Vigreaux column, *E*, on the side arm 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° and the desired heating rate may be maintained. The distillate is collected in a 150-mL beaker, *G*, or other suitable container.

Procedure: Unless otherwise directed, transfer about 100 mg of the sample, previously dried at 105° for 2 h and accurately weighed, 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. Distill 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 2 *N* sulfuric acid, 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 color, confirming the endpoint 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) to the sodium thiosulfate titration (Y_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 methylcellulose (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 hydroxypropoxyl content of the sample, in milligrams, by the formula

$$75.0 \times [N_1(V_a - V_m) - kN_2(Y_a - Y_m)]$$

in which N_1 is the exact normality of the 0.02 *N* sodium hydroxide solution, N_2 is the exact normality of the 0.02 *N* sodium thiosulfate solution, and $k = V_bN_1/Y_bN_2$.

The percentage of substitution, by weight, of hydroxypropoxyl groups, determined as directed above, may be converted to molecular substitution per glucose unit by reference to *Fig. 28*.

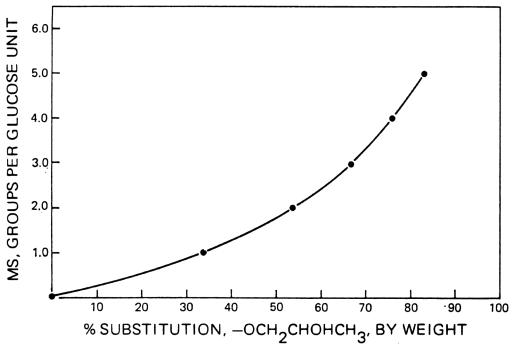

FIGURE 28 Chart for Converting Percentage of Substitution, by Weight, of Hydroxypropoxyl Groups to Molecular Substitution per Glucose Unit.

METHOXYL DETERMINATION

Apparatus: The apparatus for methoxyl determination, as shown in *Fig. 29*, consists of a boiling flask, *A*, fitted

with a capillary side arm to provide an inlet for carbon dioxide and connected to a column, *B*, which separates aqueous hydriodic acid from the more volatile methyl iodide. After the methyl iodide passes through a suspension of aqueous red phosphorus in the scrubber trap, *C*, it is absorbed in the bromine–acetic acid absorption tube, *D*. The carbon dioxide is introduced from a device arranged to minimize pressure fluctuations and connected to the apparatus by a small capillary containing a small plug of cotton.

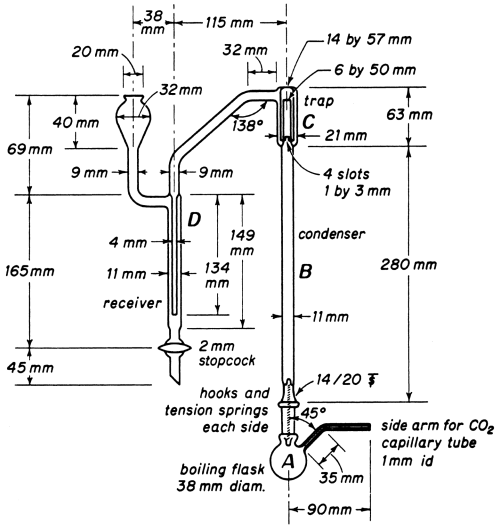

FIGURE 29 Distillation Apparatus for Methoxyl Determination.

Reagents

Acetic Potassium Acetate: Dissolve 100 g of potassium acetate in 1000 mL of a mixture consisting of 900 mL of glacial acetic acid and 100 mL of acetic anhydride.

Bromine–Acetic Acid Solution: On the day of use, dissolve 5 mL of bromine in 145 mL of the *Acetic Potassium Acetate* solution.

Hydriodic Acid: Use special-grade hydriodic acid suitable for alkoxyl determinations, or purify reagent grade as follows: Distill over red phosphorus in an all-glass apparatus, passing a slow stream of carbon dioxide through the apparatus until the distillation is terminated and the receiving flask has completely cooled.

[**CAUTION—** Use a safety shield, and conduct the distillation in a fume hood.]

Collect the colorless, or almost colorless, constant-boiling acid distilling between 126° and 127°. Store the acid in a cool, dark place in small, brown, glass-stoppered bottles previously flushed with carbon dioxide and finally sealed with paraffin.

Procedure: Fill trap *C* half-full with a suspension of about 60 mg of red phosphorus in 100 mL of water, introduced through the funnel on tube *D* and the side arm that connects with the trap at *C*. Rinse tube *D* and the side arm with water, collecting the rinsings in trap *C*, then charge absorption tube *D* with 7 mL of *Bromine–Acetic Acid Solution*. Place the sample, previously accurately weighed in a tared gelatin capsule, into the boiling flask *A*, along with a few glass beads or boiling stones, then add 6 mL of *Hydriodic Acid*. Connect the flask to the condenser, using a few drops of the acid to seal the junction, and begin passing the carbon dioxide through the apparatus at the rate of about two bubbles per second. Heat the flask in an oil bath at 150°, continue the reaction for 40 min, and drain the contents of absorption tube *D* into a 500-mL Erlenmeyer flask containing 10 mL of a 1:4 solution of sodium acetate. Rinse tube *D* with water, collecting the rinsings in the flask, and dilute with water to about 125 mL. Discharge the red-brown color of bromine by adding formic acid,

dropwise with swirling, then add 3 drops in excess. Usually a total of 12 to 15 drops of formic acid is required. Allow the flask to stand for 3 min, add 15 mL of 2 *N* sulfuric acid and 3 g of potassium iodide, and titrate immediately with 0.1 *N* sodium thiosulfate, adding starch TS near the endpoint. Perform a blank determination with the same quantities of the same reagents, including the gelatin capsule, and in the same manner, and make any necessary correction. Each milliliter of 0.1 *N* sodium thiosulfate is equivalent to 0.517 mg (517 µg) of methoxyl groups ($-OCH_3$).

NITROGEN DETERMINATION (Kjeldahl Method)

[**CAUTION—** Provide adequate ventilation in the laboratory, and do not permit accumulation of exposed mercury.]

[Note—All reagents should be nitrogen free, where available, or otherwise very low in nitrogen content.]

Method I

Use this method unless otherwise directed in the individual monograph. It is not applicable to certain nitrogen-containing compounds that do not yield their entire nitrogen upon digestion with sulfuric acid.

Nitrites and Nitrates Absent

Unless otherwise directed, transfer from 700 mg to 2.2 g of sample into a 500- to 800-mL Kjeldahl digestion flask of hard, moderately thick, well-annealed glass, wrapping the sample, if solid or semisolid, in nitrogen-free filter paper to facilitate the transfer if desired. Add 700 mg of mercuric oxide or 650 mg of metallic mercury, 15 g of powdered potassium sulfate or anhydrous sodium sulfate, and 25 mL of 93% to 98% sulfuric acid. (If a sample weight greater than 2.2 g is used, increase the sulfuric acid by 10 mL for each additional gram of sample.) Place the flask in an inclined position, and heat gently until frothing ceases, adding a small amount of paraffin, if necessary, to reduce frothing.

[**CAUTION—** The digestion should be conducted in a fume hood, or the digestion apparatus should be equipped with a fume exhaust system.]

Boil briskly until the solution clears, and then continue boiling for 30 min longer (or for 2 h for samples containing organic material). Cool, add about 200 mL of water, mix, and then cool to below 25°. Add 25 mL of sulfide or thiosulfate solution (40 g of K_2S, 40 g of Na_2S, or 80 g of $Na_2S_2O_3 \cdot 5H_2O$ in 1000 mL of water), and mix to precipitate the mercury. Add a few granules of zinc to prevent bumping, tilt the flask, and cautiously pour sodium hydroxide pellets or a 2:5 solution of sodium hydroxide down the inside of the flask so that it forms a layer under the acid solution, using a sufficient amount (usually about 25 g of solid NaOH) to make the mixture strongly alkaline. Immediately connect the flask to a distillation apparatus consisting of a Kjeldahl connecting bulb and a condenser, the delivery tube of which extends well beneath the surface of a measured excess of 0.5 *N* hydrochloric or sulfuric acid contained in a 500-mL flask. Add from 5 to 7 drops of methyl red indicator (1 g of methyl red in 200 mL of alcohol) to the receiver flask. Rotate the Kjeldahl flask to mix its contents thoroughly, and then heat until all of the ammonia has distilled, collecting at least 150 mL of distillate. Wash the tip of the delivery tube, collecting the washings in the receiving flask, and titrate the excess acid with 0.5 *N* sodium hydroxide. Perform a blank determination, substituting 2 g of sucrose for the sample, and make any necessary correction (see *General Provisions*). Each milliliter of 0.5 *N* acid consumed is equivalent to 7.003 mg of nitrogen.

[Note—If the substance to be determined is known to have a low nitrogen content, 0.1 *N* acid and alkali may be used, in which case each milliliter of 0.1 *N* acid consumed is equivalent to 1.401 mg of nitrogen.]

Nitrites and Nitrates Present:

[Note—This procedure is not applicable to liquids or to materials having a high chlorine-to-nitrate ratio.] Unless otherwise directed, transfer from 700 mg to 2.2 g of sample into a Kjeldahl flask, and add 40 mL of 93% to 98% sulfuric acid containing 2 g of salicylic acid. Mix thoroughly by shaking, and then allow to stand for 30 min or more, with occasional shaking. Add 5 g of $Na_2S_2O_3 \cdot 5H_2O$, or 2 g of zinc dust (as an impalpable powder, not granules or filings), shake, and allow to stand for 5 min. Heat over a low flame until frothing ceases, then remove the heat, add 700 mg of mercuric oxide (or 650 mg of metallic mercury) and 15 g of powdered potassium sulfate (or anhydrous sodium sulfate), and boil briskly until the solution clears. Continue boiling for 30 min longer (or for 2 h for samples containing organic material), and then continue as directed under *Nitrates and Nitrates Absent*, beginning with "Cool, add about 200 mL of water…"

Method II (Semimicro)

[Note—Automated instruments may be used in place of this manual method, provided the automated equipment has been properly calibrated.]

Transfer an accurately weighed or measured quantity of sample, equivalent to about 2 or 3 mg of nitrogen, into the digestion flask of a semimicro Kjeldahl apparatus. Add 1 g of a 10:1 powdered mixture of potassium sulfate:cupric sulfate, using a fine jet of water to wash down any material adhering to the neck of the flask, and then pour 7 mL of sulfuric acid down the inside wall of the flask to rinse it. Cautiously add down the inside of the flask 1 mL of 30% hydrogen peroxide, swirling the flask during the addition.

[**CAUTION—** Do not add any peroxide during the digestion.]

Heat over a free flame or an electric heater until the solution has attained a clear blue color and the walls of the flask are free from carbonized material. Cautiously add 20 mL of water, cool, then add through a funnel 30 mL of a 2:5 solution of sodium hydroxide, and rinse the funnel with 10 mL of water. Connect the flask to a steam distillation apparatus, and immediately begin the distillation with steam. Collect the distillate in 15 mL of a 1:25 solution of boric acid to which has been added 3 drops of methyl red–methylene blue TS and enough water to cover the end of the condensing tube. Continue passing the steam until 80 to 100 mL of distillate has been collected, then remove the absorption flask, rinse the end of the condenser tube with a small quantity of water, and titrate with 0.01 *N* sulfuric acid. Each milliliter of 0.01 *N* acid is equivalent to 140 µg of nitrogen.

When more than 2 or 3 mg of nitrogen is present in the measured quantity of the substance to be determined, 0.02 or 0.1 *N* sulfuric acid may be used in the titration if at least 15 mL of titrant is required. If the total dry weight of the material taken is greater than 100 mg, increase proportionately the quantities of sulfuric acid and sodium hydroxide added before distillation.

Add the following:

▲SPECTROPHOTOMETRIC IDENTIFICATION TESTS

Spectrophotometric tests contribute meaningfully toward the identification of many compendial chemical substances. The test procedures that follow are applicable to substances that absorb IR and/or UV radiation. The IR absorption spectrum of a substance, compared with that obtained concomitantly for the corresponding USP Reference Standard, provides perhaps the most conclusive evidence of the identity of the substance that can be realized from any single test. The UV absorption spectrum, on the other hand, does not exhibit a high degree of specificity. Conformance with both IR absorption and UV absorption test specifications, leaves little

doubt, if any, regarding the identity of the specimen under examination.

Infrared Spectra: This test is used for comparison of an IR spectrum for a sample specimen with a reference spectrum provided in the individual monograph.

Sample specimens should be prepared using the same technique as that used for the provided spectra. Unless otherwise noted in the individual monograph or spectrum caption, the spectra for liquid substances were obtained on neat liquids contained in fixed-volume sodium chloride cells or between salt plates. Spectra for solid substances were obtained on a potassium bromide pellet or a mineral oil (Nujol or equivalent) dispersion, as indicated in individual monographs or spectrum caption.

Infrared Absorption: This test is used for comparison of the IR spectrum of a sample specimen with that of a physical USP Reference Standard.

Sample and USP Reference Standard specimens should both be prepared for analysis using the same technique, as directed in the individual monographs, which use the below letter designations. Sample and USP Reference Standard specimens should be used as either dried or undried specimens as directed on the Reference Standard Label.

Record the spectra of the sample and USP Reference Standard specimens over the range of about 2.6 µm to 15 µm (3800 cm^{-1} to 650 cm^{-1}) unless otherwise specified in the individual monograph.

Designation	Specimen Preparation Technique
A	Intimately in contact with an internal reflection element for attenuated total reflectance (ATR) analysis
E	Pressed as a thin sample against a suitable plate for IR microscopic analysis
F	Suspended neat between suitable (for example sodium chloride or potassium bromide) plates
K	Mixed intimately with potassium bromide and compressed into a translucent pellet
M	Finely ground and dispersed in mineral oil
S	A solution of designated concentration, prepared as directed in the individual monograph, and examined in 0.1-mm cells (unless otherwise specified in the individual monograph)

[Note—A and E techniques can be used as alternative methods for K, M, F, and S where testing is performed qualitatively and the reference standard spectra are similarly obtained.]

Ultraviolet Absorption: The test is used for comparison of the UV spectrum of a sample specimen with that of a physical USP Reference Standard.

Sample and USP Reference Standard specimens should be prepared using the solvents and concentrations specified in the individual monograph.

Record the spectra of the sample and USP Reference Standard solutions concomitantly using 1-cm cells over the spectral range of 200 to 400 nm, unless otherwise indicated in the individual monograph. Calculate absorptivities and/or absorbance ratios where these criteria are included in an individual monograph. Unless otherwise specified, absorbances indicated for these calculations are those measured at the maximum absorbance at the wavelength specified in the individual monograph. Where the absorbance is to be measured at a wavelength other than that of maximum absorbance, the abbreviations (min) and (sh) are used to indicate a minimum and shoulder, respectively, in an absorbance spectrum.

SULFUR (by Oxidative Microcoulometry) (Based on ASTM D3120)[5]

[Note—All reagents used in this test should be reagent grade; water should be of high purity, and gases must be high-purity grade.]

Apparatus: Use the Dohrmann Microcoulometric Titrating System (MCTS-30), or equivalent (shown in *Fig. 30*), unless otherwise specified in an individual monograph. It consists of a constant rate injector, *A*, a pyrolysis furnace, *B*, a quartz pyrolysis tube, *C*, a granular-tin scrubber, *D*, a titration cell, *E*, and a microcoulometer with a digital readout, *F*.

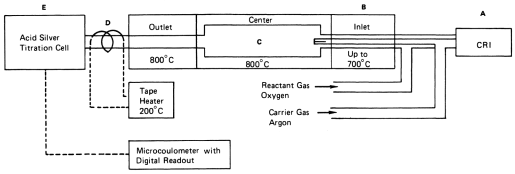

FIGURE 30 Microcoulometric Titrating System for the Determination of Sulfur in Hexanes.

Granular-Tin Scrubber: Place 5 g of 20- to 30-mesh granular reagent-grade tin between quartz-wool plugs in an elongated 18/9-12/5 standard-taper adaptor that connects the pyrolysis tube and the titration cell.

Microcoulometer: Must have variable attenuation; gain control; and be capable of measuring the potential of the sensing-reference electrode pair, and comparing this potential with a bias potential, amplifying the potential difference, and applying the amplified difference to the working-auxiliary electrode pair to generate a titrant. Also the microcoulometer output voltage signal must be proportional to the generating current.

Pyrolysis Furnace: The sample should be pyrolyzed in an electric furnace having at least two separate and independently controlled temperature zones, the first being an inlet section that can maintain a temperature sufficient to volatilize all the organic sample. The second zone is a pyrolysis section that can maintain a temperature sufficient to pyrolyze the organic matrix and oxidize all the organically bound sulfur. A third outlet temperature zone is optional.

Pyrolysis Tube: Must be fabricated from quartz and constructed in such a way that a sample, which is vaporized completely in the inlet section, is swept into the pyrolysis zone by an inert gas where it mixes with oxygen and is burned. The inlet end of the tube shall hold a septum for syringe entry of the sample and side arms for the introduction of oxygen and inert gases. The center or pyrolysis section should be of sufficient volume to ensure complete pyrolysis of the sample.

Sampling Syringe: A microlitre syringe of 10-µL capacity capable of accurately delivering 1 to 10 µL of sample into the pyrolysis tube. Three-inch × 24-gauge needles are recommended to reach the inlet zone of the pyroloysis furnace.

Titration Cell: Must contain a sensor-reference pair of electrodes to detect changes in triiodide ion concentration and a generator anode–cathode pair of electrodes to maintain constant triiodide ion concentration and an inlet for a gaseous sample from the pyrolysis tube. The sensor electrode shall be platinum foil and the reference electrode platinum wire in saturated triiodide half-cell. The generator anode and cathode half-cell shall also be platinum. The titration cell shall be placed on a suitable magnetic stirrer.

Preparation of Apparatus: Carefully insert the quartz pyrolysis tube into the furnace, attach the tin scrubber, and connect the reactant and carrier-gas lines. Add the *Cell Electrolyte Solution* (see below) to the titration cell, and flush the cell several times. Maintain an electrolyte level of 3.2 to 6.6 mm above the platinum electrodes. Place the titration cell on a magnetic stirrer, and connect the cell inlet to the tin scrubber outlet. Position the platinum-foil electrodes (mounted on the movable cell head) so that the gas-inlet flow is parallel to

the electrodes with the generator anode adjacent to the generator cathode. Assemble and connect the coulometer in accordance with the manufacturer's instructions. Double-wrap the adaptor containing the tin scrubber with heating tape and turn the heating tape on. Adjust the flow of the gases, the pyrolysis furnace temperature, the titration cell, and the coulometer to the desired operating conditions. Typical operating conditions are as follows:

Reactant gas flow (oxygen), cm^3/min	200
Carrier-gas flow (Ar, He), cm^3/min	40
Furnace temperature, °C Inlet zone	700 (maximum)
Pyrolysis zone	800 to 1000
Outlet zone	800 (maximum)
Tin-scrubber temperature, °C	200
Titration cell	Stirrer speed set to produce slight vortex
Coulometer	
Bias voltage, mV	160
Gain	50
Constant Rate Injector, µL/s	0.25

The tin scrubber must be conditioned to sulfur, nitrogen, and chlorine before quantitative analysis can be achieved. A solution containing 10 mg/kg butyl sulfide, 100 mg/kg pyridine, and 200 mg/kg chlorobenzene in isooctane has proven an effective conditioning agent. With a fresh scrubber installed and heated, two 30-µL samples of this conditioning agent injected at a flow rate of 0.5 µL/s produce a steadily increasing response, with final conditioning indicated by a constant reading from the offset during the second injection.

Reagents

Argon or Helium (Argon preferred)**:** High-purity grade, used as the carrier gas. Two-stage gas regulators must be used.

*Cell Electrolyte Solution***:** Dissolve 0.5 g of potassium iodide and 0.6 g of sodium azide in 500 mL of high-purity water, add 5 mL of glacial acetic acid and dilute to 1 L. Store in a dark bottle or in a dark place and prepare fresh at least every 3 months.

*Oxygen***:** High-purity grade, used as the reactant gas.

*Iodine***:** Resublimed, 20-mesh or less, for saturated reference electrode.

Sulfur Standard (approximately 100 mg/kg)**:** Transfer 0.1569 g of *n*-butyl sulfide, accurately weighed, into a tared 500-mL volumetric flask. Dilute to the mark with isooctane, and reweigh. Calculate the sulfur concentration (S), in percent, by the formula

$$S = W_b/W_s \times 2.192 \times 10^5$$

in which W_b is the weight of *n*-butyl sulfide and W_s is the weight of the solution.

Calibration: Prepare a calibration standard (approximately 5 mg/kg) by pipetting 5 mL of *Sulfur Standard* into a 10-mL volumetric flask and diluting with isooctane to volume. Fill and clamp the syringe onto the constant-

rate injector, push the sliding carriage forward to penetrate the septum with the needle, and zero the meter in case of long-term drift in the automatic baseline zero circuitry. Switch S_1 automatically starts the stepper-motor syringe drive and initiates the analysis cycle. At 2.5 min (before the 3-min meter hold point) set the digital meter with the scan potentiometer to correspond to the sulfur content of the known standard to the nearest 0.01 mg/kg. At the 3-min point, the number displayed on the meter stops, the plunger drive block is retracted to its original position, as preset by switch S_2, and a baseline re-equilibration period equal to the injection period elapses before a ready light and a beeper indicate that a new sample may be injected. Repeat the *Calibration* step a total of at least four times.

Procedure: Rinse the syringe several times with sample; then fill it, clamp it onto the constant-rate injector, push the sliding carriage forward to penetrate the septum with the needle, and zero the meter. Turn on switch S_1 to start the stepper-motor syringe drive automatically and initiate the analysis cycle. After the 3-min hold point, the number displayed on the meter corresponds to the sulfur content of the injected sample.

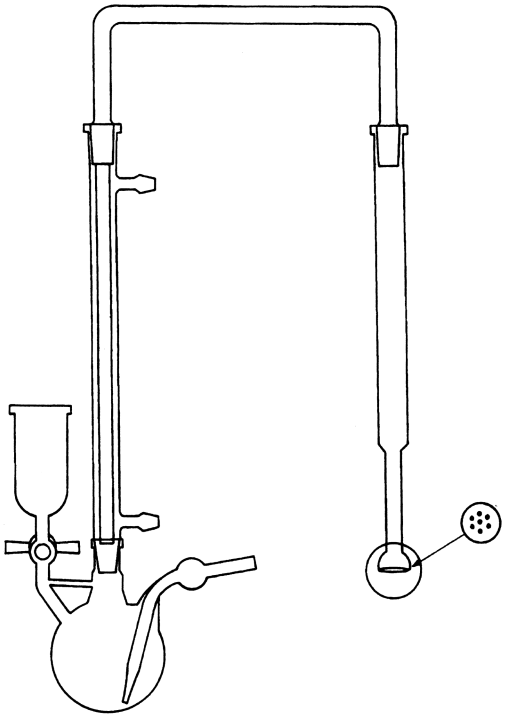

FIGURE 31 Raney Nickel Reduction Apparatus.

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[1] If the sample fails the test because of known or suspected interference, another aliquot may be run on a 6-ft × 2-mm (id) column, or equivalent, of 0.2% Carbowax 1500 on Carbopak C, operating at 100° isothermal, with 20 mL/min of helium carrier flow. Under these conditions, the 1,4-dioxane elutes in about 4 min.

[2] If a sample solution was prepared initially to ensure sample homogeneity, this is the weight of the original sugar digested (not the weight of the solution).

[3] Suitable nickel standards are available from, e.g. Fisher Scientific, Fair Lawn, NJ (nickel, reference standard solution, 1000 ppm ± 1%, certified, application: for atomic absorption) or RICCA Chemical Company, Arlington, TX (nickel standard, 1000 ppm Ni, for atomic absorption).

[4] To be used or sold for use to color food that is marketed in the United States, color additives must be from batches that have been certified by the U.S. Food and Drug Administration (FDA). If color additives are not from FDA-certified batches, they are not permitted color additives for food use in the United States, even if they are compositionally equivalent. The FD&C names can be applied only to FDA-certified batches of these color additives.

[5] Adapted from ASTM D3120 Standard Test Method for Trace Quantities of Sulfur in Light Liquid Petroleum Hydrocarbons by Oxidative Microcoulometry. The original ASTM method is available in its entirety from ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428. Phone: 610-832-9585, Fax: 610-832-9555, Email: service@astm.org, Website: www.astm.org.

BRIEFING

APPENDIX VII: FATS AND RELATED SUBSTANCES, *FCC 6* page 1146 and *FCC Forum* [June 2009]. On the basis of comments received for the proposed new monographs for *DHA Algal Oil, Crypthecodinium Type* and *DHA Algal Oil, Schizochytrium Type*, and for the purpose of harmonizing these two proposed monographs with the *USP-NF* proposed monographs for *Crypthecodinium Cohnii Oil* and *Schizochytrium Oil* in *Pharmacopeial Forum* 35(4) [Jul.–Aug. 2009], it is proposed to add the following two tests. See Briefings under *DHA Algal Oil, Crypthecodinium Type* and *DHA Algal Oil, Schizochytrium Type*.

1. A separate method for determining fatty acid composition [*Fatty Acid Composition (Saturated, cis-Monounsaturated, and cis-Polyunsaturated) in Oils Containing Long Chain Polyunsaturated Fatty Acids*] is proposed based on AOCS methods Ce 1i-07 and Ce 2-66. Comments received indicate that this method works better for oils containing long chain polyunsaturated fatty acids than the existing *Fatty Acid Composition* method in *Appendix VII*.
2. A method for determining *Anisidine Value* is proposed. This method is consistent with the test for *Anisidine Value* appearing in the general test chapter *Fats and Fixed Oils* ⟨ 401 ⟩ in *USP 32-NF27.*

(FIEC: K. Bowman) C79051

APPENDIX VII: FATS AND RELATED SUBSTANCES

ACETYL VALUE

Change to read:

(Based on AOCS Method Cd 4-40▲1▲*FCC7*)

The acetyl value is defined as the number of mg of potassium hydroxide required to neutralize the acetic acid obtained by saponifying 1 g of the acetylated sample.

Acetylation: Boil 50 mL of the oil or melted fat with 50 mL of freshly distilled acetic anhydride for 2 h under a reflux condenser. Pour the mixture into a beaker containing 500 mL of water, and boil for 15 min, bubbling a stream of nitrogen or carbon dioxide through the mixture to prevent bumping. Cool slightly, remove the water, add another 500 mL of water, and boil again. Repeat for a third time with another 500-mL portion of water, and remove the wash water, which should be neutral to litmus. Transfer the acetylated fat to a separator, and wash with two 200-mL portions of warm water, separating as much as possible of the wash water each time. Transfer the washed sample to a beaker, add 5 g of anhydrous sodium sulfate, and let stand for 1 h, agitating occasionally to assist drying. Pass the oil through a dry filter paper, preferably in an oven at 100° to 110°, and keep the filtered oil in the oven until it is completely dry. The acetylated product should be a clear, brilliant oil.

Saponification: Weigh accurately from 2 to 2.5 g each of the acetylated oil and of the original, untreated sample into separate 250-mL Erlenmeyer flasks. Add to each flask 25.0 mL of 0.5 N alcoholic potassium hydroxide, and continue as directed in the *Procedure* under *Saponification Value*, in this Appendix, beginning

with “Connect an air condenser. . . .” Record the saponification value of the untreated sample as S, and that of the acetylized oil as S′, then calculate the acetyl value of the sample by the formula:

$$\text{Result} = (S' - S)/(1.000 - 0.00075S)$$

ACID VALUE

Change to read:

(Based on AOCS Methods Te 1a-64▲1 ▲*FCC7* and Cd 3d-63▲1 ▲*FCC7*)

The acid value is defined as the number of mg of potassium hydroxide required to neutralize the fatty acids in 1 g of the test substance.

Method I (Commercial Fatty Acids)

Unless otherwise directed, weigh accurately about 5 g of the sample into a 500-mL Erlenmeyer flask, and dissolve it in 75 to 100 mL of hot alcohol, previously boiled and neutralized to phenolphthalein TS with sodium hydroxide. Agitation and further heating may be necessary to effect complete solution of the sample. Add 0.5 mL of phenolphthalein TS, and titrate immediately, while shaking, with 0.5 N sodium hydroxide to the first pink color that persists for at least 30 s. Calculate the acid value by the formula:

$$\text{Result} = 56.1V \times N/W$$

in which V is the volume, in mL, and N is the normality, respectively, of the sodium hydroxide solution; and W is the weight, in g, of the sample taken.

Method II (Animal Fats and Vegetable and Marine Oils)

Prepare a solvent mixture consisting of equal parts, by volume, of isopropyl alcohol and toluene. Add 2 mL of a 1% solution of phenolphthalein in isopropyl alcohol to 125 mL of the mixture, and neutralize with alkali to a faint but permanent pink color. Weigh accurately the appropriate amount of well-mixed liquid sample indicated in the table below, dissolve it in the neutralized solvent mixture, warming if necessary, and shake vigorously while titrating with 0.1 N potassium hydroxide to the first permanent pink color of the same intensity as that of the neutralized solvent before mixing with the sample. Calculate the acid value by the formula:

$$\text{Result} = 56.1V \times N/W$$

in which V is the volume, in mL, and N is the normality, respectively, of the potassium hydroxide solution; and W is the weight, in g, of the sample taken.

Acid Value	Sample Weight (g)
0–1	20
1–4	10
4–15	2.5
15–75	0.5
75 and over	0.1

Add the following:

▪ANISIDINE VALUE

The Anisidine Value is defined as 100 times the optical density measured in a 1-cm cell of a solution containing 1 g of the substance to be examined in 100 mL of a mixture of solvents and reagents according to the method below. [Note—Carry out the operations as rapidly as possible, avoiding exposure to actinic light.]

Reagents and Solutions

Test Solution A: Dissolve 0.500 g of the substance to be examined in isooctane, and dilute with isooctane to 25.0 mL.

Test Solution B: To 5.0 mL of *Test Solution A* add 1.0 mL of a 2.5 g/L solution of *p*-anisidine in glacial acetic acid, shake, and store protected from light.

Standard Solution: To 5.0 mL of isooctane add 1.0 mL of a 2.5 g/L solution of *p*-anisidine in glacial acetic acid, shake, and store protected from light.

Procedure: Measure the absorbance of *Test Solution A* at 350 nm using isooctane as the blank. Measure the absorbance of *Test Solution B* at 350 nm exactly 10 min after its preparation, using the *Standard Solution* as the compensation liquid. Calculate the *Anisidine Value* using the formula:

$$\text{Result} = 25 \times (1.2A_S - A_B)/m$$

where A_S is the absorbance of *Test Solution B* at 350 nm; A_B is the absorbance of *Test Solution A* at 350 nm; and m is the weight, in g, of the substance to be examined in *Test Solution A*.

■1S (*FCC7*)

CHLOROPHYLL

Change to read:

(Based on AOCS Method Cc 13d-55▲1 ▲*FCC7*)

Use a reliable spectrophotometer with a sample holder equilibrated at 44° ± 3° to obtain absorbance values at 630, 670, and 710 nm. Calculate the concentration of chlorophyll (C) using the following equation:

$$C = [A_{670} - (A_{630}/2) - (A_{710}/2)]/(K \times b)$$

in which C is the concentration of chlorophyll, in mg/kg; A is the absorbance at the wavelength indicated by the subscript; K is the constant for the specific spectrophotometer being used and is equal to 0.1016 for the Beckman Model DU; and b is the optical pathlength through the sample, in cm.

COLD TEST

Change to read:

(Based on AOCS Method Cc 11-53▲1 ▲*FCC7*)

Filter a sample (200 to 300 mL), and transfer to a clean, dry bottle. Fill the bottle completely, and insert a cork

stopper. Seal with paraffin, and equilibrate at 25° in a water bath so that it is completely covered. Next, immerse the bottle in an ice and water bath so it is completely covered. Monitor the bath during the test and replenish the ice frequently to keep the bath at 0°.

After 5.5 h remove the bottle from the bath. The sample must be clear; fat crystals or cloudiness must be totally absent.

COLOR

Change to read:

(AOCS-Wesson) (Based on AOCS Method Cc 13b-45▲1▲*FCC7*)

Apparatus: Use a Lovibond tintometer or the equivalent and a set of color comparison glasses that conform to the AOCS-Wesson Tintometer Color Scale (available from the National Institute of Standards and Technology). A minimum set of glasses consists of

Red	0.1	0.2	0.3	0.4	0.5	0.6	0.8	0.9
	1.0	2.0	2.5	3.0	3.5	4.0	5.0	6.0
	7.0	7.6	8.0	9.0	10.0	11.0	12.0	16.0
	20.0							
Yellow	1.0	2.0	3.0	5.0	10.0	15.0	20.0	35.0
	50.0	70.0						

For making color comparisons, use color tubes of clear, colorless glass with a smooth, flat, polished bottom (length 154 mm; id 19 mm; od 22 mm), and marked to indicate liquid columns of 25.4 and 133.35 mm.

Procedure: Add 0.1 g of diatomaceous earth to a 60-g sample, agitate for 2.5 min at room temperature (or 10° to 15° above the melting point if the sample is not liquid), and filter. Adjust the temperature to 25° to 35° (or not more than 100 above the melting point), and fill the color tube to the desired mark. Place the tube in the tintometer (in a dark booth or cabinet), and match the sample color as closely as possible with a standard glass.

FATTY ACID COMPOSITION

Change to read:

(Based on AOCS Methods Ce 1-62▲1▲*FCC7* and Ce 1b-89▲1▲*FCC7*)

Apparatus: Use a suitable gas chromatograph (*see Appendix IIA*) equipped with a flame ionization detector (FID) and containing either a 3.05-m × 2- or 4-mm id glass column packed with preconditioned 10%, by weight, DEGS-PS on 100- to 120-mesh diatomaceous earth (Chromosorb WHP, or equivalent) or a 30-m × 0.20- to 0.35-mm id capillary fused silica column, or equivalent, containing a suitable stationary phase.

Operating Conditions: The operating conditions may vary with the instrument used, but a suitable chromatogram may be obtained using a temperature program 180° to 215°; inlet temperature (injector), 300°; detector, 300°; and a suitable carrier gas flow.

Standard Solutions: Run through the chromatograph a commercially available standard containing a mixture of fatty-acid methyl esters. Fatty acids and methyl esters with a wide range of carbon numbers and double-bond configurations can be purchased. The calculated concentration should compare to that claimed within ±2 σ, where σ is the standard deviation calculated from at least 10 replicate determinations, preferably made over a period of several days.

Determine that the system is functioning properly: inject into the chromatograph a suitable number of samples of the standard to ensure that the resolution factor, R, defining the efficiency of the separation between methyl stearate and methyl oleate is 0.9 or greater. Calculate R by the equation:

$$R = 2(t_2 - t_1)/(w_2 + w_1)$$

in which t_2 and t_1 are the retention times of peak 2 and peak 1, respectively, and w_2 and w_1 are the corresponding widths of the bases of the peaks obtained by extrapolating relatively straight sides of the peaks to the baseline. Baseline separation of the various components in both the standard and the sample preparations is desirable.

Change to read:

Sample Preparation (for fats and oils) (Based on AOCS Method Ce 2-66▲1 ▲*FCC7*)**:** Introduce 100 to 1000 mg of the fat into a 50- or 125-mL reaction flask. Add 4 to 10 mL of 0.5 N methanolic sodium hydroxide, and add a boiling chip. Attach a condenser, and heat the mixture on a steam bath until the fat globules go into solution. This step should take 5 to 10 min. Add 5 to 12 mL of 12.5% boron fluoride–methanol reagent (this reagent contains 125 g of boron fluoride per L of methanol and is available commercially) through the condenser, and boil for 2 min. Add 2 to 5 mL of heptane through the condenser, and boil for 1 min longer. Remove from heat, remove condenser, and add about 15 mL of saturated sodium chloride solution. Stopper the flask, and shake vigorously for 15 s. Transfer about 1 mL of the heptane solution into a test tube and add a small amount of anhydrous sodium sulfate. The dry heptane solution may then be injected directly into a gas chromatograph.

The methyl esters should be analyzed as soon as possible. They may be kept in an atmosphere of nitrogen in a screw-cap vial at 2° for 24 h. For longer storage, they should be sealed in a glass ampule, subjected first to a vacuum and then backfilled with nitrogen and stored at -20° (freezer).

Procedure: Inject an appropriate volume (0.1 µL to 1.0 µL) of sample into the chromatograph. If an automated system is used, follow the manufacturer's instructions; if calculations are to be done manually, proceed as follows:

Calculate the area percent of each component (C_N) by the equation:

$$C_N = [A_N/T_S] \times 100$$

in which A_N is the area of the peak corresponding to component C_N, and T_S is the total area for all detected components [$T_S = \Sigma A_N$].

Add the following:

▪FATTY ACID COMPOSITION (SATURATED,

cis-MONOUNSATURATED, and *cis*-POLYUNSATURATED) IN OILS CONTAINING LONG CHAIN POLYUNSATURATED FATTY ACIDS

(Based on AOCS Methods Ce 1i-07 and Ce 2-66[1])

Apparatus: Use a gas chromatograph (*see Appendix IIA*) suitable for use with capillary columns, a temperature-controlled split/splitless injector operated in split mode, and a flame-ionization detector (FID). The capillary GC column should be of fused silica, 30-m × 0.25-mm, with a 0.25-µm coating of polyethylene glycol (PEG)[2].

Operating Conditions: The carrier gas should be gas chromatography grade hydrogen or helium (99.99% or better purity) that has been dried, and from which the oxygen has been removed using suitable filters. Do not use nitrogen as a carrier gas for this method. The flame gases should be gas chromatography grade hydrogen and air and the make-up gas should be gas chromatography grade nitrogen or helium. Use a 78.5-mm × 4-mm (i.d.) × 6.3-mm (o.d.) base deactivated precision injection port split liner with glass wool[3]. The injection port should be operated at 235°. The detector should be operated at 325°. The column (oven) temperature should be held at 170° initially, with a 1°/min ramp and a final temperature of 225°. When a hydrogen carrier gas is used, the column head pressure is 77.9 kPa (11.3 psi) with a constant flow rate of 1.2 mL/min, a linear velocity of 43 cm/s, and a split ratio of 100:1. When helium is used as the carrier gas, the column head pressure is 226 kPa (32.77 psi) with a constant flow rate of 2.4 mL/min, a linear velocity of 53 cm/s, and a split ratio of 100:1. [Note—These conditions may not be appropriate for the determination of very long chain fatty acids (25:0 and greater).]

Reagents and Solutions

Internal Standard Solution: 2.0 mg/mL of USP Tritricosanoin RS in chloroform. [Note—Care must be taken to prevent the loss of chloroform during use and storage. This solution is stable indefinitely if precautions are taken to eliminate the loss of chloroform and, therefore, a change in the concentration of the solution. Store the solution in a refrigerator in a well-sealed amber bottle when not in use.]

System Suitability Preparation: USP Menhaden Oil RS

Standard Solution: Prepare a 20 mg/mL solution of USP FAME Standard Mixture RS in either *n*-heptane or *n*-hexanes as follows: dilute 100 mg of USP FAME Standard Mixture RS in 5 mL of solvent, rinsing the ampule containing the standard with the solvent to ensure complete and homogeneous transfer of the mixture.

Sample Preparation (for fats and oils): Transfer sufficient *Internal Standard Solution* into a 50- or 125-mL reaction flask so that the concentration in the final solution, after the oil is added, is 0.05–0.10 mg of internal standard per 1 mg of oil. Evaporate the chloroform (from the *Internal Standard Solution*) from the flask, then introduce 100–1000 mg of the oil to the reaction flask. Add 4 to 10 mL of 0.5 N methanolic sodium hydroxide, and add a boiling chip. Attach a condenser, and heat the mixture on a steam bath until the fat globules go into solution. This step should take 5 to 10 min. Add 5 to 12 mL of 12.5% boron fluoride–methanol reagent (this reagent contains 125 g of boron fluoride per L of methanol and is available commercially) through the condenser, and boil for 2 min. [Note—Addition of antioxidants such as pyrogallol or BHT at a level of 1 mg/mg of sample may help protect highly unsaturated fatty acids from oxidation during methylation.] Add 2 to 5 mL of heptane through the condenser, and boil for 1 min longer. Remove from heat, remove condenser, and add about 15 mL of saturated sodium chloride solution. Stopper the flask, and shake vigorously for 15 s. Dilute the fatty acid methyl ester (FAME) so obtained in *n*-heptane or *n*-hexanes to a concentration of approximately

15–20 mg/mL of FAME in solvent.

System Suitability: Proceed as directed under *Sample Preparation* using the *System Suitability Preparation* in place of the sample oil. Using a microsyringe suitable for gas chromatography (10-µL), inject 1 µL of the fatty acid methyl esters obtained from the preparation into the chromatograph and record the resulting chromatogram. Compare the chromatogram to one obtained using commercially-available authentic standards of fatty acid methyl esters, if needed, to identify the peaks. Baseline separation should be obtained between 23:0 (the internal standard) and 6*c*, 9*c*, 12*c*, 15*c*, 18*c*-21:5 (21:5n-3). Baseline separation should also be obtained between 24:0 and 4*c*, 7*c*, 10*c*, 13*c*, 16*c*, 19*c*-22:6 (DHA or 22:6n-3), which should be almost baseline resolved from the 24:1 isomers.

Theoretical and Empirical Correction Factors: Theoretical correction factors may be calculated as directed below or may be available from several reference sources. Empirical correction factors are determined from the analysis of the *Standard Solution*. Using the microsyringe described under *System Suitability*, inject 1 µL of the *Standard Solution* into the chromatograph and record the resulting chromatogram. Calculate the theoretical correction factors (TCF) for each fatty acid using the equation:

$$TCF_X = MW_X/(N_X - 1) \times (AWC) \times (1.3344)$$

in which TCF_X is the theoretical flame ionization detector response factor for fatty acid X (as the methyl ester) with respect to 23:0 FAME internal standard; MW_X is the molecular weight of component X; N_X is the number of carbon atoms in the fatty acid methyl ester of component X; AWC is the atomic weight of carbon (12.011); and 1.3344 is the TCF for 23:0 FAME.

Empirical correction factors are required for long chain polyunsaturated fatty acid fatty acid methyl esters of 20 carbons or more and three or more double bonds of which standards are readily available. Using the certificate of analysis for USP FAME Standard Mixture RS, which should list both the purity (P) and amount (Amt_{FAMEx}) of each fatty acid methyl ester used to make up the standard, calculate the actual amount ($AAmt_{FAMEx}$) of each fatty acid methyl ester using the equation

$$AAmt_{FAMEx} = P \times Amt_{FAMEx}$$

The response factor (RF) for each peak is determined using the equation:

$$RF_{FAMEx} = Area_{FAMEx}/AAmt_{FAMEx}$$

where $Area_{FAMEx}$ is the peak area of the fatty acid methyl ester obtained from the chromatogram.

Each RF is then made relative to the 23:0 RF using the equation:

$$RRF_{FAMEx} = RF_{FAMEx}/RF_{23:0}$$

The empirical correction factor (ECF) for each FAME is then calculated by taking the inverse of the RRF using the equation:

$$ECF_{FAMEx} = 1/RRF_{FAMEx}$$

Procedure: Using the microsyringe described under *System Suitability*, inject 1 µL of the *Sample Preparation* into the chromatograph, and record the resulting chromatogram.

Calculate the amount, in g, of individual fatty acids, expressed as FAME (W_{FAMEx}) and triacylglycerol (W_{TAGx}) equivalents using the equations:

$$W_{FAMEx} = (A_X \times W_{TAG\text{-}IS} \times F \times R_X)/A_{IS}$$

$$W_{TAGx} = W_{FAMEx} \times F_{TAGx}$$

in which A_X is the peak area count for fatty acid X from the chromatogram obtained; $W_{TAG\text{-}IS}$ is the weight of 23:0 internal standard (in g) added to the oil; F is a factor for converting the weight of the internal standard (which is 23:0 triacylglycerol) from the triacylglycerol form to its corresponding weight of the fatty acid methyl ester form (1.0037); R_X is the theoretical correction factor (TCF) or empirical correction factor (ECF) for the fatty acid methyl esters relative to 23:0 methyl ester internal standard determined; A_{IS} is the peak area count for the internal standard; and F_{TAGx} is the conversion factor for fatty acid methyl esters to triacyl glycerols for individual fatty acids (from the table below). The TCF should be applied to the analytical data for optimum accuracy and to minimize variation between laboratories because of differences in calculating response factors. TCFs are also used for fatty acids where standards are not available. ECFs are needed due to the large deviation from TCFs for long chain polyunsaturated fatty acids of 20 carbons or more and three or more double bonds.

Calculation of Total Fat: Calculate the amount of total fat in the sample tested (sum of all fatty acids; expressed as triacylglycerols) using the equation:

$$\text{Total fat (g/100 g portion of test sample)} = (\Sigma W_{TAG}/W_{TS}) \times 100$$

where W_{TS} is the weight of the sample, in g.

Calculation of Individual Fatty Acids: Calculate the weight, in g, of each individual fatty acid (W_X) using the equation:

$$W_X \text{ (per g of sample)} = W_{FAMEx} \times F_{FAx}$$

where F_{FAx} is the factor for conversion of the fatty acid methyl ester to its corresponding fatty acid (from the table below).

Calculation of Saturated Fats: Calculate the weight of saturated fats (sum of all saturated fatty acids) using the equation:

$$\text{Saturated fat (g/100 g portion of test sample)} = (\Sigma \text{ Saturated } W_X/W_{TS}) \times 100$$

where Σ Saturated W_X is the sum of all saturated fatty acids (4:0; 5:0; 6:0; 7:0; 8:0; 9:0; 10:0; *iso* 10:0; 11:0; 12:0; *anteiso* and *iso* 12:0; 13:0; *anteiso* and *iso* 13:0; 14:0; *anteiso* and *iso* 14:0; 15:0; *anteiso* and *iso* 15:0; 2,6,10,14-tetramethyl 15:0; 16:0; *anteiso* and *iso* 16:0; 3,7,11,15-tetramethyl 16:0; 17:0; *anteiso* and *iso* 17:0; 18:0; *anteiso* and *iso* 18:0; 19:0; *anteiso* 19:0; 20:0; *iso* 20:0; 21:0; *iso* 21:0; 22:0; 24:0; 25:0; 26:0; 27:0; 28:0; 29:0; and 31:0).

Calculation of *cis*-Monounsaturated Fat: Calculate the weight of *cis*-monounsaturated fat (fatty acids containing one double bond) using the equation:

$$\textit{cis}\text{-Monounsaturated fat (g/100 g portion of test sample)} = (\Sigma \textit{ cis}\text{-monounsaturated } W_X/W_{TS}) \times 100$$

where Σ *cis*-monounsaturated W_X is the sum of all *cis*-monounsaturated fatty acids.

Calculation of *cis*-Polyunsaturated Fat: Calculate the weight of *cis*-polyunsaturated fat (fatty acids containing two or more double bonds) using the equation:

cis-Monounsaturated fat (g/100 g portion of test sample) = (Σ *cis*-polyunsaturated W_X/W_{TS}) × 100

where Σ *cis*-polyunsaturated W_X is the sum of all *cis*-polyunsaturated fatty acids.

Calculation of EPA and DHA: Calculate the weight of EPA and DHA using the equation:

EPA or DHA (g/100 g portion of test sample) = (ΣEPA or DHA W_X/W_{TS}) × 100

Factors for Converting FAME to FA and TAG Equivalents

Fatty Acid[a]	F_{FAx}	F_{TAGx}	Fatty Acid[a]	F_{FAx}	F_{TAGx}
4:0	0.8626	0.9868	20:0	0.9570	0.9959
5:0	0.8792	0.9884	20:1	0.9568	0.9959
6:0	0.8922	0.9897	20:2	0.9565	0.9958
7:0	0.9027	0.9907	20:3	0.9562	0.9958
8:0	0.9114	0.9915	20:4	0.9560	0.9958
9:0	0.9186	0.9922	20:5	0.9557	0.9958
10:0	0.9247	0.9928	21:0	0.9588	0.9961
10:1	0.9239	0.9927	21:5	0.9576	0.9959
11:0	0.9300	0.9933	22:0	0.9604	0.9962
11:1	0.9293	0.9932	22:1	0.9602	0.9962
12:0	0.9346	0.9937	22:2	0.9600	0.9962
12:1	0.9339	0.9937	22:3	0.9598	0.9961
13:0	0.9386	0.9941	22:4	0.9595	0.9961
13:1	0.9380	0.9941	22:5	0.9593	0.9961
14:0	0.9421	0.9945	22:6	0.9590	0.9961
14:1	0.9416	0.9944	23:0 (IS)	0.9619	0.9964
15:0[b]	0.9453	0.9948	23:5	0.9609	0.9963
Tetra Methyl 15:0	0.9551	0.9957	24:0	0.9633	0.9965
15:1	0.9449	0.9947	24:1	0.9631	0.9965
16:0[c]	0.9481	0.9950	24:3	0.9628	0.9964
Tetra Methyl 16:0	0.9570	0.9959	24:4	0.9626	0.9964
16:1	0.9477	0.9950	24:5	0.9624	0.9964
16:2	0.9473	0.9950	24:6	0.9621	0.9964
16:3	0.9469	0.9949	25:0	0.9646	0.9966
16:4	0.9465	0.9949	26:0	0.9658	0.9967
17:0	0.9507	0.9953	26:5	0.9650	0.9966
17:1	0.9503	0.9952	26:6	0.9648	0.9966
18:0	0.9530	0.9955	27:0	0.9670	0.9968
18;1	0.9527	0.9955	28:0	0.9680	0.9969
18:2	0.9524	0.9954	28:7	0.9670	0.9968
18:3	0.9520	0.9954	28:8	0.9668	0.9968
18:4	0.9517	0.9954	29:0	0.9690	0.9970
18:5	0.9514	0.9953	30:0	0.9700	0.9971
19:0	0.9551	0.9957	31:0	0.9708	0.9972
19:1	0.9548	0.9957			

[a] Only one factor is given for all positional and geometric isomers and for branched-chain FAME, as the factors are dependent only on the content of carbon to which hydrogen is bonded.

[b] 3,7,11-trimethyldodceanoic acid (TMDD) has equivalent F_{FAx} and F_{TAGx} to 15:0.

[c] 4,8,12-trimethyltridecanoic acid (TMTD) has equivalent F_{FAx} and F_{TAGx} to 16:0.

■1S (*FCC7*)

FREE FATTY ACIDS

Change to read:

(Based on AOCS Method Ca 5a-40▲1▲*FCC7*)

Unless otherwise directed, accurately weigh the appropriate amount of the sample, indicated in the table below, into a 250-mL Erlenmeyer flask or other suitable container. Add 2 mL of phenolphthalein TS to the specified amount of hot alcohol, neutralize with alkali to the first faint, but permanent, pink color, and then add the hot, neutralized alcohol to the sample container. Titrate with the appropriate normality of sodium hydroxide, shaking vigorously, to the first permanent pink color of the same intensity as that of the neutralized alcohol. The color must persist for at least 30 s. Calculate the percentage of free fatty acids (FFA) in the sample by the formula:

$$\text{Result} = VNe/W$$

in which V is the volume and N is the normality of the sodium hydroxide used; W is the weight of the sample, in g; and e is the equivalence factor given in the monograph.

FFA Range (%)	Grams of Sample	Milliliters of Alcohol	Strength of NaOH
0.00–0.2	56.4 ± 0.2	50	0.1 N
0.2–1.0	28.2 ± 0.2	50	0.1 N
1.0–30.0	7.05 ± 0.05	75	0.25 N
30.0–50.0	7.05 ± 0.05	100	0.25–1.0 N
50.0–100	3.525 ± 0.001	100	1.0 N

FREE GLYCERIN OR PROPYLENE GLYCOL

Change to read:

(Based on AOCS Method Ca 14-56▲1▲*FCC7*)

Reagents and Solutions: Use the *Periodic Acid Solution*, *Potassium Iodide Solution*, and *Chloroform* as described under *1-Monoglycerides*, in this Appendix.

Procedure: To the combined aqueous extracts obtained as directed under *1-Monoglycerides*, 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. Continue as directed in the *Procedure* under *1-Monoglycerides*, beginning with "... and allow to stand for at least 30 min but no longer than 90 min."

Calculation: Calculate the percentage of free glycerin in the original sample by the formula:

$$\text{Result} = (b - S) \times N \times 2.30/W$$

or calculate the percentage of free propylene glycol by the formula:

$$\text{Result} = (b - S) \times N \times 3.81/W$$

in which 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; 2.30 is the molecular weight of glycerin divided by 40; and 3.81 is the molecular weight of propylene glycol divided by 20.

[Note— If the aqueous extract contains more than 20 mg of glycerin or 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.]

HEXANE-INSOLUBLE MATTER

If the sample is plastic or semisolid, soften a portion by warming it at a temperature not exceeding 60°, and then mix it thoroughly. Transfer 100 g of well-mixed sample into a 1500-mL wide-mouth Erlenmeyer flask, add 1000 mL of solvent hexane, and shake until the sample is dissolved. Pass the resulting solution through a 600-mL Corning "C" porosity, or equivalent, filtering funnel that previously has been dried at 105° for 1 h, cooled in a desiccator, and weighed. Wash the flask with two successive 250-mL portions of solvent hexane, and pass the washings through the filter. Dry the funnel at 105° for 1 h, cool to room temperature in a desiccator, and weigh. From the gain in weight of the funnel, calculate the percentage of the hexane-insoluble matter in the sample.

HYDROXYL VALUE

Change to read:

(Based on AOCS Methods Cd 4-40▲1▲*FCC7* and Cd 13-60▲1▲*FCC7*)

The hydroxyl value is defined as the number of mg of potassium hydroxide equivalent to the hydroxyl content of 1 g of the unacetylated sample.

Method I

Proceed as directed under *Acetyl Value*, in this Appendix, but calculate the hydroxyl value by the formula:

$$\text{Result} = (S' - S)/(1.000 - 0.00075S')$$

Method II

Unless otherwise directed, accurately weigh the appropriate amount of the sample indicated in the table below, transfer it into a 250-mL glass-stoppered Erlenmeyer flask, and add 5.0 mL of pyridine–acetic anhydride reagent (mix 3 volumes of freshly distilled pyridine with 1 volume of freshly distilled acetic anhydride).

Hydroxyl Value	Sample Weight (g)
0–20	10
20–50	5
50–100	3
100–150	2
150–200	1.50
200–250	1.25
250–300	1
300–350	0.75

Pipet 5 mL of the pyridine–acetic anhydride reagent into a second 250-mL flask for the reagent blank. Heat the flasks for 1 h on a steam bath under reflux condensers, then add 10 mL of water through each condenser, heat for 10 min longer, and allow the flasks to cool to room temperature. Add 15 mL of *n*-butyl alcohol, previously neutralized to phenolphthalein TS with 0.5 N alcoholic potassium hydroxide, through the condenser, then remove the condensers, and wash the sides of the flasks with 10 mL of *n*-butyl alcohol. To each flask add 1 mL of phenolphthalein TS, and titrate to a faint pink endpoint with 0.5 N alcoholic potassium hydroxide, recording the mL required for the sample as S and that for the blank as B. To correct for free acid, mix about 10 g of the sample, accurately weighed, with 10 mL of freshly distilled pyridine, previously neutralized to phenolphthalein, add 1 mL of phenolphthalein TS, and titrate to a faint endpoint with 0.5 N alcoholic potassium hydroxide, recording the mL required as A. Calculate the hydroxyl value by the formula:

$$\text{Result} = [B + (WA/C) - S] \times 56.1N/W$$

in which W and C are the weights, in g, of the samples taken for acetylation and for the free acid determination, respectively; and N is the exact normality of the alcoholic potassium hydroxide.

IODINE VALUE

Change to read:

(Based on AOCS Method Cd 1d-92▲1▲*FCC7*)

The iodine value is a measure of unsaturation and is expressed as the number of g of iodine absorbed, under the prescribed conditions, by 100 g of the test substance.

Modified Wijs Method (Acetic Acid/Cyclohexane Method)

Wijs Solution: Dissolve 13 g of resublimed iodine in 1000 mL of glacial acetic acid. Pipet 10.0 mL of this solution into a 250-mL flask, add 20 mL of potassium iodide TS and 100 mL of water, and titrate with 0.1 N sodium thiosulfate, adding starch TS near the endpoint. Record the volume required as A. Set aside about 100 mL of the iodine–acetic acid solution for future use. Pass chlorine gas, washed and dried with sulfuric acid, through the remainder of the solution until a 10.0-mL portion requires not quite twice the volume of 0.1 N sodium thiosulfate consumed in the titration of the original iodine solution. A characteristic color change occurs when the desired amount of chlorine has been added. Alternatively, *Wijs Solution* may be prepared by dissolving 16.5 g of iodine monochloride, ICl, in 1000 mL of glacial acetic acid. Store the solution in amber bottles sealed with paraffin until ready for use, and use within 30 days.

Total Halogen Content: Pipet 10.0 mL of *Wijs Solution* into a 500-mL Erlenmeyer flask containing 150

mL of recently boiled and cooled water and 15 mL of potassium iodide TS. Titrate immediately with 0.1 N sodium thiosulfate, recording the volume required as B.

Halogen Ratio: Calculate the I/Cl ratio by the formula:

$$\text{Result} = A/(B - A)$$

The halogen ratio must be between 1.0 and 1.2. If the ratio is not within this range, the halogen content can be adjusted by adding the original solution or by passing more chlorine through the solution.

[Note— *Wijs Solution* is commercially available.]

Procedure: The appropriate weight of the sample, in g, is calculated by dividing the number 25 by the expected iodine value. Melt the sample, if necessary, and pass it through a dry filter paper. Transfer the accurately weighed quantity of sample into a clean, dry, 500-mL glass-stoppered bottle or flask containing 20 mL of glacial acetic acid/cyclohexane, 1:1, v/v, and pipet 25.0 mL of *Wijs Solution* into the flask. The excess of iodine should be between 50% and 60% of the quantity added, that is, between 100% and 150% of the quantity absorbed. Swirl, and let stand in the dark for 1.0 h where the iodine value is <150 and for 2.0 h where the iodine value is ≥150. Add 20 mL of potassium iodide TS and 100 mL of recently boiled and cooled water, and titrate the excess iodine with 0.1 N sodium thiosulfate, adding the titrant gradually and shaking constantly until the yellow color of the solution almost disappears. Add starch TS, and continue the titration until the blue color disappears entirely. Toward the end of the titration, stopper the container and shake it violently so that any iodine remaining in solution in the glacial acetic acid/cyclohexane, 1:1, solution may be taken up by the potassium iodide solution. Concomitantly, conduct two determinations on blanks in the same manner and at the same temperature. Calculate the iodine value by the formula:

$$\text{Result} = (B - S) \times 12.69N/W$$

in which B − S represents the difference between the volumes of sodium thiosulfate required for the blank and for the sample, respectively; N is the normality of the sodium thiosulfate; and W is the weight, in g, of the sample taken.

MELTING RANGE

Fats of animal and vegetable origin do not exhibit a sharp melting point. For the purpose of this test, melting range is defined as the range of temperature in which the sample becomes a perfectly clear liquid after first passing through a stage of gradual softening, during which it may become opalescent.

Apparatus: Use any suitable commercial or other apparatus. Use melting-point capillary tubes—id, 1 mm; od, 2 mm; length, 50 to 80 mm; and open at both ends.

Change to read:

Procedure

Capillary Method (Based on AOCS Method Cc 1-25▲1 ▲*FCC7*): Melt the sample and pass it through filter paper; the sample must be absolutely dry. Dip three capillary tubes in the liquid sample so that the oil stands approximately 10 mm high in the tubes, and fuse the end of the tube containing the sample without burning it. Place the tubes containing the liquid sample in a beaker, and equilibrate them at least 16 h at 4° to 10° in a

refrigerator. Determine the melting range, using a temperature increase of 0.5° per min when within 10° of the anticipated melting point. The melting ranges of the three samples should be no more than 0.5° apart.

1-MONOGLYCERIDES

Change to read:

(Based on AOCS Method Cd 11-57▲1▲*FCC7*)

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 continue as directed in the *Procedure*, beginning with "... allow to stand at least 1 min ..." The difference between the volume of 0.1 N sodium thiosulfate required in the titrations with and without the chloroform is not greater than 0.5 mL.

Change to read:

Procedure: Melt the sample, if not liquid, at a temperature not higher than 10° above its melting point, and mix thoroughly. Transfer an accurately weighed portion of the sample, equivalent to about 150 mg of 1-monoglycerides, into a 100-mL beaker (or weigh a sample equivalent to 20 mg of glycerin or 30 mg of propylene glycol if only *Free Glycerin or Propylene Glycol* is to be determined), 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 s, 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. Retain the combined aqueous extracts for the determination of *Free Glycerin or Propylene Glycol* (in this Appendix). Transfer the chloroform to a 500-mL glass-stoppered Erlenmeyer flask, and add 50.0 mL of *Periodic Acid Solution* to this flask and to each of two blank flasks containing 50 mL of chloroform and 10 mL of water. Swirl the flasks during the addition of the reagent, 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 0.1 N sodium thiosulfate, using a magnetic stirrer to keep the solution thoroughly mixed, to the disappearance of the brown iodine color, then add 2 mL of starch TS and continue the titration to the disappearance of the blue color. Calculate the percentage of 1-monoglycerides~~▲1▲2▲*FCC7*~~■4■1S (*FCC7*) in the sample by the formula:

$$\text{Result} = (B - S) \times N \times 17.927/W$$

in which 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 sample; N is the exact normality of the sodium thiosulfate; W is the weight, in g, of the sample taken; and 17.927 is the molecular weight of glyceryl monostearate divided by 20.

TOTAL MONOGLYCERIDES

Preparation of Silica Gel: Place about 10 g of 100- to 200-mesh silica gel of a grade suitable for chromatographic work in a tared weighing bottle, cap immediately, and weigh accurately. Remove the cap, dry at 200° for 2 h, cap immediately, and cool for 30 min. Raise the cap momentarily to equalize the pressure, then weigh again, reheat for 5 min at 200°, cool, and reweigh. Repeat this 5-min drying cycle until two consecutive weights agree within 10 mg. Calculate the percentage of water in the original silica gel (A) by the formula:

$$\text{Result} = (\text{loss in wt}/\text{sample wt}) \times 100$$

then calculate the amount of water required to adjust the water content to 5% by the formula:

$$\text{Result} = W \times (5 - A)/95$$

in which W is the weight, in g, of the undried sample to be used.

Accurately weigh the appropriate amount of the undried silica gel to be used in the determination, transfer to a suitable blender or mixer, and add the calculated amount of water to give a final water content of 5% ± 0.1%. Blend for 1 h to ensure complete water distribution, and store in a sealed container. Determine the water content of the adjusted silica gel as directed above, and readjust if necessary.

[Note—Each new lot of silica gel should be checked for suitability by the analysis of a monoglyceride of known composition.]

Sample Preparation: [**CAUTION—** To avoid rearrangement of partial glycerides, use extreme caution in applying heat to samples, and do not heat above 50°.]

*Samples Melting Below 50°***:** Melt the sample, if necessary, by warming for short periods below 50°, not exceeding a total of 30 min.

*Samples Melting Above 50°***:** Grind about 10 g in a mortar and pestle, chilling solid samples, if necessary, in carbon dioxide.

Weigh accurately about 1 g of the prepared sample into a 100-mL beaker, add 15 mL of chloroform, and warm, if necessary, to effect solution. Use only minimal heat, and do not heat above 40°.

Preparation of Chromatographic Column: Connect a 19- × 290-mm chromatographic tube, equipped with an outer 19/22 standard-taper joint at the top and a coarse, fritted-glass disk and inner 19/22 standard-taper joint at the bottom, with an adapter consisting of an outer 19/22 joint connected to a Teflon stopcock. Do not grease the joints. Weigh 30 g of the prepared silica gel into a 150-mL beaker, add 50 to 60 mL of petroleum ether, and stir slowly with a glass rod until all air bubbles are expelled. Transfer the slurry to the column through a powder funnel, and open the stopcock, allowing the liquid level to drop to about 2 cm above the silica gel. Transfer any silica gel slurry remaining in the beaker into the column with a minimum amount of petroleum ether, then rinse the funnel and sides of the column. Drain the solvent through the stopcock until the level drops to 2 cm above the silica gel, and remove the powder funnel.

Procedure: Carefully add the *Sample Preparation* to the prepared column. Open the stopcock, and adjust the flow rate to about 2 mL/min, discarding the eluate. Rinse the sample beaker with 5 mL of chloroform, and add the rinsing to the column when the level drops to 2 cm above the silica gel. Never allow the column to become dry on top, and maintain a flow rate of 2 mL/min throughout the elution. Avoid interruptions during elution as

they may cause pressure buildup and result in leakage through the stopcock or cracks in the silica gel packing.

Attach a 250-mL reservoir separator, provided with a Teflon stopcock and a 19/22 standard-taper drip tip inner joint, to the column. Add 200 mL of benzene, elute, and discard the eluate, which contains the triglycerides fraction. When the level of benzene drops to 2 cm above the silica gel, add 200 mL of a 1:10 mixture of ether in benzene, elute, and discard the eluate, which contains the diglycerides and the free fatty acid fraction. When all of the ether–benzene solvent has been added from the separator and the level in the column drops to 2 cm above the silica gel, add from 250 to 300 mL of ether, and collect the monoglyceride fraction in a tared flask. Rinse the tip of the column into the flask with a few mL of ether, and evaporate to dryness on a steam bath under a stream of nitrogen or dry air. Cool for at least 15 min, weigh, then reheat on the steam bath for 5 min in the same manner. Cool, reweigh, and repeat the 5-min evaporation, cooling, and reweighing procedures until two consecutive weights agree within 2 mg. The weight of the residue represents the total monoglycerides in the sample taken.

OXYETHYLENE DETERMINATION

Apparatus: The apparatus for oxyethylene group determination is shown in *Fig. 35*. It consists of a boiling flask, *A*, fitted with a capillary side tube to provide an inlet for carbon dioxide and connected by a condenser with trap *B*, which contains an aqueous suspension of red phosphorus. The first absorption tube, *C*, contains a silver nitrate solution to absorb ethyl iodide. Absorption tube *D* is fitted with a 1.75-mm spiral rod (23 turns, 8.5-mm rise per turn), which is required to provide a longer contact of the evolved ethylene with the bromine solution. A standard-taper adapter and stopcock are connected to tube *D* to permit the transfer of the bromine solution into a titration flask without loss. A final trap, *E*, containing a potassium iodide solution, collects any bromine swept out by the flow of carbon dioxide.

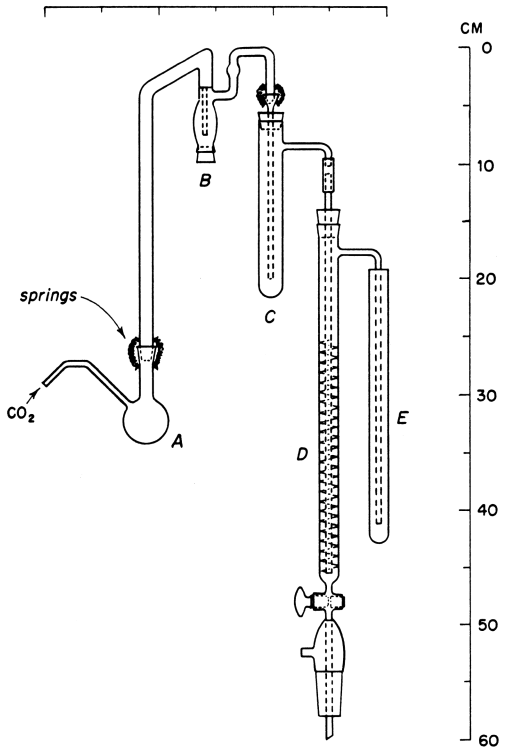

FIGURE 35 Apparatus for Oxyethylene Determination.

Dimensions of the apparatus not readily determined from *Fig. 35* are as follows: carbon dioxide inlet capillary, 1-mm id; flask *A*, 28-mm diameter, 12/18 standard-taper joint; condenser, 9-mm id; inlet to trap *B*, 2-mm id; inlet to trap *C*, 7/15 standard-taper joint, 2-mm id; trap *C*, 14-mm id; trap *D*, inner tube, 8-mm od, 2-mm opening at bottom of spiral; outer tube, approximately 12.5-mm id; side arm 7 cm from top of inserted spiral, 3.5-mm id, 2-mm opening at bottom.

Reagents

Hydriodic Acid: Use special-grade hydriodic acid suitable for alkoxyl determinations, or purify reagent-grade as follows: Distill over red phosphorus in an all-glass apparatus, passing a slow stream of carbon dioxide through the apparatus until the distillation is terminated and the receiving flask has completely cooled. [**CAUTION—** Use a safety shield, and conduct the distillation in a hood.]

Silver Nitrate Solution: Dissolve 15 g of silver nitrate in 50 mL of water, mix with 400 mL of alcohol, and add a few drops of nitric acid.

Bromine–Bromide Solution: Add 1 mL of bromine to 300 mL of glacial acetic acid saturated with dry potassium iodide (about 5 g). Fifteen mL of this solution requires about 40 mL of 0.05 N sodium thiosulfate. Store in a brown bottle in a dark place, and standardize at least once a day during use.

Procedure: Fill trap *B* with enough of a suspension of 60 mg of red phosphorus in 100 mL of water to cover the inlet tube. Pipet 10 mL of the *Silver Nitrate Solution* into tube *C* and 15 mL of the *Bromine–Bromide Solution* into tube *D*, and place 10 mL of a 1:10 solution of potassium iodide in trap *E*. Transfer an accurately weighed quantity of the sample specified in the monograph into the reaction flask, *A*, and add 10 mL of *Hydriodic Acid* along with a few glass beads or boiling stones. Connect the flask to the condenser, and begin passing carbon dioxide through the apparatus at the rate of about one bubble per s. Heat the flask in an oil bath at 140° to 145°, and continue the reaction at this temperature for at least 40 min. Heating should be continued until the cloudy reflux in the condenser becomes clear and until the supernatant liquid in the silver nitrate tube, *C*, is almost completely clarified. Five min before the reaction is terminated, heat the *Silver Nitrate Solution* in tube *C* in a hot water bath at 50° to 60° to expel any dissolved olefin. At the completion of the decomposition, cautiously disconnect tubes *D* and *C* in the order named, then disconnect the carbon dioxide source and remove the oil bath. Connect tube *D* to a 500-mL iodine flask containing 150 mL of water and 10 mL of a 1:10 solution of potassium iodide, run the *Bromine–Bromide Solution* into the flask, and rinse the tube and spiral with water. Add the potassium iodide solution from trap *E* to the flask, rinsing the side arm and tube with a few mL of water, stopper the flask, and allow to stand for 5 min. Add 5 mL of 2 N sulfuric acid, and titrate immediately with 0.05 N sodium thiosulfate, using 2 mL of starch TS for the endpoint. Transfer the silver nitrate solution from tube *C* into a flask, rinsing the tube with water, dilute with water to 150 mL, and heat to boiling. Cool, and titrate with 0.05 N ammonium thiocyanate, using 3 mL of ferric ammonium sulfate TS as the indicator. Perform a blank determination. Calculate the percentage of oxyethylene groups (—CH_2CH_2O—), as ethylene, by the formula:

$$\text{Result} = (B - S) \times N \times 2.203/W$$

in which $B - S$ represents the difference between the volumes of sodium thiosulfate required for the blank and the sample solution, respectively; N is the normality of the sodium thiosulfate; W is the weight, in g, of the sample taken; and 2.203 is an equivalence factor for oxyethylene. Calculate the percentage of oxyethylene groups, as ethyl iodide, by the formula:

$$\text{Result} = (B' - S') \times N' \times 4.405/W$$

in which $B' - S'$ represents the difference between the volumes of ammonium thiocyanate required for the blank and the sample solution, respectively; N' is the normality of the ammonium thiocyanate; and 4.405 is an equivalence factor for oxyethylene. The sum of the values so obtained represents the percentage of oxyethylene groups in the sample taken.

PEROXIDE VALUE

Unless otherwise indicated in the monograph, use *Method I: Acetic Acid–Isooctane Method.*

The peroxide value is defined as the number of milliequivalents of peroxide per 1000 g of sample that oxidizes potassium iodide under the given test conditions.

Change to read:

Method 1: Acetic Acid–Isooctane Method (Based on AOCS Method Cd 8b-90▲1▲*FCC7*)

Solutions

Acetic Acid–Isooctane Solution: Mix 3 volumes of glacial acetic acid with 2 volumes of isooctane. [Note—Use a fume hood at all times, and avoid inhalation, injestion, and skin contact.]

Saturated Potassium Iodide Solution: Dissolve an excess of potassium iodide in recently boiled water. Prepare fresh daily, and make certain the solution remains saturated during use.

Starch Indicator Solution: Make a paste with 1 g of starch and a small amount of cold water, and add it, while stirring to 200 mL of boiling water. Remove from heat within a few seconds, and cool. If desired, add salicylic acid (1.25 g/L) as a preservative. The solution may be kept refrigerated at 4° to 10° for not more than 3 weeks. Test the solution for sensitivity before use by placing 5 mL of the solution in 100 mL of water and adding 0.05 mL of 0.1 N potassium iodide solution. The deep blue color produced must be discharged by 0.05 mL of 0.1 N sodium thiosulfate. If the solution fails the test, prepare a fresh starch solution.

Procedure: Transfer 5.00 ± 0.05 g of sample, accurately weighed, into a 250-mL Erlenmeyer flask fitted with a glass stopper, and add 50 mL of *Acetic Acid–Isooctane Solution*. Swirl to dissolve the sample, and add 0.5 mL of *Saturated Potassium Iodide Solution*. Allow the sample solution to stand, agitating it occasionally, for exactly 1 min, and immediately add 30 mL of water. Titrate with 0.1 N sodium thiosulfate solution, adding the solution gradually while constantly agitating until the yellow iodine color has almost disappeared. Add 0.5 mL of a 10% sodium lauryl sulfate solution, and then add approximately 0.5 mL of *Starch Indicator Solution*. Continue the titration while constantly agitating, especially near the endpoint to liberate all of the iodine from the solvent layer. Add 0.1 N thiosulfate solution dropwise until the blue color just disappears. If the titration is less than 0.5 mL using 0.1 N sodium thiosulfate, repeat the determination using 0.01 N sodium thiosulfate. Conduct a blank determination (see *General Provisions*), and make any necessary correction. Calculate the peroxide value by the formula:

$$\text{Result} = [(S - B) \times N \times 1000]/W$$

in which S is the volume, in milliliters, of 0.1 N sodium thiosulfate consumed by the sample; B is the volume, in milliliters, of 0.1 N sodium thiosulfate consumed by the blank; N is the normality of the sodium thiosulfate solution; and W is the weight, in grams, of the sample taken.

Change to read:

Method II (Alternatively, follow the AOCS Method Cd 8-53▲1 ▲*FCC7*) [Note—To make the solutions referenced below, please see the Fifth Edition, pages 974 (0.1 N sodium thiosulfate solution, dilute to 1:1 v/v) and 969 (starch TS).]

Accurately weigh about 10 g of sample, add 30 mL of a 3:2 mixture of glacial acetic acid:chloroform, and mix. Add 1 mL of a saturated solution of potassium iodide, mix, and allow it to stand for 10 min. Add 100 mL of water, begin titrating with 0.05 N sodium thiosulfate, adding starch TS as the endpoint is approached, and continue the titration until the blue starch color has just disappeared. Perform a blank determination (see *General Provisions*), and make any necessary correction. Calculate the peroxide value, as milliequivalents of peroxide per kilogram of sample, by the formula:

$$\text{Result} = S \times N \times 1000/W$$

in which S is the net volume, in milliliters, of sodium thiosulfate solution required for the sample; N is the exact normality of the sodium thiosulfate solution; and W is the weight, in grams, of the sample taken.

REICHERT-MEISSL VALUE

Change to read:

(Based on AOCS Method Cd 5-40▲1 ▲*FCC7*)

The Reichert-Meissl value is a measure of soluble volatile fatty acids (chiefly butyric and caproic). It is expressed in terms of the number of mL of 0.1 N sodium hydroxide required to neutralize the fatty acids obtained from a 5-g sample under the specified conditions of the method.

Apparatus: Use a glass distillation apparatus of the same dimensions and construction as that shown in *Fig. 36*.

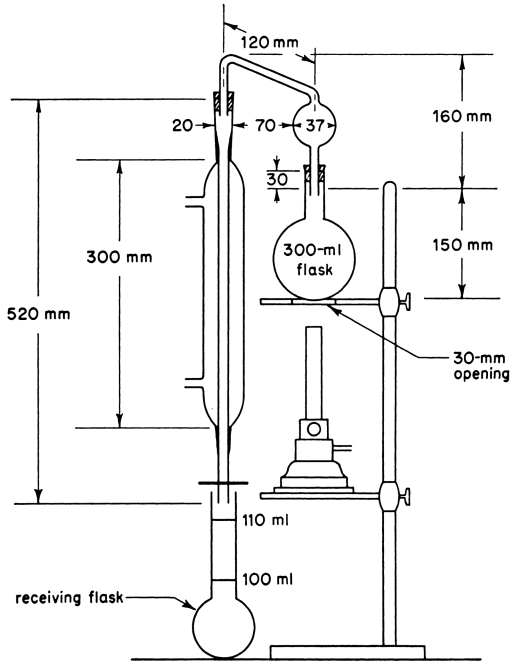

FIGURE 36 Reichert-Meissl Distillation Apparatus. [Note—A suitable heating mantle may be substituted for the burner.]

Reagents

Sodium Hydroxide Solution: Prepare a solution containing 50.0% by weight of NaOH, and protect from contact with carbon dioxide. Allow the solution to settle, and use only the clear liquid.

Glycerin–Sodium Hydroxide Mixture: Add 20 mL of the *Sodium Hydroxide Solution* to 180 mL of glycerin.

Procedure: Unless otherwise directed, accurately weigh about 5 g of the sample, previously melted if necessary, into the 300-mL distillation flask. Add 20.0 mL of the *Glycerin–Sodium Hydroxide Mixture*, and heat until the sample is completely saponified, as indicated by the mixture becoming perfectly clear. Shake the flask gently if any foaming occurs. Add 135 mL of recently boiled and cooled water, dropwise at first to prevent foaming, then add 6 mL of 1:5 sulfuric acid and a few pieces of pumice stone or silicon carbide. Rest the flask on a piece of heat-proof board having a center hole 5 cm in diameter, and begin the distillation, regulating the flame so as to collect 110 mL of distillate in 30 ± 2 min (measure time from the passage of the first drop of distillate from the condenser to the receiving flask), letting the distillate drip into the flask at a temperature not higher than 20°.

When 110 mL has distilled, disconnect the receiving flask, and remove the flame. Mix the contents of the flask with gentle shaking, and immerse almost completely for 15 min in water cooled to 15°. Filter the distillate through dry, 9-cm, moderately retentive paper (S & S No. 589 White Ribbon, or equivalent), add phenolphthalein TS, and titrate 100 mL of the filtrate with 0.1 N sodium hydroxide to the first pink color that remains unchanged for 2 to 3 min. Perform a blank determination using the same quantities of the same reagents, and calculate the Reichert-Meissl value by the formula:

$$\text{Result} = 1.1 \times (S - B)$$

in which S is the volume of 0.1 N sodium hydroxide required for the sample, and B is the volume required for the blank.

SAPONIFICATION VALUE

Change to read:

(Based on AOCS Methods Tl 1a-64▲1▲*FCC7* and Cd 3-25▲1▲*FCC7*)

The saponification value is defined as the number of mg of potassium hydroxide required to neutralize the free acids and saponify the esters in 1 g of the test substance.

Procedure: Melt the sample, if necessary, and pass it through a dry filter paper to remove any traces of moisture. Unless otherwise directed, weigh accurately into a 250-mL flask a sample of such size that the titration of the sample solution after saponification will require between 45% and 55% of the volume of 0.5 N hydrochloric acid required for the blank, and add to the flask 50.0 mL of 0.5 N alcoholic potassium hydroxide. Connect an air condenser, at least 65 cm in length, to the flask, and reflux gently until the sample is completely saponified (usually 30 min to 1 h). Cool slightly, wash the condenser with a few mL of water, add 1 mL of phenolphthalein TS, and titrate the excess potassium hydroxide with 0.5 N hydrochloric acid. Heat the contents of the flask to boiling, again titrate to the disappearance of any pink color that may have developed, and record the total volume of acid required. Perform a blank determination using the same amount of 0.5 N alcoholic potassium hydroxide. Calculate the saponification value by the formula:

$$\text{Result} = 56.1(B - S) \times N/W$$

in which B − S represents the difference between the volumes of 0.5 N hydrochloric acid required for the blank and the sample, respectively; N is the normality of the hydrochloric acid; and W is the weight, in g, of the sample taken.

[Note—A "masked phenolphthalein indicator" may be used with off-color materials. Prepare the indicator by dissolving 1.6 g of phenolphthalein and 2.7 g of methylene blue in 500 mL of alcohol, and adjust the pH with alcoholic alkali solution so that the greenish blue color is faintly tinged with purple. The color change, when going from acid to alkali, is from green to purple.]

SOAP

Prepare a solvent mixture consisting of equal parts, by volume, of benzene and methanol, add bromophenol blue TS, and neutralize with 0.5 N hydrochloric acid, or use neutralized acetone as the solvent. Accurately weigh the amount of sample specified in the individual monograph, dissolve it in 100 mL of the neutralized solvent mixture, and titrate with 0.5 N hydrochloric acid to a definite yellow endpoint. Calculate the percentage of soap in the sample by the formula:

$$\text{Result} = VNe/W$$

in which 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 given in the monograph.

SPECIFIC GRAVITY

The specific gravity of a fat or oil is determined at 25°, except when the substance is a solid at that temperature, in which case the specific gravity is determined at the temperature specified in the monograph, and is referred to water at 25°.

Clean a suitable pycnometer by filling it with a saturated solution of chromic acid (CrO_3) in sulfuric acid and allowing it to stand for at least 4 h. Empty the pycnometer, rinse it thoroughly, then fill it with recently boiled water, previously cooled to about 20°, and place in a constant-temperature bath at 25°. After 30 min, adjust the level of water to the proper point on the pycnometer, and stopper. Remove the pycnometer from the bath, wipe dry with a clean cloth free from lint, and weigh. Empty the pycnometer, rinse several times with alcohol and then with ether, allow to dry completely, remove any ether vapor, and weigh. Determine the weight of the contained water at 25° by subtracting the weight of the pycnometer from its weight when full.

Pass the oil or melted sample through filter paper to remove any impurities and the last traces of moisture, and cool to a few degrees below the temperature at which the determination is to be made. Fill the clean, dry pycnometer with the sample, and place it in the constant-temperature bath at the specified temperature. After 30 min, adjust the level of the oil to the mark on the pycnometer, insert the stopper, wipe dry, and weigh. Subtract the weight of the empty pycnometer from its weight when filled with the sample, and divide the difference by the weight of the water contained at 25°. The quotient is the specific gravity at the temperature of observation, referred to water at 25°.

STABILITY

STABILITY

Delete the following:

▲(Active Oxygen Method)▲*FCC7*

Add the following:

▲Unless otherwise indicated in the monograph, use *Method I: Active Oxygen Method.*▲*FCC7*

Change to read:

▲**Method I: Active Oxygen Method** (Based on AOCS Method Cd 12-57[1])▲*FCC7*

Fat stability is the time, in h, required for a sample of fat or oil to attain a peroxide value of 100. This period of time is determined by interpolation between two measurements and is assumed to be an index of resistance to rancidity.

[**CAUTION—** All equipment must be scrupulously clean. Do not use chromic acid or other acidic cleaning agents. All receptacles in the heater must be calibrated for temperature under the exact conditions of the test. During the test, the temperature must be monitored in a sample tube containing the recommended quantity of oil.]

Apparatus: Use a suitable heating block and aeration apparatus, such as shown in JAOCS *33* (1956), pp. 628–630.

Sampling: Remove samples from large containers or processing equipment with sampling devices only of stainless steel, aluminum, nickel, or glass. Solid fat samples should be taken at least 5 cm from the walls of large containers and 2.5 cm from the walls of small containers. If liquid oil is to be poured from a container, clean the spout or lip with an acetone-moistened cloth. Under no circumstances should samples be taken from containers equipped with plastic or enameled tops or paper or wax liners.

Procedure: Unless already completely liquid, the sample should be melted at a temperature not more than 10° above its melting point. Pour 20 mL into each of two or more sample tubes ensuring that the sample does not contact the tube where the stopper will later fit. Insert the aeration tube assembly so that the end of the air delivery tube is 5 cm below the surface of the sample. Place the sample tube in a container of vigorously boiling water for 5 min (during this time adjust the air flow rate from the manifold). Remove the tube, wipe dry, and transfer immediately to the constant-temperature heater, maintained at 97.8° ± 0.2°, and connect the aeration tube to the manifold. Determine to the nearest h the time required for the sample to attain a *Peroxide Value* (in this appendix) of 100 milliequivalents (meq) as follows: With 1-g samples determine when the peroxide value is approximately 75 meq and 125 meq, then perform the test on four 5-g samples determining the peroxide value in duplicate at the times corresponding to 75 and 125 meq. Make a second determination on two 5-g samples exactly 1 h after the first pair. Plot these values against aeration time; the AOM stability value in h is given where the line crosses 100 meq.

Add the following:

▲**Method II: Oil Stability Index** (Based on AOCS Method Cd 12b-92[1])

The oil stability index (OSI) is an indication of the resistance of a fat or oil to oxidation. It is measured as the length of time required, under specific accelerated oxidation conditions, before a rapid acceleration of oxidation occurs for a test oil or fat, also called the “induction period”.▲*FCC7*

Add the following:

▲**Apparatus:** The Oxidative Stability Instrument,[3■5]■1S (*FCC7*) Rancimat Model 617,[4■6]■1S (*FCC7*) or equivalent[5■7]■1S (*FCC7*) · ▲*FCC7*

Add the following:

▲**Sampling:** Samples should be kept cool and in the dark. Remove samples from large containers with clean sampling devices only of stainless steel, aluminum, nickel, or glass. Solid fat samples should be taken at least 5 cm from walls of large containers and 2.5 cm from walls of small containers. If liquid oil is to be poured, clean the spout with a clean, acetone-moistened cloth. Samples should be protected from heat, light, and air before analysis. ▲*FCC7*

Add the following:

▲**Procedure:** Fill the conductivity tubes with 50 mL of water (deionized or distilled with conductivity <5 µS-cm^{-1}) and attach the probes. Verify that the water conductivity in the tube is 25 µS-cm^{-1} or less and that the conductivity is constant. Unless already completely liquid, the sample should be melted at a temperature not more than 10° above its melting point. Carefully place 5.0 ± 0.2 g sample (Oxidative Stability and Rancimat instruments without an insert), or 2.5 ± 0.2 g sample (Rancimat instruments fitted with disposable inserts) into the bottom of the reaction tube, avoiding coating the sides of the tubes and contamination of samples. Connect tubing from the air manifold to the conductivity measurement tube, adjusting aeration tubes to within 5 mm of bottom of both the reaction and the conductivity tubes. Adjust the airflow to 2.5 ± 0.2 mL/s unless otherwise indicated in the individual monograph. Set the instrument to the temperature indicated in the individual monograph.

Use a computer or multichannel strip chart recorder to monitor the conductivity of each probe. Plot water conductivity versus time in h. Calculate the OSI value of the sample as the time in h which corresponds to the inflection point of the conductivity versus time curve. This time-based end point can be determined either by a micro-processor-computed slope/change algorithm or a maximum of the second derivative, or by the tangential method.

[Note—Improper temperature control is the most likely source of error for this procedure. Temperature calibration of the instrument should be performed before sample analysis by checking the actual temperature of the sample in the bath. Temperatures during analysis should be maintained within at least ±0.1°. For temperature calibration of the Rancimat instrument, a NIST traceable calibrated platinum resistance (RTD) digital thermometer can be used.[6■8]■1S (*FCC7*)]

[Note—Water in the effluent trap must not exceed 25° to minimize loss of formic acid.]

[Note—Trace-metal contamination of glassware will cause accelerated oxidation; rinse water should be tested; chromate cleaning solutions should not be used; only detergents without surface-active agents should be used for cleaning.]

[Note—Reaction tube cleaning procedures for instruments that do not utilize disposable glassware, or for precautionary cleaning of disposable glassware are described elsewhere[5■7]■1S (*FCC7*).]

▲*FCC7*

Add the following:

▲TOCOPHEROLS

(Based on AOCS Method Ce 8-89[1])

[Note—All solutions containing tocopherols should be stored in low-actinic glassware, or suitably protected from light.]

Standard Stock Solutions: Prepare individual standard stock solutions of α-, β-, γ-, and δ-tocopherol, unless otherwise directed in the monograph, according to the following general procedure.

Transfer 10 mg of suitable tocopherol standard into a 100-mL volumetric flask, and dilute with hexane to volume. Pipet 10 mL of this solution into an amber glass-round-bottom flask, and remove all hexane using a suitable rotary evaporator at a temperature not higher than 40°. Restore atmospheric pressure with nitrogen and remove the flask from the evaporator as soon as all solvent has been removed. Dissolve remaining tocopherol residue with 10 mL of methanol. Measure the absorbance (A) of this solution using a suitable spectrophotometer at the wavelength specified in the table below for the individual tocopherol being measured. Calculate the concentration (µg/mL) of the individual tocopherol in the solution by the following formula:

$$\text{Result} = A/(a \times b)$$

in which A is the absorbance of the solution at the measured wavelength; a is the absorptivity of the individual tocopherol from the table below; b is the cell path length (cm).

Tocopherol	Measurement Wavelength (nm)	Absorptivity ($mL \cdot \mu g^{-1} \cdot cm^{-1}$)
α-tocopherol	292	0.0076
β-tocopherol	296	0.0089
γ-tocopherol	298	0.0091
δ-tocopherol	298	0.0087

[Note—*Standard Stock Solutions* can be stored for up to one week under refrigerated conditions in low-actinic glassware or suitably protected from light.]

Standard Solution: Prepare a mixed tocopherol standard solution, unless otherwise directed in the monograph, as follows. Mix appropriate volumes of the individual tocopherol *Standard Stock Solutions* and dilute with hexane to give a solution containing between 1 and 5 µg/mL of each tocopherol.

[Note—A more concentrated *Standard Solution* may be necessary if UV detection is used.]

[Note—The *Standard Solution* should be prepared fresh daily and stored under refrigerated conditions in low-actinic glassware or suitably protected from light.]

Test Solution: Accurately weigh 2 g of sample into a 25-mL volumetric flask. Add hexane to dissolve the *Test Sample*, and dilute with hexane to volume.

[Note—If a fluorescence detector is used, it may be necessary to further dilute this test solution before analysis.]

[Note—The *Test Solution* should be prepared and analyzed on the same day, and stored in low-actinic glassware or suitably protected from light.]

Apparatus: Use a suitable high-performance liquid chromatography system (see *Chromatography,* Appendix IIA) equipped with either a UV (292 nm) or fluorescence (290 nm excitation and 330 nm emission) detector, or as directed in the monograph. Use a 250 × 4 mm analytical column packed with a 5-µm microparticulate

silica[7■9]■1S (*FCC7*).

Operating Conditions

[Note—New columns should be washed and conditioned for 10 min with methanol, then dicholormethane, followed by hexane at 1 mL/min. All columns should be equilibated, if necessary with mobile phase for 30 min before analysis.]

Mobile phase: Isopropanol in hexane (0.5:99.5, v/v)

Flow rate: About 1 mL/min

Injection volume: About 20 µL

System Suitability: Inject the *Standard Solution* into the chromatograph and measure the peak responses on the resulting chromatograms. The retention time for α-tocopherol is not less than 5 min. The resolution, R, between β- and γ-tocopherol is not less than 1.0. The relative standard deviation for the α-tocopherol peak area for replicate injections is no more than 5%.

Procedure: Inject the *Test Solution* into the chromatograph and measure the peak responses on the resulting chromatograms. The relative retention times for α-, β-, γ-, and δ-tocopherol are approximately 1.0, 1.6, 3.0, and 1.7, respectively. Separately calculate the concentration, in mg/kg, of each tocopherol in the portion of the sample taken by the following formula:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U)$$

in which r_U is the peak response for the tocopherol from the *Test Solution*; r_S is the peak response for the tocopherol from the *Standard Solution*; C_S is the concentration of tocopherol in the *Standard Solution* (µg/mL); and C_U is the concentration of sample in the *Test Solution* (g/mL).

▲*FCC7*

UNSAPONIFIABLE MATTER

Change to read:

(Based on AOCS Method Ca 6a-40[▲1]▲*FCC7*)

This procedure determines those substances frequently found dissolved in fatty materials that cannot be saponified by alkali hydroxides but that are soluble in the ordinary fat solvents.

Procedure: Accurately weigh 5.0 g of the sample into a 250-mL flask, add a solution of 2 g of potassium hydroxide in 40 mL of alcohol, and boil gently under a reflux condenser for 1 h or until saponification is complete. Transfer the contents of the flask to a glass-stoppered extraction cylinder (approximately 30 cm in length, 3.5 cm in diameter, and graduated at 40, 80, and 130 mL). Wash the flask with sufficient alcohol to make a volume of 40 mL in the cylinder, and complete the transfer with warm and then cold water until the total volume is 80 mL. Finally, wash the flask with a few mL of petroleum ether, add the washings to the cylinder, cool the contents of the cylinder to room temperature, and add 50 mL of petroleum ether.

Insert the stopper, shake the cylinder vigorously for at least 1 min, and allow both layers to become clear.

Siphon the upper layer as completely as possible without removing any of the lower layer, collecting the ether fraction in a 500-mL separator. Repeat the extraction and siphoning at least six times with 50-mL portions of petroleum ether, shaking vigorously each time. Wash the combined extracts, with vigorous shaking, with 25-mL portions of 10% alcohol until the wash water is neutral to phenolphthalein, and discard the washings. Transfer the ether extract to a tared beaker, and rinse the separator with 10 mL of ether, adding the rinsings to the beaker. Evaporate the ether on a steam bath just to dryness, and dry the residue to constant weight, preferably at 75° to 80° under a vacuum of not more than 200 mm Hg, or at 100° for 30 min. Cool in a desiccator, and weigh to obtain the uncorrected weight of unsaponifiable matter.

Determine the quantity of fatty acids in the residue as follows: Dissolve the residue in 50 mL of warm alcohol (containing phenolphthalein TS and previously neutralized with sodium hydroxide to a faint pink color), and titrate with 0.02 N sodium hydroxide to the same color. Each mL of 0.02 N sodium hydroxide is equivalent to 5.659 mg of fatty acids, calculated as oleic acid.

Subtract the calculated weight of fatty acids from the weight of the residue to obtain the corrected weight of unsaponifiable matter in the sample.

VOLATILE ACIDITY

Modified Hortvet-Sellier Method

Apparatus: Assemble a modified Hortvet-Sellier distillation apparatus as shown in *Fig. 37*, using a sufficiently large (approximately 38- × 203-mm) inner Sellier tube and large distillation trap.

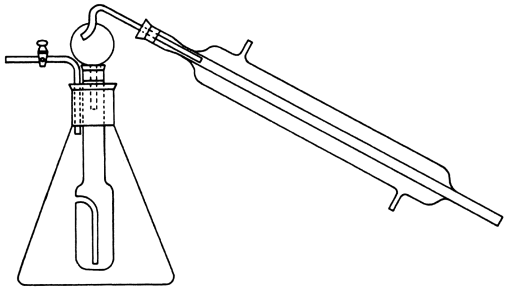

FIGURE 37 Modified Hortvet-Sellier Distillation Apparatus.

Procedure: Transfer the amount of sample, accurately weighed, specified in the monograph into the inner tube of the assembly, and insert the tube in the outer flask containing about 300 mL of recently boiled hot water. To the sample add 10 mL of approximately 4 N perchloric acid [35 mL (60 g) of 70% perchloric acid in 100 mL of water], and connect the inner tube to a water-cooled condenser through the distillation trap. Distill by heating the outer flask so that 100 mL of distillate is collected within 20–25 min. Collect the distillate in 100-mL portions, add phenolphthalein TS to each portion, and titrate with 0.5 N sodium hydroxide. Continue the distillation until a 100-mL portion of the distillate requires no more than 0.5 mL of 0.5 N sodium hydroxide for neutralization.
[**CAUTION—** Do not distill to dryness.]

Calculate the weight, in mg, of volatile acids in the sample taken by the formula:

$$\text{Result} = V \times e$$

in which V is the total volume, in mL, of 0.5 N sodium hydroxide consumed in the series of titrations and e is the equivalence factor given in the monograph.

[1] Full text of method available from the American Oil Chemists' Society (AOCS) at www.aocs.org.

[2] Supelco SUPELCOWAX®-10, Restek FAMEWAX™, Agilent HP-INNOwax™, Varian CP-WAX™ 52 CB, Agilent Carbowax™-20M, Supelco Omegawax™ 320, or equivalent.

[3] Restek part no. 21022-211.5, SGE part no. 092002, or equivalent.

~~1▲2▲*FCC7*■4■~~ 1S (*FCC7*) The monoglyceride may be calculated to some monoester other than glyceryl monostearate by dividing the molecular weight of the monoglyceride by 20 and substituting the value so obtained for 17.927 in the formula, using 17.80, for example, in calculating to the monooleate.

~~3■5■~~ 1S (*FCC7*) Ommion Inc., Rockland, MA, USA (manufactured under license from Archer Daniel Midland Co., Decatur, IL, USA).

~~4■6■~~ 1S (*FCC7*) Brinkmann Instruments, Inc., Westbury NY, USA (subsidiary of Sybron Corporation).

~~5■7■~~ 1S (*FCC7*) Suitable alternative equipment is described in AOCS Official Method Cd 12b-92. Official Methods and Recommended Practices of the American Oil Chemists' Society. American Oil Chemists' Society, 5th Edn, Champaign, IL.

~~6■8■~~ 1S (*FCC7*) Omega Engineering, Inc. (Stanford, CT) provides such a thermometer. A custom 3 wire 2 mm × 7 mm sensor probe is required for calibration of sample temperature with air flowing through the sample.

~~7■9■~~ 1S (*FCC7*) LiChrosorb SI 60, Spherisorb S5W, or equivalent.

BRIEFING

Appendix XII: Microbiological Tests. On the basis of comments received for the development of three proposed new *FCC* monographs (*5'-Adenylic Acid*, *5'-Cytidylic Acid* and *Disodium 5'-Uridylate*), it is proposed to add the following new appendix to the *FCC*—See briefing under *Disodium 5'-Uridylate*. The proposed test procedures are based on *Microbial Enumeration Tests* ⟨ 61 ⟩ and *Tests for Specified Microorganisms* ⟨ 62 ⟩ in the *First Supplement* to *USP 32–NF 27*. These chapters were recently harmonized through the Pharmacopeial Discussion Group (PDG) process with the European and Japanese pharmacopoeias. Interested parties are encouraged to submit comments to Jeff Moore, Ph.D., jm@usp.org.

(FIEC: J. Moore) C82098

Add the following:

▪APPENDIX XII: MICROBIOLOGICAL TESTS

A. MEDIA AND REAGENTS

The following solutions and culture media have been found satisfactory for the purposes for which they are prescribed in the tests for microbial contamination in this appendix. Other media may be used provided that their suitability can be demonstrated.

Stock Buffer Solution Transfer 34 g of potassium dihydrogen phosphate to a 1000-mL volumetric flask, dissolve in 500 mL of water, adjust with sodium hydroxide to a pH of 7.2 ± 0.2, add water to volume, and mix. Dispense in containers, and sterilize. Store at a temperature of 2° to 8°.

Phosphate Buffer Solution pH 7.2 Prepare a mixture of water and *Stock Buffer Solution* (800:1 v/v), and sterilize.

Buffered Sodium Chloride-Peptone Solution pH 7.0

Potassium dihydrogen phosphate	3.6 g
Disodium hydrogen phosphate dihydrate	7.2 g (equivalent to 0.067 M phosphate)
Sodium chloride	4.3 g
Peptone (meat or casein)	1.0 g
Water	1000 mL

Sterilize in an autoclave using a validated cycle.

Enterobacteria Enrichment Broth Mossel

Pancreatic digest of gelatin	10.0 g
Glucose monohydrate	5.0 g
Dehydrated ox bile	20.0 g
Potassium dihydrogen phosphate	2.0 g
Disodium hydrogen phosphate dihydrate	8.0 g
Brilliant green	15 mg
Water	1000 mL

Adjust the pH so that after heating it is 7.2 ± 0.2 at 25°. Heat at 100° for 30 min, and cool immediately.

Rappaport Vassiliadis Salmonella Enrichment Broth

Soya peptone	4.5 g
Magnesium chloride hexahydrate	29.0 g
Sodium chloride	8.0 g
Dipotassium phosphate	0.4 g
Potassium dihydrogen phosphate	0.6 g
Malachite green	0.036 g
Water	1000 mL

Dissolve, warming slightly. Sterilize in an autoclave using a validated cycle, at a temperature not exceeding 115°. The pH is to be 5.2 ± 0.2 at 25° after heating and autoclaving.

Sabouraud Dextrose Agar

Dextrose	40.0 g
Mixture of peptic digest of animal tissue and pancreatic digest of casein (1:1)	10.0 g
Agar	15.0 g
Water	1000 mL

Adjust the pH so that after sterilization it is 5.6 ± 0.2 at 25°. Sterilize in an autoclave using a validated cycle.

Soybean-Casein Digest Agar

Pancreatic digest of casein	15.0 g
Papaic digest of soybean	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Water	1000 mL

Adjust the pH so that after sterilization it is 7.3 ± 0.2 at 25°. Sterilize in an autoclave using a validated cycle.

Soybean-Casein Digest Broth

Pancreatic digest of casein	17.0 g
Papaic digest of soybean	3.0 g
Sodium chloride	5.0 g
Dibasic hydrogen phosphate	2.5 g
Glucose monohydrate	2.5 g
Water	1000 mL

Adjust the pH so that after sterilization it is 7.3 ± 0.2 at 25°. Sterilize in an autoclave using a validated cycle.

Violet Red Bile Glucose Agar

Yeast extract	3.0 g
Pancreatic digest of gelatin	7.0 g
Bile salts	1.5 g
Sodium chloride	5.0 g
Glucose monohydrate	10.0 g
Agar	15.0 g
Neutral red	30 mg
Crystal violet	2 mg
Water	1000 mL

Adjust the pH so that after heating it is 7.4 ± 0.2 at 25°. Heat to boiling; do not heat in an autoclave.

Xylose Lysine Deoxycholate Agar

Xylose	3.5 g
L-Lysine	5.0 g
Lactose monohydrate	7.5 g
Sucrose	7.5 g
Sodium chloride	5.0 g
Yeast extract	3.0 g
Phenol red	80 mg
Agar	13.5 g
Sodium deoxycholate	2.5 g
Sodium thiosulfate	6.8 g
Ferric ammonium citrate	0.8 g
Water	1000 mL

Adjust the pH so that after heating it is 7.4 ± 0.2 at 25°. Heat to boiling, cool to 50°, and pour into Petri dishes. Do not heat in an autoclave.

B. MICROBIOLOGICAL ENUMERATION TESTS

The tests described in section B of this appendix are used for quantitative enumeration of mesophilic bacteria and fungi that may grow under aerobic conditions in food ingredients.

The tests are designed primarily to determine whether a food ingredient complies with an established specification for microbiological quality. When used for such purposes, follow the instructions given below, including the number of samples to be taken, and interpret the results as stated.

The methods are not applicable to products containing viable microorganisms as active ingredients. Alternative microbiological procedures, including automated methods, may be used, provided that their equivalence to the FCC method has been demonstrated. [Note—In preparing for and in applying the tests, observe aseptic precautions in handling the specimens.]

TOTAL AEROBIC MICROBIAL COUNT

Method I (Plate Count Method)

Sample Preparation

Unless otherwise directed in the monograph, dissolve or dilute 10 g or 10 mL of sample to be examined in 100 mL of *Buffered Sodium Chloride-Peptone Solution pH 7.0, Phosphate Buffer Solution pH 7.2*, or *Soybean-Casein Digest Broth*. If necessary, adjust to a pH of 6 to 8. Further dilutions, where necessary, are prepared with the same diluent.

Procedure

Negative Control To verify testing conditions, a negative control is performed using the chosen diluent in place of the test preparation. There must be no growth of microorganisms.

Analysis To 9-cm Petri dishes add 1 mL of the *Sample Preparation* and 15–20 mL of *Soybean-Casein Digest Agar* which is not more than 45°. Prepare at least 2 Petri dishes for each level of dilution. Incubate the plates at 30° to 35° for 3–5 days.

Interpretation of Results

Select the plates corresponding to a given dilution and showing the highest number of colonies less than 250. The total aerobic microbial count (TAMC) is considered to be equal to the number of CFU found. If colonies of fungi are detected, they are counted as part of the TAMC. Take the arithmetic mean of the counts and calculate the number of CFU per g or per mL of product.

TOTAL YEASTS AND MOLDS COUNT

Method I (Plate Count Method)

Sample Preparation

Unless otherwise directed in the monograph, dissolve or dilute 10 g or 10 mL of sample to be examined in 100 mL of *Buffered Sodium Chloride-Peptone Solution pH 7.0, Phosphate Buffer Solution pH 7.2*, or *Soybean-Casein Digest Broth*. If necessary, adjust to a pH of 6–8. Further dilutions, where necessary, are prepared with the same diluent.

Procedure

Negative Control To verify testing conditions, a negative control is performed using the chosen diluent in place of the test preparation. There must be no growth of microorganisms.

Analysis To 9-cm Petri dishes add 1 mL of the *Sample Preparation* and 15–20 mL of *Sabouraud Dextrose Agar*, which is not more than 45°. Prepare at least two Petri dishes for each level of dilution. Incubate the plates at 30° to 35° for 3–5 days.

Interpretation of Results

Select the plates corresponding to a given dilution and showing the highest number of colonies less than 50. The total combined yeasts and molds count (TYMC) is considered to be equal to the number of CFU found. If colonies of bacteria are detected, they are counted as part of the TYMC. Take the arithmetic mean of the counts and calculate the number of CFU per g or per mL of sample.

C. TESTS FOR ABSENCE OF SPECIFIC MICROORGANISMS

The tests described in section C of this appendix are used for determining the absence of objectionable microorganisms in food ingredients. [Note—In preparing for and in applying the tests, observe aseptic precautions in handling the specimens.]

BILE-TOLERANT GRAM-NEGATIVE BACTERIA

Sample Preparation Unless otherwise directed in the monograph, dissolve a sample of not less than 1 g in *Soybean-Casein Digest Broth* at a sample/broth ratio of 1/10, mix, and incubate at 20° to 25° for a time sufficient to resuscitate the bacteria but not sufficient to encourage multiplication of the organisms (usually 2 h but not more than 5 h).

Procedure Unless otherwise directed in the monograph, inoculate *Enterobacteria Enrichment Broth Mossel* with a volume of *Sample Preparation* corresponding to 1 g of sample. Incubate at 30° to 35° for 24–48 h. Subculture on plates of *Violet Red Bile Glucose Agar*. Incubate at 30° to 35° for 18–24 h.

Interpretation of Results The product complies with the test if there is no growth of colonies.

ENTEROBACTER SAKAZAKII (CRONOBACTER SPP.)

Sample Preparation Proceed as directed in the test for *Bile-Tolerant Gram-Negative Bacteria* above.

Procedure Proceed as directed in the test for *Bile-Tolerant Gram-Negative Bacteria* above. Growth of yellow-pigmented colonies indicates the possible presence of *E. sakazakii*. This is confirmed by a negative oxidase test in conjunction with other commercially available identification tests[1] .

Interpretation of Results If the identification of a colony identified on *Violet Red Bile Glucose Agar* leads to *E. sakazakii*, then the test is considered as positive. If the identification leads to other bacteria than *E. sakazakii*, then the test is considered as negative. If there is no growth on the *Violet Red Bile Glucose Agar* plate, then the test is considered as negative.

SALMONELLA SPP.

Sample Preparation Dissolve the quantity of sample, as directed in the monograph, in a volume of *Soybean-Casein Digest Broth*, as directed in the monograph, mix, and incubate at 30° to 35° for 18–24 h.

Procedure Transfer 0.1 mL of the *Sample Preparation* to 10 mL of *Rappaport Vassiliadis Salmonella Enrichment Broth*, and incubate at 30° to 35° for 18–24 h. Subculture on plates of *Xylose Lysine Deoxycholate Agar*. Incubate at 30° to 35° for 18–48 h. The possible presence of *Salmonella* is indicated by the growth of well-developed, red colonies, with or without black centers. This is confirmed by commercially available identification tests.

Interpretation of Results The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

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[1] API ID 32 E (bioMérieux, Durham, NC), or equivalent.