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.
Acetaldehyde, FCC 6 page 10; \(\alpha\)-Amylcinnamaldehyde, FCC 6 page 50; Benzaldehyde, FCC 6 page 81;
Butyraldehyde, FCC 6 page 121; Camphene, FCC 6 page 154; \(\beta\)-Caryophyllene, FCC 6 page 180; Cinnamaldehyde, FCC 6 page 196; Citral, FCC 6 page 207; Citronellal, FCC 6 page 209; Cuminc Aldehyde, FCC 6 page 231; Cyclamen Aldehyde, FCC 6 page 234; \((E),(E)\)-2,4-Decadienal, FCC 6 page 244; Decanal, FCC 6 page 246; \((E)\)-2-Decenal, FCC 6 page 247; \((Z)\)-4-Decenal, FCC 6 page 248; Diacetyl, FCC 6 page 255; 3,4-Dimethyl 1,2-Cyclopentadione, FCC 6 page 272; 2,6-Dimethyl-5-heptenal, FCC 6 page 274; \((E)\)-2-Dodecen-1-al, FCC 6 page 287; 2-Ethylbutyraldehyde, FCC 6 page 308; Furfural, FCC 6 page 383; \((E),(E)\)-2,4-Heptadienal, FCC 6 page 428; Heptanal, FCC 6 page 430; 2,3-Heptandione, FCC 6 page 431; Hexanal, FCC 6 page 438; \((E)\)-2-Hexenal-1-al, FCC 6 page 439; Hydroxycitronellal, FCC 6 page 464; 4-Hydroxy-2,5-dimethyl-3(2H)-furanone, FCC 6 page 466; Isobutyaldehyde, FCC 6 page 505; Lauryl Aldehyde, FCC 6 page 530; \(\alpha\)-Limonene, FCC 6 page 545; \(\beta\)-Limonene, FCC 6 page 546; 2-Methyl Butanal, FCC 6 page 615; 3-Methyl Butanal, FCC 6 page 615; \(\alpha\)-Methylcinnamaldehyde, FCC 6 page 619; 5-Methyl Furfural, FCC 6 page 627; 5-Methyl-2-isopropyl-2-hexenal, FCC 6 page 634; 5-Methyl 2-Phenyl 2-Hexenal, FCC 6 page 644; 3-Methylthiopropionaldehyde, FCC 6 page 649; 2-Methylundecanal, FCC 6 page 650; Myrcene, FCC 6 page 660; \((E),(E)\)-2,4-Nonadienal, FCC 6 page 679; \((E),(Z)\)-2,6-Nonadienal, FCC 6 page 680; Nonanal, FCC 6 page 683; \((E)\)-2-Nonenal, FCC 6 page 685; Octanal, FCC 6 page 693; \((E)\)-2-Octenal-1-al, FCC 6 page 695; 2,3-Pentanedione, FCC 6 page 730; \(\alpha\)-Phellandrene, FCC 6 page 739; Phenylacetalddehyde, FCC 6 page 747; 2-Phenylpropionaldehyde, FCC 6 page 757; 3-Phenylpropionaldehyde, FCC 6 page 758; \(\alpha\)-Pinene, FCC 6 page 766; \(\beta\)-Pinene, FCC 6 page 766; Propionaldehde, FCC 6 page 824; Salicylaldehyde, FCC 6 page 858; \(\alpha\)-Terpinene, FCC 6 page 953; \(\gamma\)-Terpinene, FCC 6 page 953; Tolualdehyde, Mixed Isomers, FCC 6 page 980; \(p\)-Tolualdehyde, FCC 6 page 981; 2-Tridecenal, FCC 6 page 987; 3,5,5,-Trimethyl Hexenal, FCC 6 page 989; Undecanal, FCC 6 page 996; 1,3,5-Undecatriene, FCC 6 page 998; 10-Undecenal, FCC 6 page 999; Valeraldehyde, FCC 6 page 1003.

On the basis on comments received, it is proposed to add a statement to the Description section for flavoring agents susceptible to oxidation, allowing the use of a suitable antioxidant. Addition of such substances would be required to comply with the “Added Substances” requirement in the General Provisions and Requirements, FCC 6 page 1. Interested parties are encouraged to submit comments by October 1, 2008 to Jeff Moore, Ph.D., e-mail jm@usp.org.

(FIEC: J. Moore)  C64747

Acetaldehyde

Acetic Aldehyde

Ethanal
DESCRIPTION

*Change to read:* Acetaldehyde occurs as a flammable, colorless liquid. It may contain a suitable antioxidant.  

**Odor** Pungent, ethereal  

**Solubility** Miscible in alcohol, organic solvents, water  

**Boiling Point** ~ 21°C  

**Function** Flavored agent

IDENTIFICATION  
- **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.

ASSAY  
- **PROCEDURE** Proceed as directed under M-2b, Appendix XI.  
  
  **Acceptance criteria:** NLT 99.0% of C₂H₄O

SPECIFIC TESTS  
- **ACID VALUE, M-15, Appendix XI**  
  
  **Acceptance criteria:** NMT 5.0  

- **Specific Gravity** Determine at 0°C ± 0.05°C by means of a hydrometer calibrated to give the apparent specific
gravity at 0\(^\circ\)/20\(^\circ\) (see General Provisions).

**Acceptance criteria:** Between 0.804 and 0.811

**OTHER REQUIREMENTS**

- **RESIDUE ON EVAPORATION, M-16, Appendix XI**

  **Acceptance criteria:** 0.006%

**Auxiliary Information**—Staff Liaison: Kristie Bowman, Senior Scientific Associate

Expert Committee: (FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 10

Phone Number: 1-301-816-8356
**BRIEFING**

α-Amylcinnamaldehyde, *FCC 6 page 50—See briefing under Acetaldehyde.*  
(FIEC: J. Moore) C64747

**α-Amylcinnamaldehyde**

Amylcinnamaldehyde

\[
\begin{align*}
C_{14}H_{18}O
\end{align*}
\]

**DESCRIPTION**

*Change to read:*  
α-Amylcinnamaldehyde occurs as a yellow liquid. It may contain a suitable antioxidant.  
**Odor** Strong, floral, jasmine on dilution, spicy  
**Solubility** Soluble in most fixed oils; insoluble or practically insoluble in glycerin, propylene glycol  
**Boiling Point** \( \sim 285^\circ \)  
**Solubility in Alcohol, Appendix VI**  
One mL dissolves in 5 mL of 80% alcohol.  
**Function** Flavoring agent

**IDENTIFICATION**

- **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.
ASSAY

• **Procedure** Proceed as directed under M-1b, Appendix XI.
  
  **Acceptance criteria:** NLT 97.0% of C₁₄H₁₈O (sum of two isomers) and NLT 90% of the main isomer

SPECIFIC TESTS

• **ACID VALUE, M-15, Appendix XI**
  
  **Acceptance criteria:** NMT 5.0

• **REFRACTIVE INDEX, Appendix II (at 20°)**
  
  **Acceptance criteria:** Between 1.554 and 1.559

• **Specific Gravity** Determine at 25° by any reliable method (see General Provisions).
  
  **Acceptance criteria:** Between 0.963 and 0.968

OTHER REQUIREMENTS

• **CHLORINATED COMPOUNDS, Appendix VI**
  
  **Acceptance criteria:** Passes test.

**Auxiliary Information**— **Staff Liaison:** Kristie Bowman, Senior Scientific Associate

**Expert Committee:** (FI07) Food Ingredients Expert Committee

**FCC Sixth Edition Page 50**

**Phone Number:** 1-301-816-8356
BRIEFING

Benzaldehyde, FCC 6 page 81—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

Benzaldehyde

C₇H₆O

Formula wt 106.12
FEMA: 2127

DESCRIPTION

Change to read:
Benzaldehyde occurs as a colorless liquid with a burning taste. It may contain a suitable antioxidant.

Odor Bitter almond oil
Solubility Slightly soluble in water; miscible in alcohol, ether, most fixed oils, volatile oils

Boiling Point ~178°
Function Flavoring agent

IDENTIFICATION

- 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.
ASSAY

• **PROCEDURE** Proceed as directed under \textit{M-1b, Appendix XI.}

  Acceptance criteria: NLT 98.0% of C$_7$H$_6$O

SPECIFIC TESTS

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

  Acceptance criteria: Between 1.544 and 1.547

• **SPECIFIC GRAVITY** Determine at 25° by any reliable method (see \textit{General Provisions}).

  Acceptance criteria: Between 1.041 and 1.046

OTHER REQUIREMENTS

• **CHLORINATED COMPOUNDS**, Appendix VI

  Acceptance criteria: Passes test.

• **HYDROCYANIC ACID, M-8**, Appendix XI

  Acceptance criteria: Passes test.

**Auxiliary Information**— \textit{Staff Liaison}: Kristie Bowman, Senior Scientific Associate

\textit{Expert Committee}: (FI07) Food Ingredients Expert Committee

\textit{FCC Sixth Edition} Page 81

\textit{Phone Number}: 1-301-816-8356
BRIEFING

Butyraldehyde, FCC 6 page 121—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

Butyraldehyde

Butyl Aldehyde

\[ \text{C}_4\text{H}_8\text{O} \]

Formula wt 72.11
FEMA: 2219

DESCRIPTION

**Change to read:**
Butyraldehyde occurs as a colorless, mobile liquid. It may contain a suitable antioxidant. 1S (FCC 6)

**Odor** Pungent, nutty

**Solubility** 1 mL dissolves in 15 mL water; miscible in alcohol, ether

**Boiling Point** ~74.8°

**Function** Flavoring agent

IDENTIFICATION

- **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.

ASSAY

- **Procedure** Proceed as directed under M-2c, Appendix XI.

  **Acceptance criteria:** NLT 98.0% of C\textsubscript{4}H\textsubscript{8}O
SPECIFIC TESTS

• **Acid Value, M-15**, Appendix XI (Use methyl red TS as the indicator.)
  
  **Acceptance criteria:** NMT 5.0

• **Refractive Index, Appendix II** (at 20°)
  
  **Acceptance criteria:** Between 1.381 and 1.387

• **Specific Gravity** Determine at 25° by any reliable method (see General Provisions).
  
  **Acceptance criteria:** Between 0.797 and 0.802

OTHER REQUIREMENTS

• **Distillation Range, Appendix IIB**
  
  **Acceptance criteria:** Between 72° and 80° (First 95%)

• **Para-Butyraldehyde, M-1b, Appendix XI**
  
  **Acceptance criteria:** NMT 2.5%

• **Water, Water Determination, Method I, Appendix IIB**
  
  **Acceptance criteria:** NMT 0.5%

**Auxiliary Information**— **Staff Liaison:** Kristie Bowman, Senior Scientific Associate

**Expert Committee:** (FI07) Food Ingredients Expert Committee

**FCC Sixth Edition Page 121**

**Phone Number:** 1-301-816-8356
Calcium Lignosulfonate, FCC 6 page 136; Sodium Lignosulfonate, FCC 6 page 893. On the basis of data and comments received, it is proposed to correct the viscometer model used in the Viscosity of a 50% Solution test procedure. Since under the conditions of this test procedure, lignosulfonate solutions behave as non-Newtonian fluids, this test procedure measures relative viscosities. For accurate and reproducible results, it is therefore critical for the analyst for to use the prescribed test conditions including rpm, spindle, and temperature. It is also proposed to indicate in the test procedure that it measures relative viscosity. Interested parties are encouraged to submit comments to Jeff Moore, Ph.D.

Calcium Lignosulfonate

CAS: [8061-52-7]

DESCRIPTION

Calcium Lignosulfonate occurs as a brown, amorphous polymer. It is obtained from the spent sulfite and sulfate pulping liquor of wood or from the sulfate (Kraft) pulping process. It may contain up to 30% reducing sugars. It is soluble in water, but not in any of the common organic solvents. The pH of a 1:100 aqueous solution is between approximately 3 and 11.

Function Binder; dispersant

Packaging and Storage Store in well-closed containers.

IDENTIFICATION

• A. CALCIUM, Appendix IIIA
  Sample solution: 0.15 mg/mL
  Acceptance criteria: Passes tests.

• B. PROCEDURE
  Sample: 100 mg
  Analysis: Dissolve the Sample in 50 mL of water. Add 1 mL each of 10% acetic acid and 10% sodium nitrite solution and mix by swirling. Allow the solution to stand for 15 min at room temperature.
  Acceptance criteria: A brown color appears.

• C. ULTRAVIOLET ABSORPTION
  Sample solution: 0.1 mg/mL (pH 5)
  Acceptance criteria: A peak is observed between 275 and 280 nm.

ASSAY

• SULFONATE SULFUR
  Sample: 1.0 g
  Analysis: Dissolve the Sample in 400 mL of water in a beaker. Direct a gentle stream of nitrogen gas over the liquid’s surface. Add 10 mL of nitric acid, and swirl the solution thoroughly until the reaction subsides. Add 10 mL of 70% perchloric acid, and swirl thoroughly again. [CAUTION: Handle perchloric acid in an appropriate fume hood.] Place the uncovered beaker on a hot plate, and heat the contents vigorously until the center of the bottom of the beaker becomes clear. Remove the beaker, and cool it to room temperature. Add 5 mL of hydrochloric acid, and heat it again until white fumes evolve. After cooling the beaker, dilute the solution to approximately 100 mL with water, adjust to pH 6 ± 0.2 with 10% sodium hydroxide, and heat the solution to boiling. Add 15 mL of 10% barium chloride solution, and leave the solution overnight in a fresh beaker in a steam bath at 90° to 95°. Filter through ashless filter paper.
(Whatman No. 42, or equivalent), and wash the precipitate with 200 mL of warm water. Transfer the paper and precipitate into a tared crucible. Heat the crucible slowly on a Bunsen burner to expel moisture. Place the crucible and contents in a muffle furnace at 850° for 1 h. Let the crucible cool in a desiccator, and then weigh the residue to the nearest 0.0001 g. Calculate the percent sulfonate sulfur by the formula:

\[(R/S) \times 13.7\]

\[R = \text{Weight (g) of the residue}\]
\[S = \text{Weight (g) of the sample taken}\]

Acceptance criteria: NLT 5.0% sulfonate sulfur

IMPURITIES
Inorganic Impurities
- Lead, Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace, Method I, Appendix IIIB
  Acceptance criteria: NMT 1 mg/kg

SPECIFIC TESTS
- Calcium
  Strontium chloride solution: While stirring, add 164.7 g of 60% perchloric acid to 500 mL of water contained in a 1-L beaker. [CAUTION: Handle perchloric acid in an appropriate fume hood.] Then, while stirring, add 15.2 g of strontium chloride hexahydrate, stirring until solution is complete. Transfer the solution into a 1-L volumetric flask, and dilute to volume at room temperature with water. Mix thoroughly.
  Standard solution: 0.7 mg/mL of calcium, prepared from a certified Calcium Standard Solution (NIST, or equivalent). [Note: Store the Standard solution in polyethylene bottles because of its instability in glass.]
  Sample: 1 g, previously dried
  Sample solution: Dilute the Sample to 10 mL and mix. If the solution is not particle-free, filter through a 0.45-µm disposable Millipore filter, discarding the first few mL of filtrate. Pipet 5 mL of Strontium chloride solution into a 50-mL volumetric flask and add 5.0 mL of the filtrate or clear solution. Dilute to volume with water, and mix well.
  Analysis: Using a suitably calibrated atomic absorption spectrophotometer, determine the absorbance of the Standard solution and the Sample solution at 422.7 nm.
  Acceptance criteria: The absorbance of the Sample solution is not greater than that of the Standard solution. (NMT 7.0%)
- Loss on Drying, Appendix IIC (105° for 24 h)
  Acceptance criteria: NMT 10.0%
- Reducing Sugars
  Copper reagent solution: [Note: Solution must be prepared several days in advance of use.] Dissolve 28 g of anhydrous dibasic sodium phosphate and 40 g of potassium sodium tartrate tetrahydrate in 700 mL water. Add 100 mL of 1 N sodium hydroxide and 8 g of copper sulfate pentahydrate, followed by 180 g of anhydrous sodium sulfate. Add 0.7134 g of potassium iodate and dilute to 1 L. Allow to stand for several days, then filter the clear top part of the solution through a medium-porosity, sintered-glass funnel.
  Lead subacetate solution: Dissolve 80 g of lead subacetate in 220 mL of water. Stir overnight, and filter through Whatman No. 42 filter paper, or equivalent. Dilute the supernatant solution to a specific gravity of 1.254 with freshly boiled water.
Dibasic sodium phosphate solution: 190 mg/mL dibasic sodium phosphate heptahydrate, made to 100 mL

Standard solution: 280 µg/mL dried dextrose, made to 500 mL

Sample solution: Dissolve 1 g of sample in 150 mL of water and adjust the pH to between 6.9 and 7.2 with sodium hydroxide solution or acetic acid.

Analysis: To the Sample solution, add Lead subacetate solution in increments until no further precipitation is observed. Bring the volume to 250.0 mL with water, and mix well. Centrifuge the mixture, pipet 10 mL of the supernatant into a 50-mL volumetric flask, and dilute to about 35 mL with water. Add 2 mL or more of Dibasic sodium phosphate solution until no further precipitation forms. Dilute to 50 mL with water, and mix. Centrifuge at 2100 × gravity for 10 min. Pipet 5 mL of supernatant solution into a test tube containing exactly 5 mL of Copper reagent solution, and mix. Loosely plug the tube, and place it in a boiling water bath for 40 min ± 10 s. At the end of the heating period, cool the tube immediately in cold water. Add 2 mL of 2.5% potassium iodide solution and 1.5 mL of 2 N sulfuric acid. Mix well, and titrate with 0.005 N sodium thiosulfate, using starch as the indicator, and note the volume of 0.005 N sodium thiosulfate consumed as VS. Perform a corresponding blank titration using 5 mL of water and 5 mL of Copper reagent solution and record the volume of 0.005 N sodium thiosulfate consumed as VB.

Repeat the entire procedure using 5 mL of Standard solution and 5 mL of Copper reagent solution, noting the volume of 0.005 N sodium thiosulfate consumed as VD. Perform a corresponding blank titration using 5 mL of water and 5 mL of Copper reagent solution; record the volume of 0.005 N sodium thiosulfate consumed as VB. Calculate the percent reducing sugars by the formula:

\[35(V_B - V_S)/(V_B - V_D)\]

\[V_B - V_S = \text{Volume (mL)} \text{ of 0.005 N sodium thiosulfate consumed by the 5-mL aliquot of Sample solution}\]

\[V_B - V_D = \text{Volume (mL)} \text{ of 0.005 N sodium thiosulfate consumed by 5 mL of Standard solution}\]

Acceptance criteria: NMT 30.0%

• Residue on Ignition (Sulfated Ash), Appendix IIC
  Sample: 1 g
  Acceptance criteria: NMT 20.0%

Change to read:

• Viscosity of a 50% Solution
  Sample: 200 g, on the dried basis
  Analysis: Dissolve the Sample in 200 mL of water contained in a 500-mL beaker. Equilibrate the solution at 25°C, and measure its [relative viscosity]1S (FCC 6) with a Brookfield viscometer A (model LVG, or equivalent) or RVT (or equivalent) 1S (FCC 6) using a number 2 spindle at 20 rpm.

Acceptance criteria: NMT 3000 centipoises

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
FCC Sixth Edition Page 136
Phone Number: 1-301-816-8288
BRIEFING

Calcium Silicate, FCC 6 page 149. On the basis of comments received, it is proposed to revise the Description to distinguish two different processes used to manufacture this material, based on the source of silica. Based on Fluoride analysis data received for the Diatomaceous earth-based product, which indicated that the current test procedure was not suitable for the analysis of this product, it is also proposed to revise the sample extraction step to include two different procedures, depending on which silica source was used to manufacture the product. The existing sample extraction procedure in FCC 6 would be utilized for the Precipitated or other silica-based product, which is the type of material that this procedure was developed for when it first appeared in the First Supplement to FCC 4 page 11 in 1997. The second sample preparation procedure would be a new procedure developed for the Diatomaceous earth-based product, which improves the repeatability and accuracy of this procedure for this material type. Additionally, to support these two sample preparation procedures, it is proposed to add a labeling requirement to indicate which source of silica was used to manufacture the product. Interested parties are encouraged to submit comments to Jeff Moore, Ph.D. (FIEC: J. Moore) C65278

Calcium Silicate
INS: 552
CAS: [1344-95-2]

DESCRIPTION

Change to read:
Calcium Silicate occurs as a white to off-white, free-flowing powder that remains so after absorbing relatively large amounts of water or other liquids. It is a hydrous or anhydrous silicate with varying proportions of CaO and SiO_2. It is manufactured by two distinct processes identified by the form of silica used, either diatomaceous earth or precipitated silica. Diatomaceous earth-based products are produced through hydrothermal reaction processes, which combine natural, or flux-calcined diatomaceous earth with hydrated lime to produce synthetic mineral forms of gyrolite and tobermorite. Precipitated or other silica-based products are produced by reacting sodium silicate and calcium oxide. It is insoluble in water, but it forms a gel with mineral acids. The pH of 1:20 aqueous slurry is between 8.4 and 12.5.

Function Anticaking agent; filter aid
Packaging and Storage Store in well-closed containers.

IDENTIFICATION
• Calcium, Appendix IIIA
  Sample solution: Mix 500 mg of sample with 10 mL of 2.7 N hydrochloric acid, filter, and neutralize the filtrate to litmus paper with 6 N ammonium hydroxide.
  Acceptance criteria: Passes tests.
• Silica
  Analysis: Prepare a bead by fusing a few crystals of sodium ammonium phosphate on a platinum loop in the flame of a Bunsen burner. Place the hot, transparent bead in contact with a sample, and again fuse.
  Acceptance criteria: Silica floats about in the bead, producing, upon cooling, an opaque bead with a weblike structure.

ASSAY
• Silicon Dioxide
Sample: 400 mg

Analysis: Transfer the Sample into a beaker, add 5 mL of water and 10 mL of perchloric acid, and heat until dense, white fumes of perchloric acid evolve. [CAUTION: Handle perchloric acid in an appropriate fume hood.] Cover the beaker with a watch glass, and continue to heat for 15 min longer. Allow to cool, add 30 mL of water, filter, and wash the precipitate with 200 mL of hot water. Retain the combined filtrate and washings for use in the assay for Calcium Oxide. Transfer the filter paper and its contents into a platinum crucible, heat slowly to dryness, and then heat sufficiently to char the filter paper. After cooling, add a few drops of sulfuric acid, and then ignite at about 1300° to constant weight. Moisten the residue with 5 drops of sulfuric acid, add 15 mL of hydrofluoric acid, heat cautiously on a hot plate until all of the acid is driven off, and ignite to constant weight at a temperature not lower than 1000°. [CAUTION: Handle hydrofluoric acid in an appropriate fume hood.] Cool in a desiccator, and weigh. The loss in weight is equivalent to the amount of SiO₂ in the sample taken.

Acceptance criteria: The result should conform to the representations of the vendor.

**Calcium Oxide**

Sample solution: The retained combined filtrate and washings from the assay for Silicon Dioxide above

Analysis: Using 1 N sodium hydroxide, neutralize the Sample solution to litmus and add, while stirring, about 30 mL of 0.05 M disodium EDTA from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue indicator. Continue the titration with the disodium EDTA to a blue endpoint. Each mL of 0.05 M disodium EDTA is equivalent to 2.804 mg of CaO.

Acceptance criteria: The result should conform to the representations of the vendor.

**Impurities**

Change to read:

Inorganic Impurities

- **Fluoride**
  
  [NOTE: Store all fluoride solutions in plastic containers.]

  **0.2 N EDTA/0.2 N TRIS solution:** Transfer 18.6 g of disodium ethylenediaminetetraacetate (EDTA) and 6.05 g of tris-(hydroxymethyl)aminomethane (TRIS), into a single 250-mL beaker. Add 200 mL of hot, deionized water, and stir until dissolved. Adjust the pH to 7.5 to 7.6 by adding 5 N sodium hydroxide. Cool the solution, and adjust the pH to 8.0 with 5 N sodium hydroxide. Transfer the solution into a 250-mL volumetric flask and dilute to volume with deionized water. Mix well and store in a plastic container.

  **Standard stock solution:** (1000 mg/kg fluoride) Dissolve 2.210 g of sodium fluoride in 50 mL of deionized water. Transfer the solution into a 1-L volumetric flask and dilute to volume.

  **Standard solutions:** (1 mg/kg and 10 mg/kg fluoride) [NOTE: Prepare on the day of use.] Pipet 10 mL of the Standard stock solution into a 100-mL volumetric flask, dilute to volume with deionized water, and mix. Pipet 10 mL and 1 mL of this solution into separate 100-mL volumetric flasks and dilute each to volume with deionized water.

Sample solution:

- **Precipitated or other silica-based product:**
  
  Transfer 5 g of sample into a 150-mL Teflon beaker. Add 40 mL of deionized water and 20 mL of 1 N hydrochloric acid. Heat to near boiling for 1 min while stirring continuously. Cool the beaker in an ice bath, transfer its contents into a 100-mL volumetric flask, and dilute to volume with deionized water.
  
  [NOTE: The sample does not dissolve completely.]

- **Diatomaceous earth-based product:** Transfer 5 g of sample into a 150-mL Teflon beaker. Add 60 mL of deionized water and stir for 1 min. Transfer the beaker contents into a 100-mL volumetric flask, and dilute...
to volume with deionized water. [NOTE: The sample does not dissolve completely.] Decant the supernate into two 50-mL centrifuge tubes and centrifuge until the solution is clear, usually less than 30 min.

**Calibration curve:** Pipet 20 mL of each of the two Standard solutions into separate 100-mL plastic beakers. Add 10 mL of 0.2 N EDTA/0.2 N TRIS solution to each beaker. Measure the potential (mV) of each solution with a suitable fluoride-selective, ion-indicating electrode and a calomel reference electrode connected to a pH meter capable of measuring potentials with a reproducibility of ±0.2 mV (Orion model 96-09 combination fluoride electrode, or equivalent). Generate a standard curve by plotting the logarithms of the fluoride ion concentrations (mg/kg) of the Standard solutions versus the potential (mV) or calibrate an Orion Expandable Ion Analyzer EA-940 (or an equivalent instrument) for a direct concentration reading.

**Analysis:** Pipet a 20-mL aliquot of Sample solution into a 100-mL plastic beaker, add 10 mL of 0.2 N EDTA/0.2 N TRIS solution, and measure the solution potential as described for the Calibration curve (above). From the measured potential of the Sample solution, calculate the concentration (mg/kg) of fluoride ion using the Calibration curve.

**Acceptance criteria:** NMT 10 mg/kg

**Lead**

**Standard stock solution:** (100 µg/mL lead ion) [NOTE: Prepare and store this solution in glass containers that are free from lead salts.] Dissolve 159.8 mg of ACS reagent-grade Lead Nitrate in 100 mL of water containing 1 mL of nitric acid. Dilute to 1000.0 mL with water and mix.

**Standard solution:** 0.25 µg/mL of lead, prepared on the day of use from the Standard stock solution

**Sample solution:** Transfer 5.0 g of sample into a 250-mL beaker, add 50 mL of 0.5 N hydrochloric acid, cover with a watch glass, and heat slowly to boiling. Boil gently for 15 min, cool, and let the undissolved material settle. Decant the supernatant liquid through Whatman No. 4, or equivalent, filter paper into a 100-mL volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the slurry and beaker with three 10-mL portions of hot water, decanting each washing through the filter paper into the flask. Finally, wash the filter paper with 15 mL of hot water, cool the filtrate to room temperature, dilute to volume with water, and mix.

**Analysis:** Using a suitable atomic absorption spectrophotometer set at 217 nm, separately aspirate and read the absorbances of the Standard solution and Sample solution which has been zeroed with water.

**Acceptance criteria:** The absorbance of the Sample solution is not more than that of the Standard solution (NMT 5 mg/kg).

**SPECIFIC TESTS**

- **Loss on Drying, Appendix IIC (105 °C for 2 h)**
  
  **Acceptance criteria:** The result should conform to the representations of the vendor.

- **Loss on Ignition**
  
  **Sample:** 1 g, previously dried at 105 °C for 2 h
  
  **Analysis:** Transfer the Sample into a suitable tared crucible, and ignite at 900 °C to constant weight.
  
  **Acceptance criteria:** The result should conform to the representations of the vendor.

**OTHER REQUIREMENTS**

*Add the following:*

- **Labeling** If it is the Diatomaceous earth-based product, it is so labeled.

**Auxiliary Information—Staff Liaison:** Kristie Bowman, Senior Scientific Associate

**Expert Committee:** (FI07) Food Ingredients Expert Committee
Camphene, FCC 6 page 154—See briefing under Acetaldehyde.

(FIEC: J. Moore)  C64747

Camphene

\[ \text{C}_{10}\text{H}_{16} \]

Formula wt 136.24
FEMA: 2229

DESCRIPTION

Change to read:
Camphene occurs as a colorless crystalline mass. It may contain a suitable antioxidant. 1S (FCC 6)

Odor Camphoraceous-oily
Solubility Soluble in alcohol; miscible in most fixed oils; insoluble or practically insoluble in water
Boiling Point ~ 159°
Function Flavoring agent

ASSAY
• Procedure Proceed as directed under M-1a, Appendix XI.
  Acceptance criteria: NLT 80.0% of C\textsubscript{10}H\textsubscript{16}

OTHER REQUIREMENTS
• SOLIDIFICATION POINT, Appendix IIB
  Acceptance criteria: NLT 40°

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
FCC Sixth Edition Page 154
Phone Number: 1-301-816-8288
BRIEFING

Carrageenan. Because there is no existing FCC monograph for this food ingredient, a new monograph is proposed based on a combination of the Carrageenan and Processed Eucheuma Seaweed monographs (PES) from the 2001 and 2007 sessions of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the FCC Carrageenan monograph in the Second Supplement to the 3rd Edition of the FCC (FCC 3-S2), and on the basis of data and comments received, as indicated by the monograph sections below.

1. Synonyms and chemical information: JECFA 2007 Carrageenan and PES monographs
2. Description: JECFA 2007 Carrageenan and PES monographs and FCC 3-S2 Carrageenan monograph
3. Function: JECFA 2007 Carrageenan and PES monographs
4. Packaging and Storage: Comment received
5. Infrared Spectra: Data and comments received
6. Predominate Polysaccharides: JECFA 2007 Carrageenan and PES monographs, and ID tests A and B from FCC 3-S2 Carrageenan monograph
7. Arsenic: FCC 3-S2 Carrageenan monograph
9. Total Inorganic Impurities: A limit for this impurity is not yet available. The Committee will set a limit as low as practically possible for food-grade Carrageenan. Interested parties are encouraged to submit data to support an appropriate limit in a timely manner.
10. Residual Solvents and Ash (Total): JECFA 2007 Carrageenan and PES monographs
11. Acid-Insoluble Ash: FCC 3-S2
12. Acid-Insoluble Matter and Loss on Drying: JECFA 2007 Carrageenan and PES monographs
13. Microbial Limits: JECFA 2007 Carrageenan and PES monographs adapted to FCC test procedures
14. pH: JECFA 2007 Carrageenan and PES monographs
15. Sulfate: Adapted based on comments and data received from the JECFA 2007 Carrageenan and PES monographs to an acid-insoluble matter corrected basis
16. Viscosity of a 1.5% Solution: JECFA 2007 Carrageenan and PES monographs and FCC 3-S2

Interested parties are encouraged to submit comments to Jeff Moore, Ph.D., jm@usp.org.

(FIEC: J. Moore) C64603

Add the following:

- Carrageenan

Irish moss (from *Chondrus spp.*)

Eucheuma (from *Eucheuma spp.*)

Iridophycan (from *Iridaea spp.*)

Hypnean (from *Hypnea spp.*)

Processed Eucheuma Seaweed, PES, PNG-carrageenan, and Semi-refined carrageenan (from *E. spinosum* or *E. cottonii*)

INS: 407
DESCRIPTION
Carrageenan occurs as a yellow or tan to white, coarse to fine powder. It is obtained from certain members of the class Rhodophyceae (red seaweeds). The principal commercial sources of carrageenans are the following families and genera of the class Rhodophyceae:

- Furcellariaceae such as Furcellaria;
- Gigartinaceae such as Chondrus, Gigartina, Iridaea;
- Hypnaeceae such as Hypnea;
- Phyllophoraceae such as Phyllophora, Gymnogongrus, Ahnfeltia;
- Solieriaeae such as Eucheuma, Anatheca, Meristotheca.

Carrageenan is a hydrocolloid consisting mainly of the ammonium, calcium, magnesium, potassium, and sodium sulfate esters of galactose and 3,6-anhydrogalactose polysaccharides. These hexoses are alternately linked $\alpha$-(1→3) and $\beta$-(1→4) in the copolymer. The relative proportions of cations existing in carrageenan may be changed during processing to the extent that one may become predominant.

The prevalent polysaccharides in carrageenan are designated as kappa-, iota-, and lambda-carrageenan. Kappa-carrageenan is mostly the alternating polymer of D-galactose-4-sulfate and 3,6-anhydro-D-galactose; iota-carrageenan is similar except that the 3,6-anhydrogalactose is sulfated at carbon 2. Between kappa-carrageenan and iota-carrageenan, there is a continuum of intermediate compositions differing in degree of sulfation at carbon 2. In lambda-carrageenan, the alternating monomeric units are mostly D-galactose-2-sulfate (1→3-linked) and D-galactose-2,6-disulfate (1→4-linked).

Carrageenan may be obtained from any of the cited seaweeds by extraction into water or aqueous dilute alkali. It may be recovered by alcohol precipitation, by drum drying, or by precipitation in aqueous potassium chloride and subsequent freezing. Additionally, carrageenan may be obtained by extracting the cleaned seaweed with alkali for a short time at elevated temperatures. The material is then thoroughly washed with water to remove residual salts followed by purification, drying and milling to a powder. Carrageenan obtained by this method contains a higher percentage of algal cellulose. The alcohols used during recovery and purification of carrageenan are restricted to methanol, ethanol, and isopropanol.

Carrageenan is insoluble in ethanol but it is soluble in water at 80°C, forming a viscous clear or cloudy and slightly opalescent solution that flows readily. Some samples form a cloudy viscous suspension in water. Carrageenan disperses in water more readily if first moistened with alcohol, glycerol, or a saturated solution of glucose or sucrose in water.

Articles of commerce may include sugars for standardization purposes, salts to obtain specific gelling or thickening characteristics, or emulsifiers carried over from drum-drying processes.

Function Thickener, gelling agent, stabilizer, emulsifier

Packaging and Storage Store in well-closed containers.

IDENTIFICATION
- Infrared Spectra, Spectrophotometric Identification Tests, Appendix IIIC

Sample preparation:

[NOTE: If the carrageenan sample does not contain standardizing salts or sugars, the following purification step is not necessary.]

Disperse 1 g of carrageenan in 250 mL of cold water, heat the mixture at 90°C for 10 min, cool it to 60°C, and dissolve 1 g of potassium chloride into the solution. Coagulate the mixture with 2 volumes of isopropyl alcohol, then recover, wash, and dry the purified carrageenan. Disperse 0.5 g of the purified carrageenan...
sample in 250 mL of cold water, heat the mixture at 90° for 10 min, and cool it to 60°. Cast films 0.5 mm thick (when dry) on a suitable non-stick surface such as Teflon or a plastic petri dish. Alternatively, use films cast on a potassium bromide plate, care being taken to avoid moisture.

**Acceptance criteria:**

**All types:** The spectrum of the sample exhibits strong, broad absorption bands, typical of all polysaccharides, in the 1000 cm\(^{-1}\) to 1100 cm\(^{-1}\) region. Maxima are at 1065 cm\(^{-1}\) and 1020 cm\(^{-1}\) for gelling and non-gelling types, respectively.

**Kappa-type** (see appropriate spectrum below):

- Low ester sulfate absorbance at 1220–1260 cm\(^{-1}\)
- Strong 3,6-AG absorbance at 930–935 cm\(^{-1}\)
- Strong galactose-4-sulfate absorbance at 840–850 cm\(^{-1}\)
- No 3,6-AG-2-sulfate absorbance at 800–805 cm\(^{-1}\)

**Iota-type** (see appropriate spectrum below):

- Same as kappa-type except strong ester sulfate absorbance at 1220–1260 cm\(^{-1}\) and strong 3,6-AG-2-sulfate absorbance at 800–805 cm\(^{-1}\)

**Lambda-type** (see appropriate spectrum below):

- Strong ester sulfate absorbance 1220–1260 cm\(^{-1}\)
- Weak to no 3,6AG absorbance at 930–935 cm\(^{-1}\)
- Strong galactose-2-sulfate absorbance at 825–830 cm\(^{-1}\)
- Strong galactose-6-sulfate absorbance at 810–820 cm\(^{-1}\)
• **Predominant Polysaccharides**
  
  **Sample**: 4 g
  
  **Analysis**: Transfer the Sample to a flask containing 200 mL of water and heat the mixture in a water bath at 80°, with constant stirring, until dissolved. Replace any water lost by evaporation and allow the solution to cool to room temperature. [NOTE: The solution becomes viscous and may form a gel.] To 50 mL of the solution or gel, add 200 mg of potassium chloride; then reheat, mix well, and cool.
  
  **Acceptance criteria**:  
  - **kappa type**: A short-textured “brittle” gel forms.  
  - **iota type**: A compliant “elastic” gel forms.  
  - **lambda type**: The solution does not gel.

**IMPURITIES**

Inorganic Impurities

- **Arsenic, Arsenic Limit Test, Appendix IIIB**  
  **Sample solution**: Prepare as directed for organic compounds.  
  **Acceptance criteria**: NMT 3 mg/kg

- **Cadmium**
  [Note: Throughout this test, use distilled, deionized water.]
  
  **Standard stock solution**: 10 µg/mL cadmium prepared by diluting a commercially available standard solution
  
  **Standard solutions**: 0.05, 0.1, 0.2, 0.4, and 0.6 µg/mL of cadmium; from **Standard stock solution**
  
  **Sample**: 7.5 g  [NOTE: The sample should be powdered and dry.]
  
  **Sample solution**:  
  [CAUTION: Handle perchloric acid in an appropriate fume hood.] Transfer the Sample to a 250-mL Erlenmeyer flask and wet it with 10 mL of water; add 25 mL of nitric acid. As soon as any initial reaction subsides, heat gently on a hot plate set at 100–150° for about 1 h or until most of the dark fumes that form are evolved. Swirl the flask occasionally. Cool the flask and add 5 mL of perchloric acid. Salt-like particles are visible at this stage. Resume heating the flask on the hot plate at 100–150° until the digest is yellow or colorless; this takes about 1 h. Do not allow the solution to dry; if necessary add 2–3 mL of nitric acid. Cool the digest and wash the insides of the flask with 5 mL of water and swirl the flask. Add 2 mL of hydrochloric acid to complete the digestion. Resume heating the solution on the hot plate at 100–150° until brown fumes are no longer visible and the solution is white to yellowish in color. Again, do not allow the solution to dry; if necessary add 2–3 mL of nitric acid. Cool the solution. It will become slightly viscous and salt-like particles will be visible. Add 10 mL of water to the flask, while washing the sides. Transfer the viscous solution to a 50-mL volumetric flask, dilute to volume with water, and mix. Filter the salt-like particles from the solution using two layers of Whatman no. 5 filter paper (or equivalent).

  **Reagent blank**: Use the same quantities of reagents as used to prepare the Sample solution, but omitting the Sample.
Analysis: Use any suitable atomic absorption spectrophotometer equipped with a Boling-type burner, an air-acetylene flame, and a hollow-cathode cadmium lamp. 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 228.8 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 concentration of lead (µg/mL). Calculate the concentration (mg/kg) of cadmium in the Sample using the following formula:

\[ C/W \times 50 \]

\[ C = \text{The concentration (µg/mL) of cadmium in the Sample solution determined from the standard curve} \]
\[ W = \text{The weight of Sample taken (g)} \]
\[ 50 = \text{The sample dilution factor} \]

Acceptance criteria: NMT 2 mg/kg

- Lead

[NOTE: Throughout this test, use distilled, deionized water.]

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

Standard solutions: 0.1, 0.2, 0.4, 0.8, and 1.6 µg/mL of lead: from Standard stock solution

Sample: 7.5 g [NOTE: The sample should be powdered and dry.]

Sample solution: [CAUTION: Handle perchloric acid in an appropriate fume hood.] Transfer the Sample to a 250-mL Erlenmeyer flask and wet it with 10 mL of water; add 25 mL of nitric acid. As soon as any initial reaction subsides, heat gently on a hot plate set at 100–150° for about 1 h or until most of the dark fumes that form are evolved. Swirl the flask occasionally. Cool the flask and add 5 mL of perchloric acid. Salt-like particles are visible at this stage. Resume heating the flask on the hot plate at 100–150° until the digest is yellowish or colorless; this takes about 1 h. Do not allow the solution to dry; if necessary add 2–3 mL of nitric acid. Cool the digest and wash the insides of the flask with 5 mL of water and swirl the flask. Add 2 mL of hydrochloric acid to complete the digestion. Resume heating the solution on the hot plate at 100–150° until brown fumes are no longer visible and the solution is white to yellowish in color. Again, do not allow the solution to dry; if necessary add 2–3 mL of nitric acid. Cool the solution. It will become slightly viscous and salt-like particles will be visible. Add 10 mL of water to the flask, while washing the sides. Transfer the viscous solution to a 50-mL volumetric flask, dilute to volume with water, and mix. Filter the salt-like particles from the solution using two layers of Whatman no. 5 filter paper (or equivalent).

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

Analysis: Use any suitable atomic absorption spectrophotometer equipped with a lead electrodeless discharge lamp (EDL) and an air-acetylene flame. 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 283.3 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 concentration of lead (µg/mL). Calculate the concentration (mg/kg) of lead in the Sample using the following formula:

\[ C/W \times 50 \]
C = The concentration (µg/mL) of lead in the Sample solution determined from the standard curve. 

W = The weight of Sample taken (g) 

50 = The sample dilution factor

Acceptance criteria: NMT 5 mg/kg

MERCURY

[NOTE: Throughout this test, use distilled, deionized water.] 
[CAUTION: Handle perchloric acid in an appropriate fume hood.] 

Reagent solutions:

Sodium borohydride solution: 0.4% solution prepared by first dissolving 2.5 g of sodium hydroxide in water and then adding and dissolving 2.0 g of sodium borohydride (>98%) followed by dilution with water to 500 mL. [NOTE: Prepare immediately before use.]

5 M Hydrochloric acid: Dilute 417 mL of hydrochloric acid to 1 L.

Standard stock solution: 1 mg/mL mercury prepared by diluting a commercially available standard solution.

Standard solutions: 10, 25, 50, 100, 200 ng/mL of mercury: from Standard stock solution

Sample: 5 g [NOTE: The sample should be powdered and dry.]

Sample solution: Transfer the Sample to a 250-mL Erlenmeyer flask and wet it with 5 mL of water; add 10 mL of nitric acid–perchloric acid (1:1) solution. As soon as any initial reaction subsides, heat gently on a hot plate set at 100–150°C for about 1 h until all of the dark fumes that form are evolved and the solution turns yellowish or colorless. Swirl the flask occasionally. Salt-like particles are visible at this stage. Do not allow the solution to dry. Cool the solution. It will become slightly viscous and salt-like particles will be visible. Rinse down the sides of the flask with 5 mL of water and allow the solution to stand overnight to facilitate elimination of dissolved gas. Transfer the viscous solution to a 50-mL volumetric flask, dilute to volume with water, and mix. Filter the salt-like particles from the solution using two layers of Whatman no. 5 filter paper (or equivalent). Transfer the filtrate to a 100-mL Erlenmeyer flask and, in an ultrasonic bath, sonicate the flask for 10 minutes or until bubbles no longer form on the surface; this indicates that all dissolved gas has been removed.

Reagent blank: Use the same quantities of reagents as used to prepare the Sample solution.

Cold-Vapor Atomic Absorption Method

Instrument: Use any suitable atomic absorption spectrophotometer equipped with a hydride vapor generator (e.g., Shimadzu Model 6601F or equivalent) or atomic vapor assembly. [NOTE: Integral to the hydride generator is a reactor tube or coil and a peristaltic pump with dual tubing channels: one channel for the Sample solution and one for the two Reagent solutions. Flow control is determined by tubing size and tubing clamps. Flow rates are measured at the exit of the hydride generator. The hydride generator manifold is where the three solutions are mixed and pass into the reactor coil to generate atomic mercury, which is carried into the absorbance cell of the instrument.]

Lamp: Mercury at 253.7 nm

Purge gas: Argon

Pump calibration: Calibrate (using water) the peristaltic pump so that it will provide a flow rate of the Sample solution of 8 mL/min and a combined flow rate for the two Reagent solutions of 2 mL/min. [NOTE: The combined flow rate is achieved with a single pump setting.]

Analysis: Set the spectrophotometer to previously established optimum conditions at 253.7 nm. Transfer suitable quantities of the two Reagent solutions into separate graduated cylinders. Insert separate aspirator tubing leading from the peristaltic pump into each of the Reagent solutions and into the Sample solution contained in the 100-mL Erlenmeyer flask. Start the flow of argon gas (tank outlet pressure: 3.2 ±
0.2 kg/cm²) through the hydride vapor generator of the spectrophotometer. Start the pump to initiate flow of the three solutions into the hydride generator manifold. Measure the absorbance for the Sample solution. Repeat for the Reagent blank and for each of the Standard solutions.

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 concentration of mercury (ng/mL). Calculate the concentration (mg/kg) of mercury in the Sample using the following formula:

\[
(C/W) \times (50/1000)
\]

Where:
- \(C\) = The concentration (ng/mL) of mercury in the Sample solution determined from the standard curve
- \(W\) = The weight of Sample taken (g)
- 50 = The sample dilution factor
- 1000 = ng/g to mg/kg conversion factor

**Acceptance criteria:** NMT 1 mg/kg

- **Total Inorganic Impurities**
  - **Acceptance criteria:** The sum of the results from the tests for Arsenic, Cadmium, Lead, and Mercury is NMT [To come].

**Organic Impurities**

- **Residual Solvents**
  - **Internal standard solution:** Add 50.0 mL of water to a 50 mL injection vial and seal. Weigh and inject 15 µL of 3-methyl-2-pentanone through the septum and reweigh to within 0.01 mg.
  - **Blank:** A sample with very low solvent content [NOTE: Depending on the solvent or solvents used in the purification and recovery of the Sample, more than one solvent may be present.]
  - **Blank solution:** Weigh 0.20 g of the Blank into an injection vial. Add 5.0 mL of water and 1.0 mL of the Internal standard solution. Heat the vial at 60°C for 10 min and shake it vigorously for 10 sec.
  - **Standard solution:** Weigh 0.20 g of the Blank into an injection vial. Add 5.0 mL of water and 1.0 mL of the Internal standard solution. Weigh the vial to within 0.01 mg. Inject 4 µL each of ethanol, isopropanol, and methanol through the septum, reweighing the vial between the addition of each solvent. Heat the vial at 60°C for 10 min and shake it vigorously for 10 sec.
  - **Sample:** 5 g
  - **Sample solution:** Disperse 1 mL of a suitable antifoam emulsion, such as Dow-Corning G-10 or equivalent, in 200 mL of water contained in a 1000-mL 24/40 round-bottom distilling flask. Add the Sample and shake the flask for 1 h on a wrist-action mechanical shaker. Connect the flask to a fractionating column and distill about 100 mL, adjusting the heat so that the foam does not enter the column. Quantitatively transfer the distillate to a 200-mL volumetric flask, fill to the mark with water and shake the flask to mix. Weigh 8.0 g of this solution into an injection vial. Add 1.0 mL of the Internal standard solution. Heat the vial at 60°C for 10 min and shake it vigorously for 10 sec.

**Chromatographic system, Appendix IIA**

- **Mode:** Head space gas chromatography
- **Detector:** Flame-ionization detector
- **Column:** Fused silica, (0.8 m × 0.53 mm id) coated with DB-wax (1 µm thickness) coupled with fused silica column, (30 m × 0.53 mm id) coated with DB-1 (5 µm thickness)
- **Temperature**
Oven: 35° for 5 min; 5°/min to 90°; 6 min at 90°
Injection port: 140°
Detector: 300°

Headspace sampling conditions
  Sample heating temperature: 60°
  Sample heating time: 10 min
  Syringe temperature: 70°
  Transfer temperature: 80°
Carrier gas: Helium
Flow rate: 5 mL/min (208 kPa)
Injection volume: 1.0 mL

Analysis: Inject equal volumes from the Sample solution, Blank solution, and Standard solution into the chromatograph, record the chromatograms, and determine the peak areas. [NOTE: The approximate retention times for ethanol, methanol, isopropanol, and 3-methyl-2-pentanone are 2.81, 2.93, 5.23, and 16.90 min respectively.]

Calculations: For each solvent being analyzed, determine the calibration factor, C, from the following equation:

\[ C = 50D/(E(F - G)) \]

\( D \) = The weight (mg) of solvent in the Standard solution
\( E \) = The weight (mg) of internal standard in the Standard solution
\( F \) = The relative peak area for the solvent in the Standard solution
\( G \) = The relative peak area for the same solvent in the Blank solution

For each solvent being analyzed, its weight (mg) in the Sample solution is given by the formula:

\[ ABC/50 \]

\( A \) = The relative peak area of the solvent
\( B \) = The weight (mg) of internal standard
\( C \) = The calibration factor for the solvent

For each solvent being analyzed, its percentage in the Sample is given by the formula:

\[ 0.1 w/W \]

\( w \) = The weight (mg) of the solvent in the Sample solution
\( W \) = The weight (g) of the sample taken

Acceptance criteria: NMT 0.1% of ethanol, isopropanol, or methanol, singly or in combination

SPECIFIC TESTS
• Ash (Total)
Sample: 2 g of Sample 1 from the procedure for determination of Sulfate (below).

Analysis: Transfer the Sample to a previously ignited, tared silica or platinum crucible. Heat the Sample with a suitable infrared lamp, increasing the intensity gradually, until the Sample is completely charred; continue heating for an additional 30 min. Transfer the crucible with the charred Sample into a muffle furnace and ignite at about 550° for 1 h. Cool in a desiccator and weigh. Repeat the ignition in the muffle furnace until a constant weight is obtained. If a carbon-free ash is not obtained after the first ignition, moisten the charred spot with a 10% solution of ammonium nitrate and dry under an infrared lamp. Repeat the ignition step. Calculate the percentage of total ash of the sample using the formula:

\[
\frac{W_2}{W_1} \times 100\%
\]

\[W_1 = \text{The weight of Sample (g)}\]
\[W_2 = \text{The weight of ash determined (g)}\]

[NOTE: Retain the ash for the Acid-Insoluble Ash test.]

Acceptance criteria: NLT 15% and NMT 40%, on the dried basis

- **Acid-Insoluble Ash**, Appendix IIC

  Sample: Use the ash from the test for Ash (Total)

  Analysis: Ignite to constant weight at 800° ± 25°

  Acceptance criteria: NMT 1%

- **Acid-Insoluble Matter**

  Sample: 2 g of Sample 1 obtained from the procedure for determination of Sulfate (below).

  Analysis: Transfer the Sample into a 250-mL beaker containing 150 mL of water and 1.5 mL of sulfuric acid TS. Cover the beaker with a watch glass and heat the mixture on a steam bath for 6 h, rubbing down the wall of the beaker frequently with a rubber-tipped stirring rod and replacing any water lost by evaporation. Weigh 500 mg of a suitable acid-washed filter aid, pre-dried at 105° for 1 h, to the nearest 0.1 mg, add the filter aid to the sample solution, and filter it through a tared sintered-glass filter crucible. Wash the residue several times with hot water, dry the crucible and its contents at 105° for 3 h, cool in a desiccator, and weigh. The difference between the total weight and the weight of the filter aid plus crucible is the weight of the Acid-insoluble matter. Calculate as a percentage.

  Acceptance criteria: NMT 15%

- **Loss on Drying**, Appendix IIC (105° to constant weight)

  Acceptance criteria: NMT 12%

- **Microbial Limits**

  [NOTE: Current methods for the following tests may be found by accessing the US Food and Drug Administration's Bacteriological Analytical Manual (BAM) online at www.cfsan.fda.gov.]

  Acceptance criteria:

  - **Aerobic plate count**: NMT 5,000 CFU/g
  - **Salmonella spp.**: Negative per test
  - **E. coli**: Negative in 1 g

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

  Sample solution: 1:100 suspension

  Acceptance criteria: Between 8 and 11

- **Sulfate**

  Sample 1: Disperse 15 g of a sample of product in 500 mL of 60% w/w isopropanol/water at room
temperature. Stir gently for 4 h. Filter through ash-free filter paper and discard the filtrate. Wash the material remaining on the filter paper with two 15-mL portions of 60% isopropanol/water. Dry the material at 105°C to constant weight.

**Sample 2:** 1 g of Sample 1  
[NOTE: Retain the remainder of Sample 1 for determination of Ash (Total), Acid-insoluble matter, and Viscosity of a 1.5% solution.]

**Sample solution:** Transfer Sample 2 to a 100-mL long-necked round-bottom flask. Add 50 mL of 0.2 N hydrochloric acid. Fit a condenser, preferably one with at least 5 condensing bulbs, to the flask and reflux for 1 h. Add 25 mL of a 10% (by volume) hydrogen peroxide solution and resume refluxing for about 5 h or until the solution becomes completely clear.

**Analysis:** Transfer the Sample solution to a 600-mL beaker, bring to a boil, and add dropwise 10 mL of a 10% barium chloride solution. Heat the reaction mixture for 2 h on a boiling water bath. Filter the mixture through ash-free slow-filtration filter paper. Wash with boiling distilled water until the filtrate is free from chloride. Dry the filter paper and contents in a drying oven. Gently burn and ash the paper at 800°C in a tared porcelain or silica crucible until the ash is white. Cool in a desiccator. Weigh the cooled crucible containing the ash. Calculate the percentage ester sulfate, %ES, in the sample taken using the following equation:

\[
%ES = \left(\frac{W_2}{W_1}\right) \times 0.4116 \times 100\%
\]

\[W_1 = \text{The weight (g) of Sample 2 taken}\]
\[W_2 = \text{The weight (g) of the ash (barium sulfate)}\]

Calculate the acid-insoluble matter corrected percentage of ester sulfate, %ES_C, in the sample taken using the following equation:

\[
%ES_C = \frac{%ES}{1 - \left(\frac{\%AIM}{100}\right)}
\]

\[%AIM = \text{The percent Acid-Insoluble Matter (determined above).}\]

**Acceptance criteria:** NLT 20% and NMT 40% (as ester sulfate), on the washed, dried, and acid-insoluble matter corrected basis

**Viscosity of a 1.5% Solution**

**Sample:** 7.5 g of Sample 1 obtained from the procedure for determination of Sulfate (above)

**Sample solution:** Transfer the Sample into a tared, 600-mL tall-form (Berzelius) beaker, and disperse it with agitation for 10 to 20 min in 450 mL of deionized water. Add sufficient water to bring the final weight to 500 g and heat in a water bath with continuous agitation, until a temperature of 80°C is reached (20–30 min). Add water to adjust for loss by evaporation, cool to 76–77°C, and heat in a constant temperature bath at 75°C.

**Analysis:** Pre-heat the bob and guard of a Brookfield LVF or LVT viscometer to approximately 75°C in water. Dry the bob and guard and attach them to the viscometer, which should be equipped with a No.1 spindle (19 mm in diameter, approximately 65 mm in length) and capable of rotating at 30 rpm. Adjust the height of the bob in the Sample solution, start the viscometer rotating at 30 rpm and, after six complete revolutions of the viscometer, take the viscometer reading on the 0–100 scale. Record the result in centipoises, obtained by multiplying the reading on the scale by the factor given by the Brookfield...
manufacturer. [NOTE: If the viscosity is very low, increased precision may be obtained by using the Brookfield UL (ultra low) adapter or equivalent, in which case the viscometer reading on the 0–100 scale should be multiplied by 0.2 to obtain the viscosity in centipoises. On the other hand, samples of some types of carrageenan may be too viscous to read when a No. 1 spindle is used. Such samples obviously pass the specification, but if a viscosity reading is desired for other reasons, use a No. 2 spindle and take the reading on the 0–100 scale or on the 0–500 scale.]

Acceptance criteria: NLT 5 cP at 75°

1 In the United States, only the following seaweed species from the families Gigartinaceae and Solieriaceae are authorized as sources of carrageenan intended for use in foods (Title 21 US Code of Federal Regulations Part 172, section 620 (21 CFR 172.620)): Chondrus crispus, C. ocellatus, Eucheuma cottonii, E. spinosum, Gigartina acicularis, G. pistillata, G. radula, and G. stellata.

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
Phone Number: 1-301-816-8288
**BRIEFING**

β-Caryophyllene, FCC 6 page 180—See briefing under Acetaldehyde.

(FIEC: J. Moore) C64747

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**β-Caryophyllene**

\[
\text{C}_{15}\text{H}_{24}
\]

Formula wt 204.36
FEMA: 2252

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**DESCRIPTION**

*Change to read:*

β-Caryophyllene occurs as a colorless to slightly yellow, oily liquid. It may contain a suitable antioxidant.

- Odor Woody, spicy
- Solubility Soluble in alcohol, ether; insoluble or practically insoluble in water
- Boiling Point ~ 256°
- Solubility in Alcohol, Appendix VI
  - One mL dissolves in 6 mL of 95% alcohol to give a clear solution.
- Function Flavoring agent

**IDENTIFICATION**

- **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.
ASSAY

• **Procedure** Proceed as directed under *M-1a*, Appendix XI.
  
  **Acceptance criteria:** NLT 80.0% of $C_{15}H_{24}$

SPECIFIC TESTS

• **REFRACTIVE INDEX**, Appendix II (at 20°)
  
  **Acceptance criteria:** Between 1.498 and 1.504

• **Specific Gravity** Determine at 25° by any reliable method (see *General Provisions*).
  
  **Acceptance criteria:** Between 0.897 and 0.910

OTHER REQUIREMENTS

• **ANGULAR ROTATION, Optical (Specific) Rotation**, Appendix IIIB (Use a 100-mm tube.)
  
  **Acceptance criteria:** Between $-5^\circ$ and $-10^\circ$

• **PHENOLS, M-1b**, Appendix XI
  
  **Acceptance criteria:** NMT 3.0%

**Auxiliary Information**— **Staff Liaison:** Kristie Bowman, Senior Scientific Associate

*Expert Committee:* (FI07) Food Ingredients Expert Committee

**FCC Sixth Edition Page 180**

**Phone Number:** 1-301-816-8356
BRIEFING

**Cetylpyridinium Chloride.** Because there is no existing FCC monograph for this food ingredient, a new monograph based on the *Cetylpyridinium Chloride* monograph on page 1716 of *USP 31* is proposed.

1. Since the FCC does not use the nonspecific “Heavy Metals” test procedure found in the *USP* monograph, the *Flame Atomic Absorption Spectrophotometric General Test* procedure for lead is proposed. A limit for lead is not yet available. The Committee will set a lead limit as low as practicable for food-grade Cetylpyridinium Chloride. Interested parties are encouraged to develop and validate methods for use in industrial settings that are sensitive enough to detect lead in the amounts typically present in food-grade Cetylpyridinium Chloride, and to propose such methods to the committee in a timely manner.

2. The *Organic Volatile Impurities* test procedure and acceptance criteria are based on the *USP 31* monograph. Interested parties are encouraged to develop and validate improved methods and provide data to support appropriate acceptance criteria for food-grade Cetylpyridinium Chloride in a timely manner.

3. The committee recognizes the inherent safety issues with the *Pyridine* test procedure and encourages interested parties to develop and validate improved methods and provide data to support appropriate acceptance criteria for food-grade Cetylpyridinium Chloride in a timely manner.

Interested parties are encouraged to submit comments and possible methods of analysis to Jeff Moore, Ph.D., at jm@usp.org.

(FIEC: J. Moore) C64508

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Add the following:

- **Cetylpyridinium Chloride**

Pyridinium, 1-hexadecyl-, chloride, monohydrate

1-Hexadecylpyridinium chloride, monohydrate

C_{21}H_{38}ClN·H_2O

Formula wt 358.00
CAS: [6004-24-6]

**DESCRIPTION**

Cetylpyridinium chloride occurs as a white powder with a pyridine-like odor. It is freely soluble in water, ethanol, and chloroform, but it is insoluble in diethyl ether.

**Function** Antimicrobial agent

**Packaging and Storage** Store in well-closed containers.

**IDENTIFICATION**

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

  Reference standard: USP Cetylpyridinium Chloride 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, Spectrophotometric Identification Tests, Appendix III C

Standard solution: 40 µg/mL USP Cetylpyridinium Chloride RS

Sample solution: 40 µg/mL

Acceptance criteria: The ultraviolet absorption spectrum of the Sample solution exhibits maxima and minima at the same wavelengths as those of the Standard solution.

CHLORIDE, Appendix III A

Sample solution: 2 mg/mL

Analysis: Test a 10-mL volume of the Sample solution.

Acceptance criteria: The solution becomes turbid; a white, curdy precipitate does not form.

ASSAY

PROCEDURE

0.02 M Sodium tetraphenylborate solution: [Note: Prepare this solution just before use.] Dissolve an amount of sodium tetraphenylborate, equivalent to 6.845 g of NaB(C\(_6\)H\(_5\))\(_4\), in water to make 1000 mL.

Pipet two 75-mL portions of the solution into separate beakers and, to each, add 1 mL of acetic acid and 25 mL of water. To each beaker add, slowly and with constant stirring, 25 mL of potassium biphthalate solution (50 mg/mL), and allow to stand for 2 h. Filter one of the mixtures through a filtering crucible, and wash the precipitate with cold water. Transfer the precipitate to a container, add 50 mL of water, shake the container intermittently for 30 min, filter, and use the filtrate as the saturated potassium tetraphenylborate solution in the following standardization procedure. Filter the second mixture through a tared filtering crucible and wash the precipitate with three 5-mL portions of saturated potassium tetraphenylborate solution. Dry the precipitate at 105°C for 1 h. Each g of potassium tetraphenylborate is equivalent to 955.1 mg of sodium tetraphenylborate. Calculate the molarity from the following formula:

\[
\frac{(W_K \times 0.9551 \times 0.075)}{342.22}
\]

\(W_K\) = Weight (g) of potassium tetraphenylborate

Sample solution: Transfer 200 mg of sample to a glass-stoppered, 250-mL graduated cylinder containing 75 mL of water. Add 10 mL of chloroform, 0.4 mL of bromophenol blue solution (500 µg/mL), and 5 mL of a freshly prepared solution of sodium bicarbonate (4.2 mg/mL).

Analysis: Titrate the Sample solution with 0.02 M Sodium tetraphenylborate solution until the blue color disappears from the chloroform layer. Add the last portions of the 0.02 M Sodium tetraphenylborate solution dropwise, agitating vigorously after each addition. Each mL of 0.02 M Sodium tetraphenylborate solution is equivalent to 6.800 mg of C\(_{21}\)H\(_{38}\)ClN.

Acceptance criteria: NLT 99.0% and NMT 102.0% of C\(_{21}\)H\(_{38}\)ClN, calculated on the anhydrous basis.

IMPURITIES

Inorganic Impurities

LEAD, Lead Limit Test, Flame Atomic Absorption Spectrophotometric Method, Appendix IIIB

Acceptance criteria: [To come]

Organic Impurities

ORGANIC VOLATILE IMPURITIES

Standard solution: 12.0 µg/mL methylene chloride, 7.6 µg/mL 1,4-dioxane, 1.6 µg/mL trichloroethylene,
and 1.2 µg/mL chloroform. [Note: Use water free of organics. Prepare fresh daily.]

**Sample solution**: 20 mg/mL. [Note: Use water free of organics.]

**Chromatographic system**, Appendix IIA

- **Mode**: Gas chromatography
- **Detector**: Flame-ionization
- **Column**: 0.53-mm × 30-m fused silica analytical column coated with a 3.0-µm stationary phase containing 6% of cyanopropylphenyl–94% dimethylpolysiloxane, and a 0.53-mm × 5-m silica guard column deactivated with phenylmethyl siloxane

- **Column temperature**: 20 min at 40°C; increase rapidly to 240°C; maintain at 240°C for 20 min
- **Injection port temperature**: 140°C
- **Detector temperature**: 260°C
- **Carrier gas**: Helium
- **Flow rate**: 35 cm/s linear velocity
- **Injection volume**: About 1 µL

**System suitability**

- **Sample**: *Standard solution*
- **Suitability requirement 1**: The peaks in the chromatograms are fully resolved, 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**: Inject the *Sample solution* into the chromatograph, record the chromatograms, and measure the peak responses. Identify, on the basis of retention times, the peaks that are present. The identities and peak responses in the chromatogram may be established as being from the organic volatile impurities used to prepare the *Standard solution* or from some other volatile impurity eluting with a comparable retention time as determined by mass spectrometric relative abundance procedures or by the use of a second validated column containing a different stationary phase.

**Acceptance criteria**

- **Chloroform**: NMT 60 mg/kg
- **1,4-Dioxane**: NMT 380 mg/kg
- **Methylene chloride**: NMT 600 mg/kg
- **Trichloroethylene**: NMT 80 mg/kg

**Pyridine**

- **Analysis**: Dissolve 1 g of sample in 10 mL of sodium hydroxide solution (100 mg/mL) without heating.
- **Acceptance criteria**: The odor of pyridine is not immediately perceptible.

**SPECIFIC TESTS**

- **Acidity**
  - **Analysis**: Dissolve 500 mg of sample in 50 mL of water, add several drops of phenolphthalein TS, and titrate with 0.20 N sodium hydroxide.
  - **Acceptance criteria**: NMT 2.5 mL of titrant is required for neutralization.

- **Melting Range or Temperature**, Procedure for Class I, Appendix IIB [Note: Omit the preliminary drying step.]
  - **Acceptance criteria**: Between 80°C and 84°C

- **Residue on Ignition (Sulfated Ash)**, Appendix IIC
  - **Sample**: 1–2 g
  - **Analysis**: Proceed as directed, but igniting at 600 ± 50°C until the residue is completely incinerated.
  - **Acceptance criteria**: NMT 0.2%, calculated on the anhydrous basis
• Water, Water Determination, Method I, Appendix IIB
  Acceptance criteria: Between 4.5% and 5.5%

1 DB-624 (Agilent), or equivalent

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
Phone Number: 1-301-816-8288
BRIEFING

Cinnamaldehyde, FCC 6 page 196—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

Cinnamaldehyde

Cinnamal

Cinnamic Aldehyde

\[ \text{C}_9\text{H}_8\text{O} \]

Formula wt 132.16
FEMA: 2286

DESCRIPTION

Change to read:
Cinnamaldehyde occurs as a yellow, strongly refractive liquid. It may contain a suitable antioxidant. 1S (FCC 6)

Odor: Cinnamon, burning aromatic taste

Solubility: Miscible in alcohol, chloroform, ether, fixed and volatile oils; 1 g dissolves in 700 mL water.

Boiling Point: ~248°

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

Function: Flavoring agent

IDENTIFICATION

• 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.
Cinnamaldehyde

ASSAY
- **Procedure** Proceed as directed under M-1b, Appendix XI.
  - **Acceptance criteria:** NLT 98.0% of C\textsubscript{9}H\textsubscript{8}O

SPECIFIC TESTS
- **ACID VALUE,** M-15, Appendix XI
  - **Acceptance criteria:** NMT 10.0
- **REFRACTIVE INDEX,** Appendix II (at 20°)
  - **Acceptance criteria:** Between 1.619 and 1.623
- **Specific Gravity** Determine at 25° by any reliable method (see General Provisions).
  - **Acceptance criteria:** Between 1.046 and 1.050

OTHER REQUIREMENTS
- **CHLORINATED COMPOUNDS,** Appendix VI
  - **Acceptance criteria:** Passes test.

**Auxiliary Information**— *Staff Liaison:* Kristie Bowman, Senior Scientific Associate
*Expert Committee:* (FI07) Food Ingredients Expert Committee
*FCC Sixth Edition Page:* 196
*Phone Number:* 1-301-816-8356
Citral, *FCC 6* page 207—See briefing under *Acetaldehyde.*

(FIEC: J. Moore) C64747

**Citral**

Mixture of Geranial [(E)-3,7-dimethyl-2,6-octadien-1-al] and Neral [the (Z) isomer]

![Chemical structures](image)

(a) Geranial

(b) Neral

C\(_{10}\)H\(_{16}\)O

Formula wt 152.24

FEMA: 2303

**DESCRIPTION**

*Change to read:*

Citral occurs as a pale yellow liquid. It may contain a suitable antioxidant. 1S (*FCC 6*)

**Odor** Strong, lemon

**Solubility** Soluble in most fixed oils, mineral oil, propylene glycol; insoluble or practically insoluble in glycerin

**Boiling Point** \(\sim 228^\circ\)

**Solubility in Alcohol**, Appendix VI

- One mL dissolves in 7 mL of 70% alcohol.

**Function** Flavored agent

**IDENTIFICATION**

- **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.
ASSAY

- Procedure Proceed as directed under M-1b, Appendix XI.
  
  Acceptance criteria: NLT 96.0% of C₁₀H₁₆O (sum of neral and geranial)

SPECIFIC TESTS

- REFRACTIVE INDEX, Appendix II (at 20°)
  
  Acceptance criteria: Between 1.486 and 1.490

- Specific Gravity Determine at 25° by any reliable method (see General Provisions).
  
  Acceptance criteria: Between 0.885 and 0.891

Auxiliary Information— Staff Liaison: Kristie Bowman, Senior Scientific Associate

Expert Committee: (FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 207

Phone Number: 1-301-816-8356
Citronellal, FCC 6 page 209—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

Citronellal

3,7-Dimethyl-6-octen-1-al

\[ \text{C}_{10}\text{H}_{18}\text{O} \]

**DESCRIPTION**

*Change to read:*

Citronellal occurs as a colorless to slightly yellow liquid. It may contain a suitable antioxidant. 1S (FCC 6)

**Odor** Intense lemon-citronella-rose

**Solubility** Soluble in alcohol, most fixed oils; slightly soluble in propylene glycol; insoluble or practically insoluble in glycerin, water

**Boiling Point** \( \sim 206^\circ \)

**Solubility in Alcohol**, Appendix VI

One mL dissolves in 5 mL of 70% alcohol, and remains clear on dilution.

**Function** Flavoring agent

**IDENTIFICATION**

- **INFRARED SPECTRA**, *Spectrophotometric Identification Tests*, Appendix III C

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

**ASSAY**

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

**Acceptance criteria:** NLT 85.0% of aldehydes as \( \text{C}_{10}\text{H}_{18}\text{O} \) (one isomer)
SPECIFIC TESTS

• **ACID VALUE, M-15, Appendix XI**
  Acceptance criteria: NMT 3.0

• **REFRACTIVE INDEX, Appendix II (at 20°)**
  Acceptance criteria: Between 1.446 and 1.456

• **Specific Gravity** Determine at 25° by any reliable method (see General Provisions).
  Acceptance criteria: Between 0.850 and 0.860

OTHER REQUIREMENTS

• **ANGULAR ROTATION, Optical (Specific) Rotation, Appendix IIIB (Use a 100-mm tube.)**
  Acceptance criteria: Between –1° and +11°

Auxiliary Information—Staff Liaison: Kristie Bowman, Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
FCC Sixth Edition Page 209
Phone Number: 1-301-816-8356
BRIEFING

Copovidone. Because there is no existing FCC monograph for this food ingredient, a new monograph is being proposed.

1. The following test procedures and acceptance criteria were based on the Copovidone Stage 4 harmonization draft proposal published in PF 32(6) [Nov.–Dec. 2006] for public comment: Identification tests A and B, Aldehydes, Hydrazine, Monomers, Peroxides, Clarity and Color, Loss on Drying, and Residue on Ignition. The Aldehydes test was corrected from the Stage 4 harmonization draft to indicate that the first and second absorbance values are determined before and after the addition of the Aldehyde dehydrogenase solution instead of before and after the addition of the NAD solution, as indicated in the Stage 4 draft. The column information in the Monomers test procedure was based on information and comments received.

2. The synonyms for the chemical names, the Description, Function, and Packaging and Storage sections were developed based on comments and information received.

3. The test procedure for K-value was adapted from the Stage 4 harmonization draft to the K-value procedure in the Polyvinylpyrrolidone monograph in FCC 6, and the acceptance criteria were based on the Stage 4 harmonization draft.

4. The Lead test procedure was adapted from the Polyvinylpyrrolidone monograph in FCC 6, and the acceptance criteria were based on information and comments received.

Interested parties are encouraged to submit comments to Jeff Moore, Ph.D.
(FIEC: J. Moore)  C63436

Add the following:

- **Copovidone**

PVP/VA Copolymer

Poly(1-vinyl-2-pyrrolidone)/(vinyl acetate) Copolymer

1-Vinyl-2-pyrrolidone polymer with vinyl acetate

Acetic acid ethenyl ester polymer with 1-ethenyl-2-pyrrolidone

Copolyvidone

\[(C_6H_9NO)_n/(C_4H_6O_2)_m\]
DESCRIPTION
Copovidone occurs as a white to yellowish-white powder or as flakes. It is a copolymer of 1-vinyl-2-pyrrolidone
and vinyl acetate in the mass proportion of 3:2. It is freely soluble in water, in alcohol, and in methylene
chloride, but practically insoluble in ether. It is hygroscopic. The pH of a 1:10 aqueous solution is between 3
and 7.
Function Coating for fresh and fresh-cut fruits and vegetables, film-forming agent, binder, and crystallization
inhibitor
Packaging and Storage Store in tight containers.

IDENTIFICATION

A. Procedure
Sample solution: 20 mg/mL
Analysis: To 5 mL of the Sample solution, add a few drops of iodine TS.
Acceptance criteria: A deep red color is produced.

B. Infrared Absorption, Spectrophotometric Identification Tests, Appendix IIIC
Reference standard: USP Copovidone 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.

ASSAY

A. Nitrogen Determination, Method II, Appendix IIIC
Sample: 100 mg
Bromocresol green–methyl red solution: Dissolve 0.15 g of bromocresol green and 0.1 g of methyl red in
180 mL of alcohol, and dilute with water to 200 mL.
Analysis: Modify the procedure as follows: in the wet-digestion step, use 5 g of a 33:1:1 mixture of
potassium sulfate–cupric sulfate–titanium dioxide instead of the 10:1 potassium sulfate–cupric sulfate
mixture; and omit the use of hydrogen peroxide. Heat until the solution has a clear, yellow-green color and
the sides of the flask are free from carbonaceous material. Then heat for an additional 45 min and continue
as directed, beginning with "Cautiously add 20 mL of water, cool, then...". Use Bromocresol green–methyl
red solution instead of methyl red–methylene blue TS. Titrate the distillate with 0.05 N sulfuric acid until
the color of the solution changes from green through pale grayish-blue to pale grayish red-purple. Perform
a blank determination, and make any necessary correction. Each mL of 0.05 N sulfuric acid is equivalent
to 0.7004 mg of nitrogen.
Acceptance criteria: NLT 7.0% and NMT 8.0%, calculated on the dried basis

B. Copolymerized Vinyl Acetate
Sample: 2 g
Analysis: Transfer the Sample into a 250-mL borosilicate glass flask, add 25 mL of 0.5 N alcoholic
potassium hydroxide and a few glass beads, and heat under reflux for 30 min. Add 1 mL of phenolphthalein
TS, and titrate the excess 0.5 N alcoholic potassium hydroxide immediately (while still hot) with 0.5 N
hydrochloric acid. Perform a blank determination and make any necessary correction. Calculate the
percentage of copolymerized vinyl acetate in the sample taken by the formula:

$$0.1 \times \frac{(86.09/56.11) \times (56.11 \times N_A) \times (V_B - V_U)}{W}$$
86.09 = Molecular weight of vinyl acetate
56.11 = Molecular weight of potassium hydroxide
\( N_A \) = Actual normality of the alcoholic potassium hydroxide
\( V_B \) = Volume (mL) of 0.5 N hydrochloric acid consumed for the blank determination
\( V_U \) = Volume (mL) of 0.5 N hydrochloric acid consumed for the Sample determination
\( W \) = Weight (g) of Sample taken

Acceptance criteria: NLT 35.3% and NMT 42.0%, calculated on the dried basis

IMPURITIES

Inorganic Impurities

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

Organic Impurities

• **Aldehydes**

  **Pyrophosphate buffer, 0.05 M**: Transfer 8.7 g of potassium pyrophosphate into a 500-mL volumetric flask, and dissolve in 400 mL of water. Adjust, if necessary, to a pH of 9.0 with 1 N potassium hydroxide, dilute to volume, and mix.

  **Aldehyde dehydrogenase solution**: Transfer a quantity of lyophilized aldehyde dehydrogenase (Sigma A550, or equivalent) equivalent to 70 units into a glass vial, dissolve it in 10.0 mL of water, and mix.

  [Note: This solution is stable for 8 h at 4°C.]

  **NAD solution**: 4.0 mg/mL of nicotinamide adenine dinucleotide in Pyrophosphate buffer

  **Standard solution**: Dissolve 0.140 g of acetaldehyde ammonia trimer trihydrate in 200.0 mL of water, and mix. Pipet 1.0 mL of this solution into a 100-mL volumetric flask, dilute with Pyrophosphate buffer to volume, and mix.

  **Sample solution**: Transfer 1 g of sample into a 100-mL volumetric flask, dissolve it in 50 mL of Pyrophosphate buffer, dilute to volume with Pyrophosphate buffer, and mix. Stopper the flask loosely, heat at 60°C for 1 h, and cool to room temperature.

  **Blank**: Water

  **Analysis**: Pipet 0.5 mL each of the Standard solution, the Sample solution, and Blank into separate 1-cm cells. Add 2.5 mL of Pyrophosphate buffer and 0.2 mL of NAD solution to each cell. Cover the cells to exclude oxygen. Mix them by inversion and allow them to stand for 2 to 3 min at 22°C ± 2°C. Using a suitable spectrophotometer, measure the absorbances of the solutions at 340 nm. Add 0.05 mL of Aldehyde dehydrogenase solution to each cell. Stopper the cells tightly and mix by inversion. Allow them to stand for 5 min at 22°C ± 2°C. Measure the absorbances of the solutions as before. Calculate the percentage of Aldehydes (as acetaldehyde) in the sample taken by the formula:

\[
10\left(\frac{C}{W}\right)\left[\frac{(A_{U2} - A_{U1}) - (A_{B2} - A_{B1})}{(A_{S2} - A_{S1}) - (A_{B2} - A_{B1})}\right]
\]

\( C \) = Concentration (mg/mL) of acetaldehyde in the Standard solution calculated from the weight of the acetaldehyde–ammonia trimer trihydrate with the factor of 0.72

\( W \) = Weight (g) of sample taken to prepare the Sample solution
\[ A_{U1} = \text{Absorbance of the solution obtained from the Sample solution, before the Aldehyde dehydrogenase solution was added} \]
\[ A_{S1} = \text{Absorbance of the solution obtained from the Standard solution before the Aldehyde dehydrogenase solution was added} \]
\[ A_{B1} = \text{Absorbance of the solution obtained from the Blank, before the Aldehyde dehydrogenase solution was added} \]
\[ A_{U2} = \text{Absorbance of the solution obtained from the Sample solution, after the Aldehyde dehydrogenase solution was added} \]
\[ A_{S2} = \text{Absorbance of the solution obtained from the Standard solution after the Aldehyde dehydrogenase solution was added} \]
\[ A_{B2} = \text{Absorbance of the solution obtained from the Blank, after the Aldehyde dehydrogenase solution was added} \]

**Acceptance criteria:** NMT 0.05% (as acetaldehyde)

• **Hydrazine, Thin-Layer Chromatography, Appendix IIA**

**Salicylaldazine standard solution:** 9 µg/mL of salicylaldazine in toluene

**Sample:** An amount of sample equivalent to 2.5 g on the dried basis

**Sample solution:** Transfer the Sample into a 50-mL centrifuge tube, add 25 mL of water, and mix to dissolve. Add 500 µL of a 50 mg/mL solution of salicylaldehyde in methanol, swirl, and heat in a water bath at 60° for 15 min. Allow the solution to cool, add 2.0 mL of toluene, insert a stopper in the tube, shake the tube vigorously for 2 min, and centrifuge. The clear upper layer is the Sample solution.

**Adsorbent:** 0.25-mm layer of dimethylsilanized chromatographic silica gel mixture with fluorescent indicator

**Developing solvent system:** Methanol and water (2:1)

**Application volume:** 10 µL

**Detection/Visualization:** UV 365 nm

**Analysis:** Spot the Sample solution and the Salicylaldazine standard solution onto the plate. Following development, locate the spots on the plate by examination under UV light. Salicylaldazine appears as a fluorescent spot having an \( R_F \) value of about 0.3.

**Acceptance criteria:** The fluorescence of any salicylaldazine spot from the Sample solution is not more intense than that produced by the spot obtained from the Salicylaldazine standard solution (NMT 1 mg/kg).

• **Monomers (1-Vinyl-2-Pyrrolidone, Vinyl Acetate, and 2-Pyrrolidone)**

**Solution A:** Acetonitrile, methanol, and water (1:1:18)

**Solution B:** Acetonitrile, methanol, and water (9:1:10)

**Standard stock solution:** 0.5 mg/mL 1-vinyl-2-pyrrolidone, 0.5 mg/mL vinyl acetate, and 3 mg/mL of 2-pyrrolidone in methanol

**Standard solution:** 0.25 µg/mL 1-vinyl-2-pyrrolidone, 0.25 µg/mL vinyl acetate, and 1.5 µg/mL of 2-pyrrolidone prepared by diluting the Standard stock solution with Solution A

**Sample solution:** Transfer 250 mg of sample into a 10-mL volumetric flask, add 1 mL of methanol, mix ultrasonically to dissolve, dilute with water to volume, and mix. If necessary, filter this solution to remove undissolved particles.

**Chromatographic system,** Appendix IIA

**Mode:** High-performance liquid chromatography

**Detectors:** UV 205 nm and 235 nm

**Column:** 4-mm × 250-mm stainless steel, packed with octadecylsilane silica gel (5 µm particle diameter)\(^1\), with 4-mm × 30-mm guard column with the same packing\(^2\)
Column temperature: 30°
Flow rate: About 1.0 mL/min
Injection volume: About 10 µL
Mobile phase: See gradient table (below).

<table>
<thead>
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<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
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System suitability

Sample: Standard solution
Resolution: NLT 2.0 between 2-pyrrolidone, vinyl acetate, and 1-vinyl-2-pyrrolidone peaks
Relative standard deviation: NMT 2.0% for each analyte for replicate injections

Analysis: Separately inject the Standard solution and the Sample solution into the chromatograph, record the chromatograms, and measure the responses for the 2-pyrrolidone, vinyl acetate, and 1-vinyl-2-pyrrolidone peak areas. [Note: The order of elution is 2-pyrrolidone, vinyl acetate, and 1-vinyl-2-pyrrolidone.] [Note: After each injection of the Sample solution, wash the polymeric material from the guard column by passing the mobile phase (100% Solution A) through the column backwards for about 30 min at the same flow rate.] Calculate the content of the three monomers in the sample taken using the following equations:

1-Vinyl-2-pyrrolidone (mg/kg) = \( (r_{UA}/r_{SA}) \times (C_{SA}/C_U) \times 1000 \)

Vinyl acetate (mg/kg) = \( (r_{UB}/r_{SB}) \times (C_{SB}/C_U) \times 1000 \)

2-Pyrolidone (%) = \( (r_{UC}/r_{SC}) \times (C_{SC}/C_U) \times (1/1000) \times 100\%

\( r_{UA}, r_{UB}, r_{UC} \) = Peak area responses from the Sample solution for 1-vinyl-2-pyrrolidone, vinyl acetate, and 2-pyrolidone, respectively
\( r_{SA}, r_{SB}, r_{SC} \) = Peak area responses from the Standard solution for 1-vinyl-2-pyrrolidone, vinyl acetate, and 2-pyrolidone, respectively
\( C_{SA}, C_{SB}, C_{SC} \) = Concentrations of 1-vinyl-2-pyrrolidone, vinyl acetate, and 2-pyrolidone, respectively in the Standard solution (µg/mL)
\( C_U \) = Sample solution concentration (mg/mL)
1000 = Correction factor to convert units from mg/g to mg/kg
1/1000 = Correction factor to convert units from µg/mg to µg/µg

Acceptance criteria
1-Vinyl-2-pyrrolidone: NMT 10 mg/kg
Vinyl acetate: NMT 10 mg/kg
2-Pyrrolidone: NMT 0.5%

**Peroxides**

**Sample stock solution:** 40 mg/mL

**Titanium trichloride solution:** 150 mg/mL titanium trichloride in 10% hydrochloric acid

**Titanium trichloride–sulfuric acid solution:** Mix carefully 20 mL of *Titanium trichloride solution* in 13 mL of sulfuric acid. Add sufficient 30% hydrogen peroxide to produce a yellow color. Heat until white fumes are evolved, allow to cool, and dilute with water. Repeat the evaporation and addition of water until a colorless solution is obtained. Dilute with water to 100 mL.

**Sample solution:** Transfer 25.0 mL of the *Sample stock solution* to a 50-mL beaker, add 2 mL of *Titanium trichloride–sulfuric acid solution*, and mix. Allow to stand for 30 min at room temperature.

**Blank solution:** Transfer 25.0 mL of *Sample stock solution* to a 50-mL beaker, add 2 mL of 13% sulfuric acid, and mix.

**Analysis:** Measure the absorbances of the *Sample solution* and *Blank solution* in a 1-cm cell at the wavelength of maximum absorbance (about 405 nm), using a suitable spectrophotometer.

**Acceptance criteria:** The blank-corrected absorbance is not more than 0.35 (corresponding to not more than 0.04%, expressed as hydrogen peroxide).

**SPECIFIC TESTS**

**Clarity and Color**

**Sample solution:** 100 mg/mL

**Acceptance criteria:** The *Sample solution* is clear or slightly opalescent and colorless to pale yellow or pale red.

**K-Value**

[Note: The molecular weight of the sample is characterized by its viscosity in aqueous solution, relative to that of water, expressed as a K-Value.]

**Sample:** An amount of sample equivalent to 1.0 g on the dried basis

**Sample solution:** Transfer the *Sample* into a 100-mL volumetric flask, dissolve it in about 50 mL of water, dilute with water to volume, mix thoroughly, and allow it to stand for 1 h. Filter the solution. Pipet 15 mL of filtrate into a clean, dry Ubbelholde-type viscometer, and place the viscometer in a water bath maintained at 25°C ± 0.2°C.

**Analysis:** After allowing the viscometer and the *Sample solution* to warm in the water bath for 10 min, draw the solution by means of very gentle suction up through the capillary until the meniscus is above the upper etched mark. Release suction, and after the meniscus reaches the upper etched mark, begin timing the flow through the capillary. Record the exact time when the meniscus reaches the lower etched mark, and calculate the flow time to the nearest 0.01 s. Repeat this operation until at least three readings are obtained. The readings must agree within 0.1 s; if not, repeat the determination with additional 15-mL portions of the *Sample solution* after recleaning the viscometer with sulfuric acid–dichromate cleaning solution or with a suitable laboratory cleaning compound that will remove oils, greases, waxes, and other impurities. Calculate the average flow time and then obtain the flow time in a similar manner for 15 mL of water. Calculate the relative viscosity, z, of the *Sample* by dividing the average flow time of the *Sample solution* by that of the water sample, and then calculate the K-Value by the formula:

\[
\frac{\sqrt{300c \log z + (c+1.5c \log z)^2 + 1.5c \log z - c}}{(0.15c + 0.003c^2)}
\]
\[ c = \text{Weight (g) of the sample, calculated on the dried basis, in each 100.0 g of solution} \]
\[ z = \text{Relative viscosity} \]

**Acceptance criteria:** NLT 90.0% and NMT 110.0% of the \( K\)-value stated on the label

- **LOSS ON DRYING,** Appendix IIC (105\(^\circ\)C for 3h)
  - **Acceptance criteria:** NMT 5.0%
- **RESIDUE ON IGNITION (SULFATED ASH),** Appendix IIC
  - **Sample:** 2 g
  - **Analysis:** Proceed as directed, but igniting at 600 ± 50\(^\circ\)C for 30 min.
  - **Acceptance criteria:** NMT 0.1%

**OTHER REQUIREMENTS**
- **LABELING** Indicate the nominal \( K\)-value of the product.

1. **Auxiliray Information**

---

\(^1\) Aquasil C18 (Thermo-Hypersil), or equivalent

\(^2\) Nucleosil 120-5 C18 (Machery-Nagel), or equivalent

**Auxiliary Information**— *Staff Liaison:* Jeffrey Moore, Ph.D., Senior Scientific Associate  
*Expert Committee:* (FI07) Food Ingredients Expert Committee  
*Phone Number:* 1-301-816-8288
Cuminic Aldehyde, *FCC 6 page 231—See briefing under Acetaldehyde.*
(FIEC: J. Moore) C64747

**Cuminic Aldehyde**

Cuminal

Cuminaldehyde

*p*-Cuminic Aldehyde

*p*-Isopropylbenzaldehyde

\[ \text{C}_{10}\text{H}_{12}\text{O} \]

Formula wt 148.20
FEMA: 2341

**DESCRIPTION**

*Change to read:*
Cuminic Aldehyde occurs as a colorless to pale yellow liquid. It may contain a suitable antioxidant. 1S (*FCC 6*)

**Odor** Strong, pungent, cumin oil

**Solubility** Soluble in alcohol, ether; insoluble or practically insoluble in water

**Boiling Point** ~236°C

**Solubility in Alcohol,** Appendix VI
One mL dissolves in 4 mL of 70% alcohol.

**Function** Flavoring agent

**IDENTIFICATION**

- **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.
ASSAY
• **Procedure** Proceed as directed under M-2a, Appendix XI.
  
  **Acceptance criteria:** NLT 95.0% of \(\text{C}_{10}\text{H}_{12}\text{O}\)

SPECIFIC TESTS
• **ACID VALUE, M-15, Appendix XI**
  
  **Acceptance criteria:** NMT 5.0

• **REFRACTIVE INDEX, Appendix II (at 20\(^{\circ}\))**
  
  **Acceptance criteria:** Between 1.528 and 1.534

• **Specific Gravity** Determine at 25\(^{\circ}\) by any reliable method (see General Provisions).
  
  **Acceptance criteria:** Between 0.975 and 0.980

OTHER REQUIREMENTS
• **CHLORINATED COMPOUNDS, Appendix VI**
  
  **Acceptance criteria:** Passes test.

Auxiliary Information—Staff Liaison: Kristie Bowman, Senior Scientific Associate

*Expert Committee:* (FL07) Food Ingredients Expert Committee

*FCC Sixth Edition Page 231*

*Phone Number:* 1-301-816-8356
BRIEFING

Cyclamen Aldehyde, *FCC 6* page 234—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

Cyclamen Aldehyde

2-Methyl-3-(p-isopropylphenyl)propionaldehyde

\[
\text{C}_{13}\text{H}_{18}\text{O}
\]

**DESCRIPTION**

*Change to read:*

Cyclamen Aldehyde occurs as a colorless to pale yellow liquid. It may contain a suitable antioxidant.

- **Odor**: Strong, floral
- **Solubility**: Soluble in most fixed oils; insoluble or practically insoluble in glycerin, propylene glycol
- **Boiling Point**: ~270°
- **Solubility in Alcohol**, Appendix VI
  - One mL dissolves in 3 mL of 80% alcohol.
- **Function**: Flavoring agent

**IDENTIFICATION**

- **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.

![Infrared Spectrum](image)
ASSAY

- **Procedure** Proceed as directed under M-1b, Appendix XI.
  
  **Acceptance criteria**
  
  **Sum of two isomers:** NLT 90.0% of C_{13}H_{18}O
  
  **Major isomer:** NLT 85.0% of C_{13}H_{18}O

SPECIFIC TESTS

- **ACID VALUE,** M-15, Appendix XI
  
  **Acceptance criteria:** NMT 5.0

- **REFRACTIVE INDEX,** Appendix II (at 20°)
  
  **Acceptance criteria:** Between 1.503 and 1.508

- **Specific Gravity** Determine at 25° by any reliable method (see General Provisions).
  
  **Acceptance criteria:** Between 0.946 and 0.952

**Auxiliary Information—** **Staff Liaison:** Kristie Bowman, Senior Scientific Associate

**Expert Committee:** (Fl07) Food Ingredients Expert Committee

**FCC Sixth Edition Page 234**

**Phone Number:** 1-301-816-8356
(E),(E)-2,4-Decadienal, FCC 6 page 244—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

(E),(E)-2,4-Decadienal

trans,trans-2,4-Decadienal

\[ \text{C}_{10} \text{H}_{16} \text{O} \]

Formula wt 152.24
FEMA: 3135

DESCRIPTION

Change to read:

(E),(E)-2,4-Decadienal occurs as a yellow liquid. It may contain a suitable antioxidant.

Odor Powerful, oily, chicken fat

Solubility Soluble in alcohol, most fixed oils; insoluble or practically insoluble in water

Boiling Point \(-104^\circ\) (7 mm Hg)

Solubility in Alcohol, Appendix VI
One mL dissolves in 1 mL of 95% ethanol.

Function Flavoring agent

IDENTIFICATION

• 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.

ASSAY

• Procedure Proceed as directed under M-1a, Appendix XI.
  Acceptance criteria: NLT 89.0% of C\(_{10}\)H\(_{16}\)O (sum of two isomers)

SPECIFIC TESTS

• REFRACTIVE INDEX, Appendix II (at 20\(^\circ\))
  Acceptance criteria: Between 1.514 and 1.519
• **Specific Gravity** Determine at 25° by any reliable method (see General Provisions).
  
  **Acceptance criteria:** Between 0.866 and 0.876

**Auxiliary Information**— **Staff Liaison:** Kristie Bowman, Senior Scientific Associate

**Expert Committee:** (FI07) Food Ingredients Expert Committee

**FCC Sixth Edition Page 244**

**Phone Number:** 1-301-816-8356
BRIEFING

Decanal, FCC 6 page 246—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

Decanal

Aldehyde C-10

Capraldehyde

\[
\text{C}_{10}\text{H}_{20}\text{O}
\]

Formula wt 156.27
FEMA: 2362

DESCRIPTION

Change to read:
Decanal occurs as a colorless to light yellow liquid. It may contain a suitable antioxidant. 1S (FCC 6)

Odor Fatty, floral-orange on dilution

Solubility Miscible in alcohol, most fixed oils, propylene glycol (may be turbid); insoluble or practically insoluble in glycerin, water

Boiling Point \(-209^\circ\)

Function Flavoring agent

IDENTIFICATION

• 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.

ASSAY

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

Acceptance criteria: NLT 92.0% of C\(_{10}\)H\(_{20}\)O
SPECIFIC TESTS

- **ACID VALUE, M-15**, Appendix XI
  
  **Acceptance criteria**: NMT 10.0

- **REFRACTIVE INDEX**, Appendix II (at 20°)
  
  **Acceptance criteria**: Between 1.426 and 1.430

- **Specific Gravity** Determine at 25° by any reliable method (see General Provisions).
  
  **Acceptance criteria**: Between 0.823 and 0.832

**Auxiliary Information**— *Staff Liaison*: Kristie Bowman, Senior Scientific Associate

*Expert Committee*: (Fl07) Food Ingredients Expert Committee

*FCC Sixth Edition* Page 246

*Phone Number*: 1-301-816-8356
BRIEFING

(E)-2-Decenal, FCC 6 page 247—See briefing under Acetaldehyde.  
(FIEC: J. Moore) C64747

(E)-2-Decenal

trans-2-Decenal

\[
\text{C}_{10}\text{H}_{18}\text{O}
\]

Formula wt 154.25  
FEMA: 2366

DESCRIPTION

Change to read:

(E)-2-Decenal occurs as a slightly yellow liquid. It may contain a suitable antioxidant.  

Odor Orange, wax  
Solubility Soluble in alcohol, most fixed oils; insoluble or practically insoluble in water  
Boiling Point – 229°C  
Solubility in Alcohol, Appendix VI  
One mL dissolves in 1 mL of 95% ethanol.  
Function Flavoring agent

IDENTIFICATION

• 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.

![Infrared Spectrum](image)

(E)-2-Decenal

ASSAY

• Procedure Proceed as directed under M-1a, Appendix XI.  
  Acceptance criteria: NLT 92.0% of C\textsubscript{10}H\textsubscript{18}O (one isomer)

SPECIFIC TESTS

• REFRACTIVE INDEX, Appendix II (at \(20^\circ\))  
  Acceptance criteria: Between 1.452 and 1.457
• **Specific Gravity** Determine at 25° by any reliable method (see *General Provisions*).
  
  **Acceptance criteria:** Between 0.836 and 0.846

**Auxiliary Information**— *Staff Liaison:* Kristie Bowman, Senior Scientific Associate

*Expert Committee:* (FI07) Food Ingredients Expert Committee

*FCC Sixth Edition* Page 247

*Phone Number:* 1-301-816-8356
(Z)-4-Decenal, FCC 6 page 248—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

(Z)-4-Decenal

cis-4-Decenal

\[
\text{C}_{10}\text{H}_{18}\text{O}
\]

Formula wt 154.25
FEMA: 3264

DESCRIPTION

Change to read:
(Z)-4-Decenal occurs as a colorless to slightly yellow liquid. It may contain a suitable antioxidant. 1S (FCC 6)

Odor Orange, fatty

Solubility Soluble in alcohol, most fixed oils; insoluble or practically insoluble in water

Boiling Point $78^\circ$ to $80^\circ$ (10 mm Hg)

Solubility in Alcohol, Appendix VI
One mL dissolves in 1 mL of 95% ethanol.

Function Flavoring agent

IDENTIFICATION

• 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.

ASSAY

• Procedure Proceed as directed under M-1a, Appendix X.
  Acceptance criteria: NLT 90.0% of C_{10}H_{18}O

SPECIFIC TESTS
- **REFRACTIVE INDEX**, Appendix II (at 20°)
  
  **Acceptance criteria:** Between 1.442 and 1.447

- **Specific Gravity** Determine at 25° by any reliable method (see *General Provisions*).
  
  **Acceptance criteria:** Between 0.843 and 0.850

**Auxiliary Information**— *Staff Liaison*: Kristie Bowman, Senior Scientific Associate

*Expert Committee:* (FI07) Food Ingredients Expert Committee

*FCC Sixth Edition Page 248*

*Phone Number:* 1-301-816-8356
Diacetyl, FCC 6 page 255—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

Diacetyl
2,3-Butanedione
Dimethyldiketone
Dimethylglyoxal

\[
\text{\ce{H3C\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-\text}{\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-\text}{\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}\text{\tiny-}}}}}}}}}}
\]

\[
\text{C}_4\text{H}_6\text{O}_2
\]

Formula wt 86.09
FEMA: 2370

DESCRIPTION

Change to read:
Diacetyl occurs as a yellow to yellow-green liquid. ■ It may contain a suitable antioxidant. ■1S (FCC 6)

Odor Powerful, buttery in very dilute solution
Solubility Soluble in glycerin, water; miscible in alcohol, most fixed oils, propylene glycol
Boiling Point ~88°
Function Flavoring agent

IDENTIFICATION

• Infrared Spectra, Spectrophotometric Identification Tests, Appendix III C
  Acceptance criteria: The spectrum of the sample exhibits relative maxima at the same wavelengths as those of the spectrum below.
ASSAY
• **Procedure** Proceed as directed under M-1b, Appendix XI.
  **Acceptance criteria:** NLT 95.0% of C₄H₆O₂

SPECIFIC TESTS
• **Refractive Index,** Appendix II (at 20°)
  **Acceptance criteria:** Between 1.393 and 1.397

• **Specific Gravity** Determine at 25° by any reliable method (see General Provisions).
  **Acceptance criteria:** Between 0.979 and 0.985

OTHER REQUIREMENTS
• **Solidification Point,** Appendix IIIB
  **Acceptance criteria:** Between −2.0° and −4.0°

Auxiliary Information— **Staff Liaison:** Kristie Bowman, Senior Scientific Associate
**Expert Committee:** (Fl07) Food Ingredients Expert Committee
**FCC Sixth Edition Page 255**
**Phone Number:** 1-301-816-8356
3,4-Dimethyl 1,2-Cyclopentadione, FCC 6 page 272—See briefing under Acetaldehyde.

(FIEC: J. Moore) C64747

3,4-Dimethyl 1,2-Cyclopentandione

\[ C_7H_{10}O_2 \]

Formula wt 126.16

FEMA: 3268

**DESCRIPTION**

*Change to read:

3,4-Dimethyl 1,2-Cyclopentandione occurs as a pale yellow to orange crystal. It may contain a suitable antioxidant. \( \text{1S (FCC 6)} \)

**Odor** Maple

**Solubility** Slightly soluble in propylene glycol; insoluble or practically insoluble in vegetable oils, water

**Boiling Point** ~142°

**Solubility in Alcohol**, Appendix VI

One g dissolves in 3 mL of 95% ethanol.

**Function** Flavored agent

**IDENTIFICATION**

- **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.
ASSAY
• Procedure Proceed as directed under M-1b, Appendix XI.
  Acceptance criteria: NLT 95.0% of C₇H₁₀O₂

OTHER REQUIREMENTS
• MELTING RANGE OR TEMPERATURE, Appendix IIIB
  Acceptance criteria: Between 64.0° and 72.0°

Auxiliary Information—Staff Liaison: Kristie Bowman, Senior Scientific Associate
Expert Committee: (Fl07) Food Ingredients Expert Committee
FCC Sixth Edition Page 272
Phone Number: 1-301-816-8356
2,6-Dimethyl 5-heptenal, *FCC 6* page 274—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

2,6-Dimethyl-5-heptenal

![Chemical structure of 2,6-Dimethyl-5-heptenal](image)

C₉H₁₆O

Formula wt 140.23
FEMA: 2389

**DESCRIPTION**

*Change to read:*

2,6-Dimethyl-5-heptenal occurs as a pale yellow liquid. It may contain a suitable antioxidant.

**Odor** Melon

**Solubility** Soluble in vegetable oils; slightly soluble in propylene glycol; insoluble or practically insoluble in water

**Boiling Point** \(\sim 116^\circ\) to \(124^\circ\) (100 mm Hg)

**Solubility in Alcohol**, Appendix VI One mL dissolves in 1 mL of 95% alcohol.

**Function** Flavoring agent

**IDENTIFICATION**

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

**ASSAY**

- **Procedure** Proceed as directed under *M-2d*, Appendix XI.

  **Sample:** 1 g

  **Analysis:** Use 14.01 as the equivalence factor (e).  

  **Acceptance criteria:** NLT 85.0% of C₉H₁₆O
SPECIFIC TESTS

• **Acid Value, M-15, Appendix XI**
  
  **Acceptance criteria:** NMT 5.0

• **Refractive Index, Appendix II (at 20°)**
  
  **Acceptance criteria:** Between 1.442 and 1.447

• **Specific Gravity** Determine at 25° by any reliable method (see General Provisions).
  
  **Acceptance criteria:** Between 0.848 and 0.854

**Auxiliary Information—** Staff Liaison: Kristie Bowman, Senior Scientific Associate

*Expert Committee: (Fl07) Food Ingredients Expert Committee*

*FCC Sixth Edition Page 274*

*Phone Number: 1-301-816-8356*
BRIEFING

(E)-2-Dodecen-1-al, FCC 6 page 287—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

(E)-2-Dodecen-1-al

trans-2-Dodecen-1-al

\[
\text{C}_{12}\text{H}_{22}\text{O}
\]

DESCRIPTION

(E)-2-Dodecen-1-al occurs as a slightly yellow liquid. It may contain a suitable antioxidant. 1S (FCC 6)

Odor Fatty, citrus
Solubility Soluble in alcohol, most fixed oils; insoluble or practically insoluble in water
Boiling Point \(\sim 272^\circ\)
Solubility in Alcohol, Appendix VI

Function Flavoring agent

IDENTIFICATION

• 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.

\[(E)-2-\text{Dodecen-1-al}\]

ASSAY

• PROCEDURE Proceed as directed under M-1a, Appendix XI.

Acceptance criteria: NLT 93.0% of \text{C}_{12}\text{H}_{22}\text{O}

SPECIFIC TESTS

• REFRACTIVE INDEX, Appendix II (at \(20^\circ\))

Acceptance criteria: Between 1.454 and 1.460

• SPECIFIC GRAVITY Determine at \(25^\circ\) by any reliable method (see General Provisions).

Acceptance criteria: Between 0.839 and 0.849
BRIEFING

Ethoxyquin, *FCC 6* page 300. On the basis of comments received, it is proposed to correct the GC column specifications in the *p-Phenetidine* test procedure. Interested parties are encouraged to submit comments to Jeff Moore, Ph.D., email jm@usp.org.

(FIEC: J. Moore) C63642

Ethoxyquin

6-Ethoxy-1,2-dihydro-2,4-trimethylquinoline

![Chemical structure of Ethoxyquin](image)

$C_{14}H_{19}NO$

Formula wt 217.31

INS: 324

CAS: [91-53-2]

DESCRIPTION

Ethoxyquin occurs as a clear yellow to red liquid that may darken with age without affecting its antioxidant activity. It is a mixture consisting predominantly of the monomer ($C_{14}H_{19}NO$). It also contains dimers and other polymers of $C_{14}H_{19}NO$. Its specific gravity is about 1.02, and its refractive index is about 1.57.

Function Antioxidant

Packaging and Storage Store in tightly closed carbon steel or black iron (not rubber, neoprene, or nylon) containers or in polypropylene or polyethylene drums or lined drums in a cool, dark place. Prolonged exposure to sunlight causes polymerization.

IDENTIFICATION

• **Procedure**
  
  **Sample solution:** 1 mg in 10 mL of acetonitrile
  
  **Analysis:** View under short-wavelength UV light.
  
  **Acceptance criteria:** The *Sample solution* exhibits a strong fluorescence.

ASSAY

• **Procedure**
  
  **Sample:** 200 mg
  
  **Analysis:** Transfer the *Sample* into a 150-mL beaker containing 50 mL of glacial acetic acid and immediately titrate with 0.1 N perchloric acid in glacial acetic acid, determining the endpoint potentiometrically.

  **[CAUTION:** Handle perchloric acid in an appropriate fume hood. ] Perform a blank determination (see *General Provisions*) and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 21.73 mg of $C_{14}H_{19}NO$.

  **Acceptance criteria:** NLT 91.0% $C_{14}H_{19}NO$
IMPURITIES
Inorganic Impurities
• Lead, Lead Limit Test, Flame Atomic Absorption Spectrophotometric Method, Appendix IIIB
  Sample: 10 g
  Acceptance criteria: NMT 2 mg/kg

Change to read:
Organic Impurities
• Ethoxyquin-related impurities (low-boiling monomers and high-boiling dimers, trimers, and oligomers of Ethoxyquin)
  Analysis: Calculate the quantity, in percentage, of related impurities by the formula:

\[ 100 - (\%\text{Assay} + \%\text{p-Phenetidine}) \]

Acceptance criteria: NMT 8.0%
• p-Phenetidine

Standard solution:  [CAUTION: Perform all steps in a fume hood and away from a source of ignition. Wear appropriate protective equipment, including gloves. ]
Transfer approximately 200 mg of p-phenetidine and approximately 200 mg of diphenyl ether, both accurately weighed, into a 4-dram bottle, add 10 mL of toluene and 5 drops of 10% sodium hydroxide, cap, and shake vigorously to dissolve. Prepare in triplicate.  [NOTE: Both the p-phenetidine and the diphenyl ether must be of known purity. Unless the reagent supplier’s reported purity is certified quantitative and traceable, determine the purity of a reagent standard by conducting an area percent profile by injecting 0.1 µL on the same column and at conditions analogous to those described below. The area percent corresponding to the standard in the chromatograph represents its purity.]

Sample preparation: Transfer 0.1 g of sample into a 4-dram vial. Add between 0.010 and 0.015 g of diphenyl ether, accurately weighed, 10 mL of toluene, and 5 drops of 10% sodium hydroxide solution; cap the vial; and shake well. Allow the vial to stand until the caustic layer settles to the bottom, and filter the neutralized sample through a 0.45-µm polytetrafluoroethylene (PTFE) filter, or equivalent.

Chromatographic system, Appendix IIA
  Mode: Gas chromatography (HP 6890, or equivalent)
  Detector: Flame-ionization detector (FID)
  Column: 30-m × 0.25-mm (edFid\textsubscript{1S}(FCC 6)) GC capillary column (DB-5MS, or equivalent) having a film thickness of 0.25 µm
  Temperatures
    Injector: 250°C
    Detector: 280°C
    Column: Initially 50°C with 1 min hold-time, linear gradient increase of 10°C/min to 280°C, final hold-time at 280°C of 10 min
  Carrier gas: Helium
  Flow rates
    Carrier gas: 2.3 mL/min
    Makeup: 50 mL/min
    Hydrogen (to burner): 45 mL/min
    Air (to burner): 450 mL/min
Split flow rate: 232 mL/min
Total flow rate: 237 mL/min
Injection size: 1.0 µL
Injector type: Split injector port

**Standardization:** Inject the **Standard solution** into the chromatograph. Calculate the \( p \)-phenetidine factor (\( F \)) by the formula:

\[
(A_{DE} \times W_{PF} \times P_{PF})/(A_{PF} \times W_{DE} \times P_{DE})
\]

\( A_{DE} \) = Area response for diphenyl ether
\( A_{PF} \) = Area response for \( p \)-phenetidine
\( W_{PF} \) = Weight (g) of \( p \)-phenetidine
\( W_{DE} \) = Weight (g) of diphenyl ether
\( P_{PF} \) = Purity of \( p \)-phenetidine
\( P_{DE} \) = Purity of diphenyl ether

**Analysis:** Inject the **Sample preparation** into the gas chromatograph and calculate the content of \( p \)-phenetidine, in percentage, by the formula:

\[
(A_P \times W_D \times F)/(W_S \times A_D)
\]

\( A_P \) = Area of the \( p \)-phenetidine peak
\( A_D \) = Area of the diphenyl ether peak
\( W_D \) = Weight (g) of diphenyl ether in the **Sample preparation**
\( W_S \) = Weight (g) of the sample taken
\( F \) = \( p \)-phenetidine factor (calculated above)

**Acceptance criteria:** NMT 3.0%

**Auxiliary Information**— **Staff Liaison:** Jeffrey Moore, Ph.D., Senior Scientific Associate
**Expert Committee:** (Fl07) Food Ingredients Expert Committee
**FCC Sixth Edition Page 300**
**Phone Number:** 1-301-816-8288
2-Ethylbutyraldehyde, FCC 6 page 308—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

2-Ethylbutyraldehyde

\[
\begin{align*}
\text{C}_6\text{H}_{12}\text{O} \\
\text{Formula wt 100.16} \\
\text{FEMA: 2426}
\end{align*}
\]

DESCRIPTION

*Change to read:* 2-Ethylbutyraldehyde occurs as a colorless, mobile liquid. It may contain a suitable antioxidant. 1S (FCC 6)

**Odor** Pungent

**Solubility** Miscible in alcohol, ether; 1 mL dissolves in 50 mL water

**Boiling Point** ~117\(^\circ\)

**Function** Flavored agent

ASSAY

- **PROCEDURE** Proceed as directed under M-1b, Appendix XI.
  - **Acceptance criteria:** NLT 95.0% of C\(_6\)H\(_{12}\)O

SPECIFIC TESTS

- **ACID VALUE, M-15,** Appendix XI
  - **Acceptance criteria:** NMT 2.0

- **REFRACTIVE INDEX,** Appendix II (at 20\(^\circ\))
  - **Acceptance criteria:** Between 1.398 and 1.404

- **Specific Gravity** Determine at 25\(^\circ\) by any reliable method (see General Provisions).
  - **Acceptance criteria:** Between 0.808 and 0.814

OTHER REQUIREMENTS

- **DISTILLATION RANGE,** Appendix IIB
Acceptance criteria: NLT 95% between 100° and 120°

Auxiliary Information— Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
FCC Sixth Edition Page 308
Phone Number: 1-301-816-8288
BRIEFING

Ethyl Cellulose, FCC 6 page 310. On the basis of comments received, it is proposed to harmonize the temperature at which the Residue on Ignition test is performed to that listed in the USP–NF and European Pharmacopoeia. Interested parties are encouraged to comment.

(FIEC : K. Bowman) C65285

Ethyl Cellulose

Modified Cellulose, EC

INS: 462

CAS: [9004-57-3]

DESCRIPTION

Ethyl Cellulose occurs as a free-flowing, white to light tan powder. It is heat-labile, and exposure to high temperatures (240°C) causes color degradation and loss of properties. It is practically insoluble in water, in glycerin, and in propylene glycol, but is soluble in varying proportions in certain organic solvents, depending on the ethoxyl content. Ethyl Cellulose containing less than 46% to 48% of ethoxyl groups is freely soluble in tetrahydrofuran, in methyl acetate, in chloroform, and in aromatic hydrocarbon–alcohol mixtures. Ethyl Cellulose containing 46% to 48% or more of ethoxyl groups is freely soluble in alcohol, in methanol, in toluene, in chloroform, and in ethyl acetate. A 1:20 aqueous suspension is neutral to litmus.

Function Protective coating; binder; filler

Packaging and Storage Store in well-closed containers.

IDENTIFICATION

• PROCEDURE
  Sample solution: Dissolve 5 g of sample in 95 g of an 80:20 (w/w) mixture of toluene–ethanol.
  Analysis: Pour a few mL of Sample solution onto a glass plate, and allow the solvent to evaporate.
  Acceptance criteria: The Sample solution is clear, stable, and slightly yellow, and following the Analysis, a thick, tough, clear, flammable film remains.

ASSAY

• PROCEDURE
  Sample: Place about 50 mg of sample, previously dried, in a tared gelatin capsule.
  Analysis: Transfer the capsule and its contents into the boiling flask of a methoxyl determination apparatus, and proceed as directed under Methoxyl Determination, Appendix IIIC. Each mL of 0.1 N sodium thiosulfate is equivalent to 751 mg of ethoxyl groups (–OC₂H₅)
  Acceptance criteria: NLT 44.0% and NMT 50.0% of ethoxyl groups (–OC₂H₅), on the dried basis (equivalent to NMT 2.6 ethoxyl groups per anhydroglucose unit).

IMPURITIES

Inorganic Impurities

• Lead, Lead Limit Test, Appendix IIIB
  Sample solution: Prepare as directed for organic compounds, using a 2 g sample.
  Control: 6 µg of Pb (6 mL of Diluted Standard Lead Solution)
  [NOTE: Alternatively, determine as directed for Flame Atomic Absorption Spectrophotometric Method under...]

FCC Forum - June 2008 1
**Lead Limit Test, Appendix IIIB, using a 10-g sample.**

**Acceptance criteria:** NMT 3 mg/kg

**SPECIFIC TESTS**

- **Loss on Drying,** Appendix IIC (105°C for 2 h)
  
  **Acceptance criteria:** NMT 3.0%.

**Change to read:**

- **Residue on Ignition (Sulfated Ash),** Appendix IIC

  **Sample:** 1 g

  ▬**Analysis** Proceed as directed, but igniting at 600°C ± 50°C. 1S (FCC 6)

  **Acceptance criteria:** NMT 0.5%. ▲FCC 6

- **Viscosity, Viscosity of Methylcellulose,** Appendix IIB

  **Solvent systems:** For Ethyl Cellulose containing less than 46% to 48% of ethoxyl groups, prepare a solvent consisting of a 60:40 (w/w) mixture of toluene–alcohol; for Ethyl Cellulose containing 46% to 48% or more of ethoxyl groups, prepare a solvent consisting of an 80:20 (w/w) mixture of toluene–alcohol.

  **Sample solution:** Transfer 5.0 g of previously dried sample into a bottle containing 95 ± 0.5 g of the appropriate solvent system. Shake or tumble the bottle until the sample is completely dissolved, and then adjust the temperature of the solution to 25 ± 0.1°C.

  **Analysis:** Determine the viscosity of the **Sample solution** as directed, but make all determinations at 25°C instead of at 20°C.

  **Acceptance criteria**

  - **Ethyl Cellulose labeled as 10 centipoises or more:** NLT 90% and NMT 110% of the viscosity stated on the label
  - **Ethyl Cellulose labeled as 10 centipoises or less:** NLT 80% and NMT 120% of the viscosity stated on the label

**Auxiliary Information—** **Staff Liaison:** Jeffrey Moore, Ph.D., Senior Scientific Associate

**Expert Committee:** (Fl07) Food Ingredients Expert Committee

**FCC Sixth Edition Page 310**

**Phone Number:** 1-301-816-8288
BRIEFING

Ferrous Sulfate, *FCC 6* page 369. On the basis of comments received, it is proposed to revise the *Mercury* test by adding instructions for preparation of the *Sample solution* for this test. Preparation of the sample using the current instructions contained in *Mercury Limit Test, Method II*, Appendix IIIB does not allow the analyst to test to the limit specified in this monograph.

(FIEC: K. Bowman) C65274

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**Ferrous Sulfate**  
**FeSO₄·7H₂O**

*Formula wt 278.02*  
*CAS: [7782-63-0]*

**DESCRIPTION**

Ferrous Sulfate occurs as pale, blue-green crystals or granules that are efflorescent in dry air. In moist air, it oxidizes readily to form a brown-yellow, basic ferric sulfate. A 1:10 aqueous solution has a pH of about 3.7.

One g dissolves in 1.5 mL of water at 25° and in 0.5 mL of boiling water. It is insoluble in alcohol.

**Function** Nutrient

**Packaging and Storage** Store in tight containers.

**IDENTIFICATION**

- **Iron (Ferrous Salts), Appendix III**  
  **Acceptance criteria:** Passes tests.

- **Sulfate, Appendix IIIA**  
  **Acceptance criteria:** Passes tests.

**ASSAY**

- **Procedure**
  
  **Sample:** 1 g

  **Analysis:** Dissolve the *Sample* in a mixture of 25 mL of 2 N sulfuric acid and 25 mL of recently boiled and cooled water. Titrate with 0.1 N ceric sulfate, using orthophenanthroline TS, as the indicator. Perform a blank determination (see *General Provisions*), and make any necessary correction. Each mL of 0.1 N ceric sulfate is equivalent to 27.80 mg of FeSO₄·7H₂O.

  **Acceptance criteria:** NLT 99.5% and NMT 104.5% FeSO₄·7H₂O

**IMPURITIES**

*Change to read:*

**Inorganic Impurities**

- **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 in
water

Triocetylphosphine oxide solution: 50 mg/mL of triocetylphosphine oxide in 4-methyl-2-pentanone

[CAUTION: This reagent 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: 100 µg/mL of lead prepared as follows: 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 to volume with water.

Standard solution: Transfer 1.0 mL of Standard stock solution into a 100-mL volumetric flask, dilute to volume with water, 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 Triocetylphosphine 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 layer to separate. The organic solvent layer is the Standard solution and contains 0.4 µg/mL of lead.

Blank solution: 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 Triocetylphosphine 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 layer to separate. The organic solvent layer remaining is the Blank solution and contains 0.0 µg/mL of lead.

Sample solution: Add 1.0 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 Triocetylphosphine 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 solution.

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 solution, the Standard solution, and the Sample solution.

Acceptance criteria: The absorbance of the Blank solution is NMT 20% of the difference between the absorbance of the Standard solution and the absorbance of the Blank solution. The absorbance of the Sample solution does not exceed that of the Standard solution. (NMT 2 mg/kg)

- MERCURY, Mercury Limit Test, Method II, Appendix IIIB

 parfait

Sample solution: Prepare as directed using 3 g of sample.

Acceptance criteria: NMT 1 mg/kg

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate

Expert Committee: (FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 369

Phone Number: 1-301-816-8288
Ferrous Sulfate, Dried, FCC 6 page 370. On the basis of comments received, it is proposed to revise the Mercury test by adding instructions for preparation of the Sample solution for this test. Preparation of the sample using the current instructions contained in Mercury Limit Test, Method II, Appendix IIIIB does not allow the analyst to test to the limit specified in this monograph.

(FIEC: K. Bowman) C65275

Ferrous Sulfate, Dried
FeSO$_4$·xH$_2$O

Formula wt, anhydrous 151.91
CAS: [7720-78-7]

DESCRIPTION
Ferrous Sulfate, Dried occurs as a gray-white to buff colored powder consisting primarily of FeSO$_4$·H$_2$O, with varying amounts of FeSO$_4$·4H$_2$O. It dissolves slowly in water, but is insoluble in alcohol.

Function Nutrient

Packaging and Storage Store in tight containers.

IDENTIFICATION
- Iron (Ferrous Salts), Appendix III
  Acceptance criteria: Passes tests.
- Sulfate, Appendix IIIA
  Acceptance criteria: Passes tests.

ASSAY
- Procedure
  Sample: 1 g
  Analysis: Dissolve the Sample in a mixture of 25 mL of 2 N sulfuric acid and 25 mL of recently boiled and cooled water. Titrate with 0.1 N ceric sulfate, using orthophenanthroline TS, as the indicator. Perform a blank determination (see General Provisions), and make any necessary correction. Each mL of 0.1 N ceric sulfate is equivalent to 15.19 mg of FeSO$_4$.
  Acceptance criteria: NLT 86.0% and NMT 89.0% FeSO$_4$

IMPURITIES

Change to read:
Inorganic Impurities
- 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 in water
**Trioctylphosphine oxide solution:** 50 mg/mL of trioctylphosphine oxide in 4-methyl-2-pentanone

[CAUTION: This reagent 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:** 100 µg/mL of lead prepared as follows: 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 to volume with water.

**Standard solution:** Transfer 1.0 mL of Standard stock solution into a 100-mL volumetric flask, dilute to volume with water, 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 layer to separate. The organic solvent layer is the Standard solution and contains 0.4 µg/mL of lead.

**Blank solution:** 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 layer to separate. The organic solvent layer remaining is the Blank solution and contains 0.0 µg/mL of lead.

**Sample solution:** Add 1.0 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 solution.

**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 solution, the Standard solution, and the Sample solution.

**Acceptance criteria:** The absorbance of the Blank solution is NMT 20% of the difference between the absorbance of the Standard solution and the absorbance of the Blank solution. The absorbance of the Sample solution does not exceed that of the Standard solution. (NMT 2 mg/kg)

**Mercury, Mercury Limit Test, Method II, Appendix IIIB**

**Sample solution:** Prepare as directed using 3 g of sample.

**Acceptance criteria:** NMT 1 mg/kg

### SPECIFIC TESTS

**Insoluble Residue**

**Sample:** 2 g

**Analysis:** Dissolve the Sample in 20 mL of freshly boiled 1:100 sulfuric acid, heat to boiling, and then digest in a covered beaker on a steam bath for 1 h. Filter through a tared filtering crucible, wash thoroughly, and dry at 105°.

**Acceptance criteria:** The weight of the insoluble residue is NMT 1 mg. (NMT 0.05%)
**BRIEFING**

**Food Starch, Modified, FCC 6 page 374—See briefing under Food Starch, Unmodified.** Interested parties are encouraged to submit comments to Jeff Moore, Ph.D., email jm@usp.org.

(FIEC: J. Moore) C65041

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**Food Starch, Modified**

Modified Food Starch

Food Starch–Modified

**DESCRIPTION**

Food Starch, Modified usually occurs as white or nearly white powders; as intact granules; and if pregelatinized (that is, subjected to heat treatment in the presence of water), as flakes, amorphous powders, or coarse particles. Modified food starches are products of the treatment of any of several grain- or root-based native starches (for example, corn, sorghum, wheat, potato, tapioca, and sago), with small amounts of certain chemical agents that modify the physical characteristics of the native starches to produce desirable properties.

Starch molecules are polymers of anhydroglucose and occur in both linear and branched form. The degree of polymerization and, accordingly, the molecular weight of the naturally occurring starch molecules vary radically. Furthermore, they vary in the ratio of branched-chain polymers (amylopectin) to linear-chain polymers (amylose), both within a given type of starch and from one type to another. These factors, in addition to any type of chemical modification used, significantly affect the viscosity, texture, and stability of the starch sols.

Starch is chemically modified by mild degradation reactions or by reactions between the hydroxyl groups of the native starch and the reactant selected. One or more of the following processes are used: mild oxidation (bleaching), moderate oxidation, acid and/or enzyme depolymerization, monofunctional esterification, polyfunctional esterification (cross-linking), monofunctional etherification, alkaline gelatinization, and certain combinations of these treatments. These methods of preparation can be used as a basis for classifying the starches thus produced (see Other Requirements, below). Generally, however, the products are called Modified Food Starch, or Food Starch-Modified.

Modified food starches are insoluble in alcohol, in ether, and in chloroform. If not pregelatinized, they are practically insoluble in cold water. Upon heating in water, the granules usually begin to swell at temperatures between 45° and 80°, depending on the botanical origin and the degree of modification. They gelatinize completely at higher temperatures. Pregelatinized starches hydrate in cold water.

**Function** Thickener; colloidal stabilizer; binder

**Packaging and Storage** Store in well-closed containers.

**IDENTIFICATION**

- **Iodine Stain**
  
  **Sample suspension:** 1 g in 20 mL of water
  
  **Analysis:** To the Sample suspension, add a few drops of iodine TS.
  
  **Acceptance criteria:** A dark blue to red color appears.

- **Copper Reduction**
  
  **Sample:** 2.5 g
  
  **Analysis:** Place the Sample in a boiling flask, add 10 mL of 3% hydrochloric acid and 70 mL water, mix, reflux for about 3 h, and cool. Add 0.5 mL of the resulting solution to 5 mL of hot alkaline cupric tartrate TS.
Acceptance criteria: A copious red precipitate forms.

**Microscopy**

**Analysis:** Examine a portion of the sample with a polarizing microscope in polarized light under crossed Nicol prisms.

**Acceptance criteria:** The typical polarization cross is observed, except in the case of pregelatinized starches.

**Impurities**

**Inorganic Impurities**

- **Lead,**  *Lead Limit Test, Flame Atomic Absorption Spectrophotometric Method,* Appendix IIIB
  
  **Sample:** 5 g

  **Acceptance criteria:** NMT 1 mg/kg

- **Sulfur Dioxide,** Appendix X

  **Acceptance criteria:** NMT 0.005%

**Specific Tests**

- **Crude Fat,** Appendix X

  **Acceptance criteria:** NMT 0.15%

- **Loss on Drying,** Appendix IIC (120 °C for 4 h, in a vacuum oven not exceeding 100 mm Hg)

  **Sample:** 5 g

  **Acceptance criteria**

  - Cereal starch: NMT 15.0%
  - Potato starch: NMT 21.0%
  - Sago starch: NMT 18.0%
  - Tapioca starch: NMT 18.0%

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

  **Sample suspension:** Mix 20 g of sample with 80 mL of water and agitate continuously at a moderate rate for 5 min. For pregelatinized starches, suspend 3 g of sample in 97 mL of water. 

  **Acceptance criteria:** Between 3.0 and 9.0

*Change to read:*

- **Protein**

  **Sample:** 10 g

  **Analysis:** Transfer the sample into an 800-mL Kjeldahl flask, and add 10 g of anhydrous potassium sulfate or anhydrous sodium sulfate, 300 mg of copper selenite or mercuric oxide, and 60 mL of sulfuric acid.

  Gently heat the mixture, keeping the flask inclined at about a 45° angle, and after frothing has ceased, boil briskly until the solution remains clear for about 1 h. Cool the solution, add 30 mL of water, mix, and cool again. Cautiously pour about 75 mL (or enough to make the mixture strongly alkaline) of a 2:5 aqueous solution of sodium hydroxide down the inside of the flask so that it forms a layer under the acid solution; then add a few pieces of granular zinc. 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 an accurately measured excess of 0.1 N sulfuric acid contained in a 50-mL-500-mL flask. Gently rotate the contents of the Kjeldahl flask to mix and then distill until all ammonia has passed into the absorbing acid solution (about 250 mL of distillate). Titrate the excess acid
with 0.1 N sodium hydroxide, using 0.25 mL of methyl red and methylene blue TS as the indicator. Perform a blank determination (see General Provisions), substituting pure sucrose or dextrose for the sample, and make any necessary correction. Each mL of 0.1 N sulfuric acid consumed is equivalent to 1.401 mg of nitrogen.

Calculate the percent of nitrogen in the sample, and then calculate the percent of protein in starches obtained from corn by multiplying the percent of nitrogen by 6.25, or in starches obtained from wheat, by 5.7. Other factors may be applied as necessary for starches obtained from other sources.

Acceptance criteria: NMT 0.5%; except in modified high-amylose starches, NMT 1%

OTHER REQUIREMENTS

• **LABELING** Indicate the presence of sulfur dioxide if the residual concentration is greater than 10 mg/kg.

  [NOTE: The modified food starches listed below according to method of preparation must meet all of the above requirements under Identification, Impurities, and Specific Tests, in addition to any requirements for Residuals Limitation listed in the tables below. The maximum limits for reagents appearing in the Treatment column are for information only and pertain to the requirements of the U.S. Food and Drug Administration (see Title 21 of the U.S. Code of Federal Regulations, Part 172 (21 CFR 172.892: Food Starch, Modified)). For treatments for which a maximum reagent limit is not specified, the amount of reagent used should not exceed that reasonably required to accomplish the intended modification.]

• **TESTS**

  The following Tests apply to those entries as specifically cited under Residuals Limitation in the tables below:

  • **ACETYL GROUPS**, Appendix X
  • **MANGANESE**, Appendix IIIA
  • **PHOSPHATE**, Phosphorus, Appendix IIIA
  • **PROPYLENE CHLOROHYDRIN**, Appendix X

• **ALKALINE GELATINIZATION (GELATINIZED STARCH)**

<table>
<thead>
<tr>
<th>Treatment to Produce Gelatinized Starch</th>
<th>Residuals Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hydroxide, not to exceed 1%</td>
<td>—</td>
</tr>
</tbody>
</table>

• **DEPOLYMERIZATION (THIN-BOILING, OR ACID-MODIFIED STARCH)** This treatment results in partial depolymerization, causing a reduction in viscosity.

<table>
<thead>
<tr>
<th>Treatment to Produce Thin-Boiling Starch</th>
<th>Residuals Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrochloric acid and/or sulfuric acid</td>
<td>—</td>
</tr>
<tr>
<td>Alpha-amylase enzyme</td>
<td>The resulting nonsweet nutritive saccharide polymer has a dextrose equivalent of less than 20</td>
</tr>
</tbody>
</table>

• **ETHERIFICATION AND ESTERIFICATION (STARCH ETHER-ESTERS)**

<table>
<thead>
<tr>
<th>Treatment to Produce Hydroxypropyl Distarch Phosphate</th>
<th>Residuals Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorus oxychloride, not to exceed 0.1%; propylene oxide, not to exceed 10%</td>
<td>NMT 3 mg/kg of propylene chlorohydrin</td>
</tr>
</tbody>
</table>

• **ETHERIFICATION WITH OXIDATION (OXIDIZED STARCH ETHERS)**
## Treatment to Produce Oxidized Hydroxypropyl Phosphate Starch

<table>
<thead>
<tr>
<th>Residuals Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorine, as sodium hypochlorite, not to exceed 0.055 lb. (25 g) of chlorine per lb. (454 g) of dry starch; active oxygen obtained from hydrogen peroxide, not to exceed 0.45%; and propylene oxide, not to exceed 25%</td>
</tr>
<tr>
<td>NMT 1 mg/kg of propylene chlorohydrin</td>
</tr>
</tbody>
</table>

### Residuals Limitation
- **Mild Oxidation (Bleached Starch)** The starches resulting from mild oxidation are not altered chemically; in all cases, extraneous color bodies are oxidized, solubilized, and removed by washing and filtration.

### Treatment to Produce Bleached Starch

<table>
<thead>
<tr>
<th>Residuals Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active oxygen obtained from hydrogen peroxide, and/or peracetic acid, not to exceed 0.45% of active oxygen</td>
</tr>
<tr>
<td>Ammonium persulfate, not to exceed 0.075%, and sulfur dioxide, not to exceed 0.05%</td>
</tr>
<tr>
<td>Chlorine, as sodium hypochlorite, not to exceed 0.0082 lb. (3.72 g) of chlorine per lb. (454 g) of dry starch</td>
</tr>
<tr>
<td>Chlorine, as calcium hypochlorite, not to exceed 0.036% of dry starch</td>
</tr>
<tr>
<td>Potassium permanganate, not to exceed 0.2%</td>
</tr>
<tr>
<td>Sodium chlorite, not to exceed 0.5%</td>
</tr>
</tbody>
</table>

### Treatment to Produce Oxidized Starch

<table>
<thead>
<tr>
<th>Residuals Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorine, as sodium hypochlorite, not to exceed 0.055 lb. (25 g) of chlorine per lb. (454 g) of dry starch</td>
</tr>
<tr>
<td>NMT 1 mg/kg of propylene chlorohydrin</td>
</tr>
</tbody>
</table>

### Treatment to Produce Starch Acetate

<table>
<thead>
<tr>
<th>Residuals Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic anhydride of vinyl acetate</td>
</tr>
<tr>
<td>NMT 2.5% of acetyl groups introduced into finished product</td>
</tr>
</tbody>
</table>

### Residuals Limitation
- **Moderate Oxidation (Oxidized Starch)** The maximum specified treatment introduces about 1 carboxyl group per 28 anhydroglucose units. The starch is whitened, and its molecular weight and viscosity are reduced.

### Treatment to Produce Oxidized Starch

<table>
<thead>
<tr>
<th>Residuals Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorine, as sodium hypochlorite, not to exceed 0.055 lb. (25 g) of chlorine per lb. (454 g) of dry starch</td>
</tr>
<tr>
<td>NMT 1 mg/kg of propylene chlorohydrin</td>
</tr>
</tbody>
</table>

### Treatment to Produce Starch Esterification (Starch Esters)
The starch esters are named individually, depending on the method of preparation.
<table>
<thead>
<tr>
<th>Treatment to Produce Acetylated Distarch Adipate</th>
<th>Residuals Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipic anhydride, not to exceed 0.12%, and acetic anhydride</td>
<td>NMT 2.5% of acetyl groups introduced into finished product</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment to Produce Starch Phosphate</th>
<th>Residuals Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monosodium orthophosphate</td>
<td>NMT 0.4% of phosphate (calculated as P)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment to Produce Starch Octenyl Succinate</th>
<th>Residuals Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octenyl succinic anhydride, not to exceed 3%, followed by treatment with beta-amylase enzyme</td>
<td>—</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment to Produce Starch Sodium Octenyl Succinate</th>
<th>Residuals Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octenyl succinic anhydride, not to exceed 3%</td>
<td>—</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment to Produce Starch Aluminium Octenyl Succinate</th>
<th>Residuals Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octenyl succinic anhydride, not to exceed 2%, and aluminum sulfate, not to exceed 2%</td>
<td>—</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment to Produce Distarch Phosphate</th>
<th>Residuals Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorus oxychloride, not to exceed 0.1%</td>
<td>—</td>
</tr>
<tr>
<td>Sodium trimetaphosphate</td>
<td>NMT 0.04% of phosphate (calculated as P)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment to Produce Phosphated Distarch Phosphate</th>
<th>Residuals Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium tripolyphosphate and sodium trimetaphosphate</td>
<td>NMT 0.4% of phosphate (calculated as P)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment to Produce Acetylated Distarch Phosphate</th>
<th>Residuals Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorus oxychloride, not to exceed 0.1%, followed by either acetic anhydride, not to exceed 8%, or vinyl acetate, not to exceed 7.5%</td>
<td>NMT 2.5% of acetyl groups introduced into finished product</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment to Produce Starch Sodium Succinate</th>
<th>Residuals Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinic anhydride, not to exceed 4%</td>
<td>—</td>
</tr>
</tbody>
</table>

**MONOFUNCTIONAL ETERIFICATION**

<table>
<thead>
<tr>
<th>Treatment to Produce Hydroxypropyl Starch</th>
<th>Residuals Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propylene oxide, not to exceed 25%</td>
<td>NMT 1 mg/kg of propylene chlorohydrin</td>
</tr>
</tbody>
</table>

**Auxiliary Information**— *Staff Liaison*: Jeffrey Moore, Ph.D., Senior Scientific Associate
BRIEFING

Food Starch, Unmodified, FCC 6 page 376; Food Starch, Modified, FCC 6 page 374. On the basis on comments received, it is proposed in the Protein test procedure to revise the water reagent and distillation receiver flask volumes to be consistent with those appearing in FCC 2 page 329. Interested parties are encouraged to submit comments to Jeff Moore, Ph.D., email jm@usp.org.

Food Starch, Unmodified

DESCRIPTION

Food Starch, Unmodified occurs as white or nearly white powders; as intact granules; and if pregelatinized, as flakes, powders, or coarse particles. Food starches are extracted from any of several grain or root crops, including corn (maize), sorghum, wheat, potato, tapioca, sago, and arrowroot and hybrids of these crops such as waxy maize and high-amylose maize. They are chemically composed of one or a mixture of two glucose polysaccharides (amylose and amylopectin), the composition and relative proportions of which are characteristic of the plant source. Food starches are generally produced by extraction from the plant source using wet-milling processes in which the starch is liberated by grinding aqueous slurries of the raw material. The extracted starch may be subjected to other nonchemical treatments such as purification, extraction, physical treatments, dehydration, heating, and minor pH adjustment during further processing steps. Food starch may be pregelatinized by heat treatment in the presence of water or made cold-water swelling.

Food starches are insoluble in alcohol, in ether, and in chloroform. If they are not treated to be pregelatinized or cold-water swelling, then they are practically insoluble in cold water. Pregelatinized and cold-water swelling starches hydrate in cold water. When heated in water, the granules usually begin to swell at temperatures between 45° and 80°, depending on the botanical origin of the starch. They gelatinize completely at higher temperatures.

Function Thickener; colloidal stabilizer; binder.

Packaging and Storage Store in well-closed containers.

IDENTIFICATION

• A. Procedure
  
  Sample: 1 g
  
  Analysis: Suspend the Sample in 20 mL of water and add a few drops of iodine TS.
  
  Acceptance criteria: A dark blue to red color appears.

• B. Procedure
  
  Sample: 2.5 g
  
  Analysis: Place the Sample in a boiling flask, add 10 mL of 3% hydrochloric acid and 70 mL of water, mix, reflux for about 3 h, and cool. Add 0.5 mL of the resulting solution to 5 mL of hot alkaline cupric tartrate TS.
  
  Acceptance criteria: A copious, red precipitate forms.

• C. Microscopy
  
  Analysis: Examine a portion of sample with a polarizing microscope in polarized light under crossed Nicol prisms.
  
  Acceptance criteria: The typical polarization cross is observed, except in the case of pregelatinized starches.

IMPURITIES
Inorganic Impurities

  
  **Acceptance criteria:** NMT 1 mg/kg

- **Sulfur Dioxide**, *Sulfur Dioxide Determination*, Appendix X

  **Sample:** 25 g

  **Acceptance criteria:** NMT 0.005%

SPECIFIC TESTS

- **Crude Fat**, Appendix X
  
  **Acceptance criteria:** NMT 0.15%

- **Loss on Drying**, Appendix IIC (120° for 4 h in a vacuum oven not exceeding 100 mm Hg)

  **Sample:** 5 g

  **Acceptance criteria**
  - Cereal starch: NMT 15.0%
  - Potato starch: NMT 21.0%
  - Sago and Tapioca starch: NMT 18.0%

- **pH of Dispersions**, *pH Determination*, Appendix IIB

  **Sample:** 20 g (Use 3 g for pregelatinized starches.)

  **Analysis:** Mix the Sample with 80 mL of water (Use 97 mL for pregelatinized starches.) and agitate the suspension continuously at a moderate rate for 5 min. [NOTE: The water used for sample dispersion should not require more than 0.05 mL of 0.1 N acid or alkali per 200 mL of sample to obtain the methyl red or phenolphthalein endpoint, respectively.]

  **Acceptance criteria:** Between 3.0 and 9.0

*Change to read:*

- **Protein**

  **Sample:** 10 g

  **Analysis:** Transfer the Sample into an 800-mL Kjeldahl flask. Add 10 g of anhydrous potassium sulfate or anhydrous sodium sulfate, 300 mg of copper selenite or mercuric oxide, and 60 mL of sulfuric acid. Gently heat the mixture, keeping the Kjeldahl flask inclined at about a 45° angle and, after frothing has ceased, boil briskly until the solution remains clear for about 1 h. Cool, add 30 mL of water, mix, and cool again. Cautiously pour about 75 mL (or enough to make the mixture strongly alkaline) of a 2 : 5 aqueous solution of sodium hydroxide down the inside of the flask so that it forms a layer under the acid solution, and then add a few pieces of granular zinc. 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 an accurately measured excess of 0.1 N sulfuric acid contained in a 50-mL flask. Gently rotate the contents of the Kjeldahl flask to mix, and distill until all ammonia has passed into the absorbing acid solution (about 250 mL of distillate). Titrate the excess acid with 0.1 N sodium hydroxide, using 0.25 mL of methyl red and methylene blue TS as the indicator. Perform a blank determination (see General Provisions), substituting pure sucrose or dextrose for the sample and make any necessary correction. Each mL of 0.1 N sulfuric acid consumed is equivalent to 1.401 mg of nitrogen. Calculate the percent nitrogen in the sample, and then calculate the percent protein in starches obtained from corn by multiplying the percent of nitrogen by 6.25, or in starches obtained from wheat, by 5.7.

  **Acceptance criteria:** NMT 0.5%; except in high-amylose and other hybrid starches, NMT 1%
OTHER REQUIREMENTS
• **Labeling** Indicate the presence of sulfur dioxide if the residual concentration is greater than 10 mg/kg (0.001%).

**Auxiliary Information**— *Staff Liaison:* Jeffrey Moore, Ph.D., Senior Scientific Associate  
*Expert Committee:* (FI07) Food Ingredients Expert Committee  
*FCC Sixth Edition Page 376*  
*Phone Number:* 1-301-816-8288
Fructooligosaccharides, Short Chain, FCC 6, page 378. On the basis of comments received, it is proposed to revise the Description to remove references to fructooligosaccharide materials with a degree of polymerization (DP) above four which are not supported by the current Assay test procedure. It is also proposed based on comments received to revise the method of manufacture description to replace the term “synthesized” with “produced,” which more accurately reflects the nature of the fermentation process used to produce scFOS’s with DP’s of 2–4 from sucrose. Interested parties are encouraged to submit comments on this proposal as well as improved methods which may accommodate fructooligosaccharide with DP’s above four, to Jeff Moore, Ph.D. at jm@usp.org. (FIEC: J. Moore) C58608

Fructooligosaccharides, Short Chain
csFOS

**DESCRIPTION**

*Change to read:* Fructooligosaccharides, Short Chain (scFOS), are indigestible carbohydrates synthesized from sucrose and fructose through an enzymatic process or from Inulin by partial enzymatic hydrolysis. These carbohydrates are a mixture of polysaccharides consisting of a sucrose molecule (glucose-fructose disaccharide, GF₃) linked to one (GF₂), two (GF₃), or three (GF₄) additional fructose units added by β-1 glycosidic linkages to the fructose unit of sucrose for the synthesized scFOS. The scFOS from Inulin consists of oligosaccharides with the same structure but a slightly larger range of polymerization (from GF₂ to GF₃ and from F₂ to F₃). They are very soluble in hot and cold water, and almost insoluble in most organic solvents.

**Function** Bulking agent; source of dietary fiber; sweetener; prebiotic

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

**IDENTIFICATION**

- **Procedure**
  - **Acetate buffer (pH 4.5 ± 0.05):** Transfer 22 mL of 0.2 M sodium acetate and 28 mL of 0.2 M acetic acid into a 100-mL volumetric flask, and dilute to volume with water.
  - **Standard solution:** 3.0 mg/mL of GF₂, 4.5 mg/mL of GF₃, 0.50 mg/mL of GF₄ scFOS Reference Standards
Sample stock solution: 10 mg/mL using a sample previously dried to constant weight

Digested sample solution: Transfer 10 mL of Acetate buffer and 10 mL of the Sample stock solution into a 25-mL volumetric flask. Add 150 units of Fructozyme SP230 enzyme (Novozymes, Denmark), or equivalent. Digest for 30 min at 60°C, cool, and dilute to volume with water.

Mobile phase: Acetonitrile–water (65–70% to 35–30%)

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography

Detector: Refractive index  [Note: Use a detector with a sensitivity of $8 \times 10^{-5}$.

Column: 25-cm × 4-mm (id) 5-µm LiChrospher 100 NH2 column (Merck Corp.), or equivalent

Column temperature: 35°C

Flow rate: 1 mL/min

Run time: 12 min

Injection volume: 20 µL

Sample loop: 20 µL

Analysis: Separately inject the Digested sample solution and the Standard solution into the chromatograph and record the chromatograms. Determine the percentage of fructose and the percentage of glucose in the Digested sample solution using the following formula:

$$100 \left( \frac{C_{ST} \times A_{SA}}{A_{ST} \times W} \right)$$

$C_{ST} =$ Concentration of fructose or glucose in the Standard solution (mg/100 mL)

$A_{SA} =$ Area of the corresponding sugar peak in the chromatogram of the Digested sample solution

$A_{ST} =$ Area of the corresponding sugar peak in the chromatogram of the Standard solution

$W =$ Weight of sample (g) contained in each 100 mL of the Sample stock solution

Correct the percent fructose and percent glucose results for the mono- and disaccharide content (obtained in the Assay below), and for moisture.

Acceptance criteria: The sample releases greater than 67% fructose and less than 33% glucose upon enzymatic digestion.

ASSAY

• Procedure

Mobile phase: Acetonitrile–water (65–70% to 35–30%)

Standard solution: 3.0 mg/mL of GF$_2$, 4.5 mg/mL of GF$_3$, 0.50 mg/mL of GF$_4$ scFOS Reference Standards (Waco Pure Chemical Industries, Ltd., Osaka, Japan, or equivalent) and 0.50 mg/mL each of fructose, glucose, and sucrose in water

Sample stock solution: 10 mg/mL using a sample previously dried to constant weight

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography

Detector: Refractive index  [Note: Use a detector with a sensitivity of $8 \times 10^{-5}$.

Column: 25-cm × 4-mm (id) 5-µm LiChrospher 100 NH2 column (Merck Corp.), or equivalent
Column temperature: 35°C
Flow rate: 1 mL/min
Run time: 12 min
Injection volume: 20 µL
Sample loop: 20 µL

Analysis: Separately inject the Sample solution and the Standard solution into the chromatograph and record the area responses for each scFOS. Calculate the percentage of each scFOS, from trimers to nonamers, in the sample taken using the formula:

\[100 \times \frac{C_{ST} \times A_{SA}}{A_{ST} \times W}\]

\(C_{ST}\) = Concentration of the scFOS of interest in the Standard solution (mg/100 mL)
\(A_{SA}\) = Area of the corresponding sugar peak in the chromatogram of the Sample solution
\(A_{ST}\) = Area of the corresponding sugar peak in the chromatogram of the Standard solution (for oligomers without a specific standard, use the average area response of the peaks of the standards)
\(W\) = Weight of sample (g) contained in each 100 mL of the Sample solution

Calculate the total percentage of scFOS in the sample by adding the individual percentages of each scFOS, from trimers to nonamers.

Acceptance criteria: NLT 85.0% scFOS (≥ 30.0% trimer, ≥ 45.0% tetramer, and ≥ 5.0% pentamer and larger), with the remainder being glucose, fructose, and sucrose, 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

SPECIFIC TESTS
- Residue on Ignition (Sulfated Ash), Appendix IIC
  Analysis: Ignite sample at 525°C for 2 h.
  Acceptance criteria: NMT 0.1%
- Total Solids, Water Determination, Karl Fischer Titrimetric Method, Appendix IIB
  Analysis: Calculate the percent Total Solids by the formula:

\[(W_U - W_W) \times 100/W_U\]

\(W_U\) = Weight of the sample taken (mg)
\(W_W\) = Weight of the water determined (mg)
Acceptance criteria: NLT 95.0%

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
FCC Sixth Edition Page 378
Phone Number: 1-301-816-8288
Furfural, *FCC 6* page 383—See briefing under *Acetaldehyde*.
(FIEC: J. Moore) C64747

Furfural

2-Furaldehyde

Pyromucic Aldehyde

![Pyromucic Aldehyde](image)

\[ \text{C}_5\text{H}_4\text{O}_2 \]

Formula wt 96.09
FEMA: 2489

**DESCRIPTION**

*Change to read:*

Furfural occurs as a colorless to yellow oily liquid that turns red-brown on long storage. It may contain a suitable antioxidant. 1S (FCC 6)

**Odor** Sweet, bready

**Solubility** Soluble in vegetable oils; slightly soluble in propylene glycol, water

**Boiling Point** $-162^\circ$

**Solubility in Alcohol**, Appendix VI

One mL dissolves in 1 mL of 95% ethanol.

**Function** Flavoring agent

**IDENTIFICATION**

- **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.
ASSAY
- **Procedure** Proceed as directed under M-1b, Appendix XI.
  - **Acceptance criteria:** NLT 96.0% of \( \text{C}_5\text{H}_4\text{O}_2 \)

SPECIFIC TESTS
- **ACID VALUE, M-15, Appendix XI**
  - **Acceptance criteria:** NMT 1.0
- **REFRACTIVE INDEX, Appendix II (at 20°)**
  - **Acceptance criteria:** Between 1.522 and 1.528
- **Specific Gravity** Determine at 25° by any reliable method (see General Provisions).
  - **Acceptance criteria:** Between 1.154 and 1.158

**Auxiliary Information** — **Staff Liaison:** Kristie Bowman, Senior Scientific Associate
**Expert Committee:** (FI07) Food Ingredients Expert Committee
**FCC Sixth Edition Page 383**
**Phone Number:** 1-301-816-8356
BRIEFING

Gum Arabic, *FCC 6* page 425. On the basis of comments and data received, it is proposed to increase the ignition temperature used in the test procedure for Ash (Total) from the General Test procedure default of “about 550°” to 675 ± 25°. This higher temperature is consistent with that used in the same test procedure for the NF 25 Acacia monograph. It would also shorten the time to constant weight for this test procedure, a potential benefit to the analyst. Interested parties are encouraged to submit comments to Jeff Moore, Ph.D., at jm@usp.org.

(FIEC: J. Moore) C62877

Gum Arabic

Acacia

INS: 414

CAS: [9000-01-5]

DESCRIPTION

Gum Arabic occurs as a dried, gummy exudation obtained from the stems and branches of *Acacia senegal* (L.) Willdenow or of related species of *Acacia* (Fam. Leguminosae). The unground product occurs as white or yellow-white, spheroidal tears of varying size or in angular fragments. It is also available commercially as white to yellow-white flakes, granules, or powder. One g dissolves in 2 mL of water, forming a solution that flows readily and is acid to litmus. It is insoluble in alcohol.

Function Stabilizer; emulsifier

Packaging and Storage Store in well-closed containers.

IDENTIFICATION

• PROCEDURE
  
  Sample solution: 20 mg/mL
  
  Analysis: Add 0.2 mL of diluted lead subacetate TS to 10 mL of cold Sample solution.
  
  Acceptance criteria: A flocculent or curdy, white precipitate forms immediately.

IMPURITIES

Inorganic Impurities

• ARSENIC, Arsenic Limit Test, Appendix IIIB
  
  Sample solution: Prepare as directed for organic compounds.
  
  Acceptance criteria: NMT 3 mg/kg

• LEAD, Lead Limit Test, Appendix IIIB
  
  Sample solution: Prepare as directed for organic compounds.
  
  Control: 5 µg Pb (5 mL of Diluted Standard Lead Solution)
  
  Acceptance criteria: NMT 5 mg/kg

SPECIFIC TESTS

• ASH (ACID-INSOLUBLE), Appendix IIC
  
  Acceptance criteria: NMT 0.5%

Change to read:
• **Ash (Total), Appendix IIC**
  
  **Analysis:** Proceed as directed, but ignite at 675 ± 25°C. 1S (FCC 6)
  
  **Acceptance criteria:** NMT 4.0%

• **Insoluble Matter**
  
  **Sample:** 5 g
  
  **Analysis:** Dissolve the Sample in about 100 mL of water contained in a 250-mL Erlenmeyer flask, add 10 mL of 2.7 N hydrochloric acid and boil gently for 15 min. Filter the hot solution by suction through a tared filtering crucible and wash the residue thoroughly with hot water. Dry the residue at 105°C for 2 h, and weigh.
  
  **Acceptance criteria:** NMT 1.0%

• **Loss on Drying, Appendix IIC (105°C for 5 h)**
  
  [NOTE: Powder unground samples sufficiently to pass through a No. 40 sieve, and mix well before weighing and drying.]
  
  **Acceptance criteria:** NMT 15.0%

• **Starch or Dextrin**
  
  **Sample solution:** 20 mg/mL
  
  **Analysis:** Boil the Sample solution, cool, and add a few drops of iodine TS.
  
  **Acceptance criteria:** No blue or red color appears.

• **Tannin-Bearing Gums**
  
  **Sample solution:** 20 mg/mL
  
  **Analysis:** Add about 0.1 mL of ferric chloride TS to 10 mL of Sample solution.
  
  **Acceptance criteria:** No black coloration or precipitate forms.

**Auxiliary Information**— *Staff Liaison:* Jeffrey Moore, Ph.D., Senior Scientific Associate

*Expert Committee:* (FI07) Food Ingredients Expert Committee

*FCC Sixth Edition Page 425*

*Phone Number:* 1-301-816-8288
BRIEFING

(E),(E)-2,4-Heptadienal, FCC 6 page 428—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

(E),(E)-2,4-Heptadienal

trans,trans-2,4-Heptadienal

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{O} \\
\text{C}_7\text{H}_{10}\text{O} & \\
\text{Formula wt} 110.16 & \text{FEMA: 3164}
\end{align*}
\]

DESCRIPTION

**Change to read:**

(E),(E)-2,4-Heptadienal occurs as a slightly yellow liquid. It may contain a suitable antioxidant.

**Odor** Fatty, green

**Solubility** Soluble in alcohol, most fixed oils; insoluble or practically insoluble in water

**Boiling Point** \( \sim 100^\circ \text{C} \) (35 mm Hg)

**Solubility in Alcohol**, Appendix VI

One mL dissolves in 1 mL of 95% ethanol.

**Function** Flavoring agent

IDENTIFICATION

- **INFRARED SPECTRA**, *Spectrophotometric Identification Tests, Appendix III C*

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

  ![Infrared Spectrum](image)

  \((E),(E)-2,4-\text{Heptadienal}\)

ASSAY

- **Procedure** Proceed as directed under M-1a, Appendix XI

  **Acceptance criteria:** NLT 92.0% of \( \text{C}_7\text{H}_{10}\text{O} \) (sum of isomers)

SPECIFIC TESTS

- **REFRACTIVE INDEX**, Appendix II (at \(20^\circ\))
Acceptance criteria: Between 1.531 and 1.537

- **Specific Gravity** Determine at 25° by any reliable method (see General Provisions).
  
  Acceptance criteria: Between 0.878 and 0.888

Auxiliary Information—Staff Liaison: Kristie Bowman, Senior Scientific Associate

Expert Committee: (FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 428

Phone Number: 1-301-816-8356
Heptanal, FCC 6 page 430—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

Heptanal
Aldehyde C-7

Heptaldehyde

\[
\text{C}_7\text{H}_{14}\text{O}
\]

Formula wt 114.19
FEMA: 2540

DESCRIPTION

Change to read:
Heptanal occurs as a colorless to slightly yellow liquid. It may contain a suitable antioxidant. \(1S\) (FCC 6)

Odor Penetrating, oily

Solubility Slightly soluble in water; miscible in alcohol, ether, most fixed oils

Boiling Point \(153^\circ\)

Solubility in Alcohol, Appendix VI
One mL dissolves in 2 mL of 70% alcohol to give a clear solution.

Function Flavoring agent

IDENTIFICATION

• 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.

ASSAY

• Procedure Proceed as directed under M-1b, Appendix XI.
Acceptance criteria: NLT 92.0% of C$_7$H$_{14}$O

SPECIFIC TESTS

• ACID VALUE, M-15, Appendix XI
  Acceptance criteria: NMT 10.0

• REFRACTIVE INDEX, Appendix II (at 20°)
  Acceptance criteria: Between 1.412 and 1.420

• Specific Gravity Determine at 25° by any reliable method (see General Provisions).
  Acceptance criteria: Between 0.815 and 0.820

Auxiliary Information—Staff Liaison: Kristie Bowman, Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
FCC Sixth Edition Page 430
Phone Number: 1-301-816-8356
**BRIEFING**

2,3-Heptandione, *FCC 6* page 431—See briefing under *Acetaldehyde*.
(FIEC: J. Moore) C64747

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**2,3-Heptandione**

Acetyl Valeryl

\[
\text{C}_7\text{H}_{12}\text{O}_2
\]

*Formula wt 128.17  
FEMA: 2543*

**DESCRIPTION**

*Change to read:*

2,3-Heptandione occurs as a yellow liquid. ■ It may contain a suitable antioxidant. ■ 1S (*FCC 6*)

**Odor** Butter, cheese

**Solubility** Soluble in propylene glycol, vegetable oils; insoluble or practically insoluble in water

**Boiling Point** $-64\degree$ (18 mm Hg)

**Solubility in Alcohol, Appendix VI**

One mL dissolves in 1 mL of 95% ethanol.

**Function** Flavoring agent

**IDENTIFICATION**

- **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.

---

**ASSAY**

- **Procedure** Proceed as directed under *M-1b, Appendix XI*. 
Acceptance criteria: NLT 97.0% of C$_7$H$_{12}$O$_2$

SPECIFIC TESTS

- **REFRACTIVE INDEX**, Appendix II (at 20°)
  
  Acceptance criteria: Between 1.411 and 1.418

- **Specific Gravity** Determine at 25° by any reliable method (see General Provisions).
  
  Acceptance criteria: Between 0.916 and 0.923

Auxiliary Information—Staff Liaison: Kristie Bowman, Senior Scientific Associate

Expert Committee: (FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 431

Phone Number: 1-301-816-8356
Hexanal, FCC 6 page 438—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

Hexanal

Caproic Aldehyde

Hexaldehyde

Aldehyde C-6

\[ \text{C}_6\text{H}_{12}\text{O} \]

Formulas wt 100.16
FEMA: 2557

DESCRIPTION

Change to read:
Hexanal occurs as an almost colorless liquid. It may contain a suitable antioxidant. 1S (FCC 6)

Odor Fatty-green, grassy
Solubility Very slightly soluble in water; miscible in alcohol, propylene glycol, most fixed oils
Boiling Point \( -131^\circ \)
Function Flavoring agent

ASSAY

• Procedure Proceed as directed under M-1a, Appendix XI.
  Acceptance criteria: NLT 97.0% of \text{C}_6\text{H}_{12}\text{O}

SPECIFIC TESTS

• ACID VALUE, M-15, Appendix XI
  Acceptance criteria: NMT 10.0

• REFRACTIVE INDEX, Appendix II (at 20\(^{\circ}\))
  Acceptance criteria: Between 1.402 and 1.407

• SPECIFIC GRAVITY Determine at 25\(^{\circ}\) by any reliable method (see General Provisions).
  Acceptance criteria: Between 0.808 and 0.817

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (FJ07) Food Ingredients Expert Committee
FCC Sixth Edition Page 438
(E)-2-Hexen-1-al, FCC 6 page 439—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

(E)-2-Hexen-1-al

trans-2-Hexen-1-al

\[ \text{C}_6\text{H}_{10}\text{O} \]

Formula wt 98.14
FEMA: 2560

DESCRIPTION

Change to read:
(E)-2-Hexen-1-al occurs as a pale yellow liquid. It may contain a suitable antioxidant.

Odor Strong, fruity-green, vegetable
Solubility Soluble in alcohol, propylene glycol, most fixed oils; very slightly soluble in water
Boiling Point \( \sim 47^\circ \text{C} \) (17 mm Hg)
Solubility in Alcohol, Appendix VI
One mL dissolves in 1 mL of 95% ethanol.
Function Flavoring agent

ASSAY

• Procedure Proceed as directed under M-1a, Appendix XI.
  Acceptance criteria: NLT 92.0% of \( \text{C}_6\text{H}_{10}\text{O} \)

SPECIFIC TESTS

• REFRACTIVE INDEX, Appendix II (at 20\(^\circ\))
  Acceptance criteria: Between 1.445 and 1.449

• Specific Gravity Determine at 25\(^\circ\) by any reliable method (see General Provisions).
  Acceptance criteria: Between 0.841 and 0.850

Auxiliary Information— Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (F107) Food Ingredients Expert Committee
FCC Sixth Edition Page 439
Phone Number: 1-301-816-8288
BRIEFING

4-Hydroxy-2,5-dimethyl-3(2H)-furanone, FCC 6 page 466—See briefing under Acetaldehyde.
(FIEC: J. Moore)   C64747

4-Hydroxy-2,5-dimethyl-3(2H)-furanone

\[
\begin{align*}
\text{C}_6\text{H}_8\text{O}_3
\end{align*}
\]

Formula wt 128.13
FEMA: 3174

DESCRIPTION

*Change to read:
4-Hydroxy-2,5-dimethyl-3(2H)-furanone occurs as a white to pale yellow solid. It may contain a suitable antioxidant. 1S (FCC 6)

*Odor* Fruity, caramel, burnt sugar

*Solubility* Soluble in propylene glycol, vegetable oils; insoluble or practically insoluble in water

*Solubility in Alcohol, Appendix VI*
One g dissolves in 1 mL of 95% alcohol.

*Function* Flavored agent

IDENTIFICATION

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

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

\[
\text{4-Hydroxy-2,5-dimethyl-3(2H)-furanone}
\]

ASSAY

- **Procedure** Proceed as directed under M-1a, Appendix XI

  *Acceptance criteria:* NLT 98.0% of C6H8O3 in a suitable solvent
BRIEFING

**Hydroxycitronellal, FCC 6 page 464**—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

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**Hydroxycitronellal**

7-Hydroxy-3,7-dimethyl Octanal

![Chemical Structure](image)

C₁₀H₂₀O₂

**Formula wt 172.27**
FEMA: 2583

**DESCRIPTION**

*Change to read:*

Hydroxycitronellal occurs as a colorless liquid. It may contain a suitable antioxidant.

**Odor** Sweet, floral, lily

**Solubility** Soluble in most fixed oils, propylene glycol; insoluble or practically insoluble in glycerin

**Boiling Point** −241°C

**Solubility in Alcohol, Appendix VI**

One mL dissolves in 1 mL of 50% alcohol.

**Function** Flavored agent

**IDENTIFICATION**

- **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.

![Infrared Spectrum](image)

**ASSAY**

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

  **Acceptance criteria:** NLT 95.0% of C₁₀H₂₀O₂

**SPECIFIC TESTS**
• **ACID VALUE, M-15**, Appendix XI
  Acceptance criteria: NMT 5.0

• **REFRACTIVE INDEX**, Appendix II (at 20°)
  Acceptance criteria: Between 1.447 and 1.450

• **Specific Gravity** Determine at 25° by any reliable method (see General Provisions).
  Acceptance criteria: Between 0.918 and 0.923

**Auxiliary Information**—Staff Liaison: Kristie Bowman, Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
FCC Sixth Edition Page 464
Phone Number: 1-301-816-8356
BRIEFING

Iron, Carbonyl, FCC 6 page 478. On the basis of comments and data received, it is proposed to revise the Assay procedure to give instructions for using an automated titrator instead of performing the titration manually with a visual endpoint in order to improve the precision and accuracy of the test.

(FIEC: K. Bowman) C58897

Iron, Carbonyl

Fe

Atomic wt 55.85
CAS: [7439-89-6]

DESCRIPTION

Iron, Carbonyl occurs as a dark gray powder. It is elemental iron produced by the decomposition of iron pentacarbonyl. When viewed under a microscope having a magnifying power of 500 diameters or greater, it appears as spheres built up with concentric shells. It is stable in dry air.

Function Nutrient

Packaging and Storage Store in well-closed containers.

IDENTIFICATION

• Ferrous Salts, Iron, Appendix IIIA
  
  Sample solution: Dissolve a sample in a dilute mineral acid. [Note: Hydrogen is evolved, and solutions of the corresponding salts are formed.]

  Acceptance criteria: Passes test.

ASSAY

Change to read:

• Procedure
  
  Sample: 200 mg
  Analysis:

  Transfer the Sample into a 300-mL Erlenmeyer flask, add 50 mL of 2 N sulfuric acid, and close the flask with a stopper containing a Bunsen valve (made by inserting a glass tube connected to a short piece of rubber tubing with a slit on the side and a glass rod inserted in the other end and arranged so that gases can escape but air cannot enter). Heat on a steam bath until the iron is dissolved, cool the solution, dilute it with 50 mL of recently boiled and cooled water, add 2 drops of ortho-phenanthroline TS and titrate with 0.1 N ceric sulfate until the red color changes to a weak blue. Each mL of 0.1 N ceric sulfate is equivalent to 5.585 mg of Fe.

  Transfer the Sample into a 300-mL Erlenmeyer flask, add 50 mL of 2 N sulfuric acid, and close the flask with a stopper containing a Bunsen valve (made by inserting a glass tube connected to a short piece of rubber tubing with a slit on the side and a glass rod inserted in the other end and arranged so that gases can escape but air cannot enter). Heat on a steam bath to completely dissolve the Sample. [Note: The solution will be clear.] Remove the flask from the steam bath and allow the solution to cool at room temperature with the stopper in place.

  Add a stir bar and 50 mL of recently boiled and cooled water to the flask. Using an automated titrator equipped with a platinum indicating electrode and a silver–silver chloride reference electrode (or an equivalent combination electrode), titrate the solution with 0.1 N ceric sulfate VS through the inflection point of the titration curve. Perform a blank determination (see General Provisions). Each mL of 0.1 N ceric
sulfate VS is equivalent to 5.85 mg of Fe.

**Acceptance criteria:** NLT 98.0% of Fe

### IMPURITIES

#### Inorganic Impurities

- **Arsenic, Arsenic Limit Test, Appendix III B**
  
  **Sample solution:** Dissolve 1.0 g of sample in 25 mL of 2 N sulfuric acid, heat on a steam bath until the evolution of hydrogen ceases, cool, and dilute to 35 mL with water.
  
  **Acceptance criteria:** NMT 3 mg/kg

- **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:** Transfer 20 g of ascorbic acid and 38.5 g of sodium iodide to a 200-mL volumetric flask. Dissolve in and dilute to volume with water, and mix.

**Trioctylphosphine oxide solution:** Transfer 5.0 g of trioctylphosphine oxide to a 100-mL volumetric flask. Dissolve in and dilute to volume with 4-methyl-2-pentanone, and mix. [**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.]

**Lead nitrate stock solution:** Transfer 159.8 mg of reagent-grade lead nitrate [Pb(NO$_3$)$_2$] into a 1000-mL volumetric flask. Dissolve it in 100 mL of water containing 1 mL of nitric acid, dilute to volume with water, and mix. (100 µg lead/mL)

**Standard solution:** Transfer 5.0 mL of **Lead nitrate stock solution** into a 100-mL volumetric flask, dilute to volume with water, and mix. Transfer 2.0 mL of the resulting solution into a 50-mL beaker. Add 8 mL of hydrochloric acid and 2 mL of nitric acid. Place a ribbed watch glass over the beaker, and evaporate to dryness on a steam bath. Add 10 mL of 9 N hydrochloric acid, and transfer the resulting solution, with the aid of 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**; shake the flask for 30 s and allow the layers to separate. Add water to bring the organic solvent layer into the neck of the flask, shake the flask again, and allow the layers to separate. The organic solvent layer contains 2.0 µg of lead/mL, and is the **Standard solution**.

**Blank solution:** Into a 50-mL beaker, add 8 mL of hydrochloric acid and 2 mL of nitric acid. Place a ribbed watch glass over the beaker, and evaporate to dryness on a steam bath. Add 10 mL of 9 N hydrochloric acid, and transfer the resulting solution, with the aid of 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**, shake the flask for 30 s, and allow the layers to separate. Add water to bring the organic solvent layer into the neck of the flask, shake the flask again, and allow the layers to separate. The organic solvent layer contains 0.0 µg of lead/mL, and is the **Blank solution**.

**Sample solution:** Transfer 1.0 g of sample to a 50 mL beaker and cover it with a ribbed watch glass. Slowly add 8 mL of hydrochloric acid and 2 mL of nitric acid, keeping the beaker covered as much as possible. After the initial reaction subsides, evaporate to dryness on a steam bath, cool, and dissolve the residue in 10 mL of 9 N hydrochloric acid, warming if necessary to effect solution. Cool, and transfer the resultant solution, with the aid of about 10 mL of water, into a 50-mL volumetric flask. Add 20 mL of **Ascorbic acid–sodium iodide solution** and 5 mL of **Trioctylphosphine oxide solution**; shake the flask for 30 s and allow the layers to separate. Add water to bring the organic solvent layer into the neck of the flask, shake the flask
again, and allow the layers to separate. The organic solvent layer is the Sample solution.

**Analysis:** Concomitantly determine the absorbance of the Blank solution, the Standard solution, and the Sample solution at the lead emission line at 283.3 nm, with a suitable atomic absorption spectrophotometer equipped with a lead hollow-cathode lamp and an air–acetylene flame, using 4-methyl-2-pentanone to set the instrument to zero. In a suitable analysis, the absorbance of the Blank solution is not more than 20% of the difference between the absorbance of the Standard solution and that of the Blank solution.

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

### Mercury

**Dithizone stock solution:** Dissolve 30 mg of dithizone in 1000 mL of chloroform, add 5 mL of alcohol, and mix (0.03 mg/mL). [Note: Store in a refrigerator in a dark bottle; prepare fresh monthly.]

**Dithizone extraction solution:** On the day of use, dilute 30 mL of Dithizone stock solution to 100 mL with chloroform.

**Hydroxylamine hydrochloride solution:** Dissolve 20 g of hydroxylamine hydrochloride in sufficient water to make about 65 mL. Transfer the solution into a separatory funnel, add a few drops of thymol blue TS, and then add ammonia solution until a yellow color appears. Add 10 mL of 1 : 25 sodium diethylthiocarbamate solution, 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 cupric sulfate solution. 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 to 100 mL with water, and mix.

**Mercury stock solution:** Transfer 33.8 mg of mercuric chloride into a 100-mL volumetric flask, dilute to volume with 1 N hydrochloric acid, and mix (250 µg Hg/mL).

**Diluted standard mercury solution:** Transfer 4.0 mL of Mercury stock solution into a 250-mL volumetric flask, dilute to volume with 1 N hydrochloric acid, and mix (4 µg Hg/mL).

**Sodium citrate solution:** Dissolve 250 g of sodium citrate dihydrate in 1000 mL of water.

**Sample solution:** Transfer 2 g of sample into a 250-mL beaker, add 20 mL of 1 : 2 nitric acid, and digest on a steam bath for about 45 min. Add 5 mL of 1 : 3 hydrochloric acid and continue heating on the steam bath until the sample is dissolved. Cool to room temperature and filter, if necessary, through a medium-porosity filter paper. Wash the paper with a few mL of water, add 40 mL of Sodium citrate solution and 1 mL of Hydroxylamine hydrochloride solution to the filtrate, and adjust the pH to 1.8 with ammonia solution.

**Control:** Transfer 1.0 mL of Diluted standard mercury solution (4 µg Hg) into a 250-mL beaker, add 20 mL of 1 : 2 nitric acid, and digest on a steam bath for about 45 min. Add 5 mL of 1 : 3 hydrochloric acid and continue heating on the steam bath until the sample is dissolved. Cool to room temperature and filter, if necessary, through a medium-porosity filter paper. Wash the paper with a few mL of water, add 40 mL of Sodium citrate solution and 1 mL of Hydroxylamine hydrochloride solution to the filtrate, and adjust the pH to 1.8 with ammonia solution.

**Analysis:** [Note: Because mercuric dithizonate is light sensitive, this procedure should be performed in subdued light.] Transfer the Control and the Sample solution into separate 250-mL separatory funnels, and treat both solutions as follows: Extract with 5 mL of Dithizone extraction solution, shaking the mixtures vigorously for 1 min. Drain carefully, collecting the chloroform in another separatory funnel. If the chloroform does not show a pronounced green color caused by excess reagent, add another 5 mL of the extraction solution, shake the mixture again, and drain into the separatory funnel. Continue the extraction with 5-mL portions, if necessary, collecting each successive extract in the second funnel, until the final chloroform layer contains dithizone in marked excess. Add 15 mL of 1 : 3 hydrochloric acid to the combined chloroform extracts, shake the mixture vigorously for 1 min, and discard the chloroform. Extract with 2 mL of chloroform, drain carefully, and discard the chloroform. Add 1 mL of 0.05 M disodium EDTA.
and 2 mL of 6 N acetic acid to the aqueous layer. Slowly add 5 mL of 6 N ammonia solution and cool the separatory funnel. Transfer the solution into a 150-mL beaker, adjust the pH to 1.8 with 6 N ammonia solution or 1:10 nitric acid, using a pH meter, and return the solution to the funnel. Add 5.0 mL of Dithizone extraction solution, and shake the mixture vigorously for 1 min. Allow the layers to separate, insert a plug of cotton into the stem of the funnel, and collect the dithizone extract in a test tube. Determine the absorbance of each solution in 1-cm cells at 490 nm with a suitable spectrophotometer, using chloroform as the blank.

**Acceptance criteria:** The absorbance of the Sample solution does not exceed that of the Control (NMT 2 mg/kg).

### SPECIFIC TESTS

- **Acid-Insoluble Substances**
  - **Sample:** 1 g
  - **Analysis:** Dissolve the Sample in 25 mL of 2 N sulfuric acid, and heat on a steam bath until the evolution of hydrogen ceases. Filter through a tared filter crucible, wash the residue with water until free from sulfate, dry at 105° for 1 h, let cool, and weigh.
  - **Acceptance criteria:** NMT 0.2%

- **Sieve Analysis, Sieve Analysis of Granular Metal Powders, Appendix IIIC**
  - **Acceptance criteria:** NLT 100% passes through a 200-mesh sieve and NLT 95% passes through a 325-mesh sieve.

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1 Metrohm 751 GPD Titrino automatic, PC-based titrator with Titrino Workcell software (Brinkmann, Hauppauge, NY), or equivalent automated titrator

**Auxiliary Information—Staff Liaison:** Jeffrey Moore, Ph.D., Senior Scientific Associate

**Expert Committee:** (FI07) Food Ingredients Expert Committee

**FCC Sixth Edition Page 478**

**Phone Number:** 1-301-816-8288
Iron, Reduced, FCC 6 page 482. On the basis of comments and data received, it is proposed to revise the acceptance criteria for **Sieve Analysis** to reflect the current particle size range of this material being used in food fortification. It is also proposed, on the basis of comments and data received, to revise the acceptance criteria and corresponding test procedures for the **Assay, Acid-Insoluble Substances, Arsenic, Lead, and Mercury** to reflect current manufacturing capabilities. Interested parties are encouraged to submit comments to Jeff Moore, Ph.D., at jm@usp.org.

(FIEC: J. Moore) C65197

Iron, Reduced
Fe

**Atomic wt**: 55.85  
**CAS**: [7439-89-6]

**DESCRIPTION**
Iron, Reduced occurs as a gray-black powder. It is elemental iron obtained by a chemical process. It is lusterless or has not more than a slight luster. When viewed under a microscope having a magnifying power of 100 diameters, it appears as an amorphous powder, free from particles having a crystalline structure. It is stable in dry air.

**Function** Nutrient

**Packaging and Storage** Store in well-closed containers.

**IDENTIFICATION**
- **Ferrous Salts, Iron, Appendix IIIA**
  - **Sample solution**: Dissolve a sample in a dilute mineral acid. [Note: Hydrogen is evolved, and solutions of the corresponding salts are formed.]
  - **Acceptance criteria**: Passes test.

**ASSAY**

**Change to read:**
- **PROCEDURE**
  - **Sample**: 200 mg
  - **Analysis**: Transfer the Sample into a 300-mL Erlenmeyer flask, add 50 mL of 2 N sulfuric acid, and close the flask with a stopper containing a Bunsen valve (made by inserting a glass tube connected to a short piece of rubber tubing with a slit on the side and a glass rod inserted in the other end and arranged so that gases can escape but air cannot enter). Heat on a steam bath until the iron is dissolved, cool the solution, dilute it with 50 mL of recently boiled and cooled water, add 2 drops of orthophenanthroline TS and titrate with 0.1 N ceric sulfate until the red color changes to a weak blue. Each mL of 0.1 N ceric sulfate is equivalent to 5.585 mg of Fe.
  - **Acceptance criteria**: NLT 96.0% of Fe

**IMPURITIES**

**Change to read:**
- **Inorganic Impurities**
• **Arsenic, Arsenic Limit Test, Appendix IIIB**

**Sample solution:** Dissolve 1.0 g of sample in 25 mL of 2 N sulfuric acid, heat on a steam bath until the evolution of hydrogen ceases, cool, and dilute to 35 mL with water.

**Acceptance criteria:** NMT 8 mg/kg 3 mg/kg 1S (FCC 6)

• **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:** Transfer 20 g of ascorbic acid and 38.5 g of sodium iodide to a 200-mL volumetric flask. Dissolve in and dilute to volume with water, and mix.

**Trioctylphosphine oxide solution:** Transfer 5.0 g of trioctylphosphine oxide to a 100-mL volumetric flask. Dissolve in and dilute to volume with 4-methyl-2-pentanone, and mix. [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.]

**Lead nitrate stock solution:** Transfer 159.8 mg of reagent-grade lead nitrate \([\text{Pb(NO}_3\text{)}_2]\) into a 1000-mL volumetric flask. Dissolve it in 100 mL of water containing 1 mL of nitric acid, dilute to volume with water, and mix. (100 µg Pb/mL)

**Standard solution:** Transfer 5.0 mL of Lead nitrate stock solution into a 100-mL volumetric flask, dilute to volume with water, and mix. Transfer 2.0 mL of the resulting solution into a 50-mL beaker. Add 8 mL of hydrochloric acid and 2 mL of nitric acid. Place a ribbed watch glass over the beaker, and evaporate to dryness on a steam bath. Add 10 mL of 9 N hydrochloric acid, and transfer the resulting solution, with the aid of 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 Trioclyrophosphate oxide solution, shake the flask for 30 s, and allow the layers to separate. Add water to bring the organic solvent layer into the neck of the flask, shake the flask again, and allow the layers to separate. The organic solvent layer contains 2.0 µg of lead/mL, and is the Standard solution.

**Blank solution:** Into a 50-mL beaker, add 8 mL of hydrochloric acid and 2 mL of nitric acid. Place a ribbed watch glass over the beaker, and evaporate to dryness on a steam bath. Add 10 mL of 9 N hydrochloric acid, and transfer the resulting solution, with the aid of 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 Trioclyrophosphate oxide solution, shake the flask for 30 s, and allow the layers to separate. Add water to bring the organic solvent layer into the neck of the flask, shake the flask again, and allow the layers to separate. The organic solvent layer contains 0.0 µg of lead/mL, and is the Blank solution.

**Sample solution:** Transfer 4.0 g 2.5 g 1S (FCC 6) of sample to a 50 mL beaker and cover it with a ribbed watch glass. Slowly add 8 mL of hydrochloric acid and 2 mL of nitric acid, keeping the beaker covered as much as possible. After the initial reaction subsides, evaporate to dryness on a steam bath, cool, and dissolve the residue in 10 mL of 9 N hydrochloric acid, warming if necessary to effect solution. Cool, and transfer the resultant solution, with the aid of about 10 mL of water, into a 50-mL volumetric flask. Add 20 mL of Ascorbic acid–sodium iodide solution and 5 mL of Trioclyrophosphate oxide solution, shake the flask for 30 s, and allow the layers to separate. Add water to bring the organic solvent layer into the neck of the flask, shake the flask again, and allow the layers to separate. The organic solvent layer is the Sample solution.

**Analysis:** Concomitantly determine the absorbance of the Blank solution, the Standard solution, and the Sample solution at the lead emission line at 283.3 nm, with a suitable atomic absorption spectrophotometer equipped with a lead hollow-cathode lamp and an air–acetylene flame, using 4-methyl-
2-pentanone to set the instrument to zero. In a suitable analysis, the absorbance of the Blank solution is not more than 20% of the difference between the absorbance of the Standard solution and that of the Blank solution.

**Acceptance criteria:** The absorbance of the Sample solution does not exceed that of the Standard solution. (NMT 4.0 mg/kg)

- **Mercury**
  - **Dithizone stock solution:** Dissolve 30 mg of dithizone in 1000 mL of chloroform, add 5 mL of alcohol, and mix. (0.03 mg/mL) [Note: Store in a refrigerator in a dark bottle; prepare fresh monthly.]
  - **Dithizone extraction solution:** On the day of use, dilute 30 mL of Dithizone stock solution to 100 mL with chloroform.
  - **Hydroxylamine hydrochloride solution:** Dissolve 20 g of hydroxylamine hydrochloride in sufficient water to make about 65 mL. Transfer the solution into a separatory funnel, add a few drops of thymol blue TS, and then add ammonia solution until a yellow color appears. Add 10 mL of 1:25 sodium diethyldithiocarbamate solution, 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 cupric sulfate solution. 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 to 100 mL with water, and mix.
  - **Mercury stock solution:** Transfer 33.8 mg of mercuric chloride into a 100-mL volumetric flask, dilute to volume with 1 N hydrochloric acid, and mix. (250 µg Hg/mL)
  - **Diluted standard mercury solution:** Transfer 2.0 mL of Mercury stock solution (5 µg Hg) into a 250-mL beaker, add 20 mL of 1:2 nitric acid, and digest on a steam bath for about 45 min. Add 5 mL of 1:3 hydrochloric acid and continue heating on the steam bath until the sample is dissolved. Cool to room temperature and filter, if necessary, through a medium-porosity filter paper. Wash the paper with a few mL of water, add 20 mL of Sodium citrate solution and 1 mL of Hydroxylamine hydrochloride solution to the filtrate, and adjust the pH to 1.8 with ammonia solution.
  - **Control:** Transfer 1.0 mL of Diluted standard mercury solution (5 µg Hg) into a 250-mL beaker, add 20 mL of 1:2 nitric acid, and digest on a steam bath for about 45 min. Add 5 mL of 1:3 hydrochloric acid and continue heating on the steam bath until the sample is dissolved. Cool to room temperature and filter, if necessary, through a medium-porosity filter paper. Wash the paper with a few mL of water, add 20 mL of Sodium citrate solution and 1 mL of Hydroxylamine hydrochloride solution to the filtrate, and adjust the pH to 1.8 with ammonia solution.

**Analysis:** [Note: Because mercuric dithizonate is light sensitive, this procedure should be performed in subdued light.] Transfer the Control and the Sample solution into separate 250-mL separatory funnels, and treat both solutions as follows: Extract with 5 mL of Dithizone extraction solution, shaking the mixtures vigorously for 1 min. Drain carefully, collecting the chloroform in another separatory funnel. If the chloroform does not show a pronounced green color caused by excess reagent, add another 5 mL of the extraction solution, shake the mixture again, and drain into the separatory funnel. Continue the extraction with 5-mL portions, if necessary, collecting each successive extract in the second funnel, until the final chloroform layer contains dithizone in marked excess. Add 15 mL of 1:3 hydrochloric acid to the combined chloroform extracts, shake the mixture vigorously for 1 min, and discard the chloroform. Extract with 2 mL of chloroform, drain carefully, and discard the chloroform. Add 1 mL of 0.05 M disodium EDTA and 2 mL of...
6 N acetic acid to the aqueous layer. Slowly add 5 mL of 6 N ammonia solution and cool the separatory
funnel. Transfer the solution into a 150-mL beaker, adjust the pH to 1.8 with 6 N ammonia solution or 1:10
nitric acid, using a pH meter, and return the solution to the funnel. Add 5.0 mL of Dithizone extraction
solution, and shake the mixture vigorously for 1 min. Allow the layers to separate, insert a plug of cotton
into the stem of the funnel, and collect the dithizone extract in a test tube. Determine the absorbance of
each solution in 1-cm cells at 490 nm with a suitable spectrophotometer, using chloroform as the blank.
Acceptance criteria: The absorbance of the Sample solution does not exceed that of the Control. (NMT 6
mg/kg) 2 mg/kg 1S (FCC 6)

SPECIFIC TESTS

Change to read:
• Acid-Insoluble Substances
  Sample: 1.0 g
  Analysis: Dissolve the Sample in 25 mL of 2 N sulfuric acid, and heat on a steam bath until the evolution of
  hydrogen ceases. Filter through a tared filter crucible, wash the residue with water until free from sulfate,
  dry at 105° for 1 h, let cool, and weigh.
  Acceptance criteria: The weight of the residue does not exceed 42.5 mg. (NMT 1.25%) 5.0 mg. (NMT
  0.50%) 1S (FCC 6)

Change to read:
• Sieve Analysis, Sieve Analysis of Granular Metal Powders, Appendix IIC
  Acceptance criteria: NLT 100% passes through a 100-mesh sieve and NLT 95% passes through a 325-
mesh sieve 1S (FCC 6)

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
FCC Sixth Edition Page 482
Phone Number: 1-301-816-8288
BRIEFING

Isobutyraldehyde, FCC 6 page 505—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

Isobutyraldehyde

\[
\text{C}_4\text{H}_8\text{O}
\]

Formula wt 72.11
FEMA: 2220

DESCRIPTION

*Change to read:*

Isobutyraldehyde occurs as a colorless, mobile liquid. It may contain a suitable antioxidant. 1S (FCC 6)

**Odor** Sharp, pungent

**Solubility** Miscible in alcohol, ether; 1 mL dissolves in 125 mL water.

**Boiling Point** −64°C

**Function** Flavoring agent

IDENTIFICATION

**Infrared Spectra**, *Spectrophotometric Identification Tests, Appendix III C*

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

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

**Acceptance criteria:** NLT 98.0% of C₄H₈O

**SPECIFIC TESTS**

- **Acid Value, M-15, Appendix XI**
  
  [Note: Use methyl red TS as the indicator.]

  **Acceptance criteria:** NMT 5.0

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

  **Acceptance criteria:** Between 0.783 and 0.788

**Auxiliary Information—** Staff Liaison: Kristie Bowman, Senior Scientific Associate

**Expert Committee:** (FI07) Food Ingredients Expert Committee

**FCC Sixth Edition Page 505**

**Phone Number:** 1-301-816-8356
**BRIEFING**

**Isomalt.** Because there is no existing FCC monograph for this food ingredient, a new monograph is being proposed based on the Isomalt monographs from the 46th Joint FAO/WHO Expert Committee on Food Additives (1996) and NF 26.

1. The chemical information, Description, Function, and Packaging and Storage are based on the JECFA Isomalt monograph and on data and comments received.
2. The Thin-Layer Chromatography Identification test procedure and acceptance criteria are based on the NF 26 monograph.
3. The Assay and Mannitol and Sorbitol test procedures are based on the NF 26 Isomalt monograph, while the acceptance criteria are based on the JECFA Isomalt monograph and on data and comments received.
4. The Lead and Nickel test procedures were adapted from the JECFA Isomalt monograph to the corresponding General Test procedures used in FCC, and the acceptance criteria were based on the JECFA Isomalt monograph.
5. The Reducing Sugars, Water, and Residue on Ignition test procedures and acceptance criteria are based on the JECFA Isomalt monograph.
6. The Labeling requirement is based on the NF monograph.

Interested parties are encouraged to submit comments to Jeff Moore, Ph.D., email jm@usp.org.

(FIEC: J. Moore) C64696

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**Add the following:**

- Isomalt

Hydrogenated isomaltulose

INS: 953

CAS: [64519-82-0]

**DESCRIPTION**

Isomalt occurs as an odorless, white, crystalline slightly hygroscopic substance. It is a mixture of hydrogenated mono- and disaccharides whose principal components are the disaccharides 6-O-α-glucopyranosyl-D-sorbitol (1,6-GPS), formula wt, 344.32, and 1-O-α-D-glucopyranosyl-D-mannitol dihydrate (1,1-GPM), formula wt, 380.32. Isomalt is soluble in water; very slightly soluble in ethanol.

**Function** Sweetener, bulking agent, anticaking agent, glazing agent

**Packaging and Storage** Store in well-closed containers.

**IDENTIFICATION**

- **Thin-Layer Chromatography**, Appendix IIA
  - **Adsorbent**: 0.25-mm layer of chromatographic silica gel mixture containing a fluorescent indicator having optimal intensity at 254 nm
  - **Developing solvent system**: Ethyl acetate, pyridine, water, acetic acid, and propionic acid (10:10:2:1:1)
  - **Standard solution**: 5 mg/mL of USP Isomalt RS
  - **Sample solution**: 5 mg/mL
Application volume: 1 µL

Analysis: Separately apply each of the Standard solution and the Sample solution to the chromatographic plate and thoroughly dry the starting points in warm air. Develop over 10 cm using the Developing solvent system, dry the plate in a current of hot air, and dip for 3 s in a 1 g/L solution of sodium periodate. Dip the plate for 3 s in a mixture of absolute ethanol, sulfuric acid, acetic acid, and anisaldehyde (90:5:1:1). Dry the plate in a current of hot air until colored spots become visible. The background color may be brightened by exposure to warm steam. Examine in daylight.

Acceptance criteria: The chromatograms obtained from the Standard solution and the Sample solution show principal spot(s) similar in position and color.

ASSAY

• A. Procedure

Mobile phase Degassed water

Standard solution: 20 mg/mL USP Isomalt RS

Sample solution: 20 mg/mL

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography

Detector: Refractive index

Column: 7.8-mm × 30-cm analytical column1 and a 4.6-mm × 3-cm guard column, both packed with a strong cation-exchange resin consisting of sulfonated cross-linked styrene–divinylbenzene copolymer in the calcium form, about 9 µm in diameter

Column temperature: 80 ± 1°

Flow rate: About 0.5 mL/min

Injection volume: About 20 µL

System suitability

Sample: Standard solution

Resolution: NLT 2.0 between 1,1-GPM and 1,6-GPS

Relative standard deviation: NMT 2.0% determined from the 1,6-GPS and 1,1-GPM peak responses, for replicate injections

Analysis: Separately inject equal volumes of the Standard solution and Sample solution into the chromatograph, record the chromatograms, and measure the responses for the 1,6-GPS and 1,1-GPM peaks. [Note: The relative retention times for 1,6-GPS and 1,1-GPM are 1.2 and 1.0, respectively.]

Separately calculate the percentages of 1,6-GPS and 1,1-GPM in the sample taken by the following formula:

\[
\left( \frac{r_U}{r_S} \right) \times \left( \frac{C_S}{C_U} \right) \times 100\%
\]

\( r_U \) = Peak response for 1,6-GPS or 1,1-GPM from the Sample solution

\( r_S \) = Peak response for 1,6-GPS or 1,1-GPM from the Standard solution

\( C_S \) = Concentration of 1,6-GPS or 1,1-GPM in the Standard solution (mg/mL) [Calculated based on the declared 1,6-GPS or 1,1-GPM content of USP Isomalt RS]

\( C_U \) = Concentration of sample in the Sample solution (mg/mL)

Calculate the percentage of total hydrogenated mono- and disaccharides (%THS) in the sample taken using the following equation:
A = The sum of the percentages of 1,6-GPS and 1,1-GPM in the sample taken calculated above
B = The sum of the percentages of mannitol and sorbitol in the sample, determined separately in the below Mannitol and Sorbitol test procedure

**Acceptance criteria:** NLT 98.0% of total hydrogenated mono- and disaccharides (%THS) and NLT 86% of the mixture of 1,6-GPS and 1,1-GPM, calculated on the anhydrous 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, Method II, Appendix IIIB
  - Acceptance criteria: NMT 2 mg/kg

Organic Impurities

- **MANNITOL AND SORBITOL**
  - **Mobile phase** Degassed water
  - **Standard solution**: 0.1 mg/mL each of USP Sorbitol RS and USP Mannitol RS
  - **Resolution solution**: 20 mg/mL, 0.1 mg/mL, and 0.1/mL of USP Isomalt RS, USP Sorbitol RS, and USP Mannitol RS, respectively
  - **Sample solution**: 20 mg/mL
  - **Chromatographic system**, Appendix IIA
    - **Mode**: High-performance liquid chromatography
    - **Detector**: Refractive index
    - **Column**: 7.8-mm × 30-cm analytical column and a 4.6-mm × 3-cm guard column, both packed with a strong cation-exchange resin consisting of sulfonated cross-linked styrene–divinylbenzene copolymer in the calcium form, about 9 µm in diameter
    - **Column temperature**: 80 ± 1°C
    - **Flow rate**: About 0.5 mL/min
    - **Injection volume**: About 20 µL
  - **System suitability**
    - **Sample**: Resolution solution
    - **Resolution**: NLT 2.0 between 1,1-GPM and 1,6-GPS
  - **Analysis**: Separately inject equal volumes of the Standard solution and Sample solution into the chromatograph, record the chromatograms, and measure the responses for the for 1,6-GPS and 1,1-GPM.
    - [Note: The typical retention time of 1,1-GPM is about 12.3 minutes; the relative retention times are about 1.2 for 1,6-GPS, about 1.6 for mannitol, about 2.0 for sorbitol, and 1.0 for 1,1-GPM.]
  - Separately calculate the percentages of mannitol and sorbitol in the sample taken by the following formula:
    \[
    \left(\frac{r_U}{r_S}\right) \times \left(\frac{C_S}{C_U}\right) \times 100\%
    \]
    \(r_U\) = Peak response for mannitol or sorbitol from the Sample solution
    \(r_S\) = Peak response for the mannitol or sorbitol from the Standard solution
$C_S =$ Concentration of mannitol or sorbitol in the Standard solution (mg/mL)

$C_U =$ Concentration of sample in the Sample solution (mg/mL)

**Acceptance criteria:** NMT 3% mannitol and NMT 6% sorbitol

- **Reducing Sugars**

  **Alkaline tartrate solution:** Dissolve 34.6 g of potassium sodium tartrate (Rochelle salt) and 10 g of sodium hydroxide in water, dilute to 100 mL, let stand two days, and filter through glass wool.

  **Sample:** 7 g

  **Analysis:** Dissolve the Sample in 35 mL of water in a 400-mL beaker, and mix. Add 25 mL of cupric sulfate TS and 25 mL of Alkaline tartrate solution. Cover the beaker with glass and heat the mixture at such a rate that it comes to a boil in approximately 4 min and boils for exactly 2 min. Filter the precipitated cuprous oxide through a tared Gooch crucible previously washed with hot water, ethanol, and ether, and dried at $100^\circ$ for 30 min. Thoroughly wash the collected cuprous oxide on the filter with hot water, then with 10 mL of ethanol, and finally with 10 mL of ether, and dry at $100^\circ$ for 30 min. Weigh the filter containing the cuprous oxide.

  **Acceptance criteria:** The weight of the cuprous oxide does not exceed 50 mg (NMT 0.3% (as glucose)).

**SPECIFIC TESTS**

- **Water, Water Determination, Appendix IIB**

  **Acceptance criteria:** NMT 7.0%

- **Residue on Ignition (Sulfated Ash), Appendix IIC**

  **Sample:** 5 g

  **Acceptance criteria:** NMT 0.05%

---

1 Aminex Carbohydrate HPX-87C (BioRad), or equivalent

**Auxiliary Information— Staff Liaison:** Jeffrey Moore, Ph.D., Senior Scientific Associate

*Expert Committee:* (FI07) Food Ingredients Expert Committee

*Phone Number:* 1-301-816-8288
Lauryl Aldehyde, *FCC 6* page 530—See briefing under *Acetaldehyde*.  
(FIEC: J. Moore)  
C64747

Lauryl Aldehyde

Aldehyde C-12

Dodecanal

\[ \text{C}_{12}\text{H}_{24}\text{O} \]

*Formula wt 184.32*  
*FEMA: 2615*

**DESCRIPTION**

*Change to read:*

Lauryl Aldehyde occurs as a colorless to light yellow liquid (that can solidify at room temperature). It may contain a suitable antioxidant.  

*Odor*  
Fatty

*Solubility*  
Soluble in alcohol, most fixed oils, propylene glycol (may be turbid); insoluble or practically insoluble in glycerin, water

*Boiling Point*  
\( \sim 249^\circ \)

*Function*  
Flavoring agent

**IDENTIFICATION**

- **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.

![Infrared Spectrum](image)

Lauryl Aldehyde

**ASSAY**

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

  *Acceptance criteria:* NLT 92.0% of \( \text{C}_{12}\text{H}_{24}\text{O} \)
SPECIFIC TESTS

• ACID VALUE, M-15, Appendix XI
  Acceptance criteria: NMT 10.0

• REFRACTIVE INDEX, Appendix II (at 20°)
  Acceptance criteria: Between 1.433 and 1.439

• SPECIFIC GRAVITY Determine at 25° by any reliable method (see General Provisions).
  Acceptance criteria: Between 0.826 and 0.836

Auxiliary Information—Staff Liaison: Kristie Bowman, Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
FCC Sixth Edition Page 530
Phone Number: 1-301-816-8356
**BRIEFING**

**d-Limonene, FCC 6 page 545—See briefing under Acetaldehyde.**

(FIEC: J. Moore) C64747

**d-Limonene**

d-\(\text{p}\)-Mentha-1,8-diene

Cine

![Chemical structure of d-limonene]

\[C_{10}H_{16}\]

**Formula wt 136.24**

FEMA: 2633

**DESCRIPTION**

*Change to read:*
d-Limonene occurs as a colorless liquid. It may contain a suitable antioxidant. 1S (FCC 6)

**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** \(\sim 177^\circ\)

**Function** Flavoring agent

**IDENTIFICATION**
• **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.

![Infrared Spectrum](image)

**ASSAY**

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

  **Acceptance criteria:** NLT 93.0% of C\textsubscript{10}H\textsubscript{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**— Staff Liaison: Kristie Bowman, Senior Scientific Associate  
*Expert Committee:* (FI07) Food Ingredients Expert Committee  
FCC Sixth Edition Page 545  
Phone Number: 1-301-816-8356
**BRIEFING**

*1-Limonene, FCC 6 page 546*—See briefing under *Acetaldehyde.*

(FIEC: J. Moore) C64747

**1-Limonene**

1-p-Mentha-1,8-diene

![Chemical Structure](image)

C\(_{10}\)H\(_6\)

**DESCRIPTION**

*Change to read:*

1-Limonene occurs as a colorless liquid. It may contain a suitable antioxidant.**1S (FCC 6)**

**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\(_6\)

**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 X
  Acceptance criteria: NMT 5.0

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
FCC Sixth Edition Page 546
Phone Number: 1-301-816-8288
Maltitol, *FCC 6* page 574; Maltitol Syrup, *FCC 6* page 575; Mannitol, *FCC 6* page 583; Sorbitol, *FCC 6* page 919; Sorbitol Solution, *FCC 6* page 920. On the basis of comments and data received, it is proposed to replace the existing Reducing Sugars specification for Maltitol with the reducing sugar specification under the Sorbitol FCC monograph. The current cuprous oxide gravimetric method is the test formerly used for the other polyols listed above but was revised with the *FCC* Fifth Edition. The newly proposed test, which involves a titration with sodium thiosulfate, is utilized by all other polyol monographs listed above. In addition, it is proposed to revise the volume of diluted acetic acid TS in the monographs listed above in order to be consistent within both *FCC* and *USP–NF*. Interested parties are encouraged to comment.

(FIEC: K. Bowman) C57077

### Maltitol

D-Maltitol

Hydrogenated Maltose

\( \alpha\)-D-Glucopyranosyl-1,4-D-glucitol

![Maltitol Structural Formula](image)

\[ \text{C}_{12}\text{H}_{24}\text{O}_{11} \]

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 sodium metaperiodate
Spray reagent B: 20 mg/mL 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 USP Maltitol RS
  Sample solution: Transfer 0.7 g of sample into a 50-mL volumetric flask, dilute to volume with water, 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 maltotritol, 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 by the formula:

  \[
  50 \times C \times \left(\frac{r_U}{r_S}\right)
  \]

  $C$ = 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: NLT 92.0% and NMT 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 under Assay, but use the following calculation.
  
  Calculate the quantity, in mg, of maltitol and other hydrogenated saccharides in the Sample solution by the formula:

  \[ 50 \times C \times (r_U/r_S) \]

  \( C \) = 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). \( \text{1S (FCC 6)} \)
  
  Sample: 21 g

  Analysis: Dissolve the Sample in 35 mL of water in a 400 mL beaker and mix. Add 25 mL of cupric sulfate TS and 25 mL of alkaline tartrate TS. Cover the beaker with a watch glass, heat the mixture at such a rate that it comes to a boil in approximately 4 min, and boil for exactly 2 min. Filter the precipitated cuprous oxide through a tared, sintered-glass filter crucible previously washed with hot water, ethanol, and ether and dried at 100°C for 30 min. Thoroughly wash the collected cuprous oxide on the filter with hot water, then with 10 mL of ethanol, and finally with 10 mL of ether, and dry at 100°C for 30 min. 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. \( \text{1S (FCC 6)} \)

  **Acceptance criteria**: The weight of the cuprous oxide does not exceed 30 mg (NMT 0.3%) NLT 12.8 mL of 0.05 N Sodium thiosulfate VS is required (NMT 0.3% reducing sugars, as glucose) \( \text{1S (FCC 6)} \)

- **Residue on Ignition (Sulfated Ash), Method I (for solids)**, Appendix IIC
Sample: 2 g

**Acceptance criteria:** NMT 0.1%

- **Water**, *Water Determination*, Appendix II B
  **Acceptance criteria:** NMT 1.5%

**Auxiliary Information**— *Staff Liaison:* Jeffrey Moore, Ph.D., Senior Scientific Associate

*Expert Committee:* (FI07) Food Ingredients Expert Committee

*FCC Sixth Edition* Page 574

*Phone Number:* 1-301-816-8288
BRIEFING

Maltitol Syrup, FCC 6 page 575—See briefing under Maltitol. Interested parties are encouraged to comment.
(FIEC: K. Bowman) C65161

Maltitol Syrup

Hydrogenated Glucose Syrup

INS: 965

DESCRIPTION
Maltitol Syrup occurs as a clear, colorless, syrupy liquid. It is a water solution of a hydrogenated, partially
hydrolyzed starch containing maltitol, sorbitol, and hydrogenated oligo- and polysaccharides. It is miscible with
water and with glycerin, and slightly miscible with alcohol.

Function Humectant; texturizing agent; stabilizer; sweetener

Packaging and Storage Store in well-closed containers.

IDENTIFICATION

A. Procedure
   Sample: 18.7 mg/mL
   Analysis: Transfer 3 mL of the Sample solution into a 15-cm test tube, add 3 mL of a freshly prepared 1:10
catechol solution, and mix. Add 6 mL of sulfuric acid, mix again, then gently heat the tube in a flame for
about 30 s.
   Acceptance criteria: A deep pink or wine red color appears.

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 from the Assay (below).

ASSAY

A. Procedure
   Mobile phase: Degassed water
   Standard solution: 10.0 mg/g USP Maltitol RS and 1.6 mg/g USP Sorbitol RS
   Sample solution: 20 mg/g
   Chromatographic system, Appendix IIA
   Mode: High-performance liquid chromatography
   Detector: Refractive index detector
   Column: 10-cm × 7.8-mm column containing packing L34 (Bio-Rad Laboratories), or equivalent
   Temperature:
      Column: 60° ± 2°
      Detector: About 35° (Maintain at constant temperature.)
   Flow rate: About 0.5 mL/min
   Injection volume: About 10 µL
   System suitability
      Sample: Standard solution
      Suitability requirement 1: The relative standard deviation for replicate injections is NMT 2.0%.
      Suitability requirement 2: The tailing factor for maltitol and sorbitol is NMT 1.2.
Analysis: Separately inject the Sample solution and the Standard 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 relative retention times are about 0.38 for maltotriitol, 0.48 for maltitol, and 1.0 for sorbitol.]

Separately calculate the percentages, on the anhydrous basis, of maltitol and sorbitol in the portion of sample taken by the formula:

\[
[(C_S / C_U) \times (r_U / r_S) \times 10,000] / (100 - w)
\]

- \( C_S \): Concentration (mg/g) of the appropriate USP RS in the Standard solution
- \( C_U \): Concentration (mg/g) of sample in the Sample solution
- \( r_U \): Peak response of the corresponding analyte obtained from the chromatogram of the Sample solution
- \( r_S \): Peak response of the corresponding analyte obtained from the chromatogram of the Standard solution
- \( w \): Percent water as determined in the test for Water (below)

Acceptance criteria:
- D-Maltitol: NLT 50.0%, by weight, calculated on the anhydrous basis
- D-Sorbitol: NMT 8.0%, calculated on the anhydrous basis

IMPURITIES

Inorganic Impurities
- Lead, Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace Method, Method I, Appendix IIIB
  Acceptance criteria: NMT 1 mg/kg, calculated on the anhydrous basis
- Nickel, Nickel Limit Test, Appendix IIIB
  Acceptance criteria: NMT 1 mg/kg, calculated on the anhydrous basis

Change to read:

Organic Impurities
- Reducing Sugars
  0.05 N iodine: Dilute 0.1 N iodine VS 1:1(v/v) with water.
  0.05 N sodium thiosulfate: Dilute 0.1 N sodium thiosulfate VS 1:1(v/v) with water.
  Sample: Amount equivalent to 3.3 g on the anhydrous basis
Analysis: Add 3 mL of water, 20.0 mL of alkaline cupric citrate TS and a few glass beads to the Sample.
  Heat so that boiling begins after 4 min, and maintain the boiling for 3 min. Cool rapidly and add 490 mL 1S (FCC 6) of diluted acetic acid TS, 60 mL of water 1S (FCC 6) and 20.0 mL of 0.05 N iodine. 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. Use 2 mL of starch TS, added toward the end of the titration, as an indicator.
  Acceptance criteria: NLT 12.8 mL of 0.05 N sodium thiosulfate is required for the titration. (NMT 0.3%, on the anhydrous basis, as glucose)
SPECIFIC TESTS

• **pH**, *pH Determination*, Appendix IIB
  
  **Sample solution:** 140 mg/g in carbon dioxide-free water
  
  **Acceptance criteria:** Between 5.0 and 7.5

• **Residue on Ignition (Sulfated Ash), Method II (for Liquids), Appendix IIC**
  
  **Sample:** 2 g
  
  **Acceptance criteria:** NMT 0.1%, calculated on the anhydrous basis

• **Water**, *Water Determination*, Appendix IIB
  
  **Acceptance criteria:** NMT 31.5%

**Auxiliary Information**— *Staff Liaison:* Jeffrey Moore, Ph.D., Senior Scientific Associate

*Expert Committee:* (FI07) Food Ingredients Expert Committee

*FCC Sixth Edition Page 575*

*Phone Number:* 1-301-816-8288
Manganese Sulfate, FCC 6 page 583; Phosphoric Acid, FCC 6 page 761; Sodium Metaphosphate, Insoluble, FCC 6 page 897; Sodium Phosphate, Dibasic, FCC 6 page 900; Sodium Phosphate, Monobasic, FCC 6 page 901; Sodium Polyphosphates, Glassy, FCC 6 page 903; Sodium Pyrophosphate, FCC 6 page 906; Stannous Chloride, FCC 6 page 926; Zinc Oxide, FCC 6 page 1033; Zinc Sulfate, FCC 6 page 1033. It is proposed to delete the sample size for the Lead test procedure based on proposed changes to this General Test procedure which would accommodate testing to different limits using different standard solution concentrations, see briefing under Appendix IIIB, Lead Limit Test, APDC Extraction Method. Interested parties are encouraged to submit comments to Jeff Moore, Ph.D., at jm@usp.org.

Manganese Sulfate
MnSO₄·H₂O

DESCRIPTION
Manganese Sulfate occurs as a pale pink, granular powder. It is freely soluble in water, but is insoluble in alcohol.

Function Nutrient

Packaging and Storage Store in well-closed containers.

IDENTIFICATION

• A. MANGANESE, Appendix IIIA
  Sample solution: 100 mg/mL
  Acceptance criteria: Passes test.

• B. SULFATE, Appendix IIIA
  Sample solution: 100 mg/mL
  Acceptance criteria: Passes tests.

ASSAY

• PROCEDURE
  Sample solution: 16 mg/mL
  Analysis: Transfer a 25.0-mL portion of the Sample solution into a 400-mL beaker, and add 10 mL of 100 mg/mL hydroxylamine hydrochloride solution, 25 mL of 0.05 M disodium EDTA measured from a buret, 25 mL of ammonia–ammonium chloride buffer TS, and 5 drops of eriochrome black TS. Heat the solution to between 55° and 65° and titrate with 0.05 M disodium EDTA from the buret to a blue endpoint. Each mL of 0.05 M disodium EDTA is equivalent to 8.450 mg of MnSO₄·H₂O.
  Acceptance criteria: NLT 98.0% and NMT 102.0% of MnSO₄·H₂O

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

- Lead, Lead Limit Test, APDC Extraction Method, Appendix IIIB
  Sample: 5 g

- 1S (FCC 6)
  Acceptance criteria: NMT 4 mg/kg

- Selenium, Selenium Limit Test, Method II, Appendix IIIB
  Sample: 200 mg
  Acceptance criteria: NMT 0.003%

SPECIFIC TESTS

- Loss on Heating
  Sample: 1 g
  Analysis: Heat the Sample, in a crucible tared in a stoppered weighing bottle, to constant weight at 400° to 500°. Cool in a desiccator, transfer to the stoppered weighing bottle, and weigh.
  Acceptance criteria: Between 10.0% and 13.0%

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
FCC Sixth Edition Page 583
Phone Number: 1-301-816-8288
Mannitol, *FCC 6* page 583—See briefing under Maltitol. In addition to this change, it is proposed to further revise the Reducing Sugars test by moving the content from the Notes into the test. Interested parties are encouraged to comment.

(FIEC: K. Bowman) C65164

**Mannitol**

D-Mannitol

Mannite

1,2,3,4,5,6-Hexanohexol

\[
\text{C}_6\text{H}_{14}\text{O}_6
\]

INS: 421

CAS: [69-65-8]

**DESCRIPTION**

Mannitol occurs as a white, crystalline powder or as free-flowing granules consisting of D-mannitol and a small quantity of sorbitol. It is soluble in water and in pyridine, very slightly soluble in alcohol, and practically insoluble in chloroform and ether.

**Function** Nutritive sweetener; texturizing agent

**Packaging and Storage** Store in well-closed containers.

**IDENTIFICATION**

- **Procedure**
  
  **Sample solution:** 13.3 mg/mL
  
  **Analysis:** Transfer 3 mL of the *Sample solution* into a 15-cm test tube, add 3 mL of a freshly prepared 100 mg/mL catechol solution, and mix. Add 6 mL of sulfuric acid, mix again, then gently heat the tube in a flame for about 30 s.
  
  **Acceptance criteria:** A deep pink or wine red color appears.

- **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 from the *Assay* (below).

**ASSAY**

- **Procedure**
  
  **Mobile phase:** Degassed water
Resolution solution: 4.8 mg/g each of sorbitol and USP Mannitol RS
Standard solution: 4.8 mg/g USP Mannitol RS
Sample solution: 5 mg/g in water
Chromatographic system, Appendix IIA
  Mode: High-performance liquid chromatography
  Detector: Refractive index
  Column: 10-cm × 7.8-mm column containing packing L34 (Bio-Rad Laboratories), or equivalent
  Column temperature: 50\(^\circ\) ± 2\(^\circ\), isothermal
  Detector temperature: 35\(^\circ\)
  Flow rate: About 0.7 mL/min
  Injection volume: About 10 µL
System suitability
  Sample: Resolution solution and Standard solution
  Suitability requirement 1: The resolution, \(R\), between sorbitol and mannitol from the Resolution solution is NLT 2.0.
  Suitability requirement 2: The relative standard deviation for replicate injections is NMT 2.0\% for the Standard solution.
Analysis: Separately inject the Sample solution and the Standard solution into the chromatograph, record the chromatograms, and measure the responses for the major peaks. [Note: The relative retention times are about 0.6 for mannitol and 1.0 for sorbitol.]
Calculate the percentage of \(\text{C}_6\text{H}_{14}\text{O}_6\) in the sample taken by the formula:

\[
\frac{[(C_S/C_U) \times (r_U/r_S) \times 10,000]/(100 - w)}
\]

\(C_S\) = Concentration (mg/g) of the Standard solution
\(C_U\) = Concentration (mg/g) of the Sample solution
\(r_U\) = Peak responses obtained from the chromatogram of the Sample solution
\(r_S\) = Peak responses obtained from the chromatogram of the Standard solution
\(w\) = Percent loss on drying determined below for the sample

Acceptance criteria: NLT 96.0\% and NMT 101.5\% of \(\text{C}_6\text{H}_{14}\text{O}_6\), 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
    Acceptance criteria: NMT 1 mg/kg

SPECIFIC TESTS
  • Loss on Drying, Appendix IIC (105\(^\circ\) for 4 h)
    Acceptance criteria: NMT 0.3\%
• pH, pH Determination Appendix IIB
  Sample: 10% (w/w) solution in carbon dioxide-free water
  Acceptance criteria: Between 4.0 and 7.5

Change to read:
• Reducing Sugars
  0.05 N Iodine VS: Dilute 0.1 N iodine VS with water (1:1).
  0.05N Sodium thiosulfate VS: Dilute 0.1 N sodium thiosulfate VS with water (1:1).

Sample: 3.3 g
Analysis: Dissolve the Sample in 25 mL of water with the aid of gentle heat. Cool the solution and add 20 mL alkaline cupric citrate TS and a few glass beads. Heat the solution 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 and 60 mL of water. While continuously shaking the solution, 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.

Acceptance criteria: NLT 12.8 mL of 0.05 N Sodium thiosulfate VS is required for the titration (NMT 0.3% of reducing sugars, as glucose)

• Residue on Ignition (Sulfated Ash), Appendix IIC
  Sample: 1.5 g
  Acceptance criteria: NMT 0.1%

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
FCC Sixth Edition Page 583
Phone Number: 1-301-816-8288
2-Methyl Butanal, *FCC 6* page 615—See briefing under *Acetaldehyde*.
(FIEC: J. Moore)  C64747

2-Methyl Butanal

\[
\text{C}_5\text{H}_{10}\text{O}
\]

**Formula wt 86.13**

**FEMA: 2691**

**DESCRIPTION**

*Change to read:*

2-Methyl Butanal occurs as a colorless to pale yellow liquid. It may contain a suitable antioxidant.

**Odor:** Chocolate

**Solubility** Soluble in propylene glycol, vegetable oils; insoluble or practically insoluble in water

**Boiling Point** $-93^\circ$

**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$_5$H$_{10}$O

**SPECIFIC TESTS**

- **ACID VALUE, M-15,** Appendix XI
  - **Acceptance criteria:** NMT 10.0

- **REFRACTIVE INDEX,** Appendix II (at 20$^\circ$)
  - **Acceptance criteria:** Between 1.388 and 1.393

- **Specific Gravity** Determine at 25$^\circ$ by any reliable method (see *General Provisions*).
  - **Acceptance criteria:** Between 0.799 and 0.804

**Auxiliary Information** — *Staff Liaison:* Jeffrey Moore, Ph.D., Senior Scientific Associate

*Expert Committee:* (FI07) Food Ingredients Expert Committee
3-Methyl Butanal, FCC 6 page 615—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

3-Methyl Butanal

Isovaleraldehyde

\[
\begin{align*}
&\text{CH}_3 \\
&\text{H}_3\text{C} \\
&\text{C}_5\text{H}_{10}\text{O}
\end{align*}
\]

Formula wt 86.13
FEMA: 2692

DESCRIPTION

Change to read:
3-Methyl Butanal occurs as a colorless to pale yellow liquid. It may contain a suitable antioxidant. 1S (FCC 6)

Odor Chocolate

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

Boiling Point ~93°

Solubility in Alcohol, Appendix VI
One mL dissolves in 1 mL of 95% ethanol.

Function Flavoring agent

IDENTIFICATION

• 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.

ASSAY

• Procedure Proceed as directed under M-1b, Appendix XI.
Acceptance criteria: NLT 97.0% of C₅H₁₀O

SPECIFIC TESTS

• ACID VALUE, M-15, Appendix XI
  Acceptance criteria: NMT 10.0

• REFRACTIVE INDEX, Appendix II (at 20°)
  Acceptance criteria: Between 1.388 and 1.391

• Specific Gravity Determine at 25° by any reliable method (see General Provisions).
  Acceptance criteria: Between 0.795 and 0.802

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
FCC Sixth Edition Page 615
Phone Number: 1-301-816-8288
**DESCRIPTION**

*Change to read:*

α-Methylcinnamaldehyde occurs as a yellow liquid. It may contain a suitable antioxidant. 1S (FCC 6)

**Odor** Cinnamon

**Solubility** Soluble in most fixed oils, propylene glycol; insoluble or practically insoluble in glycerin

**Boiling Point** \(\sim 148^\circ\) (27 mm Hg)

**Solubility in Alcohol**, Appendix VI

One mL dissolves in 3 mL of 70% alcohol, and remains clear on dilution.

**Function** Flavoring agent

**IDENTIFICATION**

- **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.
Acceptance criteria: NLT 97.0% of C\textsubscript{10}H\textsubscript{10}O (one major isomer)

SPECIFIC TESTS

- **ACID VALUE, M-15, Appendix XI**
  Acceptance criteria: NMT 5.0

- **REFRACTIVE INDEX, Appendix II (at 20°)**
  Acceptance criteria: Between 1.602 and 1.607

- **Specific Gravity** Determine at 25° by any reliable method (see General Provisions).
  Acceptance criteria: Between 1.035 and 1.039

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (Fl07) Food Ingredients Expert Committee
FCC Sixth Edition Page 619
Phone Number: 1-301-816-8288
BRIEFING

5-Methyl Furfural, FCC 6 page 627—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

5-Methyl Furfural

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{O} \\
\text{O} & \quad \text{H}
\end{align*}
\]

\( \text{C}_6\text{H}_6\text{O}_2 \)

Formula wt 110.11
FEMA: 2702

DESCRIPTION

Change to read:
5-Methyl Furfural occurs as a yellow to brown liquid. It may contain a suitable antioxidant.\textsuperscript{1S (FCC 6)}

Odor Nutty, caramel

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

Boiling Point \( \sim 187^\circ \)

Solubility in Alcohol, Appendix VI

One mL dissolves in 1 mL of 95% ethanol.

Function Flavoring agent

IDENTIFICATION

• 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.

  ![Infrared Spectrum](image)

ASSAY

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

  Acceptance criteria: NLT 97.0% of \( \text{C}_6\text{H}_6\text{O}_2 \)
SPECIFIC TESTS

• ACID VALUE, M-15, Appendix XI
  Acceptance criteria: NMT 5.0

• REFRACTIVE INDEX, Appendix II (at 20°)
  Acceptance criteria: Between 1.525 and 1.535

• Specific Gravity Determine at 25° by any reliable method (see General Provisions).
  Acceptance criteria: Between 1.095 and 1.110

Auxiliary Information— Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
FCC Sixth Edition Page 627
Phone Number: 1-301-816-8288
BRIEFING

5-Methyl-2-isopropyl-2-hexenal, FCC 6 page 634—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

5-Methyl-2-isopropyl-2-hexenal

Isodihydrolavandulal

\[
\text{C}_{10}\text{H}_{18}\text{O}
\]

Formula wt 154.25
FEMA: 3406

DESCRIPTION

\text{Change to read:}
5-Methyl-2-isopropyl-2-hexenal occurs as a slightly yellow liquid. \(\) It may contain a suitable antioxidant.

\(\text{1S (FCC 6)}\)

\text{Odor} Herbaceous, woody, fruity, chocolate

\text{Solubility} Soluble in alcohol, most fixed oils; insoluble or practically insoluble in water, propylene glycol

\text{Boiling Point} \sim 73^\circ\text{C} (10 \text{ mm Hg})

\text{Function} Flavoring agent

IDENTIFICATION

- INFRARED SPECTRA, Spectrophotometric Identification Tests, Appendix IIIC

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

ASSAY

- \text{Procedure} Proceed as directed under M-1a, Appendix XI.
Acceptance criteria: NLT 90.0% of C\textsubscript{10}H\textsubscript{18}O (sum of isomers)

SPECIFIC TESTS

- REFRACTIVE INDEX, Appendix II (at 20°)
  Acceptance criteria: Between 1.448 and 1.454

- SPECIFIC GRAVITY Determine at 25° by any reliable method (see General Provisions).
  Acceptance criteria: Between 0.842 and 0.850

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
FCC Sixth Edition Page 634
Phone Number: 1-301-816-8288
BRIEFING

5-Methyl 2-Phenyl 2-Hexenal, FCC 6 page 644—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

5-Methyl 2-Phenyl 2-Hexenal

\[
\begin{align*}
\text{CH}_3 & \quad \text{CH}_3 \\
\text{H}_3\text{C} & \quad \text{C} \\
\text{H}_3\text{C} & \quad \text{O} \\
\end{align*}
\]

\[\text{C}_{13}\text{H}_{16}\text{O}\]

Formula wt 188.27
FEMA: 3199

DESCRIPTION

*Change to read:*
5-Methyl 2-Phenyl 2-Hexenal occurs as a colorless to pale yellow liquid. It may contain a suitable antioxidant.

- 1S (FCC 6)

*Odor* Cocoa

*Solubility* Soluble in propylene glycol, vegetable oils; insoluble or practically insoluble in water

*Boiling Point* \(\sim 89^\circ\text{C}\) (26 mm Hg)

*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 92.0% of \(\text{C}_{13}\text{H}_{16}\text{O}\) (sum of (E)- and (Z)-isomers)

SPECIFIC TESTS

- *ACID VALUE, M-15, Appendix XI*
  
  *Acceptance criteria:* NMT 4.0

- *REFRACTIVE INDEX, Appendix II (at 20\(^\circ\))*
  
  *Acceptance criteria:* Between 1.529 and 1.536

- *Specific Gravity* Determine at 25\(^\circ\) by any reliable method (see General Provisions).
  
  *Acceptance criteria:* Between 0.963 and 0.979

Auxiliary Information—*Staff Liaison:* Jeffrey Moore, Ph.D., Senior Scientific Associate
3-Methylthiopropionaldehyde, *FCC 6* page 649—See briefing under *Acetaldehyde*.

(FIEC: J. Moore) C64747

### 3-Methylthiopropionaldehyde

Methional

![Chemical structure of 3-Methylthiopropionaldehyde](image)

C₄H₈O₅S

**Formula wt 104.17**  
FEMA: 2747

### DESCRIPTION

*Change to read:*

3-Methylthiopropionaldehyde occurs as a colorless to pale yellow liquid. ■ It may contain a suitable antioxidant.  
■ 1S (FCC 6)

**Odor** Meaty potato

**Boiling Point** ~ 165° to 166°

**Solubility in Alcohol,** Appendix VI  
One mL dissolves in 1 mL of 95% alcohol.

**Function** Flavoring agent

### IDENTIFICATION

- **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.

![Infrared spectrum](image)

### ASSAY

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

  **Acceptance criteria:** NLT 98.0% of C₄H₈O₅S
SPECIFIC TESTS

• **REFRACTIVE INDEX**, Appendix II (at 20°)
  
  **Acceptance criteria**: Between 1.484 and 1.493

• **Specific Gravity** Determine at 25° by any reliable method (see *General Provisions*).
  
  **Acceptance criteria**: Between 1.038 and 1.048

**Auxiliary Information**— *Staff Liaison*: Jeffrey Moore, Ph.D., Senior Scientific Associate

*Expert Committee*: (FI07) Food Ingredients Expert Committee

*FCC Sixth Edition Page 649*

*Phone Number*: 1-301-816-8288
BRIEFING

2-Methylundecanal, FCC 6 page 650—See briefing under Acetaldehyde. 
(FIEC: J. Moore) C64747

2-Methylundecanal

Aldehyde C-12 MNA

Methyl n-Nonyl Acetaldehyde

\[
\text{Formula wt 184.32} \\
\text{FEMA: 2749}
\]

DESCRIPTION

Change to read:

2-Methylundecanal occurs as a colorless to slightly yellow liquid. It may contain a suitable antioxidant.

1S (FCC 6)

Odor Fatty

Solubility Soluble in most fixed oils, alcohol, propylene glycol (may be turbid); insoluble or practically insoluble in glycerin

Boiling Point \( \sim 171^\circ \)

Function Flavoring agent

IDENTIFICATION

• 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.

ASSAY

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

Acceptance criteria: NLT 94.0% of \( \text{C}_{12}\text{H}_{24}\text{O} \)
SPECIFIC TESTS

• **ACID VALUE, M-15, Appendix XI**
  Acceptance criteria: NMT 10.0

• **REFRACTIVE INDEX, Appendix II (at 20°)**
  Acceptance criteria: Between 1.431 and 1.436

• **SPECIFIC GRAVITY** Determine at 25° by any reliable method (see General Provisions).
  Acceptance criteria: Between 0.822 and 0.830

**Auxiliary Information**— *Staff Liaison*: Jeffrey Moore, Ph.D., Senior Scientific Associate

*Expert Committee*: (F107) Food Ingredients Expert Committee

*FCC Sixth Edition Page 650*

*Phone Number*: 1-301-816-8288
**BRIEFING**

**Myrcene, FCC 6 page 660**—See briefing under Acetaldehyde.

(FIEC: J. Moore) C64747

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**Myrcene**

7-Methyl-3-methylene-1,6-octadiene

![Chemical Structure](image)

\[ C_{10}H_{16} \]

**Formula wt 136.24**

**FEMA: 2762**

---

**DESCRIPTION**

*Change to read:*

Myrcene occurs as a colorless to pale yellow liquid. It may contain a suitable antioxidant.

**Odor** Sweet, balsamic

**Solubility** Soluble in alcohol, most fixed oils; insoluble or practically insoluble in water

**Boiling Point** \( \sim 167^\circ \)

**Function** Flavoring agent

---

**ASSAY**

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

**Acceptance criteria:** NLT 90.0% of \( C_{10}H_{16} \)

---

**SPECIFIC TESTS**

**REFRACTIVE INDEX,** Appendix II (at \( 20^\circ \))

**Acceptance criteria:** Between 1.466 and 1.471

**SPECIFIC GRAVITY** Determine at \( 25^\circ \) by any reliable method (see General Provisions).

**Acceptance criteria:** Between 0.789 and 0.793

---

**OTHER REQUIREMENTS**

**PEROXIDE VALUE,** M-11, Appendix XI

**Acceptance criteria:** NMT 50.0

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**Auxiliary Information**— *Staff Liaison:* Jeffrey Moore, Ph.D., Senior Scientific Associate

*Expert Committee:* (FI07) Food Ingredients Expert Committee

*FCC Sixth Edition Page 660*

*Phone Number:* 1-301-816-8288
(E),(E)-2,4-Nonadienal, FCC 6 page 679—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

(E),(E)-2,4-Nonadienal

trans,trans-2,4-Nonadienal

\[ \text{C}_9\text{H}_{14}\text{O} \]

Formula wt 138.21
FEMA: 3212

DESCRIPTION

*Change to read:* (E),(E)-2,4-Nonadienal occurs as a slightly yellow liquid. It may contain a suitable antioxidant.

**Odor** Strong, fatty, floral  
**Solubility** Soluble in alcohol, most fixed oils; insoluble or practically insoluble in water  
**Boiling Point** \(-97^\circ\) (10 mm Hg)  
**Solubility in Alcohol**, Appendix VI  
One mL dissolves in 1 mL of 95% ethanol.  
**Function** Flavoring agent

IDENTIFICATION

* 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.

ASSAY

* **Procedure** Proceed as directed under M-1a, Appendix XI.  
**Acceptance criteria:** NLT 89.0% of C\(_9\)H\(_{14}\)O (one major isomer)

SPECIFIC TESTS

* **REFRACTIVE INDEX**, Appendix II (at 20\(^\circ\))  
**Acceptance criteria:** Between 1.517 and 1.523
- **Specific Gravity** Determine at 25° by any reliable method (see General Provisions).
  
  **Acceptance criteria:** Between 0.865 and 0.880

**Auxiliary Information—** Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate

*Expert Committee:* (FI07) Food Ingredients Expert Committee

*FCC Sixth Edition Page 679*

*Phone Number:* 1-301-816-8288
BRIEFING

\((E),(Z)-2,6-\text{Nonadienal}, \text{ FCC} \ 6 \text{ page 680—See briefing under Acetaldehyde.} \)
(FIEC: J. Moore) C64747

\((E),(Z)-2,6-\text{Nonadienal} \)

trans,cis-2,6-Nonadienal

\[
\begin{align*}
\text{C}_9\text{H}_{14}\text{O} \\
\text{Formula wt} 138.21 \\
\text{FEMA: 3377}
\end{align*}
\]

DESCRIPTION

\textit{Change to read:}

\((E),(Z)-2,6-\text{Nonadienal} \) occurs as a slightly yellow liquid. It may contain a suitable antioxidant. \(1S \text{ (FCC 6)} \)

\textbf{Odor} Powerful, violet, cucumber

\textbf{Solubility} Soluble in alcohol, most fixed oils; insoluble or practically insoluble in water

\textbf{Boiling Point} \(-94^\circ \text{ (18 mm Hg)} \)

\textbf{Solubility in Alcohol, Appendix VI}

One mL dissolves in 1 mL of 95% ethanol.

\textbf{Function} Flavoring agent

IDENTIFICATION

\textbf{• INFRARED SPECTRA, Spectrophotometric Identification Tests, Appendix IIIC}

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

\begin{center}
\includegraphics[width=0.5\textwidth]{spectrum.png}
\end{center}

\((E),(Z)-2,6-\text{Nonadienal} \)

ASSAY

\textbf{• Procedure} Proceed as directed under M-1a, Appendix XI

\textbf{Acceptance criteria}

\textbf{Sum of two isomers:} NLT 96.0% of \(\text{C}_9\text{H}_{14}\text{O}\)

\textbf{Major isomer:} NLT 90% of \(\text{C}_9\text{H}_{14}\text{O}\)
SPECIFIC TESTS

- **REFRACTIVE INDEX**, Appendix II (at 20°)
  
  **Acceptance criteria:** Between 1.470 and 1.475

- **Specific Gravity** Determine at 25° by any reliable method (see General Provisions).
  
  **Acceptance criteria:** Between 0.850 and 0.870

**Auxiliary Information**— *Staff Liaison:* Jeffrey Moore, Ph.D., Senior Scientific Associate

*Expert Committee:* (FI07) Food Ingredients Expert Committee

_FCC Sixth Edition_ Page 680

_Phone Number:_ 1-301-816-8288
BRIEFING

Nonanal, FCC 6 page 683—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

Nonanal

Aldehyde C-9

Pelargonic Aldehyde

\[
\text{C}_9\text{H}_{18}\text{O}
\]

Formula wt 142.24
FEMA: 2782

DESCRIPTION

*Change to read:*

Nonanal occurs as a colorless to light yellow liquid. It may contain a suitable antioxidant. 1S (FCC 6)

**Odor** Fatty; citrus–rose on dilution

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

**Boiling Point** ~93\(^\circ\) (23 mm Hg)

**Function** Flavoring agent

IDENTIFICATION

- **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.

  ![Infrared Spectrum of Nonanal](image)

ASSAY

- **Procedure** Proceed as directed under M-1b, Appendix XI.
  
  **Acceptance criteria:** NLT 92.0% of C\(_9\)H\(_{18}\)O

SPECIFIC TESTS

- **ACID VALUE**, M-15, Appendix XI
  
  **Acceptance criteria:** NMT 10.0
• **REFRACTIVE INDEX**, Appendix II (at 20°)
  
  **Acceptance criteria:** Between 1.422 and 1.429

• **SPECIFIC GRAVITY** Determine at 25° by any reliable method (see General Provisions).
  
  **Acceptance criteria:** Between 0.820 and 0.830

**Auxiliary Information**—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate

*Expert Committee:* (FI07) Food Ingredients Expert Committee

*FCC Sixth Edition Page 683*

*Phone Number:* 1-301-816-8288
(E)-2-Nonenal, FCC 6 page 685—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

(E)-2-Nonenal

trans-2-Nonenal

\[
\text{C}_9\text{H}_{16}\text{O}
\]

Formula wt 140.22
FEMA: 3213

DESCRIPTION

Change to read:
(E)-2-Nonenal occurs as a white to slightly yellow liquid. It may contain a suitable antioxidant.

Odor Fatty, violet

Solubility Soluble in alcohol, most fixed oils; insoluble or practically insoluble in water

Boiling Point \( \sim 88^\circ \) (12 mm Hg)

Solubility in Alcohol, Appendix VI
One mL dissolves in 1 mL of 95% ethanol.

Function Flavoring agent

IDENTIFICATION

• 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.

ASSAY

• Procedure Proceed as directed under M-1a, Appendix XI.
  Acceptance criteria: NLT 92.0% of C\(_9\)H\(_{16}\)O (one major isomer)

SPECIFIC TESTS

• REFRACTIVE INDEX, Appendix II (at 20\(^\circ\))
Acceptance criteria: Between 1.450 and 1.460

- **Specific Gravity** Determine at 25° by any reliable method (see General Provisions).
  
  **Acceptance criteria:** Between 0.840 and 0.850

**Auxiliary Information**— *Staff Liaison:* Jeffrey Moore, Ph.D., Senior Scientific Associate

*Expert Committee:* (FI07) Food Ingredients Expert Committee

*FCC Sixth Edition* Page 685

*Phone Number:* 1-301-816-8288
BRIEFING

Octanal, **FCC 6 page 693**—See briefing under *Acetaldehyde*.
(FIEC: J. Moore) C64747

Octanal

Aldehyde C-8

Caprylic Aldehyde

\[ \text{C}_8\text{H}_{16}\text{O} \]

**Formula wt 128.21**

FEMA: 2797

**DESCRIPTION**

*Change to read:*

Octanal occurs as a colorless to light yellow liquid. It may contain a suitable antioxidant. 1S (FCC 6)

**Odor** Fatty-orange

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

**Boiling Point** 171°

**Function** Flavoring agent

**IDENTIFICATION**

- **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.

![Infrared spectrum of Octanal](image)

**ASSAY**

- **PROCEDURE** Proceed as directed under M-1b, Appendix XI.
  
  **Acceptance criteria:** NLT 92.0% of C₈H₁₆O

**SPECIFIC TESTS**

- **ACID VALUE, M-15, Appendix XI**
  
  **Acceptance criteria:** NMT 10.0
• **REFRACTIVE INDEX**, Appendix II (at 20°)
  *Acceptance criteria:* Between 1.417 and 1.425

• **SPECIFIC GRAVITY** Determine at 25° by any reliable method (see *General Provisions*).
  *Acceptance criteria:* Between 0.810 and 0.830

**Auxiliary Information**— *Staff Liaison:* Jeffrey Moore, Ph.D., Senior Scientific Associate
*Expert Committee:* (FI07) Food Ingredients Expert Committee
*FCC Sixth Edition* Page 693
*Phone Number:* 1-301-816-8288
BRIEFING

(E)-2-Octen-1-ol, FCC 6 page 695—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

(E)-2-Octen-1-al

trans-2-Octen-1-al

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{C}_8\text{H}_{14}\text{O} \\
\text{trans-2-Octen-1-al} & \\
\text{Formula wt} & \text{126.20} \\
\text{FEMA:} & \text{3215}
\end{align*}
\]

DESCRIPTION

Change to read:

(E)-2-Octen-1-al occurs as a slightly yellow liquid. It may contain a suitable antioxidant.

Odor Fatty, green
Solubility Soluble in alcohol, most fixed oils; slightly soluble in water
Boiling Point \(-84^\circ\) (19 mm Hg)
Function Flavoring agent

IDENTIFICATION

• 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.

\[(E)-2-Octen-1-al\]

ASSAY

• PROCEDURE Proceed as directed under M-1a, Appendix XI (as (E)-isomer).
  Acceptance criteria: NLT 92.0% of C\(_8\)H\(_{14}\)O

SPECIFIC TESTS

• REFRACTIVE INDEX, Appendix II (at 20°)
  Acceptance criteria: Between 1.450 and 1.455

• SPECIFIC GRAVITY Determine at 25° by any reliable method (see General Provisions).
Acceptance criteria: Between 0.830 and 0.850

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
FCC Sixth Edition Page 695
Phone Number: 1-301-816-8288
BRIEFING

2,3-Pentanedione, FCC 6 page 730—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

2,3-Pentanedione

Acetyl Propionyl

\[
\text{C}_5\text{H}_8\text{O}_2
\]

Formula wt 100.12
FEMA: 2841

DESCRIPTION

Change to read:
2,3-Pentanedione occurs as a yellow to yellow-green liquid. It may contain a suitable antioxidant. \(1\S\text{ (FCC 6)}\)

Odor Penetrating, buttery on dilution

Solubility Miscible in alcohol, propylene glycol, most fixed oils; insoluble or practically insoluble in glycerin, water

Boiling Point \(\sim 108^\circ\)

Solubility in Alcohol, Appendix VI
One mL dissolves in 3 mL of 50% alcohol.

Function Flavoring agent

IDENTIFICATION

• INFRARED SPECTRA, Spectrophotometric Identification Tests, Appendix III C

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

ASSAY
• **Procedure** Proceed as directed under *M-1b, Appendix XI*.  
  **Acceptance criteria:** NLT 93.0% of C$_5$H$_8$O$_2$

**SPECIFIC TESTS**

• **REFRACTIVE INDEX, Appendix II (at 20°)**  
  **Acceptance criteria:** Between 1.402 and 1.406

• **Specific Gravity** Determine at 25° by any reliable method (see *General Provisions*).  
  **Acceptance criteria:** Between 0.952 and 0.962

**Auxiliary Information**— *Staff Liaison:* Jeffrey Moore, Ph.D., Senior Scientific Associate  
*Expert Committee:* (Fl07) Food Ingredients Expert Committee  
*FCC Sixth Edition Page 730*  
*Phone Number:* 1-301-816-8288
**BRIEFING**

α-Phellandrene, *FCC 6* page 739—See briefing under Acetaldehyde.

(FIEC: J. Moore) C64747

α-Phellandrene

*p*-Mentha-1,5-diene

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{CH}_3 \\
\text{CH}_3 & \\
\end{align*}
\]

\( \text{C}_{10}\text{H}_{16} \)

**DESCRIPTION**

*Change to read:*

α-Phellandrene occurs as a colorless to slightly yellow liquid. It may contain a suitable antioxidant. \( 1 \text{S (FCC 6)} \)

**Odor** Herbaceous; minty background

**Solubility** Soluble in alcohol; insoluble or practically insoluble in water

**Solubility in Alcohol, Appendix VI**

One mL dissolves in 1 mL of 95% alcohol to give a clear solution.

**Function** Flavoring agent

**IDENTIFICATION**

- **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.

  ![Infrared Spectrum](image)

  **α-Phellandrene**

**SPECIFIC TESTS**
• **REFRACTIVE INDEX**, Appendix II (at 20°)
  Acceptance criteria: Between 1.471 and 1.477

• **Specific Gravity** Determine at 25° by any reliable method (see General Provisions).
  Acceptance criteria: Between 0.835 and 0.865

**OTHER REQUIREMENTS**

• **Angular Rotation**, Optical (Specific) Rotation, Appendix IIB (Use a 100-mm tube.)
  Acceptance criteria: Between −80° and −120°

**Auxiliary Information**—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate

Expert Committee: (FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 739

Phone Number: 1-301-816-8288
Phenylacetaldehyde, *FCC 6* page 747—See briefing under *Acetaldehyde*.

(FIEC: J. Moore)  C64747

Phenylacetaldehyde

α-Toluic Aldehyde

\[ \text{C}_8\text{H}_8\text{O} \]

**Formula wt 120.15**

**FEMA: 2874**

**DESCRIPTION**

*Change to read:*

Phenylacetaldehyde occurs as a colorless to slightly yellow, oily liquid; becomes more viscous on aging. It may contain a suitable antioxidant. 1S (FCC 6)

**Odor** Harsh; hyacinth on dilution

**Solubility** Soluble in most fixed oils, propylene glycol; insoluble or practically insoluble in glycerin

**Boiling Point** \( -195^\circ \)

**Solubility in Alcohol, Appendix VI**

One mL dissolves in 2 mL of 80% alcohol.

**Function** Flavoring agent

**IDENTIFICATION**

- **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.

**ASSAY**

- **Procedure** Proceed as directed under *M-1b, Appendix XI*. 
Acceptance criteria: NLT 90.0% of C₉H₈O₈

SPECIFIC TESTS

• **ACID VALUE, M-15, Appendix XI**
  Acceptance criteria: NMT 5.0

• **REFRACTIVE INDEX, Appendix II (at 20°)**
  Acceptance criteria: Between 1.525 and 1.545

• **Specific Gravity** Determine at 25° by any reliable method (see General Provisions).
  Acceptance criteria: Between 1.025 and 1.045

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
FCC Sixth Edition Page 747
Phone Number: 1-301-816-8288
2-Phenylpropionaldehyde, FCC 6 page 757—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

2-Phenylpropionaldehyde

Hydratropic Aldehyde

α-Methyl Phenylacetaldehyde

\[
\text{C}_9\text{H}_{10}\text{O}
\]

Formula wt 134.18
FEMA: 2886

DESCRIPTION

Change to read:
2-Phenylpropionaldehyde occurs as a water-white to pale yellow liquid. It may contain a suitable antioxidant.

1S (FCC 6)

Odor Floral

Solubility Soluble in most fixed oils; slightly soluble in propylene glycol; insoluble or practically insoluble in glycerin

Boiling Point \(\sim 222^\circ\)

Function Flavoring agent

IDENTIFICATION

• 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.
ASSAY

• PROCEDURE Proceed as directed under M-1b, Appendix XI.
   Acceptance criteria: NLT 95.0% of C₉H₁₀O

SPECIFIC TESTS

• ACID VALUE, M-15, Appendix XI
   Acceptance criteria: NMT 5.0

• REFRACTIVE INDEX, Appendix II (at 20°)
   Acceptance criteria: Between 1.515 and 1.520

• SPECIFIC GRAVITY Determine at 25° by any reliable method (see General Provisions).
   Acceptance criteria: Between 0.998 and 1.006

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
FCC Sixth Edition Page 757
Phone Number: 1-301-816-8288
3-Phenylpropionaldehyde

Hydrocinnamaldehyde

Phenylpropyl Aldehyde

\[ 
\text{C}_9\text{H}_{10}\text{O} 
\]

**DESCRIPTION**

*Change to read:*

3-Phenylpropionaldehyde occurs as a colorless to slightly yellow liquid. It may contain a suitable antioxidant.  
*1S (FCC 6)*

**Odor** Strong, pungent, floral, hyacinth

**Solubility** Miscible in alcohol, ether; insoluble or practically insoluble in water

**Boiling Point** 97° to 98° (12 mm Hg)

**Solubility in Alcohol,** Appendix VI

One mL dissolves in 7 mL of 60% alcohol, and remains clear on dilution.

**Function** Flavouring agent

**IDENTIFICATION**

* 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.
ASSAY

• **Procedure** Proceed as directed under M-1b, Appendix XI.
  
  **Acceptance criteria:** NLT 90.0% of aldehydes

SPECIFIC TESTS

• **ACID VALUE, M-15**, Appendix XI
  
  **Acceptance criteria:** NMT 10.0.

• **REFRACTIVE INDEX**, Appendix II (at 20°)
  
  **Acceptance criteria:** Between 1.520 and 1.532

• **Specific Gravity** Determine at 25° by any reliable method (see *General Provisions*).
  
  **Acceptance criteria:** Between 1.010 and 1.020

OTHER REQUIREMENTS

• **CHLORINATED COMPOUNDS**, Appendix VI
  
  **Acceptance criteria:** Passes test.

**Auxiliary Information**— *Staff Liaison*: Jeffrey Moore, Ph.D., Senior Scientific Associate
*Expert Committee*: (FI07) Food Ingredients Expert Committee

*FCC Sixth Edition Page 758*

*Phone Number: 1-301-816-8288*
BRIEFING

Phosphoric Acid, FCC 6 page 761.

1. On the basis of comments received, it is proposed to revise the Fluoride test procedure by deleting the Control. Use of the existing Control would not allow the analyst to test to the limit required for this monograph. The use of the existing control described in Fluoride Limit Test, Method IV, Appendix IIIB is now required. It is proposed to revise the Fluoride test procedure by adding an Analysis section directing analysts to use Buffer Solution B which is a new buffer solution being proposed for addition to Fluoride Limit Test, Method IV, Appendix IIIB. See briefing under Appendix III, Chemical Tests and Determinations for the proposal to add this buffer solution.

2. It is also proposed to revise the Lead test procedure—see briefing under Manganese Sulfate. Additionally, it should be noted that the sample size referenced in FCC 5 and 6 for this test procedure (10 g), was an error as it did not test to the 3 mg/kg limit. Interested parties are encouraged to submit comments to Kristie Bowman.

(FIEC: K. Bowman) C62645; C65234

Phosphoric Acid

Orthophosphoric Acid

\[ \text{H}_3\text{PO}_4 \]  

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
- **\text{PHOSPHATE}, Appendix IIIA**
  - Sample solution: 100 mg/mL
  - Acceptance criteria: Passes tests.

ASSAY
- **\text{PROCEDURE**
  - Sample: 1.5 g
  - Analysis: Transfer the Sample into a tared glass-stoppered flask and dilute to 120 mL with water. 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$_3$PO$_4$.
  - 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**
  - **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
  - **Control:** 0.2 mL of 50 mg/kg Fluoride Limit Solution
  - **Analysis:** Proceed as directed using Buffer Solution B. 1S (FCC 6)
  - **Acceptance criteria:** NMT 10 mg/kg

- **Lead, Lead Limit Test, APDC Extraction Method, Appendix IIIB**
  - **Sample:** 10 g
  - **Acceptance criteria:** NMT 3 mg/kg

**OTHER REQUIREMENTS**
• **LABELING** Indicate the percent or the percent range of phosphoric acid (H₃PO₄).

**Auxiliary Information**— *Staff Liaison:* Kristie Bowman, Senior Scientific Associate  
*Expert Committee:* (FI07) Food Ingredients Expert Committee  
*FCC Sixth Edition* Page 761  
*Phone Number:* 1-301-816-8356
BRIEFING

α-Pinene, FCC 6 page 766—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

α-Pinene

2,6,6-Trimethylbicyclo(3.1.1)hept-2-ene

2-Pinene

1-α-Pinene

C₁₀H₁₆  

Formula wt 136.24  
FEMA: 2902

DESCRIPTION

Change to read:

α-Pinene occurs as a colorless liquid. It may contain a suitable antioxidant.  

Odor Fresh, piney  
Solubility Soluble in alcohol, most fixed oils; insoluble or practically insoluble in water  
Boiling Point —155°C  
Solubility in Alcohol, Appendix VI  
One mL dissolves in 3 mL of 95% ethanol.  
Function Flavoring agent

ASSAY

• Procedure Proceed as directed under M-1a, Appendix XI.
Acceptance criteria: NLT 97.0% of C_{10}H_{16}

SPECIFIC TESTS

• **REFRACTIVE INDEX**, Appendix II (at 20°)
  
  Acceptance criteria: Between 1.464 and 1.468

• **Specific Gravity** Determine at 25° by any reliable method (see General Provisions).
  
  Acceptance criteria: Between 0.855 and 0.860

OTHER REQUIREMENTS

• **ANGULAR ROTATION, Optical (Specific) Rotation**, Appendix II B
  
  Acceptance criteria: Between −20° and −50°

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
FCC Sixth Edition Page 766
Phone Number: 1-301-816-8288
**BRIEFING**

\[ \beta\text{-Pinene}, \text{ FCC 6 page 766—See briefing under Acetaldehyde.} \]

(FIEC: J. Moore) C64747

\[ \beta\text{-Pinene} \]

6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane

\[
\begin{align*}
\text{CH}_2 & \\
\text{H}_3\text{C} & \\
\text{H}_3\text{C} & \\
\end{align*}
\]

\[ \text{C}_{10}\text{H}_{16} \]

Formula wt 136.24

FEMA: 2903

**DESCRIPTION**

*Change to read:*

\[ \beta\text{-Pinene occurs as a colorless liquid. It may contain a suitable antioxidant.} \]

*Odor* Resinous-piney

*Solubility* Soluble in most fixed oils; insoluble or practically insoluble in water, propylene glycol

*Boiling Point* \( \sim 165^\circ \)

*Solubility in Alcohol*, Appendix VI

One mL dissolves in 3 mL of 95% ethanol.

*Function* Flavoring agent

**ASSAY**

- **Procedure** Proceed as directed under M-1a, Appendix XI.
  - **Acceptance criteria:** NLT 97.0% of \( \text{C}_{10}\text{H}_{16} \)

**SPECIFIC TESTS**

- **REFRACTIVE INDEX**, Appendix II (at 20°)
Acceptance criteria: Between 1.477 and 1.481

- **Specific Gravity** Determine at 25° by any reliable method (see General Provisions).
  
  Acceptance criteria: Between 0.867 and 0.871

**OTHER REQUIREMENTS**

- **ANGULAR ROTATION, Optical (Specific) Rotation, Appendix II B**

  Acceptance criteria: Between $-15^\circ$ and $-30^\circ$

**Auxiliary Information**—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate

*Expert Committee:* (F107) Food Ingredients Expert Committee

*FCC Sixth Edition* Page 766

*Phone Number:* 1-301-816-8288
BRIEFING

Polyvinylpolypyrrolidone, FCC 6 page 794.

1. On the basis of comments received, it is proposed to revise the title of this monograph to improve nomenclature consistency for this material among FCC, USP–NF, and other compendia. It is also proposed to revise the Description and synonyms list accordingly. To aid with the transition, monographs with both titles (Polyvinylpolypyrrolidone and Crospovidone) would appear in the First Supplement to FCC 6 with an explanatory note, and one monograph with the title of Crospovidone would appear in FCC 7, with appropriate cross-references, in the index. It should be noted that FCC specifications for this material apply equally to substances bearing the title names, and to substances bearing the synonyms, as described under the General Provisions to FCC 6 page 1.

2. For the NOTE in the Identification test procedure, it is proposed to replace the reference to polyvinylpyrrolidone with a reference to povidone (See briefing under Polyvinylpyrrolidone).

3. On the basis of comments received, it is proposed to reduce the current acceptable levels of vinylpyrrolidinone in the test for Unsaturation (As Vinylpyrrolidinone) from 1000 ppm to 10 ppm or less, in order to minimize the safety risks associated with higher levels of the monomer. The proposed limit is consistent with the Stage 4 Pharmacopeial Discussion Group (PDG) harmonization draft proposal. Also, it is proposed to replace the current titrimetric test procedure for Unsaturation (As Vinylpyrrolidinone) with a high-performance liquid chromatography test procedure consistent with the Stage 4 PDG harmonization draft proposal, but using a C18 column, as indicated in the European Pharmacopoeia 6.1 monograph.

4. On the basis of comments received, it is proposed to revise the porosity of the filter media in the test procedure for Water Soluble Substances to make it consistent with the Crospovidone monographs in NF 25 and European Pharmacopoeia 6.1. Also, it is proposed to revise the acceptance criteria for the Water Soluble Substances test to make it consistent with the Crospovidone monographs in NF 25 and the European Pharmacopoeia 6.1.

Interested parties are encouraged to submit comments to Jeff Moore, Ph.D.
(FIEC: J. Moore) C65002

Polyvinylpolypyrrolidone
Crospovidone 1S (FCC 6)

PVPP

1-Vinyl-2-pyrrolidone Crosslinked Insoluble Polymer
DESCRIPTION

Change to read:
Polyvinylpolypyrrolidone [Crosplidone 1S (FCC 6)] 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

Change to read:

• 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 1S (FCC 6), 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: NLT 11.0% and NMT 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 (SULFAED ASH), Appendix IIC
  Sample: 2 g
  Acceptance criteria: NMT 0.4%

Change to read:

• UNSATURATION (AS VINYLPYRROLIDONE)
  Sample: 4 g
  Analysis: Suspend the Sample in 30 mL of water, stir for 15 min, and filter through a sintered glass filter having porosity between 9 and 15 µm, collecting the filtrate in a 250 mL flask. Wash the residue with 100 mL of water, add 500 mg of sodium acetate to the combined filtrates, and titrate with 0.1 N iodine until the color of iodine no longer fades. Add an additional 3.0 mL of 0.1 N iodine, allow to stand for 10 min, and titrate the excess iodine with 0.1 N sodium thiosulfate, adding 3 mL of starch TS as the endpoint is approached. Perform a blank determination (see General Provisions), and make any necessary correction.
  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 II A
  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
**Sample:** 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 vinylpyrrolidone in the sample by the formula:

$$1000\left(\frac{C}{W}\right)\left(\frac{r_U}{r_S}\right)$$

- $C =$ Concentration (mg/mL) of vinylpyrrolidone in the Standard solution
- $W =$ Weight (mg) of sample taken to prepare the Sample solution
- $r_U =$ Peak area response for vinylpyrrolidone obtained from the Sample solution
- $r_S =$ Peak area responses for vinylpyrrolidone obtained from the Standard solution

**Acceptance criteria:**
- NMT 0.72 mL of 0.1 N sodium thiosulfate is consumed (NMT 0.1%)
- NMT 0.001%

**Acceptance criteria:**
- Water, Water Determination, Appendix IIB
- 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 on a filter screen with a porosity of 2.5 µm, then on a filter screen with a porosity of 0.8 µm. Filter through a membrane filter having a 0.45-µm porosity, protected against clogging by superimposing a membrane filter having a 3-µm porosity. Evaporate the filtrate over a water bath until dry.

**Acceptance criteria:** The residue left by evaporating the filtrate is less than 50 mg. (NMT 0.5%)

**Auxiliary Information—Staff Liaison:** Kristie Bowman, Senior Scientific Associate

*Expert Committee:* (FI07) Food Ingredients Expert Committee

*FCC Sixth Edition Page 794*

*Phone Number:* 1-301-816-8356
**BRIEFING**

**Polyvinylpyrrolidone,** *FCC* 6 page 795. On the basis of comments received, it is proposed to revise the title of this monograph to improve nomenclature consistency for this material among *FCC, USP–NF,* and other compendia. It is also proposed to revise the *Description* and synonyms list accordingly. To aid with the transition, monographs with both titles (*Polyvinylpyrrolidone* and *Povidone*) would appear in the *First Supplement to FCC* 6 with an explanatory note, and one monograph with the title of *Povidone* would appear in *FCC* 7 with appropriate cross-references in the index. It should be noted that *FCC* specifications for this material apply equally to substances bearing the title names and to substances bearing the synonyms as described under the *General Provisions to FCC* 6, page 1. Interested parties are encouraged to submit comments to Jeff Moore, Ph.D., email jm@usp.org.

(FIEC: J. Moore) C65002

---

**Change to read:**

*Polyvinylpyrrolidone* $\rightarrow$ *Povidone* 1S (*FCC* 6)

**Change to read:**

PVP

*Povidone*

$\rightarrow$

*Polyvinylpyrrolidone*

1S (*FCC* 6)

Poly[1-(2-oxo-1-pyrrolidinyl)ethylene]

![Chemical Structure](image)

$(\text{C}_6\text{H}_9\text{NO})_x$

Lower mol wt range product $\sim 40,000$

Higher mol wt range product $\sim 360,000$

INS: 1201

CAS: [9003-39-8]
DESCRIPTION

Change to read:
Polyvinylpyrrolidone (Povidone 1S (FCC 6)) occurs as a white to tan powder. It is a polymer of purified 1-vinyl-2-pyrrolidone produced catalytically. It is soluble in water, in alcohol, and in chloroform, and is insoluble in ether. The pH of a 1:20 aqueous solution is between 3 and 7.

Function Clarifying agent; separation/filtration aid; stabilizer; bodying agent; tableting aid; dispersant; coating on fresh fruit

Packaging and Storage Store in tight containers.

IDENTIFICATION

- A. Procedure
  Sample solution: 20 mg/mL
  Analysis: Add 20 mL of 1 N hydrochloric acid and 5 mL of potassium dichromate TS to 10 mL of Sample solution.
  Acceptance criteria: An orange-yellow precipitate forms.

- B. Procedure
  Sample solution: 20 mg/mL
  Sample: Add 5 mL of Sample solution to 75 mg of cobalt nitrate and 300 mg of ammonium thiocyanate dissolved in 2 mL of water, mix, and make the resulting solution acid with 2.7 N hydrochloric acid.
  Acceptance criteria: A pale blue precipitate forms.

- C. Procedure
  Sample solution: 5 mg/mL
  Sample: Add a few drops of iodine TS to 5 mL of Sample solution.
  Acceptance criteria: A deep red color appears.

ASSAY

- Nitrogen, Nitrogen Determination, Method II, Appendix IIIC
  Sample: 100 mg
  Analysis: In the wet-digestion step, omit the use of hydrogen peroxide, and use 5 g of a 33:1:1 mixture of potassium sulfate–cupric sulfate–titanium dioxide instead of the 10:1 potassium sulfate–cupric sulfate mixture. Heat until a clear, light green solution appears, heat for an additional 45 min, and continue as directed, beginning with “Cautiously add 20 mL of water, cool, then…,” except use 70 mL of water instead of 20.
  Acceptance criteria: NLT 11.5% and NMT 12.8% as nitrogen (N), 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 2 mg/kg

Change to read:

Organic Impurities
- Aldehydes (as Acetaldehyde)
  Phosphate buffer: Transfer 50.0 g of potassium pyrophosphate into a 500-mL volumetric flask, and dissolve in 400 mL of water. Adjust, if necessary, to a pH of 9.0 with 1 N hydrochloric acid, dilute to volume, and
mix.

**Aldehyde dehydrogenase solution:** Transfer a quantity of lyophilized aldehyde dehydrogenase (Sigma A550, or equivalent) equivalent to 70 units into a glass vial, dissolve it in 10.0 mL of water, and mix.

[Note: This solution is stable for 8 h at 4°.]

**NAD solution:** 4.0 mg/mL of nicotinamide adenine dinucleotide in Phosphate buffer

**Standard stock solution:** 1 mg/mL of acetaldehyde in water [Note: Store at 4° for about 20 h.]

**Standard solution:** 0.01 mg/mL of acetaldehyde in water: from Standard stock solution

**Sample solution:** Transfer 2 g of sample into a 100-mL volumetric flask, dissolve it in 50 mL of Phosphate buffer, dilute to volume with Phosphate buffer, and mix. Stopper the flask loosely, heat at 60° for 1 h, and cool to room temperature.

**Analysis:** Pipet 0.5 mL each of the Standard solution, the Sample solution, and water (the reagent blank) into separate 1-cm cells. Determine the absorbances of the solutions at 340 nm, using water as the reference. Add 2.5 mL of Phosphate buffer and 0.2 mL of NAD solution to each cell. Cover the cells to exclude oxygen. Mix by inversion, and allow them to stand for 2 to 3 min at 22° ± 2°. Determine the absorbances of the solutions as before. Calculate the percentage of aldehydes, as acetaldehyde, in the sample by the formula:

\[
10(C / W)\left\{\left(\frac{A_{U2} - A_{U1}}{A_{S2} - A_{S1}}\right) - \left(\frac{A_{B2} - A_{B1}}{A_{B2} - A_{B1}}\right)\right\}
\]

- **C** = Concentration (mg/mL) of acetaldehyde in the Standard solution
- **W** = Weight (g) of sample taken to prepare the Sample solution
- **A_{U1}** = Absorbance of the solution obtained from the Sample solution, before the Phosphate buffer and NAD solution were added
- **A_{S1}** = Absorbance of the solution obtained from the Standard solution, before the Phosphate buffer and NAD solution were added
- **A_{B1}** = Absorbance of the solution obtained from the water reagent blank, before the Phosphate buffer and NAD solution were added
- **A_{U2}** = Absorbance of the solution obtained from the Sample solution, after the Phosphate buffer and NAD solution were added
- **A_{S2}** = Absorbance of the solution obtained from the Standard solution, after the Phosphate buffer and NAD solution were added
- **A_{B2}** = Absorbance of the solution obtained from the water reagent blank, after the Phosphate buffer and NAD solution were added

**Acceptance criteria:** NMT 0.05%

**Hydrazine, Thin-Layer Chromatography, Appendix II**

**Salicylaldazine standard solution:** Dissolve 300 mg of hydrazine sulfate in 5 mL of water, add 1 mL of glacial acetic acid and 2 mL of a freshly prepared 20% (v/v) solution of salicylaldehyde in isopropyl alcohol, mix, and allow the solution to stand until a yellow precipitate forms. Extract the mixture with two 15-mL portions of methylene chloride. Combine the methylene chloride extracts, and dry over anhydrous sodium sulfate. Decant the methylene chloride solution, and evaporate it to dryness. Recrystallize the residue of salicylaldazine from a 60:40 mixture of warm toluene and methanol by cooling. Filter, and dry the crystals in a vacuum. The crystals have a melting range of 213° to 219°, but the range between the beginning and
end of melting is not to exceed 10°. Prepare a salicylaldazine solution in toluene containing 9.38 µg/mL.

**Sample solution:** Transfer 2.5 g of sample into a 50-mL centrifuge tube, add 25 mL of water, and mix to dissolve. Add 500 µL of a 1:20 solution of salicylaldehyde–methanol, swirl, and heat in a water bath at 60° for 15 min. Allow the solution to cool, add 2.0 mL of toluene, insert a stopper in the tube, shake vigorously for 2 min, and centrifuge.

**Adsorbent:** 0.25-mm layer of dimethylsilanized chromatographic silica gel mixture

**Developing solvent system:** Methanol and water (2 : 1)

**Application volume:** 10 µL

**Detection/Visualization:** UV 365 nm

**Analysis:** Spot the clear upper toluene layer from the Sample solution and the Salicylaldazine standard solution onto the plate. Following development, locate the spots on the plate by examination under UV light. Salicylaldazine appears as a fluorescent spot having an $R_f$ value of about 0.3.

**Acceptance criteria:** The fluorescence of any salicylaldazine spot from the Sample solution is not more intense than that produced by the spot obtained from the Salicylaldazine standard solution (NMT 1 mg/kg).

- **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:** Transfer 250 mg of sample into a 10-mL volumetric flask, dilute to volume with Mobile phase, and mix.

**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 vinylpyrrolidione is about 10 min.

**Injection volume:** About 50 µL

**System suitability**

- **Sample:** 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 by the formula:

$$1000(C/W)(r_U / r_S)$$
\( C = \text{Concentration (mg/mL) of vinylpyrrolidinone in the Standard solution} \)

\( W = \text{Weight (mg) of sample taken to prepare the Sample solution} \)

\( r_U = \text{Peak area response for vinylpyrrolidinone obtained from the Sample solution} \)

\( r_S = \text{Peak area responses for vinylpyrrolidinone obtained from the Standard solution} \)

\( \text{Acceptance criteria: NMT} 0.001\% \)

**SPECIFIC TESTS**

- **K-VALUE**
  
  [Note: The molecular weight of the sample is characterized by its viscosity in aqueous solution, relative to that of water, expressed as a K-Value.]

**Sample:** An amount of sample equivalent to 1 g on the anhydrous basis

**Sample solution:** Transfer the Sample into a 100-mL volumetric flask, dissolve it in about 50 mL of water, dilute to volume with water, mix thoroughly, and allow it to stand for 1 h. Filter the solution. Pipet 15 mL of filtrate into a clean, dry Ubbelohde-type viscometer, and place the viscometer in a water bath maintained at 25° ± 0.2°.

**Analysis:** After allowing the viscometer and the Sample solution to warm in the water bath for 10 min, draw the solution by means of very gentle suction up through the capillary until the meniscus is above the upper etched mark. Release suction, and after the meniscus reaches the upper etched mark, begin timing the flow through the capillary. Record the exact time when the meniscus reaches the lower etched mark, and calculate the flow time to the nearest 0.01 s. Repeat this operation until at least three readings are obtained. The readings must agree within 0.1 s; if not, repeat the determination with additional 15-mL portions of the Sample solution after recleaning the viscometer with sulfuric acid–dichromate cleaning solution or with a suitable laboratory cleaning compound that will remove oils, greases, waxes, and other impurities. Calculate the average flow time and then obtain the flow time in a similar manner for 15 mL of water. Calculate the relative viscosity, \( z \), of the sample by dividing the average flow time of the Sample solution by that of the water sample, and then calculate the K-Value by the formula:

\[
\frac{\sqrt{300c\log z + (c + 1.5c\log z)^2 + 1.5c\log z - c}}{0.15c + 0.003c^3}
\]

\( c = \text{Weight (g) of the sample, on the anhydrous basis, in each 100.0 g of solution} \)

\( z = \text{Relative viscosity} \)

**Acceptance criteria:**

- **Lower-molecular-weight-range product:** Between 27 and 32
- **Higher-molecular-weight-range product:** Between 81 and 97
- **Residue on Ignition (Sulfated Ash), Appendix IIC**
  
  **Sample:** 2 g
  
  **Acceptance criteria:** NMT 0.1%
• **Water**, *Water Determination*, Appendix IIB
  
  **Acceptance criteria**: NMT 5.0%

**OTHER REQUIREMENTS**

• **Labeling** Indicate the K-value or the K-value range.

**Auxiliary Information**— *Staff Liaison*: Kristie Bowman, Senior Scientific Associate  
*Expert Committee*: (FI07) Food Ingredients Expert Committee  
FCC Sixth Edition Page 795  
*Phone Number*: 1-301-816-8356
BRIEFING

Propionaldehyde, FCC 6 page 824—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

Propionaldehyde

\[
\text{C}_3\text{H}_6\text{O}
\]

Formula wt 58.08
FEMA: 2923

DESCRIPTION

*Change to read:*
Propionaldehyde occurs as a colorless, mobile liquid. It may contain a suitable antioxidant. [1S (FCC 6)]

**Odor** Sharp, pungent

**Solubility** Miscible in alcohol, ether, water

**Boiling Point** ~49°C

**Function** Flavoring agent

IDENTIFICATION

• **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.

ASSAY

• **Procedure** Proceed as directed under M-2c, Appendix XI.
  
  **Acceptance criteria:** NLT 97.0% of C\(_3\)H\(_6\)O

SPECIFIC TESTS
• **ACID VALUE, M-15,** Appendix XII
  Acceptance criteria: NMT 5.0

• **SPECIFIC GRAVITY** Determine at 25° by any reliable method (see *General Provisions*).
  Acceptance criteria: Between 0.800 and 0.805

**OTHER REQUIREMENTS**

• **DISTILLATION RANGE,** Appendix IIIB
  Acceptance criteria: 46° to 50° (first 97%) 

• **WATER,** *Water Determination, Method I,* Appendix IIIB
  [Note: Use freshly distilled pyridine as solvent.]
  Acceptance criteria: NMT 2.5%

**Auxiliary Information—** Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
FCC Sixth Edition Page 824
Phone Number: 1-301-816-8288
**BRIEFING**

Pullulan. Because there is no existing FCC monograph for this food ingredient, a new monograph is proposed based on the following:

1. The chemical information and Description are based on the Pullulan monograph from the 65th session (2005) of the Joint FAO/WHO Expert Committee on Food Additives (JECFA).
2. The Function and Packaging and Storage sections are based on comments received.
3. The Identification test C; Loss on Drying; Mono-, Di-, and Oligosaccharides; and Viscosity test procedures and acceptance criteria are based on the JECFA monograph.
4. The Assay test procedure and acceptance criterion are based on comments and data received.
5. The Lead test procedure was adapted from the JECFA monograph to a similar FCC general test procedure, and the acceptance criterion is based on comments and data received.
6. The Identification tests A and B; Nitrogen Content; and pH test procedures and acceptance criteria are based on the NF Pullulan monograph proposal in *Pharmacopeial Forum* 33(5) page 975.
7. The Residue on Ignition test procedure was adapted from the NF proposal, while the acceptance criterion is based on comments and data received.

Interested parties are encouraged to submit comments by October 1, 2008 to Jeff Moore, Ph.D., e-mail jrm@usp.org.
(FIEC: J. Moore) C55605

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**Add the following:**

- Pullulan

Poly[6-\(\alpha\)-D-glucopyranosyl-(1\(\rightarrow\)4)-\(\alpha\)-D-glucopyranosyl-(1\(\rightarrow\)4)-\(\alpha\)-D-glucopyranosyl-(1\(\rightarrow\) ]

\[(C_{16}H_{30}O_{15})_n\]

INS: 1204

---

**DESCRIPTION**

Pullulan occurs as a white to off-white odorless powder. It is a neutral simple polysaccharide produced by the fermentation of hydrolyzed starch using *Aureobasidium pullulans*. After completion of the fermentation, the fungal cells are removed by microfiltration, the filtrate is heat-sterilized and pigments and other impurities are removed by adsorption and ion exchange chromatography. It is a linear glucan consisting predominately of \(\alpha\)-(1\(\rightarrow\)6)-linked maltotriose subunits. It may also contain some maltotetraose subunits. It is freely soluble in water, and practically insoluble in dehydrated alcohol.

**Function** Glazing agent; film-forming agent; thickener

**Packaging and Storage** Store in well-closed containers.
IDENTIFICATION

• **A. Procedure**
  
  **Analysis:** In small increments, dissolve 10 g of sample in 100 mL of water with stirring.
  
  **Acceptance criteria:** A viscous solution is produced. [Note: Retain this solution for Identification test B.]

• **B. Depolymerization with Pullulanase**
  
  **Sample:** 10 mL of the viscous solution obtained in Identification test A
  
  **Pullulanase solution:** Prepare a solution of pullulanase containing 10 units per mL as directed in Enzyme Assays, Pullulanase Activity, Appendix V.
  
  **Analysis:** Mix the Sample with 0.1 mL of Pullulanase solution, and allow the mixture to incubate at 25°C for about 20 min.
  
  **Acceptance criteria:** A significant loss of viscosity is observed.

• **C. Procedure**
  
  **Sample solution:** 20 mg/mL
  
  **Analysis:** To 10 mL of Sample solution, add 2 mL of polyethylene glycol 600.
  
  **Acceptance criteria:** A white precipitate immediately forms.

ASSAY

• **Procedure**
  
  **Analysis:** Calculate the percentage of glucan in the sample using the following formula:

\[
100 - \left[ L + \frac{R}{1.3} + (N \times 6.25) + C \right]
\]

\[
L = \text{Percent Loss on Drying (see below)}
\]
\[
R = \text{Percent Residue on Ignition (Sulfated Ash) (see below)}
\]
\[
N = \text{Percent Nitrogen Content (see below)}
\]
\[
C = \text{Percent Mono-, Di-, and Oligosaccharides (see below)}
\]

1.3 = Sulfate correction factor

6.25 = Nitrogen to protein conversion factor

**Acceptance criteria:** More than 90%

IMPURITIES

Inorganic Impurities

• **Lead,** Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace Method, Method I, Appendix IIIB
  
  **Acceptance criteria:** Less than 1 mg/kg

SPECIFIC TESTS

• **Loss on Drying,** Appendix IIIC (90°C for 6 h in vacuum)
  
  **Acceptance criteria:** Less than 6.0%

• **Mono-, Di-, and Oligosaccharides**
  
  **Standard solution:** 200 µg/mL of glucose
  
  **Sample stock solution:** 8 mg/mL using a previously dried sample
  
  **Sample solution:** Transfer 1.0 mL of the Sample stock solution to a centrifuge tube. Add 0.1 mL of saturated potassium chloride solution. Add 3 mL of methanol, shake the mixture vigorously for about 20 s, and centrifuge at 11,000 rpm for 10 min. Use the supernatant as the Sample solution.
Blank: Water

Anthrone solution: 2 mg/mL anthrone in 75% (v/v) sulfuric acid, freshly prepared

Analysis: Immerse three test tubes in ice water, each tube containing 5 mL of Anthrone solution. Separately transfer 0.2 mL of the Standard solution, the Sample solution, and the Blank to the test tubes. Mix each tube immediately, and then place them in a water bath at 90° for 10 min. Remove the tubes and cool them under cold running water. Using a suitable spectrophotometer, measure the absorbances at 620 nm of the Sample solution, the Standard solution, and the Blank solution. Calculate the percentage of mono-, di-, and oligosaccharides in the sample taken expressed as glucose using the following formula:

\[
(A_U / A_S) \times (C_S / C_U) \times 4.1 \times 100
\]

- \(A_U\) = Absorbance of the Sample solution minus the absorbance of the Blank solution
- \(A_S\) = Absorbance of the Standard solution minus the absorbance of the Blank solution
- \(C_U\) = Concentration of Sample stock solution (mg/mL)
- \(C_S\) = Concentration of Standard solution (mg/mL)
- 4.1 = Dilution factor for the Sample solution

Acceptance criteria: Less than 10.0%, on the dried basis

- **Nitrogen Content, Nitrogen Determination, Appendix IIIC**
  
  **Sample:** 3 g, previously dried
  
  **Analysis:** Determine as directed using 12 mL of sulfuric acid for the decomposition and 40 mL of the sodium hydroxide (400 mg/mL) solution.
  
  **Acceptance criteria:** Less than 0.05%

- **pH, pH Determination, Appendix IIB**
  
  **Sample solution:** 1.0 g in 10 mL of freshly boiled and cooled water
  
  **Acceptance criteria:** Between 4.5 and 6.5

- **Residue on Ignition (Sulfated Ash), Appendix IIC**
  
  **Sample:** 2.0 g
  
  **Analysis:** Moisten the Sample with 1 mL of sulfuric acid and ignite at 600° ± 50°.
  
  **Acceptance criteria:** Less than 1.5%

- **Viscosity, Appendix IIB**
  
  **Sample:** 10.0 g, previously dried
  
  **Sample solution:** Dissolve the Sample in water to make 100 g of solution.
  
  **Analysis:** Use an Ubbelohde viscometer and perform the test at 30.0 ± 0.1°.
  
  **Acceptance criteria:** Between 100 and 180 mm²s⁻¹

\[1S (FCC 6)\]

Auxiliary Information— *Staff Liaison:* Jeffrey Moore, Ph.D., Senior Scientific Associate

*Expert Committee:* (FI07) Food Ingredients Expert Committee

*Phone Number:* 1-301-816-8288

Salicylaldehyde

\[
\text{C}_7\text{H}_6\text{O}_2
\]

**Formula wt** 122.12  
**FEMA:** 3004

**DESCRIPTION**

*Change to read:*
Salicylaldehyde occurs as a colorless to yellow liquid. It may contain a suitable antioxidant. 1S (FCC 6)

**Odor** Phenolic

**Solubility** Soluble in propylene glycol, vegetable oils; insoluble or practically insoluble in water

**Boiling Point** ~197°C

**Solubility in Alcohol,** Appendix VI
One mL dissolves in 1 mL of 95% ethanol.

**Function** Flavoring agent

**IDENTIFICATION**

- **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.
ASSAY

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

**Acceptance criteria:** NLT 97.0% of C_7H_6O_2

SPECIFIC TESTS

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

[Note: Use phenol red TS as the indicator.]

**Acceptance criteria:** NMT 10.0

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

**Acceptance criteria:** Between 1.570 and 1.576

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

**Acceptance criteria:** Between 1.159 and 1.170

**Auxiliary Information**—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate

Expert Committee: (FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 858

Phone Number: 1-301-816-8288
**BRIEFING**

**Sodium Carboxymethyl Cellulose, Enzymatically Hydrolyzed.** Because there is currently no FCC monograph for this food ingredient, a new monograph is being proposed. This proposed new monograph is based on the Sodium Carboxymethyl Cellulose, Enzymatically Hydrolyzed monograph from the 51st Joint Expert Committee on Food Additives (JECFA), 1998, with the exception of the Assay, which is based on the Assay in the Cellulose Gum monograph in the Food Chemicals Codex, Sixth Edition. Interested parties are encouraged to submit comments to Kristie Bowman, Scientific Liaison.

(FIEC: K. Bowman) C64347

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**Add the following:**

**Sodium Carboxymethyl Cellulose, Enzymatically Hydrolyzed**

Enzymatically Hydrolyzed Carboxymethyl Cellulose, Sodium

CMC-ENZ

Cellulose Gum, Enzymatically Hydrolyzed

![Chemical structure](image)

INS: 469

**DESCRIPTION**

Sodium Carboxymethyl Cellulose (CMC), Enzymatically Hydrolyzed occurs as a white or slightly yellowish or grayish granular or fibrous powder. It is the odorless and slightly hygroscopic product of the sodium salt of a carboxymethyl ether of cellulose, which has been partially hydrolyzed by enzymatic treatment with food-grade cellulase \([Trichoderma longibrachiatum\) (formerly reesei)]. The total content of mono- and disaccharides is typically about 7.5%. It is soluble in water and insoluble in ether.

**Function** Thickener, stabilizer

**Packaging and Storage** Store in well-closed containers.

**IDENTIFICATION**

- **A. PROCEDURE**
  - **Sample solution:** 1 mg/mL
  - **Analysis:** Shake the Sample solution.
  - **Acceptance criteria:** No layer of foam appears (distinction from other cellulose ethers).

- **B. PROCEDURE**
  - **Sample solution:** 5 mg/mL
  - **Analysis:** Add 5 mL of a 50 mg/mL solution of copper sulfate (or aluminum sulfate) to 5 mL of the Sample solution.
  - **Acceptance criteria:** A precipitate forms (distinction from other cellulose ethers).

- **C. PROCEDURE**
Sample solution: 10 mg/mL

1-Naphthol solution: 1 g of 1-naphthol in 25 mL of methanol

Analysis: Dilute 1 mL of the Sample solution with 1 mL of water in a small test tube. Add 5 drops of 1-naphthol solution to the test tube, tilt the tube, and carefully add 2 mL of sulfuric acid down the side of the tube so that it forms a lower layer.

Acceptance criteria: A red-purple color develops at the liquid–liquid interface.

• D. Viscosity

Sample solution: 60 g of sample in 40 mL of water, with continuous stirring; refrigerate the solution at 4°C for several hours before testing

Analysis: Using a Bohlin viscometer or equivalent instrument, measure the viscosity of the Sample solution at 25°C using a shear rate of 147 sec⁻¹.

Acceptance criteria: NLT 2500 mPa (corresponds to an average molecular weight of 5000 Da) [Note: This test distinguishes enzymatically hydrolyzed CMC from non-hydrolyzed CMC.]

ASSAY

• Percent Sodium Chloride

Sample solution: Transfer 5 g of sample into a 250-mL beaker, add 50 mL of water and 5 mL of 30% hydrogen peroxide, and heat on a steam bath for 20 min, stirring occasionally to ensure complete dissolution.

Analysis: Cool the Sample solution, add 100 mL of water and 10 mL of nitric acid, and titrate with 0.05 N silver nitrate to a potentiometric endpoint, using a silver/calomel (AgCl) electrode set, and stirring constantly. Calculate the percent sodium chloride in the sample by the formula:

\[
(584.4 \times V \times N)/(100 - b)W
\]

584.4 = Calculation factor including the equivalence factor for sodium chloride, percentage factor, and conversion of sample weight from g to mg

V = Volume of the silver nitrate (mL)

N = Normality of the silver nitrate

b = Percent Loss on Drying, determined separately (below)

W = Weight of the sample (g) taken

• Percent Sodium Glycolate

Standard stock solution: 1 mg/mL of glycolic acid (using glycolic acid previously dried in a desiccator at room temperature overnight) [Note: Use this solution within 30 days.]

Standard solutions: Transfer 1.0, 2.0, 3.0, and 4.0 mL, respectively, of the Standard solution into separate 100-mL volumetric flasks, add sufficient water to each flask to make 5 mL, then add 5 mL of glacial acetic acid, and dilute to volume with acetone.

Sample solution: Transfer 500 mg of sample into a 100-mL beaker, moisten thoroughly with 5 mL glacial acetic acid followed by 5 mL of water, and stir with a glass rod until dissolution is complete (usually about 15 min). While stirring, slowly add 50 mL of acetone, then add 1 g of sodium chloride, and stir for several minutes to ensure complete precipitation of the cellulose gum. Filter through a soft, open-textured paper, previously wetted with a small amount of acetone, and collect the filtrate in a 100-mL volumetric flask. Use an additional 30 mL of acetone to facilitate transfer of the solids and to wash the filter cake, then dilute to volume with acetone, and mix.

Blank solution: 5% each of glacial acetic acid and water in acetone
Analysis: Transfer 2.0 mL of the Sample solution, 2.0 mL of each of the Standard solutions, and 2.0 mL of the Blank solution into separate 25-mL volumetric flasks. Place the uncovered flasks in a boiling water bath for exactly 20 min to remove the acetone, remove from the bath, and cool. Add to each flask 5.0 mL of 2,7-dihydroxynaphthalene TS, mix thoroughly, add an additional 15 mL, and again mix thoroughly. Cover the mouth of each flask with a small piece of aluminum foil. Place the flasks upright in a boiling water bath for 20 min, then remove from the bath, cool, dilute to volume with sulfuric acid, and mix. Using a suitable spectrophotometer, determine the absorbance of the solutions prepared from the Sample solution and from the Standard solutions at 540 nm against the solution prepared from the Blank solution. Prepare a standard curve using the absorbance obtained from each of the Standard solutions. From the standard curve and the absorbance of the Sample solution, determine the weight \((w)\), in mg, of glycolic acid in the sample taken, and calculate the percent sodium glycolate in the sample by the formula:

\[
(12.9 \times w)/(100 - b)W
\]

12.9 = Calculation factor including conversion of glycolic acid to sodium glycolate, percentage factor, and conversion of sample weight from g to mg

\(b\) = Percent Loss on Drying, determined separately (below)

\(W\) = Weight of the sample (g) taken

\(w\) = Weight of glycolic acid in the Sample, as determined from the standard curve

- **Percent Enzymatically Hydrolyzed Sodium Carboxymethyl Cellulose**
  - **Calculation:** Calculate the Percent Enzymatically Hydrolyzed Sodium Carboxymethyl Cellulose by subtracting from 100 the Percent Sodium Chloride and Percent Sodium Glycolate determined above.
  - **Acceptance criteria:** NLT 99.5% (including mono- and di-saccharides), calculated on the dried basis

**Impurities**

Inorganic Impurities

- **Lead, Lead Limit Test, Flame Atomic Absorption Spectrophotometric Method, Appendix IIIB**
  - **Sample:** 3 g
  - **Acceptance criteria:** NMT 3 mg/kg

**Specific Tests**

- **Degree of Substitution**
  - **Electrode system:** Use a standard glass electrode and a calomel electrode modified as follows: Discard the aqueous potassium chloride solution contained in the electrode, rinse and fill with the supernatant liquid obtained by shaking thoroughly 2 g each of potassium chloride and silver chloride (or silver oxide) with 100 mL of methanol, then add a few crystals of potassium chloride and silver chloride (or silver oxide) to the electrode.
  - **Sample:** 200 mg, previously dried
  - **Analysis:** Add 75 mL of glacial acetic acid to the Sample contained in a 250-mL glass stoppered Erlenmeyer flask, connect the flask with a water-cooled condenser, and reflux gently on a hot plate for 2 h. Cool, transfer the solution to a 250-mL beaker with the aid of 50 mL of glacial acetic acid, and titrate with 0.1 N perchloric acid in dioxane while stirring with a magnetic stirrer. [**CAUTION:** Handle perchloric acid in an appropriate fume hood.] Determine the endpoint potentiometrically with a pH meter equipped with the Electrode system described. Record the mL of 0.1 N perchloric acid versus mV (0- to 700-mV range), and continue the titration to a few mL beyond the endpoint. Plot the titration curve and read the volume (\(A\)), in mL, of 0.1 N perchloric acid at the inflection point. Calculate the degree of substitution by the formula:
\[
\frac{(162 \times V \times N)}{W} - \frac{(80 \times V \times N)}{}
\]

162 = The molecular weight of one anhydroglucose unit
\(V\) = Volume of perchloric acid used in the titration (mL)
\(N\) = The normality of the perchloric acid titrant solution
\(W\) = Weight of the sample taken (mg)
80 = The molecular weight of one sodium carboxymethyl group

Acceptance criteria: NLT 0.2 and NMT 1.50 carboxymethyl groups (–CH\(_2\)COOH) per anhydroglucose unit, on the dried basis

- Loss on Drying, Appendix IIC (105° to constant weight)
  Acceptance criteria: NMT 12%

- pH, pH Determination, Appendix IIB
  Sample solution: 10 mg/mL
  Acceptance criteria: Between 6.0 and 8.5

- Residual Enzyme Activity
  Sample solution: 200 mg/mL
  CMC solution: 50 mg/mL of sodium carboxymethyl cellulose (Use a CMC that gives a viscosity of 25–50 mPa for a 2% solution.)

Analysis: To 20 g of the CMC solution, add 2 g of the Sample solution. Using a Bohlin viscometer or equivalent, follow the viscosity of the mixture for 10 minutes at 25°, using a shear rate of 147 sec\(^{-1}\).

Perform a blank determination using 2 g of water in place of the Sample solution.

Acceptance criteria: The change in viscosity in the CMC solution to which Sample solution is added is not greater than that of the blank determination.

1S (FCC 6)

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
Phone Number: 1-301-816-8288
Sodium Lignosulfonate, FCC 6 page 893—See briefing under Calcium Lignosulfonate. Interested parties are encouraged to submit comments to Jeff Moore, Ph.D., email jm@usp.org.

**Sodium Lignosulfonate**

**CAS:** [8061-51-6]

**DESCRIPTION**

Sodium Lignosulfonate occurs as a brown, amorphous polymer. It is obtained from the spent sulfite and sulfate pulping liquor of wood or from the sulfate (Kraft) pulping process. It may contain up to 30% reducing sugars. It is soluble in water, but not in any of the common organic solvents. The pH of a 1:100 aqueous solution is approximately between 3 and 11.

**Function** Binder; dispersant; boiler water additive

**Packaging and Storage** Store in well-closed containers.

**IDENTIFICATION**

- **A. Sodium**, Appendix IIIA
  
  **Sample solution:** 0.15 mg/mL
  
  **Acceptance criteria:** Passes test.

- **B. Procedure**
  
  **Sample:** 100 mg
  
  **Analysis:** Dissolve the Sample in 50 mL of water. Add 1 mL each of 10% acetic acid and 10% sodium nitrite solutions. Mix the solution by swirling, and allow it to stand for 15 min at room temperature.
  
  **Acceptance criteria:** A brown color appears.

- **C. Ultraviolet Absorption**
  
  **Sample solution:** 0.1 mg/mL at pH 5
  
  **Acceptance criteria:** The spectrum of the Sample solution exhibits a peak between 275 and 280 nm.

**ASSAY**

- **Sulfonate Sulfur**
  
  **Sample:** 1.0 g
  
  **Analysis:** Dissolve the Sample in 400 mL of water in a beaker. Direct a gentle stream of nitrogen gas over the liquid's surface. Add 10 mL of nitric acid and swirl the solution thoroughly until the reaction subsides. Add 10 mL of 70% perchloric acid and swirl thoroughly again. **[CAUTION: Handle perchloric acid in an appropriate fume hood.]** Place the uncovered beaker on a hot plate and heat the contents vigorously until the center of the bottom of the beaker becomes clear. Remove the beaker and cool to room temperature. Add 5 mL of hydrochloric acid and heat again until white fumes evolve. After cooling, dilute the solution to approximately 100 mL with water, adjust to pH 6 ± 0.2 with 10% sodium hydroxide, and heat the solution to boiling. Add 15 mL of 10% barium chloride solution and leave the solution overnight in a fresh beaker in a steam bath at 90° to 95°. Filter the solution through ashless filter paper (Whatman No. 42, or equivalent) and wash the precipitate with 200 mL of warm water. Transfer the paper and precipitate into a tared crucible. Heat the crucible slowly on a Bunsen burner to expel moisture. Place the crucible and contents in a muffle furnace at 850° for 1 h. Let the crucible cool in a desiccator, and then weigh the residue to the nearest 0.0001 g. Calculate the percent sulfonate sulfur by the formula:
\( R = \text{Weight of the residue (g)} \)
\( S = \text{Weight of the sample taken (g)} \)

**Acceptance criteria:** NLT 5.0% sulfonate sulfur

### IMPURITIES

#### Inorganic Impurities

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

  **Acceptance criteria:** NMT 1 mg/kg

### SPECIFIC TESTS

- **LOSS ON DRYING**, Appendix IIC (105°C for 24 h)
  
  **Acceptance criteria:** NMT 10.0%

- **REDUCING SUGARS**

  **Copper reagent solution:** [Note: This solution must be prepared several days in advance of use.]

  Dissolve 28 g of anhydrous dibasic sodium phosphate and 40 g of potassium sodium tartrate tetrahydrate in 700 mL of water. Add 100 mL of 1 N sodium hydroxide and 8 g of copper sulfate pentahydrate. Add 180 g of anhydrous sodium sulfate. Then, add 0.7134 g of potassium iodate and dilute to 1 L. Allow to stand for several days, then filter the clear top part of the solution through a medium-porosity, sintered-glass funnel.

  **Lead subacetate solution:** Dissolve 80 g of lead subacetate in 220 mL of water. Stir overnight, and filter through Whatman No. 42 filter paper, or equivalent. Dilute the supernatant solution to a specific gravity of 1.254 with freshly boiled water.

  **Dextrose standard solution:** 280 µg/mL of dried dextrose, made to 500 mL

  **Dibasic sodium phosphate solution:** 190 mg/mL of dibasic sodium phosphate heptahydrate, made to 100 mL

  **Sample solution:** Dissolve 1 g of sample in 150 mL of water and adjust the pH to between 6.9 and 7.2 with sodium hydroxide solution or acetic acid. Add Lead subacetate solution in increments until no further precipitation is observed. Bring the volume to 250.0 mL with water, and mix well. Centrifuge the mixture, pipet 10 mL of the supernatant into a 50-mL volumetric flask, and dilute to about 35 mL with water. Add 2 mL or more of Dibasic sodium phosphate solution until no further precipitation forms. Dilute to 50 mL with water, and mix. Centrifuge at 2100 × gravity for 10 min. The supernatant is the Sample solution.

  **Analysis:** Pipet 5 mL of the Sample solution into a test tube containing exactly 5 mL of Copper reagent solution and mix. Loosely plug the tube and place it in a boiling water bath for 40 min ± 10 s. At the end of the heating period, cool the tube immediately in cold water. Add 2 mL of 2.5% potassium iodide solution and 1.5 mL of 2 N sulfuric acid. Mix well and titrate with 0.005 N sodium thiosulfate, using starch as the indicator. Record the volume of 0.005 N sodium thiosulfate consumed as \( V_S \). Run a corresponding blank titration, \( V_B \), using 5 mL of water and 5 mL of Copper reagent solution. Repeat the entire procedure using 5 mL of Dextrose standard solution and 5 mL of Copper reagent solution, and noting the volume of 0.005 N sodium thiosulfate consumed as \( V_D \). Run a corresponding blank titration, \( V_{B_D} \), using 5 mL of water and 5 mL of Copper reagent solution. Calculate the percent reducing sugars by the formula:

\[
35(V_B - V_D)(V_B - V_{D_B})
\]
\[ V_B - V_S = \text{Quantity of 0.005 N sodium thiosulfate (mL) consumed by the 5-mL aliquot of Sample solution} \]
\[ V_B - V_D = \text{Quantity of 0.005 N sodium thiosulfate (mL) consumed by 5 mL of Dextrose standard solution} \]

**Acceptance criteria:** NMT 30.0%

- **Residue on Ignition (Sulfated Ash), Appendix IIC**
  - **Sample:** 1 g
  - **Acceptance criteria:** NMT 20.0%

- **Sodium**
  - **Standard solution:** 2 µg/mL prepared by diluting a certified 1000-ppm Sodium Standard Solution (Mallinckrodt or equivalent) quantitatively and stepwise with deionized water. Store the Standard solution in polyethylene bottles because of its instability in glass.
  - **Sample solution:** Transfer 1.00 ± 0.05 g of a previously dried sample into a silica or porcelain dish. Ash in a muffle furnace at 246° to 260° for 2 to 4 h. Allow the ash to cool, and dissolve it in 5 mL of 20% hydrochloric acid, warming the solution, if necessary, to completely dissolve the residue. Filter the solution through acid-washed filter paper into a 500-mL volumetric flask. Wash the filter paper with hot water, dilute to volume with water, and mix. Prepare a 1:100 aqueous dilution of this solution to obtain the final Sample solution.
  - **Analysis:** Using a suitably calibrated atomic absorption spectrophotometer, determine the absorbance of the Standard solution and Sample solution at 589.0 nm.
  - **Acceptance criteria:** The absorbance of the Sample solution is not greater than that of the Standard solution (NMT 10.0%).

**Change to read:**

- **Viscosity of a 50% Solution**
  - **Sample:** An amount of sample equivalent to 200 g calculated on the dried basis
  - **Analysis:** Dissolve the Sample in 200 mL of water contained in a 500-mL beaker. Equilibrate the solution at 25° and measure its relative viscosity with a Brookfield viscometer (Model LVG, or equivalent) (Model RVT, or equivalent) \( \eta_1^S \) (FCC 6), using a number 2 spindle at 20 rpm.
  - **Acceptance criteria:** NMT 3000 centipoises

**Auxiliary Information— Staff Liaison:** Jeffrey Moore, Ph.D., Senior Scientific Associate
**Expert Committee:** (FI07) Food Ingredients Expert Committee
**FCC Sixth Edition Page 893**
**Phone Number:** 1-301-816-8288
**BRIEFING**

**Sodium Metaphosphate, Insoluble, FCC 6 page 897**—See briefing under *Manganese Sulfate*. Interested parties are encouraged to submit comments to Jeff Moore, Ph.D., at jm@usp.org.

(FIEC: J. Moore) C65193

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**Sodium Metaphosphate, Insoluble**

Insoluble Sodium Polyphosphate

IMP

Maddrell's Salt

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**DESCRIPTION**

Sodium Metaphosphate, Insoluble, occurs as a white, crystalline powder. It is a high-molecular-weight sodium polyphosphate composed of two long metaphosphate chains (NaPO$_3$) that spiral in opposite directions about a common axis. The Na$_2$O:P$_2$O$_5$ ratio is about 1 : 1. It is practically insoluble in water but dissolves in mineral acids and in solutions of potassium and ammonium (but not sodium) chlorides. The pH of a 1 : 3 slurry in water is about 6.5.

**Function** Emulsifier; sequestrant; texturizer

**Packaging and Storage** Store in tight containers.

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**IDENTIFICATION**

- **A. Procedure**
  - **Sample:** 1 g
  - **Analysis:** Finely powder the *Sample* and add it slowly to 100 mL of a 50 mg/mL solution of potassium chloride in water while stirring vigorously.
  - **Acceptance criteria:** A gelatinous mass forms.

- **B. Phosphates, Appendix IIIA**
  - **Sample solution:** Mix 500 mg of sample with 10 mL of nitric acid and 50 mL of water, boil for about 30 min and cool. [NOTE: Retain remaining solution for Identification test C (below)].
  - **Acceptance criteria:** Passes tests.

- **C. Sodium, Appendix IIIA**
  - **Sample solution:** Solution prepared for Identification test B (above)
  - **Acceptance criteria:** Passes tests.

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**ASSAY**

- **Procedure**
  - **Sample:** 800 mg
  - **Analysis:** Transfer the *Sample* to a 400-mL beaker, add 100 mL of water and 25 mL of nitric acid, cover with a watch glass, and boil for 10 min on a hot plate. Rinse any condensate from the watch glass into the beaker, cool the solution to room temperature, transfer it quantitatively to a 500-mL volumetric flask, dilute to volume with water, and mix thoroughly. Pipet 20.0 mL of this solution into a 500-mL Erlenmeyer flask, add 100 mL of water, and heat just to boiling. While stirring, add 50 mL of quimociac TS, then cover with a
watch glass, and boil for 1 min in a well-ventilated hood. Cool to room temperature, swirling occasionally while cooling, then filter through a tared, sintered-glass filter crucible of medium porosity, and wash with five 25-mL portions of water. Dry at about 225° for 30 min, cool, and weigh. Each mg of precipitate thus obtained is equivalent to 32.074 µg of P₂O₅.

Acceptance criteria: NLT 68.7% and NMT 70.0% of P₂O₅

IMPURITIES

Change to read:
Inorganic Impurities

• ARSENIC, Arsenic Limit Test, Appendix IIIB
  Sample solution: 1 g in 15 mL of 2.7 N hydrochloric acid
  Acceptance criteria: NMT 3 mg/kg
• FLUORIDE, Fluoride Limit Test, Method III, Appendix IIIB
  Acceptance criteria: NMT 0.005%
• LEAD, Lead Limit Test, APDC Extraction Method, Appendix IIIB
  Sample: 5 g
  Acceptance criteria: NMT 4 mg/kg

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
FCC Sixth Edition Page 897
Phone Number: 1-301-816-8288
Sodium Phosphate, Dibasic, *FCC* 6 page 900—See briefing under *Manganese Sulfate*. Interested parties are encouraged to submit comments to Jeff Moore, Ph.D., at jm@usp.org.

(FIEC: J. Moore) C65193

**Sodium Phosphate, Dibasic**

Disodium Monohydrogen Phosphate

Disodium Phosphate

\[ \text{Na}_2\text{HPO}_4 \]

Formula wt, anhydrous 141.96

\[ \text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O} \]

Formula wt, dihydrate 177.99

INS: 339(ii)

CAS: anhydrous [7558-79-4]

CAS: dihydrate [10028-24-7]

**DESCRIPTION**

Sodium Phosphate, Dibasic occurs as a white, crystalline powder or as granules. It may be anhydrous or contain two molecules of water of hydration. The anhydrous form is hygroscopic. Both forms are freely soluble in water and insoluble in alcohol.

**Function** Emulsifier; texturizer; buffer; nutrient

**Packaging and Storage** Store in tightly closed containers.

**IDENTIFICATION**

- **Phosphate**, Appendix IIIA
  - Sample solution: 50 mg/mL
  - Acceptance criteria: Passes test.
- **Sodium**, Appendix IIIA
  - Sample solution: 50 mg/mL
  - Acceptance criteria: Passes test.

**ASSAY**

- **Procedure**
  - Sample: 6.5 g, previously dried at 105° for 4 h
  - 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 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) of 1 N hydrochloric acid consumed by the sample using the equation:

\[
A = 50 - x
\]
\[ x = \text{The volume (mL) of 1 N sodium hydroxide used in the titration} \]

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 142.0 mg of \( \text{Na}_2\text{HPO}_4 \). When \( A \) is greater than \( B \), each mL of the volume \( 2B - A \) of 1 N sodium hydroxide is equivalent to 142.0 mg of \( \text{Na}_2\text{HPO}_4 \).

**Acceptance criteria:** NLT 98.0% of \( \text{Na}_2\text{HPO}_4 \), 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**
  
  **Sample:** 5 g

  **1S (FCC 6)**

  **Acceptance criteria:** NMT 4 mg/kg

**SPECIFIC TESTS**

- **Loss on Drying, Appendix IIC (120° for 4 h)**
  
  **Acceptance criteria**
  
  - **Anhydrous:** NMT 5.0%
  
  - **Dihydrate:** Between 18.0% and 22.0%

- **Insoluble Substances**
  
  **Sample:** 10 g
  
  **Analysis:** Dissolve the Sample in 100 mL of hot water and filter 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%

**Auxiliary Information**— **Staff Liaison:** Jeffrey Moore, Ph.D., Senior Scientific Associate

**Expert Committee:** (FI07) Food Ingredients Expert Committee

**FCC Sixth Edition** Page 900

**Phone Number:** 1-301-816-8288
**BRIEFING**

*Sodium Phosphate, Monobasic, FCC 6 page 901*—See briefing under *Manganese Sulfate*. Interested parties are encouraged to submit comments to Jeff Moore, Ph.D., email jm@usp.org.

(FIEC: J. Moore) C65235

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**Sodium Phosphate, Monobasic**

Monosodium Phosphate

Sodium Biphosphate

Monosodium Dihydrogen Phosphate

\[ \text{Na}_2\text{HPO}_4 \]

\[ \text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O} \]

**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**

- **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 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. 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 NaH₂PO₄.

**Acceptance criteria:** NLT 98.0% and NMT 103.0% NaH₂PO₄, 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
  - **Sample:** 5 g
  - **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 filter the solution through a tared filtering crucible (not glass). Wash the insoluble residue with hot water, dry at 105\(^\circ\) for 2 h, cool, and weigh.
  - **Acceptance criteria:** NMT 0.2%

- **Loss on Drying**, Appendix IIC (60\(^\circ\) for 1 h, then 105\(^\circ\) 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**— **Staff Liaison:** Kristie Bowman, Senior Scientific Associate

**Expert Committee:** (FI07) Food Ingredients Expert Committee

**FCC Sixth Edition Page 901**

**Phone Number:** 1-301-816-8356
Sodium Polyphosphates, Glassy, FCC 6 page 903—See briefing under Manganese Sulfate. Interested parties are encouraged to submit comments to Jeff Moore, Ph.D., at jm@usp.org.

Sodium Polyphosphates, Glassy

Sodium Hexametaphosphate

Sodium Tetrapolyphosphate

Graham's Salt

\[
\begin{align*}
\text{Na}^+ & \quad \text{O} \quad \text{P} \\
\text{Na}^+ & \quad \text{O} \quad \text{P} \\
x & \quad \text{O} \quad \text{P} \\
\text{Na}^+ & \quad \text{O} \\
\text{Na}^+ & \quad \text{O} \\
\end{align*}
\]

INS: 452(i)  CAS: [68915-31-1]  CAS: [10361-03-2]

DESCRIPTION

**Change to read:**

Sodium Polyphosphates, Glassy, occur as colorless or white, transparent platelets, granules, or powders. They belong to a class consisting of several amorphous, water-soluble polyphosphates composed of linear chains of metaphosphate units \((\text{NaPO}_3)x\) for which \(x \geq 2\), terminated by \(\text{Na}_2\text{PO}_4\)-groups. They are usually identified by their \(\text{Na}_2\text{O}/\text{P}_2\text{O}_5\) ratio or their \(\text{P}_2\text{O}_5\) content. The \(\text{Na}_2\text{O}/\text{P}_2\text{O}_5\) ratios vary from about 1.5 to 1.0 for the higher molecular weight sodium polyphosphates, for which \(x = 20\) to 100 or more. Glassy Sodium Polyphosphates are very soluble in water. The pH of their solutions varies from about 3.0 to 9.0.

**Function** Emulsifier; sequestrant; texturizer

**Packaging and Storage** Store in tightly closed containers.

IDENTIFICATION

- **A. Sodium, Appendix IIIA**
  - Sample solution: 50 mg/mL
  - Acceptance criteria: Passes tests.
- **B. Procedure**
  - Sample: 100 mg
  - Analysis: Dissolve the Sample in 5 mL of hot 1.7 N nitric acid, warm on a steam bath for 10 min, and cool.
    - Neutralize to litmus paper with 1 N sodium hydroxide, and add silver nitrate TS.
  - Acceptance criteria: A yellow precipitate forms that is soluble in 1.7 N nitric acid.
ASSAY

• **PROCEDURE**
  
  **Sample**: 800 mg

  **Sample solution**: Transfer the Sample into a 400-mL beaker, add 100 mL of water and 25 mL of nitric acid, cover with a watch glass, and boil for 10 min on a hot plate. Rinse any condensate from the watch glass into the beaker, cool the solution to room temperature, transfer it quantitatively into a 500-mL volumetric flask, dilute to volume with water, and mix thoroughly.

  **Analysis**: Pipet 20.0 mL of Sample solution into a 500-mL Erlenmeyer flask, add 100 mL of water, and heat just to boiling. While stirring, add 50 mL of quinomiac TS, cover with a watch glass, and boil for 1 min in a well-ventilated hood. Cool to room temperature, swirling occasionally while cooling, then pass through a tared, sintered-glass filter crucible of medium porosity, and wash with five 25-mL portions of water. Dry at about 225° for 30 min, cool, and weigh. Each mg of precipitate thus obtained is equivalent to 32.074 µg of P₂O₅.

  **Acceptance criteria**: Between 60.0% and 71.0% of P₂O₅

IMPURITIES

Change to read:

Inorganic Impurities

• **Arsenic**, Arsenic Limit Test, Appendix IIIB
  
  **Sample solution**: 1 g of sample in 35 mL of water

  **Acceptance criteria**: NMT 3 mg/kg

• **Fluoride**, Fluoride Limit Test, Method III, Appendix IIIB
  
  **Sample**: 2 g

  **Acceptance criteria**: NMT 0.005%

• **Lead**, Lead Limit Test, APDC Extraction Method, Appendix IIIB
  
  **Sample**: 5 g

  **1S (FCC 6)**

  **Acceptance criteria**: NMT 4 mg/kg

SPECIFIC TESTS

• **Insoluble Substances**
  
  **Sample**: 10 g

  **Analysis**: Dissolve 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 0.1%

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate

*Expert Committee*: (Fl07) Food Ingredients Expert Committee

FCC Sixth Edition Page 903

Phone Number: 1-301-816-8288
**BRIEFING**

*Sodium Pyrophosphate, FCC 6* page 906—See briefing under *Manganese Sulfate*. Interested parties are encouraged to submit comments to Jeff Moore, Ph.D.

(FIEC: J. Moore) C65193

**Sodium Pyrophosphate**

Tetrasodium Diphosphate

Tetrasodium Pyrophosphate

\[ \text{Na}_4\text{P}_2\text{O}_7 \]

Formula wt, anhydrous 265.90

\[ \text{Na}_4\text{P}_2\text{O}_7\cdot10\text{H}_2\text{O} \]

Formula wt, decahydrate 446.06

INS: 450(iii)

CAS: anhydrous [7722-88-5]

CAS: decahydrate [13472-36-1]

**DESCRIPTION**

Sodium Pyrophosphate occurs as colorless or white crystals or as a white, crystalline or granular powder. It is anhydrous or contains 10 molecules of water of hydration. The decahydrate effloresces slightly in dry air. It is soluble in water, but is insoluble in alcohol. The pH of a 1:100 aqueous solution is about 10.

**Function** Emulsifier; buffer; nutrient; sequestrant; texturizer

**Packaging and Storage** Store in tight containers.

**IDENTIFICATION**

- **A. Sodium**, Appendix IIIA
  - **Sample solution**: 50 mg/mL
  - **Acceptance criteria**: Passes tests.

- **B. Procedure**
  - **Sample solution**: Dissolve 100 mg of sample in 100 mL of 1.7 N nitric acid.
    - **Analysis 1**: Add 0.5 mL of the *Sample solution* to 30 mL of quimociac TS.
    - **Analysis 2**: Heat the remaining portion of *Sample solution* (~99.5 mL) for 10 min at 95°C, then add 0.5 mL of the solution to 30 mL of quimociac TS.
    - **Acceptance criteria**: A yellow precipitate does not form in *Analysis 1*, but does form immediately in *Analysis 2*.

**ASSAY**

- **Procedure**
  - **Sample**: Amount equivalent to 500 mg of anhydrous \( \text{Na}_4\text{P}_2\text{O}_7 \)
  - **Analysis**: Dissolve the *Sample* in 100 mL of water contained in a 400-mL beaker. Using a pH meter, adjust the pH of the solution to 3.8 with hydrochloric acid, then add 50 mL of a 1:8 solution of zinc sulfate (125 g of \( \text{ZnSO}_4\cdot7\text{H}_2\text{O} \) dissolved in water, diluted to 1000 mL, filtered, and adjusted to pH 3.8), and allow the mixture to stand for 2 min. Titrate the liberated acid with 0.1 N sodium hydroxide until the pH of 3.8 is again
reached. After each addition of sodium hydroxide near the endpoint, allow time for any precipitated zinc hydroxide to redissolve. Each mL of 0.1 N sodium hydroxide is equivalent to 13.30 mg of Na₄P₂O₇.

**Acceptance criteria:** NLT 95.0% and NMT 100.5% of Na₄P₂O₇, calculated on the ignited basis.

**IMPURITIES**

**Change to read:**

Inorganic Impurities

- **ARSENIC, Arsenic Limit Test, Appendix IIIIB**
  
  **Sample solution:** 1 g of sample in 35 mL of water
  
  **Acceptance criteria:** NMT 3 mg/kg

- **FLUORIDE**
  
  [NOTE: Prepare and store all solutions in plastic containers.]
  
  **Buffer solution:** Dissolve 73.5 g sodium citrate in water, made to 250 mL.
  
  **Standard stock solution:** 1.1052 mg/mL USP Sodium Fluoride RS
  
  **Standard solution:** Transfer 20.0 mL of the *Standard stock solution* to a 100-mL volumetric flask containing 50 mL of *Buffer solution*, dilute to volume with water, and mix (100 µg/mL fluoride ion).
  
  **Sample solution:** Transfer 2.0 g of sample to a beaker containing a plastic-coated stirring bar, add 20 mL of water and 2.0 mL of hydrochloric acid, and stir until the sample is dissolved. Add 50.0 mL of *Buffer solution* and sufficient water to make 100 mL.
  
  **Electrode system:** Use a fluoride-specific, ion-indicating electrode and a silver-silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of ±0.2 mV.
  
  **Standard response line:** Transfer 50.0 mL of *Buffer solution* and 2.0 mL of hydrochloric acid into a beaker and add water to make 100 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, stir for 15 min, and read the potential (mV). Continue stirring, and at 5-min intervals, add 100 µL, 100 µL, 300 µL, and 500 µL of *Standard solution*, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride ion concentrations (0.1, 0.2, 0.5, and 1.0 µg/mL) versus potential, in mV.
  
  **Analysis:** Rinse and dry the electrodes, insert them into the *Sample solution*, stir for 5 min, and read the potential (mV). From the measured potential and the *Standard response line*, determine the concentration, C (µg/mL), of fluoride ion in the *Sample solution*. Calculate the percentage of fluoride in the sample taken by the formula:

\[
C \times 0.005
\]

**Acceptance criteria:** NMT 0.005%

- **LEAD, Lead Limit Test, APDC Extraction Method, Appendix IIIIB**
  
  **Sample:** 5 g
  
  **Acceptance criteria:** NMT 4 mg/kg

**SPECIFIC TESTS**

- **INSOLUBLE SUBSTANCES**
  
  **Sample:** 10 g
  
  **Analysis:** Dissolve the *Sample* in 100 mL of hot water, and filter the solution 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:** Dry a sample at 110° for 4 h, and then ignite it at about 800° for 30 min.

**Acceptance criteria**

- **Anhydrous:** NMT 0.5%
- **Decahydrate:** Between 38.0% and 42.0%

**Auxiliary Information**— **Staff Liaison:** Jeffrey Moore, Ph.D., Senior Scientific Associate

**Expert Committee:** (FI07) Food Ingredients Expert Committee

**FCC Sixth Edition** Page 906

**Phone Number:** 1-301-816-8288
BRIEFING

Sorbitol, FCC 6 page 919—See briefing under Maltitol. Interested parties are encouraged to comment.
(FIEC: K. Bowman) C65167

Sorbitol

D-Sorbitol

D-Glucitol

D-Sorbite

1,2,3,4,5,6-Hexahexol

Sorbitol occurs as a white powder, as granules, or as crystalline masses. It is very soluble in water; slightly soluble in ethanol, in methanol, and in acetic acid; and insoluble in ether. It is hygroscopic.

Function Humectant; texturizing agent; nutritive sweetener

Packaging and Storage Store in tight containers.

IDENTIFICATION

• A. PROCEDURE
  Sample solution: 13.33 mg/mL
  Analysis: Transfer 3 mL of the Sample solution into a 15-cm test tube, add 3 mL of a freshly prepared 1:10 catechol solution, and mix. Add 6 mL of sulfuric acid, mix again, then gently heat the tube in a flame for about 30 s.
  Acceptance criteria: A deep pink or wine red color appears.

• 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 obtained in the Assay (below).

ASSAY

• PROCEDURE
  Mobile phase: Degassed water
  Standard solution: 4.8 mg/g USP Sorbitol RS
**System suitability solution:** 4.8 mg/g each of USP Mannitol RS and USP Sorbitol RS

**Sample solution:** 5 mg/g

**Chromatographic system,** Appendix IIA

- **Mode:** High-performance liquid chromatography
- **Detector:** Refractive index
- **Column:** 10 cm × 7.8-mm; containing packing L34 (Bio-Rad Laboratories), or equivalent
- **Column temperature:** 50° ± 2°
- **Detector temperature:** 35°
- **Flow rate:** About 0.7 mL/min
- **Injection size:** About 10 µL

**System suitability**

**Samples:** System suitability solution and Standard solution

**Suitability requirement 1:** Resolution, R, between the sorbitol and mannitol in the System suitability solution is NLT 2.0

**Suitability requirement 2:** Relative standard deviation for three replicate injections of the Standard solution is NMT 2.0%

**Analysis:** Separately inject volumes of the Standard solution and Sample solution into the chromatograph and measure the responses for the major peaks on the resulting chromatograms.  [NOTE: Approximate relative retention times for mannitol and sorbitol are 0.6 and 1.0, respectively.]  Calculate the percentage of C₆H₁₄O₆ (on the anhydrous basis) in the sample taken by the formula:

\[
\frac{((C_S/C_U) \times (r_U/r_S) \times 10,000)}{(100 - w)}
\]

- **C₅** = Concentration of USP Sorbitol RS in the Standard solution (mg/g)
- **Cₚ** = Concentration of sample in the Sample solution (mg/g)
- **rₚ** = Peak response obtained with the Sample solution
- **rₛ** = Peak response obtained with the Standard solution
- **w** = Percent water as determined under Water (below)

**Acceptance criteria:** NLT 91.0% and NMT 100.5% of D-Sorbitol (C₆H₁₄O₆), calculated on the anhydrous basis

**IMPURITIES**

**Inorganic Impurities**

- **Lead,** Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace Method, Method I, Appendix III B
  - **Sample:** 10 g
  - **Acceptance criteria:** NMT 1 mg/kg
- **Nickel,** Nickel Limit Test, Appendix III B
  - **Acceptance criteria:** NMT 1 mg/kg

**Change to read:**

**Organic Impurities**
• Reducing Sugars
  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).

Sample: 3.3 g

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 490 mL of 40 mL of diluted acetic acid TS, 60 mL of water, 10 mL (FCC 6) of 0.05 N Iodine VS, 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.

Acceptance criteria: NLT 12.8 mL of 0.05 N Sodium thiosulfate VS is required (NMT 0.3% reducing sugars, as glucose)

SPECIFIC TESTS
• pH, pH Determination, Appendix IIB
  Sample: 10% w/w solution of sample in carbon dioxide-free water
  Acceptance criteria: Between 3.5 and 7.0

• Residue on Ignition (Sulfated Ash), Method I (for Solids), Appendix IIC
  Sample: 2 g
  Acceptance criteria: NMT 0.1%

• Water, Water Determination, Appendix IIB
  Acceptance criteria: NMT 1.5%

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate

Expert Committee: (FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 919

Phone Number: 1-301-816-8288
Sorbitol Solution, FCC 6 page 920—See briefing under Maltitol. Interested parties are encouraged to comment.
(FIEC: K. Bowman)  C65169

Sorbitol Solution
INS: 420

DESCRIPTION
Sorbitol Solution occurs as a clear, colorless, syrupy liquid. It is a water solution of sorbitol (C₆H₁₄O₆) containing a small amount of mannitol and other isomeric polyhydric alcohols. It is miscible with water, with ethanol, with glycerin, and with propylene glycol. It sometimes separates into crystalline masses.

Function  Humectant; texturizing agent; nutritive sweetener

Packaging and Storage  Store in well closed containers.

IDENTIFICATION
• A. Procedure
  Sample solution: 1.4 g of sample in 75 mL of water
  Analysis: Transfer 3 mL of the Sample solution into a 15-cm test tube, add 3 mL of a freshly prepared 1:10 catechol solution, and mix. Add 6 mL of sulfuric acid, mix again, then gently heat the tube in a flame for about 30 s.
  Acceptance criteria: A deep pink or wine red color appears.
• 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 obtained in the Assay (below).

ASSAY
• Procedure
  Mobile phase: Degassed water
  Standard solution: 4.8 mg/g USP Sorbitol RS
  System suitability solution: 4.8 mg/g each of USP Mannitol RS and USP Sorbitol RS
  Sample solution: 6 mg/g
  Chromatographic system, Appendix II A
    Mode: High-performance liquid chromatography
    Detector: Refractive index
    Column: 10 cm × 7.8-mm, containing packing L34 (Bio-Rad Laboratories), or equivalent
    Column temperature: 50° ± 2°
    Detector temperature: 35°
    Flow rate: About 0.7 mL/min
    Injection size: About 10 µL
  System suitability
    Samples: System suitability solution and Standard solution
    Suitability requirement 1: Resolution, R, between the sorbitol and mannitol in the System suitability solution is NLT 2.0.
    Suitability requirement 2: Relative standard deviation for three replicate injections of the Standard solution is NMT 2.0%.
  Analysis: Separately inject volumes of the Standard solution and Sample solution into the chromatograph
and measure the responses for the major peaks on the resulting chromatograms. [NOTE: Approximate relative retention times for mannitol and sorbitol are 0.6 and 1.0, respectively.] Calculate the percentage of \( C_6H_{14}O_6 \) in the portion of the sample taken by the formula:

\[
100\left(\frac{C_S}{C_U}\right)\left(\frac{r_U}{r_S}\right)
\]

\( C_S \) = Concentration of USP Sorbitol RS in the Standard solution (mg/g)

\( C_U \) = Concentration of sample in the Sample solution (mg/g)

\( r_U \) = Peak response obtained with the Sample solution

\( r_S \) = Peak response obtained with the Standard solution

**Acceptance criteria:** NLT 64.0% sorbitol (\( C_6H_{14}O_6 \))

**IMPURITIES**

**Inorganic Impurities**

- **Lead, Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace Method, Method I, Appendix IIIB**
  
  **Sample:** 10 g

  **Acceptance criteria:** NMT 1 mg/kg, calculated on the anhydrous basis

- **Nickel, Nickel Limit Test, Appendix IIIB**
  
  **Acceptance criteria:** NMT 1 mg/kg, calculated on the anhydrous basis

**Change to read:**

**Organic Impurities**

- **Reducing Sugars**

  **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).

  **Sample:** Amount equivalent to 3.3 g of sorbitol on the anhydrous basis

  **Analysis:** To the Sample, add 3 mL of water, 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 400 mL of 1S (FCC 6) of diluted acetic acid TS, 60 mL of water, 1S (FCC 6) 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.

  **Acceptance criteria:** NLT 12.8 mL of 0.05 N Sodium thiosulfate VS is required (NMT 0.3% reducing sugars, calculated on the anhydrous basis).

**SPECIFIC TESTS**

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

  **Sample:** 14% w/w solution of sample in carbon dioxide-free water

  **Acceptance criteria:** Between 5.0 and 7.5

- **Residue on Ignition (Sulfated Ash), Method II (for Liquids), Appendix IIC**

  **Sample:** 2 g

  **Acceptance criteria:** NMT 0.1%, calculated on the anhydrous basis
• Water, Water Determination, Appendix IIB
  
  **Acceptance criteria:** Between 28.5% and 31.5%

**Auxiliary Information—** Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate  
Expert Committee: (FI07) Food Ingredients Expert Committee  
FCC Sixth Edition Page 920  
Phone Number: 1-301-816-8288
**BRIEFING**

Noncrystallizing Sorbitol Solution. Because there is no existing FCC monograph for this food ingredient, it is proposed to add a new monograph based on the NF 18 Second Supplement and NF 26 Noncrystallizing Sorbitol Solution monographs, the FCC 6 monograph for Sorbitol Solution, a compositionally similar material, and based on comments and data received.

1. The INS, Description, Function, and Packaging and Storage are based on data and comments received.
2. The Identification tests A and B procedures and acceptance criteria are based on the NF 26 Noncrystallizing Sorbitol Solution monograph.
3. The Assay test procedure is based on the Assay procedure in FCC 6 for Sorbitol Solution, a compositionally similar material. The acceptance criteria are based on comments and data received.
4. The Lead and Nickel test procedures and acceptance criteria are based on comments and data received.
5. The Reducing Sugars, pH, Residue on Ignition, and Water test procedures are based on the same test procedures in the FCC 6 monograph for Sorbitol Solution, a compositionally similar material. The acceptance criteria are based on comments and data received.
6. The Total Sugars test procedure is based on the NF 18 Second Supplement monograph and is proposed to aid in distinguishing this material from sorbitol solution, a compositionally similar material. The acceptance criteria are based on comments and data received.

Interested parties are encouraged to submit comments by October 1, 2008 to Jeff Moore, Ph.D., at jm@usp.org. (FIEC: J. Moore) C64695

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**Add the following:**

- **Noncrystallizing Sorbitol Solution**
  - INS: 420

**DESCRIPTION**

Noncrystallizing Sorbitol Solution occurs as a clear, colorless, syrupy liquid. It is an aqueous solution of hydrogenated saccharides, consisting primarily of sorbitol and lesser amounts of hydrogenated mono-, di-, and polysaccharides. It is miscible with water, with ethanol, with glycerin, and with propylene glycol.

**Function** Humectant; texturizing agent; nutritive sweetener

**Packaging and Storage** Store in well-closed containers.

**IDENTIFICATION**

- **A. Procedure**
  - **Sample solution**: 1.4 g of sample in 75 mL of water
  - **Analysis**: Transfer 3 mL of the Sample solution into a 15-cm test tube, add 3 mL of a freshly prepared 1:10 catechol solution, and mix. Add 6 mL of sulfuric acid, mix again, then gently heat the tube in a flame for about 30 s.
  - **Acceptance criteria**: A deep pink or wine red color appears.

- **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 obtained in the Assay (below).

**ASSAY**
**PROCEDURE**

**Mobile phase:** Degassed water

**Standard solution:** 4.8 mg/g USP Sorbitol RS

**System suitability solution:** 4.8 mg/g each of USP Mannitol RS and USP Sorbitol RS

**Sample solution:** 10 mg/g

**Chromatographic system,** Appendix IIA

- **Mode:** High-performance liquid chromatography
- **Detector:** Refractive index
- **Column:** 10 cm × 7.8-mm, 9 µm with a lead ionic stationary phase on a sulfonated divinyl benzene–styrene copolymer

- **Column temperature:** 50° ± 2°

- **Detector temperature:** 35°

- **Flow rate:** About 0.7 mL/min

- **Injection size:** About 10 µL

**System suitability**

- **Samples:** System suitability solution and Standard solution

  **Suitability requirement 1:** Resolution, $R_s$ between the sorbitol and mannitol in the System suitability solution is NLT 2.0

  **Suitability requirement 2:** Relative standard deviation for three replicate injections of the Standard solution 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: Approximate relative retention times for mannitol and sorbitol are 0.6 and 1.0, respectively.] Calculate the percentage of sorbitol, C$_6$H$_{14}$O$_6$, on the anhydrous basis in the portion of the sample taken by the formula:

$$100 \left( \frac{C_S}{C_U} \right) \left( \frac{r_U}{r_S} \right) \left( 100 - W \right)$$

- $C_S$ = Concentration of USP Sorbitol RS in the Standard solution (mg/g)
- $C_U$ = Concentration of sample in the Sample solution (mg/g)
- $r_U$ = Peak response for sorbitol obtained with the Sample solution
- $r_S$ = Peak response for sorbitol obtained with the Standard solution
- $W$ = Percentage water determined separately in the test for Water, below

**Acceptance criteria:** NLT 64.0% sorbitol (C$_6$H$_{14}$O$_6$) on the anhydrous basis

**IMPURITIES**

**Inorganic Impurities**

- **Lead**

  [NOTE: Use deionized ultra-filtered water throughout this test procedure.]

  **Digester solution (aqua regia):** To 1200 mL of water add 360 mL of hydrochloric acid and 240 mL of nitric acid.

  **Diluent:** 20 mL/L nitric acid
**Internal standard solution:** 2 µg/mL yttrium, prepared by diluting a commercially prepared yttrium reference standard solution with Diluent.

**Standard stock solution:** 10 µg/mL lead prepared by diluting a commercially prepared lead ICP standard with Diluent.  
[NOTE: Prepare this solution fresh every two months.]

**Standard solutions:** 50, 100, and 200 ng/mL lead in Diluent: from Standard stock solution.  
[NOTE: Prepare these solutions fresh weekly.]

**Sample:** 10.0 g, on the anhydrous basis

**Sample solution:** Add the Sample into a 125-mL Erlenmeyer flask. Add 40 mL of Digester solution and place on a hotplate. Heat the solution for about 20 min, being careful to prevent the solution from boiling over. The solution will turn a dark caramel color. Transfer into a clean, dry, 50-mL volumetric flask with washings of Diluent. Dilute to volume with Diluent. Filter the sample into a 15-mL centrifuge tube, using a 10-mL B-D syringe, fitted with a 0.45-µm syringe filter.

**Apparatus:** Use a suitable inductively coupled plasma-optical emission spectrometer (ICP-OES) configured in an axial optical alignment. This method was developed using a Perkin Elmer Optima 3100 ICP-OES unit. 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. The instrument parameters are as follows: Set the ultraviolet detector to scan lead at 220.353 nm and yttrium at 371.029 nm. Set the sample read time to 20 s minimum and 50 s maximum. Three replicate scans are taken with the integration set to one point per peak. Set the forward power from the RF generator to 1500 watts. Use an argon plasma feed gas flow of 15 L/min with the auxiliary gas (shear gas) set to flow at 0.5 L/min. Use a gem cone nebulizer with a nebulization gas flow rate of 0.55 L/min. The sample is delivered to the spray chamber by a multi-channel peristaltic pump set to deliver sample at a rate of 2.00 mL/min. The Internal standard is added in-line via a mixing block between the sample probe and spray chamber. Samples are flushed through the system for 45 s at a rate of 4.0 mL/min prior to analysis. A 45-second 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 30 s at a rate of 4.0 mL/min.

**Analysis:** Generate a calibration curve using Diluent as a Blank and the Standard solutions as follows: Scan the Internal standard solution while running the blank to measure the intensity of the yttrium emission. Hold this value constant throughout the remainder of the test. Separately scan the Blank and the Standard solutions for lead and yttrium. Normalize the yttrium intensity to the value of the Internal standard solution.  
[NOTE: The Internal standard is added in-line via a mixing block between the sample probe and spray chamber.] Also apply this normalization factor to the lead intensity, which is then referred to as the corrected lead intensity. A calibration curve is constructed by plotting the corrected lead intensity versus the known concentrations of the Standard solutions. The correlation coefficient for the best-fit line should not be less than 0.999. Similarly, analyze the Sample solution on the ICP. The intensity of the emission of the Sample solution is plotted on the calibration curve and the concentration is extrapolated against the x-axis. Calculate the concentration (mg/kg) of lead in the Sample taken using the following equation:

\[
(C/W) \times (50/1000)
\]

- \(C\) = Concentration (ng/mL) of lead in the Sample solution determined from the standard curve
- \(W\) = Weight (g) of Sample taken
- 50 = Sample dilution factor
- 1000 = ng to µg conversion factor
Acceptance criteria: NMT 1 mg/kg, calculated on the anhydrous basis

**Nickel**

[NOTE: Use deionized ultra-filtered water throughout this test procedure.]

**Digester solution (aqua regia):** To 1200 mL of water add 360 mL of hydrochloric acid and 240 mL of nitric acid.

**Diluent:** 20 mL/L nitric acid

**Internal standard solution:** 2 µg/mL yttrium, prepared by diluting a commercially prepared yttrium reference standard solution with Diluent.

**Standard stock solution:** 10 µg/mL nickel prepared by diluting a commercially prepared nickel ICP standard with Diluent. [NOTE: Prepare this solution fresh every two months.]

**Standard solutions:** 50, 100, and 200 ng/mL lead in Diluent: from Standard stock solution. [Note: Prepare these solutions fresh weekly.]

**Sample:** 10.0 g, on the anhydrous basis

**Sample solution:** Add the Sample into a 125-mL Erlenmeyer flask. Add 40 mL of Digester solution and place on a hotplate. Heat the solution for about 20 min, being careful to prevent the solution from boiling over. The solution will turn a dark caramel color. Transfer into a clean, dry, 50-mL volumetric flask with washings of Diluent. Dilute to volume with Diluent. Filter the sample into a 15-mL centrifuge tube, using a 10-mL B-D syringe, fitted with a 0.45-µm syringe filter.

**Apparatus:** Use a suitable inductively coupled plasma-optical emission spectrometer (ICP-OES) configured in an axial optical alignment. This method was developed using a Perkin Elmer Optima 3100 ICP-OES unit. 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. The instrument parameters are as follows: Set the ultraviolet detector to scan nickel at 232.005 nm and yttrium at 371.029 nm. Set the sample read time to 10 s minimum and 50 s maximum. Three replicate scans are taken with the integration set to one point per peak. Set the forward power from the RF generator to 1500 watts. Use an argon plasma feed gas flow of 15 L/min with the auxiliary gas (shear gas) set to flow at 0.5 L/min. Use a gem cone nebulizer with a nebulization gas flow rate of 0.55 L/min. The sample is delivered to the spray chamber by a multi-channel peristaltic pump set to deliver sample at a rate of 1.00 mL/min. The Internal standard is added in-line via a mixing block between the sample probe and spray chamber. Samples are flush through the system for 30 s at a rate of 4.0 mL/min prior to analysis. A 60-second 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 30 s at a rate of 4.0 mL/min.

**Analysis:** Generate a calibration curve using Diluent as a Blank and the Standard solutions as follows: Scan the Internal standard solution while running the blank to measure the intensity of the yttrium emission. Hold this value constant throughout the remainder of the test. Separately scan the Blank and the Standard solutions for nickel and yttrium. Normalize the yttrium intensity to the value of the Internal standard solution. [NOTE: The Internal standard solution is added in-line via a mixing block between the sample probe and spray chamber.] Also apply this normalization factor to the nickel intensity, which is then referred to as the corrected nickel intensity. A calibration curve is constructed by plotting the corrected nickel intensity versus the known concentrations of the Standard solutions. The correlation coefficient for the best-fit line should not be less than 0.999.

Similarly, analyze the Sample solution on the ICP. The intensity of the emission of the Sample solution is plotted on the calibration curve and the concentration is extrapolated against the x-axis. Calculate the concentration (mg/kg) of nickel in the Sample taken using the following equation:

\[
(C/W) \times (50/1000)
\]
C = Concentration (ng/mL) of nickel in the Sample solution determined from the standard curve

W = Weight (g) of Sample taken

50 = Sample dilution factor

1000 = ng to µg conversion factor

Acceptance criteria: NMT 1 mg/kg, calculated on the anhydrous basis

Organic Impurities

• Reducing Sugars

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)

Sample: Amount equivalent to 3.3 g, on the anhydrous basis

Analysis: To the Sample, add 3 mL of water, 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.

Acceptance criteria: NLT 12.8 mL of 0.05 N Sodium thiosulfate VS is required (equivalent to NMT 0.3% reducing sugars (as glucose), calculated on the anhydrous basis)

SPECIFIC TESTS

• pH, pH Determination Appendix IIIB

Sample: 14% w/w solution of sample in carbon dioxide-free water

Acceptance criteria: Between 5.0 and 7.5

• Residue on Ignition (Sulfated Ash), Method II, Appendix IIIC

Sample: 2 g

Acceptance criteria: NMT 0.1%, calculated on the anhydrous basis

• Total Sugars

Cupric sulfate–iodide solution: Dissolve 81 g of potassium citrate monohydrate, 92 g of potassium oxalate, and 74 g of potassium carbonate in hot water, and dilute with water to 600 mL (Solution A). Dissolve 25 g of cupric sulfate in hot water, and dilute with water to 200 mL. Combine this solution with Solution A, and mix for 30 min (Solution B). Dissolve 0.4 g of sodium hydroxide in about 100 mL of water. Dissolve 3.4 g of potassium iodate and 50 g of potassium iodide in this sodium hydroxide solution, and dilute with water to 200 mL (Solution C). Add Solution C to Solution B, and stir for at least 2 h.

Sample: An amount equivalent to 0.25 g on the anhydrous basis

Analysis: Transfer the Sample to a 300-mL conical flask. While swirling the solution, add 35 mL of 3 N sulfuric acid and a few glass beads. Connect a suitable condenser to the flask, bring to a boil on a hot plate within 3 min, and gently reflux the solution for 15 min. Remove the flask from the hot plate and cool it in a 20°C water bath for at least 5 min. Add 5 drops of phenolphthalein TS and 20.0 mL of 5 N sodium hydroxide, and mix. Neutralize the solution with 1 N hydroxide (about 4.5 mL) to a pink endpoint. Adjust the volume of the solution with water to 50 mL, and add 50.0 mL of Cupric sulfate–iodide solution. Connect a suitable condenser to the flask, heat on a hot plate adjusted to bring the solution to boil within 3 min, and gently reflux the solution for 5 min. Remove the flask from the hot plate, and cool it in a water bath at 20°C for 15 to 25 min. Do not overcool. Slowly add 25 mL of 5 N sulfuric acid, and swirl gently to mix. [NOTE: Foaming may occur when the 5 N sulfuric acid is added.] Titrate the liberated iodine with 0.1 N
sodium thiosulfate VS to a pale green color. Add 1 mL of starch TS, mix, and continue the titration to a
pale green-blue endpoint. Perform a blank determination. Calculate the titration difference, based on an
anhydrous 0.5 g sample, by the formula:

\[(V_B - V_U) \times (0.5/W) \times (0.1/N_A)\]

- \(V_B\) = Volume (mL) of sodium thiosulfate VS required for the blank
- \(V_U\) = Volume (mL) of sodium thiosulfate VS required for the Sample
- 0.5 = Theoretical sample weight (g) on the anhydrous basis
- 0.1 = Theoretical normality of the sodium thiosulfate VS
- \(W\) = Weight (g) of the Sample taken on the anhydrous basis
- \(N_A\) = Actual normality of the sodium thiosulfate VS

**Acceptance criteria:** The titration difference is NLT 5.8 mL, corresponding to NLT 7.0% total sugars (as
glucose) on the anhydrous basis

• **WATER, Water Determination, Appendix IIIB**

  **Acceptance criteria:** NMT 31.5%

\(^1\) Aminex Fast Carbohydrate Analytical Column (Bio-Rad Laboratories), or equivalent

**Auxiliary Information**— **Staff Liaison:** Jeffrey Moore, Ph.D., Senior Scientific Associate

**Expert Committee:** (FI07) Food Ingredients Expert Committee

**Phone Number:** 1-301-816-8288
Stannous Chloride, FCC 6 page 926—See briefing under Manganese Sulfate. Interested parties are encouraged to submit comments to Jeff Moore, Ph.D., email jm@usp.org.

(FIEC: J. Moore) C65193

Stannous Chloride

Tin Dichloride

\( \text{SnCl}_2 \)

Formula wt, anhydrous 189.60

\( \text{SnCl}_2 \cdot 2\text{H}_2\text{O} \)

Formula wt, dihydrate 225.63

INS: 521

CAS: anhydrous [7772-99-8]

CAS: dihydrate [10025-69-1]

DESCRIPTION

Stannous Chloride occurs as white or colorless crystals. It is anhydrous or contains two molecules of water of hydration. It is very soluble in water, and it is soluble in alcohol and in glacial acetic acid.

Function Reducing agent; antioxidant

Packaging and Storage Store in well-closed containers.

IDENTIFICATION

- **A. CHLORIDE, Appendix IIIA**
  - Sample solution: 50 mg/mL
  - Acceptance criteria: Passes test.

- **B. PROCEDURE**
  - Sample solution: 50 mg/mL in 2.7 N hydrochloric acid
  - Analysis: Add mercuric chloride TS dropwise to the Sample solution.
  - Acceptance criteria: A white or gray-white precipitate forms.

ASSAY

- **PROCEDURE**
  - Sample: 2 g
  - Analysis: Transfer the Sample into a 250-mL volumetric flask, dissolve it in 15 mL of hydrochloric acid, dilute to volume with water, and mix. Transfer 50.0 mL of this solution into a 500-mL flask, add 5 g of sodium potassium tartrate, and mix. Make the solution alkaline to litmus with a cold saturated solution of sodium bicarbonate and titrate at once with 0.1 N iodine, using starch TS as the indicator. Each mL of 0.1 N iodine is equivalent to 9.48 mg of SnCl\(_2\) or 11.28 mg of SnCl\(_2\)·2H\(_2\)O.

  - Acceptance criteria
    - Anhydrous: NLT 99.0% and NMT 101.0% of SnCl\(_2\)
    - Dihydrate: NLT 98.0% and NMT 102.2% of SnCl\(_2\)·2H\(_2\)O

IMPURITIES
Change to read:
Inorganic Impurities

• **IRON**
  - **Sample**: Residue obtained in the test for *Substances Not Precipitated by Sulfide* (below)
  - **Sample solution**: Add 3 mL of 1:2 hydrochloric acid to the Sample, cover the Sample with a watch glass, and digest on a steam bath for 15 min. Remove the cover, and evaporate to dryness on the steam bath. Dissolve the residue in a few mL of water and 8 mL of hydrochloric acid, dilute to 100 mL with water, and mix. Add 2 mL of hydrochloric acid, 46 mL of water, 40 mg of ammonium persulfate crystals, and 3 mL of ammonium thiocyanate TS to 2.0 mL of this solution.
  - **Control solution**: Combine 2.0 mL of *Iron Standard Solution* (20 µg iron; see *Standard Solutions for the Preparation of Controls and Standards, Solutions and Indicators*), with the same quantities of reagents used in the preparation of the Sample solution.
  - **Acceptance criteria**: Any red or pink color produced by the Sample solution does not exceed that of the Control solution. (NMT 0.005%)

• **LEAD**, Lead Limit Test, APDC Extraction Method, Appendix IIIB
  - **Sample**: 5 g
  - **1S (FCC 6)**
  - **Acceptance criteria**: NMT 4 mg/kg

• **SULFATE**
  - **Sample**: 5 g
  - **Analysis**: Dissolve the Sample in 5 mL of hydrochloric acid and dilute to 50 mL with water. Filter if not clear and heat the filtrate or clear solution to boiling. Add 5 mL of barium chloride TS, digest in a covered beaker on a steam bath for 2 h, and allow to stand overnight.
  - **Acceptance criteria**: No precipitate forms.

SPECIFIC TESTS

• **SOLUBILITY IN HYDROCHLORIC ACID**
  - **Sample**: 5 g
  - **Analysis**: Dissolve the Sample in a mixture of 5 mL of hydrochloric acid and 5 mL of water. Heat to 40°, if necessary, to aid in dissolution.
  - **Acceptance criteria**: The Sample dissolves completely, and the solution is clear.

• **SUBSTANCES NOT PRECIPITATED BY SULFIDE**
  - **Sample**: 20 g
  - **Analysis**: Transfer the Sample into a 250-mL beaker, and add 50 mL of a solution prepared by carefully adding 75 mL of bromine to 425 mL of 48% hydrobromic acid. Then, add 1 mL of sulfuric acid, and mix to aid in dissolution. Place the beaker on a hot plate, and volatilize the tin slowly, with gentle boiling, to fumes of sulfur trioxide. Cool, add 30 mL of water, and pass hydrogen sulfide gas through the solution for about 5 min. Filter through Whatman No. 42 filter paper, or equivalent, into a weighed platinum dish, and wash with three small portions of a 1% solution of sulfuric acid saturated with hydrogen sulfide. Carefully evaporate to dryness on a hot plate, and heat in a furnace at 800° ± 25° for 13 min. Cool in a desiccator for at least 30 min, and weigh. [Note: Retain the residue for the Iron test (above).] Calculate the percentage of substances not precipitated by sulfide by the formula:

\[
\text{Percentage} = \frac{A}{B} \times 100
\]
\[ A = \text{Weight (g) of the residue} \]
\[ B = \text{Weight (g) of the Sample taken} \]

**Acceptance criteria:** NMT 0.05%

**Auxiliary Information**  
*Staff Liaison:* Jeffrey Moore, Ph.D., Senior Scientific Associate  
*Expert Committee:* (FI07) Food Ingredients Expert Committee  
*FCC Sixth Edition Page 926*  
*Phone Number:* 1-301-816-8288
BRIEFING

Tara Gum. Because there is no existing FCC monograph for this food ingredient, a new monograph is proposed based on the following:

1. The chemical information and Description were based on the 1986 Joint FAO/WHO Expert Committee (JECFA) monograph for Tara Gum.
2. The Function and Packaging and Storage were based on comments received.
3. The Gel, Microscopic Examination, Viscosity, and Starch test procedures and acceptance criteria were based on the JECFA monograph.
4. The Arsenic test procedure and limit were adapted from the FCC 6 Locust (Carob) Bean Gum monograph, a galactomannan similar to Tara Gum. The Committee intends to set an Arsenic limit as low as practical for food-grade Tara Gum. Interested parties are encouraged to submit improved methods of analysis and supporting data on this specification in a timely manner.
5. The Lead test procedure was adapted from the FCC 6 Guar Gum monograph, a galactomannan similar to Tara Gum, while the limit was based on the JECFA monograph. The Committee intends to set a Lead limit as low as practical for food-grade Tara Gum. Interested parties are encouraged to submit improved methods of analysis and supporting data on this specification in a timely manner.
6. The Protein, Starch, and Acid-Insoluble Matter test procedures and acceptance criteria were based on the JECFA monograph.
7. The Ash test procedure and acceptance criteria were based on comments and data received.
8. The Galactomannans test procedure and limit were adapted from the FCC 6 Locust (Carob) Bean Gum monograph, a galactomannan similar to Tara Gum. The Committee intends to set Galactomannans acceptance criteria as high as practical for food-grade Tara Gum. Interested parties are encouraged to submit improved methods of analysis and supporting data on this specification in a timely manner.
9. The Loss on Drying test procedure was based on those used in the FCC 6 monographs for Locust (Carob) Bean Gum and Guar Gum, galactomannans similar to Tara Gum, while the acceptance criteria were based on comments and data received.

Interested parties are encouraged to submit comments to Jeff Moore, Ph.D (FIEC: J. Moore) C64547

Add the following:

- Tara Gum

Peruvian carob

INS: 417

CAS: [39300-88-4]

DESCRIPTION

Tara gum occurs as a white to tan, nearly odorless powder obtained by grinding the endosperm of the seeds of the tara tree, Caesalpinia spinosa (Fam. Leguminosae). The gum consists of polysaccharides of high molecular weight composed mainly of galactomannans. The principal component consists of a linear chain of \((1\rightarrow4)\)-\(\beta\)-D-mannopyranose (mannose) units with \(\alpha\)-D-galactopyranose (galactose) units attached by \((1\rightarrow6)\) linkages. The ratio of mannose to galactose in tara gum is approximately 3:1. Tara gum is water-soluble, but insoluble in ethanol.
**IDENTIFICATION**

- **Gel Test**
  - **Analysis:** To an aqueous solution of the sample add small amounts of sodium borate.
  - **Acceptance criteria:** A gel is formed.

- **Microscopic Examination**
  - **Analysis:** Prepare an aqueous solution of 0.5% iodine and 1% potassium iodide. Add some ground sample to the solution and transfer a portion of this mixture onto a glass slide and examine under a microscope.
  - **Acceptance criteria:** Tara gum contains groups of round to pear-shaped cells; their contents are yellow to brown. [Note: Guar gum cells are similar in form but markedly larger in size. Carob bean gum shows long, stretched tubiform cells, separate or slightly interspaced and can be easily distinguished from tara gum.]

- **Viscosity**
  - **Sample:** 2 g
  - **Analysis:** Transfer the sample into a 400-mL beaker and moisten it thoroughly with about 4 mL of isopropanol. Add, with vigorous stirring, 200 mL of water and continue stirring until the gum is completely and uniformly dispersed and an opalescent, moderately viscous solution forms. [Note: This solution is more viscous than a carob bean gum solution when prepared in the same manner.] Transfer 100 mL of this solution into another 400-mL beaker, heat the mixture in a boiling water-bath for about 10 min and cool to room temperature.
  - **Acceptance criteria:** A marked increase in viscosity is observed.

**IMPURITIES**

**Inorganic Impurities**

- **Arsenic, Arsenic Limit Test, Appendix IIIB**
  - **Sample solution:** Prepare as directed for organic compounds.
  - **Acceptance criteria:** NMT 3 mg/kg

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

**Organic Impurities**

- **Protein, Nitrogen Determination, Method I, Appendix IIIC**
  - **Sample:** 3.5 g
  - **Analysis:** Transfer the sample into a 500-mL Kjeldahl flask. The percentage of nitrogen determined multiplied by 5.7 gives the percentage of protein in the sample.
  - **Acceptance criteria:** NMT 3.5%

- **Starch**
  - **Sample solution:** 100 mg/mL
  - **Analysis:** Add a few drops of iodine TS.
  - **Acceptance criteria:** No blue color develops.

**SPECIFIC TESTS**

- **Ash (Total), Appendix IIC**
  - **Analysis:** Proceed as directed, but ignite at 675 ±25°.
  - **Acceptance criteria:** NMT 1.0%

- **Acid-Insoluble Matter**
Sample: 2 g

**Analysis:** Transfer the Sample into a 250-mL beaker containing 150 mL of water and 1.5 mL of sulfuric acid. Cover the beaker with a watch glass and heat the mixture on a steam bath for 6 h, rubbing down the wall of the beaker frequently with a rubber-tipped stirring rod and replacing any water lost by evaporation. Weigh 500 mg of a suitable acid-washed filter aid, pre-dried at 105° for 1 h, to the nearest 0.1 mg, add the filter aid to the sample solution, and filter it through a tared sintered-glass filter crucible. Wash the residue several times with hot water, dry the crucible and its contents at 105° for 3 h, cool in a desiccator, and weigh. The difference between the total weight and the weight of the filter aid plus crucible is the weight of the Acid-insoluble matter. Calculate as a percentage.

**Acceptance criteria:** NMT 2.0%

• **GALACTOMANNANS**
  
  **Analysis:** Determine the difference between 100 and the sum of the percentages of Acid-Insoluble Matter, Ash (Total), Loss on Drying, and Protein.

  **Acceptance criteria:** NLT 75%

• **Loss on Drying**, Appendix IIC (105° for 5 h)
  
  **Acceptance criteria:** NMT 12.0%

Auxiliary Information—**Staff Liaison:** Jeffrey Moore, Ph.D., Senior Scientific Associate

**Expert Committee:** (FI07) Food Ingredients Expert Committee

**Phone Number:** 1-301-816-8288
TBHQ, *FCC 6* page 950. On the basis of comments received, it is proposed to revise the tert-Butyl-p-benzoquinone impurity test procedure to replace use of the existing USP tert-Butyl-p-benzoquinone RS with reagent-grade material. Comments submitted indicated a lack of chemical stability for this compound under typical storage conditions and a lack of widespread usage in industry. Interested parties are encouraged to submit comments and potential improved analytical methods for this test procedure to Jeff Moore, Ph.D., at jm@usp.org.

(FIEC: J. Moore) C65282

**TBHQ**

tert-Butylhydroquinone

Mono-tert-butylhydroquinone

![Chemical structure of TBHQ](image_url)

**DESCRIPTION**

TBHQ occurs as a white, crystalline solid. It is soluble in alcohol and in ether, and it is practically insoluble in water.

**Function** Antioxidant

**Packaging and Storage** Store in well-closed containers.

**IDENTIFICATION**

- **Procedure**
  - **Analysis:** Dissolve a few mg of sample in 1 mL of methanol, and add a few drops of a 25% solution of dimethylamine in water.
  - **Acceptance criteria:** A pink to red color appears.

**ASSAY**

- **Procedure**
  - **Sample:** 170 mg, previously ground to a fine powder
  - **Analysis:** Transfer Sample, into a 250-mL wide-mouth Erlenmeyer flask, and dissolve in 10 mL of methanol. To the flask, add 150 mL of water, 1 mL of 1 N sulfuric acid, and 4 drops of diphenylamine indicator (3 mg...
of p-diphenylaminesulfonic acid sodium salt per mL of 0.1 N sulfuric acid), and titrate with 0.1 N ceric sulfate to the first complete color change from yellow to red-violet. Record the volume, in mL, of 0.1 N ceric sulfate required as V. Calculate the percentage, A, of C\textsubscript{10}H\textsubscript{14}O\textsubscript{2} in the Sample, uncorrected for hydroquinone and 2,5-di-tert-butylhydroquinone, by the formula:

\[
N = \text{Exact normality of the ceric sulfate solution} \\
0.1 \text{ mL} = \text{Volume of ceric sulfate solution consumed by the primary oxidation products of TBHQ ordinarily present in the Sample} \\
W = \text{Weight (g) of Sample taken}
\]

If hydroquinone (HQ) and 2,5-di-tert-butylhydroquinone (DTBHQ) are present in the Sample, they will be included in the titration. Calculate the corrected percentage of C\textsubscript{10}H\textsubscript{14}O\textsubscript{2} in the Sample by the formula:

\[
A (\%HQ \times 1.51) (\%DTBHQ \times 0.75)
\]

\%HQ = Percent hydroquinone, determined below in the test for 2,5-Di-tert-butylhydroquinone and Hydroquinone
\%DTBHQ = Percent 2,5-Di-tert-butylhydroquinone, determined below in the test for 2,5-Di-tert-butylhydroquinone and Hydroquinone

Acceptance criteria: NLT 99.0% of C\textsubscript{10}H\textsubscript{14}O\textsubscript{2}

**IMPURITIES**

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

**Change to read:**

**Organic Impurities**
- **TERT-BUTYL-p-BENZOQUINONE**
  - **Standard solution**: Transfer 10 mg of USP Monotertiary-butyl-p-benzoquinone RS\textsuperscript{1} into a 10-mL volumetric flask, dissolve in and dilute to volume with chloroform, and mix.
  - **Sample solution**: Transfer 1 g of sample, previously reduced to a fine powder in a high-speed blender, into a 10-mL volumetric flask, dilute to volume with chloroform, and shake for 5 min to extract the tert-butyl-p-benzoquinone. Filter through a Millipore filter (UHWP01300), or equivalent, before use.
  - **Analysis**: Use a suitable double-beam IR spectrophotometer and matched 0.4-mm liquid sample cells with calcium fluoride windows. Fill the reference cell with chloroform and the sample cell with **Standard solution**, place the cells in the respective reference and sample beams of the spectrophotometer, and record the spectrum from 1600 to 1775 cm\textsuperscript{-1}. Draw a background line on the spectrum from 1612 to 1750 cm\textsuperscript{-1}, and determine the net absorbance (A\textsubscript{S}) of the **Standard solution** at 1659 cm\textsuperscript{-1}. Similarly, obtain the
spectrum of the Sample solution, and determine its net absorbance ($A_U$) at 1659 cm$^{-1}$. Calculate the percentage of tert-butyl-p-benzoquinone in the sample taken by the formula:

$$100\left(\frac{A_U}{A_S}\right)\left(\frac{W_S}{W_U}\right)$$

$W_S =$ The exact$^{\text{1S (FCC 6)}}$ Weight (mg) of USP Monotertiary-butyl-p-benzoquinone RSmonotertiary-butyl-p-benzoquinone$^{\text{1S (FCC 6)}}$ taken to prepare the Standard solution

$W_U =$ The exact$^{\text{1S (FCC 6)}}$ Weight (mg) of the sample taken to prepare the Sample solution

**Acceptance criteria:** NMT 0.2%

- **2,5-Di-tert-butylhydroquinone and Hydroquinone**

  **HQ stock solution:** 1 mg/mL hydroquinone in pyridine
  **DTBHQ stock solution:** 1 mg/mL 2,5-di-tert-butylhydroquinone in pyridine
  **Methyl benzoate stock solution:** 1 mg/mL methyl benzoate in pyridine

  **Standard solutions:** Prepare four HQ standard solutions as follows: Add 0.50, 1.00, 2.00, and 3.00 mL of HQ stock solution into separate 10-mL volumetric flasks, then add 2.00 mL of Methyl benzoate stock solution to each, dilute to volume with pyridine, and mix.

  Prepare four DTBHQ Standard solutions as follows: Add 0.50, 1.00, 2.00, and 3.00 mL of DTBHQ stock solution into separate 10-mL volumetric flasks, then add 2.00 mL of Methyl benzoate stock solution to each, dilute to volume with pyridine, and mix.

  Prepare the trimethylsilyl derivative of each standard solution as follows: Add 9 drops of standard solution to a 2-mL serum vial, cap the vial, evacuate with a 50-mL gas syringe, add 250 µL of N,O-bistrimethylsilylacetamide, and heat at about 80$^\circ$C for 10 min.

  **Sample solution:** Transfer 1 g of sample into a 10-mL volumetric flask, add 2.00 mL of Methyl benzoate stock solution, dilute to volume with pyridine, and mix. Add 9 drops of the resulting solution to a 2-mL serum vial, cap the vial, evacuate with a 50-mL gas syringe, add 250 µL of N,O-bistrimethylsilylacetamide, and heat at about 80$^\circ$C for 10 min.

  **Chromatographic system,** Appendix IIA

  **Mode:** Gas chromatography
  **Detector:** Thermal conductivity detector (F and M Model 810, or equivalent)
  **Column:** 0.61-m × 6.35-mm (od) stainless steel column (Perkin Elmer Instruments, or equivalent) packed with 20% Silicone SE-30, by weight, and 80% Diatoport S (60-to 80-mesh), or equivalent materials
  **Column temperature:** From 100$^\circ$C to 270$^\circ$C, heated at a rate of 15$^\circ$/min
  **Injection port temperature:** 300$^\circ$C
  **Carrier gas:** Helium
  **Flow rate:** 100 mL/min
  **Injection volume:** 10 µL

  **Analysis:** [Note: Use bridge current of 140 mA, and sensitivity, 1× for the integrator (Infotronics CRS-100, or equivalent) and 2× for the recorder.] Chromatograph each Standard solution in duplicate, and plot the concentration ratio of HQ standard solution to Methyl benzoate stock solution (x-axis) against the response ratio of HQ standard solution to Methyl benzoate stock solution (y-axis). Plot the same relationships between DTBHQ standard solution and the Methyl benzoate stock solution. Chromatograph duplicate aliquots of Sample solution. The approximate peak times, in min, are methyl...
benzoate, 2.5; TMS derivative of HQ, 5.5; TMS derivative of tert-butylhydroquinone, 7.3; TMS derivative of DTBHQ, 8.4.

Determine the peak areas (response) of interest by automatic integration or manual triangulation. Calculate the response ratio of HQ standard solution and DTBHQ standard solution to Methyl benzoate stock solution. From the calibration curves, determine the concentration ratio of HQ standard solution and DTBHQ standard solution to Methyl benzoate stock solution, and calculate the percentages of hydroquinone and of 2,5-di-tert-butylhydroquinone in the sample by the formula:

\[ Y \times I \times 10/S \]

- **Acceptance criteria:** NMT 0.2% 2,5-Di-tert-butylhydroquinone; NMT 0.1% Hydroquinone

• **Toluene**

  **Standard solution:** 50 µg/mL toluene in octyl alcohol

  **Sample solution:** 0.2 g/mL sample in octyl alcohol

  **Chromatographic system,** Appendix IIA

  **Mode:** Gas chromatography

  **Detector:** Flame ionization detector (F and M Model 810, or equivalent)

  **Column:** 3.66-m × 3.18-mm (od) stainless steel column, or equivalent, packed with 10% Silicone SE-30, by weight, and 90% Diatoport S (60- to 80-mesh), or equivalent materials

  **Column temperature:** From 70°C to 280°C, heated at a rate of 15°C/min and held

  **Injection port temperature:** 275°C

  **Oven temperature:** 300°C

  **Carrier gas:** Helium

  **Flow rate:** 50 mL/min

  **Injection volume:** 5 µL

  **Analysis:** [Note: Use hydrogen and air settings of 20 psi for each.] Inject Standard solution into the chromatograph, and measure the height of the toluene peak (\(H_R\)) on the chromatogram. The toluene retention time is 3.3 min; other peaks are of no interest in this analysis. Similarly, obtain the chromatogram of Sample solution, and measure the height of the toluene peak (\(H_S\)). Calculate the percentage of toluene in the sample by the formula:

\[ 100(H_S/H_R)(C_R/C_S) \]

- **Acceptance criteria:** NMT 0.0025%

**SPECIFIC TESTS**
• **Melting Range or Temperature**, Appendix IIB

**Acceptance criteria:** Between 126.5° and 128.5°

**Change to read:**

• **Ultra Violet Absorbance (Polynuclear Hydrocarbons)**

**Sample preparation:** Dissolve 1 g of L-ascorbic acid in 100 mL of ethanol and 100 mL of water contained in a 500-mL separator (S-1). Transfer about 50 g of sample into the separator, shake to dissolve, then add 50 mL of isooctane, and extract for 3 min. After the phases have separated, drain the lower, aqueous phase into a second 500-mL separator (S-2), then after 1 min of further separating, drain the lower phase into separator S-2. Add a second 50-mL portion of isooctane to the aqueous solution in S-2, and repeat the extraction procedure as previously described, drawing off the lower, aqueous phase into a third 500-mL separator (S-3). Add a third 50-mL portion of isooctane to the aqueous solution in S-3, and repeat the extraction procedure as previously described, drawing off and discarding the lower, aqueous phase. Extract the solutions in S-1, S-2, and S-3 with two 100-mL portions of a 0.5% solution of 75 : 25 ethanol and water–ascorbic acid. Shake each mixture for 3 min, allow the phases to separate, and discard the lower, aqueous phases. Next, extract each isooctane solution with two 100-mL portions of a 5% solution of ethanol in water, and discard the lower, aqueous phases. Finally, wash each solution twice with 100 mL of water, and discard the washings.

Lightly pack a standard-size chromatographic tube with 100 g of anhydrous sodium sulfate, and wash the packed column with 75 mL of isooctane, discarding the washings. Filter the isooctane solution from S-1 through the column, and collect the filtrate in a 500-mL distillation flask. Wash S-1 with the isooctane solution contained in S-2, then pour the solution onto the column, collecting the filtrate in the flask. Wash S-2 and S-1, successively, with the isooctane solution in S-3, and filter the solution through the column as before. Wash S-3, S-2, and S-1, in that order and in tandem, with two successive 25-mL portions of isooctane, and pass the washings individually through the column and into the flask. Let the column drain completely.

Add 2 mL of hexadecane and 2 boiling stones to the 500-mL distillation flask containing the combined isooctane extracts, and attach the flask to a suitable vacuum distillation assembly. Evacuate the assembly to about one-third atmosphere, then immerse the flask in a steam bath, and distill the solvent. When isooctane stops dripping into the receiver, turn off the vacuum, wash down the walls of the flask with 5 mL of isooctane added through the top of the distillation head, then replace the thermometer and again evacuate. The isooctane should distill over in about 1 min. At the end of this distillation, add another 5-mL portion of isooctane, and repeat the stripping procedure. Quantitatively wash the residue from the distillation flask into a 50-mL volumetric flask with isooctane, dilute to volume with isooctane, and mix.

**Analysis:** Determine the ultraviolet absorption spectrum of the Sample preparation in a 5-cm silica cell from 400 nm to 250 nm with a suitable spectrophotometer, using isooctane as the blank. Determine the absorbance of a solvent control by following the above procedure in every detail, but with the sample omitted. From the sample spectrum determine the maximum absorbance per cm pathlength in each of the following wavelength intervals: (a) 280 to 289 nm; (b) 290 to 299 nm; (c) 300 to 359 nm; and (d) 360 to 400 nm. Calculate the maximum net absorbance/cm in each interval by subtracting from the sample absorbance the corresponding absorbance per cm of the solvent control.

**Acceptance criteria:** The following net absorbance values are not exceeded at the indicated intervals: (a) 0.15; (b) 0.12; (c) 0.08; and (d) 0.02.

**Auxiliary Information—** Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate

**Expert Committee:** (FI07) Food Ingredients Expert Committee
BRIEFING

α-Terpine,  

FCC 6 page 953—See briefing under 

Acetaldehyde.

(FIEC:  J. Moore)  C64747

α-Terpine

1-Methyl-4-(1-methylethyl)-1,3-cyclohexadiene

C_{10}H_{16}  

Formula wt 136.24

FEMA:  3558

DESCRIPTION

Change to read:

α-Terpine occurs as a colorless liquid. It may contain

a suitable antioxidant. (FCC)

Odor  Lemon

Solubility  Soluble in alcohol, most fixed oils; insoluble

or practically insoluble in water

Boiling Point  \( \sim 173^\circ \)

Solubility in Alcohol, Appendix VI

One mL dissolves in 2 mL of 95% ethanol.

Function  Flavoring agent

ASSAY

• PROCEDURE  Proceed as directed under M-1a, Appen-
dix XI.

Acceptance criteria:  NLT 89.0% of C_{10}H_{16}

SPECIFIC TESTS

• REFRACTIVE INDEX, Appendix II (at 20°)

Acceptance criteria:  Between 1.475 and 1.480

• SPECIFIC GRAVITY  Determine at 25° by any reliable

method (see General Provisions).

Acceptance criteria:  Between 0.833 and 0.838
**Briefing**

**γ-Terpinene, FCC 6 page 953**—See briefing under Acetaldehyde.

(FIEC: J. Moore) C64747

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**γ-Terpinene**

1-Methyl-4-(1-methylethyl)-1,4-cyclohexadiene

\[ \text{C}_{10}\text{H}_{16} \quad \text{Formula wt 136.24} \]

FEMA: 3559

**Odor**  Herbaceous, citrus

**Solubility**  Soluble in alcohol, most fixed oils; insoluble or practically insoluble in water

**Boiling Point**  \( \sim 182^\circ \)

**Solubility in Alcohol**, Appendix VI  
One mL dissolves in 3 mL of 95% ethanol.

**Function**  Flavoring agent

**ASSAY**

- **PROCEDURE**  Proceed as directed under M-1a, Appendix XI.
  - Acceptance criteria:  NLT 95.0% of \( \text{C}_{10}\text{H}_{16} \)

**SPECIFIC TESTS**

- **Refractive Index**, Appendix II  (at 20°)
  - Acceptance criteria:  Between 1.473 and 1.477

- **Specific Gravity**  Determine at 25° by any reliable method (see General Provisions).
  - Acceptance criteria:  Between 0.841 and 0.845

**DESCRIPTION**

*Change to read:*  γ-Terpinene occurs as a colorless liquid.  ■ It may contain a suitable antioxidant.  ■■ (FCC)

---

\[ \text{Date: 28-APR-2008} \quad \text{Time: 15:28} \]

\[
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\]
BRIEFING

Tolualdehyde, Mixed Isomers, *FCC 6* page 980—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

Tolualdehyde, Mixed Isomers

Tolyl Aldehyde, mixed isomers

Methylbenzaldehyde

\[
\text{C}_8\text{H}_8\text{O}
\]

Formula wt 120.15
FEMA: 3068

DESCRIPTION

Tolualdehyde, Mixed Isomers occurs as a colorless liquid. It may contain a suitable antioxidant. 1S (FCC 6)

Odor Cherry

Boiling Point \(-198^\circ\)

Solubility in Alcohol, Appendix VI

One mL dissolves in 1 mL of 95% alcohol.

Function Flavored agent

IDENTIFICATION

• 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.
ASSAY

• Procedure Proceed as directed under M-1b, Appendix XI
  Acceptance criteria: NLT 94.0% of C₉H₁₈O (sum of three isomers)

SPECIFIC TESTS

• ACID VALUE, M-15, Appendix XI
  Acceptance criteria: NMT 5.0

• REFRACTIVE INDEX, Appendix II (at 20°)
  Acceptance criteria: Between 1.540 and 1.548

• Specific Gravity Determine at 25° by any reliable method (see General Provisions).
  Acceptance criteria: Between 1.019 and 1.029

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
FCC Sixth Edition Page 980
Phone Number: 1-301-816-8288
p-Tolualdehyde, FCC 6 page 981—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

p-Tolualdehyde

p-Tolyl Aldehyde

p-Methylbenzaldehyde

\[
\text{C}_8\text{H}_9\text{O}
\]

Formula wt 120.15
FEMA: 3068

DESCRIPTION

Change to read:
p-Tolualdehyde occurs as a colorless liquid. It may contain a suitable antioxidant. 1S (FCC 6)

Odor Cherry

Boiling Point −83° to 85° (11 mm Hg)

Solubility in Alcohol, Appendix VI
One mL dissolves in 1 mL of 95% alcohol.

Function Flavoring agent

ASSAY

• Procedure Proceed as directed under M-1b, Appendix XI.
  Acceptance criteria: NLT 97.0% of C\(_8\)H\(_9\)O

SPECIFIC TESTS

• ACID VALUE, M-15, Appendix XI
  Acceptance criteria: NMT 5.0

• REFRACTIVE INDEX, Appendix II (at 20°)
  Acceptance criteria: Between 1.542 and 1.548

• Specific Gravity Determine at 25° by any reliable method (see General Provisions).
  Acceptance criteria: Between 1.012 and 1.018
**BRIEFING**

**Trehalose, FCC 6 page 983.** On the basis of comments received, it is proposed to make the following revisions.

1. It is proposed to delete the CAS number for anhydrous trehalose since only the dihydrate material is currently in commerce. It is also proposed to revise the chemical structure to reflect the dihydrate material, consistent with the structure appearing in *FCC 5* page 486.

2. For the *Color in Solution, pH, and Turbidity of a 30% Solution* test procedures, it is proposed to correct the *Sample solution* concentrations to reflect those in *FCC 5* page 486, and to delete the *Note* and reference to use of a refractometer to confirm the concentrations of these solutions.

Interested parties are encouraged to submit comments to Jeff Moore, Ph.D., e-mail jm@usp.org.

(FIEC: J. Moore) C64985

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**Trehalose**

*Change to read:*

\(\alpha\)-D-Glucopyranosyl-\(\alpha\)-D-glucopyranoside, dihydrate

\[C_{12}H_{22}O_{11} \cdot 2H_2O\]

**DESCRIPTION**

Trehalose occurs as a nonhygroscopic, white, crystalline powder. It is obtained through enzymatic conversion of food-grade starch into a stable, nonreducing disaccharide with two glucose molecules linked in an \(\alpha,\alpha-1,1\) configuration. The powder is freely soluble or readily dispersible in water. Trehalose is typically used in its dihydrate form.

**Function** Humectant; nutritive sweetener, stabilizer; thickener; texturizer
Packaging and Storage  Store in tight containers in a dry place.

IDENTIFICATION
• Microscopic Examination
  Analysis: Observe a sample with a light microscope at 50×.
  Acceptance criteria: The sample is composed of colorless, rectangular crystals with a prismatic structure.

ASSAY
• Procedure
  Sample: 3 g
  Sample stock solution: 30 mg/mL, filtered through a 0.45-µm membrane filter
  Sample solution: Combine 3.7 mL of Sample stock solution with 10 mL of acetonitrile.
  Standard stock solution: 40 mg/mL Trehalose\(^1\) reference standard (on an anhydrous basis).
    [NOTE: Determine the water content of the Trehalose reference standard as directed under Water Determination, Appendix IIIB. Using the water content, calculate the weight of Trehalose reference standard needed to prepare the solution.]
  Standard solution: Combine 3.7 mL of Standard stock solution with 10 mL of acetonitrile.
  Mobile phase: Acetonitrile and water [73:27] (v/v)
  Chromatographic system, Appendix IIA
    Mode: High-performance liquid chromatography
    Detector: Differential refractometer
    Column: 300-mm × 10-mm (id); (Shodex Ionpack KS 801, or equivalent)
  Column temperature: 35\(^{\circ}\)
  Injection volume: 20 µL
  Analysis: Filter the Standard solution and Sample solution through a 0.45-µm membrane filter and separately inject the solutions into the chromatograph. Calculate the percent Trehalose in the sample using the equation:

  \[
  \%\text{Trehalose} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_S}{C_U} \right) \times 100\%
  \]

  \(r_U\) = Peak response from the Sample solution
  \(r_S\) = Peak response from the Standard solution
  \(C_S\) = Concentration of Standard solution (mg/mL)
  \(C_U\) = Concentration of Sample solution (mg/mL)

  Acceptance criteria: NLT 98.0%, calculated on the dried basis

IMPURITIES
Inorganic Impurities
• Lead, Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace Method, Method I, Appendix IIIB
  Sample: 5 g
  Acceptance criteria: NMT 0.1 mg/kg
SPECIFIC TESTS

Change to read:

• **Color in Solution**

  Sample solution:
  0.5 mg/mL in recently boiled water. [NOTE: Confirm the concentration as 30% ± 1% using a suitable refractometer.] Dissolve 33 g of sample in 67 g of recently boiled water. \(1\)S (FCC 6)

  **Analysis:** Determine the absorbance of the Sample solution at 420 nm and 720 nm using a 1-cm cuvette. Calculate *Color in solution* by the following formula:

  \[
  A_{420} - A_{720}
  \]

  \(A_{420}\) = Absorbance at 420 nm

  \(A_{720}\) = Absorbance at 720 nm

  **Acceptance criteria:** NMT 0.100

• **Loss on Drying, Appendix IIC (60°C for 5 h)**

  **Acceptance criteria:** NMT 1.5%

Change to read:

• **pH, pH Determination, Appendix IIB**

  Sample solution:
  0.5 mg/mL in recently boiled water. [NOTE: Confirm the concentration as 30% ± 1% using a suitable refractometer.] Dissolve 33 g of sample in 67 g of recently boiled water. \(1\)S (FCC 6)

  **Acceptance criteria:** Between 4.5 and 6.5

• **Residue on Ignition (Sulfated Ash), Appendix IIC**

  **Sample:** 5 g

  **Acceptance criteria:** NMT 0.05%.

Change to read:

• **Turbidity of a 30% Solution**

  Sample solution:
  0.5 mg/mL in recently boiled water. [NOTE: Confirm the concentration as 30% ± 1% using a suitable refractometer.] Dissolve 33 g of sample in 67 g of recently boiled water. \(1\)S (FCC 6)

  **Analysis:** Using a suitable spectrophotometer, determine the absorbance of the Sample solution at 720 nm.

  **Acceptance criteria:** NMT 0.050


**Auxiliary Information—Staff Liaison:** Jeffrey Moore, Ph.D., Senior Scientific Associate

**Expert Committee:** (FI07) Food Ingredients Expert Committee

**FCC Sixth Edition** Page 983

**Phone Number:** 1-301-816-8288
BRIEFING

2-Tridecenal, *FCC 6* page 987—See briefing under *Acetaldehyde*.
(FIEC: J. Moore) C64747

2-Tridecenal

\[ \text{C}_{13}\text{H}_{24}\text{O} \]

Formula wt 196.33
FEMA: 3082

DESCRIPTION

*Change to read:*

2-Tridecenal occurs as a white or slightly yellow liquid. It may contain a suitable antioxidant. 1S (FCC 6)

**Odor** Oily, citrus

**Solubility** Soluble in alcohol, most fixed oils; insoluble or practically insoluble in water

**Solubility in Alcohol, Appendix VI**

One mL dissolves in 1 mL of 95% ethanol.

**Function** Flavoring agent

IDENTIFICATION

*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.

ASSAY

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

**Acceptance criteria:** NLT 92.0% of *C*\textsubscript{13}\textsubscript{H}\textsubscript{24}\textsubscript{O}

SPECIFIC TESTS

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

**Acceptance criteria:** Between 1.455 and 1.460

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

**Acceptance criteria:** Between 0.842 and 0.862

**Auxiliary Information—Staff Liaison:** Jeffrey Moore, Ph.D., Senior Scientific Associate
3,5,5-Trimethyl Hexenal, *FCC 6* page 989—See briefing under Acetaldehyde.
(FIEC: J. Moore) C64747

3,5,5-Trimethyl Hexanal

\[
\begin{align*}
\text{C}_9\text{H}_{18}\text{O} \\
\text{Formula wt 142.24} \\
\text{FEMA: 3524}
\end{align*}
\]

**DESCRIPTION**

*Change to read:*

3,5,5-Trimethyl Hexanal occurs as a colorless to pale yellow liquid. It may contain a suitable antioxidant.

**Odor** Melon, green

**Boiling Point** \(\sim 67^\circ\) (2.5 mm Hg)

**Function** Flavoring agent

**ASSAY**

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

  **Acceptance criteria:** NLT 97.0%

**SPECIFIC TESTS**

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

  **Acceptance criteria:** NMT 5.0

- **REFRACTIVE INDEX,** Appendix II (at 20\(^\circ\))

  **Acceptance criteria:** Between 1.419 and 1.424

- **SPECIFIC GRAVITY** Determine at 25\(^\circ\) by any reliable method (see General Provisions).

  **Acceptance criteria:** Between 0.817 and 0.823

**Auxiliary Information**— *Staff Liaison:* Jeffrey Moore, Ph.D., Senior Scientific Associate

*Expert Committee:* (FI07) Food Ingredients Expert Committee

*FCC Sixth Edition Page 989*

*Phone Number:* 1-301-816-8288
**BRIEFING**

Undecanal, FCC 6 page 996—See briefing under Acetaldehyde.

(FIEC: J. Moore) C64747

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**Undecanal**

Aldehyde C-11 Undecyclic

\( n \text{-Undecyl Aldehyde} \)

\[ C_{11}H_{22}O \]

Change to read:

Undecanal occurs as a colorless to slightly yellow liquid. It may contain a suitable antioxidant.\(^1\)\(^S\) (FCC 6)

Odor Sweet, fatty, floral

Solubility Soluble in most fixed oils, propylene glycol; insoluble or practically insoluble in glycerin, water

Boiling Point \( \sim223^\circ \)

Function Flavoring agent

**IDENTIFICATION**

- **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.

**ASSAY**

- **PROCEDURE** Proceed as directed under M-1b, Appendix XI.
  
  Acceptance criteria: NLT 92.0% of \( C_{11}H_{22}O \)

**SPECIFIC TESTS**

- **ACID VALUE, M-15, Appendix XI**
Acceptance criteria: NMT 10.0

• **REFRACTIVE INDEX**, Appendix II (at 20°)
  Acceptance criteria: Between 1.430 and 1.435

• **Specific Gravity** Determine at 25° by any reliable method (see General Provisions).
  Acceptance criteria: Between 0.825 and 0.832

**Auxiliary Information**— *Staff Liaison:* Jeffrey Moore, Ph.D., Senior Scientific Associate
*Expert Committee:* (Fi07) Food Ingredients Expert Committee
*FCC Sixth Edition* Page 996
*Phone Number: 1-301-816-8288*
BRIEFING

1,3,5-Undecatriene, FCC 6 page 998—See briefing under Acetaldehyde.
(FIEC: J. Moore)  C64747

1,3,5-Undecatriene

\[ \text{C}_{11}\text{H}_{18} \]

Formula wt 150.26
FEMA: 3795

DESCRIPTION

Change to read:
1,3,5-Undecatriene occurs as a clear, colorless to pale yellow liquid. It may contain a suitable antioxidant.

- Odor Oily, waxy, peppery
- Boiling Point \(-88^\circ\) (1 mm Hg)
- Solubility in Alcohol, Appendix VI
  One mL dissolves in 25 mL of 95% alcohol.
- Function Flavoring agent

IDENTIFICATION

- 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.

<table>
<thead>
<tr>
<th>1,3,5-Undecatriene</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Infrared Spectra" /></td>
</tr>
</tbody>
</table>

ASSAY

- Procedure Proceed as directed under M-1b, Appendix XI
  Acceptance criteria: NLT 90% of \( \text{C}_{11}\text{H}_{18} \) (sum of isomers)

SPECIFIC TESTS

- REFRACTIVE INDEX, Appendix II (at 20\(^\circ\))
  Acceptance criteria: Between 1.508 and 1.517
- Specific Gravity Determine at 25\(^\circ\) by any reliable method (see General Provisions).
Acceptance criteria: Between 0.787 and 0.793

Auxiliary Information— Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
FCC Sixth Edition Page 998
Phone Number: 1-301-816-8288
10-Undecenal

10-Undecenal occurs as a colorless to light yellow liquid. It may contain a suitable antioxidant.\(^{1s}\) (FCC 6)

**Odor** Fatty; rose on dilution

**Solubility** Soluble in most fixed oils, propylene glycol; insoluble or practically insoluble in glycerin, water

**Boiling Point** \(\sim 235^\circ\)

**Function** Flavoring agent

**IDENTIFICATION**

- **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.

**ASSAY**

- **Procedure** Proceed as directed under *M-1b, Appendix XI*
  
  **Acceptance criteria**: NLT 90.0% of \(\text{C}_{11}\text{H}_{20}\text{O}\)

**SPECIFIC TESTS**

- **ACID VALUE, M-15, Appendix XI**
  
  **Acceptance criteria**: NMT 6.0
• **REFRACTIVE INDEX**, Appendix II (at 20°)
  Acceptance criteria: Between 1.441 and 1.447

• **Specific Gravity** Determine at 25° by any reliable method (see *General Provisions*).
  Acceptance criteria: Between 0.840 and 0.850

** Auxiliary Information —** Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate
Expert Committee: (FI07) Food Ingredients Expert Committee
FCC Sixth Edition Page 999
Phone Number: 1-301-816-8288
Valeraldehyde, FCC 6 page 1003—See briefing under Acetaldehyde. (FIEC: J. Moore) C64747

Valeraldehyde

Valeraldehyde occurs as a colorless to pale yellow liquid. It may contain a suitable antioxidant. 

Odor Chocolate

Boiling Point \( \sim 103^\circ \)

Solubility in Alcohol, Appendix VI

One mL dissolves in 1 mL of 95% alcohol.

Function Flavoring agent

**IDENTIFICATION**

- **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.

**ASSAY**

- **Procedure** Proceed as directed under M-2a, Appendix XI.
  
  **Acceptance criteria:** NLT 97.0% of \( C_5H_{10}O \)

**SPECIFIC TESTS**

- **ACID VALUE, M-15, Appendix XI**
Acceptance criteria: NMT 5.0

- **REFRACTIVE INDEX**, Appendix II (at 20°)
  Acceptance criteria: Between 1.390 and 1.395

- **Specific Gravity** Determine at 25° by any reliable method (see General Provisions).
  Acceptance criteria: Between 0.805 and 0.809

**Auxiliary Information**— *Staff Liaison*: Jeffrey Moore, Ph.D., Senior Scientific Associate
*Expert Committee*: (FI07) Food Ingredients Expert Committee

*FCC Sixth Edition* Page 1003

*Phone Number*: 1-301-816-8288
BRIEFING

Zinc Oxide, *FCC 6* page 1033—See briefing under *Manganese Sulfate*. Interested parties are encouraged to submit comments to Jeff Moore, Ph.D., at jm@usp.org.

(FIEC: J. Moore) C65193

Zinc Oxide
ZnO

**DESCRIPTION**

Zinc Oxide occurs as a fine, white, amorphous powder. It gradually absorbs carbon dioxide from the air. It is insoluble in water and in alcohol, and is soluble in dilute acids and in strong bases.

**Function** Nutrient

**Packaging and Storage** Store in well-closed containers.

**IDENTIFICATION**

- **A. Zinc**, Appendix IIIA
  - **Sample solution**: Dissolve a sample in a slight excess of 3 N hydrochloric acid.
  - **Acceptance criteria**: Passes tests.

- **B. Procedure**
  - **Analysis**: Strongly heat a sample.
  - **Acceptance criteria**: A yellow color forms that disappears on cooling.

**ASSAY**

- **Procedure**
  - **Sample**: 1.5 g, freshly ignited
  - **Analysis**: Dissolve the Sample and 2.5 g of ammonium chloride in 50 mL of 1 N sulfuric acid with the aid of gentle heat, if necessary. When solution is complete, add methyl orange TS and titrate the excess sulfuric acid with 1 N sodium hydroxide. Each mL of 1 N sulfuric acid is equivalent to 40.69 mg of ZnO.
  - **Acceptance criteria**: NLT 99.0% of ZnO, on the ignited basis

**IMPURITIES**

*Change to read:*

**Inorganic Impurities**

- **Cadmium**, *Cadmium Limit Test*, Appendix IIIIB
  - **Sample solution**: Transfer 5 g of sample into a 50-mL volumetric flask, dissolve the sample in a minimum volume of 2:3 hydrochloric acid, dilute to volume with water, and mix.
  - **Acceptance criteria**: NMT 3 mg/kg

- **Lead**, *Lead Limit Test, APDC Extraction Method*, Appendix IIIIB
  - **Sample**: 2 g
  - **Acceptance criteria**: NMT 10 mg/kg

- **Substances not Precipitated by Sulfide**
  - **Sample**: 2 g
Analysis: Transfer the Sample into a 200-mL flask and dissolve it in 20 mL of 1:4 acetic acid. Dilute to about 150 mL with water, and mix. Precipitate the zinc completely with ammonium sulfide TS, dilute to volume with water, and mix. Filter through a dry filter, discarding the first portion of filtrate, and collect 100 mL of the subsequent filtrate. Add a few drops of sulfuric acid to the collected filtrate, and evaporate to dryness on a steam bath in a tared dish. Ignite cautiously until the ammonium salts are volatilized, ignite to constant weight at $800^\circ \pm 25^\circ$, cool, and weigh.

Acceptance criteria: The weight of the residue does not exceed 5 mg. (NMT 0.5%)

SPECIFIC TESTS

• **Alkalinity**
  
  **Sample:** 2 g
  
  **Analysis:** Suspend the Sample in 20 mL of water, boil for 1 min, filter, and add 0.1 mL of phenolphthalein TS to the filtrate.
  
  **Acceptance criteria:** No red color appears.

• **Loss on Ignition, Appendix IIIB**
  
  **Sample:** 2 g
  
  **Analysis:** Ignite at $800^\circ \pm 25^\circ$ to constant weight.
  
  **Acceptance criteria:** NMT 1.0%

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate

Expert Committee: (FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 1033

Phone Number: 1-301-816-8288
Zinc Sulfate, FCC 6 page 1033—See briefing under Manganese Sulfate. Interested parties are encouraged to submit comments to Jeff Moore, Ph.D.

(FIEC: J. Moore) C65193

Zinc Sulfate
ZnSO₄·H₂O

Formula wt, monohydrate 179.45

ZnSO₄·7H₂O

Formula wt, heptahydrate 287.54
CAS: monohydrate [7446-19-7]
CAS: heptahydrate [7446-20-0]

DESCRIPTION
Zinc Sulfate occurs as colorless, transparent prisms or small needles, or as a granular, crystalline powder. It contains one or seven molecules of water of hydration. The monohydrate loses water at temperatures above 238°C; the heptahydrate effloresces in dry air at room temperature. Its solutions are acid to litmus. The monohydrate is soluble in water and practically insoluble in alcohol. One g of the heptahydrate dissolves in about 0.6 mL of water and in about 2.5 mL of glycerin; it is insoluble in alcohol.

Function Nutrient

Packaging and Storage Store in tight containers.

IDENTIFICATION
• SULFATE, Appendix IIIA
  Sample solution: 50 mg/mL
  Acceptance criteria: Passes tests.
• ZINC, Appendix IIIA
  Sample solution: 50 mg/mL
  Acceptance criteria: Passes tests.

ASSAY
• PROCEDURE
  Sample: 175 mg of the monohydrate, or 300 mg of the heptahydrate
  Analysis: Dissolve the Sample in 100 mL of water, and add 5 mL of ammonia–ammonium chloride buffer TS and 0.1 mL of eriochrome black TS. Titrate with 0.05 M disodium EDTA until the solution turns deep blue. Each mL of 0.05 M disodium EDTA is equivalent to 8.973 mg of ZnSO₄·H₂O, or 14.38 mg of ZnSO₄·7H₂O.
  Acceptance criteria
  Monohydrate: NLT 98.0% and NMT 100.5% of ZnSO₄·H₂O
  Heptahydrate: NLT 99.0% and NMT 108.7% of ZnSO₄·7H₂O

IMPURITIES
• ALKALIES AND ALKALINE EARTHS
  Sample: 2 g
  Analysis: Transfer the Sample into a 200-mL volumetric flask, dissolve the sample in about 150 mL of water, and precipitate the zinc completely with ammonium sulfide TS. Dilute to volume with water, and mix. Filter
the contents of the flask through a dry filter, rejecting the first portion of the filtrate, and add a few drops of sulfuric acid to 100 mL of the subsequent filtrate. Evaporate to dryness in a tared dish, ignite to constant weight, cool, and weigh.

Acceptance criteria: The weight of the residue does not exceed 5 mg. (NMT 0.5%)

- Cadmium, Cadmium Limit Test, Appendix IIIB
  Acceptance criteria: NMT 2 mg/kg

Change to read:

- Lead, Lead Limit Test, APDC Extraction Method, Appendix IIIB
  Sample: 5 g
  Acceptance criteria: NMT 4 mg/kg

- Mercury, Mercury Limit Test, Appendix IIIB
  Sample preparation: Dissolve 400 mg of sample in 10 mL of water in a small beaker, add 1 mL of 1:5 sulfuric acid solution, and 1 mL of 1:25 potassium permanganate solution. Cover the beaker, boil for a few seconds, and cool.
  Acceptance criteria: NMT 5 mg/kg

- Selenium, Selenium Limit Test, Method I, Appendix IIIB
  Sample: 200 mg
  Acceptance criteria: NMT 0.003%

SPECIFIC TESTS

- Acidity
  Sample solution: 50 mg/mL
  Analysis: Add methyl orange TS to the Sample solution.
  Acceptance criteria: No pink color appears.

Auxiliary Information—Staff Liaison: Jeffrey Moore, Ph.D., Senior Scientific Associate

Expert Committee: (FI07) Food Ingredients Expert Committee

FCC Sixth Edition Page 1033

Phone Number: 1-301-816-8288
APPENDIX II: PHYSICAL TESTS
AND DETERMINATIONS

A. CHROMATOGRAPHY
[NOTE: Chromatographic separations may also be characterized according to the type of instrumentations or apparatus used. The types of chromatography that may be used in the Food Chemicals Codex are column, thin-layer, gas, and high-pressure or high-performance liquid chromatography. The Committee on Food Chemicals Codex recognizes that the field of chromatography continues to advance. Accordingly, the use of equivalent or improved systems is acceptable with appropriate validation.]

For the purposes of the Food Chemicals Codex, chromatography is defined as an analytical technique whereby a mixture of chemicals may be separated by virtue of their differential affinities for two immiscible phases. One of these, the stationary phase, consists of a fixed bed of small particles with a large surface area, while the other, the mobile phase, is a gas or liquid that moves constantly through, or over the surface of, the fixed phase. Chromatographic systems achieve their ability to separate mixtures by selectively retarding the passage of some compounds through the stationary phase while permitting others to move more freely. Therefore, the chromatogram may be evaluated qualitatively by determining the $R_f$, or retardation factor, for each of the eluted substances. The $R_f$ is a measure of that fraction of its total elution time that any compound spends in the mobile phase. Because this fraction is directly related to the fraction of the total amount of the solute that is in the mobile phase, the $R_f$ can be expressed as:

$$R_f = \frac{V_m C_m}{V_m C_m + V_s C_s}$$

in which $V_m$ and $V_s$ are the volumes of the mobile and stationary phase, respectively, and $C_m$ and $C_s$ are the concentrations of the solute in either phase at any time. This can be simplified to:

$$R_f = \frac{V_m}{V_m + KV_s}$$

in which $K = C_s/C_m$ and is an equilibrium constant that indicates this differential affinity of the solute for the phases. Alternatively, a new constant, $k$, the capacity factor, may be introduced, giving another form of the expression:

$$R_f = \frac{1}{1 + k}$$
in which \( k = \frac{KV_s}{Vm} \). The capacity factor, \( k \), which is normally constant for small samples, is a parameter that expresses the ability of a particular chromatographic system to interact with a solute. The larger the \( k \) value, the more the sample is retarded.

Both the retardation factor and the capacity factor may be used for qualitative identification of a solute or for developing strategies for improving separation. In terms of parameters easily obtainable from the chromatogram, the \( R_F \) is defined as the ratio of the distance traveled by the solute band to the distance traveled by the mobile solvent in a particular time. The capacity factor, \( k \), can be evaluated by the expression:

\[
\frac{t_f}{t_o}
\]

in which \( t_f \) is the retention time, is the elapsed time from the start of the chromatogram to the elution maximum of the solute, and \( t_o \) is the retention time of a solute that is not retained by the chromatographic system.

Retardation of the solutes by the stationary phase may be achieved by one or a combination of mechanisms. Certain substances, such as alumina or silica gel, interact with the solutes primarily by adsorption, either physical adsorption, in which the binding forces are weak and easily reversible, or chemisorption, in which strong bonding to the surface can occur. Another important mechanism of retardation is partition, which occurs when the solute dissolves in the stationary phase, usually a liquid coated as a thin layer on the surface of an inert particle or chemically bonded to it. If the liquid phase is a polar substance (e.g., polyethylene glycol) and the mobile phase is nonpolar, the process is termed normal-phase chromatography. When the stationary phase is nonpolar (e.g., octadecylsilane) and the mobile phase is polar, the process is reversed-phase chromatography. For the separation of mixtures of ionic species, insoluble polymers called ion exchangers are used as the stationary phase. Ions of the solutes contained in the mobile phase are adsorbed onto the surface of the ion exchanger while at the same time displacing an electrically equivalent amount of less strongly bound ions to maintain the electroneutrality of both phases. The chromatographic separation of mixtures of large molecules such as proteins may be accomplished by a mechanism called size exclusion chromatography. The stationary phases used are highly cross-linked polymers that have imbibed a sufficient amount of solvent to form a gel. The separation is based on the physical size of the solvated solutes; those that are too large to fit within the interstices of the gel are eluted rapidly, while the smaller molecules permeate into the pores of the gel and are eluted later. In any chromatographic separation, more than one of the above mechanisms may be occurring simultaneously.

Chromatographic separations may also be characterized according to the type of instrumentation or apparatus used. The types of chromatography that may be used in the FCC are column, thin-layer, gas, and high-performance liquid chromatography.

**COLUMN CHROMATOGRAPHY**

**Apparatus** The equipment needed for column chromatography is not elaborate, consisting only of a cylindrical glass or Teflon tube that has a restricted outflow orifice. The dimensions of the tube are not critical and may vary from 10 to 40 mm in inside diameter and from 100 to 600 mm in length. For a given separation, greater efficiency may be obtained with a long narrow column, but the resultant flow rate will be lower. A fritted-glass disk may be seated in the end of the tube to act as a support for the packing material. The column is fitted at the end with a stopcock or other flow-restriction device to control the rate of delivery of the eluant.

**Procedure** The stationary phase is introduced into the column either as a dry powder or as a slurry in the
mobile phase. Because a homogeneous bed free of void spaces is necessary to achieve maximum separation efficiency, the packing material is introduced in small portions and allowed to settle before further additions are made. Settling may be accomplished by allowing the mobile phase to flow through the bed, by tapping or vibrating the column if a dry powder is used, or by compressing each added portion using a tamping rod. The rod can be a solid glass, plastic, or metal cylinder whose diameter is slightly smaller than that of the column, or it can be a thinner rod onto the end of which has been attached a disk of suitable diameter. Ion-exchange resins and exclusion polymers are never packed as dry powders because after introduction of the mobile phase, they will swell and create sufficient pressure to shatter the column. When the packing has been completed, the sample is introduced onto the top of the column. If the sample is soluble, it is dissolved in a minimum amount of the mobile phase, pipetted onto the column, and allowed to percolate into the top of the bed. If it is not soluble or if the volume of solution is too large, it may be mixed with a small amount of the column packing. This material is then transferred to the chromatographic tube to form the top of the bed. The chromatogram is then developed by adding the mobile phase to the column in small portions and allowing it to percolate through the packed bed either by gravity or under the influence of pressure or vacuum. Development of the chromatogram takes place by selective retardation of the components of the mixture as a result of their interaction with the stationary phase. In column chromatography, the stationary phase may act by adsorption, partition, ion exchange, exclusion of the solutes, or a combination of these effects.

When the development is complete, the components of the sample mixture may be detected and isolated by either of two procedures. The entire column may be extruded carefully from the tube, and if the compounds are colored or fluorescent under ultraviolet light, the appropriate segments may be cut from the column using a razor blade. If the components are colorless, they may be visualized by painting or spraying a thin longitudinal section of the surface of the chromatogram with color-developing reagents. The chemical may then be separated from the stationary phase by extraction with a strong solvent such as methanol and subsequently quantitated by suitable methods.

In the second procedure, the mobile phase may be allowed to flow through the column until the components of the mixture successively appear in the effluent. This eluate may be collected in fractions and the mobile phase evaporated if desired. The chemicals present in each fraction may then be determined by suitable analytical techniques.

PAPER CHROMATOGRAPHY

In this type of chromatography, the stationary phase ordinarily consists of a sheet of paper of suitable texture and thickness. The paper used is made from highly purified cellulose, which has a great affinity for water and other polar solvents since it has many hydroxyl functional groups. The tightly bound water acts as the stationary phase, and therefore the mechanism that predominates is liquid-liquid or partition chromatography. Adsorption of solutes to the cellulose surface may also occur, but this is of lesser importance. Papers especially impregnated to permit ion-exchange or reverse-phase chromatography are also available.

**Apparatus**

The essential equipment for paper chromatography consists of the following:

*Vapor-Tight Chamber*  The chamber is constructed preferably of glass, stainless steel, or porcelain. It is provided with inlets for the addition of solvent or for releasing internal pressure, and it is designed to permit observation of the progress of the chromatographic run without being opened. Tall glass cylinders are convenient if they are made vapor-tight with suitable covers and a sealing compound.

*Supporting Rack*  The rack serves as a support for the solvent troughs and antisiphoning rods. It is constructed of a corrosion-resistant material about 5 cm shorter than the inside height of the chamber.
Solvent Troughs  The troughs, made of glass, are designed to be longer than the width of the chromatographic sheets and to contain a volume of solvent greater than that required for one chromatographic run.

Antisiphoning Rods  Constructed of heavy glass, the rods are placed on the rack and arranged to run outside of, parallel to, and slightly above the edge of the glass trough.

Chromatographic Sheets  Special chromatographic filter paper is cut to length approximately equal to the height of the chamber. The sheet is at least 2.5 cm wide but not wider than the length of the trough. A fine pencil line is drawn horizontally across the filter paper at a distance from one end such that when the sheet is suspended from the antisiphoning rods with the upper end of the paper resting in the trough and the lower portion hanging free into the chamber, the line is located a few cm below the rods. Care is necessary to avoid contaminating the paper by excessive handling or by contact with dirty surfaces.

Procedure for Descending Chromatography  Separation of substances by descending chromatography is accomplished by allowing the mobile phase to flow downward on the chromatographic sheet. The substance or substances to be analyzed are dissolved in a suitable solvent. Convenient volumes of the resulting solution, normally containing 1 to 20 µg of the compound, are placed in 6- to 10-mm spots along the pencil line not less than 3 cm apart. If the total volume to be applied would produce spots of a diameter greater than 6 to 10 mm, it is applied in separate portions to the same spot, each portion being allowed to dry before the next is added.

The spotted chromatographic sheet is suspended in the chamber by use of the antisiphoning rod and an additional heavy glass rod that holds the upper end of the sheet in the solvent trough. The bottom of the chamber is covered with a mixture containing both phases of the prescribed solvent system. It is important to ensure that the portion of the sheet hanging below the rods is freely suspended in the chamber without touching the rack or the chamber walls. The chamber is sealed to allow equilibration (saturation) of the chamber and the paper with solvent vapor. Any excess pressure is released as necessary. For large chambers, equilibration overnight may be necessary.

A volume of the mobile phase in excess of the volume required for complete development of the chromatogram is saturated with the immobile phase. After equilibration of the chamber, the prepared mobile solvent is introduced into the trough through the inlet. The inlet is closed, and the mobile phase is allowed to travel down the paper the desired distance. Precautions must be taken against allowing the solvent to run down the sheet when opening the chamber and removing the chromatogram. The location of the solvent front is quickly marked, and the sheets are dried.

The chromatogram is observed and measured directly or after suitable development to reveal the location of the spots of the isolated components of the mixture.

Procedure for Ascending Chromatography  In ascending chromatography, the lower edge of the sheet (or strip) is dipped into the mobile phase to permit the mobile phase to rise on the chromatographic sheet. The test materials are applied to the chromatographic sheet as directed under Procedure for Descending Chromatography. Enough of both phases of the solvent mixture to cover the bottom of the chamber is added. Empty solvent troughs are placed on the bottom of the chamber, and the chromatographic sheet is suspended so that the end near which the spots have been added hangs free inside the empty trough.

The chamber is sealed, and equilibration is allowed to proceed as described under Procedure for Descending Chromatography. Then the solvent is added through the inlet to the trough in excess of the quantity of solvent required for complete moistening of the chromatographic sheet. The chamber is resealed. When the solvent front has reached the desired height, the chamber is opened and the sheet is removed, the location of the
solvent front is quickly marked, and the sheet is dried.

Small cylinders may be used without troughs so that only the mobile phase is placed on the bottom. The chromatographic sheet is suspended during equilibration with the lower end just above the solvent, and chromatography is started by lowering the sheet so that it touches the solvent.

**Detection of Chromatographic Bands** After the chromatogram has been fully developed, the bands corresponding to the various solutes may be detected by means similar to those described in *Column Chromatography*. If the compounds are colored or fluorescent under ultraviolet light, they may be visualized directly. Colorless compounds may be detected by spraying the paper with color-developing reagents. The bands corresponding to the individual components can be cut from the paper, and the chemical substances eluted from the cellulose by the use of a strong solvent such as methanol.

**Identification of Solutes** Since the chromatographic mobilities of the solutes may change from run to run due to varying experimental conditions, presumptive identification of a substance should be based on comparison with a reference standard. The $R_F$ values of the unknown substance and the standard on the same chromatogram must be identical. Alternatively, the ratio between the distances traveled by a given compound and a reference substance, the $R_r$ value, must be 1.0. Identification may also be made by missing a small amount of the reference substance with the unknown and chromatographing. The resulting chromatogram should contain only one spot. Definitive identification of solutes may be achieved by eluting them from the paper and subjecting them to IR, NMR, or mass spectrometry.

**THIN-LAYER CHROMATOGRAPHY**

In thin-layer chromatography (TLC), the stationary phase is a uniform layer of a finely divided powder that has been coated on the surface of a glass or plastic sheet and that is held in place by a binder. The capacity of the system is dependent on the thickness of the layer, which may range from 0.1 to 2.0 mm. The thinner layers are used primarily for analytical separations, while the thicker layers, because of their greater sample-handling ability, are useful for preparative work.

Substances that are used as coatings in TLC include silica gel, alumina, cellulose, and reversed-phase packings. Separations occur because of adsorption of the solutes from the mobile phase onto the surface of the thin layer. However, adsorption of water from the air or solvent components from the mobile phase can give rise to partition or liquid–liquid chromatography. Specially coated plates are available that permit ion-exchange or reversed-phase separations.

**Apparatus** Acceptable apparatus and materials for thin-layer chromatography consist of the following:

- **Glass Plates** Flat glass plates of uniform thickness throughout their areas. The most common sizes are 20, 10, and 5 cm × 20 cm. (Aluminum plates also are commonly used.)

- **Aligning Tray** An aligning tray or other suitable flat surface is used to align and hold plates during application of the adsorbent.

- **Adsorbent** The adsorbent may consist of finely divided adsorbent materials for chromatography. It can be applied directly to the glass plate, or it can be bonded to the plate by means of plaster of Paris or with starch paste. Pretreated chromatographic plates are available commercially.

- **Spreader** A suitable spreading device that, when moved over the glass plate, applies a uniform layer of adsorbent of the desired thickness over the entire surface of the plate.
**Storage Rack**  A rack of convenient size to hold the prepared plates during drying and transportation.

**Developing Chamber**  A glass chamber that can accommodate one or more plates and can be properly closed and sealed. It is fitted with a plate-support rack that can support the plates when the lid of the chamber is in place.

[NOTE: Preformed TLC plates available commercially may also be used.]

**Procedure**  Clean the plates scrupulously, as by immersion in a chromic acid cleansing mixture, rinse them with copious quantities of water until the water runs off the plates without leaving any visible water or oily spots, and dry.

Arrange the plate or plates on the aligning tray, and secure them so that they will not slip during the application of the adsorbent. Mix an appropriate quantity of adsorbent and liquid, usually water, which when shaken for 30 s gives a smooth slurry that will spread evenly with the aid of a spreader. Transfer the slurry to the spreader, and apply the coating at once before the binder begins to harden. Move the spreader smoothly over the plates from one end of the tray to the other. Remove the spreader, and wipe away excess slurry.

Allow the plates to set for 10 min, and then place them in the storage rack and dry at 105° for 30 min or as directed in the individual monograph. Store the finished plates in a desiccator.

Equilibrate the atmosphere in the Developing Chamber by placing in it a volume of the mobile phase in excess of that required for complete development of the chromatogram, cover the chamber with its lid, and allow it to stand for at least 30 min.

Apply the Sample Solution and the Standard Solution at points about 1.5 cm apart and about 2 cm from the lower edge of the plate (the lower edge is the first part over which the spreader moves in the application of the adsorbent layer), and allow to dry. A template will aid in determining the spot points and the 10- to 15-cm distance through which the solvent front should move.

Arrange the plate on the supporting rack (sample spots on the bottom), and introduce the rack into the developing chamber. The solvent in the chamber must be deep enough to reach the lower edge of the adsorbent, but must not touch the spot points. Seal the cover in place, and maintain the system until the solvent ascends to a point 10 to 15 cm above the initial spots; this usually requires 15 min to 1 h. Remove the plates, and dry them in air. Measure and record the distance of each spot from the point of origin. If so directed, spray the spots with the reagent specified, observe, and compare the sample with the standard chromatogram.

**Detection and Identification**  Detection and identification of solute bands is done by methods essentially the same as those described in Column Chromatography. However, in TLC an additional method called fluorescence quenching is also used. In this procedure, an inorganic phosphor is mixed with the adsorbent before it is coated on the plate. When the developed chromatogram is irradiated with ultraviolet light, the surface of the plate fluoresces with a characteristic color, except in those places where ultraviolet-absorbing solutes are situated. These quench the fluorescence and are detectable as dark spots.

Detection with an ultraviolet light source suitable for observations with short (254-nm) and long (360-nm) ultraviolet wavelengths may be called for in some cases.

**Quantitative Analysis**  Two methods are available if quantitation of the solute is necessary. In the first, the bands are detected and their positions marked. Those areas of adsorbent containing the compounds of interest are scraped from the surface of the plate into a centrifuge tube. The chemicals are extracted from the adsorbent with the aid of a suitable strong solvent, the suspension is centrifuged, and the supernatant layer is subjected to appropriate methods of quantitative analysis.

The second method involves the use of a scanning densitometer. This is a spectrophotometric device that
directs a beam of monochromatic radiation across the surface of the plate. After interaction with the solutes in the adsorbent layer, the radiation is detected as transmitted or reflected light and a recording of light intensity versus distance traveled is produced. The concentration of a particular species is proportional to the area under its peak and can be determined accurately by comparison with standards.

**GAS CHROMATOGRAPHY**

The distinguishing features of gas chromatography are a gaseous mobile phase and a solid or immobilized liquid stationary phase. Liquid stationary phases are available in packed or capillary columns. In the packed columns, the liquid phase is deposited on a finely divided, inert solid support, such as diatomaceous earth or porous polymer, which is packed into a column that typically has a 2- to 4-mm id and is 1 to 3 m long. In capillary columns, which contain no particles, the liquid phase is deposited on the inner surface of the fused silica column and may be chemically bonded to it. In gas–solid chromatography, the solid phase is an active adsorbent, such as alumina, silica, or carbon, packed into a column. Polyaromatic porous resins, which are sometimes used in packed columns, are not coated with a liquid phase.

When a volatile compound is introduced into the carrier gas and carried into the column, it is partitioned between the gas and stationary phases by a dynamic countercurrent distribution process. The compound is carried down the column by the carrier gas, retarded to a greater or lesser extent by sorption and desorption in the stationary phase. The elution of the compound is characterized by the partition ratio, $k$, a dimensionless quantity also called the capacity factor. It is equivalent to the ratio of the time required for the compound to flow through the column (the retention time) to the retention time of a nonretarded compound. The value of the capacity factor depends on the chemical nature of the compound; the nature, amount, and surface area of the liquid phase; and the column temperature. Under a specified set of experimental conditions, a characteristic capacity factor exists for every compound. Separation by gas chromatography occurs only if the compounds concerned have different capacity factors.

**Apparatus** A gas chromatograph consists of a carrier gas source, an injection port, column, detector, and recording device. The injection port, column, and detector are carefully temperature controlled. The typical carrier gas is helium or nitrogen, depending on the column and detector in use. The gas is supplied from a high-pressure cylinder and passes through suitable pressure-reducing valves to the injection port and column. Compounds to be chromatographed, either in solution or as gases, are injected into the gas stream at the injection port. Depending on the configuration of the apparatus, the test mixture may be injected directly into the column or be vaporized in the injection port and mixed into the flowing carrier gas before entering the column.

Once in the column, compounds in the test mixture are separated by virtue of differences in their capacity factors, which in turn depend on their vapor pressure and degree of interaction with the stationary phase. The capacity factor, which governs resolution and retention times of components of the test mixture, is also temperature dependent. The use of temperature-programmable column ovens takes advantage of this dependence to achieve efficient separation of compounds differing widely in vapor pressure.

As resolved compounds emerge from the column, they pass through a detector, which responds to the amount of each compound present. The type of detector to be used depends on the nature of the compounds to be analyzed, and is specified in the individual monograph. Detectors are heated above the maximum column operating temperature to prevent condensation of the eluting compounds.

Detector output is recorded as a function of time, producing a chromatogram, which consists of a series of peaks on a time axis. Each peak represents a compound in the vaporized test mixture, although some peaks
may overlap. The elution time is characteristic of the individual compounds (qualitative analysis), and the peak area is a function of the amount present (quantitative analysis).

Injectors Sample injection devices range from simple syringes to fully programmable automatic injectors. The amount of sample that can be injected into a capillary column without overloading is small compared with the amount that can be injected into a packed column, and may be less than the smallest amount that can be manipulated satisfactorily by syringe. Capillary columns are therefore used with injectors able to split samples into two fractions, a small one that enters the column and a large one that goes to waste (split injector). Such injectors may also be used in a splitless mode for analyses of trace or minor components. Purge and trap injectors are equipped with a sparging device by which volatile compounds in solution are carried into a low-temperature trap. When sparging is complete, trapped compounds are thermally desorbed into the carrier gas by rapid heating of the temperature-programmable trap.

Headspace injectors are equipped with a thermostatically controlled sample-heating chamber. Solid or liquid samples in tightly closed containers are heated in the chamber for a fixed period of time, allowing the volatile components in the sample to reach an equilibrium between the nongaseous phase and the gaseous or headspace phase.

After this equilibrium has been established, the injector automatically introduces a fixed amount of the headspace in the sample container into the gas chromatograph.

Columns Capillary columns, which are usually made of fused silica, have a 0.2- to 0.53-mm id and are 5 to 30 m long. The liquid or stationary phase is 0.1 to 1.0 µm thick, although nonpolar stationary phases may be up to 5 µm thick. Packed columns, made of glass or metal, are 1 to 3 m long, with a 2- to 4-mm id. Those used for analysis typically have liquid phase loadings of about 5% (w/w) on a solid support.

Supports for analysis of polar compounds on low-capacity, low-polarity liquid phase columns must be inert to avoid peak tailing. The reactivity of support materials can be reduced by silanizing before coating with liquid phase. Acid-washed, flux-calcined diatomaceous earth is often used for drug analysis. Support materials are available in various mesh sizes, with 80- to 100-mesh and 100- to 120-mesh being more commonly used with 2- to 4-mm columns. Because of the absence of a solid support, capillary compounds are much more inert than packed columns.

Retention time and the peak efficiency depend on the carrier gas flow rate; retention time is also directly proportional to column length, while resolution is proportional to the square root of the column length. For packed columns, the carrier gas flow rate is usually expressed in milliliters per minute at atmospheric pressure and room temperature. It is measured at the detector outlet with a soap film flow meter while the column is at operating temperature. Unless otherwise specified in the individual monograph, flow rates for packed columns are 60 to 75 mL/min for 4-mm id columns and ~30 mL/min for 2-mm id columns.

For capillary columns, linear flow velocity is often used instead of flow rate. This is conveniently determined from the length of the column and the retention time of a dilute methane sample, provided a flame-ionization detector is in use. Typical linear velocities are 20 to 60 cm/s for helium. At high operating temperatures there is sufficient vapor pressure to result in a gradual loss of liquid phase, a process called “bleeding.”

Detectors Flame-ionization detectors are used for most analyses, with lesser use made of thermal conductivity, electron-capture, nitrogen–phosphorus, and mass spectrometric detectors. For quantitative analyses, detectors must have a wide linear dynamic range: the response must be directly proportional to the amount of compound present in the detector over a wide range of concentrations. Flame-ionization detectors
have a wide linear range (~10^6) and are sensitive to organic compounds. Unless otherwise specified in individual monographs, flame-ionization detectors with either helium or nitrogen carrier gas are to be used for packed columns, and helium is used for capillary columns.

The thermal conductivity detector detects changes in the thermal conductivity of the gas stream as solutes are eluted. Although its linear dynamic range is smaller than that of the flame-ionization detector, it is quite rugged and occasionally used with packed columns, especially for compounds that do not respond to flame-ionization detectors.

The alkali flame-ionization detector, sometimes called an NP or nitrogen—phosphorus detector, contains a thermionic source, such as an alkali-metal salt or a glass element containing rubidium or other metal, that results in the efficient ionization of organic nitrogen and phosphorus compounds. It is a selective detector that shows little response to hydrocarbons.

The electron-capture detector contains a radioactive source (usually 63Ni) of ionizing radiation. It exhibits an extremely high response to compounds containing halogens and nitro groups but little response to hydrocarbons. The sensitivity increases with the number and atomic weight of the halogen atoms.

Data Collection Devices  Modern data stations receive the detector output, calculate peak areas, and print chromatograms, complete with run parameters and peak data. Chromatographic data may be stored and reprocessed, with integration and other calculation variables being changed as required. Data stations are used also to program the chromatograph, controlling most operational variables and providing for long periods of unattended operation.

Data can also be collected for manual measurement on simple recorders or on integrators whose capabilities range from those providing a printout of peak areas to those providing chromatograms with peak areas and peak heights calculated and data stored for possible reprocessing.

Procedure  Capillary columns must be tested to ensure that they comply with the manufacturers’ specifications before they are used. These tests consist of the following injections: a dilute methane sample to determine the linear flow velocity; a mixture of alkanes (e.g., C_{14}, C_{15}, and C_{16}) to determine resolution; and a polarity test mixture to check for active sites on the column. The latter mixture may include a methyl ester, an unsaturated compound, a phenol, an aromatic amine, a diol, a free carboxylic acid, and a polycyclic aromatic compound, depending on the samples to be analyzed.

Packed columns must be conditioned before use until the baseline and other characteristics are stable. This may be done by operation at a temperature above that called for by the method or by repeated injections of the compound or mixture to be chromatographed. A suitable test for support inertness should be done. Very polar molecules (like free fatty acids) may require a derivatization step.

Before any column is used for assay purposes, a calibration curve should be constructed to verify that the instrumental response is linear over the required range and that the curve passes through the origin. If the compound to be analyzed is adsorbed within the system, the calibration curve will intersect the abscissa at a nonzero value. This may result in error, particularly for compounds at low concentrations determined by a procedure based on a single reference point. At high concentrations, the liquid phase may be overloaded, leading to loss of peak height and symmetry.

Assays require quantitative comparison of one chromatogram with another. A major source of error is irreproducibility in the amount of sample injected, notably when manual injections are made with a syringe. The effects of variability can be minimized by addition of an internal standard, a noninterfering compound present at the same concentration as in the sample and standard solutions. The ratio of peak response of the analyte to that of the internal standard is compared from one chromatogram to another. Where the internal
standard is chemically similar to the substance being determined, there is also compensation for minor variations in column and detector characteristics. In some cases, the internal standard may be carried through the sample preparation procedure before gas chromatography to control other quantitative aspects of the assay. Automatic injectors greatly improve the reproducibility of sample injections and reduce the need for internal standards.

Many monographs require that system suitability requirements be met before samples are analyzed, see System Suitability below.

**HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY**

High-performance liquid chromatography (HPLC) is a separation technique based on a solid stationary phase and a liquid mobile phase. Separations are achieved by partition, adsorption, exclusion, or ion-exchange processes, depending on the type of stationary phase used. HPLC has distinct advantages over gas chromatography for the analysis of nonvolatile organic compounds. Compounds to be analyzed are dissolved in a liquid, and most separations take place at room temperature.

As in gas chromatography, the elution time of a compound can be described by the capacity factor, $k$, which depends on the chemical nature of the composition and flow rate of the mobile phase, and the composition and surface area of the stationary phase. Column length is an important determinant of resolution. Only compounds having different capacity factors can be separated by HPLC.

**Apparatus** A liquid chromatograph consists of one, two, or more reservoirs containing the mobile phase, a pump to force the mobile phase through the system at high pressure, an injector to introduce the sample into the mobile phase, a chromatographic column, a detector, and a data collection device such as a computer, integrator, or recorder. Short, 3-, 5-, 10-, and 25-cm, small-bore columns containing densely packed particles of stationary phase provide for the rapid exchange of compounds between the mobile and stationary phases. In addition to receiving and reporting detector output, computers are used to control chromatographic settings and operations, thus providing for long periods of unattended operation.

**Pumping Systems** HPLC pumping systems deliver metered amounts of mobile phase from the solvent reservoirs to the column through high-pressure tubing and fittings. Modern systems consist of one or more computer-controlled metering pumps that can be programmed to vary the ratio of mobile phase components, as is required for gradient chromatography, or to mix isocratic mobile phases (i.e., mobile phases having a fixed ratio of solvents). However, the proportion of ingredients in premixed isocratic mobile phases can be more accurately controlled than in those delivered by most pumping systems. Operating pressures up to 5000 psi with delivery rates up to about 10 mL/min are typical. Pumps used for quantitative analysis should be constructed of materials inert to corrosive mobile phase components and be capable of delivering the mobile phase at a constant rate with minimal fluctuations over extended periods of time.

**Injectors** After dissolution in mobile phase or other suitable solution, compounds to be chromatographed are injected into the mobile phase, either manually by syringe or loop injectors, or automatically by autosamplers. The latter consist of a carousel or rack to hold sample vials with tops that have a pierceable septum or stopper and an injection device to transfer sample from the vials to a calibrated, fixed-volume loop from which it is loaded into the chromatograph. Some autosamplers can be programmed to control sample volume, the number of injections and loop rinse cycles, the interval between injections, and other operating variables. Some valve systems incorporate a calibrated sample loop that is filled with test solution for transfer to the column in the mobile phase. In other systems, test solution is transferred to a cavity by syringe and then switched into the mobile phase.
Columns For most analyses, separation is achieved by partition of compounds in the test solution between the mobile and stationary phases. Systems consisting of polar stationary phases and nonpolar mobile phases are described as normal phase, while the opposite arrangement, polar mobile phases and nonpolar stationary phases, is called reversed-phase chromatography. Partition chromatography is almost always used for hydrocarbon-soluble compounds of a molecular weight that is less than 1000. The affinity of a compound for the stationary phase, and thus its retention time on the column, is controlled by making the mobile phase more or less polar. Mobile phase polarity can be varied by the addition of a second, and sometimes a third or even a fourth, component.

Stationary phases for modern, reversed-phase liquid chromatography typically consist of an organic phase chemically bound to silica or other materials. Particles are usually 3, 5, or 10 µm in diameter, but sizes may range up to 50 µm for preparative columns. Small particles thinly coated with organic phase allow fast mass transfer and, hence, rapid transfer of compounds between the stationary and mobile phases. Column polarity depends on the polarity of the bound functional groups, which range from relatively nonpolar octadecyl silane to very polar nitrile groups.

Columns used for analytical separations usually have internal diameters of 2 to 4.6 mm; larger diameter columns are used for preparative chromatography. Columns may be heated to give more efficient separations, but only rarely are they used at temperatures above 60° because of potential stationary phase degradation or mobile phase volatility. Unless otherwise specified in the individual monograph, columns are used at an ambient temperature.

Ion-exchange chromatography is used to separate water-soluble, ionizable compounds of molecular weights that are less than 2000. The stationary phases are usually synthetic organic resins; cation-exchange resins contain negatively charged active sites and are used to separate basic substances such as amines; while anion-exchange resins have positively charged active sites for separation of compounds with negatively charged groups such as phosphate, sulfonate, or carboxylate groups. Water-soluble ionic or ionizable compounds are attracted to the resins, and differences in affinity bring about the chromatographic separation. The pH of the mobile phase, temperature, ion type, ionic concentration, and organic modifiers affect the equilibrium, and these variables can be adjusted to obtain the desired degree of separation.

In size-exclusion chromatography, columns are packed with a porous stationary phase. Molecules of the compounds being chromatographed are filtered according to size. Those too large to enter the pores pass unretained through the column (total exclusion). Smaller molecules enter the pores and are increasingly retained as molecular size decreases. These columns are typically used to remove high molecular weight matrices or to characterize the molecular weight distribution of a polymer.

Detectors Many compendial HPLC methods require the use of spectrophotometric detectors. Such a detector consists of a flow-through cell mounted at the end of the column. A beam of ultraviolet radiation passes through the flow cell and into the detector. As compounds elute from the column, they pass through the cell and absorb the radiation, resulting in measurable energy level changes. Fixed, variable, and photodiode array (PDA) detectors are widely available. Fixed wavelength detectors operate at a single wavelength, typically 254 nm, emitted by a low-pressure mercury lamp. Variable wavelength detectors contain a continuous source, such as a deuterium or high-pressure xenon lamp, and a monochromator or an interference filter to generate monochromatic radiation at a wavelength selected by the operator. Modern variable wavelength detectors can be programmed to change wavelength while an analysis is in progress. Multi-wavelength detectors measure absorbance at two or more wavelengths simultaneously. In diode array multi-wavelength detectors, continuous radiation is passed through the sample cell, then resolved into its constituent wavelengths, which are individually detected by the photodiode array. These detectors acquire absorbance data over the entire UV-visible range, thus providing the analyst with chromatograms at
multiple, selectable wavelengths and spectra of the eluting peaks. Diode array detectors usually have lower signal-to-noise ratios than fixed or variable wavelength detectors, and thus are less suitable for analysis of compounds present at low concentrations.

Differential refractometer detectors measure the difference between the refractive index of the mobile phase alone and that of the mobile phase containing chromatographed compounds as it emerges from the column. Refractive index detectors are used to detect non-UV absorbing compounds, but they are less sensitive than UV detectors. They are sensitive to small changes in solvent composition, flow rate, and temperature, so that a reference column may be required to obtain a satisfactory baseline.

Fluorometric detectors are sensitive to compounds that are inherently fluorescent or that can be converted to fluorescent derivatives either by chemical transformation of the compound or by coupling with fluorescent reagents at specific functional groups. If derivatization is required, it can be done before chromatographic separation or, alternatively, the reagent can be introduced into the mobile phase just before its entering the detector.

Potentiometric, voltammetric, or polarographic electrochemical detectors are useful for the quantitation of species that can be oxidized or reduced at a working electrode. These detectors are selective, sensitive, and reliable, but require conducting mobile phases free of dissolved oxygen and reducible metal ions. A pulseless pump must be used, and care must be taken to ensure that the pH, ionic strength, and temperature of the mobile phase remain constant. Working electrodes are prone to contamination by reaction products with consequent variable responses.

Electrochemical detectors with carbon-paste electrodes may be used advantageously to measure nanogram quantities of easily oxidized compounds, notably phenols and catechols.

**Data Collection Devices** Modern data stations receive and store detector output and print out chromatograms complete with peak heights, peak areas, sample identification, and method variables. They are also used to program the liquid chromatograph, controlling most variables and providing for long periods of unattended operation.

Data also may be collected on simple recorders for manual measurement or on stand-alone integrators, which range in complexity, from those providing a printout of peak areas to those providing a printout of peak areas and peak heights calculated and data stored for possible subsequent reprocessing.

**Procedure** The mobile phase composition significantly influences chromatographic performance and the resolution of compounds in the mixture being chromatographed. Composition has a much greater effect than temperature on the capacity factor, \( k \).

In partition chromatography, the partition coefficient, and hence the separation, can be changed by addition of another component to the mobile phase. In ion-exchange chromatography, pH and ionic strength as well as changes in the composition of the mobile phase affect capacity factors. The technique of continuously increasing mobile phase strength during the chromatographic run is called gradient elution or solvent programming. It is sometimes used to chromatograph complex mixtures of components differing greatly in their capacity factors. Detectors that are sensitive to change in solvent composition, such as the differential refractometer, are more difficult to use with the gradient elution technique.

For accurate quantitative work, high-purity, “HPLC-grade” solvents and reagents must be used. The detector must have a broad linear dynamic range, and compounds to be measured must be resolved from any interfering substances. The linear dynamic range of a compound is the range over which the detector signal response is directly proportional to the amount of the compound. For maximum flexibility in quantitative work, this range should be about three orders of magnitude. HPLC systems are calibrated by plotting peak
responses in comparison with known concentrations of a reference standard, using either an external or an internal standardization procedure.

Reliable quantitative results are obtained by external calibration if automatic injectors or autosamplers are used. This method involves direct comparison of the peak responses obtained by separately chromatographing the test and reference standard solutions. If syringe injection, which is irreproducible at the high pressures involved, must be used, better quantitative results are obtained by the internal calibration procedure where a known amount of a noninterfering compound, the internal standard, is added to the test and reference standard solutions, and the ratios of peak responses of the analyte and internal standard are compared.

Because of normal variations in equipment, supplies, and techniques, a system suitability test is required to ensure that a given operating system may be generally applicable. The main features of System Suitability tests are described below.

For information on the interpretation of results, see the section Interpretation of Chromatograms.

**Interpretation of Chromatograms**  
*Fig. 1* represents a typical chromatographic separation of two substances, 1 and 2, in which $t_{R(1)}$ and $t_{R(2)}$ are the respective retention times; $h$, $h'2$, and $W_{1/2}$ are the height, the half-height, and the width at half-height, respectively, for peak 1; and $W_1$ and $W_2$ are the respective widths of peaks 1 and 2 at the baseline. Air peaks are a feature of gas chromatograms and correspond to the solvent front in liquid chromatography.

![FIGURE 1 Chromatographic Separation of Two Substances.](image)

Chromatographic retention times are characteristic of the compounds they represent but are not unique. Coincidence of retention times of a test and a reference substance can be used as a feature in construction of an identity profile but is insufficient on its own to establish identity. Absolute retention times of a given compound vary from one chromatogram to the next. Comparisons are normally made in terms of relative retention, which is calculated by the equation:

$$\alpha = \frac{(t_{R(2)} - t_{R(0)})}{(t_{R(1)} - t_O)}$$

in which $t_{R(2)}$ and $t_{R(1)}$ are the retention times, measured from the point of injection, of the test and reference substances, respectively, determined under identical experimental conditions on the same column, and $t_O$ is the retention time of a nonretained substance, such as methane in this case, of gas chromatography.

In this and the following expressions, the corresponding retention volumes or linear separations on the chromatogram, both of which are directly proportional to retention time, may be substituted in the equations. Where the value of $t_O$ is small, $R_f$ may be estimated from the retention times measured from the point of injection $(t_{R(2)}/t_{R(1)})$.

The number of theoretical plates, $N$, is a measure of column efficiency. For Gaussian peaks, it is calculated by the equations:

$$N = 16(t_RW)^2 \text{ or } N = 5.54(t_{R/2}W)^2$$

in which $t_R$ is the retention time of the substance and $W$ is the width of the peak at its base, obtained by
extrapolating the relatively straight sides of the peak to the baseline. \( W_{1/2} \) is the peak width at half-height, obtained directly by electronic integrators. The value of \( N \) depends on the substance being chromatographed as well as the operating conditions such as mobile phase or carrier gas flow rates and temperature, the quality of the packing, the uniformity of the packing within the column, and for capillary columns, the thickness of the stationary phase film and the internal diameter and length of the column.

The separation of two components in a mixture, the resolution, \( R \), is determined by the equation:

\[
R = 2\left( t_{R(2)} - t_{R(1)} \right) / (W_2 + W_1)
\]

in which \( t_{R(2)} \) and \( t_{R(1)} \) are the retention times of the two components, and \( W_2 \) and \( W_1 \) are the corresponding widths at the bases of the peaks obtained by extrapolating the relatively straight sides of the peaks to the baseline.

Peak areas and peak heights are usually proportional to the quantity of compound eluting. These are commonly measured by electronic integrators but may be determined by more classical approaches. Peak areas are generally used but may be less accurate if peak interference occurs. For manual measurements, the chart should be run faster than usual, or a comparator should be used to measure the width at half-height and the width at the base of the peak, to minimize error in these measurements. For accurate quantitative work, the components to be measured should be separated from any interfering components. Peak tailing and fronting and the measurement of peaks on solvent tails are to be avoided (see Fig. 2). The relative standard deviation is expressed by the equation:

\[
S_R (\%) = \left( 100 / \bar{X} \right) \left[ \sum_{i=1}^{N} (X_i - \bar{X})^2 / (N-1) \right]^{1/2}
\]

in which \( S_R \) is the relative standard deviation in percent, \( \bar{X} \) is the mean of the set of \( N \) measurements, and \( X_i \) is an individual measurement. When an internal standard is used, the measurement \( X_i \) usually refers to the measurement of relative area, \( A_s \),

\[
X_i = A_s = a_r / a_i
\]

in which \( a_r \) is the area of the peak corresponding to the standard substance and \( a_i \) is the area of the peak corresponding to the internal standard. When peak heights are used, the measurement \( X_i \) refers to the measurement of relative heights, \( H_s \),

\[
X_i = H_s = h_r / h_i
\]

in which \( h_r \) is the height of the peak corresponding to the standard substance and \( h_i \) is the height of the peak corresponding to the internal standard.

![FIGURE 2 Asymmetrical Chromatographic Peak.](image)
**System Suitability**  Such tests are an integral part of gas and liquid chromatographic methods. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. The tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such.

The resolution, $R$, is a function of column efficiency, $N$, and is specified to ensure that closely eluting compounds are resolved from each other, to establish the general resolving power of the system, and to ensure that internal standards are resolved from the analyte. Column efficiency may be specified also as a system suitability requirement, especially if there is only one peak of interest in the chromatogram; however, it is a less reliable means to ensure resolution than direct measurement. Column efficiency is a measure of peak sharpness, which is important for the detection of trace components.

Replicate injections of a standard preparation used in the assay or other standard solution are compared to ascertain whether requirements for precision are met. Unless otherwise specified in the individual monograph, data from five replicate injections of the analyte are used to calculate the relative standard deviation if the requirement is 2.0% or less; data from six replicate injections are used if the relative standard deviation requirement is more than 2.0%.

The tailing factor, $T$, a measure of peak symmetry, is unity for perfectly symmetrical peaks, and its value increases as tailing becomes more pronounced. In some cases, values less than unity may be observed. As peak asymmetry increases, integration, and hence precision, becomes less reliable. The calculation is expressed by the equation:

$$\text{tailing factor} = T = W_{0.05}/2f$$

These tests are performed by collecting data from replicate injections of standard or other solutions as specified in the individual monograph. The specification of definitive parameters in a monograph does not preclude the use of other suitable operating conditions (see Procedures under Tests and Assays under General Provisions). Adjustments of operating conditions to meet system suitability requirements may be necessary.

Unless otherwise directed in the monograph, system suitability parameters are determined from the analyte peak.

To ascertain the effectiveness of the final operating system, it should be subjected to a suitability test before use and during testing whenever there is a significant change in equipment or in a critical reagent or when a malfunction is suspected.

**B. PHYSICOCHEMICAL PROPERTIES**

**DISTILLATION RANGE**

**Scope**  This method is to be used for determining the distillation range of pure or nearly pure compounds or mixtures having a relatively narrow distillation range of about $40^\circ$ or less. The result so determined is an indication of purity, not necessarily of identity. Products having a distillation range of greater than $40^\circ$ may be determined by this method if a wide-range thermometer, such as ASTM E1, 1C, 2C, or 3C, is specified in the individual monograph.
Definitions

**Distillation Range**  The difference between the temperature observed at the start of a distillation and that observed at which a specified volume has distilled, or at which the dry point is reached.

**Initial Boiling Point**  The temperature indicated by the distillation thermometer at the instant the first drop of condensate leaves the end of the condenser tube.

**Dry Point**  The temperature indicated at the instant the last drop of liquid evaporates from the lowest point in the distillation flask, disregarding any liquid on the side of the flask.

Apparatus

**Distillation Flask**  A 200-mL round-bottom distilling flask of heat-resistant glass is preferred when sufficient sample (in excess of 100 mL) is available for the test. If a sample of less than 100 mL must be used, a smaller flask having a capacity of at least double the volume of the liquid taken may be employed. The 200-mL flask has a total length of 17 to 19 cm, and the inside diameter of the neck is 20 to 22 mm. Attached about midway on the neck, approximately 12 cm from the bottom of the flask, is a side arm 10 to 12.7 cm long and 5 mm in internal diameter, which forms an angle of 70° to 75° with the lower portion of the neck.

**Condenser**  Use a straight glass condenser of heat-resistant tubing, 56 to 60 cm long and equipped with a water jacket so that about 40 cm of the tubing is in contact with the cooling medium. The lower end of the condenser may be bent to provide a delivery tube or it may be connected to a bent adapter that serves as the delivery tube.

[NOTE: All-glass apparatus with standard-taper ground joints may be used alternatively if the assembly employed provides results equal to those obtained with the flask and condenser described above.]

**Receiver**  The receiver is a 100-mL cylinder that is graduated in 1-mL subdivisions and calibrated “to contain.” It is used for measuring the sample as well as for receiving the distillate.

**Thermometer**  An accurately standardized partial-immersion thermometer having the smallest practical subdivisions (not greater than 0.2°) is recommended to avoid the necessity for an emergent stem correction. Suitable thermometers are available as the ASTM E1 Series 37C through 41C, and 102C through 107C, or as the MCA types R-1 through R-4 (see Thermometers, Appendix I).

**Source of Heat**  A Bunsen burner is the preferred source of heat. An electric heater may be used, however, if it is shown to give results comparable to those obtained with the gas burner.

**Shield**  The entire burner and flask assembly should be protected from external air currents. Any efficient shield may be employed for this purpose.

**Flask Support**  A heat-resistant board, 5 to 7 mm in thickness and having a 10-cm circular hole, is placed on a suitable ring or platform support and fitted loosely inside the shield to ensure that hot gases from the source of heat do not come in contact with the sides or neck of the flask. A second 5- to 7-mm thick heat-resistant board, 14- to 16-cm square and provided with a 30- to 40-mm circular hole, is placed on top of the first board. This board is used to hold the 200-mL distillation flask, which should be fitted firmly on the board so that direct heat is applied to the flask only through the opening in the board.

**Procedure**  [NOTE: For materials boiling below 50°, cool the liquid to below 10° before sampling, receive the distillate in a water bath cooled to below 10°, and use water cooled to below 10° in the condenser.] Measure 100 ± 0.5 mL of the liquid in the 100-mL graduate, and transfer the sample, together with an efficient
antibumping device, into the distilling flask. Do not use a funnel in the transfer or allow any of the sample to enter the side arm of the flask. Place the flask on the heat-resistant boards, which are supported on a ring or platform, and position the shield for the flask and burner. Connect the flask and condenser, place the graduate under the outlet of the condenser tube, and insert the thermometer. The thermometer should be located in the center of the neck so that the top of the contraction chamber (or bulb, if 37°C or 38°C is used) is level with the bottom of the outlet to the side arm. Regulate the heating so that the first drop of liquid is collected within 5 to 10 min. Read the thermometer at the instant the first drop of distillate falls from the end of the condenser tube, and record as the initial boiling point. Continue the distillation at the rate of 4 or 5 mL of distillate per minute, noting the temperature as soon as the last drop of liquid evaporates from the bottom of the flask (dry point) or when the specified percentage has distilled over. Correct the observed temperature readings for any variation in the barometric pressure from the normal (760 mm) by allowing 0.1°C for each 2.7 mm of variation, adding the correction if the pressure is lower, or subtracting if higher, than 760 mm.

When a total-immersion thermometer is used, correct for the temperature of the emergent stem by the formula:

\[ 0.00015 \times N(T - t) \]

in which \( N \) represents the number of degrees of emergent stem from the bottom of the stopper, \( T \) represents the observed temperatures of the distillation, and \( t \) represents the temperature registered by an auxiliary thermometer, the bulb of which is placed midway of the emergent stem, adding the correction to the observed readings of the main thermometer.

**MELTING RANGE OR TEMPERATURE**

For purposes of the FCC, the melting range or temperature of a solid is defined as those points of temperature within which or the point at which the solid coalesces and is completely melted when determined as directed below. Any apparatus or method capable of equal accuracy may be used. The accuracy should be checked frequently by the use of one or more of the six USP Melting Point Reference Standards, preferably the one that melts nearest the melting temperature of the compound to be tested.

Five procedures for the determination of melting range or temperature are given herein, varying in accordance with the nature of the substance. When no class is designated in the monograph, use the procedure for Class I.

The procedure known as the mixed melting point determination, whereby the melting range of a solid under test is compared with that of an intimate mixture of equal parts of the solid and an authentic specimen of it, may be used as a confirmatory identification test. Agreement of the observations on the original and the mixture usually constitutes reliable evidence of chemical identity.

**Apparatus** The melting range apparatus consists of a glass container for a bath of colorless fluid, a suitable stirring device, an accurate thermometer (see Appendix I), and a controlled source of heat. The bath fluid is selected consistent with the temperature required, but light paraffin is used generally, and certain liquid silicones are well adapted to the higher temperature ranges. The fluid is deep enough to permit immersion of the thermometer to its specified immersion depth so that the bulb is still about 2 cm above the bottom of the bath. The heat may be supplied electrically or by an open flame. The capillary tube is about 10 cm long, with an internal diameter of 0.8 to 1.2 mm, and with walls 0.2 to 0.3 mm thick.

The thermometer is preferably one that conforms to the specifications provided under Thermometers, Appendix I, selected for the desired accuracy and range of temperature.

**Procedure for Class I** Reduce the sample to a very fine powder, and unless otherwise directed, render it
anhydrous when it contains water of hydration by drying it at the temperature specified in the monograph, or when the substance contains no water of hydration, dry it over a suitable desiccant for 16 to 24 h. Charge a capillary glass tube, one end of which is sealed, with a sufficient amount of the dry powder to form a column in the bottom of the tube 2.5 to 3.5 mm high when packed down as closely as possible by moderate tapping on a solid surface.

Heat the bath until a temperature approximately 30° below the expected melting point is reached, attach the capillary tube to the thermometer, and adjust its height so that the material in the capillary is level with the thermometer bulb. Return the thermometer to the bath, continue the heating, with constant stirring, at a rate of rise of approximately 3°/min until a temperature 3° below the expected melting point is attained, then carefully regulate the rate to about 1° to 2°/min until melting is complete.

The temperature at which the column of the sample is observed to collapse definitely against the side of the tube at any point is defined as the beginning of melting, and the temperature at which the sample becomes liquid throughout is defined as the end of melting. The two temperatures fall within the limits of the melting range.

**Procedure for Class Ia** Prepare the sample and charge the capillary glass tube as directed for Class I. Heat the bath until a temperature 10° ± 1° below the expected melting range is reached, then introduce the charged tube, and heat at a rate of rise of 3° ± 0.5°/min until melting is complete. Record the melting range as for Class I.

**Procedure for Class Ib** Place the sample in a closed container, and cool to 10° or lower for at least 2 h. Without previous powdering, charge the cooled material into the capillary tube as directed for Class I, immediately place the charged tube in a vacuum desiccator, and dry at a pressure not exceeding 20 mm Hg for 3 h. Immediately upon removal from the desiccator, fire-seal the open end of the tube. As soon as is practicable, proceed with the determination of the melting range as follows: Heat the bath until a temperature of 10° ± 1° below the expected melting range is reached, then introduce the charged tube, and heat at a rate of rise of 3° ± 0.5°/min until melting is complete. Record the melting range as directed in Class I.

If the particle size of the material is too large for the capillary, precool the sample as directed above, then with as little pressure as possible, gently crush the particles to fit the capillary, and immediately charge the tube.

**Procedure for Class II** Carefully melt the material to be tested at as low a temperature as possible, and draw it into a capillary tube that is left open at both ends to a depth of about 10 mm. Cool the charged tube at 10°, or lower, for 24 h, or in contact with ice for at least 2 h. Then attach the tube to the thermometer by means of a rubber band, adjust it in a water bath so that the upper edge of the material is 10 mm below the water level, and heat as directed for Class I, except within 5° of the expected melting temperature, regulate the rate of rise of temperature to 0.5° to 1.0°/min. The temperature at which the material is observed to rise in the capillary tube is the melting temperature.

**Procedure for Class III** Melt a quantity of the substance slowly, while stirring, until it reaches a temperature of 90° to 92°. Remove the source of heat, and allow the molten substance to cool to a temperature of 8° to 10° above the expected melting point. Chill the bulb of an ASTM 14C thermometer (see Appendix I) to 5°, wipe it dry, and while it is still cold, dip it into the molten substance so that approximately the lower half of the bulb is submerged. Withdraw it immediately, and hold it vertically away from the heat until the wax surface dulls, then dip it for 5 min into a water bath having a temperature not higher than 16°.
Fix the thermometer securely in a test tube so that the lower point is 15 mm above the bottom of the test tube. Suspend the test tube in a water bath adjusted to about 16°, and raise the temperature of the bath at the rate of 2°/min to 30°, then change to a rate of 1°/min, and note the temperature at which the first drop of melted substance leaves the thermometer. Repeat the determination twice on a freshly melted portion of the sample. If the variation of three determinations is less than 1°, take the average of the three as the melting point. If the variation of three determinations is greater than 1°, make two additional determinations and take the average of the five.

**OPTICAL (SPECIFIC) ROTATION**

Many chemicals in a pure state or in solution are optically active in the sense that they cause incident polarized light to emerge in a plane forming a measurable angle with the plane of the incident light. When this effect is large enough for precise measurement, it may serve as the basis for an assay or an identity test. In this connection, the optical rotation is expressed in degrees, as either angular rotation (observed) or specific rotation (calculated with reference to the specific concentration of 1 g of solute in 1 mL of solution, measured under stated conditions).

Specific rotation of a liquid substance usually is expressed by the equation \([\alpha]_x^t = a/ld\), and for solutions of solid substances, expressed by the equation \([\alpha]_x^t = 100a/lpd = 100a/lc\), in which \(a\) is the corrected observed rotation, in degrees, at temperature \(t\); \(x\) is the wavelength of the light used; \(l\) is the length of the polarimeter cell, in dm; \(d\) is the specific gravity of the liquid or solution at the temperature of observation; \(p\) is the concentration of the solution expressed as the number of grams of substance in 100 g of solution; and \(c\) is the concentration of the solution expressed as the number of grams of substance in 100 mL of solution. The concentrations \(p\) and \(c\) should be calculated on the dried or anhydrous basis, unless otherwise specified. Spectral lines most frequently employed are the D line of sodium (doublet at 589.0 and 589.6 nm) and the yellow-green line of mercury at 546.1 nm. The specific gravity and the rotatory power vary appreciably with the temperature.

The accuracy and precision of optical rotatory measurements will be increased if they are carried out with due regard for the following general considerations.

Supplement the source of illumination with a filtering system capable of transmitting light of a sufficiently monochromatic nature. Precision polarimeters generally are designed to accommodate interchangeable disks to isolate the D line from sodium light or the 546.1-nm line from the mercury spectrum. With polarimeters not thus designed, cells containing suitably colored liquids may be employed as filters (see also A. Weissberger and B. W. Rossiter, *Techniques of Chemistry, Vol. I: Physical Methods of Chemistry*, Part 3, Wiley-Interscience, New York, 1972).

Pay special attention to temperature control of the solution and of the polarimeter. Make accurate and reproducible observations to the extent that differences between replicates, or between observed and true values of rotation (the latter value having been established by calibration of the polarimeter scale with suitable standards), calculated in terms of either specific rotation or angular rotation, whichever is appropriate, do not exceed one-fourth of the range given in the individual monograph for the rotation of the article being tested. Generally, a polarimeter accurate to 0.05° of angular rotation, and capable of being read with the same precision, suffices for FCC purposes; in some cases, a polarimeter accurate to 0.01°, or less, of angular rotation, and read with comparable precision, may be required.
Fill polarimeter tubes in such a way as to avoid creating or leaving air bubbles, which interfere with the passage of the beam of light. Interference from bubbles is minimized with tubes in which the bore is expanded at one end. However, tubes of uniform bore, such as semimicro- or micro-tubes, require care for proper filling. At the time of filling, the tubes and the liquid or solution should be at a temperature not higher than that specified for the determination to guard against the formation of a bubble upon cooling and contraction of the contents.

In closing tubes having removable end plates fitted with gaskets and caps, the latter should be tightened only enough to ensure a leak-proof seal between the end plate and the body of the tube. Excessive pressure on the end plate may set up strains that result in interference with the measurements. In determining the specific rotation of a substance of low rotatory power, loosen the caps and tighten them again between successive readings in the measurement of both the rotation and the zero point. Differences arising from end plate strain thus generally will be revealed and appropriate adjustments to eliminate the cause may be made.

Procedure  In the case of a solid, dissolve the substance in a suitable solvent, reserving a separate portion of the latter for a blank determination. Make at least five readings of the rotation of the solution, or of the substance itself if liquid, at 25°C or the temperature specified in the individual monograph. Replace the solution with the reserved portion of the solvent (or, in the case of a liquid, use the empty tube), make the same number of readings, and use the average as the zero point value. Subtract the zero point value from the average observed rotation if the two figures are of the same sign, or add if opposite in sign, to obtain the corrected observed rotation.

Calculation  Calculate the specific rotation of a liquid substance, or of a solid in solution, by application of one of the following formulas:

1. for liquid substances,

\[
[α]_x^t = \frac{a}{ld}
\]

2. for solutions of solids,

\[
[α]_x^t = \frac{100a}{lpd} = \frac{100a}{lc}
\]

in which \(a\) is the corrected observed rotation, in degrees, at temperature \(t\); \(x\) is the wavelength of the light used; \(l\) is the length, in dm, of the polarimeter cell; \(d\) is the specific gravity of the liquid or solution at the temperature of observation; \(p\) is the concentration of the solution expressed as the number of grams of substance in 100 g of solution; and \(c\) is the concentration of the solution expressed as the number of grams of substance in 100 mL of solution. The concentrations \(p\) and \(c\) should be calculated on the dried or anhydrous basis, unless otherwise specified.

**pH DETERMINATION**

**Principle**  The definition of pH is the negative log of the hydrogen ion concentration in moles per liter of aqueous solutions. Measure pH potentiometrically by using a pH meter or colorimetrically by using pH indicator paper.

**Scope**  This method is suitable to determine the pH of aqueous solutions. While pH meters, calibrated with
aqueous solutions, are sometimes used to make measurements in semiaqueous solutions or in nonaqueous polar solutions, the value obtained is the apparent pH value only and should not be compared with the pH of aqueous solutions. For nonpolar solutions, pH has no meaning, and pH electrodes may be damaged by direct contact with these solutions. References to the pH of nonpolar solutions or liquids usually indicate the pH of a water extract of the nonpolar liquid or the apparent pH of a mixture of the nonpolar liquid in a polar liquid such as alcohol or alcohol–water mixtures.

**Procedure [Potentiometric Method (pH Meter)]**

*Calibration*  Select two standard buffers to bracket, if possible, the anticipated pH of the unknown substances. These commercially available standards and the sample should be at the same temperature, within two degrees. Set the temperature compensator of the pH meter to the temperature of the samples and standards. Follow the manufacturer's instructions for setting temperature compensation and for adjusting the output during calibration. Rinse the electrodes with distilled or deionized water, and blot them dry with clean, absorbent laboratory tissue. Place the electrode(s) in the first standard buffer solution, and adjust the standardization control so that the pH reading matches the stated pH of the standard buffer. Repeat this procedure with fresh portions of the first buffer solution until two successive readings are within ± 0.02 pH units with no further adjustment. Rinse the electrodes, blot them dry, and place them in a portion of the second standard buffer solution. Following the manufacturer's instructions, adjust the slope control (not the standardization control) until the output displays the pH of the second standard buffer. Repeat the sequence of standardization with both buffers until pH readings are within ± 0.02 pH units for both buffers without adjustments to either the slope or standardization controls. The pH of the unknown may then be measured, using either a pH electrode in combination with a reference electrode or a single combination electrode. Select electrodes made of chemically resistant glass when measuring samples of either low or high pH.

*pH Indicator Paper*  Test papers impregnated with acid–base indicators, although less accurate than pH meters, offer a convenient way to determine the pH of an aqueous solution. They may be purchased in rolls or strips covering all or part of the pH range; papers covering a narrow part of the pH range can be sensitive to differences of 0.2 pH units. Some test papers comprise a plastic strip with small squares of test paper attached. The different squares are sensitive to different pH ranges. When using this type of test paper, wet all of the squares with the test sample to ensure a correct pH reading. Test paper can contaminate the sample being tested; therefore, do not dip it into the sample. Either use a clean glass rod to remove a drop of the test solution and place it on the test paper, or transfer a small amount of the sample to a small container, dip the test paper into this portion, and compare the developed color with the color comparison chart provided with the test paper to determine the pH of the sample.

**READILY CARBONIZABLE SUBSTANCES**

**Reagents**

*Sulfuric Acid, 95%*  Add a quantity of sulfuric acid of known concentration to sufficient water to adjust the final concentration to between 94.5% and 95.5% of H$_2$SO$_4$. Because the acid concentration may change upon standing or upon intermittent use, check the concentration frequently and either adjust solutions assaying more than 95.5% or less than 94.5% by adding either diluted or fuming sulfuric acid, as required, or discard them.
Cobaltous Chloride CS  Dissolve about 65 g of cobaltous chloride (CoCl$_2$·6H$_2$O) in enough of a mixture of 25 mL of hydrochloric acid and 975 mL of water to make 1000 mL. Pipet 5 mL of this solution into a 250-mL iodine flask; add 5 mL hydrogen peroxide TS (3%) and 15 mL of a 1:5 solution of sodium hydroxide, boil for 10 min, cool, and add 2 g of potassium iodide and 20 mL of 1:4 sulfuric acid. When the precipitate has dissolved, titrate the liberated iodine with 0.1 N sodium thiosulfate. The titration is sensitive to air oxidation and should be blanketed with carbon dioxide. Each milliliter of 0.1 N sodium thiosulfate is equivalent to 23.79 mg of CoCl$_2$·6H$_2$O. Adjust the final volume of the solution by adding enough of the mixture of hydrochloric acid and water so that each milliliter contains 59.5 mg of CoCl$_2$·6H$_2$O.

Cupric Sulfate CS  Dissolve about 65 g of cupric sulfate (CuSO$_4$·5H$_2$O) in enough of a mixture of 25 mL of hydrochloric acid and 975 mL of water to make 1000 mL. Pipet 10 mL of this solution into a 250-mL iodine flask; add 40 mL of water, 4 mL of acetic acid, and 3 g of potassium iodide; and titrate the liberated iodine with 0.1 N sodium thiosulfate, adding starch TS as the indicator. Each milliliter of 0.1 N sodium thiosulfate is equivalent to 24.97 mg of CuSO$_4$·5H$_2$O. Adjust the final volume of the solution by adding enough of the mixture of hydrochloric acid and water so that each milliliter contains 62.4 mg of CuSO$_4$·5H$_2$O.

Ferric Chloride CS  Dissolve about 55 g of ferric chloride (FeCl$_3$·6H$_2$O) in enough of a mixture of 25 mL of hydrochloric acid and 975 mL of water to make 1000 mL. Pipet 10 mL of this solution into a 250-mL iodine flask; add 15 mL of water, 5 mL of hydrochloric acid, and 3 g of potassium iodide; and allow the mixture to stand for 15 min. Dilute with 100 mL of water, and titrate the liberated iodine with 0.1 N sodium thiosulfate, adding starch TS as the indicator. Perform a blank determination with the same quantities of the same reagents and in the same manner, and make any necessary correction. Each milliliter of 0.1 N sodium thiosulfate is equivalent to 27.03 mg of FeCl$_3$·6H$_2$O. Adjust the final volume of the solution by adding the mixture of hydrochloric acid and water so that each milliliter contains 45.0 mg of FeCl$_3$·6H$_2$O.

Platinum–Cobalt CS  Transfer 1.246 g of potassium chloroplatinate (K$_2$PtCl$_6$) and 1.00 g of crystallized cobaltous chloride (CoCl$_2$·6H$_2$O) into a 1000-mL volumetric flask, dissolve in about 200 mL of water and 100 mL of hydrochloric acid, dilute to volume with water, and mix. This solution has a color of 500 APHA units. [NOTE: Use this solution only when specified in an individual monograph.]

Procedure  Unless otherwise directed, add the specified quantity of the substance, finely powdered if in solid form, in small portions to the comparison container, which is made of colorless glass resistant to the action of sulfuric acid and contains the specified volume of 95% Sulfuric Acid. Stir the mixture with a glass rod until solution is complete, allow the solution to stand for 15 min, unless otherwise directed, and compare the color of the solution with that of the specified matching fluid in a comparison container that also is of colorless glass and has the same internal and cross-section dimensions, viewing the fluids transversely against a background of white porcelain or white glass.

When heat is directed to effect solution of the substance in the 95% Sulfuric Acid, mix the sample and the acid in a test tube, heat as directed, cool, and transfer the solution to the comparison container for matching.

Matching Fluids  For purposes of comparison, a series of 20 matching fluids, each designated by a letter of the alphabet, is provided, the composition of each being as indicated in the accompanying table. To prepare the matching fluid specified, pipet the prescribed volumes of the colorimetric test solutions (CS) and water into one of the matching containers, and mix the solutions in the container.

Matching Fluids$^a$
<table>
<thead>
<tr>
<th>Matching Fluid</th>
<th>Parts of Cobaltous Chloride CS</th>
<th>Parts of Ferric Chloride CS</th>
<th>Parts of Cupric Sulfate CS</th>
<th>Parts of Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.1</td>
<td>0.4</td>
<td>0.1</td>
<td>4.4</td>
</tr>
<tr>
<td>B</td>
<td>0.3</td>
<td>0.9</td>
<td>0.3</td>
<td>8.5</td>
</tr>
<tr>
<td>C</td>
<td>0.1</td>
<td>0.6</td>
<td>0.1</td>
<td>4.2</td>
</tr>
<tr>
<td>D</td>
<td>0.3</td>
<td>0.6</td>
<td>0.4</td>
<td>3.7</td>
</tr>
<tr>
<td>E</td>
<td>0.4</td>
<td>1.2</td>
<td>0.3</td>
<td>3.1</td>
</tr>
<tr>
<td>F</td>
<td>0.3</td>
<td>1.2</td>
<td>0.0</td>
<td>3.5</td>
</tr>
<tr>
<td>G</td>
<td>0.5</td>
<td>1.2</td>
<td>0.2</td>
<td>3.1</td>
</tr>
<tr>
<td>H</td>
<td>0.2</td>
<td>1.5</td>
<td>0.0</td>
<td>3.3</td>
</tr>
<tr>
<td>I</td>
<td>0.4</td>
<td>2.2</td>
<td>0.1</td>
<td>2.3</td>
</tr>
<tr>
<td>J</td>
<td>0.4</td>
<td>3.5</td>
<td>0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>K</td>
<td>0.5</td>
<td>4.5</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>L</td>
<td>0.8</td>
<td>3.8</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>M</td>
<td>0.1</td>
<td>2.0</td>
<td>0.1</td>
<td>2.8</td>
</tr>
<tr>
<td>N</td>
<td>0.0</td>
<td>4.9</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>O</td>
<td>0.1</td>
<td>4.8</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>P</td>
<td>0.2</td>
<td>0.4</td>
<td>0.1</td>
<td>4.3</td>
</tr>
<tr>
<td>Q</td>
<td>0.2</td>
<td>0.3</td>
<td>0.1</td>
<td>4.4</td>
</tr>
<tr>
<td>R</td>
<td>0.3</td>
<td>0.4</td>
<td>0.2</td>
<td>4.1</td>
</tr>
<tr>
<td>S</td>
<td>0.2</td>
<td>0.1</td>
<td>0.0</td>
<td>4.7</td>
</tr>
<tr>
<td>T</td>
<td>0.5</td>
<td>0.5</td>
<td>0.4</td>
<td>3.6</td>
</tr>
</tbody>
</table>

\*a\ Solutions A–D, very light brown-yellow.
Solutions E–L, yellow through red-yellow.
Solutions M–O, green-yellow.
Solutions P–T, light pink.

**REFRACTIVE INDEX**

The refractive index of a transparent substance is the ratio of the velocity of light in air to its velocity in that material under like conditions. It is equal to the ratio of the sine of the angle of incidence made by a ray in air to the sine of the angle of refraction made by the ray in the material being tested. The refractive index values specified in this Codex are for the D line of sodium (589 nm) unless otherwise specified. The determination should be made at the temperature specified in the individual monograph, or at 25°C if no temperature is specified. This physical constant is used as a means for identification of, and detection of impurities in, volatile oils and other liquid substances. The Abbé refractometer, or other refractometers of equal or greater accuracy, may be employed at the discretion of the operator.
**SOLIDIFICATION POINT**

**Scope**  This method is designed to determine the solidification point of food-grade chemicals having appreciable heats of fusion. It is applicable to chemicals having solidification points between $-20^\circ$ and $+150^\circ$. Necessary modifications will be noted in individual monographs.

**Definition**  Solidification Point is an empirical constant defined as the temperature at which the liquid phase of a substance is in approximate equilibrium with a relatively small portion of the solid phase. It is measured by noting the maximum temperature reached during a controlled cooling cycle after the appearance of a solid phase.

The solidification point is distinguished from the freezing point in that the latter term applies to the temperature of equilibrium between the solid and liquid state of pure compounds.

Some chemical compounds have more than one temperature at which there may be an equilibrium between the solid and liquid state depending on the crystal form of the solid that is present.

**Apparatus**  The apparatus illustrated in Figs. 3 and 4 consists of the components described in the following paragraphs.

![FIGURE 3 Apparatus for Determination of Solidification Point.](image)
Thermometer  A thermometer having a range not exceeding $30^\circ$, graduated in $0.1^\circ$ divisions, and calibrated for 76-mm immersion should be employed. A satisfactory series of thermometers, covering a range from $-20^\circ$ to $+150^\circ$, is available as ASTM-E1 89C through 96C (see *Thermometers*, Appendix I). A thermometer should be chosen such that the solidification point is not obscured by the cork stopper of the sample container.

Sample Container  Use a standard glass 25- × 150-mm test tube with a lip, fitted with a two-hole cork stopper to hold the thermometer in place and to allow adequate stirring with a stirrer.

Air Jacket  For the air jacket, use a standard glass 38- × 200-mm test tube with a lip and fitted with a cork or rubber stopper bored with a hole into which the sample container can easily be inserted up to the lip.

Cooling Bath  Use a 2000-mL beaker or a similar, suitable container as a cooling bath. Fill it with an appropriate cooling medium such as glycerin, mineral oil, water, water and ice, or alcohol–dry ice.

Stirrer  The stirrer (Fig. 4) consists of a 1-mm in diameter (B & S gauge 18), corrosion-resistant wire bent into a series of three loops about 25 mm apart. It should be made so that it will move freely in the space between the thermometer and the inner wall of the sample container. The shaft of the stirrer should be of a convenient length designed to pass loosely through a hole in the cork holding the thermometer. Stirring may be hand operated or mechanically activated at 20 to 30 strokes/min.

Assembly  Assemble the apparatus in such a way that the cooling bath can be heated or cooled to control the desired temperature ranges. Clamp the air jacket so that it is held rigidly just below the lip, and immerse it in the cooling bath to a depth of 160 mm.

Sample Preparation  The solidification point of chemicals is usually determined as they are received. Some may be hygroscopic, however, and will require special drying. If this is necessary, it will be noted in the individual monographs.

Products that are normally solid at room temperature must be carefully melted at a temperature about $10^\circ$
above the expected solidification point. Care should be observed to avoid heating in such a way as to decompose or distill any portion of a sample.

**Procedure**  Adjust the temperature of the cooling bath to about $5^\circ$ below the expected solidification point. Fit the thermometer and stirrer with a cork stopper so that the thermometer is centered and the bulb is about 20 mm from the bottom of the sample container. Transfer a sufficient amount of the sample, previously melted if necessary, into the sample container to fill it to a depth of about 90 mm when in the molten state. Place the thermometer and stirrer in the sample container, and adjust the thermometer so that the immersion line will be at the surface of the liquid and so that the end of the bulb is $20 \pm 4$ mm from the bottom of the sample container. When the temperature of the sample is about $5^\circ$ above the expected solidification point, place the assembled sample tube in the air jacket. Allow the sample to cool while stirring, at the rate of 20 to 30 strokes/min, in such a manner that the stirrer does not touch the thermometer. Stir the sample continuously during the remainder of the test.

The temperature at first will gradually fall, then will become constant as crystallization starts and continues under equilibrium conditions, and finally will start to drop again. Some chemicals may supercool slightly below $(0.5^\circ)$ the solidification point; as crystallization begins, the temperature will rise and remain constant as equilibrium conditions are established. Other products may cool more than $0.5^\circ$ and cause deviation from the normal pattern of temperature change. If the temperature rise exceeds $0.5^\circ$ after the initial crystallization begins, repeat the test, and seed the melted compound with small crystals of the sample at $0.5^\circ$ intervals as the temperature approaches the expected solidification point. Crystals for seeding may be obtained by freezing a small sample in a test tube directly in the cooling bath. It is preferable that seed of the stable phase be used from a previous determination.

Observe and record the temperature readings at regular intervals until the temperature rises from a minimum, due to supercooling, to a maximum and then finally drops. The maximum temperature reading is the solidification point. Readings 10 s apart should be taken to establish that the temperature is at the maximum level and should continue until the drop in temperature is established.

**VISCOSITY**

Viscosity is a fluid's measured internal resistance to flow. Thick, slow-moving fluids have higher viscosities than thin, free-flowing fluids. The basic unit of measure for viscosity is the poise or Pascal second, Pa·s, in SI units. The relationship between poise and Pa·s is 1 poise = 0.1 Pa·s. Since commonly encountered viscosities are often fractions of 1 poise, viscosities are commonly expressed as centipoises (one centipoise = 0.01 poise). Poise or centipoise is the unit of measure for absolute viscosity. Kinematic viscosity also is commonly used and is determined by dividing the absolute viscosity of the test liquid by the density of the test liquid at the same temperature as the viscosity measurement and is expressed as stokes or centistokes (poise/density = stokes). The specified temperature is important: viscosity varies greatly with temperature, generally decreasing with increasing temperature.

Absolute viscosity can be determined directly if accurate dimensions of the measuring instruments are known. It is common practice to calibrate an instrument with a fluid of known viscosity and to determine the unknown viscosity of another fluid by comparison with that of the known viscosity.

Many substances, such as gums, have a variable viscosity, and most of them are less resistant to flow at higher flow (more correctly, shear) rates. In such cases, select a given set of conditions for measurement, and
consider the measurement obtained to be an apparent viscosity. Since a change in the conditions of measurement would yield a different value for the apparent viscosity of such substances, the operator must closely adhere to the instrument dimensions and conditions for measurement.

**Measuring Viscosity**  Several common methods are available for measuring viscosity. Two very common ones are the use of capillary tubes such as Ubbelohde, Ostwald, or Cannon-Fenske viscometer tubes and the use of a rotating spindle such as the Brookfield viscometer.

Determine the viscosity in capillary tubes by measuring the amount of time it takes for a given volume of liquid to flow through a calibrated capillary tube. Calibrate the capillary tube by using liquids of known viscosity. The calibration may be supplied with the viscometer tube when purchased along with specific instructions for its use. Many types of capillary viscometer tubes are available, and exact procedures will vary with the type of tube chosen. Examples of procedures are in the following sections: *Viscosity of Dimethylpolysiloxane* and *Viscosity of Methylcellulose*. In general, calibrate capillary viscometers by filling the viscometers per the manufacturer's instructions and allowing the filled tube to equilibrate to the given temperature in a constant-temperature bath. Draw the liquid to the top graduation line, and measure the time, in seconds, it takes for the liquid to flow from the upper mark to the lower mark in the capillary tube. Calculate the viscometer constant, \( k \), by the equation:

\[
k = \frac{v}{d t}
\]

in which \( v \) is the known viscosity, in centipoises, of the standard liquid; \( d \) is the density, at the specified temperature, of the liquid; and \( t \) is the time, in seconds, for the liquid to pass from the upper mark to the lower mark. It is not necessary to recalibrate the tube unless changes or repairs are made to it. To measure viscosity, introduce the unknown liquid into the viscometer tube in the same way as the calibration standard was introduced, and measure the time, in seconds, it takes for the liquid to flow from the upper mark to the lower mark. Calculate viscosity by the equation:

\[
v = k d t
\]

in which \( v \) is the viscosity to be determined, \( k \) is the viscometer constant, and \( d \) is the density of the liquid being measured.

Using rotational viscometers provides a particularly rapid and convenient method for determining viscosity. They employ a rotating spindle or cup immersed in the liquid, and they measure the resistance of the liquid to the rotation of the spindle or cup. A wide range of viscosities can be measured with one instrument by using spindles or cups of different sizes and by rotating them at different speeds. The manufacturer supplies the calibration of viscosity versus the spindle size and speed, which can be checked by using fluids of known viscosity. Take a measurement by allowing the sample to come to the desired temperature in a constant-temperature bath and immersing the spindle or cup to the depth specified by the manufacturer. Allow the spindle or cup to rotate until a constant reading is obtained. Multiply the reading by a factor supplied by the manufacturer for a given spindle or cup and given rotational speed to obtain the viscosity. The exact procedures will vary with the particular instrument. An example is given in the section on *Viscosity of Cellulose Gum*.

Another method to determine viscosity uses the falling-ball viscometer. Determine viscosity by noting the time it takes for a ball to fall through the distance between two marks on a tube filled with the unknown liquid (the tube is generally in a constant-temperature bath). Use balls of different weights to measure a wide range of viscosities. Calculate the viscosity by using manufacturer-supplied constants for the ball used. These instruments can be quite precise for Newtonian liquids, that is, liquids that do not have viscosities that vary with flow (more correctly, shear) rate.
Three specific methods are described below.

**Viscosity of Dimethylpolysiloxane**

**Apparatus** The Ubbelohde suspended level viscometer, shown in Fig. 5 is preferred to determine the viscosity of dimethylpolysiloxane. Alternatively, a Cannon-Ubbelohde viscometer may be used.

Select a viscometer having a minimum flow time of at least 200 s. Use a No. 3 size Ubbelohde, or a No. 400 size Cannon-Ubbelohde, viscometer for the range of 300 to 600 centistokes. The viscometer should be fitted with holders that satisfy the dimensional positions of the separate tubes as shown in the diagram and that hold the viscometer vertically. Filling lines in bulb A indicate the minimum and maximum volumes of liquid to be used for convenient operation. The volume of bulb B is approximately 5 mL.

**Calibration of the Viscometer** Determine the viscosity constant, C, for each viscometer by using an oil of known viscosity. Charge the viscometer by tilting the instrument about 30 degrees from the vertical, with bulb A below the capillary, and then introduce enough of the sample into tube 1 to bring the level up to the lower filling line. The level should not be above the upper filling line when the viscometer is returned to the vertical position and the sample has drained from tube 1. Charge the viscometer in such a manner that the U-tube at the bottom fills completely without trapping air.

After the viscometer has been in a constant-temperature bath (25° ± 0.2°) long enough for the sample to reach temperature equilibrium, place a finger over tube 3, and apply suction to tube 2 until the liquid reaches the center of bulb C. Remove suction from tube 2, then remove the finger from tube 3, and place it over tube 2 until the sample drops away from the lower end of the capillary. Remove the finger from tube 2, and measure the time, to the nearest 0.1 s, required for the meniscus to pass from the first timing mark (T1) to the second (T2).

Calculate the viscometer constant, C, by the equation:

\[ C = \frac{cs}{t_1} \]

in which cs is the viscosity, in centistokes, and \( t_1 \) is the efflux time, in seconds, for the standard liquid.

**Determination of the Viscosity of Dimethylpolysiloxane** Charge the viscometer with the sample in the same manner as described for the calibration procedure; determine the efflux time, \( t_2 \); and calculate the viscosity of the dimethylpolysiloxane by the formula:

\[ V = C \times t_2 \]
Viscosity of Methylcellulose

**Apparatus** Viscometers used to determine the viscosity of methylcellulose and some related compounds are illustrated in Fig. 6 and consist of three parts: a large filling tube, A; an orifice tube, B; and an air vent to the reservoir, C.

![Methylcellulose Viscometers](image)

There are two basic types of methylcellulose viscometers—one for cellulose derivatives of a range between 1500 and 4000 centipoises, and the other for less viscous ones. Each type of viscometer is modified slightly for the different viscosities.

**Calibration of the Viscometer** Determine the viscometer constant, $K$, for each viscometer by using an oil of known viscosity. Place an excess of the liquid that is to be tested (adjusted to 20°C ± 0.1°C) in the filling tube, A, and transfer it to the orifice tube, B, by gentle suction, taking care to keep the liquid free from air bubbles by closing the air vent tube, C. Adjust the column of liquid in tube B so it is even with the top graduation line. Open both tubes B and C to permit the liquid to flow into the reservoir against atmospheric pressure. [NOTE: Failure to open air vent tube C before determining the viscosity will yield false values.]

Record the time, in seconds, for the liquid to pass from the upper mark to the lower mark in tube B. Calculate the viscometer constant, $K$, from the equation:

$$K = V/dt$$

in which $V$ is the viscosity, in centipoises, of the liquid; $K$ is the viscometer constant; $d$ is the specific gravity of the liquid tested at 20°C/20°C; and $t$ is the time, in seconds, for the liquid to pass from the upper to the lower mark.

For the calibration, all values in the equation are known or can be determined except $K$, which must be solved. If a tube is repaired, it must be recalibrated to avoid obtaining significant changes in the value of $K$.

**Determination of the Viscosity of Methylcellulose** Prepare a 2% solution of methylcellulose or other cellulose derivative, by weight, as directed in the monograph. Place the solution in the proper viscometer and determine the time, $t$, required for the solution to flow from the upper mark to the lower mark in orifice tube B.
Separately determine the specific gravity, \( d \), at 20\(^\circ\)/20\(^\circ\). Viscosity, \( V = K d t \).

**Change to read:**

**Viscosity of Cellulose Gum**

**Apparatus** Use a Brookfield Model LV series viscometer, analog or digital, or equivalent type viscometer for the determination of viscosity of aqueous solutions of cellulose gum within the range of 25 to 10,000 centipoises at 25\(^\circ\). Rotational viscometers of this type have spindles for use in determining the viscosity of different viscosity types of cellulose gum. The spindles and speeds for determining viscosity within different ranges are tabulated below.

<table>
<thead>
<tr>
<th>Viscosity Range (centipoises)</th>
<th>Spindle No.</th>
<th>Speed (rpm)</th>
<th>Scale</th>
<th>Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>10–100</td>
<td>1</td>
<td>60</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>100–200</td>
<td>1</td>
<td>30</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>200–1000</td>
<td>2</td>
<td>30</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>1000–4000</td>
<td>3</td>
<td>30</td>
<td>100</td>
<td>40</td>
</tr>
<tr>
<td>4000–10,000</td>
<td>4</td>
<td>30</td>
<td>100</td>
<td>200</td>
</tr>
</tbody>
</table>

**Mechanical Stirrer** Use an agitator essentially as shown in Fig. 7 that can be attached to a variable-speed motor capable of operating at 900 ± 100 rpm under varying load conditions.

![Agitator for Viscosity of Cellulose Gum](image-url)
NOTE: The agitator may be fabricated from stainless steel (Hercules, Inc., Wilmington, Delaware, or equivalent.) or glass as shown in Fig. 7. Where this procedure is specified for viscosity measurements by reference in other monographs, equivalent three-blade agitators may be used.]  

Sample Container Use a glass jar about 152 mm deep having an od of approximately 64 mm and a capacity of about 340 g.

Water Bath Use a water bath capable of maintaining a constant temperature. Set the temperature to 25° and maintain it within ±0.2°.

Thermometer Use an ASTM Saybolt Viscosity Thermometer having a range from 19° to 27° and conforming to the requirements for Thermometer 17C as described in ASTM Specification E1.

Sample Preparation Accurately weigh an amount of sample equivalent to 4.8 g of cellulose gum on the dried basis, and record the actual quantity required, in grams, as S. Transfer an accurately measured volume of water equivalent to 240 − S g into the sample container. Position the stirrer in the sample container, allowing minimal clearance between the stirrer and the bottom of the container. Begin stirring, and slowly add the sample. Adjust the stirring speed to approximately 900 ± 100 rpm. Mix for exactly 2 h. Do not allow the stirring speed to exceed 1200 rpm. Remove the stirrer, cap the sample container, and transfer the sample container into a constant-temperature water bath, maintained at 25° ± 0.2°, for 1 h. Check the sample temperature with a thermometer at the end of 1 h to ensure that the test temperature has been reached.

Procedure Remove the sample container from the water bath, shake vigorously for 10 s, and measure the viscosity with the Brookfield viscometer, using the proper spindle and speed indicated in the accompanying table. Be sure to use the viscometer guard, and allow the spindle to rotate for 3 min before taking the reading. Calculate the viscosity, in centipoises, by multiplying the reading observed by the appropriate factor from the table.
WATER DETERMINATION

Method I (Karl Fischer Titrimetric Method) Determine the water by Method Ia, unless otherwise specified in the individual monograph.

Method Ia (Direct Titration)

Principle The titrimetric determination of water is based on the quantitative reaction of water with an anhydrous solution of sulfur dioxide and iodine in the presence of a buffer that reacts with hydrogen ions.

In the original titrimetric solution, known as Karl Fischer Reagent, the sulfur dioxide and iodine are dissolved in pyridine and methanol. Pyridine-free reagents are more commonly used now. The test specimen may be titrated with the Karl Fischer Reagent directly, or the analysis may be carried out by a residual titration procedure. The stoichiometry of the reaction is not exact, and the reproducibility of the determination depends on such factors as the relative concentrations of the Karl Fischer Reagent ingredients, the nature of the inert solvent used to dissolve the test specimen, the apparent pH of the final mixture, and the technique used in the particular determination. Therefore, an empirically standardized technique is used to achieve the desired accuracy. Precision in the method is governed largely by the extent to which atmospheric moisture is excluded from the system. The titration of water is usually carried out with the use of anhydrous methanol as the solvent for the test specimen; however, other suitable solvents may be used for special or unusual test specimens.

Substances that may interfere with the test results are ferric ion, chlorine, and similar oxidizing agents, as well as significant amounts of strong acids or bases, phosgene, or anything that will reduce iodide to iodine, poison the reagent, and show the sample to be bone dry when water may be present (false negative). 8-Hydroxyquinoline may be added to the vessel to eliminate interference from ferric ion. Chlorine interference can be eliminated with SO$_2$ or unsaturated hydrocarbon. Excess pyridine or other amines may be added to the vessel to eliminate the interference of strong acids. Excess acetic acid or other carboxylic acid can be added to reduce the interference of strong bases. Aldehydes and ketones may react with the solution, showing the sample to be wet while the detector never reaches an endpoint (false positive).

Apparatus Any apparatus may be used that provides for adequate exclusion of atmospheric moisture and for determination of the endpoint. In the case of a colorless solution that is titrated directly, the endpoint may be observed visually as a change in color from canary yellow to amber. The reverse is observed in the case of a test specimen that is titrated residually. More commonly, however, the endpoint is determined electrometrically with an apparatus employing a simple electrical circuit that serves to impress about 200 mV of applied potential between a pair of platinum electrodes (about 5 mm$^2$ in area and about 2.5 cm apart) immersed in the solution to be titrated. At the endpoint of the titration, a slight excess of the reagent increases the flow of current to between 50 and 150 microamperes for 30 s to 30 min, depending on the solution being titrated. The time is shortest for substances that dissolve in the reagent. The longer times are required for solid materials that do not readily go into solution in the Karl Fischer Reagent. With some automatic titrators, the abrupt change in current or potential at the endpoint serves to close a solenoid-operated valve that controls the buret delivering the titrant. A commercially available apparatus generally comprises a closed system consisting of one or two automatic burets and a tightly covered titration vessel fitted with the necessary electrodes and a magnetic stirrer. The air in the system is kept dry with a suitable desiccant such as phosphorus pentoxide, and the titration vessel may be purged by means of a stream of dry nitrogen or a current of dry air.

Reagent The Karl Fischer Reagent may be prepared as follows: add 125 g of iodine to a solution containing 670 mL of methanol and 170 mL of pyridine, and cool. Place 100 mL of pyridine in a 250-mL graduated
cylinder, and keeping the pyridine cold in an ice bath, pass in dry sulfur dioxide until the volume reaches 200 mL. Slowly add this solution, with shaking, to the cooled iodine mixture. Shake to dissolve the iodine, transfer the solution to the apparatus, and allow the solution to stand overnight before standardizing. One milliliter of this solution, when freshly prepared, is equivalent to approximately 5 mg of water, but it deteriorates gradually; therefore, standardize it within 1 h before use, or daily in continual use. Protect the solution from light while in use. Store any bulk stock of the solution in a suitably sealed, glass-stoppered container, fully protected from light and under refrigeration.

A commercially available, stabilized solution of a Karl Fischer-type reagent may be used. Commercially available reagents containing solvents or bases other than pyridine and/or alcohols other than methanol also may be used. These may be single solutions or reagents formed in situ by combining the components of the reagents present in two discrete solutions. The diluted Karl Fischer Reagent called for in some monographs should be diluted as directed by the manufacturer. Either methanol, or another suitable solvent such as ethylene glycol monomethyl ether, may be used as the diluent.

**Test Preparation**  Unless otherwise specified in the individual monograph, use an accurately weighed or measured amount of the specimen under test estimated to contain 10 to 250 mg of water.

Where the monograph specifies that the specimen under test is hygroscopic, accurately weigh a sample of the specimen into a suitable container. Use a dry syringe to inject an appropriate volume of methanol, or other suitable solvent, accurately measured, into the container and shake to dissolve the specimen. Dry the syringe, and use it to remove the solution from the container and transfer it to a titration vessel prepared as directed under Procedure. Repeat the procedure with a second portion of methanol, or other suitable solvent, accurately measured; add this washing to the titration vessel; and immediately titrate. Determine the water content, in milligrams, of a portion of solvent of the same total volume as that used to dissolve the specimen and to wash the container and syringe, as directed under Standardization of Water Solution for Residual Titrations, and subtract this value from the water content, in milligrams, obtained in the titration of the specimen under test.

**Standardization of the Reagent**  Place enough methanol or other suitable solvent in the titration vessel to cover the electrodes, and add sufficient Karl Fischer Reagent to give the characteristic color or 100 ± 50 microamperes of direct current at about 200 mV of applied potential. Pure methanol can make the detector overly sensitive, particularly at low ppm levels of water, causing it to deflect to dryness and slowly recover with each addition of reagent. This slows down the titration and may allow the system to actually pick up ambient moisture during the resulting long titration. Adding chloroform or a similar nonconducting solvent will retard this sensitivity and can improve the analysis.

For determination of trace amounts of water (less than 1%), quickly add 25 µL (25 mg) of pure water, using a 25- or 50-µL syringe, and titrate to the endpoint. The water equivalence factor \( F \), in milligrams of water per milliliter of reagent, is given by the formula:

\[
25/V
\]

in which \( V \) is the volume, in milliliters, of the Karl Fischer Reagent consumed in the second titration.

For the precise determination of significant amounts of water (more than 1%), quickly add between 25 and 250 mg (25 to 250 µL) of pure water, accurately weighed by difference from a weighing pipet or from a precalibrated syringe or micropipet, the amount of water used being governed by the reagent strength and the buret size, as referred to under Volumetric Apparatus. Titrate to the endpoint. Calculate the water equivalence factor, \( F \), in milligrams of water per milliliter of reagent by the formula:

\[
W/V
\]

in which \( W \) is the weight, in milligrams, of the water, and \( V \) is the volume, in milliliters, of the Karl Fischer Reagent required.
Procedure

Unless otherwise specified, transfer 35 to 40 mL of methanol or other suitable solvent to the titration vessel, and titrate with the Karl Fischer Reagent to the electrometric or visual endpoint to consume any moisture that may be present. (Disregard the volume consumed because it does not enter into the calculations.) Quickly add the Test Preparation, mix, and again titrate with the Karl Fischer Reagent to the electrometric or visual endpoint. Calculate the water content of the specimen, in milligrams, by the formula:

\[
SF
\]

in which \( S \) is the volume, in milliliters, of the Karl Fischer Reagent consumed in the second titration, and \( F \) is the water equivalence factor of the Karl Fischer Reagent.

Method Ib (Residual Titration)

Principle

See the information in the section entitled Principle under Method Ia. In the residual titration, add excess Karl Fischer Reagent to the test specimen, allow sufficient time for the reaction to reach completion, and titrate the unconsumed Karl Fischer Reagent with a standard solution of water in a solvent such as methanol. The residual titration procedure is generally applicable and avoids the difficulties that may be encountered in the direct titration of substances from which the bound water is released slowly.

Apparatus, Reagent, and Test Preparation

Use those in Method Ia.

Standardization of Water Solution for Residual Titration

Prepare a Water Solution by diluting 2 mL of pure water to 1000 mL with methanol or another suitable solvent. Standardize this solution by titrating 25.0 mL with the Karl Fischer Reagent, previously standardized as directed under Standardization of the Reagent. Calculate the water content, in milligrams per milliliter, of the Water Solution with the formula:

\[
\frac{VF}{25}
\]

in which \( V \) is the volume of the Karl Fischer Reagent consumed, and \( F \) is the water equivalence factor of the Karl Fischer Reagent. Determine the water content of the Water Solution weekly, and standardize the Karl Fischer Reagent against it periodically as needed. Store the Water Solution in a tightly capped container.

Procedure

Where the individual monograph specifies the water content is to be determined by Method Ib, transfer 35 to 40 mL of methanol or other suitable solvent into the titration vessel, and titrate with the Karl Fischer Reagent to the electrometric or visual endpoint. Quickly add the Test Preparation, mix, and add an accurately measured excess of the Karl Fischer Reagent. Allow sufficient time for the reaction to reach completion, and titrate the unconsumed Karl Fischer Reagent with standardized Water Solution to the electrometric or visual endpoint. Calculate the water content of the specimen, in milligrams, with the formula:

\[
F(X' - XR)
\]

in which \( F \) is the water equivalence factor of the Karl Fischer Reagent; \( X' \) is the volume, in milliliters, of the Karl Fischer Reagent added after introduction of the specimen; \( X \) is the volume, in milliliters, of standardized Water Solution required to neutralize the unconsumed Karl Fischer Reagent; and \( R \) is the ratio \( V/25 \) (milliliters of Karl Fischer Reagent/milliliters of Water Solution), determined from the Standardization of Water Solution for Residual Titration.

Method Ic (Coulometric Titration)

Principle

Use the Karl Fischer reaction in the coulometric determination of water. In this determination, iodine is not added in the form of a volumetric solution, but is produced in an iodide-containing solution by
anodic oxidation. The reaction cell usually consists of a large anode compartment and a small cathode compartment that are separated by a diaphragm. Other suitable types of reaction cells (e.g., without diaphragms) may be used. Each compartment has a platinum electrode that conducts current through the cell. Iodine, which is produced at the anode electrode, immediately reacts with the water present in the compartment. When all the water has been consumed, an excess of iodine occurs, which can be detected potentiometrically, thus indicating the endpoint. Pre-electrolysis, which can take several hours, eliminates moisture from the system. Therefore, changing the Karl Fischer Reagent after each determination is not practical. Individual determinations may be carried out in succession in the same reagent solution. A requirement for this method is that each component of the test specimen be compatible with the other components and that no side reactions take place. Samples may be transferred into the vessel as solids or as solutions by means of injection through a septum. Gases can be introduced into the cell by means of a suitable gas inlet tube. For the water determination of solids, another common technique is to dissolve the solid in a suitable solvent and then inject a portion of this solution into the cell. In the case of insoluble solids, water may be extracted using suitable solvents, and then the extracts injected into the coulometric cell. Alternatively, an evaporation technique may be used in which the sample is heated in a tube and the water is evaporated and carried into the cell by means of a stream of dry, inert gas. Precision in the method is predominantly governed by the extent to which atmospheric moisture is excluded from the system. Control of the system may be monitored by measuring the amount of baseline drift. The titration of water in solid test specimens is usually carried out with the use of anhydrous methanol as the solvent. Other suitable solvents may be used for special or unusual test specimens. This method is particularly suited to chemically inert substances such as hydrocarbons, alcohols, and ethers. In comparison with the volumetric Karl Fischer titration, coulometry is a micro-method. The method uses extremely small amounts of current. It is predominantly used for substances with a very low water content (0.1% to 0.0001%).

**Apparatus**  Any commercially available apparatus consisting of an absolutely tight system fitted with the necessary electrodes and a magnetic stirrer is appropriate. The instrument's microprocessor controls the analytical procedure and displays the results. Calibration of the instrument is not necessary as the current consumed can be measured absolutely. Proper operation of the instrument can be confirmed by injecting 1 µL of water into the vessel. The instrument should read 1000 µg of water on reaching the endpoint.

**Reagent**  See Reagent under Method Ia.

**Test Preparation**  Using a dry syringe, inject an appropriate volume of test specimen estimated to contain 0.5 to 5 mg of water, accurately measured, into the anolyte solution. The sample may also be introduced as a solid, accurately weighed, into the anolyte solution. Perform coulometric titration, and determine the water content of the specimen under test.

Alternatively, when the specimen is a suitable solid, dissolve an appropriate quantity, accurately weighed, in anhydrous methanol or another suitable solvent, and inject a suitable portion into the anolyte solution.

When the specimen is an insoluble solid, extract the water by using a suitable anhydrous solvent from which an appropriate quantity, accurately weighed, may be injected into the anolyte solution. Alternatively use an evaporation technique.

**Procedure**  Quickly inject the Test Preparation, or transfer the solid sample, into the anolyte, mix, and perform the coulometric titration to the electrometric endpoint. Read the water content of the Test Preparation directly from the instrument's display, and calculate the percent that is present in the substance.

### Method II (Toluene Distillation Method)

**Principle**  This method determines water by distillation of a sample with an immiscible solvent, usually
**Apparatus**  Use a glass distillation apparatus (see Fig. 8) provided with 24/40 ground-glass connections. The components consist of a 500-mL short-neck, round-bottom flask connected by means of a trap to a 400-mm water-cooled condenser. The lower tip of the condenser should be about 7 mm above the surface of the liquid in the trap after distillation conditions have been established (see Procedure).

**FIGURE 8 Moisture Distillation Apparatus.**

The trap should be constructed of well-annealed glass, the receiving end of which is graduated to contain 5 mL and subdivided into 0.1-mL divisions, with each 1-mL line numbered from 5 mL beginning at the top. Calibrate the receiver by adding 1 mL of water, accurately measured, to 100 mL of toluene contained in the distillation flask. Conduct the distillation, and calculate the volume of water obtained as directed in the Procedure. Add another milliliter of water to the cooled apparatus, and repeat the distillation. Continue in this manner until five 1-mL portions of water have been added. The error at any indicated capacity should not exceed 0.05 mL. The source of heat is either an oil bath or an electric heater provided with a suitable means of temperature control. The distillation may be better controlled by insulating the tube leading from the flask to the receiver. It is also advantageous to protect the flask from drafts. Clean the entire apparatus with potassium dichromate-sulfuric acid cleaning solution, rinse thoroughly, and dry completely before using.

**Procedure**  Place in the previously cleaned and dried flask a quantity of the substance, weighed accurately to the nearest 0.01 g, that is expected to yield from 1.5 to 4 mL of water. If the substance is of a pastelike consistency, weigh it in a boat of metal foil that will pass through the neck of the flask. If the substance is likely to cause bumping, take suitable precautions to prevent it. Transfer about 200 mL of ACS reagent-grade toluene into the flask, and swirl to mix it with the sample. Assemble the apparatus, fill the receiver with toluene by pouring it through the condenser until it begins to overflow into the flask, and insert a loose cotton plug in the top of the condenser. Heat the flask so that the distillation rate will be about 200 drops/min, and continue distilling until the volume of water in the trap remains constant for 5 min. Discontinue the heating, use a copper or nichrome wire spiral to dislodge any drops of water that may be adhering to the inside of the condenser tube or receiver, and wash down with about 5 mL of toluene. Disconnect the receiver, immerse it in water at 25° for
at least 15 min or until the toluene layer is clear, and then read the volume of water. Conduct a blank determination using the same volume of toluene as used when distilling the sample mixture, and make any necessary correction (see General Provisions).

C. OTHERS

ASH (Acid-Insoluble)
Boil the ash obtained as directed under Ash (Total), below, with 25 mL of 2.7 N hydrochloric acid for 5 min, collect the insoluble matter on a tared, porous-bottom porcelain filter crucible or ashless filter, wash it with hot water, ignite to constant weight at 675°C ± 25°C, and weigh. Calculate the percent acid-insoluble ash from the weight of the sample taken.
[NOTE: Avoid exposing the crucible to sudden temperature changes.]

ASH (Total)
Unless otherwise directed, accurately weigh about 3 g of the sample in a tared crucible, ignite it at a low temperature (about 550°C), not to exceed a very dull redness, until it is free from carbon, cool it in a desiccator, and weigh. If a carbon-free ash is not obtained, wet the charred mass with hot water, collect the insoluble residue on an ashless filter paper, and ignite the residue and filter paper until the ash is white or nearly so. Finally, add the filtrate, evaporate it to dryness, and heat the whole to a dull redness. If a carbon-free ash is still not obtained, cool the crucible, add 15 mL of ethanol, break up the ash with a glass rod, then burn off the ethanol, again heat the whole to a dull redness, cool it in a desiccator, and weigh.

HYDROCHLORIC ACID TABLE

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Specific gravity determinations were made at 60°F, compared with water at 60°F.

From the specific gravities, the corresponding degrees Baumé were calculated by the following formula:

$$\text{degrees Baumé} = 145 - \left( \frac{145}{\text{sp. gr.}} \right)$$

Baumé hydrometers for use with this table must be graduated by the above formula, which should always be printed on the scale.

**Allowance for Temperature**
- 10° to 15°Bé: 1/40 °Bé or 0.0002 sp. gr. for 1°F
- 15° to 22°Bé: 1/30 °Bé or 0.0003 sp. gr. for 1°F
- 22° to 25°Bé: 1/28 °Bé or 0.00035 sp. gr. for 1°F

**LOSS ON DRYING**
This procedure is used to determine the amount of volatile matter expelled under the conditions specified in the monograph. Because the volatile matter may include material other than adsorbed moisture, this test is designed for compounds in which the loss on drying may not definitely be attributable to water alone. For substances appearing to contain water as the only volatile constituent, the Direct (Karl Fischer) Titration Method, provided under Water, Appendix IIB, is usually appropriate.

**Procedure**  Unless otherwise directed in the monograph, conduct the determination on 1 to 2 g of the substance, previously mixed and accurately weighed. If the sample is in the form of large crystals, reduce the particle size to about 2 mm, quickly crushing the sample to avoid absorption or loss of moisture. Tare a glass-stoppered, shallow weighing bottle that has been dried for 30 min under the same conditions to be used in the determination. Transfer the sample to the bottle, replace the cover, and weigh the bottle and its contents. By gentle sideways shaking, distribute the sample as evenly as possible to a depth of about 5 mm for most substances and not over 10 mm in the case of bulky materials. Place the loaded bottle in the drying chamber, removing the stopper and leaving it also in the chamber, and dry at the temperature and for the length of time specified in the monograph. Upon opening the chamber, close the bottle promptly and allow it to come to room temperature, preferably in a desiccator, before weighing. Where drying in vacuum is specified in the monograph, use a pressure as low as that obtainable by an aspirating water pump (not higher than 20 mm Hg).

If the test substance melts at a temperature lower than that specified for the determination, preheat the bottle and its contents for 1 to 2 h at a temperature 5° to 10° below the melting range, then continue drying at the specified temperature for the determination. When drying the sample in a desiccator, ensure that the desiccant is kept fully effective by replacing it frequently.

**OIL CONTENT OF SYNTHETIC PARAFFIN**

**Apparatus**

*Filter Stick*  Use either a 10-mm diameter sintered-glass filter stick of 10- to 15-µm maximum pore diameter, or a filter stick made of stainless steel and having a 0.5-in. disk of 10- to 15-µm maximum pore diameter. Determine conformance with the pore diameter specified as follows: clean sintered-glass filter sticks by soaking in hydrochloric acid, or stainless steel sticks by soaking in nitric acid, wash with water, rinse with acetone, and dry in air followed by drying in an oven at 105° for 30 min.

Thoroughly wet the clean filter stick by soaking in water, and then connect it with an apparatus (see Fig. 9) consisting of a mercury-filled manometer, readable to 0.5 mm; a clean and filtered air supply; a drying bulb filled with silica gel; and a needle-valve type air pressure regulator. Apply pressure slowly from the air source, and immerse the filter just below the surface of water contained in a beaker.
[NOTE: If a head of liquid is noted above the surface of the filter after it is inserted into the water, the back pressure thus produced should be subtracted from the observed pressure when the pore diameter is calculated as directed below.]

Increase the air pressure to 10 mm below the acceptable pressure limit, and then increase the pressure at a slow, uniform rate of about 3 mm Hg per minute until the first bubble passes through the filter. This can be conveniently observed by placing the beaker over a mirror. Read the manometer when the first bubble passes off the underside of the filter. Calculate the pore diameter, in micrometers, by the formula:

\[ \frac{2180}{p} \]

in which \( p \) is the observed pressure, in millimeters, corrected for any back pressure as mentioned above.

**Filtration Assembly** Connect the Filter Stick with an air pressure inlet tube and delivery nozzle and ground-glass joint to fit a 25- × 170-mm test tube as shown in Fig. 10. If a stainless steel Filter Stick is used, make the connection to the test tube by means of a cork.

**Cooling Bath** Use a suitable insulated box having 1-in. holes in the center to accommodate any desired number of test tubes. The bath may be filled with a suitable medium such as kerosene and may be cooled by circulating a refrigerant through coils, or by using solid carbon dioxide, to produce a temperature of 30°C ± 2°F.

**Air Pressure Regulator** Use a suitable pressure-reduction valve, or other suitable regulator, that will supply air to the Filtration Assembly at the volume and pressure required to give an even flow of filtrate (see
Procedure. Connect the regulator with rubber tubing to the end of the Filter Stick in the Filtration Assembly.

**Thermometer** Use an ASTM Oil in Wax Thermometer having the range of −35° to +70°F and conforming to the requirements for an ASTM 71F thermometer (see Thermometers, Appendix I).

**Weighing Bottles** Use glass-stoppered conical bottles having a capacity of 15 mL. The bottles are used as evaporating flasks in the Procedure.

**Evaporation Assembly** The assembly consists of an evaporating cabinet capable of maintaining a temperature of 95° ± 2°F around the evaporation flasks, and air jets (4 ± 0.2 mm id) for delivering a stream of clean, dry air vertically downward into the flasks. In the Procedure below, support each jet so that the tip is 15 ± 5 mm above the surface of the liquid at the start of the evaporation. Supply the air (purified by passage through a tube of 1-cm bore packed loosely to a height of 20 cm with absorbent cotton) at the rate of 2 to 3 L/min per jet. The cleanliness of the air should be checked periodically to ensure that not more than 0.1 mg of residue is obtained when 4 mL of methyl ethyl ketone is evaporated as directed in the Procedure.

**Wire Stirrer** Use a 250-mm length of stiff iron or ni-chrome wire of about No. 20 B & S gauge. Form a 10-mm diameter loop at each end, and bend the loop at the bottom end so that the plane of the loop is perpendicular to the length of the wire.

**Sample Selection** If the sample weighs about 1 kg or less, obtain a representative portion by melting the entire sample and stirring thoroughly. For samples heavier than about 1 kg, exercise special care to ensure that a truly representative portion is obtained, noting that the oil may not be distributed uniformly throughout the sample and that mechanical operations may have expressed some of the oil.

**Procedure** Melt a representative portion of the sample in a beaker, using a water bath or oven maintained at 160° to 210°F. As soon as the sample is completely melted, thoroughly mix it by stirring. Preheat a dropper pipet, provided with a rubber bulb and calibrated to deliver 1 ± 0.05 g of molten sample, and withdraw a 1-g portion of the sample as soon as possible after it has melted. Hold the pipet in a vertical position, and carefully transfer its contents into a clean, dry test tube previously weighed to the nearest milligram. Evenly coat the bottom of the tube by swirling, allow the tube to cool, and weigh to the nearest milligram. Calculate the sample weight, in grams, and record it as B (see Calculation). Pipet 15 mL of methyl ethyl ketone (ASTM Specification D 740, or equivalent) into the tube, and immerse the tube up to the top of the liquid in a hot water or steam bath. Stir with an up-and-down motion with the wire stirrer, and continue heating and stirring until a homogeneous solution is obtained, exercising care to avoid loss of solvent by prolonged boiling.

[NOTE: If it appears that a clear solution will not be obtained, stir until any undissolved material is well dispersed so as to produce a slightly cloudy solution.]

After the sample solution is prepared, plunge the test tube into an 800-mL beaker of ice water, and continue to stir until the contents are cold. Remove the stirrer, then remove the test tube from the bath, dry the outside of the tube with a cloth, and weigh to the nearest 100 mg. Calculate the weight, in grams, of solvent in the test tube, and record it as C (see Calculation). Place the tube in the cooling bath, maintained at −30° ± 2°F, and stir continuously with the thermometer until the temperature reaches −25° ± 0.5°F, maintaining the slurry at a uniform consistency and taking precautions to prevent the sample from setting up on the walls of the tube or forming crystals.

Place the filter stick in a test tube and cool at −30° ± 2°F in the cooling bath for a minimum of 10 min. Immerse the cooled filter stick in the sample, then connect the filtration assembly, seating the ground-glass joint of the filter so as to make an airtight seal. Place an unstoppered weighing bottle, previously weighed together with the glass stopper to the nearest 0.1 mg, under the delivery nozzle of the filtration assembly.
[NOTE: Suitable precautions and proper analytical technique should be applied to ensure the accuracy of the weight of the bottle. Before determining its weight, the bottle and its stopper should have been cleaned and dried, then rinsed with methyl ethyl ketone, wiped dry on the outside, dried in the evaporation assembly for about 5 min, and cooled. Then allow it to stand for about 10 min near the balance before weighing.]

Apply air pressure to the filtration assembly, immediately collect about 4 mL of filtrate in the weighing bottle, and release the air pressure to permit the liquid to drain back slowly from the delivery nozzle. Stopper the bottle, and weigh it to the nearest 10 mg without waiting for it to come to room temperature. Remove the stopper, transfer the bottle to the evaporation assembly maintained at 95° ± 2°F, and place it under an air jet centered inside the neck, with the tip 15 ± 5 mm above the surface of the liquid. After the solvent has evaporated (usually less than 30 min), stopper the bottle, and allow it to stand near the balance for about 10 min before it is weighed to the nearest 0.1 mg. Repeat the evaporation procedure for 5-min periods until the loss between successive weighings is not more than 0.2 mg. Determine the weight of the oil residue, in grams, by subtracting the weight of the empty stoppered bottle from the weight of the stoppered bottle plus the oil residue after the evaporation procedure, and record the results as A (see Calculation). Determine the weight of solvent evaporated, in grams, by subtracting the weight of the bottle plus oil residue from the weight of the bottle plus filtrate, and record the result as D (see Calculation).

Calculation  Calculate the percent, by weight, of oil in the sample by the formula:

\[
(100 \frac{AC}{BD}) - 0.15
\]

in which 0.15 is a factor to correct for solubility of the sample in the solvent at −25°F.

RESIDUE ON IGNITION (Sulfated Ash)

Method I (for Solids)
Transfer the quantity of the sample directed in the individual monograph onto a tared 50- to 100-mL platinum dish or other suitable container, and add sufficient 2 N sulfuric acid to moisten the entire sample. Heat gently, using a hot plate, an Argand burner, or an infrared heat lamp, until the sample is dry and thoroughly charred, then continue heating until all of the sample has been volatilized or nearly all of the carbon has been oxidized, and cool. Moisten the residue with 0.1 mL of sulfuric acid, and heat in the same manner until the remainder of the sample and any excess sulfuric acid have been volatilized. To promote volatilization of sulfuric acid, add a few pieces of ammonium carbonate just before completing ignition. Finally, ignite to constant weight in a muffle furnace at 800° ± 25°F for 15 min, or longer if necessary to complete ignition, cool in a desiccator, and weigh.

Method II (for Liquids)
Unless otherwise directed, transfer the required weight of the sample onto a tared 75- to 100-mL platinum dish. Heat gently, using an Argand or Meker burner, until the sample ignites, then allow the sample to burn until it self-extinguishes. Cool, then wet the residue with 2 mL of concentrated sulfuric acid, and heat the sample over a low flame until dry. Ignite to constant weight in a muffle furnace at 800° ± 25°F for 30 min, or longer if necessary for complete ignition, cool in a desiccator, and weigh.
SIEVE ANALYSIS OF GRANULAR METAL POWDERS (Based on ASTM Designation: B 214)³

Apparatus

Sieves Use a set of standard sieves, ranging from 80-mesh to 325-mesh, conforming to the specifications in ASTM Designation: E 11 (Sieves for Testing Purposes).

Sieve Shaker Use a mechanically operated sieve shaker that imparts to the set of sieves a horizontal rotary motion of between 270 and 300 rotations/min and a tapping action of between 140 and 160 taps/min. The sieve shaker is fitted with a plug to receive the impact of the tapping device. The entire apparatus is rigidly mounted —bolted to a solid foundation, preferably of concrete. Preferably a time switch is provided to ensure the accuracy of test duration.

Procedure Assemble the sieves in consecutive order by opening size, with the coarsest sieve (80-mesh) at the top, and place a solid-collecting pan below the bottom sieve (325-mesh). Place 100.0 g of the test sample, W, on the top sieve, and close the sieve with a solid cover. Securely fasten the assembly to the sieve shaker, and operate the shaker for 15 min. Remove the most coarse sieve from the nest, gently tap its contents to one side, and pour the contents onto a tared, glazed paper. Using a soft brush, transfer onto the next finer sieve any material adhering to the bottom of the sieve and frame. Place the sieve just removed upside down on the paper containing the retained portion, and tap the sieve. Accurately weigh the paper and its contents, and record the net weight of the fraction, F, obtained. Repeat this process for each sieve in the nest and for the portion of the sample that has been collected in the bottom pan. Record the total of the fractions retained on the sieves as T and that portion collected in the pan as t. The combined total, S, of T + t is the amount of the sample, W, recovered in the test. Calculate the percent recovery by the formula:

\[
\frac{S}{W} \times 100
\]

If the percent recovery is less than 99.0%, check the condition of the sieves and for possible errors in weighing, and repeat the test. If the percent recovery is not less than 99.0%, calculate the percent retained on each sieve by the formula:

\[
\frac{F}{W} \times 100
\]

Calculate the percent through the smallest mesh sieve from the portion collected in the pan by the formula:

\[
\frac{(100 - t)}{W} \times 100
\]

SULFURIC ACID TABLE

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Specific gravity determinations were made at 60°F, compared with water at 60°F. The values given above for aqueous sulfuric acid solutions were adopted as standard in 1904 by the Manufacturing Chemists' Association of
the United States.

From the specific gravities, the corresponding degrees Baumé were calculated by the following equation:

\[ ^\circ \text{Baumé} = 145 - (145/\text{sp. gr.}) \]

Baumé hydrometers for use with this table must be graduated by the above formula, which should always be printed on the scale. Acids stronger than 66\(^\circ\) Bé should have their percentage compositions determined by chemical analysis.

---

1 Oils of known viscosities may be obtained from the Cannon Instrument Co., P.O. Box 812, State College, PA 16801. For determining the viscosity of dimethylpolysiloxane, choose an oil with a viscosity as close as possible to that of the type of sample to be tested.

2 Oils of known viscosities may be obtained from the Cannon Instrument Co., P.O. Box 812, State College, PA 16801. For determining the viscosity of methylcellulose, choose an oil that has a viscosity as close as possible to that of the type of sample to be tested.

3 Adapted from ASTM B214 Standard Test Method for Sieve Analysis of Metal Powders. 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 III: Chemical Tests and Determinations, FCC 6 page 1071. On the basis of comments received, the following changes are proposed:

1. It is proposed to revise the APDC Extraction Method under Lead Limit Test, Appendix IIIB, to enable testing to limits other than 2 mg/kg. This revision includes additional Standard Lead Solution preparations at concentrations to accommodate limits of 3, 4, and 10 mg/kg, which are currently used in the following FCC 6 monographs: Aluminum Ammonium Sulfate; Aluminum Potassium Sulfate; Aluminum Sodium Sulfate; Aluminum Sulfate; Ammonium Bicarbonate; Ammonium Carbonate; Ammonium Phosphate, Dibasic; Ammonium Phosphate, Monobasic; Ammonium Sulfate; Calcium Carbonate; Calcium Glycerophosphate; Copper Sulfate; Magnesium Sulfate; Manganese Sulfate; Phosphoric Acid; Sodium Carbonate; Sodium Hypophosphite; Sodium Metaphosphate, Insoluble; Sodium Phosphate, Dibasic; Sodium Phosphate, Monobasic; Sodium Phosphate, Tribasic; Sodium Polyphosphates, Glassy; Sodium Pyrophosphate; Sodium Trimetaphosphate; Stannous Chloride; Zinc Oxide; Zinc Sulfate. Interested parties are encouraged to submit comments to Jeff Moore, Ph.D.

2. It is proposed to revise the Dithizone Method under Lead Limit Test, Appendix IIIB, to remove the current statement, which instructs the user to not use the Dithizone Extraction Solution if it is more than 1-month old. This revision would make the reagent preparation consistent with that found in the Lead 251 general chapter in USP. Interested parties are encouraged to submit comments to Jeff Moore, Ph.D.

3. It is proposed to revise Method IV under Fluoride Limit Test, Appendix IIIB, to add a second buffer, Buffer Solution B, and a statement which explains proper usage of each of the two buffer solutions. It is further proposed to add a second slope range to the section of this test entitled Electrode Calibration, and to indicate that the existing range is appropriate for Buffer Solution B, and that the new range is appropriate for Buffer Solution A. Interested parties are encouraged to submit comments to Kristie Bowman.

(FIEC: J. Moore; K. Bowman) C65115; C65194; C65112

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.

**Sulfate**
Solutions of sulfates yield with barium chloride TS (10%) a white precipitate that is insoluble in hydrochloric and nitric acids. Sulfates yield with lead acetate TS (8%) 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

**ARSENIC LIMIT TEST**
Silver Diethylthiocarbamate 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°C 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 to 1000.0 mL with recently boiled water. Transfer 10.0 mL of this solution into a 1000-mL volumetric flask, add 10 mL of 2 N sulfuric acid, dilute to volume with recently boiled water, 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 (SnCl₂·2H₂O) 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°C 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°C ± 3°C) 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°C 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 (CdCl₂·2½H₂O), accurately weighed, into a 1000-mL volumetric flask, dissolve in and dilute to volume with water, and mix. Pipet 25 mL of this solution into a 100-mL volumetric flask, add 1 mL of hydrochloric acid, dilute to volume with water, 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 to volume with water, 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 to volume with water, 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 to volume with water, 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, filter 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 (SO₄) 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 to volume with water, 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 to 50 mL with water, 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 to volume with water, 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 to 50 mL with water, 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.

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 to 2.0 mL with water, 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 to 1000 mL with water, 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 to 100 mL with the Buffer Solution. 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 by the formula:

\[ \frac{IA}{(R-I)} \times 100 \times \left(\frac{200}{25W}\right) \]

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.

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 to volume with water, 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 to volume with water, 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 to volume with water, 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), 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 to volume with water; 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*.

*Change to read:*

**Method IV (Ion-Selective Electrode Method C)**

- **Buffer Solution A** 1S (FCC 6)  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 to 1000 mL with water.

**Fluoride Standard Solutions**

- **1000 mg/kg Fluoride Standard**  Transfer 2.2108 g of sodium fluoride, previously dried at 200 °C for 4 h, into a 1000-mL volumetric flask and dissolve in and dilute to volume with water. 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 to volume with water.

- **10 mg/kg Fluoride Standard**  Pipet 100 mL of the **50 mg/kg Fluoride Standard** into a 500-mL volumetric flask. Dilute to volume with water.

**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 to volume with water.

- **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 to volume with water.

**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 to volume with water.

- **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 to volume with water.

[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 \( {\text{1S (FCC 6)}} \) Buffer Solution, dilute to volume with water, and mix.

Electrode Calibration  Pipet 50 mL of the appropriate \( {\text{1S (FCC 6)}} \) 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°C for Buffer Solution A and in the range of 54 to 60 mV at 25°C for Buffer Solution B. If the difference in potential is not within this range, check, and, if necessary, replace the electrode, instrument, or solutions.

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 \( {\text{1S (FCC 6)}} \) 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 criterion 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, filter 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..."
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 to 100 mL with water.

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 to 1000.0 mL with water, 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 to 100.0 mL with water. 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. Do not use if more than 1 month old.

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 to 100 mL with water, 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...
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(NO₃)₂] in 100 mL of water containing 1 mL of nitric acid in a 1000-mL volumetric flask, and dilute to volume with water.

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 to volume with water.

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 to volume with water.

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 to volume with water. 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 to volume with water.

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 525º, 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 by the formula:

\[ \frac{10C}{W_S} \]

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 electrodeless 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 (m₀) (mass of Pb pg necessary to produce an integrated absorbance of 0.0044 abs-sec) as follows:

\[ m₀ = \frac{(0.0044 \text{ abs-sec})(25 \text{ pg/µL})(20 \text{ µL})}{(\text{measured 25 pg/µL abs-sec})} \]

Record and track the integrated absorbance and m₀ 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:

\[ DL = (3)(\text{s.d. blank abs-sec})(10 \text{ pg/µL})(20 \text{ µL})/(\text{abs-sec 10 ng/mL std}) \]

\[ QL = (10)(\text{s.d. blank abs-sec})(10 \text{ pg/µL})(20 \text{ µL})/(\text{abs-sec 10 ng/mL std}) \]

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:
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 to 1000.0 mL with Hydrogen Peroxide–Nitric Acid Solution, 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 to 100.0 mL with Hydrogen Peroxide–Nitric Acid Solution, 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 to volume with butanol, 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 to volume with Butanol–Nitric Acid Solution, 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 to volume with Butanol–Nitric Acid Solution, and mix. Use this solution for analysis.

**Procedure**

**Tungsten Solution**  Transfer 0.1 g of tungstic acid ($\text{H}_2\text{WO}_4$) 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:

$$\frac{10C}{W}$$

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(NO₃)₂] in 100 mL of water containing 1 mL of nitric acid in a 1000-mL volumetric flask, and dilute to volume with water.

Standard Lead Solution (2 µg/mL)  On the day of use, transfer 2.0 mL of Lead Nitrate Stock Solution into a 100-mL volumetric flask, and dilute to volume with water.

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 to volume with water. 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 to volume with water. 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 to volume with water. 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 to volume with water. The resulting solution contains 10 µg of lead per milliliter.

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 to about 100 mL with deionized water. 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 to about 200 mL with water. 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 to volume with water.

Procedure  Concomitantly determine the absorbances of the appropriate 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 to volume with water, and mix. Pipet 5.0 mL of this solution into a 50-mL volumetric flask, dilute to volume with water, 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 to volume with water, 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 to volume with 0.5 \( N \) hydrochloric acid, 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.](image)

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 \([\text{Hg(NO}_3\text{)}\cdot\text{H}_2\text{O}]\) into a 1000-mL volumetric flask, dissolve in a mixture of 100 mL of water and 2 mL of nitric acid, dilute to volume with water, 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 to volume with water, 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 to volume with water, 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 to the 60-mL mark with water. Add 2 mL of 10% stannous chloride solution (prepared fresh each week by dissolving 10 g of SnCl\(_2\)-2H\(_2\)O 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 to 100 mL with water, and mix.

Mercury Stock Solution  Transfer 135.4 mg of mercuric chloride, accurately weighed, into a 100-mL volumetric flask, dissolve in and dilute to volume with 1 N sulfuric acid, 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 to volume with 1 N sulfuric acid, 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 filter 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**

**Method I**

[NOTE:] Unless otherwise specified in the individual monograph, use 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 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 by the formula:

\[ \frac{100C}{W} \]

in which \( W \) is the weight, in g, of test specimen taken to prepare the Test Solution.

PHOSPHORUS LIMIT TEST

Reagents

Ammonium Molybdate Solution (5%)  Dissolve 50 g of ammonium molybdate tetrahydrate,
(NH₄)₆Mo₇O₂₄·4H₂O, in 900 mL of warm water, cool to room temperature, dilute to 1000 mL with water, and mix.

Ammonium Vanadate Solution (0.25%) Dissolve 2.5 g of ammonium metavanadate, NH₄VO₃, in 600 mL of boiling water, cool to 60° to 70°, and add 20 mL of nitric acid. Cool to room temperature, dilute to 1000 mL with water, and mix.

Zinc Acetate Solution (10%) Dissolve 120 g of zinc acetate dihydrate, Zn(C₂H₃O₂)₂·2H₂O, in 880 mL of water, and filter through Whatman No. 2V or equivalent filter paper before use.

Nitric Acid Solution (29%) Add 300 mL of nitric acid (sp. gr. 1.42) to 600 mL of water, and mix.

Standard Phosphorus Solution (100 µg P in 1 mL) Dissolve 438.7 mg of monobasic potassium phosphate, KH₂PO₄, in water in a 1000-mL volumetric flask, dilute to volume with water, and mix.

Standard Curve Pipet 5.0, 10.0, and 15.0 mL of the Standard Phosphorus Solution into separate 100-mL volumetric flasks. To each of these flasks, and to a fourth, blank flask, add in the order stated 10 mL of Nitric Acid Solution, 10 mL of Ammonium Vanadate Solution, and 10 mL of Ammonium Molybdate Solution, mixing thoroughly after each addition. Dilute to volume with water, mix, and allow to stand for 10 min. Determine the absorbance of each standard solution in a 1-cm cell at 460 nm, with a suitable spectrophotometer, using the blank to set the instrument 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 to volume with water, and mix. Transfer an accurately measured aliquot (V, in mL) of this solution, containing not more than 1.5 mg of phosphorus, into a 100-mL volumetric flask, and add 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
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:

$$\text{mg/kg P} = \left(\frac{a \times 200 \times 1000}{V \times W}\right)$$

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 ($\text{C}_{10}\text{H}_{10}\text{N}_2$) and 500 mg of hydroxylamine hydrochloride ($\text{NH}_2\text{OH}\cdot\text{HCl}$) in sufficient 0.1 $\text{N}$ 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 to volume with water, and mix.

Selenium Standard Solution  Pipet 5.0 mL of Selenium Stock Solution into a 200-mL volumetric flask, dilute to volume with water, 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 $\text{N}$ nitric acid, and mix.

Sample Preparation  Using a 1000-mL combustion flask and 25 mL of 0.5 $\text{N}$ 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 $\text{N}$ 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 (BaCl₂·2H₂O). 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 (CO₂). 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 to volume with water, 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 (NH₃-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:

\[
(NH_3-N/S) \times 100
\]

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.

![Typical Chromatogram for the Determination of Benzene in Hexanes Using Column No. 5.](image)

**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.

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.

Calculate a response factor for benzene \(R_b\) relative to the Internal Standard by the formula:

\[
\frac{A_i}{W_i} \times \frac{B_v}{A_b}
\]

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 to volume with the sample to be analyzed.

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:

\[
\frac{(A_b \times R_b \times W_i \times 100)}{(A_i \times S)}
\]

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**
<table>
<thead>
<tr>
<th>Column No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid phase</td>
<td>CEF</td>
<td>PEF 200</td>
<td>CEF</td>
<td>DEGS</td>
<td>TCEPE</td>
<td>TCEPE</td>
<td>DEGS</td>
</tr>
<tr>
<td>Length, ft</td>
<td>15</td>
<td>6</td>
<td>16</td>
<td>10</td>
<td>15</td>
<td>100</td>
<td>12</td>
</tr>
<tr>
<td>m</td>
<td>4.5</td>
<td>2</td>
<td>—</td>
<td>5</td>
<td>3.1</td>
<td>—</td>
<td>313.7</td>
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<tr>
<td>Diameter, in (mm) Inside</td>
<td>0.07(1.8)</td>
<td>—</td>
<td>0.07</td>
<td>0.18(4.5)</td>
<td>0.06(1.5)</td>
<td>—</td>
<td>0.01(2.54)</td>
</tr>
<tr>
<td>Outside</td>
<td>1/8(3.2)</td>
<td>1/4(6.4)</td>
<td>1/8</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1/8</td>
</tr>
<tr>
<td>Weight, percent</td>
<td>17</td>
<td>30</td>
<td>20</td>
<td>20</td>
<td>10</td>
<td>—</td>
<td>20</td>
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<tr>
<td>Solid support</td>
<td>Chromosorb P</td>
<td>Chromosorb P</td>
<td>Chromosorb P</td>
<td>Chromosorb P</td>
<td>Capillary</td>
<td>Chromosorb P</td>
<td></td>
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<tr>
<td>Mesh</td>
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<td>60–80</td>
<td>80–100</td>
<td>60–80</td>
<td>—</td>
<td>80–100</td>
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<td>Treatment</td>
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<td>none</td>
<td>AW</td>
<td>none</td>
<td>AW Sil</td>
</tr>
<tr>
<td>Inlet, deg</td>
<td>200</td>
<td>210</td>
<td>250</td>
<td>260</td>
<td>250</td>
<td>275</td>
<td>260</td>
</tr>
<tr>
<td>Detector, deg</td>
<td>200</td>
<td>155</td>
<td>250</td>
<td>200</td>
<td>175</td>
<td>250</td>
<td>240</td>
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<tr>
<td>Column, deg</td>
<td>115</td>
<td>95</td>
<td>90</td>
<td>100</td>
<td>115</td>
<td>95</td>
<td>65</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>N₂</td>
<td>He</td>
<td>He</td>
<td>He</td>
<td>N₂</td>
<td>N₂</td>
<td>He</td>
</tr>
<tr>
<td>Flow rate, cm³/min</td>
<td>30</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>1</td>
<td>3</td>
<td>52</td>
</tr>
<tr>
<td>Detector</td>
<td>FI</td>
<td>TC</td>
<td>FI</td>
<td>FI</td>
<td>FI</td>
<td>FI</td>
<td>FI</td>
</tr>
<tr>
<td>Recorder, mV</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sample, 1</td>
<td>5</td>
<td>10</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>0.8</td>
<td>5</td>
</tr>
<tr>
<td>Split</td>
<td>9 + 1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>100 + 1</td>
<td>100 – 1</td>
<td>—</td>
</tr>
<tr>
<td>Area</td>
<td>Tri</td>
<td>El</td>
<td>Di</td>
<td>Tri Plan</td>
<td>El</td>
<td>El</td>
<td>Tri</td>
</tr>
</tbody>
</table>

**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 Pentaerythritol; Tri—Triangulation.

**Retention Times in Minutes for Selected Hydrocarbons Under the Conditions for the Determination of Benzene in Hexanes**

<table>
<thead>
<tr>
<th>Column No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>3.4</td>
<td>2.0</td>
<td>6.5</td>
<td>6.7</td>
<td>5.4</td>
<td>6.1</td>
<td>6.7</td>
</tr>
<tr>
<td>Toluene</td>
<td>4.4</td>
<td>3.2</td>
<td>9.0</td>
<td>10.3</td>
<td>7.8</td>
<td>7.0</td>
<td>10.3</td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>5.4</td>
<td>5.2</td>
<td>11.5</td>
<td>14.8</td>
<td>10.8</td>
<td>8.0</td>
<td>14.8</td>
</tr>
<tr>
<td>p-m-Xylenes</td>
<td>5.8</td>
<td>—</td>
<td>12.5</td>
<td>—</td>
<td>11.4</td>
<td>8.5</td>
<td>—</td>
</tr>
<tr>
<td>o-Xylene</td>
<td>7.5</td>
<td>6.8</td>
<td>17.0</td>
<td>16.1</td>
<td>14.5</td>
<td>10.0</td>
<td>—</td>
</tr>
<tr>
<td>n-Undecane</td>
<td>3.0</td>
<td>2.8</td>
<td>3.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>n-Dodecane</td>
<td>—</td>
<td>—</td>
<td>12.8</td>
<td>8.5</td>
<td>6.5</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
COLORS

Chromium

Standards

*Standard Chromium Solution* (1000 mg/kg) Transfer 2.829 g of K$_2$Cr$_2$O$_7$, accurately weighed (National Institute of Standards and Technology No. 136) into a 1-L volumetric flask; dissolve in and dilute to volume with water.

*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 to volume with water.

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**: 1 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 to volume with water.

Transfer 5 g of the colorant to be analyzed to a 100-mL volumetric flask; dissolve in and dilute to volume with water. 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.
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 to approximately 200 mL with water. 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°C 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  
CuCl<sub>2</sub>·2H<sub>2</sub>O  Transfer 10.0 g of CuCl<sub>2</sub>·2H<sub>2</sub>O to a 1-L volumetric flask; dissolve in and dilute to volume with dimethylformamide (DMF).

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 nearly to volume with water; 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.

<table>
<thead>
<tr>
<th>Curve No.</th>
<th>Solution in Sample Cell</th>
<th>Solution in Reference Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>III</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>IVa</td>
<td>3</td>
<td>4a</td>
</tr>
<tr>
<td>IVb</td>
<td>3</td>
<td>4b</td>
</tr>
</tbody>
</table>

Calculation

\[
\% \text{ Leuco Base} = \left( \frac{(IV - III) - (II - I)}{IV - III} \right) \times 2500 \times \frac{a \times W \times r}{a \times W \times r}
\]

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 Brilliant 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 Brilliant 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 (Lindberg 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.
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 to volume with water. 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., Brilliant 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 to volume with water 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:

\[
P = \left( \frac{V \times N}{W} \right) \times 22.79
\]

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:

\[
P = \left( \frac{(V - B) \times N}{W} \right) \times 55.4
\]

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)
Pipe 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:

\[
\text{% total color} = \left( \frac{A \times 100}{a \times W} \right)
\]

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 (TiCl₃) 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 CO₂ or N₂ to maintain an inert atmosphere in which the reaction takes place; a stirrer; and the buret, \( C \).

![FIGURE 23 Titanous Chloride Titration Apparatus.](image)
Reagents and Solutions

Titanium Chloride Solution (0.1 N) Transfer 73 mL of commercially prepared 20% TiCl₃ solution into a storage bottle, and carefully add 82 mL of concentrated HCl per L of final solution. Mix well, and bubble CO₂ or N₂ 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 K₂Cr₂O₇ (National Institute of Standards and Technology No. 136) to a 1-L volumetric flask; dissolve in and dilute to volume with water.

Ammonium Thiocyanate (50%) Transfer 500 g of NH₄SCN, 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(NH₄)₂(SO₄)₂·6H₂O, ACS certified.

Standardization of the Titanium Chloride Solution Drain any standing titanium chloride (TiCl₃) 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:

\[ N = \frac{V_r \times N_r}{V_t - V_b} \]

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:

\[ T = \left[ \frac{(V_t - V_b)(W \times F_s)}{W \times F_s} \right] \times 100 \times N \]

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°C. Cool in a desiccator, and weigh. Calculate the total color, \( P \), in weight percent, by the equation:

\[
P = \left( \frac{W_p \times F}{W_s} \right) \times 100
\]

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 to volume with water.

**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\(_4\)OH 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:

\[
C = \frac{A}{(a \times b)}
\]

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 II/A) 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 \( \text{Na}_2\text{B}_4\text{O}_7 \).

**Secondary Eluant:** 0.20 \( M \) \( \text{NaClO}_4 \) in aqueous 0.01 \( M \) \( \text{Na}_2\text{B}_4\text{O}_7 \).

**Sample Size:** 20 \( \mu \text{L} \) of a 0.25\% solution.

**Flow Rate:** 0.60 \( \text{mL/min} \).

**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\° C.

**Pressure:** 1000 psi.

**Order of Elution:** (1) Cresidinesulfonic acid (CSA); (2) unknown; (3) Schaeffer's salt (SS); (4) unknown; (5) 4,4'-diazaoaminobis(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 \( \text{Na}_2\text{B}_4\text{O}_7 \).

**Secondary Eluant:** 0.10 \( M \) \( \text{NaClO}_4 \) in aqueous 0.01 \( M \) \( \text{Na}_2\text{B}_4\text{O}_7 \).

**Sample Size:** 50 \( \mu \text{L} \) of a 0.15\% solution, prepared within 13 min of injection.

**Flow Rate:** 1.00 \( \text{mL/min} \).

**Gradient:** Exponential at 4\%/min: 0.95\%.

**Temperature:** 50\° C.

**Pressure:** 1000 psi.

**Order of Elution:** (1) Phenylhydrazine-p-sulfonic acid (PHSA); (2) sulfanilic acid (SA); (3) 1-(4-sulfo phenyl)-3-ethylcarboxy-5-hydroxy pyrazolone (PY-T); (4) 1-(4-sulfo phenyl)-3-carboxy-5-hydroxy pyrazolone (EEPT); (5) 4,4'-diazaoamino-dibenzenesulfonic acid (DAADBSA).

Sunset Yellow

**Primary Eluant:** 0.01 \( M \) aqueous \( \text{Na}_2\text{B}_4\text{O}_7 \).

**Secondary Eluant:** 0.20 \( M \) \( \text{NaClO}_4 \) in aqueous 0.01 \( M \) \( \text{Na}_2\text{B}_4\text{O}_7 \).

**Sample Size:** 5 \( \mu \text{L} \) of a 1\% solution.

**Flow Rate:** 0.50 \( \text{mL/min} \).

**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\° C.

**Pressure:** 1000 psi.

**Order of Elution:** (1) Sulfanilic acid (SA); (2) Schaeffer's salt (SS); (3) 4,4'-diazaoamino-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 to volume with 0.1 \( M \) \( \text{Na}_2\text{B}_4\text{O}_7 \).

**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 to volume with 0.1 \( M \) \( \text{Na}_2\text{B}_4\text{O}_7 \).

**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 to volume with 0.1 M Na₂B₄O₇.

**Test Solutions**  Prepare at least four test solutions, each containing the colorant, and one impurity, accurately weighed, dissolved in 0.1 M Na₂B₄O₇, 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.](image)
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:

\[ m = \frac{\sum N_i C_i A_i - \sum C_i \sum A_i}{\sum N_i A_i^2 - (\sum A_i)^2} \]

\[ b = \bar{A}_i - m \bar{C}_i \]

in which \( \bar{C} \) and \( \bar{A} \) 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:

\[ r = \frac{\sum (C_i - \bar{C})(A_i - \bar{A})}{\sqrt{\sum (C_i - \bar{C})^2 \times \sum (A_i - \bar{A})^2}} \]

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, \( l \), by the equation:

\[ l = \frac{W_c}{W_s} \times 100 \]

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 to volume with water, 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:

\[ A_U \times \frac{C_S}{A_S} \]

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:

\[ \frac{100 \times C_A}{(6.25 \times N_T)} \]

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:

\[ 100 \times \frac{C_A}{S_W} \]

in which S_W is the weight, in milligrams, of the sample taken.

HYDROXYPROPOXOXYL 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$^\circ$C 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$^\circ$C 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$^\circ$C 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 \( k = \frac{V_b}{N_1Y_bN_2} \).

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.](image)

**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.](image)

**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 to about 125 mL with water. 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 (–OCH₃).

**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°C. Add 25 mL of sulfide or thiosulfate solution (40 g of K₂S, 40 g of Na₂S, or 80 g of Na₂S₂O₃·5H₂O 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 below 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 Na₂S₂O₃·5H₂O, 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 Nitrites 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

<table>
<thead>
<tr>
<th>Designation</th>
<th>Specimen Preparation Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Intimately in contact with an internal reflection element for attenuated total reflectance (ATR) analysis</td>
</tr>
<tr>
<td>E</td>
<td>Pressed as a thin sample against a suitable plate for IR microscopic analysis</td>
</tr>
<tr>
<td>F</td>
<td>Suspended neat between suitable (for example sodium chloride or potassium bromide) plates</td>
</tr>
<tr>
<td>K</td>
<td>Mixed intimately with potassium bromide and compressed into a translucent pellet</td>
</tr>
<tr>
<td>M</td>
<td>Finely ground and dispersed in mineral oil</td>
</tr>
<tr>
<td>S</td>
<td>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)</td>
</tr>
</tbody>
</table>

[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)

[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.](figure)

**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 pyrolysis 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
700 (maximum)

Inlet zone
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:

$$S = \frac{W_b}{W_s} \times 2.192 \times 10^5$$

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 to volume with isooctane. 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 S2, 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 S1 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.](image)

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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°C isothermal, with 20 nL/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.