

### 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■<sup>1S</sup>(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, ■<sup>1S</sup>(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.

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**BRIEFING**

**Acetoin Monomer**, FCC 7 page 13. Comments received indicate that the commercial monomer of acetoin usually contains some variable amount of its dimer, and thus specific gravity and refractive index determination for the monomer can be problematic and not representative. It is therefore proposed to eliminate the acceptance criteria for specific gravity and refractive index determination in the *Specific Tests* for this monograph.

(FIEC: C. Mejia) C89494

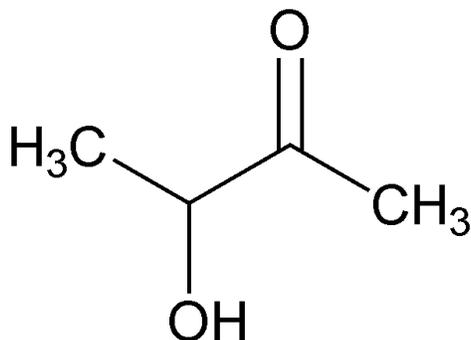
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**Acetoin Monomer**

Acetyl Methyl Carbinol

Dimethylketol

3-Hydroxy-2-butanone



C<sub>4</sub>H<sub>8</sub>O<sub>2</sub>

Formula wt 88.11  
FEMA: 2008

**DESCRIPTION****Change to read:**

Acetoin Monomer occurs as a colorless to pale yellow liquid. ■ It can contain some variable amount of its dimer.

■ 2S (FCC7)

**Odor:** Buttery

**Solubility:** Miscible in alcohol, propylene glycol, water; insoluble or practically insoluble in vegetable oils

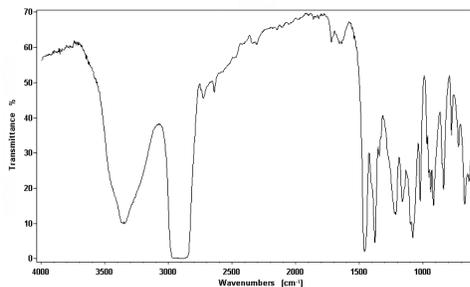
**Boiling Point:** ~ 148°

**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.



Acetoin Monomer

**ASSAY**

- **PROCEDURE:** Proceed as directed under *M-1b*, Appendix XI.  
**Acceptance criteria:** NLT 96.0% of C<sub>4</sub>H<sub>8</sub>O<sub>2</sub>

**SPECIFIC TESTS**

*Delete the following:*

- ~~■ **REFRACTIVE INDEX**, Appendix II: At 20°  
**Acceptance criteria:** Between 1.417 and 1.422 ■ 2S (FCC7)~~

*Delete the following:*

- ~~■ **SPECIFIC GRAVITY**: Determine at 25° by any reliable method (see *General Provisions*).  
**Acceptance criteria:** Between 0.995 and 1.019 ■ 2S (FCC7)~~

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

Topic/Question	Contact	Expert Committee
Monograph	Carla D Mejia, Ph.D. Scientific Liaison 1-301-816-8571	(F12010) Monographs - Food Ingredients

## BRIEFING

**Acid Hydrolysates of Proteins**, FCC 7 page 22. On the basis of comments received, it is proposed to revise the acceptance criteria in the test for *Glutamic Acid* to clarify that the basis “of the total amino acids” should be calculated on the basis of total protein as described in the test procedure under Appendix III C.

(FIEC: J. Moore) C86205

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### Acid Hydrolysates of Proteins

Acid-Hydrolyzed Proteins

Hydrolyzed Vegetable Protein (HVP)

Hydrolyzed Plant Protein (HPP)

Hydrolyzed (Source) Protein Extract

Acid-Hydrolyzed Milk Protein

### DESCRIPTION

Acid Hydrolysates of Proteins occur as liquids, pastes, powders, or granules. They are composed primarily of amino acids, small peptides (peptide chains of five or fewer amino acids), and salts resulting from the essentially complete hydrolysis of peptide bonds in edible proteinaceous materials, catalyzed by food-grade acids and/or heat. Cleavage of peptide bonds typically ranges from a low of 85% to essentially 100%. In processing, the protein hydrolysates may be treated with safe and suitable alkaline materials. The edible proteinaceous materials used as raw materials are derived from corn, soy, wheat, yeast, peanuts, rice, or other safe and suitable vegetable or plant sources, or from milk.

**Function:** Flavoring agent; flavor enhancer

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

[Note—Perform all tests on the dried basis. Evaporate liquid and paste samples to dryness in a suitable tared container; then, as for the powdered and granular forms, dry to constant weight at 105° (see *General Provisions*.).]

### ASSAY

- **TOTAL NITROGEN**, *Nitrogen Determination*, Appendix III C  
**Acceptance criteria:** NLT 4.0%

### IMPURITIES

#### Inorganic Impurities

- **LEAD**, *Lead Limit Test*, Appendix III B  
**Sample solution:** Prepare as directed for organic compounds.  
**Control:** 3 µg Pb (3 mL of *Diluted Standard Lead Solution*)  
**Acceptance criteria:** NMT 3 mg/kg, on the dried basis

#### Organic Impurities

- **3-CHLOROPROPANE-1,2-DIOL (3-MCPD)**  
**Standard stock solution:** 125 µg/mL of reagent-grade 3-chloropropane-1,2-diol (3-MCPD) in ethyl acetate  
**Diluted standard solution:** 6.25 µg/mL of 3-MCPD in ethyl acetate from the *Standard stock solution*

**Internal standard solution:** 10 µg/mL of 1-chlorotetradecane in ethyl acetate

**Standard solution A:** 2 mL of *Diluted standard solution* and 2.5 mL of *Internal standard solution* diluted to 25 mL with ethyl acetate (contains 0.5 µg/mL 3-MCPD)

**Standard solution B:** 8 mL of *Diluted standard solution* and 2.5 mL of *Internal standard solution* diluted to 25 mL with ethyl acetate (contains 2.0 µg/mL 3-MCPD)

**Standard solution C:** 16 mL of *Diluted standard solution* and 2.5 mL of *Internal standard solution* diluted to 25 mL with ethyl acetate (contains 4.0 µg/mL 3-MCPD)

**Sample stock solution:** Dissolve the sample, as needed with 20% aqueous sodium chloride, to obtain a solution with a solids content of 36%.

**Sample preparation:** Transfer a 20-g aliquot of the *Sample stock solution* into a 20-mL Extrelut NT column (EM Science, Gibbstown, NJ), or equivalent, and allow it to equilibrate for 15 min. Elute the column with 150 mL of ethyl acetate, collecting the eluent in a 250-mL short-neck, round-bottom flask with a 24/40 joint. Using a rotary evaporator at 50°, concentrate the eluent to a volume of approximately 3 mL. Add 0.5 mL of *Internal standard solution* to the eluent, transfer this mixture to a 4-dram screw-cap vial, and dilute to a volume of 5.0 mL.

**Chromatographic system, Appendix IIA**

**Mode:** Gas chromatography

**Detector:** Electrolytic conductivity detector. [Note—Operate the detector in the halogen mode.]

**Column:** 30-m × 0.53-mm (id), fused-silica column, or equivalent, coated with 1-µm Supelcowax 10 or an equivalent bonded carbowax column fitted with a 50-cm retention gap of 0.53-mm, deactivated, fused silica, or equivalent

**Temperature**

**Column:** Hold at 170° for 5 min, then increase at 5°/min to 250°, hold at 250° for 10 min

**Injector:** 225°

**Detector reactor:** 900°

**Detector base:** 275°

**Carrier gas:** Helium

**Reactant gas:** Hydrogen

**Solvent:** 1-Propanol

**Flow rate**

**Helium:** 8 mL/min

**Hydrogen:** 30 mL/min

**1-Propanol:** 0.5 mL/min through the cell or at the manufacturer's specified flow rate for the optimum operation of the detector

**Injection volume:** 1.0 µL

**Injection type:** Use a capillary injector operated in the splitless mode or a purged, packed injector with a glass insert.

[Note—Minimize contamination of the reaction tube by venting flow from the column at all times, except for the time during which compounds of interest elute.]

**Analysis:** Separately inject *Standard solution A*, *Standard solution B*, *Standard solution C*, and the *Sample preparation* into the chromatograph and record the resulting chromatograms. Calculate the response area ratios of 3-MCPD to the *Internal standard solution* for each *Standard solution*. Plot the response area ratios versus the µg of 3-MCPD in each *Standard solution* to obtain the standard curve. From the chromatogram of the *Sample preparation*, measure the response area ratio of 3-MCPD to the *Internal standard solution* and, using the standard curve, determine the amount of 3-MCPD, in µg, in the 20-g aliquot of *Sample stock solution* taken.

**Acceptance criteria:** NMT 1 mg/kg, on the dried basis

• **1,3-DICHLORO-2-PROPANOL (DCP)**

**Diluent:** Pentane and diethyl ether (85:15) (v/v)

**Stock solution:** 1 mg/mL of reagent-grade 1,3-dichloro-2-propanol (DCP) in *Diluent*

**Diluted standard solution:** 1 µg/mL of DCP in *Diluent* from the *Stock solution*

**Internal standard solution:** 1 µg/mL of trichlorobenzene in *Diluent*

**Standard solutions:** Pipet 1, 2, 3, and 4 mL portions of *Diluted standard solution*, into separate 50 mL volumetric flasks. Add 1.0 mL of *Internal standard solution* to each and dilute with *Diluent* to volume.

**Sample solution:** Dissolve 5.0 g of the sample in a minimal volume of 20% aqueous sodium chloride solution. Quantitatively transfer this solution to an Extrelut NT column (EM Science, Gibbstown, NJ), or equivalent. After 15 min, elute the column with three 20-mL portions of *Diluent*, and collect all of the eluate. Carefully evaporate the eluate to less than 4 mL. Add 1.0 mL of *Internal standard solution*, and dilute with *Diluent*, as necessary, to bring the final volume to 5.0 mL.

**Chromatographic system,** Appendix IIA

**Mode:** Gas chromatography with a split injector

**Detector:** Electrolytic conductivity detector

**Column:** 50-m × 0.2-mm (id), fused-silica column (Carbowax 20M, or equivalent) coated with dimethylpolysiloxane, or equivalent

**Temperature**

**Column:** Hold at 115° for 10 min, then increase at 30°/min to 200°, hold at 200° for 12 min

**Injector:** 250°

**Detector:** 300°

[Note—Precondition the column by heating it at 200° and the detector at 300° for 24 h.]

**Carrier gas:** Nitrogen

**Flow rate:** 8 mL/min

**Injection size:** 1.0 µL

**Analysis:** Separately inject each of the *Standard solutions* and the *Sample solution* into the chromatograph and record the resulting chromatograms. Calculate the response area ratios of DCP to *Internal standard solution* for each *Standard solution*. Plot the response area ratios versus the µg of DCP in each *Standard solution* to obtain the standard curve. From the chromatograph of the *Sample solution*, measure its response area ratio of DCP to *Internal standard solution* and, using the standard curve, determine the amount of DCP, in µg, in the sample taken.

**Acceptance criteria:** NMT 0.05 mg/kg, on the dried basis

## SPECIFIC TESTS

• **α-AMINO NITROGEN,** Appendix IIIC

**Acceptance criteria:** NLT 3.0%, on the dried basis

• **α-AMINO NITROGEN/TOTAL NITROGEN PERCENT RATIO**

**Analysis:** Calculate by the formula:

$$\text{Result} = \frac{(\text{AN} - \text{P})}{(\text{TN} - \text{P})} \times 100$$

AN = percent *α-Amino Nitrogen*, determined above

P = percent *Ammonia Nitrogen*, determined below

TN = percent *Total Nitrogen*, determined above

**Acceptance criteria:** NLT 62.0% and NMT 85.0%, when calculated on an ammonia nitrogen-free basis

- **AMMONIA NITROGEN**, Appendix IIIC

**Acceptance criteria:** NMT 1.5%, on the dried basis

**Change to read:**

- **GLUTAMIC ACID**, Appendix IIIC

**Acceptance criteria:** NMT 20.0% as glutamic acid ( $C_5H_9NO_4$ ) and NMT 35.0% of the total amino acids protein, 2S (FCC7) both on the dried basis

- **INSOLUBLE MATTER**

**Sample:** 5 g

**Analysis:** Transfer the *Sample* into a 250-mL Erlenmeyer flask, add 75 mL of water, cover the flask with a watch glass, and boil gently for 2 min. Filter the solution through a tared filtering crucible, dry at 105° for 1 h, cool, and weigh.

**Acceptance criteria:** NMT 0.5%, on the dried basis

- **POTASSIUM**

**Standard solution:** 1.91 µg/mL of potassium chloride (corresponds to 1.0 µg/mL of potassium ion)

**Sample stock solution:** Transfer 1.00 ± 0.05 g of the previously dried sample into a silica or porcelain dish.

Ash in a muffle furnace at 550° for 2 to 4 h. Allow the ash to cool, and dissolve in 5 mL of 20% hydrochloric acid, warming the solution if necessary to complete solution of the residue. Filter the solution through acid-washed filter paper into a 1000-mL volumetric flask. Wash the filter paper with hot water, dilute to volume, and mix.

**Sample solution:** 1:300 (v/v) dilution of the *Sample stock solution*

**Analysis:** Using a suitable atomic absorption spectrophotometer, determine the absorbance of the *Standard solution* and the *Sample solution* at 766.5.

**Acceptance criteria:** The absorbance of the *Sample solution* does not exceed that of the *Standard solution* (NMT 30.0%, on the dried basis).

- **SODIUM**

**Standard stock solution:** 254.2 µg/mL of sodium chloride

**Standard solution:** 12.71 ng/mL of sodium chloride from the *Standard stock solution* (corresponds to 5 ng/mL of sodium ion)

**Sample stock solution:** Transfer 1.00 ± 0.05 g of the previously dried sample into a silica or porcelain dish.

Ash in a muffle furnace at 550° for 2 to 4 h. Allow the ash to cool, and dissolve in 5 mL of 20% hydrochloric acid, warming the solution if necessary to complete solution of the residue. Filter the solution through acid-washed filter paper into a 100-mL volumetric flask. Wash the filter paper with hot water, dilute to volume, and mix.

**Sample solution:** 1:4000 (v/v) dilution of the *Sample stock solution*

**Analysis:** Using a suitable atomic absorption spectrophotometer, determine the absorbance of the *Standard solution* and the *Sample solution* at 589.0.

**Acceptance criteria:** The absorbance of the *Sample solution* does not exceed that of the *Standard solution* (NMT 20.0%, on the dried basis).

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

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(FI2010) Monographs - Food Ingredients



## BRIEFING

**Bergamot Oil, Coldpressed**, FCC 7 page 100. On the basis of comments received, the food-grade materials of commerce for this ingredient have a different *Specific Gravity* range than the current requirement in this monograph. The comments indicate that this is potentially a result of changes in the growing region where this ingredient is produced. It is therefore proposed to lower and widen the specification in the test for *Specific Gravity* for this monograph to a level which includes these materials of commerce.

(FIEC: C. Mejja) C89505

### Bergamot Oil, Coldpressed

FEMA: 2153  
CAS: [8007-75-8]

### DESCRIPTION

Bergamot Oil, Coldpressed occurs as a green to yellow-green or yellow-brown liquid with a fragrant, sweet-fruity odor. It is a volatile oil obtained by pressing, without the aid of heat, the fresh peel of the fruit of *Citrus bergamia* Risso et Poiteau (Fam. Rutaceae). It is miscible with alcohol and with glacial acetic acid. It is soluble in most fixed oils, but is insoluble in glycerin and in propylene glycol. It may contain a suitable antioxidant.

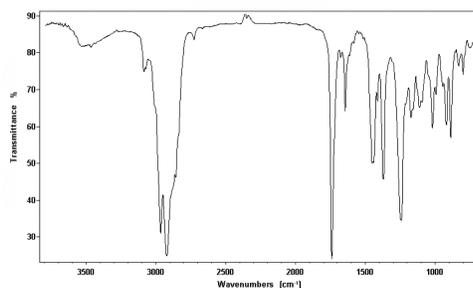
**Function:** Flavoring agent

**Packaging and Storage:** Store in a cool place protected from light in full, tight containers that are made from steel or aluminum and that are suitably lined.

### 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.



Bergamot Oil, Coldpressed

### ASSAY

- **ESTERS**, *Ester Determination*, Appendix VI

**Sample:** 2 g

**Analysis:** Heat the mixture for 30 min on a steam bath, rather than for 1 h. Use 98.15 as the equivalence factor (e) in the calculation.

**Acceptance criteria:** NLT 36.0% of esters, calculated as linalyl acetate (C<sub>12</sub>H<sub>20</sub>O<sub>2</sub>)

### SPECIFIC TESTS

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

**Acceptance criteria:** Between +12° and +30°

- **REFRACTIVE INDEX**, Appendix IIB  
 [Note—Use an Abbé or other refractometer of equal or greater accuracy.]  
**Acceptance criteria:** Between 1.465 and 1.468 at 20°
- **RESIDUE ON EVAPORATION**, Appendix VI: Heat the sample for 5 h.  
**Acceptance criteria:** NMT 6.0%
- **SOLUBILITY IN ALCOHOL**, Appendix VI  
**Acceptance criteria:** One mL of the sample dissolves in 2 mL of 90% alcohol.

**Change to read:**

- **SPECIFIC GRAVITY:** Determine by any reliable method (see *General Provisions*).  
**Acceptance criteria:** Between ~~0.875 and 0.880~~ 0.871 and 0.879, 2S (FCC7)
- **ULTRAVIOLET ABSORBANCE**, *Ultraviolet Absorbance of Citrus Oils*, Appendix VI  
**Sample:** 50 mg  
**Acceptance criteria:** The absorbance difference is NLT 0.32. [Note—The absorbance maximum occurs at 315 ±3 nm.]

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

Topic/Question	Contact	Expert Committee
Monograph	Carla D Mejia, Ph.D. Scientific Liaison 1-301-816-8571	(F12010) Monographs - Food Ingredients

## BRIEFING

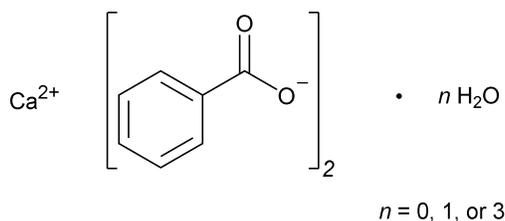
**Calcium Benzoate.** Because there is no existing *FCC* monograph for this food ingredient, a new monograph is proposed based the specifications for Calcium Benzoate prepared at the 49th Session of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The proposed *Lead* method is adapted from the *FCC* procedure used for a similar food ingredient, Sodium Benzoate. Interested parties are encouraged to submit validated HPLC or other chromatographic test procedures to replace the use of the titrimetric Assay procedure for this monograph.

(FIEC: J. Moore) C87467

### Add the following:

#### ■ Calcium Benzoate

Monocalcium Benzoate



$\text{C}_{14}\text{H}_{10}\text{CaO}_4 \cdot x\text{H}_2\text{O}$

Formula wt, anhydrous 282.31

213

CAS: [2090-05-3]

### DESCRIPTION

Calcium Benzoate occurs as white or colorless or white powder. It contains up to three molecules of water of hydration. It is sparingly soluble in water.

**Function:** Preservative; antimicrobial agent

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

### IDENTIFICATION

- **BENZOATE**, Appendix IIIA  
**Acceptance criteria:** Passes test
- **CALCIUM**, Appendix IIIA  
**Acceptance criteria:** Passes tests
- **INFRARED ABSORPTION**, *Spectrophotometric Identification Tests*, Appendix IIIC  
**Reference standard:** USP Calcium Benzoate 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

- **PROCEDURE**  
**Sample:** 600 mg

**Analysis:** Dissolve the *Sample* in a mixture of 20 mL of water and 2 mL of dilute hydrochloric acid TS, and dilute with water to 100 mL. While stirring (preferably with a magnetic stirrer), add about 30 mL of 0.05 M disodium ethylenediaminetetraacetate from a 50-mL buret, then add 15 mL of sodium hydroxide TS, 40 mg of murexide indicator preparation and 3 mL of naphthol green TS, and continue the titration until the solution is deep blue in color. Each mL of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 14.116 mg of  $C_{14}H_{10}CaO_4$ . [Note—Hydroxynaphthol blue may be used as an alternative indicator, in which case the naphthol green TS is omitted.]

**Acceptance criteria:** NLT 99.0% on the dried basis

## IMPURITIES

### Inorganic Impurities

- **FLUORIDE**, *Fluoride Limit Test, Method I or III, Appendix IIIB*

**Analysis:** Proceed as directed using a 5-g sample.

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

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

**Sample:** 10 g

**Acceptance criteria:** NMT 2 mg/kg

## SPECIFIC TESTS

- **ACIDITY OR ALKALINITY**

**Sample:** 2 g

**Analysis:** Dissolve the *Sample* in 20 mL of freshly boiled water and add phenolphthalein TS as an indicator.

**Acceptance criteria:** NMT 0.5 mL of either 0.1 N sodium hydroxide or 0.1 N hydrochloric acid is required for neutralization.

- **CHLORINATED COMPOUNDS**

**Sample:** 0.25 g

**Control:** Mix 0.5 mL of 0.1 N silver nitrate with 20 mL of dilute nitric acid TS containing 0.5 mL of 0.01 N hydrochloric acid.

**Analysis:** Dissolve the *Sample* in 10 mL of water. Acidify with nitric acid and filter off the precipitate. Mix the precipitate with 0.5 g of calcium carbonate, dry the mixture and then ignite. Take up the ignition residue in 20 mL of dilute nitric acid TS and filter. Mix the filtrate with 0.5 mL of 0.1 N silver nitrate.

**Acceptance criteria:** Any turbidity produced by the *Sample* does not exceed that produced by the *Control*. (NMT 0.07% as  $Cl_2$ )

- **LOSS ON DRYING**, Appendix IIC ( $105^\circ$  for 4 h)

**Acceptance criteria:** NMT 17.5%

- **READILY OXIDIZABLE SUBSTANCES**

**Sample:** 1 g

**Analysis:** Add 0.1 N potassium permanganate, dropwise, to a mixture of 100 mL of water and 1.5 mL of sulfuric acid heated to boiling, until a pink color persists for 30 s. Dissolve the *Sample* in the hot solution. Titrate with 0.1 N potassium permanganate to a pink color that persists for 15 s.

**Acceptance criteria:** The volume of 0.1 N potassium permanganate consumed in the titration does not exceed 0.5 mL.

- **WATER-INSOLUBLE MATTER**

**Sample:** 10 g

**Analysis:** Dissolve the *Sample* in 100 mL of hot water. Filter through a tared Gooch crucible, and wash any residue with hot water. Dry the crucible for 2 h at  $105^\circ$ . Cool, weigh, and calculate the percentage of water-

insoluble matter.

**Acceptance criteria:** NMT 0.3%

■ 2S (FCC7)

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

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(F12010) Monographs - Food Ingredients

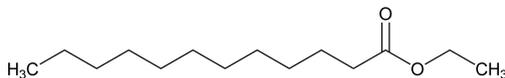
## BRIEFING

**Ethyl Laurate**, FCC 7 page 357. In an effort to modernize the *Identification* test methods in FCC, it is proposed to change the existing infrared method in the *Identification* section to one that utilizes a USP Reference Standard, instead of comparison to a printed spectrum.

(FIEC: K. Laurick) C89836

### Ethyl Laurate

Ethyl Dodecanoate



$C_{14}H_{28}O_2$

Formula wt 228.38

FEMA: 2441

### DESCRIPTION

Ethyl Laurate occurs as a colorless, oily liquid.

**Odor:** Fruity-floral

**Solubility:** Miscible in alcohol, chloroform, ether; insoluble or practically insoluble in water

**Boiling Point:**  $\sim 269^{\circ}$

**Solubility in Alcohol**, Appendix VI: One mL dissolves in 9 mL of 80% alcohol to give a clear solution.

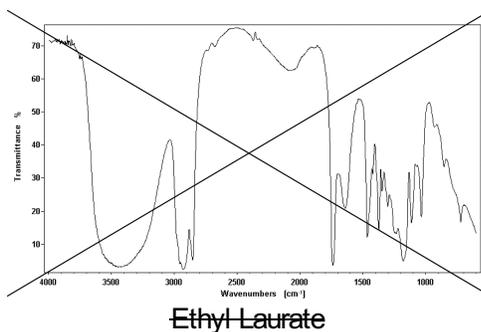
**Function:** Flavoring agent

### IDENTIFICATION

*Change to read:*

- ~~Infrared Spectra, ■ Infrared Absorption, ■ 2S (FCC7) 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.~~



■ **Reference standard:** USP Ethyl Laurate RS

**Sample and standard preparation:** *F*

**Acceptance criteria:** The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard*. ■ 2S (FCC7)

### ASSAY

- **Procedure:** Proceed as directed under *M-1b*, Appendix XI.  
**Acceptance criteria:** NLT 98.0% of C<sub>14</sub>H<sub>28</sub>O<sub>2</sub>

**SPECIFIC TESTS**

- **Acid Value,** *M-15*, Appendix XI  
**Acceptance criteria:** NMT 1.0
- **Refractive Index,** Appendix II: At 20°  
**Acceptance criteria:** Between 1.430 and 1.434
- **Specific Gravity:** Determine at 25° by any reliable method (see *General Provisions*).  
**Acceptance criteria:** Between 0.858 and 0.863

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

Topic/Question	Contact	Expert Committee
Monograph	Kristie Laurvick Scientific Liaison 1-301-816-8356	(F12010) Monographs - Food Ingredients

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**BRIEFING**

**Glycerin**, FCC 7 page 442. The following revisions to this monograph are proposed in order to enhance its ability to detect adulterated materials and to better reflect the specifications of food-grade materials currently in the marketplace.

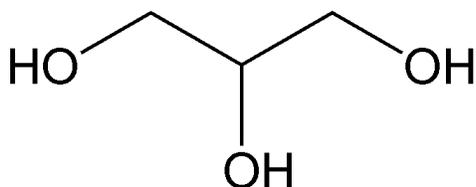
- 1 Add a Note to the *Description* stating that a GC test to identify and quantify diethylene glycol and ethylene glycol in glycerin is available in a new FCC Appendix (See Briefing under Appendix XII: *Food Ingredient Adulterants and Contaminants*). This method is not proposed as a requirement, but rather as an informational test for FCC users interested in screening food-grade glycerin for these two potential adulterants.
- 2 Add a Note to *Identification* test A to indicate how glycerin can be distinguished from diethylene glycol and ethylene glycol, using IR.
- 3 Add a new GC *Identification* test based on that in the *USP 33–NF 28 Glycerin* monograph.
- 4 On the basis of data received indicating that food-grade glycerin in the marketplace is >99% pure, revise the acceptance criteria for *Assay* and *Specific Gravity* to reflect a higher purity limit.
- 5 On the basis of data received, add a *Water* content test procedure and limit.

(FIEC: J. Moore) C86999

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**Glycerin**

Glycerol



C<sub>3</sub>H<sub>8</sub>O<sub>3</sub>

Formula wt 92.09

422

CAS: [56-81-5]

**DESCRIPTION*****Change to read:***

Glycerin occurs as a clear, colorless, viscous liquid. It is hygroscopic, and its solutions are neutral. Glycerin is miscible with water and with alcohol. It is insoluble in chloroform, in ether, and in fixed and volatile oils. ■

[Note—An informational GC method (not a monograph requirement) for the identification and quantification of diethylene glycol and ethylene glycol in glycerin is available for FCC users interested in testing food-grade materials for these potential adulterants. See *Diethylene Glycol and Ethylene Glycol in Glycerin*, Appendix XII.]

■ 2S (FCC7)

**Function:** Humectant; solvent; bodying agent; plasticizer

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

**IDENTIFICATION****Change to read:**

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

**Reference standard:** USP Glycerin RS

**Sample and standard preparation:** *F*

**Acceptance criteria:** The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard*. ■ [Note—A very strong absorption band in the glycerin spectrum at about 10.1  $\mu\text{m}$  can be useful for differentiating glycerin from diethylene glycol and ethylene glycol, which both lack this band.] ■2S (FCC7)

**Add the following:**

- ■**B. PROCEDURE**

**Standard solution:** 2.0 mg/mL of USP Glycerin RS and 0.050 mg/mL of USP Diethylene Glycol RS in methanol

**Sample solution:** 50 mg/mL in methanol

**Chromatographic system**, Appendix IIA

**Mode:** GC

**Detector:** Flame-ionization

**Column:** 0.53-mm  $\times$  30-m fused-silica analytical column coated with 3.0- $\mu\text{m}$  6% cyanopropylphenyl–94% dimethylpolysiloxane stationary phase<sup>1</sup>

**Temperature**

**Injector:** 220 $^{\circ}$

**Detector:** 250 $^{\circ}$

**Column:** See the temperature program table below.

Initial Temperature ( $^{\circ}$ )	Temperature Ramp ( $^{\circ}$ /min)	Final Temperature ( $^{\circ}$ )	Hold Time at Final Temperature (min)
100	—	100	4
100	50	120	10
100	50	220	6

**Carrier gas:** Helium

**Injection size:** 1.0  $\mu\text{L}$

**Flow rate:** 4.5 mL/min

**Injection type:** Split flow ratio is about 10:1

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements:**

**Resolution:** NLT 1.5 between diethylene glycol and glycerin

**Analysis:** Separately inject equal volumes of the *Standard solution* and the *Sample solution*. [Note—The relative retention times for diethylene glycol and glycerin are about 0.8 and 1.0, respectively.]

**Acceptance criteria:** The retention time of the glycerin peak for the *Sample solution* corresponds to that of the glycerin peak for the *Standard solution*. ■2S (FCC7)

**ASSAY**

**Change to read:**• **PROCEDURE**

**Sodium periodate solution:** Dissolve 60 g of sodium metaperiodate ( $\text{NaIO}_4$ ) in sufficient water, containing 120 mL of 0.1 N sulfuric acid, to make 1000 mL. Do not heat to dissolve the periodate. If the solution is not clear, pass through a sintered-glass filter. Store the solution in a glass-stoppered, light-resistant container. Test the suitability of this solution as follows: Pipet 10 mL into a 250-mL volumetric flask, dilute to volume, and mix. Dissolve about 550 mg of sample in 50 mL of water, and add 50 mL of the diluted *Sodium periodate solution* by pipet. For a blank, pipet 50 mL of the diluted *Sodium periodate solution* into a flask containing 50 mL of water. Allow the solutions to stand for 30 min, then add 5 mL of hydrochloric acid and 10 mL of potassium iodide TS to each, and rotate to mix. Allow to stand for 5 min, add 100 mL of water, and titrate with 0.1 N sodium thiosulfate, shaking continuously and adding starch TS near the endpoint. The ratio of the volume of 0.1 N sodium thiosulfate required for the *Sample*–periodate mixture to that required for the blank should be between 0.750 and 0.765.

**Sample:** 400 mg

**Analysis:** Transfer the *Sample* into a 600-mL beaker, dilute with 50 mL of water, add bromothymol blue TS, and acidify with 0.2 N sulfuric acid to a definite green or green-yellow color. Neutralize with 0.05 N sodium hydroxide to a definite blue endpoint free of green color. Prepare a blank containing 50 mL of water, and neutralize in the same manner. Pipet 50 mL of the *Sodium periodate solution* into each beaker, mix by swirling gently, cover with a watch glass, and allow to stand for 30 min at room temperature (not above 35°) in the dark or in subdued light. Add 10 mL of a mixture consisting of equal volumes of ethylene glycol and water to each beaker, and allow to stand for 20 min. Dilute each solution with water to about 300 mL, and titrate with 0.1 N sodium hydroxide to a pH of  $8.1 \pm 0.1$  for the *Sample* and  $6.5 \pm 0.1$  for the blank, using a pH meter previously calibrated with pH 4.0 *Acid Phthalate Standard Buffer Solution (Solutions and Indicators)*. Each mL of 0.1 N sodium hydroxide, after correction for the blank, is equivalent to 9.210 mg of glycerin ( $\text{C}_3\text{H}_8\text{O}_3$ ).

**Acceptance criteria:** NLT 95.0% ■ 99.0% ■<sub>2S (FCC7)</sub> and NMT 100.5% ■ 101.0% ■<sub>2S (FCC7)</sub> of  $\text{C}_3\text{H}_8\text{O}_3$  ■ on the as-is basis ■<sub>2S (FCC7)</sub>

**IMPURITIES****Inorganic Impurities**

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

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

**Organic Impurities**

- **FATTY ACIDS AND ESTERS**

**Sample:** 40.0 mL (50 g)

**Analysis:** Mix the *Sample* with 50 mL of recently boiled water and 5.0 mL of 0.5 N sodium hydroxide. Boil the mixture for 5 min, cool, add phenolphthalein TS, and titrate the excess alkali with 0.5 N hydrochloric acid.

**Acceptance criteria:** More than 4 mL of 0.5 N hydrochloric acid is consumed. (Limit is about 0.1% calculated as butyric acid.)

**SPECIFIC TESTS**

- **CHLORINATED COMPOUNDS (AS CL)**

**Sample:** 5.0 g

**Analysis:** Transfer the *Sample* into a dry, 100-mL round-bottom, ground-joint flask, and add 15 mL of

morpholine. Connect the flask with a ground-joint reflux condenser, and reflux the mixture gently for 3 h. Rinse the condenser with 10 mL of water, receiving the washing into the flask, and cautiously acidify with nitric acid. Transfer the solution to a suitable comparison tube, add 0.5 mL of silver nitrate TS, dilute to 50.0 mL, and mix thoroughly.

**Control:** 150 µg of chloride in an equal volume of solution containing the quantities of reagents used in the *Analysis*, but omitting the refluxing.

**Acceptance criteria:** Any turbidity produced by the *Sample* does not exceed that produced by the *Control* (NMT 0.003% as Cl).

- **COLOR**

**Sample:** 50 mL

**Control:** 0.40 mL of ferric chloride CS diluted with water to 50 mL

**Analysis:** Transfer the *Sample* and the *Control* to separate 50-mL Nessler tubes of the same diameter and color, and view the tubes downward against a white surface.

**Acceptance criteria:** The color of the *Sample* is not darker than that of the *Control*.

- **READILY CARBONIZABLE SUBSTANCES, Appendix IIB**

**Sample:** 5 mL

**Analysis:** Rinse a glass-stoppered 25-mL cylinder with 95% sulfuric acid, and allow it to drain for 10 min.

Add the *Sample* and 5 mL of 95% sulfuric acid, gently mix for 1 min at 18° to 20°, and allow to stand for 1 h.

**Acceptance criteria:** The resulting mixture has no more color than *Matching Fluid H*.

- **RESIDUE ON IGNITION**

**Sample:** 50 g

**Analysis:** Heat the *Sample* in a tared, open dish, and ignite the vapors, allowing them to burn until the sample has been completely consumed. After cooling, moisten the residue with 0.5 mL of sulfuric acid, and complete the ignition by heating for 15-min periods at 800° ± 25° to constant weight.

**Acceptance criteria:** NMT 0.01%

**Change to read:**

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

**Acceptance criteria:** NLT 1.249<sup>■</sup> 1.259<sup>■</sup> 2S (FCC7)

**Add the following:**

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

**Acceptance criteria:** NMT 1.0%<sup>■</sup> 2S (FCC7)

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<sup>1</sup> DB-624 (J & W Scientific), or equivalent.

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

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(FI2010) Monographs - Food Ingredients

## BRIEFING

**Hydrochloric Acid**, FCC 7 page 492. On the basis of comments received, a revision to add a test method and specification for *Mercury* in this food ingredient is proposed in order to ensure that mercury contamination of this ingredient is kept to a minimum, particularly when it is manufactured by the chlor-alkali process. Available data when proposing this revision did not specify the analytical method. Comments and data relevant to the test method specified and the proposed limit are encouraged.

(FIEC: K. Laurick) C89601

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### Hydrochloric Acid

HCl

Formula wt 36.46

507

CAS: [7647-01-0]

### DESCRIPTION

Hydrochloric Acid occurs as a clear, colorless or slightly yellow, corrosive liquid. It is a water solution of hydrogen chloride of varied concentrations. It is miscible with water and with alcohol. Concentrations of Hydrochloric Acid are expressed in percent by weight or may be expressed in degrees Baumé (°Bé) from which percents of Hydrochloric Acid and specific gravities may readily be derived (see *Hydrochloric Acid Table*, Appendix IIC). The usually available concentrations are 18°, 20°, 22°, and 23° Bé. Concentrations above 13° Bé (19.6%) fume in moist air, lose hydrogen chloride, and create a corrosive atmosphere. Because of these characteristics, observe suitable precautions during sampling and analysis to prevent losses.

[Note—Hydrochloric Acid is produced by various methods that might impart trace amounts of organic compounds as impurities. The manufacturer, vendor, or user is responsible for identifying the specific organic compounds that are present and for meeting the requirements for *Organic Impurities* (below). Methods are likewise provided for their determination. In applying the procedures, use any necessary standards to quantitate the organic compounds present in each specific product.

The variety of organic impurities that might conceivably be found in Hydrochloric Acid is such that it is impossible to provide a comprehensive and accurate list here. Therefore, the manufacturer, vendor, or user is responsible for establishing the suitability of such Hydrochloric Acid for its intended application in foods or food processing in accordance with the provision of *Trace Impurities* (see *General Provisions*.)]

**Function:** Acidifier

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

### IDENTIFICATION

- CHLORIDE, Appendix IIIA

**Acceptance criteria:** Passes test

### ASSAY

- PROCEDURE

**Sample preparation:** Tare a 125-mL glass-stoppered Erlenmeyer flask containing 35.0 mL of 1 N sodium hydroxide. Without the use of vacuum, partially fill a 10-mL serological pipet from near the bottom of a flask containing the sample, remove any acid adhering to the outside, and discard the first mL flowing from

the pipet. Hold the tip of the pipet just above the surface of the sodium hydroxide solution, and transfer between 2.5 and 3 mL of the sample into the flask, leaving at least 1 mL in the pipet. Stopper the flask, gently swirl to mix the contents, and accurately weigh to obtain the sample weight.

**Analysis:** Add methyl orange TS to the *Sample preparation* and titrate the excess sodium hydroxide with 1 N hydrochloric acid. Each mL of 1 N sodium hydroxide is equivalent to 36.46 mg of HCl.

**Acceptance criteria:** 97.0%–103.0% of the labeled amount of HCl, or within the range specified on the label

## IMPURITIES

### *Change to read:*

#### Inorganic Impurities

- IRON

**Sample:** 5 g (4.3 mL)

**Control:** 25 µg Fe (2.5 mL *Iron Standard Solution*, Solutions and Indicators)

**Analysis:** Dilute the *Sample* with water to 40 mL. Add 40 mg of ammonium persulfate and 10 mL of ammonium thiocyanate TS. Repeat the preceding using the *Control* in place of the *Sample*.

**Acceptance criteria:** Any red color produced by the *Sample* does not exceed that produced by the *Control*. (NMT 5 mg/kg)

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

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

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

**Acceptance criteria:** NMT 0.10 mg/kg

- 2S (FCC7)

- OXIDIZING SUBSTANCES (AS  $Cl_2$ )

**Sample:** 1 mL

**Analysis:** Transfer the *Sample* into a 30-mL test tube, dilute to 20 mL with freshly boiled and cooled water, and add 1 mL of potassium iodide TS and 1 mL of starch TS. Stopper the test tube and mix thoroughly. Prepare a *Control* consisting of 1.0 mL of 0.001 N iodine in an equal volume of water containing the same quantities of the same reagents and 1 mL of ACS reagent-grade hydrochloric acid.

**Acceptance criteria:** Any blue color produced by the *Sample* does not exceed that produced by the *Control* (NMT 0.003%).

- REDUCING SUBSTANCES (AS  $SO_3$ )

**Sample:** 1 mL

**Analysis:** Transfer 1 mL of ACS reagent-grade hydrochloric acid into a 30-mL test tube, dilute with recently boiled and cooled water to 20 mL, and add 1 mL of potassium iodide TS, 1 mL of starch TS, and 2.0 mL of 0.001 N iodine. Stopper the test tube and mix thoroughly. Add the *Sample* to the test tube.

**Acceptance criteria:** The blue color of the solution does not disappear when the *Sample* is added (NMT 0.007%).

- SULFATE

**Sample solution:** 10 mg/mL in water

**Analysis:** Transfer 5.0 mL of the *Sample solution* into a 50-mL tall-form Nessler tube, and dilute with water to 20 mL. Add a drop of phenolphthalein TS, neutralize the solution with 6 N ammonium hydroxide, and then add 1 mL of 2.7 N hydrochloric acid. Add 3 mL of barium chloride TS to the resulting clear solution, previously filtered, if necessary, dilute with water to 50 mL, and mix. Prepare a *Control* consisting of 1 mL of ACS reagent-grade hydrochloric acid and 250 µg of sulfate ( $SO_4$ ) and the same quantities of the reagents used for the sample.

**Acceptance criteria:** Any turbidity produced by the *Sample solution* does not exceed that shown in the *Control* (NMT 0.5%).

### Organic Impurities

#### • ORGANIC COMPOUNDS

[Note—Use either the *Vapor Partitioning Method* (below) or the *Solvent Extraction Method* (below) for analysis of all listed elements, except for benzene, which requires the *Vapor Partitioning Method*.]

#### Tests:

##### • Vapor Partitioning Method

[Note—This method is suitable for the determination of extractable organic compounds at 0.05 to 100 mg/kg but is most appropriate for organic compounds with a vapor pressure greater than 10 mm Hg at 25°.]

**Standard solutions:** Prepare a standard solution of each of the organic compounds to be quantitated in hydrochloric acid (known to be free of interfering impurities) at approximate concentrations of 5 mg/kg, or within ±50% of the concentrations in the samples to be analyzed.

Place a stirring bar in a 1-L volumetric flask equipped with a ground-glass stopper, and tare the combination. Fill the flask with reagent-grade hydrochloric acid so that no air space is present when the flask is stoppered, and determine the weight of the hydrochloric acid.

Calculate the volume (V), in µL, of each organic component to be added using the equation:

$$V = (C \times W)/(D \times F)$$

C = desired concentration of the organic compound to be added (mg/kg)

W = weight of the hydrochloric acid (g)

D = density of the organic compound to be added (mg/µL)

F = conversion factor, 1000 (g/kg)

Add the calculated amount of each component to the hydrochloric acid with a syringe (ensure that the syringe tip is under the solution surface), stopper the flask, and stir the solution for at least 2 h using a magnetic stirrer.

#### Chromatographic system, Appendix IIA

**Mode:** Gas chromatography

**Detector:** Flame-ionization detector

**Column:** 4-m × 2-mm (id) stainless-steel column, or equivalent, packed with 15%, by weight, methyl trifluoropropyl silicone (DCFS 1265, or QF-1, or OV-210, or SP-2401) stationary phase on 80- to 100-mesh Gas Chrom R, or the equivalent. [Note—Condition a newly packed column at 120° and with a 30-mL/min helium flow for at least 2 h (preferably overnight) before it is attached to the detector.]

**Flow rate:** 11 mL/min; with fuel gas flows optimized for the gas chromatograph and detector in use.

#### Temperature

**Column:** 105° (isothermal)

**Injection port:** 250°

**Detector:** 250°

#### Suitability requirements

**Suitability requirement 1:** The signal-to-noise ratio should be at least 10:1.

**Suitability requirement 2:** The relative standard deviation at 5 mg/kg is NMT 15% for five sample analyses.

[Note—Change the experimental conditions as necessary for optimal resolution and sensitivity.]

**Calibration (non-gaseous standards):** Dilute a 10-mL aliquot of *Standard solution* with an equal volume of water. Draw this solution into a 50-mL glass syringe. Then draw 20 mL of air into the syringe, cap with a rubber septum, and place the syringe on a shaker for 5 min. Draw 1 mL of the vapor through the septum, and inject it into the gas chromatograph. Determine a blank for each lot of reagent-grade hydrochloric acid, and calculate a response factor using the following equation:

$$R = C/(A - B)$$

- R = response factor  
 C = concentration for the standard component of interest (mg/kg)  
 A = peak area for the standard component of interest  
 B = peak area for the *Blank*

**Calibration (gaseous standards)**

[Note—Gaseous compounds present special problems in the preparation of standards. Therefore, to determine response factors for gaseous compounds use the following *Method of Multiple Extractions*.] Dilute a sample of hydrochloric acid known to contain the gaseous compound of interest with an equal volume of water. Draw 20 mL of this solution into a 50-mL glass syringe; then draw 20 mL of air into the syringe, cap with a rubber septum, and place the syringe on a shaker for 5 min. Withdraw 1 mL of the vapor through the septum, and inject it into the chromatograph. Expel the vapor phase from the 50-mL syringe, draw in another 20 mL of air, repeat the extraction, and inject another 1-mL vapor sample into the chromatograph.

Carry out the extraction and analysis on the same sample of acid six times. For each impurity, plot the area ( $A_N$ ) determined for extraction (N) against the difference between  $A_N$  and the area determined for extraction (N + 1); that is, plot  $A_N$  against ( $A_N - A_{N+1}$ ). The slope of this line is the extraction efficiency (E) for that impurity into the air.

Inject 1 mL of a 0.1% (by volume) standard gas sample of each impurity in air into the chromatograph, and determine the absolute factor ( $F_A$ ), in g, per peak area by the following equation:

$$F_A = (M \times 4.0816 \times 10^{-8})/A$$

- M = molecular weight of the compound  
 A = peak area of the compound

The concentration (C), in mg/kg, of the component of interest in the original sample is calculated by the formula:

$$C = (A \times F_A \times 1.6949 \times 10^6)/E$$

- A = peak area corresponding to the compound (as above)  
 $F_A$  = absolute factor (determined above)  
 E = extraction efficiency (determined above)

The response factor, R, is then calculated as:

$$R = C/A$$

**Analysis:** Dilute 10-mL of sample with an equal volume of water. Draw this solution into a 50-mL glass syringe. Then draw 20 mL of air into the syringe, cap with a rubber septum, and place the syringe on a shaker for 5 min. Draw 1 mL of the vapor through the septum, and inject it into the gas chromatograph.

Approximate elution times, in minutes, for some specific organic compounds are as follows:

Methane and acetylene: 1.70  
 Methyl chloride: 2.21  
 Vinyl chloride: 2.29  
 1,1,1-Trichlorofluoromethane: 2.62  
 Ethyl chloride: 2.90  
 Vinylidene chloride: 3.20  
 Methylene chloride: 3.64  
 Chloroform: 4.49  
 1,1-Dichloroethane: 4.53  
 Carbon tetrachloride: 4.86  
 1,1,1-Trichloroethane: 5.50  
 Benzene: 6.00  
 Trichloroethylene: 6.22  
 Ethylene dichloride: 6.61  
 Propylene dichloride: 8.41  
 Perchloroethylene: 9.73

[Note—Alternative columns may be required to resolve some combinations of components. Methyl chloride and vinyl chloride are resolved by a 3.7-m × 3-mm (id) squalane column, or equivalent, at 45° and a helium flow of 10 mL/min. Chloroform and 1,1-dichloroethane are resolved by a 4-m × 3-mm (id) DC 550R column, or equivalent, at 110° and a helium flow of 12 mL/min.]

Calculate the concentration, in mg/kg, of each compound by multiplying its corresponding peak area by the appropriate response factor determined in the Calibration protocol:

$$C = R \times A$$

- C = concentration of the compound of interest in the sample taken (mg/kg)  
 R = response area for the compound of interest, determined in *Calibration* (above)  
 A = peak area for the compound of interest in the chromatogram of the sample

#### • Solvent Extraction Method

[Note—This method is suitable for the determination of extractable organic compounds at 0.3 to 100 mg/kg, but is most appropriate for organic compounds with vapor pressures less than 10 mm Hg at 25°.]

**Standard solutions:** Prepare as described under the *Vapor Partitioning Method* (above).

**Chromatographic system,** Appendix IIA

**Mode:** Gas chromatography

**Detector:** Flame-ionization detector

**Column:** 4-m × 2-mm (id) stainless-steel column, or equivalent, packed with 15%, by weight, methyl trifluoropropyl silicone (DCFS 1265, or QF-1, or OV-210, or SP-2401) stationary phase on 80- to 100-mesh Gas Chrom R, or the equivalent. [Note—Condition a newly packed column at 120° and with a 30-mL/min helium flow for at least 2 h (preferably overnight) before it is attached to the detector.]

**Flow rate:** 21 mL/min; with fuel gas flows optimized for the gas chromatograph and detector in use.

**Temperature**

**Column:** 120<sup>o</sup> (isothermal)

**Injection port:** 250<sup>o</sup>

**Detector:** 250<sup>o</sup>

**Suitability requirements**

**Suitability requirement 1:** The signal-to-noise ratio should be at least 10:1.

**Suitability requirement 2:** The relative standard deviation at 5 mg/kg is NMT 15% for five sample analyses.

[Note—Change the experimental conditions as necessary for optimal resolution and sensitivity.]

**Calibration:** Accurately transfer 90 mL of *Standard solution* and 10 mL of perchloroethylene (free of interfering impurities) into a narrow-mouth, 4-oz bottle. Place the bottle in a mechanical shaker for 30 min. Separate the two phases (perchloroethylene on the bottom) and inject 3  $\mu$ L of the perchloroethylene extract into the gas chromatograph, or equivalent. Determine a *Blank* for each lot of reagent-grade hydrochloric acid and perchloroethylene by extracting the hydrochloric acid in the same way as for the *Standard solution*. Calculate a response factor using the following equation:

$$R = C/(A - B)$$

R = response factor

C = concentration for the standard component of interest  
(mg/kg)

A = peak area for the standard component of interest

B = peak area for the *Blank*

**Analysis:** Accurately transfer 90 mL of sample and 10 mL of perchloroethylene (free of interfering impurities) into a narrow-mouth, 4-oz bottle. Place the bottle in a mechanical shaker for 30 min. Separate the two phases (perchloroethylene on the bottom) and inject 3  $\mu$ L of the perchloroethylene extract into the gas chromatograph, or equivalent. Approximate elution times, in minutes, for some chlorinated organic compounds are as follows:

Vinylidene chloride: 2.94

Methylene chloride: 3.27

Chloroform: 3.83

Carbon tetrachloride: 4.07

1,1,1-Trichloroethane: 4.50

Trichloroethylene: 4.97

Ethylene dichloride: 5.26

Propylene dichloride: 6.36

Perchloroethylene: 6.95

1,1,1,2-Tetrachloroethane: 10.12

1,1,1,2,2-Tetrachloroethane: 13.70

Pentachloroethane: 16.19

[Note—To determine perchloroethylene and higher-boiling impurities, substitute methylene chloride (free of interfering impurities) for perchloroethylene in the extraction step. For higher boiling impurities such as monochlorobenzene and the three dichlorobenzenes, use a 2.74-m  $\times$  2.1-mm (id) stainless-steel column packed with 10% carbowax 20M/2% KOH on 80- to 100-mesh chromasorb W (acid washed), set at 150<sup>o</sup> and with a nitrogen flow of 35 mL/min.]

Calculate the concentration, in mg/kg, of each compound by multiplying its corresponding peak area

by the appropriate response factor determined in the *Calibration* protocol:

$$C = R \times (A - B)$$

- C = concentration of the compound of interest in the sample taken (mg/kg)
- R = response area for the compound of interest, determined in *Calibration* (above)
- A = peak area for the compound of interest in the chromatogram of the sample
- B = area obtained from a blank sample

#### Acceptance criteria

**Total non-fluorine containing organic compounds:** NMT 5 mg/kg, including NMT 0.05 mg/kg benzene

**Total fluorinated organic compounds:** NMT 0.0025%

#### SPECIFIC TESTS

- **COLOR, Readily Carbonizable Substances, Appendix IIB**

**Acceptance criteria:** A sample shows no more color than does *Matching Fluid A*.

- **DEGREES BAUMÉ**

**Sample:** 200 mL, previously cooled to a temperature below 15°

**Analysis:** Transfer the *Sample* into a 250-mL hydrometer cylinder. Insert a suitable Baumé hydrometer graduated at 0.1 °Bé intervals, adjust the temperature to 15.6°, and note the reading at the bottom of the meniscus.

**Acceptance criteria:** Within the range shown on the label or claimed by the vendor.

- **NONVOLATILE RESIDUE**

**Sample:** 1 g

**Analysis:** Transfer the *Sample* into a tared glass dish, evaporate to dryness on a steam bath, then dry at 110° for 1 h. Cool in a desiccator and weigh.

**Acceptance criteria:** The weight of the residue does not exceed 5 mg (NMT 0.5%).

- **SPECIFIC GRAVITY**

**Analysis:** Determine 15.6° with a hydrometer, or calculate from the degrees Baumé observed in *Degrees Baumé* (above).

**Acceptance criteria:** Within the range specified or implied by the vendor

#### OTHER REQUIREMENTS

- **LABELING:** Indicate the content, by weight, of Hydrochloric Acid (HCl). Alternatively, indicate the range of Hydrochloric Acid content, the range of degrees Baumé, and/or the specific gravity range.

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

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(F12010) Monographs - Food Ingredients

## BRIEFING

**Lycopene from *Blakeslea trispora*.** Because there is no existing FCC monograph for this ingredient, a new monograph is proposed based on the Lycopene from *Blakeslea trispora* monograph in the 67th Session of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 2006.

- The chromatographic methods described do not include system suitability criteria (consistent with JECFA); comments including system suitability criteria and supporting data are specifically requested.
- The Assay method requires a preparation for *Standard solution A* which includes enzymatic digestion because USP Lycopene RS has a gelatin coating. This represents a change from the JECFA assay version. Comments and data specific to the proposed method are encouraged.
- The title of this monograph is consistent with that in JECFA. Comments on other appropriate titles/synonyms are encouraged.

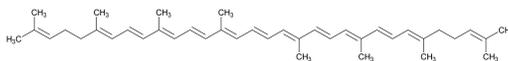
(FIEC: K. Laurick) C88330

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### Add the following:

#### ■ Lycopene from *Blakeslea trispora*

All-*trans*-lycopene



C<sub>40</sub>H<sub>56</sub>

Formula wt 536.85

160d(iii)

CAS: [502-65-8]

### DESCRIPTION

Lycopene from *Blakeslea trispora* occurs as a red crystalline powder. It is a fermentation product that accumulates inside the biomass of the fungus and is extracted and purified using suitable solvents. It is predominantly all-*trans*-lycopene, but also contains minor quantities of other carotenoids. Commercial preparations containing Lycopene from *Blakeslea trispora* are available as suspensions in edible oils or as water-dispersible powders and are stabilized using suitable food-grade antioxidants. Lycopene from *Blakeslea trispora* is freely soluble in chloroform and insoluble in water.

**Function:** Source of lycopene; color

**Packaging and Storage:** Preserve in tight, light-resistant containers under inert gas, and store in a cool place.

### IDENTIFICATION

#### • A. PRESENCE OF CAROTENOIDS

**Sample solution:** Solution in acetone

**Analysis:** To the *Sample solution*, successively add a 5% solution of sodium nitrite and 1 N sulfuric acid.

**Acceptance criteria:** The color of the *Sample solution* disappears.

#### • B. PROCEDURE

**Acceptance criteria:** The retention time of the major peak in the chromatogram of the *Sample solution* corresponds to that of the major peak in the chromatogram of *Standard solution A*, as determined in the Assay for Lycopene.

- **C. UV-Vis Absorption Spectrum**

**Sample solution:** Solution in hexane

**Acceptance criteria:** The absorption spectrum of the *Sample solution* exhibits an absorption maximum at about 470 nm.

## ASSAY

- **LYCOPENE**

**Mobile phase:** Methanol and acetonitrile (60:40)

**Standard solution A:** Transfer an amount of USP Lycopene RS equivalent to 5 mg of lycopene to a 250-mL volumetric flask, add about 60 units of bacterial alkaline protease preparation or another suitable enzyme, and about 25 mg of butylated hydroxytoluene. Add 2.50 mL of dilute ammonium hydroxide (2 in 100) in water, mix, place in an ultrasonic bath at 50° for 10 min, rotate the flask occasionally to avoid having the material stick to the glass surface, and continue until the material is dispersed with no lumps. Add 5 mL of tetrahydrofuran, 40 mL of dehydrated alcohol, mix, and place in the ultrasonic bath for about 1 min. Cool to room temperature, and dilute with *tert*-butyl methyl ether to volume. Shake vigorously, allow the precipitate to settle, and filter the supernatant for use as *Standard solution A*.

**Standard solution B:** [Note—Preparation of *Standard solution B* is required to determine the exact concentration of *Standard solution A*.] Dilute 1 mL of *Standard solution A* with hexane to 10 mL.

To determine the purity of the USP Lycopene RS used to prepare *Standard solution A*,  $P_S$ , measure the absorbance of *Standard solution B* in a 1-cm optical cell at the wavelength of maximum absorption of approximately 470 nm, using hexane as a blank. Calculate  $P_S$  by:

$$P_S = A_{MAX}/(F \times C_{SSB})$$

$A_{MAX}$  = absorbance of *Standard solution B* at the wavelength of maximum absorbance

$F$  = absorptivity of pure lycopene in hexane, 345

$C_{SSB}$  = concentration of USP Lycopene RS in *Standard solution B* (mg/mL)

[Note— $P_S$ , the purity of the USP Lycopene RS, equals 1.0 for a 100% pure standard and is less than 1.0 for a standard with purity below 100%.]

**Standard solution C:** Dilute 25 mL of *Standard solution A* with acetone to 100 mL.

**Sample stock solution:** Transfer an amount of sample equivalent to 25 mg of lycopene to a 100-mL volumetric flask and dissolve in 10 mL of methylene chloride. Dilute with hexane to volume.

**Sample solution:** Dilute 1 mL of the *Sample stock solution* with acetone to 50 mL.

**Chromatographic system,** Appendix IIA

**Mode:** Liquid chromatography

**Detector:** UV-Vis 470 nm

**Column:** 4.6-mm × 25-cm; packing of octadecylsilane chemically bonded to 5- $\mu$ m porous (300 Å pore size) silica micro-particles<sup>1</sup>

**Temperature**

**Column:** 30°

**Injector:** 10°

**Flow rate:** 1 mL/min

**Injection size:** 10  $\mu$ L

**Analysis:** Inject *Standard solution C* into the chromatograph and record the chromatogram. [Note—The

retention time of all-*trans*-lycopene is approximately 11.5–12.5 min. The relative retention time of 13-*cis*-lycopene with respect to all-*trans*-lycopene is 1.25. The relative retention times for other carotenoids with respect to all-*trans*-lycopene are 1.2 for  $\beta$ -carotene and 1.1 for  $\gamma$ -carotene.]

Record the total peak area of all-*trans*-lycopene and *cis*-lycopene isomers and calculate the response factor, RF, in AU mL/mg, for lycopene:

$$RF = r_{ST}/(C_S \times P_S)$$

$r_{ST}$  = total lycopene peak area for all-*trans*-lycopene and *cis*-lycopene, as determined from the chromatogram of the *Standard solution*

$C_S$  = concentration of *Standard solution C* (mg/mL)

$P_S$  = purity of the USP Lycopene RS, as determined above

Inject the *Sample solution* into the chromatograph and record the chromatogram, measuring the peak areas for all-*trans*-lycopene ( $r_1$ ); total lycopene (all-*trans*-lycopene + *cis*-lycopene,  $r_2$ ); other carotenoids ( $r_3$ ); and all carotenoids (all-*trans*-lycopene + *cis*-lycopene + other carotenoids,  $r_4$ ).

Calculate the percentages of total lycopene and all-*trans*-lycopene in the sample taken:

$$\text{Total lycopene} = r_2/(C_U \times RF) \times 100$$

$$\text{All-}i\text{-trans-lycopene} = (r_1/r_2) \times 100$$

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

#### Acceptance criteria

**Total lycopene:** NLT 95%

**All-*trans*-lycopene:** NLT 90%

## IMPURITIES

### Inorganic Impurities

- LEAD, *Elemental Impurities by ICP, Method I, Appendix IIIC*

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

### Organic Impurities

- RESIDUAL SOLVENTS

[Note—Perform this test to determine the amount of residual isopropanol and isobutyl acetate in the sample.]

**Internal standard:** 3-methyl-2-pentanone

**Internal standard solution:** Add 50.0 mL of methanol to a 50-mL headspace vial and seal. Weigh and inject 15  $\mu$ L of the *Internal standard* through the septum. Re-weigh the vial to within 0.01 mg.

**Blank:** Use a portion of the sample with very low solvent content.

**Blank solution:** Weigh 0.20 g of the *Blank* to a 50-mL headspace vial. Add 5.0 mL of methanol and 1.0 mL of *Internal standard solution*. Heat at 60° for 10 min and shake vigorously for 10 s.

**Standard stock solution:** Add 50.0 mL of methanol to a 50-mL headspace vial and seal. Weigh the vial and inject 50  $\mu$ L of the component of interest through the septum. Re-weigh the vial and mix the solution well.

**Standard solution:** Weigh 0.20 g of the *Blank* into a 50-mL headspace vial, and add 4.9 mL of methanol and 1.0 mL of *Internal standard solution*. Introduce 0.1 mL of the *Standard stock solution* to the vial, mix

well, and heat at 60° for 10 min. Shake vigorously for 10 s.

**Sample solution:** Weigh 0.20 g of the sample into a 50-mL headspace vial. Add 5.0 mL of methanol and 1.0 mL of the *Internal standard solution*. Heat at 60° for 10 min and shake vigorously for 10 s.

**Chromatographic system,** Appendix IIA

**Mode:** Headspace gas chromatography

**Detector:** Flame ionization

**Column:** 0.8-m × 0.53-mm (i.d.) megabore fused silica column coated with a 1-µm film of polyethylene glycol (average molecular weight about 15,000) coupled with a second 30-m × 0.53-mm (i.d.) fused silica column coated with a 5-µm film of dimethylpolysiloxane gum

**Carrier gas:** Helium

**Flow rate:** 5 mL/min (209 kPa)

**Temperature**

**Injector:** 140°

**Detector:** 300°

**Oven:** Hold at 35° for 5 min; ramp to 90° at 5°/min; hold at 90° for 6 min

**Syringe:** 70°

**Transfer:** 80°

**Headspace sampler**

**Sample heating temperature:** 60°

**Sample heating period:** 10 min

**Injection size:** 1000 µL

**Injection mode:** Split

[Note—The approximate retention time for isopropanol is 5.23 min. The approximate retention time for isobutyl acetate is not available for this system and should be determined experimentally.]

**Analysis:** Place the *Sample solution*, *Blank solution*, and *Standard solution* into the sample tray of the headspace gas chromatograph. Record the resulting chromatograms and determine the calibration factor, *C*, for each component of interest:

$$C = W_S / [W_{ISS} \times (r_S - r_{SB}) \times F_1]$$

*C* = calibration factor

*W<sub>S</sub>* = amount of the component of interest in the *Standard stock solution* (mg)

*W<sub>ISS</sub>* = amount of *Internal standard* in the 1.0-mL aliquot of *Internal standard solution* used to prepare the *Standard solution* (mg)

*r<sub>S</sub>* = relative peak area of the component of interest in the chromatogram of the *Standard solution*

*r<sub>SB</sub>* = relative peak area of the component of interest in the chromatogram of the *Blank solution*

*F<sub>1</sub>* = dilution factor for the *Stock standard solution*, 10

Calculate the amount of each component of interest in the sample taken, in mg/headspace vial:

$$\text{Result} = (r_U \times W_{ISU} \times C) / F_2$$

$r_U$  = relative peak area of the component of interest in the chromatogram of the *Sample solution*

$W_{ISU}$  = amount of *Internal standard* in the 1.0-mL aliquot of *Internal standard solution* used to prepare the *Sample solution* (mg)

$F_2$  = factor, 50

From the calculations, determine the percentage of each component of interest in the sample taken.

#### Acceptance criteria

**Isobutyl acetate:** NMT 1.0%

**Isopropanol:** NMT 0.1%

#### SPECIFIC TESTS

- **CONTENT OF OTHER CAROTENOIDS**

**Mobile phase, Sample stock solution, Sample solution, and Chromatographic system:** Prepare as directed in the Assay for *Lycopene*.

**Analysis:** Proceed as directed in the Assay for *Lycopene*.

Calculate the percentage of other carotenoids in the sample taken:

$$\text{Other carotenoids} = (r_3/r_4) \times 100$$

**Acceptance criteria:** NMT 5%

- **Loss on Drying,** Appendix IIC (40°, 4 h at 20 mm Hg)

**Acceptance criteria:** NMT 0.5%

- 2S (FCC7)

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<sup>1</sup> Vydac 281TP54, or equivalent, available from Grace Davison Discovery Sciences at [www.discoverysciences.com](http://www.discoverysciences.com)

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

Topic/Question	Contact	Expert Committee
Monograph	Kristie Laurvick Scientific Liaison 1-301-816-8356	(F12010) Monographs - Food Ingredients

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## BRIEFING

**Lycopene Extract from Tomato.** Because there is no existing *FCC* monograph for this ingredient, a new monograph is proposed based on comments and data received, and based on the Lycopene Extract from Tomato monograph in the 71st Session of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) 2009, and based on the *Tomato Extract Containing Lycopene* monograph in the *Second Supplement to USP 32–NF 27*.

- The title of this monograph is consistent with that in JECFA. Comments on other appropriate titles/synonyms are encouraged.
- The monograph for *Tomato Extract Containing Lycopene* in the *Second Supplement to USP 32–NF 27* used as a reference for this proposed monograph contains specifications and methods for analysis of pesticide residues. The *Description* section informs users who choose to test their ingredient for pesticide residues that a new method is available under *Pesticide Residues* in Appendix XIII, but such testing is not required at this time. Comments regarding the voluntary testing for pesticide residues are encouraged—See briefing under *Appendix XIII: Adulterants and Contaminants in Food Ingredients*.
- The microbiological criteria included in the *USP 32–NF 27 Second Supplement* monograph have been omitted in the proposed *FCC* monograph. *FCC*'s policy on microbiological limits can be found in *FCC 5* on page 3, and includes the following statement: “The General Policy for microbiological safety and quality of *FCC* substances is such that substances be produced, handled, and used in food processing following GMPs and applicable food safety systems. Therefore, the *FCC* does not list specific microbiological criteria for *FCC* substances other than those for which scientifically valid data are available to the committee that support the need for such criteria.” Comments regarding the omission of microbiological specifications are encouraged.
- The methods described in the *Identification* tests originate from the monograph in *USP 32–NF 27 Second Supplement*.
- Specifications proposed in the *Assay for Lycopene* are consistent with the JECFA monograph. The method used is consistent with the method in *USP 32–NF 27 Second Supplement*.
- The specifications and method proposed in *Content of Other Carotenoids and Tocopherols* are consistent with the monograph in *USP 32–NF 27 Second Supplement*. The JECFA monograph only gives a nonspecific limit for total carotenoids.
- Data submitted indicated metals analysis using inductively coupled plasma atomic emission spectroscopy (ICP-AES or ICP-OES) instrumentation. Methods given in this proposal for the analysis of *Arsenic* and *Lead* reference a new proposed test for *Elemental Impurities by ICP* in Appendix IIIC—See briefing under *Appendix III: Chemical Tests and Determinations*.
- The specifications and method proposed in *Residual Solvents* is consistent with the JECFA monograph.
- One of the *Functions* listed for this ingredient is “color”, because this item may legally be used as a color in some countries. The *Function* category is informational only. Users are responsible for independently determining the regulatory status of the food ingredients that they manufacture, sell, and use in the countries where they are operating and should not use the *FCC Function* as an endorsement for the legal use of any ingredient.

(FIEC: K. Laurick) C87962

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### **Add the following:**

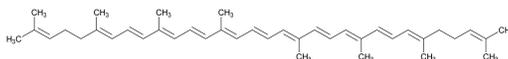
#### ■ **Lycopene Extract from Tomato**

All-trans-lycopene

Lycopene (Tomato)

Lycopene, Tomato Extract

Tomato Oleoresin Extract

C<sub>40</sub>H<sub>56</sub>

Formula wt 536.85

160d(ii)

CAS: [502-65-8]

## DESCRIPTION

Lycopene Extract from Tomato occurs as a dark-red viscous liquid. It is obtained through ethyl acetate extraction of the pulp of ripe red tomatoes (*Lycopersicon esculentum* L.) followed by removal of the solvent. The ingredient contains only the fat-soluble solids extracted from the fruit. Lycopene is the major coloring component in tomato extract; minor amounts of other carotenoid pigments may also be present. Lycopene Extract from Tomato also contains oils, fats, waxes, and flavor components naturally occurring in tomatoes. It is freely soluble in ethyl acetate and *n*-hexane; partially soluble in ethanol and acetone; and insoluble in water. Tocopherols may be added as antioxidants. While not a requirement for this monograph, users interested in analyzing this ingredient for potential pesticide residues may use the informational method found under *Pesticide Residues* in Appendix XIII.

**Function:** Antioxidant; source of lycopene; color

**Packaging and Storage:** Preserve in tight, light-resistant containers, and store in a cool place.

## IDENTIFICATION

- PRESENCE OF LYCOPENE, PHYTOFLUENE, AND PHYTOENE

**Butylated hydroxytoluene solution, Diluent, Standard solution C, Sample stock solution, and**

**Sample solution:** Proceed as directed in the *Assay for Lycopene*.

**Chromatographic system:** Proceed as directed in the test for *Content of Other Carotenoids and Tocopherols* in *Specific Tests*.

**Acceptance criteria:** The retention times for the lycopene, phytofluene, and phytoene peaks of the *Sample solution* correspond to those of *Standard solution C*, as obtained in the test for *Content of Other Carotenoids and Tocopherols* (under *System suitability*).

- RATIO OF ALL-*E*-LYCOPENE AND 5*Z*-LYCOPENE

**Butylated hydroxytoluene solution:** Proceed as directed in the *Assay for Lycopene*.

**Mobile phase:** 0.05% diisopropylethylamine in *n*-hexane; sonicate for 3–4 min

**Sample stock solution:** Proceed as directed in the *Assay for Lycopene*.

**Sample solution:** Dilute 5 mL of the *Sample stock solution* with *n*-hexane to 100 mL.

**Chromatographic system,** Appendix IIA

**Mode:** Liquid chromatography

**Detector:** UV-Vis 472 nm

**Column:** Two 4.0-mm × 25-cm columns; 5-μm packing of porous silica particles (300 Å pore size); connected in series

**Temperature:** 22°

**Flow rate:** 0.5 mL/min

**Injection size:** 10  $\mu$ L

**Analysis:** Inject the *Sample solution* into the chromatograph, record the chromatogram, and measure the peak responses of the two major peaks. [Note—The approximate relative retention times for all-*E*-lycopene and 5*Z*-lycopene are 1.00 and 1.04–1.10, respectively. The approximate retention time for the all-*E*-lycopene peak is 30–45 min.]

Calculate the ratio of the peak areas:

$$\text{Result} = r_{U1}/r_{U2}$$

$r_{U1}$  = peak response of 5*Z*-lycopene

$r_{U2}$  = peak response of all-*E*-lycopene

**Acceptance criteria:** The peak area ratio is NMT 0.10.

## ASSAY

### • LYCOPENE

**Butylated hydroxytoluene solution:** 5 mg/mL in methylene chloride

**Mobile phase:** Prepare a solution containing acetonitrile, methanol, methylene chloride, and *n*-hexane (850:100:25:25). Add 0.05% of diisopropylethylamine and sonicate for 3–4 min.

**Diluent:** Prepare a solution containing acetonitrile, methylene chloride, methanol, *n*-hexane, and butylated hydroxytoluene (600:150:150:100:0.5). Add 0.05% of diisopropylethylamine and sonicate for 3–4 min.

**Standard solution A:** Transfer an amount of USP Lycopene RS equivalent to 5 mg of lycopene into a 100-mL volumetric flask, add 60 units of bacterial alkaline protease preparation or another suitable enzyme, and 25 mg of butylated hydroxytoluene. Add 2.5 mL of dilute ammonium hydroxide (2 in 100) in water, and place in an ultrasonic bath at 50° for 10 min, rotating the flask occasionally to avoid having material stick to the glass surface. Continue ultrasonication until the material is dispersed with no lumps. Add 5 mL of tetrahydrofuran, and shake until no colored precipitate remains. Add an additional 2-mL portion of tetrahydrofuran, 40 mL of *Diluent*, and shake until the mixture is homogeneous. Dilute with *Diluent* to volume, shake vigorously, and allow to stand, if necessary, until the solid has settled.

**Standard solution B:** [Note—Preparation of *Standard solution B* is required to determine the exact concentration of *Standard solution A*.] Transfer 2.0 mL of *Standard solution A* to a 100-mL volumetric flask, add 10 mL of alcohol and 10 mL of *Butylated hydroxytoluene solution*, and dilute with *n*-hexane to volume. Prepare in triplicate.

Determine the absorbance of *Standard solution B* at the wavelength of maximum absorbance at 472 nm using a mixture of alcohol, *Butylated hydroxytoluene solution*, and *n*-hexane (10:10:80) as the blank.

Calculate the concentration of lycopene, in  $\mu$ g/mL, in *Standard solution A*:

$$\text{Result} = (A_{MAX}/F) \times 50,000$$

$A_{MAX}$  = absorbance of *Standard solution B*

F = absorptivity of pure lycopene in *n*-hexane at 472 nm, 345

**Standard solution C:** Transfer an amount of USP Tomato Extract Containing Lycopene RS equivalent to 6 mg of lycopene to a 100-mL volumetric flask, and dissolve in 1 mL of *Butylated hydroxytoluene solution* and 9 mL of methylene chloride, using a sonicator. Dilute with *Diluent* to volume (0.06 mg/mL of lycopene).

**Sample stock solution:** Warm several grams of the extract to 50° in a water bath. Mix well with a glass rod or spatula, then weigh 1–1.2 g into a 100-mL volumetric flask. Add 10 mL of *Butylated hydroxytoluene solution* and 30 mL of methylene chloride to the flask and sonicate for 1 min in order to dissolve the

sample completely. Cool to room temperature and dilute with methylene chloride to volume.

**Sample solution:** Transfer 5.0 mL of *Sample stock solution* to a 50-mL volumetric flask and dilute with *Diluent* to volume.

**Chromatographic system,** Appendix IIA

**Mode:** Liquid chromatography

**Detector:** UV-Vis 472 nm

**Column:** 4.6-mm × 25-cm; with octylsilane chemically bonded to 5-µm porous silica particles

**Temperature:** 39 ± 1 °

**Flow rate:** 0.7 mL/min

**Injection size:** 10 µL

**System suitability**

**Sample:** *Standard solution A*

**Suitability requirement 1:** The relative standard deviation is NMT 1.5% for the lycopene peak area for replicate injections.

**Analysis**

[Note—Both *Standard solution A* and *Standard solution C* will be necessary for use in *Content of Other Carotenoids and Tocopherols*, but analysts may choose which solution to use in the *Assay for Lycopene*.]

Separately inject *Standard solution A* or *Standard solution C* into the chromatograph, record the chromatograms, and measure the responses of the major lycopene peak in each chromatogram. [Note—The approximate retention time for lycopene is 6 min.]

Calculate the percentage of lycopene in the sample taken:

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

$C_S$  = concentration of lycopene in *Standard solution A* or *Standard solution C* (µg/mL)

$C_U$  = concentration of the *Sample solution* (µg/mL)

$r_U$  = peak response for lycopene obtained from the chromatogram for the *Sample solution*

$r_S$  = peak response for lycopene obtained from the chromatogram of *Standard solution A* or *Standard solution B*

**Acceptance criteria:** 5.0%–15.0%

## IMPURITIES

### Inorganic Impurities

- **ARSENIC,** *Elemental Impurities by ICP, Method I, Appendix IIIC*

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

- **LEAD,** *Elemental Impurities by ICP, Method I, Appendix IIIC*

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

### Organic Impurities

- **RESIDUAL SOLVENTS**

**Standard stock solution:** 10.00 mg/g of ethyl acetate in diethylphthalate. [Note—Use an ultrasonic bath to dissolve. This solution is stable for 2 months at room temperature.]

**Standard solution 1:** Dilute 500 mg of *Solution A* to 50.00 g with diethylphthalate (100 µg/g). [Note—Use an ultrasonic bath to dissolve. This solution is stable for 2 months at room temperature.]

**Standard solution 2:** Dilute 500 mg of *Standard solution 1* to 10.00 g (±0.1 mg) with diethylphthalate in a

pre-weighed and tared 20-mm headspace vial (5 µg/g). Insert a 12–15 mm magnetic stirrer and seal the vial.

**Standard solution 3:** Dilute 1000 mg of *Standard solution 1* to 10.00 g (±0.1 mg) with diethylphthalate in a pre-weighed and tared 20-mm headspace vial (10 µg/g). Insert a 12–15 mm magnetic stirrer and seal the vial.

**Standard solution 4:** Dilute 1750 mg of *Standard solution 1* to 10.00 g (±0.1 mg) with diethylphthalate in a pre-weighed and tared 20-mm headspace vial (17.5 µg/g). Insert a 12–15 mm magnetic stirrer and seal the vial.

**Standard solution 5:** Dilute 2500 mg of *Standard solution 1* to 10.00 g (±0.1 mg) with diethylphthalate in a pre-weighed and tared 20-mm headspace vial (25 µg/g). Insert a 12–15 mm magnetic stirrer and seal the vial.

**Sample solution:** Heat a portion of sample material to 40°–50° in a water bath while stirring mechanically.

Remove a 30-g portion of the sample material and warm to 50° in a water bath. Mix well with a glass rod or spatula and weigh 5000 mg into a pre-weighed and tared 20-mm headspace vial. Bring the weight of the sample to 10.00 g (total weight; ±0.1 mg) with diethylphthalate. Insert a 12–15 mm magnetic stirrer and seal the vial. Mix the sample using the stirrer.

**Chromatographic system, Appendix IIA**

**Mode:** Headspace gas chromatography

**Detector:** Flame ionization

**Column:** 30 m × 0.53 mm (i.d.) megabore fused silica column coated with a 3-µm film of 5% diphenyl/95% dimethyl polysiloxane

**Carrier gas:** Nitrogen

**Temperature**

**Injector:** 180°

**Detector:** 230°

**Oven:** Hold at 73° for 5 min; ramp to 160° at 25°/min; hold at 160° for 1 min. [Note—The run time is about 9.5 min.]

**Flow rate:** 4 mL/min

**Injection size:** 1000 µL

**Injection mode:** Splitless 1:6

**Analysis:** Place *Standard solutions 2, 3, 4, and 5*, and the *Sample solution* in a water bath held at 70° for exactly 2 h, stirring each solution for 1 min every 30 min. Separately inject each of the *Standard solutions* into the head-space gas chromatograph, record the peak area, and calculate the mean ratio of the standard concentration to peak area based on concentrations and peak areas of *Standard solutions 2, 3, 4, and 5*.

Inject the *Sample solution* into the chromatograph, record the peak area, and calculate the concentration of ethyl acetate, in mg/kg:

$$\text{Result} = A_U \times F \times (W_{TU}/W_U)$$

$A_U$  = peak area obtained from the chromatogram of the *Sample solution*

$F$  = mean ratio of the concentration of *Standard solutions 2, 3, 4, and 5* to the peak areas obtained on the solutions

$W_{TU}$  = total weight of the *Sample solution* prepared (g)

$W_U$  = amount of the sample used to prepare the *Sample solution* (g)

**Acceptance criteria:** NMT 50 mg/kg

## SPECIFIC TESTS

### • CONTENT OF OTHER CAROTENOIDS AND TOCOPHEROLS

**Butylated hydroxytoluene solution, Diluent, Standard solution A, Standard solution B, Standard solution C, Sample stock solution, and Sample solution:** Prepare as directed in the Assay for *Lycopene*.

**Mobile phase:** Prepare a solution containing acetonitrile, methanol, methylene chloride, and *n*-hexane (475:475:25:25). Add 0.05% of diisopropylethylamine and sonicate for 3–4 min.

**Chromatographic system,** Appendix IIA

**Mode:** Liquid chromatography

**Detector:** UV-Vis 472 nm (lycopene); 450 nm (beta-carotene); 350 nm (phytofluene); 288 nm (phytoene and tocopherol)

**Column:** 4.6-mm × 25-cm; with octadecylsilane chemically bonded to 5-μm porous silica particles

**Temperature:** 39 ± 1°

**Flow rate:** 0.6 mL/min

**Injection size:** 10 μL

#### System suitability

**Sample:** *Standard solution C*

[Note—The chromatogram obtained from *Standard solution C* should be similar to the reference chromatogram provided with the USP Tomato Extract Containing Lycopene RS. Approximate relative retention times are 0.6 for the peaks of the tocopherol isomers, 1.0 for the peak of all-*E*-lycopene, 1.5–1.7 for the peaks of beta carotene isomers, 1.6–1.8 for the peaks of the phytofluene isomers, and 1.8–2.2 for the phytoene peak.]

**Suitability requirement 1:** The relative standard deviation is NMT 2% for the peak responses for the lycopene isomers for replicate injections.

**Analysis:** Separately inject *Standard solution A* and the *Sample solution* into the chromatograph and record the chromatograms. Identify the locations of the peaks for the lycopene isomers, beta carotene isomers, phytofluene isomers, and phytoene by comparison with the reference chromatogram provided with the corresponding lot of USP Tomato Extract Containing Lycopene RS. Measure the sum of the peak responses of the lycopene isomers at 472 in *Standard solution A*. Determine the concentration of *Standard solution A* as directed in the Assay for *Lycopene*. [Note—The lycopene isomers may be resolved in more than one peak in this chromatographic system.]

In the chromatogram of the *Sample solution*, measure the sum of the peak responses of the beta carotene isomers at 450 nm, the sum of the peak responses of the phytofluene isomers at 350 nm, the response of phytoene at 288 nm, and the sum of the peak responses of all tocopherols at 288 nm.

Calculate the percentage of beta carotene in the portion of the sample taken:

$$\text{Result} = (C_S/C_U) \times (r_{U1}/r_S) \times (F_1/F_2) \times 100$$

- $C_S$  = concentration of *Standard solution A* ( $\mu\text{g/mL}$ )  
 $C_U$  = concentration of the *Sample solution* ( $\mu\text{g/mL}$ )  
 $r_{U1}$  = sum of the peak responses for the beta carotene isomers at 450 nm obtained from the chromatogram of the *Sample solution*  
 $r_S$  = sum of the peak responses for the lycopene isomers at 473 nm obtained from the chromatogram of *Standard solution A*  
 $F_1$  = absorptivity for pure lycopene, 345  
 $F_2$  = absorptivity for pure beta carotene, 259.2

Calculate the percentage of phytofluene in the portion of the sample taken:

$$\text{Result} = (C_S/C_U) \times (r_{U2}/r_S) \times (F_1/F_3) \times 100$$

- $C_S$  = concentration of *Standard solution A* ( $\mu\text{g/mL}$ )  
 $C_U$  = concentration of the *Sample solution* ( $\mu\text{g/mL}$ )  
 $r_{U2}$  = sum of the peak responses for the phytofluene isomers at 350 nm obtained from the chromatogram of the *Sample solution*  
 $r_S$  = sum of the peak responses for the lycopene isomers at 472 nm obtained from the chromatogram of *Standard solution A*  
 $F_1$  = absorptivity for pure lycopene, 345  
 $F_3$  = absorptivity for pure phytofluene, 135

Calculate the percentage of phytoene in the portion of the sample taken:

$$\text{Result} = (C_S/C_U) \times (r_{U3}/r_S) \times (F_1/F_4) \times 100$$

- $C_S$  = concentration of *Standard solution A* ( $\mu\text{g/mL}$ )  
 $C_U$  = concentration of the *Sample solution* ( $\mu\text{g/mL}$ )  
 $r_{U3}$  = peak area response for phytoene at 288 nm obtained from the chromatogram of the *Sample solution*  
 $r_S$  = sum of the peak responses for the lycopene isomers at 472 nm obtained from the chromatogram of *Standard solution A*  
 $F_1$  = absorptivity for pure lycopene, 345  
 $F_4$  = absorptivity for pure phytoene, 125

Calculate the percentage of tocopherols in the portion of the sample taken:

$$\text{Result} = (C_S/C_U) \times (r_{U4}/r_S) \times (F_1/F_5) \times 100$$

- $C_S$  = concentration of *Standard solution A* ( $\mu\text{g/mL}$ )  
 $C_U$  = concentration of the *Sample solution* ( $\mu\text{g/mL}$ )  
 $r_{U4}$  = sum of the peak responses for all the tocopherol peaks at 288 nm obtained from the chromatogram of the *Sample solution*  
 $r_S$  = sum of the peak responses for the lycopene isomers at 472 nm obtained from the chromatogram of *Standard solution A*  
 $F_1$  = absorptivity for pure lycopene, 345  
 $F_5$  = average absorptivity for tocopherols, 8.5

**Acceptance criteria**

**Phytofluene and phytoene (combined):** NLT 0.8%, on the anhydrous basis

**Beta carotene:** NLT 0.2%, on the anhydrous basis

**Tocopherols:** NLT 1.0%, on the anhydrous basis

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

**Sample:** 1–2 g

**Acceptance criteria:** NMT 1.0%

- 2S (FCC7)

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

Topic/Question	Contact	Expert Committee
Monograph	Kristie Laurvick Scientific Liaison 1-301-816-8356	(FI2010) Monographs - Food Ingredients

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**BRIEFING**

**Magnesium Ammonium Potassium Chloride, Hydrate.** Because there is no existing *FCC* monograph for this ingredient, a new monograph based on comments and data received is proposed. The method included under *Assay for Chloride* is based on AOAC Official Method 971.27, Sodium Chloride in Canned Vegetables, Method III (Potentiometric Method). Data submitted for metals impurities was generated using atomic absorption methods. Comments are encouraged regarding the use of ICP for all three metals impurities.

(FIEC: K. Laurick) C88032

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**Add the following:****■ Magnesium Ammonium Potassium Chloride, Hydrate**

Ammonium Magnesium Potassium Chloride, Hydrate

Magnesal

Magnesium Ammonium Potassium Camallite

Magnesium Ammonium Potassium Chloride, Triple Salt

$Mg_4K(NH_4)_3Cl_{12} \cdot 24H_2O$

Formula wt 1048.23  
CAS: [1044829-32-4]

**DESCRIPTION**

Magnesium Ammonium Potassium Chloride, Hydrate occurs as a white co-crystalline triple salt (solid) containing 24 waters of hydration for each unit of the magnesium/potassium/ammonium chloride complex. The method of manufacture involves dissolving stoichiometric amounts of the individual magnesium, potassium, and ammonium chloride salts in water, boiling the solution and removing the vapor formed, then cooling the mixture, crystallizing, filtering or centrifuging, drying and sieving the product. Magnesium Ammonium Potassium Chloride, Hydrate is freely soluble in water and remains dry in air at relative humidity below 70%, but may become deliquescent at higher humidity. It may contain suitable food-grade anticaking or free flowing agents, such as silicon dioxide (SiO<sub>2</sub>) or calcium silicate (Ca<sub>2</sub>SiO<sub>4</sub>). Magnesium Ammonium Potassium Chloride, Hydrate containing water insoluble anticaking or free flowing agents may produce cloudy solutions and dissolve incompletely.

**Function:** Salt substitute; flavoring agent and intensifier; dough conditioner

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

**IDENTIFICATION**

- **Ammonium**, Appendix IIIA  
**Sample solution:** 100 mg/mL  
**Acceptance criteria:** Passes test
- **Chloride**, Appendix IIIA  
**Sample solution:** 100 mg/mL  
**Acceptance criteria:** Passes test
- **Magnesium**, Appendix IIIA

**Sample solution:** 100 mg/mL

**Acceptance criteria:** Passes test

- **Potassium**, Appendix IIIA

**Sample solution:** 100 mg/mL

**Acceptance criteria:** Passes tests

- **X-RAY DIFFRACTION PATTERN**

**Sample preparation:** Prepare a random powder specimen of the sample.

**Analysis:** Record the x-ray diffraction pattern of the *Sample preparation* using a copper source and calculate the *d* values.

**Acceptance criteria:** The *Sample preparation* exhibits intense reflections at the following *d* values: 6.59Å, 3.79Å, 2.96Å, 2.34Å, and 2.10Å.

## ASSAY

[Note—In the following procedures, it may be necessary to filter solutions of the sample to avoid interference from insoluble or suspended added anticaking or free flowing agents.]

- **AMMONIUM**

**Sample:** 700 mg–2.2 g, previously dried at 60° for 2 h

**Analysis:** Proceed as directed under *Nitrogen Determination*, Appendix III C. From the mg of nitrogen determined, N, calculate the percentage of ammonium in the *Sample* by:

$$\text{Result} = N(F \times W) \times 100$$

F = factor representing the percentage of nitrogen in ammonium, by weight, 0.776

W = weight of the *Sample* taken (mg)

**Acceptance criteria:** 4.9%–5.4%

- **CHLORIDE<sup>1</sup>**

[Note—Use distilled or deionized water that is halogen-free when tested as follows: Add 1 mL of 0.1 M silver nitrate and 5 mL of HNO<sub>3</sub> (20% v/v, diluted with water) to 100 mL of the water. No more than slight turbidity is produced.]

**Solution A:** 2% nitric acid in water (v/v)

**Sodium chloride standard solution:** 5.000 mg/mL NaCl (0.0856 M); prepared from sodium chloride primary standard that has been previously dried at 110° for 2 h

**Silver nitrate standard solution:** 14.451 mg/mL AgNO<sub>3</sub> (0.0856 M). Standardize as follows: Pipet 25 mL of *Sodium chloride standard solution* into a 250-mL beaker and dilute with water to 50 mL, then add 50 mL of *Solution A*. Using the *Electrode system* specified below with a suitable pH meter and a magnetic stirrer (stir at a constant, vigorous rate without splashing), titrate the solution with *Silver nitrate standard solution*, recording the change in voltage with the incremental addition of titrant. Add a total of 50 mL of *Silver nitrate standard solution* to obtain a complete titration curve. Plot the results in mV against mL of *Silver nitrate standard solution* added. Determine the inflection point of the resulting curve and, from the volume of *Silver nitrate standard solution* used to titrate the sodium chloride, calculate the exact molarity of the *Silver nitrate standard solution*. [Note—The *Silver nitrate standard solution* should be restandardized occasionally. Recheck the end point potential occasionally, and redetermine it if any changes are made in the *Electrode system*, or if the pH meter is replaced. Store in a Pyrex container out of direct sunlight. Solution is stable in room light.]

**Electrode system:** Use an Ag billet combination electrode<sup>2</sup>, or separate indicating Ag<sup>3</sup> and glass reference<sup>4</sup>

electrodes. Before initial use and before use each day, if necessary, clean the Ag billet electrode tip with scouring powder or other suitable material and rinse thoroughly with water. Clean other electrodes as recommended by the manufacturer(s) and reclean as frequently as necessary to prevent drifting of the end point reading. Store and care for all electrodes as directed by the manufacturer(s).

**Sample solution:** Transfer 5.00 g of sample, previously dried at 60° for 2 h, to a 100-mL volumetric flask and dilute with water to volume.

**Analysis:** Transfer 5 mL of the *Sample solution* to a 250-mL beaker and dilute with water to 50 mL, then add 50 mL of *Solution A*. Using the *Electrode system* specified above with a suitable pH meter and a magnetic stirrer (stir at a constant, vigorous rate without splashing), titrate the solution with *Silver nitrate standard solution*, recording the change in voltage with the incremental addition of titrant. Add a total of 50 mL of *Silver nitrate standard solution* to obtain a complete titration curve. Plot the results in mV against mL of *Silver nitrate standard solution* added. Determine the inflection point of the resulting curve and, from the volume of *Silver nitrate standard solution* used to titrate the sodium chloride, calculate the percentage of chloride in the *Sample* by:

$$\text{Result} = [V_T \times M_T \times M_{r1}] / [F \times V_S \times C_U] \times 100$$

$V_T$  = volume of *Silver nitrate standard solution* used to titrate the sample to the inflection point (mL)

$M_T$  = exact molarity of the *Silver nitrate standard solution* used in the titration (mol/L)

$M_{r1}$  = atomic weight of chlorine, 35.453 g/mol

$F$  = conversion from mL to L, 1000 mL/L

$C_U$  = concentration of the *Sample solution* (g/mL)

$V_S$  = volume of *Sample solution* titrated, 5 mL

**Acceptance criteria:** 38.6%–42.2%

- **MAGNESIUM**

[Note—Thoroughly clean all glassware by soaking overnight in 20% nitric acid (v/v). Triple-rinse all glassware with distilled and deionized water. All water used for cleaning glassware and for preparation of solutions should have a resistance of NLT 18 megaohms.]

**Solution A:** 0.1% (w/v) lanthanum chloride; prepare by dissolving 1.33 g of lanthanum chloride heptahydrate in water and diluting with water to 500 mL. [Note—This solution is stable for up to 6 months when stored at room temperature.]

**Standard stock solution:** 1000 µg/mL magnesium; commercially prepared, certified atomic absorption standard

**Standard solutions:** Using serial dilutions of *Standard stock solution* in *Solution A*, prepare three standard solutions containing 0.05, 0.1, and 0.2 µg/mL of magnesium.

**Sample solution:** 10 mg/mL, using a sample previously dried at 60° for 2 h

**Blank:** *Solution A*

**Analysis:** Using a suitable atomic absorption spectrophotometer equipped with a lead hollow-cathode lamp set at the magnesium emission line of 285.21 nm and an air–acetylene flame and optimized according to the instrument manufacturer's instructions, concomitantly determine the absorbance of the *Blank*, the three *Standard solutions*, and the *Sample solution*. If the absorbance of the *Sample solution* is above the absorbance of the most concentrated *Standard solution*, further dilute the *Sample solution* with *Solution A* until its absorbance falls within the range of the *Standard solutions*. Correct the readings from *Standard solutions* and the final dilution of the *Sample solution* for the *Blank* reading. Plot the absorbance readings of the *Standard solutions* against the magnesium concentration, in µg/mL, and determine the magnesium

concentration of the *Sample solution* from the curve so obtained.

Calculate the percentage of magnesium in the sample by:

$$\text{Result} = (C/C_U) \times F \times 100$$

C = concentration of magnesium in the solution of the *Sample*, determined from the standard curve ( $\mu\text{g/mL}$ )

$C_U$  = concentration of sample in the final solution used for the determination ( $\text{mg/mL}$ )

F = factor converting  $\mu\text{g}$  to  $\text{mg}$ , 0.001

**Acceptance criteria:** 8.8%–9.6%

- **POTASSIUM**

[Note—Thoroughly clean all glassware by soaking overnight in 20% nitric acid (v/v). Triple-rinse all glassware with distilled and deionized water. All water used for cleaning glassware and for preparation of solutions should have a resistance of NLT 18 megaohms.]

**Solution A:** 0.5% (w/v) cesium; prepare by dissolving 1.58 g of cesium chloride in water and diluting with water to 250 mL [Note—Make fresh every 6 months.]

**Standard stock solution:** 1000  $\mu\text{g/mL}$  potassium; commercially prepared, certified atomic absorption standard

**Standard solutions:** Using serial dilutions of *Standard stock solution* in *Solution A*, prepare three standard solutions containing 0.1, 0.5, and 1.0  $\mu\text{g/mL}$  of potassium.

**Sample solution:** 10  $\text{mg/mL}$ , using a sample previously dried at 60° for 2 h

**Blank:** *Solution A*

**Analysis:** Using a suitable atomic absorption spectrophotometer equipped with a lead hollow-cathode lamp set at the potassium emission line of 766.7 nm and an air–acetylene flame and optimized according to the instrument manufacturer's instructions, concomitantly determine the absorbance of the *Blank*, the three *Standard solutions*, and the *Sample solution*. If the absorbance of the *Sample solution* is above the absorbance of the most concentrated *Standard solution*, further dilute the *Sample solution* with *Solution A* until its absorbance falls within the range of the *Standard solutions*. Correct the readings from *Standard solutions* and the final dilution of the *Sample solution* for the *Blank* reading. Plot the absorbance readings of the *Standard solutions* against the potassium concentration, in  $\mu\text{g/mL}$ , and determine the potassium concentration of the *Sample solution* from the curve so obtained.

Calculate the percentage of potassium in the sample by:

$$\text{Result} = (C/C_U) \times F \times 100$$

C = concentration of potassium in the *Sample solution*, determined from the standard curve ( $\mu\text{g/mL}$ )

$C_U$  = concentration of sample in the final solution used for the determination ( $\text{mg/mL}$ )

F = factor converting  $\mu\text{g}$  to  $\text{mg}$ , 0.001

**Acceptance criteria:** 3.5%–3.9%

## IMPURITIES

- **Cadmium**, *Elemental Impurities by ICP*, Appendix IIIC

**Acceptance criteria:** NMT 0.3  $\text{mg/kg}$

- **Lead**, *Elemental Impurities by ICP*, Appendix IIIC

**Acceptance criteria:** NMT 2  $\text{mg/kg}$

- *Mercury, Elemental Impurities by ICP, Appendix IIIC*

**Acceptance criteria:** NMT 0.5 mg/kg

■ 2S (FCC7)

<sup>1</sup> Based on AOAC Official Method 971.27, Sodium Chloride in Canned Vegetables, Method III (Potentiometric Method).

<sup>2</sup> Beckman No. 39261, or equivalent.

<sup>3</sup> Beckman No. 39261, Orion No. 94-17-BN, Fisher No. 13-639-122, or equivalent.

<sup>4</sup> Beckman No. 39419, Orion No. 90-02-00, Fisher No. 9-313-216, or equivalent.

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

Topic/Question	Contact	Expert Committee
Monograph	Kristie Laurvick Scientific Liaison 1-301-816-8356	(FI2010) Monographs - Food Ingredients

## BRIEFING

**Magnesium Phosphate, Monobasic.** Because there is no existing *FCC* monograph for this food ingredient, a new monograph based on the Monomagnesium Phosphate monograph prepared at the 69th Session of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is proposed with the following exceptions which are based on those in *FCC* monographs for similar magnesium phosphate food ingredients, such as *Magnesium Phosphate, Dibasic, Mixed Hydrates* and *Magnesium Phosphate, Dibasic, Trihydrate*:

- 1.The proposed *Sample solution* preparation for the *Magnesium* identification test.
- 2.The proposed *Analysis* for the *Phosphate* identification test.
- 3.The proposed *Arsenic* and *Lead* methods.
- 4.The proposed *Fluoride* analysis using *Method III* is modified to use a reduced volume of HCl to dissolve the sample.
- 5.The proposed *Fluoride* limit.

(FIEC: J. Moore) C89420

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### **Add the following:**

#### ■ **Magnesium Phosphate, Monobasic**

Acid Magnesium Phosphate

Magnesium Biphosphate

Magnesium Dihydrogen Phosphate

Monomagnesium Dihydrogen Phosphate

Monomagnesium Orthophosphate

Monomagnesium Phosphate

$Mg(H_2PO_4)_2 \cdot xH_2O$

Formula wt, anhydrous 218.28

Formula wt, dihydrate 254.31

Formula wt, tetrahydrate 290.34

343(i)

CAS: anhydrous [13092-66-5]

dihydrate [15609-87-7]

### **DESCRIPTION**

Magnesium Phosphate, Monobasic, occurs as a white, odorless, crystalline powder. It may contain two or four molecules of water of hydration. It is produced by partially neutralizing phosphoric acid with magnesium oxide and drying the resulting material. It is slightly soluble in water.

**Function:** Nutrient; pH control agent

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

## IDENTIFICATION

- **MAGNESIUM**, Appendix IIIA

**Sample solution:** Dissolve a 100-mg sample in 0.5 mL of 1 N acetic acid and 20 mL of water. Add 1 mL of ferric chloride TS, let the solution stand for 5 min, and filter. Use the filtrate to carry out the *Magnesium* identification test procedure in Appendix IIIA.

**Acceptance criteria:** Filtrate passes tests

- **PHOSPHATE**, Appendix IIIA

**Sample:** 200 mg

**Analysis:** Dissolve the *Sample* in 10 mL of 1.7 N nitric acid and add, dropwise, ammonium molybdate TS.

**Acceptance criteria:** A green-yellow precipitate of ammonium phosphomolybdate forms that is soluble in 6 N ammonium hydroxide.

## ASSAY

- **PROCEDURE**

**Sample:** 200 mg of the residue obtained from the *Loss on Ignition* test

**Analysis:** Transfer the *Sample* into a 250-mL beaker, dissolve in 2 mL of hydrochloric acid (16%), and add 100 mL of water. Heat the solution to 50° to 60° and add 10 mL of 0.1 M disodium EDTA from a buret. Add a magnetic stirring bar and, while stirring, adjust with 1 N sodium hydroxide to a pH of 10. Add 10 mL of ammonia–ammonium chloride buffer TS, 12 drops of eriochrome black TS, and continue the titration with 0.1 M disodium EDTA until the red color changes to green. [Note—The solution must be clear when the end point is reached.] Each mL of 0.1 M disodium EDTA consumed is equivalent to 9.14 mg of  $Mg_2P_2O_7$ .

**Acceptance criteria:** NLT 96% and NMT 102% of  $Mg_2P_2O_7$  on the ignited basis

## IMPURITIES

### Inorganic Impurities

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

**Sample solution:** 1 g in 10 mL of 2.7 N hydrochloric acid

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

- **FLUORIDE**, *Fluoride Limit Test, Method III*, Appendix IIIB

**Analysis:** Determine as directed, except in the *Procedure* use 10 mL of 1 N hydrochloric acid instead of 20 mL to dissolve the sample.

**Acceptance criteria:** NMT 25 mg/kg

- **LEAD**, *Lead Limit Test, APDC Extraction Method*, Appendix IIIB

**Acceptance criteria:** NMT 4 mg/kg

## SPECIFIC TESTS

- **LOSS ON DRYING**, Appendix IIC (105° for 4 h)

**Acceptance criteria**

**Anhydrous:** NMT 1.5%

- **LOSS ON IGNITION**

**Sample:** 2 g

**Analysis:** Ignite the *Sample* in a platinum, quartz, or porcelain dish at about 800° for 30 min, preferably in a muffle furnace. [Note—Save the residue for the Assay.]

**Acceptance criteria**

**Anhydrous:** NMT 18.5%

**Dihydrate:** NMT 33%

**Tetrahydrate:** NMT 43%

■ 2S (FCC7)

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

<b>Topic/Question</b>	<b>Contact</b>	<b>Expert Committee</b>
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(FI2010) Monographs - Food Ingredients

## BRIEFING

**Maritime Pine Extract.** Because there is no existing FCC monograph for this ingredient, a new monograph is proposed based on comments and data received and based on the Maritime Pine Extract monograph in *USP 32–NF 27*.

- Methods given for analysis of *Arsenic, Cadmium, Lead, and Mercury* reference a new test for *Elemental Impurities by ICP in Appendix IIIC*. The *Elemental Impurities by ICP* section is based on *Elemental Impurities—Procedures* 〈 233 〉, appearing in *Pharmacopeial Forum* 36(1) [Jan.–Feb. 2010]. The limits proposed for these elemental impurities are consistent with those proposed in *Elemental Contaminants in Dietary Supplements* 〈 2232 〉, also appearing in *PF* 36(1). Data submitted to support the limits for arsenic, lead, cadmium, and mercury in this new monograph proposal was generated using an ICP-MS method—See briefing under *Appendix III: Chemical Tests and Determinations*.
- The monograph for Maritime Pine Extract in *USP 32–NF 27* contains specifications and methods for analysis of pesticide residues. The *Description* informs users who choose to test their ingredient for pesticide residues that a new method is available in *Pesticide Residues* under *Appendix XIII*, but such testing is not required at this time. Comments regarding the voluntary testing for pesticide residues are encouraged—See briefing under *Appendix XIII: Adulterants and Contaminants in Food Ingredients*.
- The microbiological criteria included in the *USP 32–NF 27* monograph have been omitted in the proposed FCC monograph. FCC's policy on microbiological limits can be found in *FCC 5* on page 3, and includes the following statement: "The General Policy for microbiological safety and quality of FCC substances is such that substances be produced, handled, and used in food processing following GMPs and applicable food safety systems. Therefore, the FCC does not list specific microbiological criteria for FCC substances other than those for which scientifically valid data are available to the committee that support the need for such criteria." Comments regarding the omission of microbial specifications are encouraged.

(FIEC: K. Laurick) C87416

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### **Add the following:**

#### ■ **Maritime Pine Extract**

*Pinus pinaster* Extract

#### **DESCRIPTION**

Maritime Pine Extract occurs as a fine, brown powder. It is extracted and purified from pulverized Maritime Pine (*Pinus pinaster*) using suitable solvents. While not a requirement for this monograph, users interested in analyzing this ingredient for potential pesticide residues may use the informational method found in *Pesticide Residues* under Appendix XII.

**Function:** Source of procyanidins and other phenolic compounds

**Packaging and Storage:** Store in a tight, light-resistant container protected from excessive heat.

#### **IDENTIFICATION**

##### • **A. PROCEDURE**

**Sample solution:** Dissolve 50 mg of sample in 6 mL of a mixture of butanol and hydrochloric acid (95:5).

**Analysis:** Heat the *Sample solution* in a boiling water bath for 2 min.

**Acceptance criteria:** The *Sample solution* so treated turns dark red.

• **B. PROCEDURE**

**Solution A:** Methanol

**Solution B:** 0.1% phosphoric acid in water (w/v)

**Mobile phase:** See gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	8	92
40	34	66
45	2	98
50	2	98
52	8	92
57	8	92

**Standard solution:** 2 mg/mL of USP Maritime Pine Extract RS in *Solution A*. Pass through a membrane filter having a 0.45- $\mu$ m or finer porosity.

**Sample solution:** Add 20 mg of sample to 10 mL of *Solution A* and sonicate for 10 min to dissolve. Pass through a membrane filter having a 0.45- $\mu$ m or finer porosity, discarding the first 4 mL of filtrate.

**Chromatographic system,** Appendix IIA

**Mode:** High-performance liquid chromatography

**Detector:** UV 280 nm

**Column:** 4.6-mm  $\times$  15-cm; contains base-deactivated octylsilane bonded to porous silica particles of less than 5- $\mu$ m<sup>1</sup>

**Temperature:** 40 $^{\circ}$

**Flow rate:** 1 mL/min

**Injection size:** 10  $\mu$ L

**System suitability**

[Note—The chromatogram obtained is similar to the Reference Chromatogram provided with the USP Maritime Pine Extract RS.]

**Sample:** *Standard solution*

**Suitability requirement 1:** The resolution between taxifolin and ferulic acid is NLT 3.0.

**Suitability requirement 2:** The tailing factor for taxifolin is NMT 2.0.

**Analysis:** Separately inject the *Sample solution* and the *Standard solution* into the chromatograph, record the chromatograms, and identify the peaks for catechin, caffeic acid, taxifolin, and ferulic acid by comparing the chromatogram of the *Standard solution* with the Reference Chromatogram provided with the USP Maritime Pine Extract RS.

**Acceptance criteria:** The chromatogram of the *Sample solution* exhibits peaks for catechin, caffeic acid, taxifolin, and ferulic acid at the retention times corresponding to those in the chromatogram of the *Standard solution*.

• **C. THIN-LAYER CHROMATOGRAPHY,** Appendix IIA

**Sample solution:** 25 mg/mL in methanol

**Standard solution A:** 25 mg/mL of USP Maritime Pine Extract RS in methanol

**Standard solution B:** 1 mg/mL each of USP Ferulic Acid RS<sup>1</sup> and USP Protocatechuic Acid RS<sup>2</sup> in methanol

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 5  $\mu$ L

**Developing solvent system:** Methylene chloride, methanol, glacial acetic acid, and water (80:15:2:2)

**Spray reagent:** 5% ferric chloride in methanol

**Analysis:** Develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, dry at 110°, and examine under short-wavelength and long-wavelength UV light. The chromatograms of *Standard solution A* and *Standard solution B* exhibit bands in the middle third and upper third that correspond to protocatechuic acid and ferulic acid, respectively. Spray the plate with the *Spray reagent*, and heat at 115° for 15 min. The bands due to ferulic acid and protocatechuic acid turn grayish green. Grayish-green bands become visible in the chromatogram of *Standard solution A* above and below protocatechuic acid, indicating the presence of caffeic acid and catechin, respectively.

**Acceptance criteria:** The chromatogram of the *Sample solution* exhibits bands due to catechin, protocatechuic acid, caffeic acid, and ferulic acid that correspond in color and R<sub>F</sub> values to those in the chromatograms of *Standard solution A* and *Standard solution B*.

• **D. THIN-LAYER CHROMATOGRAPHY**, Appendix IIA

**Sample solution:** Use the *Sample solution* prepared as directed in *Identification test C*.

**Standard solution:** Use *Standard solution A* prepared as directed in *Identification test C*.

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 5 µL

**Developing solvent system:** Ethyl acetate, formic acid, and water (50:5:3)

**Spray reagent:** Phosphoric acid and alcohol (1:1), containing 1% of vanillin

**Analysis:** Develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate and dry it with the aid of a current of air.

Spray the plate with the *Spray reagent* and heat at 115° for 15 min. Three red bands appear in the middle third of the chromatogram of the *Standard solution* corresponding to two dimeric procyanidins and catechin. The chromatogram of the *Standard solution* also exhibits a blue band between the upper band due to upper dimeric procyanidins and the band due to catechin.

**Acceptance criteria:** The chromatogram of the *Sample solution* contains bands that correspond to those found in the chromatogram of the *Standard solution*.

## ASSAY

• **PROCYANIDINS**

**Solution A:** Butanol and hydrochloric acid (95:5) [Note—Prepare this solution on the day of use.]

**Solution B:** Dissolve 2 g of ferric ammonium sulfate in a mixture of 100 mL of water and 17.5 mL of hydrochloric acid. [Note—This solution can be used within 15 days of preparation.]

**Standard solution:** 95 µg/mL of procyanidins from USP Maritime Pine Extract RS in methanol

**Sample solution:** 0.125 mg/mL in methanol

**Analysis:** Transfer 1.0 mL each of the *Standard solution*, *Sample solution*, and methanol to three 10-mL vials. To each vial add 6.0 mL of *Solution A* and 0.25 mL of *Solution B*. Seal the vials with crimp caps. Mix, and heat the vials in a boiling water bath for 40 min. Quickly cool to room temperature in an ice bath. Transfer each solution, with the aid of *Solution A*, to three separate 10-mL volumetric flasks, and dilute with *Solution A* to volume.

Using a suitable spectrophotometer, determine the absorbance of the three solutions at 551 nm, using the methanol-containing solution as a blank. Calculate the percentage of total procyanidins in the portion of the sample taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

- $A_U$  = absorbance of the solution from the *Sample solution*  
 $A_S$  = absorbance of the solution from the *Standard solution*  
 $C_S$  = concentration of the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = concentration of the *Sample solution* ( $\mu\text{g/mL}$ )

**Acceptance criteria:** 65%–75%, calculated on the dried basis

## IMPURITIES

### Inorganic Impurities

- **ARSENIC**, *Elemental Impurities by ICP*, Appendix IIIC  
**Acceptance criteria:** NMT 1.5 mg/kg
- **CADMIUM**, *Elemental Impurities by ICP*, Appendix IIIC  
**Acceptance criteria:** NMT 0.5 mg/kg
- **LEAD**, *Elemental Impurities by ICP*, Appendix IIIC  
**Acceptance criteria:** NMT 1.0 mg/kg
- **MERCURY**, *Elemental Impurities by ICP*, Appendix IIIC  
**Acceptance criteria:** NMT 1.5 mg/kg

## SPECIFIC TESTS

- **ASH (TOTAL)**, Appendix IIC  
**Analysis:** Proceed as directed, heating the material to  $675 \pm 25^\circ$ .  
**Acceptance criteria:** NMT 0.7%
  - **LOSS ON DRYING**, Appendix IIC ( $110^\circ$  for 3 h)  
**Sample:** 1.0 g  
**Acceptance criteria:** NMT 8.0%
  - **WATER-INSOLUBLE SUBSTANCES**  
**Sample solution:** Add 0.5 g of sample to 50 mL of water at  $20^\circ$  and stir for 15 min.  
**Analysis:** Pass the *Sample solution* through a fine sintered glass filter, previously weighed. Dry the filter at  $110^\circ$  for 3 h, cool to room temperature, and weigh the filter. Calculate the amount of water-insoluble substances in the sample taken.  
**Acceptance criteria:** NMT 10%
- 2S (FCC7)

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<sup>1</sup> Zorbax SB-C8, or equivalent.

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<sup>1</sup> USP Ferulic Acid RS is *trans*-4-hydroxy-3-methoxycinnamic acid.

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<sup>2</sup> USP Protocatechuic Acid RS is 3,4-dihydroxybenzoic acid.

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

Topic/Question	Contact	Expert Committee
Monograph	Kristie Laurick Scientific Liaison 1-301-816-8356	(F12010) Monographs - Food Ingredients

## BRIEFING

**Mineral Oil, High Viscosity.** Because there is no existing *FCC* monograph for this food ingredient, a new monograph is proposed based on the Mineral Oil (High Viscosity) monograph prepared at the 44th Session (1995) of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and on the basis of data supplied. Suggestions related to the current test for polynuclear hydrocarbons determination and possible alternatives are especially welcomed. Interested parties are encouraged to submit comments.

(FIEC: C. Mejia) C89019

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### ***Add the following:***

#### ■ **Mineral Oil, High Viscosity**

Liquid Petrolatum

Liquid Paraffin

CAS: [8012-95-1]

905a

### **DESCRIPTION**

Mineral Oil, High Viscosity occurs as a colorless, transparent, oily liquid, free or nearly free from fluorescence. It is a mixture of refined liquid hydrocarbons, essentially paraffinic and naphthenic in nature, obtained from petroleum by solvent extraction and/or crystallization with subsequent purification by acid treatment and/or hydrogen treatment. It has an initial boiling point above 350°. Its average molecular weight is not less than 500. It is insoluble in water and sparingly soluble in alcohol, soluble in volatile oils, and miscible with most fixed oils, but not with castor oil. It may contain any antioxidant permitted in food, in an amount not greater than that required to produce its intended effect.

**Function:** Lubricant; release agent; protective coating

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

### **IDENTIFICATION**

#### • **CARBON NUMBER AT 5% DISTILLATION POINT<sup>1</sup>**

[Note—"Carbon Number" is the number of carbon atoms in a molecule.]

**System suitability solution:** 1% each of hexadecane and octadecane in *n*-octane

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

**Chromatographic system,** Appendix IIA

[Note—Use a suitable gas chromatograph. Typical conditions that may be used in the system are identified below.]

**Mode:** Gas chromatography

**Detector:** Flame ionization

**Packed column:** 5% SE-30 or equivalent

**Carrier gas:** Helium

**Column temperature:** 10°–350°, at a rate of 6.5°/min

**Detector temperature:** 370°

**Injection block temperature:** 370°

**System suitability**

**Suitability requirement 1:** The peak height for the dodecane peak is NLT 10% of full scale under the conditions used for the *Calibration solution*.

**Suitability requirement 2:** The baseline drift, when the chromatographic system is operated at the required sensitivity level to meet *Suitability requirement 1*, is NMT 1% of full scale per h for the *Calibration solution*.

**Suitability requirement 3:** Retention times have a repeatability of NMT 6 s for each component of the *Calibration solution*.

**Suitability requirement 4:** The resolution, R, determined for the *System suitability solution* is between 3 and 8 when calculated by:

$$R = 2d/(W_1 + W_2)$$

d = distance between the peak maxima of hexadecane and octadecane (mm)

W<sub>1</sub> = hexadecane peak width at baseline (mm)

W<sub>2</sub> = octadecane peak width at baseline (mm)

**Calibration curve:** Cool the column to the selected starting temperature (the retention time for the initial boiling point must be NLT 1 min) and inject the *Calibration solution*. For each component, record the retention time of the peak maximum and the peak areas. Plot the retention time of each peak versus the corresponding normal boiling point of that component, in C°, to obtain a calibration curve.

**Analysis:** Using the conditions for the *Calibration curve*, inject a sample. Record the area of each time segment at fixed time intervals NMT 1% of the retention time equivalent to a boiling point of 538° obtained from the *Calibration curve*.

Sum the area segments to obtain the cumulative area at each time interval during the run. At the point of the chromatogram where the baseline at the end first becomes steady, observe the cumulative area counts. Move back along the record until a cumulative area equal to 99.5% of the total at the steady point appears. Mark this point as the final boiling point. Observe the area counts at the start of the run until the point is reached where the cumulative area count is equal to 0.5% of the total area. Mark this point as the initial boiling point of the sample. Divide the cumulative area at each interval between the initial and final boiling points by the total cumulative area and multiply by 100. This will give the cumulative percent of the sample recovered at each time interval. Tabulate the cumulative percent recovered at each interval and the retention time at the end of the interval. Using linear interpolation, if necessary, determine the retention time associated with 5% and read the corresponding boiling temperature from the *Calibration curve*.

**Acceptance criteria:** The carbon number is NLT 28 at 5% distillation. The boiling point at 5% distillation is higher than 422°.

## IMPURITIES

### Inorganic Impurities

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

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

**SPECIFIC TESTS**• **READILY CARBONIZABLE SUBSTANCES**

**Chromic acid cleaning mixture:** Dissolve 200 g of sodium dichromate in 100 mL of water to which 1500 mL of sulfuric acid has been added, slowly with stirring.

**Sample:** 5 mL

**Control solution:** Mix 3 mL of ferric chloride CS, 1.5 mL of cobaltous chloride CS, and 0.5 mL of cupric sulfate CS in a glass-stoppered test tube that previously has been rinsed with *Chromic acid cleaning mixture*, then rinsed with water, and dried. Overlay this mixture with 5 mL of mineral oil.

**Analysis:** Place the *Sample* in a glass-stoppered test tube that previously has been rinsed with *Chromic acid cleaning mixture*, then rinsed with water, and dried. Add 5 mL of 94.5% to 94.9% sulfuric acid; while simultaneously starting a stopwatch, place the tube in a boiling water bath. After the test tube has been in the bath for 30 s, use a 3-s time span to remove it, and while holding the stopper in place, give three vigorous vertical shakes over an amplitude of about 5 in, then return it to the bath. Repeat every 30 s until exactly 10 min has passed, then remove the test tube.

**Acceptance criteria:** The *Sample* remains unchanged in color, and the acid does not become darker than the standard color of the *Control solution*.

• **SPECIFIC GRAVITY** Determine by any reliable method (see *General Provisions*).

**Acceptance criteria:** NLT that stated, or within the range claimed by the vendor

• **ULTRAVIOLET ABSORBANCE (POLYNUCLEAR HYDROCARBONS)**

**Hexane:** Use a pure grade of hexane (predominantly *n*-hexane and methylcyclopentane) having an ultraviolet absorbance not exceeding 0.10 down to 220 nm and not exceeding 0.02 down to 260 nm. The purity should be such that the *Solvent control* as defined below, has an absorbance curve, compared to water, showing no extraneous impurity peaks and no absorbance exceeding that of dimethyl sulfoxide, compared to water, at any wavelength in the range 260–400 nm, inclusive. If necessary to obtain the prescribed purities, the hexane may be passed through activated silica gel.

**Dimethyl sulfoxide:** Use a pure grade of dimethyl sulfoxide (99.9%, melting point: 18°) that has a clear, water-white appearance; has an absorbance curve, compared with water, not exceeding 1.0 at 264 nm; and shows no extraneous impurity peaks in the wavelength range up to 400 nm. Store in glass-stoppered bottles.

**Apparatus:** Use 125-mL glass-stoppered separatory funnels equipped with tetrafluoroethylene polymer stopcocks or other suitable stopcocks that will not contaminate the solvents.

**Sample preparation:** Transfer 25 mL of the sample and 25 mL of *Hexane* to a separatory funnel and mix. Add 5.0 mL of *Dimethyl sulfoxide*, shake the mixture vigorously for at least 1 min, and allow it to stand until the lower layer is clear. Completely transfer the lower layer to a second separatory funnel, add 2 mL of *Hexane*, and shake the mixture vigorously. Allow it to stand until the lower layer is clear, and then draw off the lower layer, designated as the *Sample preparation*.

**Solvent control:** In a separatory funnel, vigorously shake 5.0 mL of *Dimethyl sulfoxide* with 25 mL of *Hexane* for at least 1 min, allow it to stand until the lower layer is clear, and draw off this layer, designated as *Solvent control*.

**Standard solution:** Use a standard reference solution of naphthalene (National Institute of Standards and Technology, Standard Material No. 577 or a solution of equivalent purity) containing a concentration of 7.0 mg/mL in purified isooctane.

**Analysis:** Determine the absorbance of the *Sample preparation* and the *Solvent control* in a 1-cm cell in the range 260–400 nm, inclusive.

Determine the absorbance of the *Standard solution* at 275 nm measured against isooctane of the same spectral purity in 1-cm cells. (The absorbance will be approximately 0.30.)

[Note—Make suitable corrections of the absorbance when testing samples containing added

antioxidants.]

**Acceptance criteria:** The absorbance of the *Sample preparation* does not exceed that of the *Solvent control* at any wavelength in the specified range by more than one-third of the absorbance of the *Standard solution*.

- **Viscosity:** Determine by any reliable method (see Appendix IIB).

**Acceptance criteria:** NLT 11 centistokes at 100<sup>o</sup>

■ 2S (FCC7)

<sup>1</sup> As determined by ASTM D2887 Standard Test Method for Boiling Range Distribution of Petroleum Fractions by Gas Chromatography. The original ASTM method is available in its entirety from ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428. Phone: 610-832-9555; email: [service@astm.org](mailto:service@astm.org); website: [www.astm.org](http://www.astm.org).

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

Topic/Question	Contact	Expert Committee
Monograph	Carla D Mejia, Ph.D. Scientific Liaison 1-301-816-8571	(F12010) Monographs - Food Ingredients

## BRIEFING

### Mineral Oil, Medium and Low Viscosity, FCC 7 page 692.

- 1 On the basis of comments received, it is proposed to revise the specifications for molecular weight, carbon number at 5% distillation, and viscosity. The new proposed limits are consistent with the monograph for *Mineral Oil, Medium and Low Viscosity* prepared at the 59<sup>th</sup> Session of the FAO/WHO Joint Expert Committee on Food Additives (2002).
- 2 A new monograph is also proposed in this *Forum* to address mineral oil with different attributes, which cannot be included in this document.
- 3 A new wavelength range is also proposed in the ultraviolet absorbance analysis for polynuclear hydrocarbons, in an effort to harmonize the current method with the monograph by the Joint Expert Committee on Food Additives.

Interested parties are encouraged to submit comments. Suggestions related to the current test for polynuclear hydrocarbons determination and possible alternatives are especially welcomed. Submit comments to Carla Mejia at [cdm@usp.org](mailto:cdm@usp.org)  
(FIEC: C. Mejia) C88510

### Mineral Oil, ~~White~~ Medium and Low Viscosity ■ 2S (FCC7)

Liquid Petrolatum

Liquid Paraffin

■

White Mineral Oil

■ 2S (FCC7)

CAS: [8042-47-5]

## DESCRIPTION

### **Change to read:**

Mineral Oil, ~~White~~ Medium and Low Viscosity ■ 2S (FCC7) occurs as a colorless, transparent, oily liquid, free or nearly free from fluorescence. It is a mixture of refined liquid hydrocarbons, essentially paraffinic and naphthenic in nature, obtained from petroleum by solvent extraction and/or crystallization with subsequent purification by acid treatment and/or hydrogen treatment. It has an initial boiling point above 200°. Its average molecular weight is not less than 300. Its minimum carbon number at 5% distillation is 17 ■ between 300 and 500. ■ 2S (FCC7) It is insoluble in water and in alcohol, is soluble in volatile oils, and is miscible with most fixed oils, but not with castor oil. It may contain any antioxidant permitted in food, by the U.S. Food and Drug Administration ■ 2S (FCC7) in an amount not greater than that required to produce its intended effect.

**Function:** Defoaming agent; lubricant; ■ 2S (FCC7) release agent; ~~protective coating;~~ ■ 2S (FCC7) glazing agent; sealing agent

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

## IDENTIFICATION

### Add the following:

- **CARBON NUMBER AT 5% DISTILLATION POINT<sup>1</sup>**

[Note—"Carbon Number" is number of carbon atoms in a molecule.]

**System suitability solution:** 1% each of hexadecane and octadecane in *n*-octane.

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

**Chromatographic system,** Appendix IIA

[Note—Use a suitable gas chromatograph. Typical conditions that may be used in the system are identified below.]

**Mode:** Gas chromatography

**Detector:** Flame-ionization

**Packed column:** 5% SE-30 or equivalent

**Carrier gas:** Helium

**Temperature**

**Column:** 10<sup>o</sup>-350<sup>o</sup>, at a rate of 6.5<sup>o</sup>/min

**Detector:** 370<sup>o</sup>

**Injection block:** 370<sup>o</sup>

**System suitability**

**Suitability requirement 1:** The peak height for the dodecane peak is NLT 10% of full scale under the conditions used for the *Calibration solution*.

**Suitability requirement 2:** The baseline drift, when the *Chromatographic system* is operated at the required sensitivity level to meet *Suitability requirement 1*, is NMT 1% of full scale per hour for the *Calibration solution*.

**Suitability requirement 3:** Retention times have a repeatability of NMT 6 s for each component of the *Calibration solution*.

**Suitability requirement 4:** The resolution, *R*, determined for the *System suitability solution* is between 3 and 8 when calculated by:

$$R = 2d/(W_1 + W_2)$$

*d* = distance between the peak maxima of hexadecane and octadecane (mm)

*W*<sub>1</sub> = hexadecane peak width at baseline (mm)

*W*<sub>2</sub> = octadecane peak width at baseline (mm)

**Calibration curve:** Cool the column to the selected starting temperature (the retention time for the initial boiling point must be NLT 1 min) and inject the *Calibration solution*. Record for each component the retention time of each peak maximum and the peak areas. Plot the retention time of each peak versus the corresponding normal boiling point of that component, in <sup>o</sup>C, to obtain a calibration curve.

**Analysis:** Using the conditions for the *Calibration curve*, inject a sample. Record the area of each time segment at fixed time intervals NMT 1% of the retention time equivalent to a boiling point of 538<sup>o</sup> obtained from the *Calibration curve*.

Sum the area segments to obtain the cumulative area at each time interval during the run. At the point of

the chromatogram where the baseline at the end first becomes steady, observe the cumulative area counts. Move back along the record until a cumulative area equal to 99.5% of the total at the steady point appears. Mark this point as the final boiling point. Observe the area counts at the start of the run until the point is reached, where the cumulative area count is equal to 0.5% of the total area. Mark this point as the initial boiling point of the sample. Divide the cumulative area at each interval between the initial and final boiling points by the total cumulative area and multiply by 100. This will give the cumulative percent of the sample recovered at each time interval. Tabulate the cumulative percent recovered at each interval and the retention time at the end of the interval. Using linear interpolation, if necessary, determine the retention time associated with 5% and read the corresponding boiling temperature from the *Calibration curve*.

**Acceptance criteria:** Carbon number is between 17 and 25 at 5% distillation. The boiling point at the 5% distillation is between 287<sup>o</sup> and 422<sup>o</sup>. ■ 2S (FCC7)

## IMPURITIES

### Inorganic Impurities

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

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

## SPECIFIC TESTS

- **READILY CARBONIZABLE SUBSTANCES**

**Chromic acid cleaning mixture:** Dissolve 200 g of sodium dichromate in 100 mL of water to which 1500 mL of sulfuric acid has been added, slowly with stirring.

**Sample:** 5 mL

**Control solution:** Mix 3 mL of ferric chloride CS, 1.5 mL of cobaltous chloride CS, and 0.5 mL of cupric sulfate CS in a glass-stoppered test tube that previously has been rinsed with *Chromic acid cleaning mixture*, then rinsed with water, and dried. Overlay this mixture with 5 mL of mineral oil.

**Analysis:** Place the *Sample* in a glass-stoppered test tube that previously has been rinsed with *Chromic acid cleaning mixture*, then rinsed with water, and dried. Add 5 mL of 94.5% to 94.9% sulfuric acid; while simultaneously starting a stopwatch, place the tube in a boiling water bath. After the test tube has been in the bath for 30 s, use a 3-s time span to remove it, and while holding the stopper in place, give three vigorous vertical shakes over an amplitude of about 5 in, then return it to the bath. Repeat every 30 s until exactly 10 min has passed, then remove the test tube.

**Acceptance criteria:** The *Sample* remains unchanged in color, and the acid does not become darker than the standard color of the *Control solution*.

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

**Acceptance criteria:** NLT that stated, or within the range claimed by the vendor

### *Change to read:*

- **ULTRAVIOLET ABSORBANCE (POLYNUCLEAR HYDROCARBONS)**

**Hexane:** Use a pure grade of hexane (predominantly *n*-hexane and methylcyclopentane) having an ultraviolet absorbance not exceeding 0.10 down to 220 nm and not exceeding 0.02 down to 260 nm. The purity should be such that the *Solvent control* as defined below, has an absorbance curve, compared to water, showing no extraneous impurity peaks and no absorbance exceeding that of dimethyl sulfoxide, compared to water, at any wavelength in the range 260 to 350 ■ 420 ■ 2S (FCC7) nm, inclusive. If necessary to obtain the prescribed purities, the hexane may be passed through activated silica gel.

**Dimethyl sulfoxide:** Use a pure grade of dimethyl sulfoxide (99.9%, melting point: 18°) that has a clear, water-white appearance; has an absorbance curve, compared with water, not exceeding 1.0 at 264 nm; and shows no extraneous impurity peaks in the wavelength range up to 350–420 nm (FCC7). Store in glass-stoppered bottles.

**Apparatus:** Use 125-mL glass-stoppered separatory funnels equipped with tetrafluoroethylene polymer stopcocks or other suitable stopcocks that will not contaminate the solvents.

**Sample preparation:** Transfer 25 mL of the sample and 25 mL of *Hexane* to a separatory funnel and mix. Add 5.0 mL of *Dimethyl sulfoxide*, shake the mixture vigorously for at least 1 min, and allow it to stand until the lower layer is clear. Completely transfer the lower layer to a second separatory funnel, add 2 mL of *Hexane*, and shake the mixture vigorously. Allow it to stand until the lower layer is clear, and then draw off the lower layer, designated as the *Sample preparation*.

**Solvent control:** In a separatory funnel, vigorously shake 5.0 mL of *Dimethyl sulfoxide* with 25 mL of *Hexane* for at least 1 min, allow it to stand until the lower layer is clear, and draw off this layer, designated as *Solvent control*.

**Standard solution:** Use a standard reference solution of naphthalene (National Institute for Standards and Technology Standard Material No. 577, or a solution of equivalent purity) containing a concentration of 7.0 mg per 1000 mL in purified isooctane.

**Analysis:** Determine the absorbance of the *Sample preparation* and the *Solvent control* in a 1-cm cell in the range 260 to 350–420 nm (FCC7), inclusive.

Determine the absorbance of the *Standard solution* at 275 nm measured against isooctane of the same spectral purity in 1-cm cells. (The absorbance will be approximately 0.30.)

[Note—Make suitable corrections of the absorbance when testing samples containing added antioxidants.]

**Acceptance criteria:** The absorbance of the *Sample preparation* does not exceed that of the *Solvent control* at any wavelength in the specified range by more than one-third of the absorbance of the *Standard solution*.

**Change to read:**

- **Viscosity:** Determine by any reliable method (see Appendix IIB).

**Acceptance criteria:** NLT-3 Between 3 and 11 cSt (FCC7) at 100°

<sup>1</sup> As determined by ASTM D2887 Standard Test Method for Boiling Range Distribution of Petroleum Fractions by Gas Chromatography. The original ASTM method is available in its entirety from ASTM International, 100 Barr Harbor Drive, West Conshohocken, PA 19428. Phone: 610-832-9555, email: service@astm.org, website: www.astm.org

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

Topic/Question	Contact	Expert Committee
Monograph	Carla D Mejia, Ph.D. Scientific Liaison 1-301-816-8571	(F12010) Monographs - Food Ingredients

**BRIEFING**

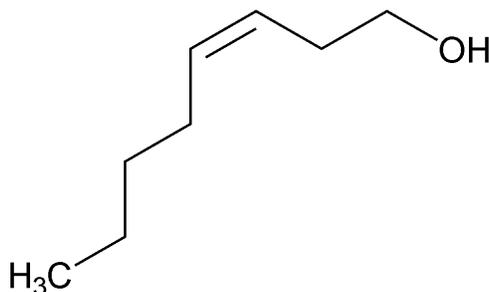
**(Z)-3-Octen-1-ol**, FCC 7 page 736. On the basis of comments and data received, it is proposed to revise the tests for *Refractive Index* and *Specific Gravity*. Since the degree of purity of the material currently commercially available is higher, the ranges for its specifications need to reflect the current higher level of purity. Additional comments and data are encouraged, especially those related to the Assay acceptance criteria.

(FIEC: C. Mejia) C89494

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**(Z)-3-Octen-1-ol**

*cis*-3-Octen-1-ol



C<sub>8</sub>H<sub>16</sub>O

Formula wt 128.21  
FEMA: 3467

**DESCRIPTION****Change to read:**

(Z)-3-Octen-1-ol occurs as a white colorless liquid (FCC7) to slightly yellow liquid.

**Odor:** Musty, mushroom

**Solubility:** Insoluble or practically insoluble in water

**Boiling Point:** -174°

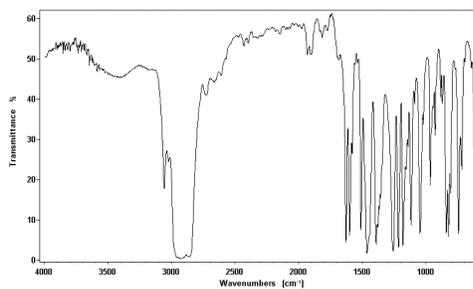
**Solubility in Alcohol**, Appendix VI: One mL dissolves in 1 mL of 95% ethanol.

**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.



(Z)-3-Octen-1-ol

**ASSAY**

- **Procedure:** Proceed as directed under *M-1a*, Appendix XI (as (Z)-isomer).  
**Acceptance criteria:** NLT 95.0% of C<sub>8</sub>H<sub>16</sub>O

**SPECIFIC TESTS**

*Change to read:*

- **Refractive Index**, Appendix II: At 20°  
**Acceptance criteria:** Between ~~1.440 and 1.446~~ 1.444 and 1.450 ■ 2S (FCC7)

*Change to read:*

- **Specific Gravity:** Determine at 25° by any reliable method (see *General Provisions*).  
**Acceptance criteria:** Between ~~0.830 and 0.850~~ 0.844 and 0.848 ■ 2S (FCC7)

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

Topic/Question	Contact	Expert Committee
Monograph	Carla D Mejia, Ph.D. Scientific Liaison 1-301-816-8571	(F12010) Monographs - Food Ingredients

## BRIEFING

**Patent Blue V.** Because there is no existing FCC monograph for this food ingredient, a new monograph is proposed based on the Patent Blue V monograph from the 69th session (2008) of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and specifications published in the European Union (EU) Commission Directive 2008/128/EC for this color. It should be noted that this color additive is not approved for use in the US, but is approved in other countries.

1. The *Visible Absorption Spectrum* Identification specification is based on that from the EU. Stakeholders are encouraged to submit additional, more modern test procedures to aid in identifying and distinguishing this ingredient from other synthetic color additives.
2. To measure *Total Color*, the JECFA monograph uses a titrimetric procedure while the EU specifies use of a spectrophotometric procedure. The proposed FCC monograph averages the results from both procedures, consistent with other color additive monographs in FCC such as *Fast Green*. It should be noted that a revision to the FCC Total Color method is also being proposed (see Briefing under Appendix III).
3. For *Inorganic Impurities*, it is proposed to use the specifications for chromium and lead from the JECFA monograph and test procedures already in FCC for these two impurities in Appendix III. Stakeholders are encouraged to submit a more modern lead procedure validated for this ingredient.
4. A test procedure and specification for *Uncombined Intermediates and Products of Side Reactions* is proposed based on that in JECFA. Stakeholders are encouraged to submit information on a supplier and appropriate concentration for standards used in this test procedure.
5. Specifications for *Loss on Drying, Chloride, and Sulfates* in the *Combined Tests* section are proposed based on those in JECFA, but using different test procedures—ones already existing in FCC Appendix IIIC for the analysis of colors.
6. Specifications for *Ether Extracts* and *Leuco Base* are proposed based on those in JECFA using FCC test procedures in FCC Appendix IIIC which are equivalent to those used in JECFA.
7. The specifications and test procedures for *Subsidiary Coloring Matters* and *Un sulfonated Primary Aromatic Amines* are based on those in JECFA. Stakeholders are encouraged to submit more modern test procedures, especially for *Subsidiary Coloring Matters*, for consideration in this monograph.
8. The specification for *Water-Insoluble Matter* is based on that in JECFA using a similar test procedure already in FCC Appendix IIIC.

(FIEC: J. Moore) C87152

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### **Add the following:**

#### ■ Patent Blue V<sup>1</sup>

Patent Blue 5

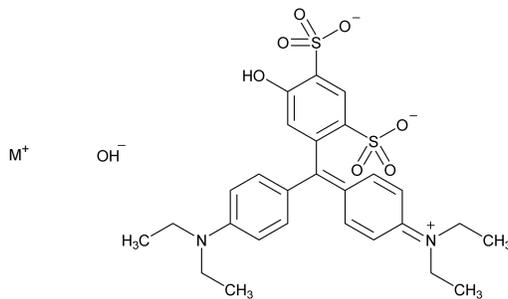
CI Food Blue 5

CI No. 42051

Class: Triarylmethane

Calcium or sodium salt of 2-[4-(4-diethylaminophenyl)(4-diethylimino-2,5-cyclohexadien-1-ylidene)methyl]-4-hydroxy-1,5-benzene disulfonate

Calcium or sodium salt of [4-[*alpha*-(4-diethyl-aminophenyl)-5-hydroxy-2,4-disulfonatophenylmethylidene]-2,5-cyclohexadien-1-ylidene] diethylammonium hydroxide inner salt



M<sup>+</sup> = 1/2 Ca<sup>2+</sup>, or Na<sup>+</sup>

Calcium salt: C<sub>27</sub>H<sub>31</sub>N<sub>2</sub>O<sub>7</sub>S<sub>2</sub>·1/2Ca

Formula wt, calcium salt 579.72

Sodium salt: C<sub>27</sub>H<sub>31</sub>N<sub>2</sub>O<sub>7</sub>S<sub>2</sub>Na

Formula wt, sodium salt 582.67

131

CAS: [3536-49-0]

## DESCRIPTION

Patent Blue V occurs as a blue powder or granules. It is principally the calcium or sodium salt of 2-[4-[*alpha*-(4-diethyl-aminophenyl)-5-hydroxy-2,4-disulfonatophenylmethylidene]-2,5-cyclohexadien-1-ylidene] diethylammonium hydroxide inner salt, and subsidiary coloring matters. Water, sodium chloride, sodium sulfate, calcium chloride, and calcium sulfate can be present as the principal uncolored components. It is soluble in water and slightly soluble in ethanol.

**Function:** Color

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

## IDENTIFICATION

### • VISIBLE ABSORPTION SPECTRUM

**Sample solution:** Dissolve a sample in water and adjust the pH to 5.

**Analysis:** Measure the absorption spectrum of the *Sample solution* using a suitable UV-visible spectrophotometer.

**Acceptance criteria:** The *Sample solution* exhibits a wavelength maximum at 638 nm

## ASSAY

### • TOTAL COLOR, *Colors, Methods I and II, Appendix IIIC* [Note—Both methods must be used.]

**Method I** (Spectrophotometric)

**Sample solution:** 10 mg/mL adjusted to pH 5

**Analysis:** Determine as directed at 638 nm using 0.2 L/(mg·cm) for the absorptivity (*a*) for Patent Blue V.

**Method II** (TiCl<sub>3</sub> Titration)

**Sample:** 1.3–1.4 g

**Analysis:** Determine as directed, except under *Procedure* use 15 g of *Sodium Bitartrate* instead of 21 g

22 g and use 150 mL of water instead of 275 mL. For the calculation, use 34.51 and 34.33 as the stoichiometric factor ( $F_S$ ) for the calcium and sodium salts of Patent Blue V, respectively.

**Acceptance criteria:** The average of results obtained from *Method I* and *Method II* is NLT 85.0% total coloring matters.

## IMPURITIES

### Inorganic Impurities

- **CHROMIUM**, *Colors*, Appendix IIIC

**Acceptance criteria:** NMT 0.005%

- **LEAD**, *Lead Limit Test*, Appendix IIIB

**Sample solution:** Prepare as directed for organic compounds.

**Control:** 2 µg Pb (2 mL of *Diluted Standard Lead Solution*)

**Acceptance criteria:** NMT 2 mg/kg

### Organic Impurities

- **UNCOMBINED INTERMEDIATES AND PRODUCTS OF SIDE REACTIONS**

**Solution A:** 10% (w/v) aqueous acetate buffer pH 4.6 prepared using 1 M sodium hydroxide, 1 M acetic acid, and water (5:10:35)

**Solution B:** Acetonitrile

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)	Flow Rate (mL/min)
0	85	15	1
12	85	15	1
25	20	80	2
28	20	80	2
40	85	15	1

**Sample solution:** 5 mg/mL in 0.02 M ammonium acetate

**Standard solution:** Solution containing 3-hydroxybenzaldehyde, 3-hydroxybenzoic acid, 3-hydroxy-4-sulfonatobenzoic acid, and *N,N*-diethylaminobenzenesulfonic acid in 0.2 M ammonium acetate

**Chromatographic system**, Appendix IIA

**Mode:** High-performance liquid chromatography

**Detector:** UV (254 nm)

**Column:** 25-cm × 4-mm C18 column (7-µm)<sup>1</sup>

**Temperature:** Ambient

**Flow rate:** See gradient table, above

**Injection volume:** 20 µL

**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. Calculate the percents of all four impurities (3-hydroxybenzaldehyde, 3-hydroxybenzoic acid, 3-hydroxy-4-sulfonatobenzoic acid, and *N,N*-diethylaminobenzenesulfonic acid) in the sample taken by the equation:

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

- $r_U$  = peak area response for the analyte in the *Sample solution*  
 $r_S$  = peak area response for the analyte in the *Standard solution*  
 $C_S$  = concentration of analyte in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of sample in the *Sample solution* (mg/mL)

#### Acceptance criteria

**3-hydroxybenzaldehyde, 3-hydroxybenzoic acid, 3-hydroxy-4-sulfonatobenzoic acid, and *N,N*-diethylaminobenzenesulfonic acid:** NMT 0.5%, combined

### SPECIFIC TESTS

#### • COMBINED TESTS

##### Tests

- Loss on Drying (Volatile Matter), *Colors*, Appendix IIIC
- Chloride, *Sodium Chloride*, *Colors*, Appendix IIIC
- Sulfates (as sodium salts), *Sodium Sulfate*, *Colors*, Appendix IIIC

**Acceptance criteria:** NMT 15.0%, combined as the sum of all three tests

#### • ETHER EXTRACTS, *Colors*, Appendix IIIC

**Acceptance criteria:** NMT 0.2%

#### • LEUCO BASE, *Colors*, Appendix IIIC

**Sample solution:** 110 µg/mL

**Analysis:** Proceed as directed using an absorptivity constant,  $a$ , of 0.200 L/(mg·cm) at 638 nm for Patent Blue V. For the ratio of the molecular weight of colorant and leuco base,  $r$ , use 0.95960 (582.15/606.66) and 0.96401 (579.14/600.76) for the sodium and calcium salts, respectively.

**Acceptance criteria:** NMT 4%

#### • SUBSIDIARY COLORING MATTERS

[Note—In this method, subsidiary coloring matters are separated from the main coloring matter of Patent Blue V by ascending paper chromatography (see *Paper Chromatography*, Appendix IIA), and extracted separately from the chromatographic paper. The absorbance of each extract is measured at the wavelength of maximum absorption for Patent Blue V (638 nm) by visible spectrophotometry. Because it is impractical to identify each subsidiary coloring matter using this procedure, and because the subsidiary coloring matters are usually minor components of food colors, the method assumes that the maximum absorbance of each subsidiary coloring matter is the same as that of the total coloring matters. The subsidiary color matters content is calculated by adding together the absorbances of the extracts in conjunction with the total coloring matters content of the sample.]

**Chromatographic apparatus:** The chromatography tank (*Figures 1 and 2*) is comprised of a glass tank (A) and cover (B); frame to support chromatography paper (C); solvent tray (D); secondary frame (E) for supporting "drapes" of the filter paper; and 20-cm × 20-cm chromatography grade paper<sup>2</sup>. Mark out the chromatography paper as shown in *Figure 3*.

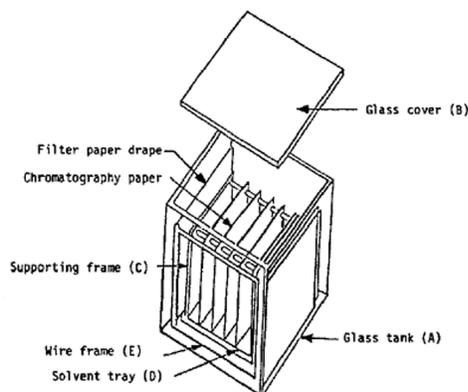


Figure 1. Assembly of the Chromatographic Apparatus

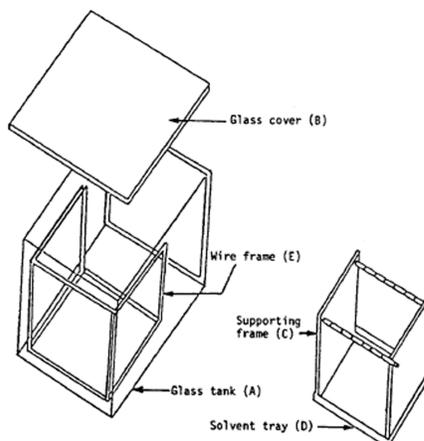


Figure 2. Components of the Chromatographic Apparatus

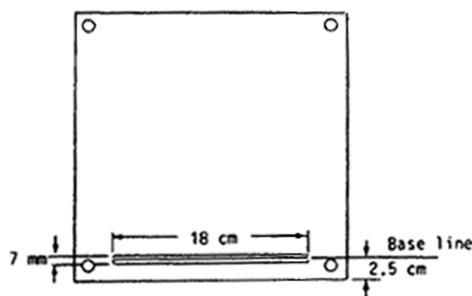


Figure 3. Method for Marking the Chromatographic Paper

**Chromatographic solvent:** Prepare of mixture of *n*-butanol:water:ethanol:ammonia (s.g. 0.880) (600:264:135:6). Shake for 2 min, allow the layers to separate, and use the upper layer as the chromatographic solvent.

**Sample solution:** 10 mg/mL sample

**Standard solution:** 0.2 mg/mL sample prepared by diluting the *Sample solution*

**Application volume:** 0.10 mL

**Analysis:** No less than 2 h before analysis, arrange the filter-paper drapes in the glass tank and pour sufficient *Chromatographic solvent* over the drapes and into the bottom of the tank to cover the bottom of the tank to a depth of 1 cm. Place the solvent tray in position and fit the cover to the tank. Using a microsyringe capable of delivering 0.1 mL with a tolerance of  $\pm 0.002$  mL, apply to separate chromatograph sheets 0.1 mL aliquots *Sample solution* and *Standard solution*, as uniformly as possible within the confines of the 18-cm  $\times$  7-mm rectangle, holding the nozzle of the microsyringe steadily in contact with

the paper. Allow the papers to dry at room temperature for 1–2 h or at 50° in a drying cabinet for 5 min followed by 15 min at room temperature. Mount the dried sheets, together with two plain sheets to act as a blanks on the supporting frame. [Note—If required, several dried sheets may be developed simultaneously.]

Pour sufficient *Chromatography solvent* into the solvent tray to bring the surface of the solvent about 1 cm below the base line of the chromatography sheets. The volume necessary will depend on the dimensions of the apparatus and should be predetermined. Put the supporting frame into position and replace the cover. Allow the solvent front to ascend approximately 17 cm above baseline, then remove the supporting frame and transfer it to a drying cabinet at 50–60° for 10–15 min. Remove the sheets from the frame.

For the *Sample solution* sheets, cut each subsidiary band from each chromatogram sheet as a strip, and cut an equivalent strip from the corresponding position of the plain (blank) sheet. For the *Standard solution* sheet, cut the entire band from the sheet, and cut an equivalent strip from the corresponding position of the plain (blank) sheet. Place each strip, subdivided into a suitable number of approximately equal portions, in a separate test tube. Add 5.0 mL of water:acetone (1:1 by vol) to each test tube, swirl for 2–3 min, add 15.0 mL of 0.05 N sodium hydrogen carbonate solution, and shake the tube to ensure mixing. Filter the colored extracts and blanks through 9-cm coarse porosity filter papers into clean test tubes and determine the absorbances of the colored extracts at 638 nm using a suitable spectrophotometer with 40-mm closed cells against a filtered mixture of 5.0 mL of water:acetone (1:1 by vol) and 15.0 mL of the 0.05 N sodium hydrogen carbonate solution. Measure the absorbances of the extracts of the blank strips at 638 nm and correct the absorbances of the colored extracts with the blank values. Calculate the percent subsidiary coloring matter in the portion of the sample taken:

$$\text{Result} = 0.2 \times D \times [(A_a + A_b + A_c \dots A_n)/A_s]$$

0.2 = dilution factor for the *Standard solution*

D = total coloring matter content of the sample, determined from the *Total Color* test above and expressed as percent.

$A_s$  = the absorbance from the *Standard solution*

$A_a + A_b + A_c \dots A_n$  = the sum of the absorbances of the subsidiary coloring matters from the *Sample solution*, corrected for the blank values

**Acceptance criteria:** NMT 2%

• **UN SULFONATED PRIMARY AROMATIC AMINES**

[Note—Under the conditions of this test, unsulfonated primary aromatic amines are extracted into toluene from an alkaline solution of the sample, re-extracted into acid, and then determined spectrophotometrically after diazotization and coupling.]

**R salt solution:** 0.05 N 2-naphthol-3,6-disulfonic acid, disodium salt

**Sodium carbonate solution:** 2 N

**Standard stock solution:** Weigh 0.100 g of redistilled aniline into a small beaker and transfer to a 100-mL volumetric flask, rinsing the beaker several times with water. Add 30 mL of 3 N hydrochloric acid and dilute to the mark with water at room temperature. Dilute 10.0 mL of this solution to 100 mL with water and mix well; 1 mL of this solution is equivalent to 0.0001 g of aniline. [Note—Prepare this *Standard stock solution* fresh.]

**Standard solutions:** Separately dilute 5-, 10-, 15-, 20-, and 25-mL aliquots of the *Standard stock solution* to 100 mL with 1 N hydrochloric acid.

**Standard blank solution:** In a 25-mL volumetric flask mix 10.0 mL of 1 N hydrochloric acid, 10.0 mL of

*Sodium carbonate solution*, 2.0 mL of *R salt solution*, and dilute with water to volume.

**Sample solution:** Add 2.0 g of sample into a separatory funnel containing 100 mL of water, rinse down the sides of the funnel with 50 mL of water, swirling to dissolve the sample, and add 5 mL of 1 N sodium hydroxide. Extract with two 50-mL portions of toluene and wash the combined toluene extracts with 10-mL portions of 0.1 N sodium hydroxide to remove traces of color. Extract the washed toluene with three 10-mL portions of 3 N hydrochloric acid and dilute the combined extract with water to 100 mL.

**Sample blank solution:** In a 25-mL volumetric flask mix 10.0 mL of *Sample solution*, 10 mL of *Sodium carbonate solution*, and 2.0 mL of *R salt solution*, and dilute with water to volume.

**Analysis:** Into separate clean dry test tubes, pipet 10.0-mL aliquots of *Sample solution* and each of the *Standard solutions*, cool for 10 min by immersion in a beaker of ice water, add 1 mL of 50% potassium bromide solution and 0.05 mL of 0.5 N sodium nitrite solution. Mix and allow the tubes to stand for 10 min in the ice water bath while the aniline is diazotized. Into each of five 25-mL volumetric flasks, measure 1 mL of *R salt solution* and 10 mL the *Sodium carbonate solution*. Separately pour each diazotized aniline solution into a 25-mL volumetric flask containing *R salt solution* and *Sodium carbonate solution*; rinse each test tube with a few drops of water. Dilute to the mark with water, stopper the flasks, mix the contents well and allow them to stand for 15 min in the dark.

Measure the absorbance of each of the coupled *Standard solutions* at 510 nm using a suitable spectrophotometer with 40-mm cells against the *Standard blank solution* as a blank. Plot a standard curve relating absorbance to weight (g) of aniline in of the *Standard solutions*.

Measure the absorbance of the coupled *Sample solution* at 510 nm using a suitable spectrophotometer with 40-mm cells against the *Sample blank solution* as a blank. From the standard curve, determine the weight (g) of aniline in each 100 mL of the *Sample solution*. Calculate the percent unsulfonated primary aromatic amine (as aniline) in the portion of the sample taken:

$$\text{Result} = W_A/W \times 100$$

$W_A$  = weight of aniline in the *Sample solution* calculated from the standard curve (g/100 mL)

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

**Acceptance criteria:** NMT 0.01%, calculated as aniline

- **WATER-INSOLUBLE MATTER**, *Colors*, Appendix IIIC

**Acceptance criteria:** NMT 0.5%

- 2S (FCC7)

<sup>1</sup> Patent Blue V is approved for use in some countries, but banned in other countries such as the United States and Australia.

<sup>1</sup> LiChrosorb RP 18 (Merck), or equivalent.

<sup>2</sup> Whatman No 1, or equivalent

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

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(F12010) Monographs - Food Ingredients

## BRIEFING

**Partially Hydrolyzed Proteins**, FCC 7 page 761. On the basis of comments received, it is proposed to reference the test procedure *Glutamic Acid, Appendix IIIC* in the test for *Glutamic Acid*, since it is the identical procedure. It is also proposed to clarify the basis for calculating the result in the test for *Glutamic Acid* (see briefing under *Acid Hydrolysates of Protein*).

(FIEC: J. Moore) C86203

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### Partially Hydrolyzed Proteins

Enzyme-Hydrolyzed (Source) Protein

Partially Hydrolyzed (Source) Protein

(Source) Peptone

Enzyme-Modified (Source) Protein

Partial Enzymatic Digest of (Source) Protein

Partial Acid Digest of (Source) Protein

429

### DESCRIPTION

Partially Hydrolyzed Proteins occur as liquid, paste, powder, or granules. They are composed of peptides and polypeptides resulting from the partial or incomplete hydrolysis of peptide bonds present in edible proteinaceous materials catalyzed by heat, food-grade proteolytic enzymes, and/or suitable food-grade acids. Their degree of hydrolysis typically ranges from 3% to 85% on the basis of peptide bond cleavage. During processing, the proteinaceous raw material may be treated with safe and suitable alkaline materials. The edible proteinaceous materials used as raw materials are derived from casein and other milk products such as whey protein; from animal tissue, including gelatin, defatted animal tissue, and egg albumen; from yeast; and from soy protein products, wheat protein products, or other suitable and safe plant sources.

[**CAUTION**— Depending on the protein source and the degree of hydrolysis, partially hydrolyzed proteins may present an allergenic risk to sensitized individuals. ]

**Function:** Binder; dough conditioner; emulsifier and emulsifier salt; flavoring agent; flavor enhancer; nutrient; fermentation aid; surface-active agent; texturizer

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

[Note—All analyses should be calculated on the dried basis, based on a sample previously dried as follows: Evaporate liquid and paste samples to dryness in a suitable tared container on a steam bath, then, as for the powdered and granular forms, dry to constant weight at 105°.]

### ASSAY

- **TOTAL NITROGEN**, *Nitrogen Determination, Appendix IIIC*  
**Acceptance criteria:** NLT 7.0%, calculated on the dried basis

### IMPURITIES

### Inorganic Impurities

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

**Sample:** 10 g

**Acceptance criteria:** NMT 2 mg/kg, calculated on the dried basis

### SPECIFIC TESTS

- **α-AMINO NITROGEN**

**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

**Sample solution:** Transfer 7 to 25 g of the sample into a 500-mL volumetric flask with the aid of several 50-mL portions of warm, ammonia-free water, dilute with water to volume, and mix.

**Analysis:** Neutralize 20.0 mL of the *Sample 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*. 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 (see *General Provisions*), using the same reagents, with 20 mL of water in place of the *Sample solution*. Each mL of 0.2 N barium hydroxide or 0.2 N sodium hydroxide is equivalent to 2.8 mg of α-amino nitrogen.

**Acceptance criteria:** NLT 90.0% and NMT 110.0% of the amount claimed on the label, calculated on the dried basis

- **α-AMINO NITROGEN/TOTAL NITROGEN PERCENT RATIO**

**Analysis:** Calculate by the formula:

$$\text{Result} = 100 \times [(\text{AN} - \text{P})/(\text{TN} - \text{P})]$$

AN = percent α-Amino Nitrogen determined above

TN = percent Total Nitrogen, determined above

P = percent Ammonia Nitrogen, determined below

**Acceptance criteria:** NLT 2.0% and NMT 62.0%, when calculated on an ammonia nitrogen-free and dried basis

- **AMMONIA NITROGEN**

[CAUTION— Provide adequate ventilation. ]

[Note—Use nitrogen-free reagents, where available, or reagents very low in nitrogen content.]

**Methyl red indicator:** 1 g of methyl red in 200 mL of alcohol

**Sample:** 0.700–2.2 g

**Analysis:** Transfer the *Sample* into a 500- to 800-mL Kjeldahl digestion flask of hard, moderately thick, well-annealed glass. [Note—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* 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 (see *General provisions*), substituting 2 g of sucrose for the *Sample*, and make any necessary correction. Each mL of 0.5 N acid consumed is equivalent to 7.003 mg of ammonia nitrogen (A).

[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 mL of 0.1 N acid consumed is equivalent to 1.401 mg of nitrogen.]

Calculate the percent of ammonia nitrogen by the formula:

$$\text{Result} = 100 \times (A/S)$$

A = weight of ammonia nitrogen (mg)

S = weight of the sample taken (mg)

**Acceptance criteria:** NMT 1.5%, calculated on the dried basis

- **ASH (TOTAL)**, Appendix IIC

**Sample:** 1 g

**Acceptance criteria:** NMT 40.0%, calculated on the dried basis

**Change to read:**

- **GLUTAMIC ACID**, ■ Appendix IIIC ■ 2S (FCC7)

**Standard stock solution:** 2.5 mg/mL reagent-grade glutamic acid prepared as follows: Weigh  $1250 \pm 2$  mg of reagent-grade glutamic acid, and place it into a 500-mL volumetric flask. Fill the flask half-full with water, and add 5 mL of hydrochloric acid to help dissolve the amino acid, dilute to volume with water, and mix.

**Standard solution:** Dilute 1 mL of the *Standard stock solution* with 4 mL of 0.2 N sodium citrate, pH 2.2, buffer. This solution contains 0.5 mg glutamic acid per mL.

**Sample solution:** Accurately weigh 5 mg of sample, and dilute it to exactly 5 mL with 0.2 N sodium citrate, pH 2.2, buffer. [Note—Remove any insoluble material by centrifugation or filtration.]

**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.

**Analysis:** Using 2-mL aliquots of both the *Standard solution* and the *Sample solution* proceed according to the apparatus manufacturer's instructions. From the chromatograms thus obtained, match the retention times produced by the *Standard solution* with those produced by the *Sample solution*, and identify the peak produced by glutamic acid.

Calculate the concentration, in mg/mL, of glutamic acid in the *Sample solution* by the equation:

$$C_A = A_U \times C_S / A_S$$

$C_A$  = concentration of glutamic acid in the *Sample solution* (mg/mL)

$A_U$  = area of the glutamic acid peak from the chromatogram of the *Sample solution*

$A_S$  = area of the glutamic acid peak from the chromatogram of the *Standard solution*

$C_S$  = concentration of the glutamic acid in the *Standard solution* (mg/mL)

Calculate the percentage of glutamic acid, on the basis of total protein, by the formula:

$$\text{Result} = (100 \times C_A) / (F \times N_T)$$

$C_A$  = concentration of glutamic acid in the *Sample solution*,  
calculated above (mg/mL)

F = conversion factor for protein and amino acids, 6.25

$N_T$  = total nitrogen, as a percentage, determined in the *Assay*

Calculate the percentage of glutamic acid in the sample by the formula:-

$$\text{Result} = 100 \times C_A / S_W$$

$S_W$  = weight of the sample taken (mg)

$C_A$  = concentration of glutamic acid in the *Sample solution*,  
calculated above (mg/mL)

■ ■ 2S (FCC7)

**Acceptance criteria:** NMT 20.0% as glutamic acid (C<sub>5</sub>H<sub>9</sub>NO<sub>4</sub>) and NMT 35.0% of the total amino acids ■  
protein, ■ 2S (FCC7) calculated on the dried basis

**OTHER REQUIREMENTS**

- **LABELING:** Indicate the source of protein, including type.

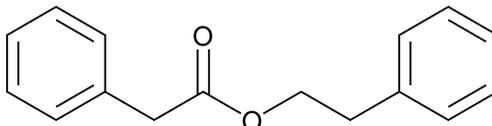
**Auxiliary Information**— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(F12010) Monographs - Food Ingredients

**BRIEFING**

**Phenethyl Phenylacetate**, FCC 7 page 784. On the basis of comments and supporting data received, it is proposed to revise the *Acceptance criteria* in the test for *Specific Gravity*. Since the temperature at which the specific gravity is measured is almost the same as the specification for the solidification point, it is proposed to lower the specification for specific gravity so that the material at the low limit of the proposed range still meets the specification for the solidification point. Additional comments are encouraged.

(FIEC: C. Mejia) C89494

**Phenethyl Phenylacetate**

C<sub>16</sub>H<sub>16</sub>O<sub>2</sub>

Formula wt 240.30  
FEMA: 2866

**DESCRIPTION**

Phenethyl Phenylacetate occurs as a colorless to slightly yellow liquid above 26°.

**Odor:** Rosy, hyacinth

**Solubility:** Soluble in alcohol; insoluble or practically insoluble in water

**Boiling Point:** ~ 325°

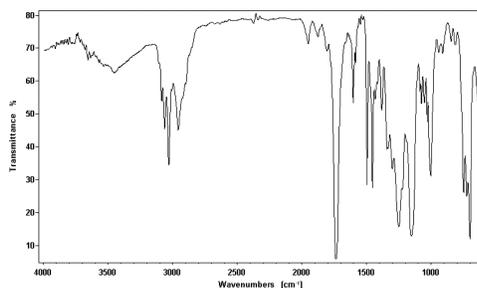
**Solubility in Alcohol**, Appendix VI: One mL dissolves in 4 mL of 90% alcohol to give a clear solution.

**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.



Phenethyl Phenylacetate

**ASSAY**

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

**Acceptance criteria:** NLT 98.0% of C<sub>16</sub>H<sub>16</sub>O<sub>2</sub>

**SPECIFIC TESTS**

- **Acid Value**, *M-15*, Appendix XI  
**Acceptance criteria:** NMT 1.0
- **Refractive Index**, Appendix II: At 20°  
 [Note—May solidify]  
**Acceptance criteria:** Between 1.548 and 1.552

**Change to read:**

- **Specific Gravity:** Determine at 25° by any reliable method (see *General Provisions*).  
**Acceptance criteria:** Between 1.079<sup>■</sup> 1.075<sup>■</sup> 2S (FCC7) and 1.082

**OTHER REQUIREMENTS**

- **Solidification Point**, Appendix IIB  
**Acceptance criteria:** NLT 26°

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

Topic/Question	Contact	Expert Committee
Monograph	Carla D Mejia, Ph.D. Scientific Liaison 1-301-816-8571	(F12010) Monographs - Food Ingredients

## BRIEFING

**Phosphoric Acid**, FCC 7 page 800 and December 2009 *FCC Forum*. On the basis of comments and data received, several revisions to this monograph are proposed.

1A revision to the Assay to replace the visual (color) endpoint of the titration with an endpoint determined from the inflection point is proposed in order to remove subjectivity from the endpoint determination.

2Revisions to the *Arsenic* and *Lead* tests are proposed to add a note to each test indicating that, as an alternate to using the Appendix methods currently referenced, analysts may use the ICP method detailed under *Cadmium*.

3Revisions to the ICP method under *Cadmium* are proposed to make it clear how to adapt the method for arsenic and lead analysis.

(FIEC: K. Laurick) C89971

### Phosphoric Acid

Orthophosphoric Acid

H<sub>3</sub>PO<sub>4</sub>

Formula wt 98.00

338

CAS: [7664-38-2]

### DESCRIPTION

Phosphoric Acid occurs as a colorless, aqueous solution, usually available in concentrations ranging from 75.0% to 85.0%. It is miscible with water and with alcohol.

**Function:** Acidifier; sequestrant

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

### IDENTIFICATION

- PHOSPHATE, Appendix IIIA
  - Sample solution:** 100 mg/mL
  - Acceptance criteria:** Passes tests

### ASSAY

#### **Change to read:**

- PROCEDURE

**Sample:** 1.5 g

**Analysis:** Transfer the *Sample* into a ~~tared glass-stoppered flask~~ 250-mL beaker, 2S (FCC7) and dilute with water to 120 mL. ~~Add 0.5 mL of thymolphthalein TS, mix, and titrate~~ Place the electrodes of a suitable pH meter into the solution and titrate, 2S (FCC7) with 1 N sodium hydroxide to the ~~first appearance of a blue color~~ inflection point occurring between pH 8.9 and 9.2. 2S (FCC7) Each mL of 1 N sodium hydroxide is equivalent to 49.00 mg of H<sub>3</sub>PO<sub>4</sub>.

**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

■ [Note—Alternatively, use the Inductively Coupled Plasma Emission Method under *Cadmium* to determine the arsenic content.] ■ 2S (FCC7)

**Sample solution:** 1 g dissolved in 35 mL of water

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

- **CADMIUM**, *Cadmium Limit Test*, Appendix IIIB

[Note—Alternately, use the Inductively Coupled Plasma Emission Method below to determine the cadmium content.]

**Apparatus:** Inductively Coupled Plasma Emission Spectrophotometer set to 226.502 nm for cadmium ■ (188.979 nm for arsenic; 220.353 nm for lead) ■ 2S (FCC7) 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 of cadmium ■ (and/or of arsenic, lead) ■ 2S (FCC7) 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 of yttrium in 2% nitric acid, from a certified stock solution

**Standard solutions:** 0.250, 0.050, and 0 µg/mL of cadmium ■ (and/or of arsenic, lead) ■ 2S (FCC7) containing 5% nitric acid; 0.100 µg/mL of 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 of yttrium. Dilute to 50 mL.

**Analysis:** Set up the instrumental method to measure the ~~area~~ ■ intensities ■ 2S (FCC7) of the 0- ■, 0.050- ■, and 0.250- ■ 2S (FCC7) µg/mL ~~Standard solution (blank) peaks and then the net intensities of the 0.050- and 0.250- µg/mL Standard solutions with~~ ■ *Standard solutions*, correcting the cadmium (and/or arsenic, lead) intensities based upon the intensity of ■ 2S (FCC7) the yttrium *Internal standard solution*. The calibration curve ■ for cadmium (and/or arsenic, lead) ■ 2S (FCC7) should be linear. Examine the spectra of the cadmium ■ (and/or arsenic, lead) ■ 2S (FCC7) 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~~ ■ measurement of the respective peak intensities. ■ 2S (FCC7)

Analyze the *Sample solution* and calculate the concentration, in µg/mL, of the cadmium ■ (and/or arsenic, lead) ■ 2S (FCC7) in the *Sample solution* ■, again correcting based upon the intensity of the yttrium internal standard. ■ 2S (FCC7) Calculate the quantity, in mg/kg, of cadmium in the sample by multiplying this value by 20.

[Note—Some sample types may naturally contain significant levels of yttrium. In these cases, choose a suitable alternative internal standard, or run the test without an internal standard. Use of the internal standard is not required, but it is helpful when there are variations in the viscosity among sample types. Samples may be prepared in higher or lower concentrations as needed. Standard concentrations may be adjusted as needed. Alternative procedures should be validated before use.]

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

- ~~FLUORIDE, Fluoride Limit Test, Method IV, Appendix IIIB~~  
**Sample:** 1 g  
~~■ Analysis:~~ Proceed as directed using *Buffer Solution B*. ■ 1S (FCC6)  
**Acceptance criteria:** NMT 10 mg/kg

- ■ FLUORIDE  
**Fluoride standard solution:** 100 µg/mL of fluoride ion; prepare by dissolving 22.2 mg of sodium fluoride, previously dried at 200° for 4 h, in sufficient water to make 100.0 mL.  
**Electrode calibration:** Determine the electrode slope, S, according to the manufacturer's instructions or according to the method described under *Fluoride Limit Test, Method IV, Appendix IIIB*.  
**Sample preparation:** Weigh 5 g of sample into a 100-mL volumetric flask, add 30 mL water, and place the uncapped flask on a hot plate capable of maintaining a temperature of 80°–90° for 10 min without boiling. (Alternatively, suspend the uncapped flask in a boiling water bath for 10 min.) Allow the contents to cool to room temperature, and dilute with water to volume.  
**Analysis:** Transfer the *Sample preparation* to a 150-mL plastic beaker with a magnetic stir bar. Place in the solution the fluoride ion and reference electrodes (or a combination fluoride electrode) of a suitable ion-selective apparatus with a magnetic stirrer. Begin stirring slowly and allow the instrument to equilibrate. Obtain the initial mV reading from the instrument, E1. Add 1 mL of the *Fluoride standard solution* to the beaker, allow the electrode to equilibrate with continued stirring, and take the final mV reading, E2. [Note—The ion-selective electrode responds more slowly than does a pH electrode, and a stable reading may not be obtained for 2–3 min. The mV displayed should be stable for 30 s before taking readings.]  
 Calculate the amount of fluoride, in mg/kg, in the sample:

$$\text{Result} = 20/[10^{(E2 - E1)/S} - 1]$$

- Acceptance criteria:** NMT 10 mg/kg
  - 1S (FCC7)
- LEAD, *Lead Limit Test, APDC Extraction Method, Appendix IIIB*
  - [Note—Alternatively, use the Inductively Coupled Plasma Emission Method under *Cadmium* to determine the lead content.] ■ 2S (FCC7)
  - Acceptance criteria:** NMT 3 mg/kg

**OTHER REQUIREMENTS**

- LABELING: Indicate the percent or the percent range of phosphoric acid (H<sub>3</sub>PO<sub>4</sub>).

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

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(F12010) Monographs - Food Ingredients

## BRIEFING

**L-Selenomethionine.** Because there is no existing *FCC* monograph for this provisional food ingredient, a new provisional monograph is proposed based on data and comments received and based on the *Selenomethionine* monograph in *USP 32–NF 27*. A provisional *FCC* monograph is one that has been developed for a food ingredient which has been self-affirmed Generally Recognized As Safe (GRAS) by a manufacturer without formal notification to the U.S. Food and Drug Administration. The *USP–NF* monograph for this article does not currently include testing of elemental impurities, so the included specification for *Lead* is based on data and comments received. The lead specification references a new ICP method for elemental impurities; comments and data on this choice of method are encouraged.

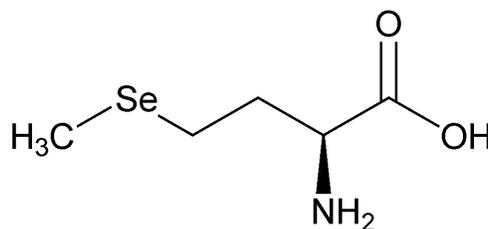
(FIEC: K. Laurick) C87443

### **Add the following:**

#### ■ L-Selenomethionine

(S)-2-Amino-4-(methylselenyl)butyric Acid

Butanoic Acid, 2-amino-4-(methylseleno)-, (S)-



$C_5H_{11}NO_2Se$

Formula wt 196.11

CAS: [1464-42-2]

### **DESCRIPTION**

L-Selenomethionine occurs as a white to off-white crystalline powder. It is soluble in hot water and has a melting point of about 260° (with decomposition).

**Function:** Nutrient; source of selenium

**Packaging and Storage:** Store in a tight container in a cool place.

### **IDENTIFICATION**

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

**Reference standard:** USP Selenomethionine 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*.

- **B. PROCEDURE**

**Acceptance criteria:** The retention times for the major peaks in the chromatogram of the *Sample solution* correspond to those in the chromatogram of the *Standard solution*, as determined in the *Assay*.

- **C. PROCEDURE**

**Acceptance criteria:** The  $R_F$  value of the principal spot obtained from the chromatogram of the *Sample solution* corresponds to that obtained from the chromatogram of the *Standard solution*, as determined in the test for *Chromatographic Purity*.

## ASSAY

### • PROCEDURE

**Mobile phase:** Dissolve 6.8 g of monobasic potassium phosphate in 1 L of water. Filter and degas, then adjust with phosphoric acid to a pH of  $2.75 \pm 0.25$ . [Note—Make adjustments if necessary.]

**System suitability solution:** Prepare a solution containing 0.8 mg/mL of USP I-Methionine RS and 0.16 mg/mL of USP Selenomethionine RS in *Mobile phase*.

**Standard solution:** 0.16 mg/mL of USP Selenomethionine RS in *Mobile phase*

**Sample solution:** 0.16 mg/mL in *Mobile phase*. Dilute sample with sonication prior to bringing to volume. Filter the volumetric solution through a 0.45- $\mu$ m membrane.

**Chromatographic system,** Appendix IIA

**Mode:** High-performance liquid chromatography

**Detector:** UV 220 nm

**Column:** 4.6-mm  $\times$  25-cm column packed with octadecylsilane chemically bonded to porous silica or ceramic particles 1.5- to 10- $\mu$ m in diameter (with polar end-capping)

**Flow rate:** 1.0 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Sample:** *System suitability solution*

**Suitability requirement 1:** The resolution between methionine and selenomethionine is NLT 3.0.

**Suitability requirement 2:** The tailing factor is NMT 2.

**Suitability requirement 3:** The relative standard deviation 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 approximate relative retention times for methionine and selenomethionine are 0.8 and 1.0, respectively.]

Calculate the percentage of  $C_5H_{11}NO_2Se$  in the portion of the sample taken:

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

$r_U$  = peak response obtained from the chromatogram of the *Sample solution*

$r_S$  = peak response obtained from the chromatogram of the *Standard solution*

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

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

**Acceptance criteria:** 97.0%–103.0%

## IMPURITIES

### Inorganic Impurities

- **LEAD,** *Elemental Impurities by ICP,* Appendix IIIC

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

- **SODIUM**

**Solution A:** Potassium chloride and water (1 in 5)

**Standard stock solution:** 10  $\mu$ g/mL of sodium in water from sodium chloride previously dried at 105 $^\circ$  for 2 h

**Standard solutions:** Transfer 2.0, 5.0, and 10.0 mL of the *Standard stock solution*, respectively, to three separate 100-mL volumetric flasks. To each flask add 2.0 mL of *Solution A* and 1.0 mL of hydrochloric acid, and dilute with water to volume. The resulting solutions contain 0.2, 0.5, and 1.0 µg/mL of sodium, respectively.

**Sample stock solution:** 10 mg/mL in water

**Sample solution:** Transfer 10.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, add 2.0 mL of *Solution A* and 1.0 mL of hydrochloric acid, and dilute with water to volume.

**Analysis:** Using a suitable atomic absorption spectrophotometer equipped with a sodium hollow-cathode lamp and an oxidizing air–acetylene flame, determine the absorbances of the *Standard solutions* and the *Sample solution* at the sodium emission line of 589 nm, using water as the blank. Plot the absorbances of the *Standard solutions* versus their concentrations, in µg/mL, of sodium, and draw the straight line best fitting the plotted points. From the standard curve so obtained, determine the concentration of sodium, in µg/mL, in the *Sample solution*.

Calculate the percentage of sodium in the portion of the sample taken:

$$\text{Result} = S/C_U \times 100$$

S = concentration of sodium in the *Sample solution*, as determined from the standard curve (µg/mL)

C<sub>U</sub> = concentration of l-Selenomethionine in the *Sample solution* (µg/mL)

**Acceptance criteria:** NMT 0.1%

#### Organic Impurities

- **CHROMATOGRAPHIC PURITY, Thin-Layer Chromatography, Appendix IIA**

**Standard solution:** Dissolve 50 mg of USP Selenomethionine RS in 2 mL of water, warming if necessary, then dilute with methanol to 10.0 mL (5 mg/mL).

**Dilute standard solution:** Dilute 1 mL of the *Standard solution* with methanol to 100 mL (50 µg/mL).

**Sample solution:** Dissolve 50 mg of the sample in 2 mL of water, warming if necessary, then dilute with methanol to 10.0 mL.

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

**Application volume:** 10 µL

**Developing solvent system:** Butanol, glacial acetic acid, and water (80:20:20)

**Spray reagent:** 200 mg of ninhydrin in 100 mL of alcohol

**Analysis:** Separately apply portions of the *Standard solution*, *Dilute standard solution*, and *Sample solution* to a suitable thin-layer chromatographic plate. Following development, remove the plate from the chromatographic chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with *Spray reagent*, and dry it at 110° for 10 min.

**Acceptance criteria:** The R<sub>F</sub> value of the principal spot obtained from the chromatogram of the *Sample solution* corresponds to that obtained from the chromatogram of the *Standard solution*, and no spot, other than the principal spot, in the chromatogram of the *Sample solution* is larger or more intense than the principal spot obtained from the *Dilute standard solution* (NMT 1.0%).

#### SPECIFIC TESTS

- **OPTICAL (SPECIFIC) ROTATION, Appendix IIB**

**Sample solution:** 10 mg/mL in hydrochloric acid

**Acceptance criteria:** [α]<sub>D</sub><sup>20</sup> between +17.0° and +19.5°

- **SELENIUM**

[ **CAUTION**— Selenium is toxic; handle with care. ]

**Standard stock solution:** 1000 µg/mL of selenium prepared as follows: Dissolve 100 mg of metallic selenium in a minimum volume of nitric acid, evaporate to dryness, add 0.2 mL of water, and evaporate to dryness once more. Repeat the addition of water and evaporation to dryness three times. Dissolve the residue obtained in 3 N hydrochloric acid, transfer to a 100-mL volumetric flask, and dilute with 3 N hydrochloric acid to volume.

**Standard solutions:** Transfer 2.0, 5.0, and 10.0 mL of the *Standard stock solution*, respectively, to three separate 100-mL volumetric flasks. Dilute the contents of each flask with water to volume. The resulting solutions contain 20, 50, and 100 µg/mL of selenium, respectively.

**Sample solution:** 125 µg/mL in water

**Analysis:** Using a suitable atomic absorption spectrophotometer equipped with a selenium hollow-cathode lamp and an air-acetylene flame, determine the absorbances of the *Standard solutions* and the *Sample solution* at the selenium emission line of 196 nm, using water as the blank. Plot the absorbances of the *Standard solutions* versus their concentrations, in µg/mL, of selenium, and draw the straight line best fitting the plotted points. From the standard curve so obtained, determine the concentration of selenium, in µg/mL, in the *Sample solution*.

Calculate the percentage of selenium in the portion of the sample taken:

$$\text{Result} = S/C_U \times 100$$

S = concentration of selenium in the *Sample solution*, as determined from the standard curve (µg/mL)

C<sub>U</sub> = concentration of l-Selenomethionine in the *Sample solution* (µg/mL)

**Acceptance criteria:** 39.0%–41.0%

■ 2S (FCC7)

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

Topic/Question	Contact	Expert Committee
Monograph	Kristie Laurvick Scientific Liaison 1-301-816-8356	(F12010) Monographs - Food Ingredients

## BRIEFING

**Sodium Iron EDTA**, FCC 7 page 942. On the basis of comments and data received, several revisions to this monograph are proposed.

- A change to the *Description* is proposed to clarify that this monograph was developed specifically to represent the trihydrate form of this article. The CAS number for the trihydrate is also added. Because of this clarification, instructions within the *Acceptance criteria* for both *Assay* methods to determine the result “on the basis of the trihydrate” will be removed since the trihydrate is the only form of the article addressed by this monograph.
- Specifications and test methods for *Free Iron*, *Sulfate*, *UV Absorbance*, and *Loss on Drying* are proposed in order to strengthen general quality specifications for this food ingredient and to distinguish the ingredient from grades not suitable for use in foods (and potentially not manufactured according to current food GMPs).
- Tighter limits for *EDTA*, *Iron*, and *pH* are proposed on the basis that they are more representative of the ingredient for use in foods as it exists on the market and on the basis of strengthening the general quality specifications for this food ingredient (by distinguishing product manufactured for use in foods from that manufactured for use in agriculture).

(FIEC: K. Laurick) C89495

### Sodium Iron EDTA

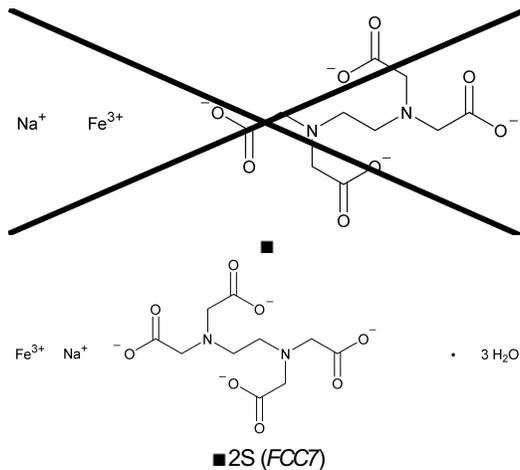
#### *Change to read:*

Sodium Iron (III) Ethylenediaminetetraacetate, Trihydrate

Ferric Sodium EDTA Trihydrate

Ferric Sodium Edetate

Sodium Feredetate



Formula wt 421.09  
 CAS: anhydrous [15708-41-5]  
 ■ trihydrate [18154-32-0] ■ 2S (FCC7)

## DESCRIPTION

### *Change to read:*

Sodium Iron EDTA occurs as an odorless, light-yellow to yellow-brown powder that is highly stable and unaffected by storage. ■ It contains three molecules of water of hydration. ■ 2S (FCC7) It is freely soluble in water.

**Function:** Nutrient

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

## IDENTIFICATION

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

**Reference standard:** USP Sodium Iron EDTA RS

**Standard and sample 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

### *Change to read:*

- **EDTA**

**0.1 M Calcium chloride solution:** Transfer 14.7 g of calcium chloride dihydrate to a 1-L volumetric flask, and dissolve in and dilute with water to volume. Standardize the solution using the following procedure.

Weigh 0.4–0.5 g of ethylenediamine–tetraacetic acid (EDTA acid) into each of three 250-mL conical flasks.

Add 100 mL of *Triethanolamine solution* to each flask, and adjust to a pH of  $12.6 \pm 0.1$  with 33% (w/v) sodium hydroxide solution. Add 10 mL of *Calmagite indicator solution* to each flask, adjust the transmission to 70%, and titrate with *0.1 M Calcium chloride solution* to the first significant color change using a photometer operated at a wavelength of 520 nm. [Note—The correct endpoint is found at the crossing of the intersection lines. It can also be determined from the first significant deviation of the first derivative dU/mV from zero.]

Calculate the exact molarity of the *0.1 M Calcium chloride solution*:

$$\text{Result} = (W_{\text{EDTA}} \times 1000) / (V \times F)$$

$W_{\text{EDTA}}$  = quantity of EDTA acid taken (g)

1000 = conversion factor (mL/L)

V = volume of titrant used (mL)

F = molecular weight of EDTA acid, 292.24

**Calmagite indicator solution:** Dissolve 100 mg of calmagite, 3-hydroxy-4-(2-hydroxy-5-methylphenylazo)-1-naphthalenesulfonic acid in 1 L of water.

**Triethanolamine solution:** Dilute 100 mL of triethanolamine with water to 1 L.

**Sample:** 0.65 g

**Analysis:** Transfer the *Sample* to a 250-mL beaker. Add 100 mL of *Triethanolamine solution* and dissolve.

Adjust to a pH of  $12.6 \pm 0.1$  with 33% (w/v) sodium hydroxide solution. [Note—The resulting solution should be clear and colorless.] Add 10 mL of *Calmagite indicator solution*, adjust the transmission to 70%, and titrate with *0.1 M Calcium chloride solution* to the first significant color change using a

photometer operated at a wavelength of 520 nm. [Note—The correct endpoint is found at the crossing of the intersection lines. It can also be determined from the first significant deviation of the first derivative dU/mV from zero.] Perform a blank determination (see *General Provisions*).

Calculate the percentage of EDTA acid:

$$\text{Result} = [(V_S - V_B) \times M \times F] / (1000 \times W_S) \times 100$$

$V_S$  = volume of 0.1 M Calcium chloride solution used for the Sample titration (mL)

$V_B$  = volume of 0.1 M Calcium chloride solution used for the blank titration (mL)

$M$  = exact molarity of the 0.1 M Calcium chloride solution

$F$  = molecular weight of EDTA acid, 292.24

1000 = conversion factor (mL/L)

$W_S$  = quantity of Sample taken (g)

**Acceptance criteria:** ~~67.0%–72.0% EDTA, calculated on the basis of the trihydrate~~ ■ 67.5%–71.5%  
■ 2S (FCC7)

### Change to read:

- IRON

**Solution A:** 1 M sodium hydrogen carbonate (NaHCO<sub>3</sub>)

**Sample:** 0.65 g

**Analysis:** Dissolve the Sample in 100 mL of water in an iodine flask. Add 10 mL of Solution A and carefully add 20 mL of concentrated hydrochloric acid. Add 15 g of potassium iodide, close the flask immediately, and mix. Allow to stand in the dark for 10 min at  $25 \pm 5^\circ$ . Titrate the liberated iodine with 0.1 N sodium thiosulfate, using starch TS as the indicator. Avoid vigorous mixing during the titration. Perform a blank determination (see *General Provisions*). Calculate the percentage of iron in the Sodium Iron EDTA:

$$\text{Result} = [(T_S - T_B) \times N \times F \times 100] / W_S$$

$T_S$  = volume of titrant used for the Sample titration (mL)

$T_B$  = volume of titrant used for the blank titration (mL)

$N$  = exact normality of the sodium thiosulfate used in the titration

$F$  = atomic weight of iron  $\times 10^{-3}$ , 0.05585

$W_S$  = quantity of Sample taken (g)

**Acceptance criteria:** ~~12.5%–13.5% iron, calculated on the basis of the trihydrate~~ ■ 13.0%–13.5% ■ 2S (FCC7)

## IMPURITIES

### Inorganic Impurities

- ARSENIC, *Arsenic Limit Test*, Appendix IIIB

**Sample solution:** Prepare as directed for organic compounds, using a 3.0-g sample.

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

- CHLORIDE

**Polyvinyl alcohol solution:** Dissolve, with heating, 2 g of polyvinyl alcohol (suitable for argentometric titrations) in 1 L of water.

**Sample:** 5.0 g

**Analysis:** Transfer the Sample to a 250-mL beaker. Add 80 mL of water, dissolve, and add 80 mL of methanol. Add, using a volumetric pipet, 5.00 mL of 0.01 M hydrochloric acid and 5 mL of Polyvinyl alcohol solution. Titrate with 0.01 M silver nitrate solution, using a combined silver/reference electrode. Perform a

blank determination (see *General Provisions*).

Calculate the concentration of chloride, in mg/kg:

$$\text{Result} = [(V_S - V_B) \times M \times F \times 1000] / W_S$$

$V_S$  = volume of titrant used for the *Sample* titration (mL)

$V_B$  = volume of titrant used for the blank titration (mL)

$M$  = exact molarity of the silver nitrate solution used in the titration

$F$  = atomic weight of chloride, 35.45

$W_S$  = quantity of *Sample* taken (g)

**Acceptance criteria:** NMT 600 mg/kg

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

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

#### **Add the following:**

- **SULFATE (AS BARIUM SULFATE)**

**Barium chloride solution:** 0.5 M; prepare by diluting 30.5 g of barium chloride dihydrate with water to 250 mL.

**Zinc chloride solution:** 1 M zinc chloride; prepare by dissolving 13.6 g of zinc chloride in water containing 1 mL of 2 M hydrochloric acid and diluting to 100 mL.

**Standard sulfate solution:** 2.113 mg/mL sodium sulfate (1.430 mg/mL sulfate)

**Solution A:** Dilute 5 mL of the *Barium chloride solution* with 55 mL of water and 20 mL of 96% ethanol. Add 0.5 mL of the *Standard sulfate solution* and mix. [Note—Prepare immediately before use.]

**Indicator solution:** 0.25% *p*-nitrophenol in water

**Sample:** 1.8 g

**Control:** Transfer 25 mL of water to a colorless clear glass test tube with a 50-mL mark. Add 1.8 mL of 5 M sodium hydroxide and 100  $\mu$ L of the *Indicator solution* to the tube. Neutralize with 2 M hydrochloric acid until the yellow solutions turn colorless. Add 2.0 mL of *Zinc chloride solution* and adjust to a pH of 2.0 with the addition of either 2 M hydrochloric acid or 2 M sodium hydroxide. Using a positive displacement (piston) pipet, transfer 300  $\mu$ L of the *Standard sulfate solution* to the test tube. Dilute the contents of the tube to 50 mL and add 5.0 mL of *Solution A*. Mix the contents of the tube and visually compare the turbidity of the solution to the solution prepared in the *Analysis*.

**Analysis:** Transfer the *Sample* to a 50-mL test tube identical to that used in the *Control* and dissolve in 30 mL of water. Carefully add 4.5 mL of 5 M sodium hydroxide and stir with a stir bar for 15 min. Remove the stir bar and dilute the solution with water to the 50-mL mark on the tube. Filter the solution<sup>1</sup> and transfer 20 mL of the filtrate to another identical test tube. Add 100  $\mu$ L of the *Indicator solution* to the tube containing the 20 mL of filtrate and neutralize the filtrate with 2 M hydrochloric acid until the yellow solution turns colorless. Add 2.0 mL of *Zinc chloride solution* to the tube and adjust to a pH of 2.0 with the addition of 2 M hydrochloric acid. Dilute the contents of the tube to 50 mL and add 5.0 mL of *Solution A*. Mix the contents of the tube and visually compare the turbidity of the solution to the solution prepared in the *Control*.

**Acceptance criteria:** The turbidity of the solution prepared in the *Analysis* is NMT the turbidity of the solution prepared in the *Control* (NMT 0.06%). ■2S (FCC7)

#### **Organic Impurities**

- **NITRILOTRIACETIC ACID**

[Note—Carry out the test protected from light.]

**Mobile phase:** Dissolve 50.0 mg of ferric sulfate pentahydrate in 50 mL of 0.5 M sulfuric acid, and add 750 mL of water. Adjust to a pH of 1.5 with 0.5 M sulfuric acid or 1 M sodium hydroxide, and add 20 mL of ethylene glycol. Dilute with water to 1 L.

**Solvent mixture:** Dissolve 10.0 g of ferric sulfate pentahydrate in 20 mL of 0.5 M sulfuric acid, and add 780 mL of water. Adjust to a pH of 2.0 with 1 M sodium hydroxide. Dilute with water to 1 L.

**Standard stock solution:** 0.40 mg/mL of USP Nitrotriacetic Acid RS in *Solvent mixture*

**Standard solution:** Transfer 1.0 mL of *Standard stock solution* and 0.1 mL of *Sample solution* to a 100-mL volumetric flask. Dilute with *Solvent mixture* to volume.

**Sample solution:** 4.0 mg/mL in *Solvent mixture*. [Note—Sonicate, if necessary, to achieve complete dissolution.]

**Chromatographic system,** Appendix IIA

**Mode:** High-performance liquid chromatography

**Detector:** UV 273 nm

**Column:** 4.6-mm × 10-cm column that contains spherical graphitized carbon for chromatography (5 μm) with a specific surface area of 120 m<sup>2</sup>/g and a pore size of 25 nm (Hypercarb, or equivalent)

**Flow rate:** 1 mL/min

**Injection size:** 20 μL

**System suitability**

**Sample:** *Standard solution* (three replicate injections)

**Suitability requirement 1:** The resolution factor between nitrotriacetic acid and sodium iron EDTA is NLT 4.0.

**Suitability requirement 2:** The relative standard deviation for nitrotriacetic acid 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 retention times for nitrotriacetic acid and sodium iron EDTA are about 5 and 10 min, respectively.]

**Acceptance criteria:** The response of the nitrotriacetic acid peak of the *Sample solution* does not exceed the response of the nitrotriacetic acid peak obtained from the *Standard solution* (NMT 0.1% nitrotriacetic acid).

## SPECIFIC TESTS

**Add the following:**

- **FREE IRON**

**Electrode system:** Combined platinum/reference electrode<sup>2</sup>; condition in a 0.1 M solution of iron (II) sulfate before use.

**Buffer solution:** 84 mg/mL of monochloroacetic acid and 76 mg/mL of sodium acetate trihydrate (pH 3)

**Starch solution:** Add, while stirring, 2 g of a suitable soluble starch to 150 mL of boiling water. Continue boiling until the solution is clear, then dilute with water to 1 L.

**Iodine solution:** 0.05 M I<sub>2</sub>; prepare from a commercially available concentrated iodine solution.

**EDTA standard solution:** 0.1 M EDTA–Na<sub>2</sub>H<sub>2</sub>; prepare from a commercially available concentrated EDTA disodium salt solution.

**Iron (III) chloride standard solution:** Dissolve 27.0 g of iron (III) chloride hexahydrate in water containing 1 mL of hydrochloric acid, then dilute with water to 1 L.

**Sample:** 5 g

**Analysis:** Transfer the *Sample* to a 250-mL beaker and dissolve in 100 mL of water. Adjust to a pH of 3 with 4 M hydrochloric acid. To the beaker, add 10 mL of the *Buffer solution* and 3 mL of the *Starch solution*.

Add a quantity of the *Iodine solution* to the beaker until the solution turns blue, then add an excess 2-mL portion of the *Iodine solution*. Titrate with standardized 0.1 M sodium thiosulfate solution, using the *Electrode system* described. Stop the titration just beyond the equivalence point. Add 5.00 mL of the *EDTA standard solution* to the beaker, then titrate a second time with the *Iron (III) chloride standard solution* to beyond the equivalence point. Perform a blank determination (see *General Provisions*). Calculate the percentage of free iron in the sample taken:

$$\text{Result} = (V_0 - V) \times C \times 55.85 \times F \times (1/M_S) \times 100$$

$V_0$  = volume of *Iron (III) chloride standard solution* used for the *Sample* titration (mL)

$V$  = volume of *Iron (III) chloride standard solution* used for the blank titration (mL)

$C$  = exact concentration of the *Iron (III) chloride standard solution* (mol/L)

$F$  = factor converting mL to L (0.001)

$M_S$  = quantity of the *Sample* used (g)

**Acceptance criteria:** NMT 0.05% ■<sub>2S</sub> (FCC7)

**Add the following:**

• ■ **LOSS ON DRYING**

**Sample:** 1.0 g

**Analysis:** Mix the *Sample* immediately before analysis. Transfer the *Sample* to a suitable halogen dryer/moisture analyzer<sup>3</sup> and dry at 170° for 30 min.

**Acceptance criteria:** 12.5%–13.5% ■<sub>2S</sub> (FCC7)

**Change to read:**

• pH, *pH Determination*, Appendix IIB

**Sample:** 10 mg/mL

**Acceptance criteria:** 3.5 ■<sub>4.5</sub> ■<sub>2S</sub> (FCC7)–5.5

**Add the following:**

• ■ **UV ABSORBANCE**

**Buffer solution:** 7.94 mg/mL of potassium dihydrogen phosphate and 1.49 mg/mL of disodium hydrogen phosphate, dihydrate

**Sample solution:** Transfer 1.0 g of the sample to a 100-mL volumetric flask and dissolve in 50 mL of water.

Add 2 mL of the *Buffer solution* to the flask, and dilute with water to volume. Dilute 1.00 mL of this solution with water to 1 L.

**Analysis:** Using a suitable spectrophotometer, measure the absorbance of the *Sample solution* at 260 nm in a 1-cm cell, using water as the blank.

**Acceptance criteria:** The absorbance of the *Sample solution* is NMT 0.240. ■<sub>2S</sub> (FCC7)

• **WATER-INSOLUBLE MATTER**, Appendix IIC

**Acceptance criteria:** NMT 0.1%

<sup>1</sup> Use Schleicher & Schuell filter paper 595 ½ No. 311.647, or equivalent, folded.

<sup>2</sup> Metrohm 6.0415.100, or equivalent.

<sup>3</sup> Mettler Toledo HG53, HG63, or equivalent.

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

Topic/Question	Contact	Expert Committee
Monograph	Kristie Laurick Scientific Liaison 1-301-816-8356	(FI2010) Monographs - Food Ingredients

*FCC Seventh Edition Page 942*

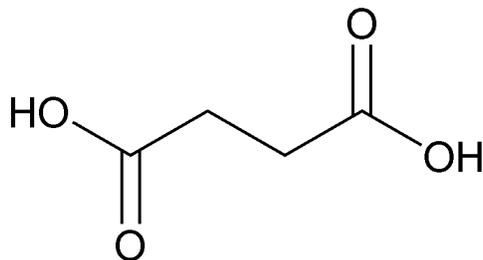
## BRIEFING

**Succinic Acid**, FCC 7 page 992. In an effort to modernize *Identification* test methods in FCC, it is proposed to replace the existing non-specific *Identification* test with a more specific infrared comparison to a USP Reference Standard.

(FIEC: K. Laurick) C89976

### Succinic Acid

Butanedioic Acid



C<sub>4</sub>H<sub>6</sub>O<sub>4</sub>

Formula wt 118.09

363

CAS: [110-15-6]

### DESCRIPTION

Succinic Acid occurs as colorless or white crystals. One g dissolves in 13 mL of water at 25°, in 1 mL of boiling water, in 18.5 mL of alcohol, and in 20 mL of glycerin.

**Function:** Buffer; neutralizing agent

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

### IDENTIFICATION

**Delete the following:**

- **PROCEDURE**

~~**Sample solution:** Saturated solution~~

~~**Analysis:** To a drop of *Sample solution* in a micro test tube, add a drop of a 0.5% solution of ammonium chloride and several mg of zinc powder. Cover the mouth of the tube with a disk of filter paper moistened with a solution of 5% p-dimethylaminobenzaldehyde and 20% trichloroacetic acid in hexane. Heat with a small flame for about 1 min.~~

~~**Acceptance criteria:** A pink to red-violet stain appears on the paper. ■ 2S (FCC7)~~

**Add the following:**

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

**Reference standard:** USP Succinic Acid RS

**Sample and standard preparation:** K

**Acceptance criteria:** The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard*. ■ 2S (FCC7)

**ASSAY**• **PROCEDURE****Sample:** 250 mg**Analysis:** Dissolve the *Sample* in 25 mL of recently boiled and cooled water, add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide to the first appearance of a faint pink color that persists for at least 30 s. Each mL of 0.1 N sodium hydroxide is equivalent to 5.905 mg of C<sub>4</sub>H<sub>6</sub>O<sub>4</sub>.**Acceptance criteria:** NLT 99.0% and NMT 100.5% of C<sub>4</sub>H<sub>6</sub>O<sub>4</sub>**IMPURITIES****Inorganic Impurities**

- *Lead, Lead Limit Test, Flame Atomic Absorption Spectrophotometric Method, Appendix IIIB*

**Sample:** 5 g**Acceptance criteria:** NMT 2 mg/kg**SPECIFIC TESTS**

- **MELTING RANGE OR TEMPERATURE**, Appendix IIB

**Acceptance criteria:** Between 185.0° and 190.0°

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

**Sample:** 8 g**Acceptance criteria:** NMT 0.025%**Auxiliary Information**— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Kristie Laurvick Scientific Liaison 1-301-816-8356	(F12010) Monographs - Food Ingredients

FCC Seventh Edition Page 992

## BRIEFING

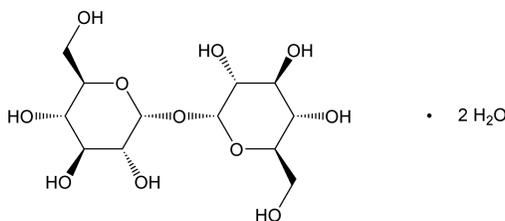
**Trehalose**, FCC 7 page 1051. On the basis of comments and supporting data received, it is proposed to harmonize the Assay method with that in the *USP 32-NF 27* monograph for this material. In conjunction with this revision, it is proposed to change the method for determining the water content of this ingredient from *Loss on Drying* to the Karl Fischer method. This change is necessary because the purity value assigned to the USP Trehalose RS used in the proposed Assay was determined using the Karl Fischer method. It is also proposed based on data to increase the water content acceptance criterion in the monograph to account for the water of crystallization measured by Karl Fischer but not by *Loss on Drying*.

Comments submitted indicate that the *Microscopic Examination* requirement in this monograph is very subjective and that other tests already in the monograph are better able to distinguish Trehalose from other similar food ingredients. On this basis, it is proposed to change *Microscopic Examination* from a monograph requirement to part of the monograph *Description* which is provided in FCC monographs for informational purposes only.

(FIEC: J. Moore) C89176

### Trehalose

$\alpha$ -D-Glucopyranosyl- $\alpha$ -D-glucopyranoside, dihydrate



$C_{12}H_{22}O_{11} \cdot 2H_2O$

Formula wt 378.33  
CAS: dihydrate [6138-23-4]

### DESCRIPTION

#### **Change to read:**

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. ■ Viewed under a light microscope at 50× magnification Trehalose appears as colorless, rectangular crystals with a prismatic structure. ■ 2S (FCC7)

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

#### **Add the following:**

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

**Reference standard:** USP Trehalose RS

**Sample and standard preparations:** M

**Acceptance criteria:** The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard*.▲FCC7

**Delete the following:**

• ~~■ 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.~~

~~■ 2S (FCC7)~~

**ASSAY**

**Change to read:**

• **PROCEDURE**

**Sample:** 3 g

~~**Sample stock solution:** 30 mg/mL, passed 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<sup>1</sup> reference standard (on an anhydrous basis). [Note —Determine the water content of the Trehalose reference standard as directed under *Water Determination*, Appendix II B. 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 II A-~~

~~**Mode:** High-performance liquid chromatography~~

~~**Detector:** Differential refractometer~~

~~**Column:** 300-mm × 4.0-mm (id); (Shodex Ionpack KS 801, or equivalent)~~

~~**Column temperature:** 35°~~

~~**Injection volume:** 20 µL~~

~~**Analysis:** Pass 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:-~~

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

~~$r_U$  = 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)~~

■ **Mobile phase:** Water

**Standard solution:** 10 mg/mL of USP Trehalose RS, calculated on the basis of the USP RS label claim

**Sample solution:** 10 mg/mL, calculated on the anhydrous basis

**Chromatographic system,** Appendix II A

**Mode:** High-performance liquid chromatography

**Detector:** Refractive index

**Column:** 8-mm × 30-cm<sup>1</sup>

**Temperature**

**Detector:** 40°

**Column:** 80°

**Flow rate:** Adjust so that the retention time of trehalose is about 15 min.

**Injection size:** 20 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirement:** The relative standard deviation of the trehalose area responses from replicate injections 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. Calculate the percentage of trehalose in the portion of the sample taken:

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

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution*, calculated based on the USP Trehalose RS label claim (mg/mL)

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

■ 2S (FCC7)

**Acceptance criteria:** NLT 98.0%, calculated on the dried ■ anhydrous ■ 2S (FCC7) 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

- **COLOR IN SOLUTION**

**Sample solution:** Dissolve 33 g of sample in 67 g of recently boiled water.

**Analysis:** Determine the absorbance of the *Sample solution* at 420 and 720 nm using a 1-cm cuvette. Calculate *Color in Solution* by the following formula:

$$\text{Result} = A_{420} - A_{720}$$

$A_{420}$  = absorbance at 420 nm

$A_{720}$  = absorbance at 720 nm

**Acceptance criteria:** NMT 0.100

**Delete the following:**

- ■ **Loss on Drying**, Appendix IIC: 60° for 5 h

**Acceptance criteria:** NMT 1.5% ■ 2S (FCC7)

**Add the following:**

- **WATER, Water Determination, Appendix IIB**  
**Acceptance criteria:** NMT 11.0% ■ 2S (FCC7)
- **pH, pH Determination, Appendix IIB**  
**Sample solution:** Dissolve 33 g of sample in 67 g of recently boiled water.  
**Acceptance criteria:** Between 4.5 and 6.5
- **RESIDUE ON IGNITION (SULFATED ASH), Appendix IIC**  
**Sample:** 5 g  
**Acceptance criteria:** NMT 0.05%

• **TURBIDITY OF A 30% SOLUTION**

**Sample solution:**

Dissolve 33 g of sample in 67 g of recently boiled water.

**Analysis:** Using a suitable spectrophotometer, determine the absorbance of the *Sample solution* at 720 nm.

**Acceptance criteria:** NMT 0.050

<sup>4</sup>Available from Hayashibara International, Inc., Suite 200, 8670 Wolff Court, Westminster, CO 80031-6953.

<sup>1</sup> Shodex SUGAR KS-801, or equivalent

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

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(F12010) Monographs - Food Ingredients

## BRIEFING

**Yeast, Autolyzed**, FCC 7 page 1094. See briefing under *Acid Hydrolysates of Protein*.  
(FIEC: J. Moore) C86205

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### Yeast, Autolyzed

Autolyzed Yeast

#### DESCRIPTION

Yeast, Autolyzed, occurs in granular, powdered, flake, or paste form. It is the concentrated, nonextracted, partially soluble digest obtained from food-grade yeasts. Solubilization is accomplished by enzyme hydrolysis or autolysis of yeast cells. Food-grade salts and enzymes may be added. Yeast, Autolyzed, contains both soluble and insoluble components derived from the whole yeast cell. It is composed primarily of amino acids, peptides, proteins, carbohydrates, fats, and salts.

**Function:** Flavoring agent; flavor enhancer; protein source; binder

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

[Note—Perform all analyses using a sample previously dried as follows: Liquid and paste samples should be evaporated to dryness on a steam bath, then, as for the powdered and granular forms, dried to constant weight at 65° (see *General Provisions*).]

#### ASSAY

- **PROTEIN**, *Nitrogen Determination*, Appendix IIIC

**Analysis:** Calculate the percent protein by the formula:

$$\text{Result} = N \times F$$

N = percent nitrogen

F = nitrogen-to-protein conversion factor, 6.25

**Acceptance criteria:** NLT 38.1% protein (NLT 6.1% nitrogen), calculated on the sodium chloride-free basis

#### IMPURITIES

##### Inorganic Impurities

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

**Sample:** 10 g

**Acceptance criteria:** NMT 2 mg/kg

- **MERCURY**, *Mercury Limit Test*, Appendix IIIB

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

#### SPECIFIC TESTS

- **α-AMINO NITROGEN/TOTAL NITROGEN (AN/TN) PERCENT RATIO**, **α-AMINO NITROGEN (AN) DETERMINATION**, Appendix IIIC and *Total Nitrogen (TN), Nitrogen Determination*, Appendix IIIC

**Analysis:** Calculate the AN/TN percent ratio, as corrected for *Ammonia Nitrogen*, according to the formula:

$$\text{Result} = [(AN - AmN)/(TN - AmN)] \times 100$$

AN = percent of  $\alpha$ -Amino Nitrogen

AmN = percent of Ammonia Nitrogen determined below

TN = percent of Total Nitrogen

**Acceptance criteria:** NLT 5.0%

- **AMMONIA NITROGEN**, *Ammonia Nitrogen (NH<sub>3</sub>-N) Determination*, Appendix IIIC

**Acceptance criteria:** NMT 1.0%, calculated on the sodium chloride-free basis

**Change to read:**

- **GLUTAMIC ACID**, Appendix IIIC

**Acceptance criteria:** NMT 13.0% as C<sub>5</sub>H<sub>9</sub>NO<sub>4</sub> calculated on the sodium chloride-free basis, and NMT 24.0% of the total amino-acids<sup>■</sup> protein<sup>■</sup> 2S (FCC7)

- **INSOLUBLE MATTER**

**Sample:** 5 g

**Analysis:** Transfer the *Sample* into a 250-mL Erlenmeyer flask, add 75 mL of water, cover the flask with a watch glass, and boil gently for 2 min. Filter the solution through a tared filtering crucible, dry the crucible at 105<sup>o</sup> for 1 h, cool, and weigh.

**Acceptance criteria:** Between 20.0% and 60.0%

- **MICROBIAL LIMITS**

[Note—Current methods for the following tests may be found in the Food and Drug Administration's Bacteriological Analytical Manual online at [www.cfsan.fda.gov](http://www.cfsan.fda.gov).]

**Acceptance criteria**

**Aerobic plate count:** NMT 50,000 cfu/g

**Coliforms:** NMT 10 cfu/g

**Salmonella:** Negative in 25 g

**Yeasts and molds:** NMT 50 cfu/g

- **POTASSIUM**

**Standard stock solution:** 200 µg/mL of potassium in deionized water prepared as follows: Transfer 38.20 mg of reagent-grade potassium chloride into a 100-mL volumetric flask, add deionized water to dissolve the salt, dilute with deionized water to volume, and mix.

**Standard solution:** 1.0 µg/mL of potassium in deionized water from the *Standard stock solution*

**Sample stock solution:** Transfer 2.33 g of the sample into a silica or porcelain dish. Ash it in a muffle furnace at 550<sup>o</sup> for 2 to 4 h. Allow the ash to cool and add 5 mL of 20% hydrochloric acid. If necessary, warm the mixture to completely dissolve the residue. Filter the solution through acid-washed filter paper into a 1000-mL volumetric flask. Wash the filter paper with hot water, dilute the solution to volume, and mix.

**Sample solution:** 1:300 dilution of the *Sample stock solution*

**Analysis:** Using a suitable spectrophotometer, measure the absorbance of the *Sample solution* and the *Standard solution* at 766.5 nm.

**Acceptance criteria:** The absorbance of the *Sample solution* does not exceed that of the *Standard solution* (NMT 13.0%).

- **SODIUM CHLORIDE**

**Standard stock solution:** 430 µg/mL of sodium chloride in deionized water

**Standard solution:** 4.3 µg/mL of sodium chloride in deionized water from the *Standard stock solution*

**Sample stock solution:** Transfer 1.0 ± 0.05 g of the sample into a silica or porcelain dish. Ash in a muffle furnace at 550<sup>o</sup> for 2 to 4 h. Allow the ash to cool and add 5 mL of 20% hydrochloric acid. If necessary,

warm the mixture to completely dissolve the residue. Filter the solution through acid-washed filter paper into a 100-mL volumetric flask. Wash the filter paper with hot water, dilute the solution to volume, and mix.

**Sample solution:** 1:100 dilution of the *Sample stock solution*

**Analysis:** Using a suitable spectrophotometer, measure the absorbance of the *Sample solution* and the *Standard solution* at 589.0 nm.

**Acceptance criteria:** The absorbance of the *Sample solution* does not exceed that of the *Standard solution* (NMT 50.0%).

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

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(F12010) Monographs - Food Ingredients

FCC Seventh Edition Page 1094

## BRIEFING

**Yeast Extract**, FCC 7 page 1097. See briefing under *Acid Hydrolysates of Protein*.  
(FIEC: J. Moore) C86205

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### Yeast Extract

Autolyzed Yeast Extract

#### DESCRIPTION

Yeast Extract occurs as a liquid, paste, powder, or granular substance. It comprises the water-soluble components of the yeast cell, the composition of which is primarily amino acids, peptides, carbohydrates, and salts. Yeast Extract is produced through the hydrolysis of peptide bonds by the naturally occurring enzymes present in edible yeasts or by the addition of food-grade enzymes. Food-grade salts may be added during processing.

**Function:** Flavoring agent; flavor enhancer

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

[Note—Perform all calculations on the dried basis. In a suitable tared container, evaporate liquid and paste samples to dryness on a steam bath, then, as for the powdered and granular forms, dry to constant weight at 105° (see *General Provisions*).]

#### ASSAY

- **PROTEIN**, *Nitrogen Determination*, Appendix IIIC

**Analysis:** Calculate the percent protein by the formula:

$$\text{Result} = N \times F$$

N = percent nitrogen

F = nitrogen-to-protein conversion factor, 6.25

**Acceptance criteria:** NLT 42.0% protein

#### IMPURITIES

##### Inorganic Impurities

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

**Sample:** 10 g

**Acceptance criteria:** NMT 2 mg/kg

- **MERCURY**, *Mercury Limit Test*, Appendix IIIB

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

#### SPECIFIC TESTS

- **α-AMINO NITROGEN/TOTAL NITROGEN (AN/TN) PERCENT RATIO**, **α-AMINO NITROGEN (AN) DETERMINATION**, Appendix IIIC and *Total Nitrogen (TN), Nitrogen Determination*, Appendix IIIC

**Analysis:** Calculate the AN/TN percent ratio, where AN is the percent of α-Amino Nitrogen and TN is the percent of Total Nitrogen.

**Acceptance criteria:** Between 15.0% and 55.0%

- **AMMONIA NITROGEN**, *Ammonia Nitrogen (NH<sub>3</sub>-N) Determination*, Appendix IIIC

**Acceptance criteria:** NMT 2.0%, calculated on the sodium chloride-free basis

**Change to read:**

• **GLUTAMIC ACID**, Appendix IIIC

**Acceptance criteria:** NMT 12.0% as  $C_5H_9NO_4$  and NMT 28.0% of the total amino acids ■ protein ■<sub>2S</sub> (FCC7)

• **INSOLUBLE MATTER**

**Sample:** 5 g

**Analysis:** Transfer the *Sample* into a 250-mL Erlenmeyer flask, add 75 mL of water, cover the flask with a watch glass, and boil gently for 2 min. Filter the solution through a tared filtering crucible, dry the crucible at 105° for 1 h, cool, and weigh.

**Acceptance criteria:** NMT 2%

• **MICROBIAL LIMITS**

[Note—Current methods for the following tests may be found in the Food and Drug Administration's Bacteriological Analytical Manual online at [www.cfsan.fda.gov](http://www.cfsan.fda.gov).]

**Acceptance criteria**

**Aerobic plate count:** NMT 50,000 cfu/g

**Coliforms:** NMT 10 cfu/g

**Salmonella:** Negative in 25 g

**Yeasts and Molds:** NMT 50 cfu/g

• **POTASSIUM**

**Standard stock solution:** 200 µg/mL of potassium in deionized water prepared as follows: Transfer 38.20 mg of reagent grade potassium chloride into a 100-mL volumetric flask, add deionized water to dissolve the salt, diluting with deionized water to volume, and mixing.

**Standard solution:** 1.0 µg/mL of potassium in deionized water from the *Standard stock solution*

**Sample stock solution:** Transfer 2.33 g of the sample into a silica or porcelain dish. Ash it in a muffle furnace at 550° for 2 to 4 h. Allow the ash to cool and add 5 mL of 20% hydrochloric acid. If necessary, warm the mixture to completely dissolve the residue. Filter the solution through acid-washed filter paper into a 1000-mL volumetric flask. Wash the filter paper with hot water, dilute the solution to volume, and mix.

**Sample solution:** 1:300 dilution of the *Sample stock solution*

**Analysis:** Using a suitable spectrophotometer, measure the absorbance of the *Sample solution* and the *Standard solution* at 766.5 nm.

**Acceptance criteria:** The absorbance of the *Sample solution* does not exceed that of the *Standard solution*. (NMT 13.0%)

• **SODIUM CHLORIDE**

**Standard stock solution:** 500 µg/mL of sodium chloride in deionized water

**Standard solution:** 5.0 µg/mL of sodium chloride in deionized water from the *Standard stock solution*

**Sample stock solution:** Transfer  $1.0 \pm 0.05$  g of the sample into a silica or porcelain dish. Ash in a muffle furnace at 550° for 2 to 4 h. Allow the ash to cool and add 5 mL of 20% hydrochloric acid. If necessary, warm the mixture to completely dissolve the residue. Filter the solution through acid-washed filter paper into a 100-mL volumetric flask. Wash the filter paper with hot water, dilute the solution to volume, and mix.

**Sample solution:** 1:100 dilution of the *Sample stock solution*

**Analysis:** Using a suitable spectrophotometer, measure the absorbance of the *Sample solution* and the *Standard solution* at 589.0 nm.

**Acceptance criteria:** The absorbance of the *Sample solution* does not exceed that of the *Standard solution*. (NMT 50.0%)

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

<b>Topic/Question</b>	<b>Contact</b>	<b>Expert Committee</b>
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(F12010) Monographs - Food Ingredients

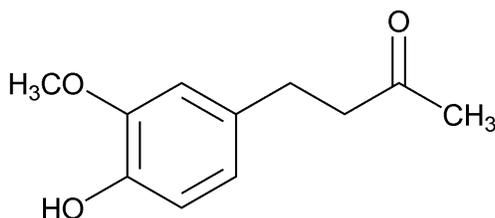
*FCC Seventh Edition Page 1097*

## BRIEFING

**Zingerone**, FCC 7 page 1101. Comments received indicate that the commercial material currently available is of higher purity than before, and thus it does not occur commonly as a liquid anymore. Therefore, its specifications for *Refractive Index* and *Specific Gravity* are no longer appropriate. On the basis of comments received, it is proposed to revise the *Description* and to eliminate the tests for *Refractive Index* and *Specific Gravity*. The addition of *Melting Range or Temperature, Appendix IIB* is also being proposed. All comments and data are encouraged, especially if they are related to the acceptance criteria in the *Assay*.

(FIEC: C. Mejia) C89494

### Zingerone



$C_{11}H_{14}O_3$

Formula wt 194.23  
FEMA: 3124

### DESCRIPTION

**Change to read:**

Zingerone occurs as a yellow to yellow-brown liquid that can solidify at room temperature ■ fused mass or crystals. ■ 2S (FCC7)

**Odor:** Spicy

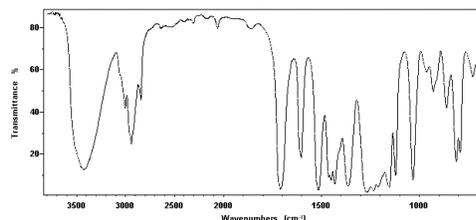
**Boiling Point:**  $\sim 290^{\circ}$

**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.



Zingerone

### ASSAY

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

**Acceptance criteria:** NLT 95.0% of  $C_{11}H_{14}O_3$

**SPECIFIC TESTS**

*Delete the following:*

- ~~■ REFRACTIVE INDEX, Appendix II: At 20°~~  
~~Acceptance criteria: Between 1.538 and 1.545 ■ 2S (FCC7)~~

*Delete the following:*

- ~~■ SPECIFIC GRAVITY: Determine at 25° by any reliable method (see General Provisions):~~  
~~Acceptance criteria: Between 1.136 and 1.140 ■ 2S (FCC7)~~

**OTHER REQUIREMENTS**

*Add the following:*

- ■ MELTING RANGE OR TEMPERATURE, Appendix IIB  
 Acceptance criteria: Between 39° and 43° ■ 2S (FCC7)

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

Topic/Question	Contact	Expert Committee
Monograph	Carla D Mejia, Ph.D. Scientific Liaison 1-301-816-8571	(F12010) Monographs - Food Ingredients

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**BRIEFING****Appendix III: Chemical Tests and Determinations, FCC 7 page 1144; and FCC Forum [Dec. 2009].**

1. In an effort to add more modern methods of analysis for metals and other elemental impurities, a revision to add two methods for *Elemental Impurities by ICP* to *Appendix IIIC* is proposed. These methods are consistent with those proposed in the new *USP* general test chapter *Elemental Impurities—Procedures* ( 233 ), appearing in *PF* 36(1) [Jan.–Feb. 2010] and are being referenced in a new proposed monograph in this *Forum*—See Briefing under *Maritime Pine Extract*. Submit comments to Kristie Laurvick at [kxb@usp.org](mailto:kxb@usp.org).
2. A revision is proposed to correct the equation used to calculate %total color in the *Total Color, Method I* test procedure in the Appendix IIIC section on *Colors*. Submit comments to Jeff Moore at [jm@usp.org](mailto:jm@usp.org).
3. In an effort to modernize test procedures in the *FCC*, specifically by eliminating the use of hazardous substances where possible, a revision to *Method I* under *Nitrogen Determination (Kjeldahl Method)* is proposed. The proposed revisions are based on the *USP 32–NF 27* method *Nitrogen Determination* ( 461 ), on the method for *Nitrogen Determination (Kjeldahl Method I)* appearing in the Joint FAO/WHO Expert Committee on Food Additives Combined Compendium of Food Specifications, Vol. 4, and on AOAC Official Method 991.20. Interested parties are encouraged to submit comments, especially about the applicability and performance of this method for different ingredient matrices, and minimum concentration of nitrites and nitrates in the matrices. Submit comments to Carla Mejia at [cdm@usp.org](mailto:cdm@usp.org).

(FIEC: K. Laurvick; C. Mejia; J. Moore) C87198; C89149 C87445

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# 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 nitroferrocyanide TS. A blue color is produced.

## Magnesium

Solutions of magnesium salts in the presence of ammonium chloride yield no precipitate with ammonium carbonate TS, but a white crystalline precipitate, which is insoluble in 6 N ammonium hydroxide, is formed on the subsequent addition of sodium phosphate TS (6%).

## Manganese

Solutions of manganous salts yield with ammonium sulfide TS a salmon colored precipitate that dissolves in acetic acid.

## Nitrate

When a solution of a nitrate is mixed with an equal volume of sulfuric acid, the mixture cooled, and a solution of ferrous sulfate superimposed, a brown color is produced at the junction of the two liquids. Brown-red fumes are evolved when a nitrate is heated with sulfuric acid and metallic copper. Nitrates do not decolorize acidified potassium permanganate TS (0.1 N) (distinction from nitrites).

## Nitrite

Nitrites yield brown-red fumes when treated with diluted mineral acids or acetic acid. A few drops of potassium iodide TS (15%) and a few drops of 2 N sulfuric acid added to a solution of nitrite liberate iodine, which colors starch TS blue.

## Peroxide

Solutions of peroxides slightly acidified with sulfuric acid yield a deep blue color on the addition of potassium dichromate TS. On shaking the mixture with an equal volume of diethyl ether and allowing the liquids to separate, the blue color is transferred to the ether layer.

## Phosphate

Neutral solutions of orthophosphates yield with silver nitrate TS (0.1 N) a yellow precipitate, which is soluble in 1.7 N nitric acid or in 6 N ammonium hydroxide. With ammonium molybdate TS, a yellow precipitate, which is soluble in 6 N ammonium hydroxide, is formed.

## Potassium

Potassium compounds impart a violet color to a nonluminous flame if not masked by the presence of small quantities of sodium. In neutral, concentrated or moderately concentrated solutions of potassium salts, sodium bitartrate TS (10%) slowly produces a white, crystalline precipitate that is soluble in 6 N ammonium hydroxide and in solutions of alkali hydroxides or carbonates. The precipitation may be accelerated by stirring or rubbing the inside of the test tube with a glass rod or by the addition of a small amount of glacial acetic acid or alcohol.

## Sodium

Dissolve 0.1 g of the sodium compound in 2 mL of water. Add 2 mL of 15% potassium carbonate, and heat to boiling. No precipitate is formed. Add 4 mL of potassium pyroantimonate TS, and heat to boiling. Allow to cool in ice water, and if necessary, rub the inside of the test tube with a glass rod. A dense precipitate is formed. Sodium compounds impart an intense yellow color to a nonluminous flame.

**Change to read:**

## Sulfate

Solutions of sulfates yield with barium chloride TS ▲▲<sub>FCC7</sub> a white precipitate that is insoluble in hydrochloric and nitric acids. Sulfates yield with lead acetate TS ▲▲<sub>FCC7</sub> 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

### ALUMINUM LIMIT TEST

[Note—The *Standard Solutions* and *Sample Solution* may be modified, if necessary, to obtain solutions of suitable concentrations adaptable to the linear or working range of the instrument.]

**Nitric Acid Diluent** Dilute 40 mL of nitric acid with water to 1000 mL.

**Standard Aluminum Solutions** Treat a quantity of aluminum wire with 6 N hydrochloric acid at 80° for a few min. Dissolve 100 mg of the treated wire in a mixture consisting of 10 mL of hydrochloric acid and 2 mL of nitric acid, by heating at 80° for about 30 min. Continue heating until the volume is reduced to about 4 mL. Cool to room temperature, and add 4 mL of water. Evaporate to about 2 mL by heating. Cool, and transfer this solution, with the aid of water, to a 100-mL volumetric flask, and dilute with water to volume (1 mg/mL aluminum). Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, and dilute with water to volume (100 µg/mL aluminum). Transfer 1.0 mL of this solution to a third 100-mL volumetric flask, and dilute with water to volume (1 µg/mL aluminum). [Note—If more diluted *Standard Aluminum Solutions* are required, transfer 1.0-, 2.0-, and 4.0-mL portions of the 1 µg/mL *Standard Aluminum Solution* to separate 100-mL volumetric flasks, dilute with *Nitric Acid Diluent* to volume, and mix. These solutions contain 0.01, 0.02, and 0.04 µg/mL of aluminum, respectively.]

**Sample Solution** Transfer the amount of sample specified in the monograph to a plastic 100-mL volumetric flask. Add 50 mL of water, and sonicate for 30 min. Add 4 mL of nitric acid, and dilute with water to volume.

**Procedure** Determine the absorbances of the *Standard Aluminum Solutions* and the *Sample Solution* at the aluminum emission line at 309.3 nm with a suitable atomic absorption spectrophotometer equipped with an aluminum hollow-cathode lamp and a flameless electrically heated furnace, using the *Nitric Acid Diluent* as the blank. Plot the absorbances of the *Standard Solutions* versus the concentration of aluminum, in µg/mL, drawing a straight line best fitting the three points. From the graph so obtained, determine the concentration, in µg/mL, of aluminum in the *Sample Solution*.

Calculate the amount of aluminum in the sample taken, in µg/g, using the following formula

$$\text{Result} = C_A/C_S$$

in which  $C_A$  is the concentration of aluminum in the *Sample Solution*, in µg/mL, obtained from the standard curve; and  $C_S$  is the concentration of the *Sample Solution*, in g/mL.

## ARSENIC LIMIT TEST

### Silver Diethyldithiocarbamate Colorimetric Method

[Note—All reagents used in this test should be very low in arsenic content.]

**Apparatus** Use the general apparatus shown in *Fig. 11* unless otherwise specified in an individual monograph. It consists of a 125-mL arsine generator flask (*a*) fitted with a scrubber unit (*c*) and an absorber tube (*e*), with a 24/40 standard-taper joint (*b*) and a ball-and-socket joint (*d*), secured with a No. 12 clamp, connecting the units. The tubing between *d* and *e* and between *d* and *c* is a capillary having an id of 2 mm and an od of 8 mm. Alternatively, an apparatus embodying the principle of the general assembly described and illustrated may be used.

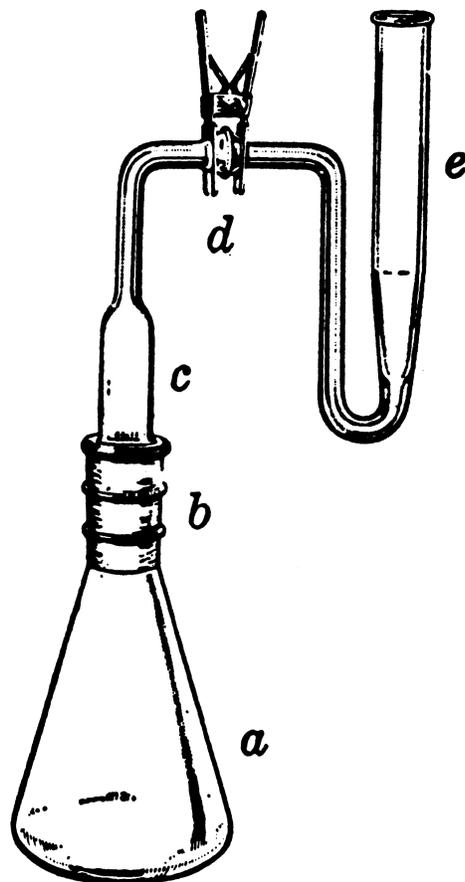


FIGURE 11 General Apparatus for Arsenic Limit Test. (Courtesy of the Fisher Scientific Co., Pittsburgh, PA.)

[Note—The special assemblies shown in Figs. 12, 13, and 14 are to be used only when specified in certain monographs.]

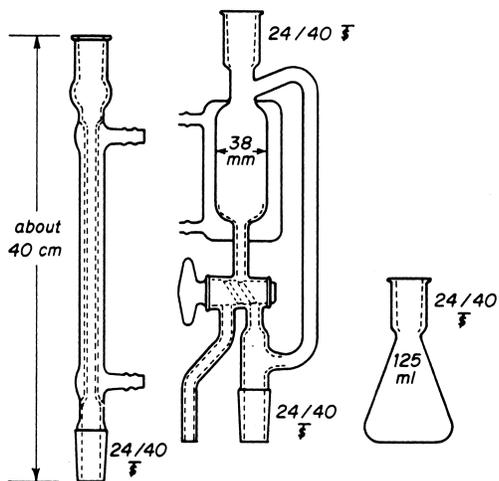


FIGURE 12 Modified Bethe Apparatus for the Distillation of Arsenic Tribromide.

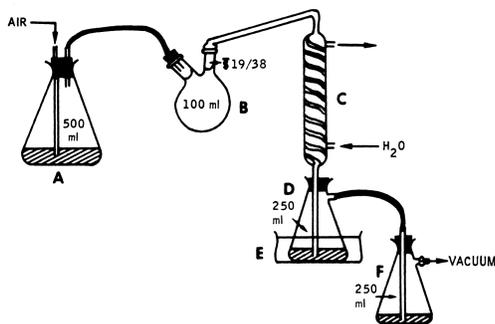


FIGURE 13 Special Apparatus for the Distillation of Arsenic Trichloride. (Flask A contains 150 mL of hydrochloric acid; flasks D and F contain 20 mL of water. Flask D is placed in an ice water bath, E.)

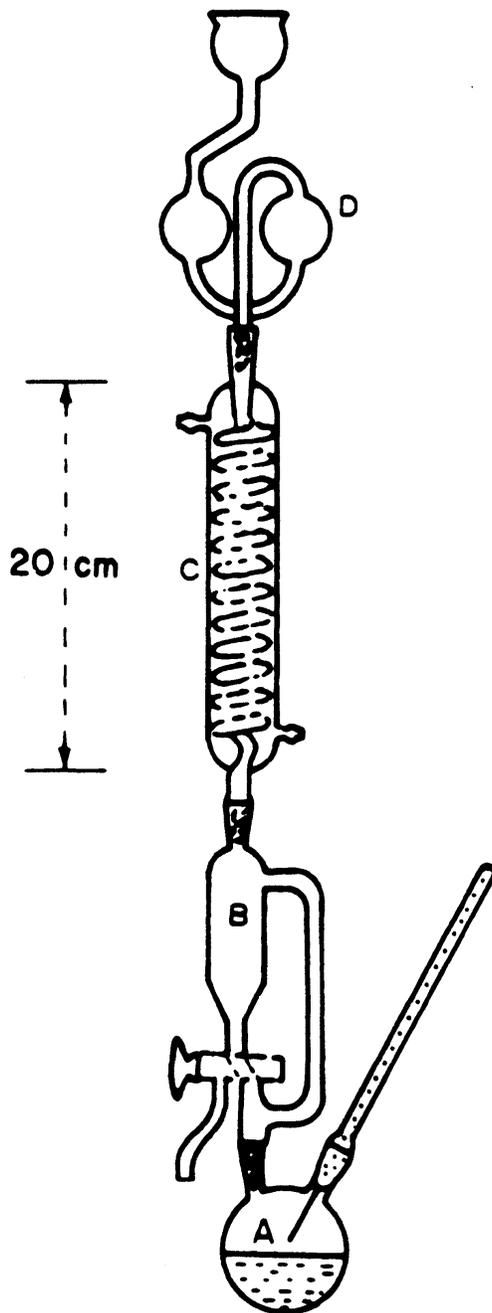


FIGURE 14 Special Apparatus for the Determination of Inorganic Arsenic. (A, 250-mL distillation flask; B,

receiver chamber, approximately 50-mL capacity; C, reflux condenser; D, splash head.)

**Standard Arsenic Solution** Accurately weigh 132.0 mg of arsenic trioxide that has been previously dried at 105° for 1 h, and dissolve it in 5 mL of a 1:5 sodium hydroxide solution. Neutralize the solution with 2 N sulfuric acid, add 10 mL in excess, and dilute with recently boiled water to 1000.0 mL. Transfer 10.0 mL of this solution into a 1000-mL volumetric flask, add 10 mL of 2 N sulfuric acid, dilute with recently boiled water to volume, and mix. Use this final solution, which contains 1 µg of arsenic in each milliliter, within 3 days.

**Silver Diethyldithiocarbamate Solution** Dissolve 1 g of ACS reagent-grade silver diethyldithiocarbamate in 200 mL of recently distilled pyridine. Store this solution in a light-resistant container and use within 1 month.

**Stannous Chloride Solution** Dissolve 40 g of stannous chloride dihydrate ( $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ ) in 100 mL of hydrochloric acid. Store the solution in glass containers and use within 3 months.

**Lead Acetate-Impregnated Cotton** Soak cotton in a saturated solution of lead acetate trihydrate, squeeze out the excess solution, and dry in a vacuum at room temperature.

**Sample Solution** Use directly as the *Sample Solution* in the *Procedure* the solution obtained by treating the sample as directed in an individual monograph. Prepare sample solutions of organic compounds in the generator flask (a), unless otherwise directed, according to the following general procedure:

[**CAUTION**— Some substances may react unexpectedly with explosive violence when digested with hydrogen peroxide. Use appropriate safety precautions at all times. ]

[Note—If halogen-containing compounds are present, use a lower temperature while heating the sample with sulfuric acid; do not boil the mixture; and add the peroxide, with caution, before charring begins to prevent loss of trivalent arsenic.]

Transfer 1.0 g of sample into the generator flask, add 5 mL of sulfuric acid and a few glass beads, and digest at a temperature not exceeding 120° until charring begins, preferably using a hot plate in a fume hood. (Additional sulfuric acid may be necessary to completely wet some samples, but the total volume added should not exceed about 10 mL.) After the acid has initially decomposed the sample, cautiously add, dropwise, hydrogen peroxide (30%), allowing the reaction to subside and reheating the sample between drops. Add the first few drops very slowly with sufficient mixing to prevent a rapid reaction, and discontinue heating if foaming becomes excessive. Swirl the solution in the flask to prevent unreacted substance from caking on the walls or bottom of the flask during digestion.

[Note—Maintain oxidizing conditions at all times during the digestion by adding small quantities of the peroxide whenever the mixture turns brown or darkens.]

Continue the digestion until the organic matter is destroyed, gradually raising the temperature of the hot plate to 250° to 300° until fumes of sulfur trioxide are copiously evolved and the solution becomes colorless or retains only a light straw color. Cool, cautiously add 10 mL of water, heat again to strong fuming, and cool. Cautiously add 10 mL of water, mix, wash the sides of the flask with a few milliliters of water, and dilute to 35 mL.

**Procedure** If the *Sample Solution* was not prepared in the generator flask, transfer to the flask a volume of the solution, prepared as directed, equivalent to 1.0 g of the substance being tested, and add water to make 35 mL. Add 20 mL of 1:5 sulfuric acid, 2 mL of potassium iodide TS, 0.5 mL of *Stannous Chloride Solution*, and 1 mL of isopropyl alcohol, and mix. Allow the mixture to stand for 30 min at room temperature. Pack the scrubber unit (c) with two plugs of *Lead Acetate-Impregnated Cotton*, leaving a small air space between the two plugs, lubricate joints b and d with stopcock grease, if necessary, and connect the scrubber unit with the absorber tube (e). Transfer 3.0 mL of *Silver Diethyldithiocarbamate Solution* to the absorber tube, add 3.0 g of granular zinc (20-mesh) to the mixture in the flask, and immediately insert the standard-taper joint (b) into the

flask. Allow the evolution of hydrogen and color development to proceed at room temperature ( $25^{\circ} \pm 3^{\circ}$ ) 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  $\mu\text{g}$  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  $\pm 2^{\circ}$  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 Belling-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 ( $\text{CdCl}_2 \cdot 2\frac{1}{2}\text{H}_2\text{O}$ ), accurately weighed, into a 1000-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Pipet 25 mL of this solution into a 100-mL volumetric flask, add 1 mL of hydrochloric acid, dilute with water to volume, and mix. Each milliliter contains 12.5  $\mu\text{g}$  of cadmium.

**Sample Solution** Transfer 10 g of sample, accurately weighed, into a 50-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

**Test Solutions** Transfer 5.0 mL of *Sample Solution* into each of five separate 25-mL volumetric flasks. Dilute the contents of *Flask 1* with water to volume, and mix. Add 1.00, 2.00, 3.00, and 4.00 mL of *Standard Solution*, to *Flasks 2, 3, 4, and 5*, respectively, then dilute each flask with water to volume, and mix. The *Test Solutions* contain, respectively, 0, 0.5, 1.0, 1.5, and 2.0  $\mu\text{g}/\text{mL}$  of cadmium.

**Procedure** Determine the absorbance of each *Test Solution* at 228.8 nm, setting the instrument to previously established optimum conditions, using water as a blank. Plot the absorbance of the *Test Solutions* versus their contents of cadmium, in micrograms per milliliter. Draw the straight line best fitting the five points, and extrapolate the line until it intercepts the concentration axis. From the intercept, determine the amount, in micrograms, of cadmium in each milliliter of the *Test Solution* containing 0 mL of the *Standard Preparation*. Calculate the quantity, in milligrams per kilogram, of cadmium in the sample by multiplying this value by 25.

## CHLORIDE AND SULFATE LIMIT TESTS

Where limits for chloride and sulfate are specified in the individual monograph, compare the *Sample Solution* and control in appropriate glass cylinders of the same dimensions and matched as closely as practicable with

respect to their optical characteristics.

If the solution is not perfectly clear after acidification, pass it through filter paper that has been washed free of chloride and sulfate. Add identical quantities of the precipitant (silver nitrate TS or barium chloride TS) in rapid succession to both the *Sample Solution* and the control solution.

Experience has shown that visual turbidimetric comparisons are best made between solutions containing from 10 to 20  $\mu\text{g}$  of chloride (Cl) ion or from 200 to 400  $\mu\text{g}$  of sulfate ( $\text{SO}_4$ ) ion in 50 mL. Weights of samples are specified on this basis in the individual monographs in which these limits are included.

## Chloride Limit Test

**Standard Chloride Solution** Dissolve 165 mg of sodium chloride in water and dilute to 100.0 mL. Transfer 10.0 mL of this solution into a 1000-mL volumetric flask, dilute with water to volume, and mix. Each milliliter of the final solution contains 10  $\mu\text{g}$  of chloride (Cl) ion.

**Procedure** Unless otherwise directed, dissolve the specified amount of the test substance in 30 to 40 mL of water; neutralize to litmus external indicator with nitric acid, if necessary; and add 1 mL in excess. Add 1 mL of silver nitrate TS to the clear solution or filtrate, dilute with water to 50 mL, mix, and allow to stand for 5 min protected from direct sunlight. Compare the turbidity, if any, with that produced similarly in a control solution containing the required volume of *Standard Chloride Solution* and the quantities of the reagents used for the sample.

## Sulfate Limit Test

**Standard Sulfate Solution** Dissolve 148 mg of anhydrous sodium sulfate in water, and dilute to 100.0 mL. Transfer 10.0 mL of this solution to a 1000-mL volumetric flask, dilute with water to volume, and mix. Each milliliter of the final solution contains 10  $\mu\text{g}$  of sulfate ( $\text{SO}_4$ ).

**Procedure** Unless otherwise directed, dissolve the specified amount of the test substance in 30 to 40 mL of water; neutralize to litmus external indicator with hydrochloric acid, if necessary; then add 1 mL of 2.7 N hydrochloric acid. Add 3 mL of barium chloride TS to the clear solution or filtrate, dilute with water to 50 mL, and mix. After 10 min compare the turbidity, if any, with that produced in a solution containing the required volume of *Standard Sulfate Solution* and the quantities of the reagents used for the sample.

## 1,4-DIOXANE LIMIT TEST

**Vacuum Distillation Apparatus** Assemble a closed-system vacuum distillation apparatus employing glass vacuum stopcocks (A, B, and C), as shown in *Fig. 15*.

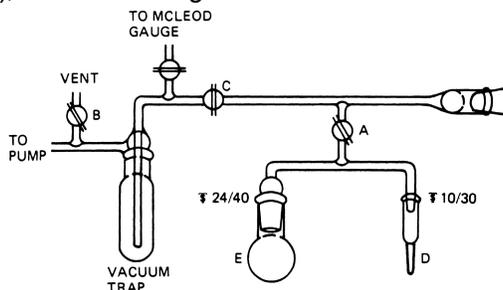


FIGURE 15 Closed-System Vacuum Distillation Apparatus for 1,4-Dioxane.

The concentrator tube (*D*) is made of borosilicate or quartz (not flint) glass, graduated precisely enough to measure the 0.9 mL or more of distillate and marked so that the analyst can accurately dilute to 2.0 mL (available as Chromaflex concentrator tube, Kontes Glass Co., Vineland, NJ, Catalog No. K42560-0000).

**Standard Preparation** Prepare a solution of 1,4-dioxane in water containing 100 µg/mL. Keep the solution refrigerated, and prepare fresh weekly.

**Sample Preparation** Transfer 20 g of the sample, accurately weighed, into a 50-mL round-bottom flask (*E*) having a 24/40 ground-glass neck. Semisolid or waxy samples should be liquefied by heating on a steam bath before making the transfer. Add 2.0 mL of water to the flask for crystalline samples, and 1.0 mL for liquid, semisolid, or waxy samples. Place a small Teflon-covered stirring bar in the flask, stopper, and stir to mix. Immerse the flask in an ice bath, and chill for about 1 min.

Wrap heating tape around the tube connecting the Chromaflex tube (*D*) and the round-bottom flask (*E*), and apply about 10 V to the tape. Apply a light coating of high-vacuum silicone grease to the ground-glass joints, and connect the Chromaflex tube to the 10/30 joint and the round-bottom flask to the 24/40 joint. Immerse the vacuum trap in a Dewar flask filled with liquid nitrogen, close stopcocks *A* and *B*, open stopcock *C*, and begin evacuating the system with a vacuum pump. Prepare a slush bath from powdered dry ice and methanol, and raise the bath to the neck of the round-bottom flask. After freezing the contents of the flask for about 10 min, and when the vacuum system is operating at 0.05 mm pressure or lower, open stopcock *A* for 20 s, and then close it. Remove the slush bath, and allow the flask to warm in air for about 1 min. Immerse the flask in a water bath at 20° to 25°, and after about 5 min warm the water in the bath to 35° to 40° (sufficient to liquefy most samples) while stirring slowly but constantly with the magnetic bar. Cool the water in the bath by adding ice, and chill for about 2 min. Replace the water bath with the slush bath, freeze the contents of the flask for about 10 min, then open stopcock *A* for 20 s, and close it. Remove the slush bath, and repeat the heating steps as before, this time reaching a final temperature of 45° to 50° or a temperature necessary to melt the sample completely. If there is any condensation in the tube connecting the round-bottom flask to the Chromaflex tube, slowly increase the voltage to the heating tape and heat until condensation disappears.

Stir with the magnetic stirrer throughout the following steps: very slowly immerse the Chromaflex tube in the Dewar flask containing liquid nitrogen. [**CAUTION**— When there is liquid distillate in the Chromaflex tube, the tube must be immersed in the nitrogen very slowly, or the tube will break. ]

Water will begin to distill into the tube. As ice forms in the tube, raise the Dewar flask to keep the liquid nitrogen level only slightly below the level of ice in the tube. When water begins to freeze in the neck of the 10/30 joint, or when liquid nitrogen reaches the 2.0-mL graduation mark on the Chromaflex tube, remove the Dewar flask and let the ice melt without heating. After the ice has melted, check the volume of water that has distilled, and repeat the sequence of chilling and thawing until at least 0.9 mL of water has been collected. Freeze the tube once again for about 2 min, and release the vacuum first by opening stopcock *B*, followed by stopcock *A*. Remove the Chromaflex tube from the apparatus, close it with a greased stopper, and let the ice melt without heating. Mix the contents of the tube by swirling, note the volume of distillate, and dilute with water to 2.0 mL, if necessary. Use this *Sample Preparation* as directed under *Chromatography*.

**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  $\mu\text{L}$ , 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*.<sup>1</sup>

## 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  $135^{\circ}$  to  $140^{\circ}$  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  $135^{\circ}$ . Add water from the funnel or introduce steam through the capillary, maintaining the temperature between  $135^{\circ}$  and  $140^{\circ}$  at all times. Continue the distillation until 100 mL of distillate has been collected. After the 100-mL portion (*Distillate A*) is collected, collect an additional 50-mL portion of distillate (*Distillate B*) to ensure that all of the fluorine has been volatilized.

Place 50 mL of *Distillate A* in a 50-mL Nessler tube. In another, similar Nessler tube, place 50 mL of water distilled through the apparatus as a control. Add to each tube 0.1 mL of a filtered 1:1000 solution of sodium alizarinsulfonate and 1 mL of a freshly prepared 1:4000 solution of hydroxylamine hydrochloride, and mix well. Add, dropwise and with stirring, either 1 N or 0.05 N sodium hydroxide, depending on the expected volume of volatile acid distilling over, to the tube containing the distillate until its color just matches that of the control, which is faintly pink. Then add to each tube 1.0 mL of 0.1 N hydrochloric acid, and mix well. From a buret, graduated in 0.05 mL, add slowly to the tube containing the distillate enough of a 1:4000 solution of thorium nitrate so that, after mixing, the color of the liquid just changes to a faint pink. Note the volume of the solution

added, then add exactly the same volume to the control, and mix. Now add to the control solution sodium fluoride TS (10 µg F per milliliter) from a buret to make the colors of the two tubes match after dilution to the same volume. Mix well, and allow all air bubbles to escape before making the final color comparison. Check the endpoint by adding 1 or 2 drops of sodium fluoride TS to the control. A distinct change in color should take place. Note the volume of sodium fluoride TS added.

Dilute *Distillate B* to 100 mL, and mix well. Place 50 mL of this solution in a 50-mL Nessler tube, and follow the procedure used for *Distillate A*. The total volume of sodium fluoride TS required for the solutions from both *Distillate A* and *Distillate B* should not exceed 2.5 mL.

## Method II (Ion-Selective Electrode Method A)

**Buffer Solution** Dissolve 36 g of cyclohexylenedinitrilotetraacetic acid (CDTA) in sufficient 1 N sodium hydroxide to make 200 mL. Transfer 20 mL of this solution (equivalent to 4 g of disodium CDTA) into a 1000-mL beaker containing 500 mL of water, 57 mL of glacial acetic acid, and 58 g of sodium chloride, and stir to dissolve. Adjust the pH of the solution to between 5.0 and 5.5 by the addition of 5 N sodium hydroxide, then cool to room temperature, dilute with water to 1000 mL, and mix.

**Procedure** Unless otherwise directed in the individual monograph, transfer 8.0 g of sample and 20 mL of water into a 250-mL distilling flask, cautiously add 20 mL of perchloric acid, and then add 2 or 3 drops of a 1:2 solution of silver nitrate and a few glass beads.

[**CAUTION**— Handle perchloric acid in an appropriate fume hood. ]

Following the directions, and observing the *Caution* and *Notes*, as given under *Method I*, distill the solution until 200 mL of distillate has been collected.

Transfer a 25.0-mL aliquot of the distillate into a 250-mL plastic beaker, and dilute with the *Buffer Solution* to 100 mL. Place the fluoride ion and reference electrodes (or a combination fluoride electrode) of a suitable ion-selective electrode apparatus in the solution. Adjust the calibration control until the indicator needle points to the center on the logarithmic concentration scale, allowing sufficient time for equilibration (about 20 min), and stirring constantly during the equilibration period and throughout the remainder of the procedure. Pipet 1.0 mL of a solution containing 100 µg of fluoride (F) ion per milliliter (prepared by dissolving 22.2 mg of sodium fluoride, previously dried at 200° for 4 h, in sufficient water to make 100.0 mL) into the beaker, allow the electrode to come to equilibrium, and record the final reading on the logarithmic concentration scale.

[*Note*—Follow the instrument manufacturer's instructions regarding precautions and interferences, electrode filling and check, temperature compensation, and calibration.]

**Calculations** Calculate the fluoride content, in milligrams per kilogram, of the sample taken:

$$\text{Result} = [A/(R - I)] \times 100 \times (200/25W)$$

in which I is the initial scale reading before the addition of the sodium fluoride solution; A is the concentration, in micrograms per milliliter, of fluoride in the sodium fluoride solution added to the sample solution; R is the final scale reading after addition of the sodium fluoride solution; and W is the original weight, in grams, of the sample.

**Change to read:**

## Method III (Ion-Selective Electrode Method B)

**Sodium Fluoride Solution** (5 µg F per milliliter) Transfer 2.210 g of sodium fluoride, previously dried at 200° for 4 h and accurately weighed, into a 400-mL plastic beaker, add 200 mL of water, and stir until dissolved. Quantitatively transfer this solution into a 1000-mL volumetric flask with the aid of water, dilute with water to volume, and mix. Store this stock solution in a plastic bottle. On the day of use, transfer 5.0 mL of the stock solution into a 1000-mL volumetric flask, dilute with water to volume, and mix.

**Calibration Curve:** Transfer 1.0, 2.0, 3.0, 5.0, 10.0, and 15.0 mL of the *Sodium Fluoride Solution* into separate 250-mL plastic beakers; add 50 mL of water, 5 mL of 1 N hydrochloric acid, 10 mL of 1 M sodium citrate, and 10 mL of 0.2 M disodium EDTA to each beaker; and mix. Transfer each solution into separate 100-mL volumetric flasks, dilute with water to volume, and mix. Transfer a 50-mL portion of each solution into separate 125-mL plastic beakers, and measure the potential of each solution with a suitable ion-selective electrode apparatus (such as the Orion Model No. 94-09, with solid-state membrane), using a suitable reference electrode (such as the Orion Model No. 90-01, with single junction). Plot the calibration curve on two-cycle semilogarithmic paper (such as K & E No. 465130) or with the use of a suitable graphing calculator or spreadsheet program, 1S (FCC7) with micrograms of F per 100 mL solution on the logarithmic scale.

**Procedure:** Transfer 1.00 g of sample into a 150-mL glass beaker, add 10 mL of water, and, while stirring continuously, slowly add 20 mL of 1 N hydrochloric acid to dissolve the sample. Boil rapidly for 1 min, then transfer into a 250-mL plastic beaker, and cool rapidly in ice water. Add 15 mL of 1 M sodium citrate and 10 mL of 0.2 M disodium EDTA, and mix. Adjust the pH to 5.5 ± 0.1 with 1 N hydrochloric acid or 1 N sodium hydroxide, if necessary; transfer into a 100-mL volumetric flask; dilute with water to volume; and mix. Transfer a 50-mL portion of this solution into a 125-mL plastic beaker, and measure the potential of the solution with the apparatus described under *Calibration Curve*. Determine the fluoride content, in micrograms, of the sample from the *Calibration Curve*. Determine the percentage of fluoride in the sample by the formula

$$\text{Result} = (C/W_S) \times 0.000001 \times 100\%$$

in which C is the content of fluoride, in µg, in the sample, determined from the *Calibration Curve*;  $W_S$  is the sample weight, in g; and 0.000001 is a factor converting micrograms to grams. 1S (FCC7)

**Change to read:**

## Method IV (Ion-Selective Electrode Method C)

[Note—Unless directed otherwise by the individual monograph, use *Buffer Solution A* for samples with a neutral to higher pH, and use *Buffer Solution B* for samples with a neutral to lower pH.]

**Buffer Solution A** Add 2 volumes of 6 N acetic acid to 1 volume of water, and adjust the pH to 5.0 with 50% potassium hydroxide solution.

**Buffer Solution B** Dissolve 150 g of sodium citrate dihydrate and 10.3 g of disodium EDTA dihydrate in 800 mL of water, adjust the pH to 8.0 with 50% sodium hydroxide solution, and dilute with water to 1000 mL.

### Fluoride Standard Solutions

*1000 mg/kg Fluoride Standard* Transfer 2.2108 g of sodium fluoride, previously dried at 200° for 4 h, into a 1000-mL volumetric flask and dissolve in and dilute with water to volume. The resulting solution contains 1000 µg of fluoride per milliliter.

*50 mg/kg Fluoride Standard* Pipet 50 mL of the *1000 mg/kg Fluoride Standard* into a 1000-mL volumetric flask. Dilute with water to volume.

*10 mg/kg Fluoride Standard* Pipet 100 mL of the *50 mg/kg Fluoride Standard* into a 500-mL volumetric flask. Dilute with water to volume.

**Fluoride Limit Solutions** (for a 1-g sample)

*50 mg/kg Fluoride Limit Solution* (1 mg/kg fluoride standard) Pipet 50 mL of the *10 mg/kg Fluoride Standard* into a 500-mL volumetric flask, and dilute with water to volume.

*10 mg/kg Fluoride Limit Solution* (0.2 mg/kg fluoride standard) Pipet 10 mL of the *10 mg/kg Fluoride Standard* into a 500-mL volumetric flask, and dilute with water to volume.

**Fluoride Limit Solutions** (for a 2-g sample)

*50 mg/kg Fluoride Limit Solution* (2 mg/kg fluoride standard) Pipet 100 mL of the *10 mg/kg Fluoride Standard* into a 500-mL volumetric flask, and dilute with water to volume.

*10 mg/kg Fluoride Limit Solution* (0.4 mg/kg fluoride standard) Pipet 20 mL of the *10 mg/kg Fluoride Standard* into a 500-mL volumetric flask, and dilute with water to volume.

[Note—Store all standard and limit solutions in plastic containers.]

**Sample Preparation** Accurately weigh the amount of sample specified in the monograph, transfer it into a 100-mL volumetric flask, and dissolve it in a minimal amount of water. Add 50.0 mL of the appropriate *Buffer Solution*, dilute with water to volume, and mix.

**Electrode Calibration** Pipet 50 mL of the appropriate *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  $\mu$ L and 1000  $\mu$ L 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 $^{\circ}$  for *Buffer Solution A* and in the range of 54 to 60 mV at 25 $^{\circ}$  for *Buffer Solution B*. If the difference in potential is not within this range, check, and, if necessary, replace the electrode, instrument, or solutions.

■ Alternatively, the electrode calibration should be performed according to the manufacturer's instructions and should comply with the manufacturer's calibration range at 25 $^{\circ}$ . If the difference in potential is not within this range, evaluate the system and equipment as necessary. ■ 1S (FCC7)

**Procedure** Transfer the entire sample into a plastic beaker. Place the electrode into the beaker, allow the solution to equilibrate for 5 min with stirring, and read the potential, in millivolts. Remove and rinse the electrode(s) with water. In another beaker, using a pipet, add 50 mL of the appropriate *Buffer Solution* followed by 50 mL of the *Fluoride Limit Solution* that best reflects the fluoride limit of the sample. Place the electrode in the beaker, equilibrate for 3 min, and read the potential in millivolts. If the potential of the *Fluoride Limit Solution* is less than that of the sample, the sample passes the test criteria for maximum acceptable fluoride level limit.

## Method V

**Lime Suspension** Carefully shake about 56 g of low-fluorine calcium oxide (about 2 mg/kg of F) with 250 mL of water, and while stirring, slowly add 250 mL of 60% perchloric acid. Add a few glass beads, and boil until copious fumes of perchloric acid evolve, then cool, add 200 mL of water, and boil again.

[CAUTION— Handle perchloric acid in an appropriate fume hood. ]

Repeat the dilution and boiling once more, cool, dilute considerably, and if precipitated silicon dioxide forms, pass through a fritted-glass filter. While stirring, pour the clear solution into 1000 mL of a 1:10 solution of

sodium hydroxide, allow the precipitate to settle, and siphon off the supernatant liquid. Remove the sodium salts from the precipitate by washing five times with water in large centrifuge bottles, shaking the mass thoroughly each time. Finally, shake the precipitate into a suspension and dilute with water to 2000 mL. Store in paraffin-lined bottles, and shake well before use.

[Note—100 mL of this suspension should give no appreciable fluoride blank when evaporated, distilled, and titrated as directed under *Method I*.]

**Procedure** Assemble the distilling apparatus as described under *Method I*, and add 1.67 g of sample, accurately weighed, and 25 mL of 1:2 sulfuric acid to the distilling flask. Distill until the temperature reaches 160°, then maintain at 160° to 165° by adding water from the funnel, collecting 300 mL of distillate. Oxidize the distillate by cautiously adding 2 or 3 mL of fluorine-free 30% hydrogen peroxide (to remove sulfates), allow to stand for a few minutes, and evaporate in a platinum dish with an excess of *Lime Suspension*. Ignite briefly at 600°, then cool and wet the ash with about 10 mL of water. Cover the dish with a watch glass, and cautiously introduce under the watch glass just sufficient 60% perchloric acid to dissolve the ash. Add the contents of the dish through the dropping funnel of a freshly prepared distilling apparatus (the distilling flask should contain a few glass beads), using a total of 20 mL of the 60% perchloric acid to dissolve the ash and transfer the solution. Add 10 mL of water and a few drops of a 1:2 solution of silver perchlorate through the dropping funnel, and continue as directed under *Method I*, beginning with “Distill until the temperature reaches 135° . . . .”

## LEAD LIMIT TEST

[Note—Unless otherwise specified in the monograph, use the *Dithizone Method* to determine lead levels.]

### Dithizone Method

**Special Reagents** Select reagents having as low a lead content as practicable, and store all solutions in containers of borosilicate glass. Rinse all glassware thoroughly with warm, 1:2 nitric acid followed by water.

**Ammonia–Cyanide Solution** Dissolve 2 g of potassium cyanide in 15 mL of ammonium hydroxide, and dilute with water to 100 mL.

**Ammonium Citrate Solution** Dissolve 40 g of citric acid in 90 mL of water, add 2 or 3 drops of phenol red TS, then cautiously add ammonium hydroxide until the solution acquires a red color. Extract it with 20-mL portions of *Dithizone Extraction Solution* until the dithizone solution retains its green color or remains unchanged.

#### Diluted Standard Lead Solution (1 µg Pb in 1 mL)

*Lead Nitrate Stock Solution* Dissolve 159.8 mg of ACS Reagent-Grade Lead Nitrate [Pb(NO<sub>3</sub>)<sub>2</sub>] in 100 mL of water containing 1 mL of nitric acid, dilute with water to 1000.0 mL, and mix. Prepare and store this solution in glass containers that are free from lead salts.

*Standard Lead Solution* On the day of use, dilute 10.0 mL of *Lead Nitrate Stock Solution* with water to 100.0 mL. Each milliliter of *Standard Lead Solution* contains the equivalent of 10 µg of lead (Pb) ion.

*Diluted Standard Lead Solution* Immediately before use, transfer 10.0 mL of *Standard Lead Solution* into a 100-mL volumetric flask, dilute with 1:100 nitric acid to volume, 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.

**Hydroxylamine Hydrochloride Solution** Dissolve 20 g of hydroxylamine hydrochloride in sufficient water to make about 65 mL, transfer the solution into a separator, add a few drops of thymol blue TS, then add ammonium hydroxide until the solution assumes a yellow color. Add 10 mL of a 1:25 solution of sodium diethyldithiocarbamate, mix, and allow to stand for 5 min. Extract the solution with successive 10- to 15-mL portions of chloroform until a 5-mL test portion of the chloroform extract does not assume a yellow color when shaken with cupric sulfate TS. Add 2.7 N hydrochloric acid until the extracted solution is pink, adding 1 or 2 drops more of thymol blue TS if necessary, then dilute with water to 100 mL, and mix.

**Potassium Cyanide Solution** Dissolve 50 g of potassium cyanide in sufficient water to make 100 mL. Remove the lead from the solution by extraction with successive portions of *Dithizone Extraction Solution* as described under *Ammonium Citrate Solution*, then extract any dithizone remaining in the cyanide solution by shaking with chloroform. Finally, dilute the cyanide solution with sufficient water so that each 100 mL contains 10 g of potassium cyanide.

**Standard Dithizone Solution** Dissolve 10 mg of dithizone in 1000 mL of chloroform, keeping the solution in a glass-stoppered, lead-free bottle suitably wrapped to protect it from light and stored in a refrigerator.

**Sample Solution** Use the solution obtained by treating the sample as directed in an individual monograph as the *Sample Solution* in the *Procedure*. Sample solutions of organic compounds are prepared, unless otherwise directed, according to the following general method: [**CAUTION**— Some substances may react unexpectedly with explosive violence when digested with hydrogen peroxide. Use appropriate safety precautions at all times. ]

Transfer 1.0 g of sample into a suitable flask, add 5 mL of sulfuric acid and a few glass beads, and digest at a temperature not exceeding 120° until charring begins, using preferably, a hot plate in a fume hood. (Additional sulfuric acid may be necessary to completely wet some samples, but the total volume added should not exceed about 10 mL.) After the sample has initially been decomposed by the acid, add with caution, dropwise, hydrogen peroxide (30%), allowing the reaction to subside and reheating between drops. The first few drops must be added very slowly with sufficient mixing to prevent a rapid reaction, and heating should be discontinued if foaming becomes excessive. Swirl the solution in the flask to prevent unreacted substance from caking on the walls or bottom of the flask during the digestion.

[Note—Add small quantities of the peroxide when the solution begins to darken.]

Continue the digestion until the organic matter is destroyed, gradually raising the temperature of the hot plate to 250° to 300° until fumes of sulfur trioxide are copiously evolved and the solution becomes colorless or retains only a light straw color. Cool, cautiously add 10 mL of water, again evaporate to strong fuming, and cool. Quantitatively transfer the solution into a separator with the aid of small quantities of water.

**Procedure** Transfer the *Sample Solution*, prepared as directed in the individual monograph, into a separator, and unless otherwise directed, add 6 mL of *Ammonium Citrate Solution* and 2 mL of *Hydroxylamine Hydrochloride Solution*. (Use 10 mL of the citrate solution when determining lead in iron salts.) Add 2 drops of phenol red TS to the separator, and make the solution just alkaline (red in color) by the addition of ammonium hydroxide. Cool the solution, if necessary, under a stream of tap water, then add 2 mL of *Potassium Cyanide Solution*. Immediately extract the solution with 5-mL portions of *Dithizone Extraction Solution*, draining each extract into another separator, until the dithizone solution retains its green color. Shake the combined dithizone solutions for 30 s with 20 mL of 1:100 nitric acid; discard the chloroform layer; add 5.0 mL of

*Standard Dithizone Solution* and 4 mL of *Ammonia–Cyanide Solution* to the acid solution; and shake for 30 s. The purple hue in the chloroform solution of the sample caused by any lead dithizonate present does not exceed that in a control, containing the volume of *Diluted Standard Lead Solution* equivalent to the amount of lead specified in the monograph, when treated in the same manner as the sample.

### Flame Atomic Absorption Spectrophotometric Method

Select reagents having as low a lead content as practicable, and store all solutions in high-density polyethylene containers. Rinse all plastic and glassware thoroughly with warm, 1:2 nitric acid followed by water.

**Lead Nitrate Stock Solution** (100 µg/mL) Dissolve 159.8 mg of reagent-grade lead nitrate [Pb(NO<sub>3</sub>)<sub>2</sub>] in 100 mL of water containing 1 mL of nitric acid in a 1000-mL volumetric flask, and dilute with water to volume.

**Standard Lead Solution** (10 µg/mL) On the day of use, transfer 10 mL of *Lead Nitrate Stock Solution* into a 100-mL volumetric flask, and dilute with water to volume.

**Diluted Standard Lead Solutions** On the day of use, prepare a set of standard lead solutions that corresponds to the lead limit specified in the monograph:

*1 mg/kg Lead Limit* (0.5, 1.0, and 1.5 µg/mL standards) On the day of use, transfer 5.0, 10.0, and 15.0 mL of *Standard Lead Solution* into three separate 100-mL volumetric flasks, add 10 mL of 3 N hydrochloric acid to each, and dilute with water to volume.

*5 mg/kg Lead Limit* (1.0, 5.0, and 10.0 µg/mL standards) On the day of use, transfer 10.0 and 50.0 mL of *Standard Lead Solution* into two separate 100-mL volumetric flasks, add 10 mL of 3 N hydrochloric acid to each, and dilute with water to volume. The final standard, 10.0 µg/mL, is taken directly from the *Standard Lead Solution*.

*10 mg/kg Lead Limit* (5.0, 10.0, and 15.0 µg/mL standards) On the day of use, transfer 5.0, 10.0, and 15.0 mL of *Lead Nitrate Stock Solution* into three separate 100-mL volumetric flasks, add 10 mL of 3 N hydrochloric acid to each, and dilute with water to volume.

**25% Sulfuric Acid Solution (by volume)** Cautiously add 100 mL of sulfuric acid to 300 mL of water with constant stirring while cooling in an ice bath.

**Sample Preparation** Transfer the sample weight as specified in the monograph, weighed to the nearest 0.1 mg, into an evaporating dish. Add a sufficient amount of *25% Sulfuric Acid Solution*, and distribute the sulfuric acid uniformly through the sample. Within a hood, place the dish on a steam bath to evaporate most of the water. Place the dish on a burner, and slowly pre-ash the sample by expelling most of the sulfuric acid. Place the dish in a muffle furnace that has been set at 525°, and ash the sample until the residue appears free from carbon. Prepare a *Sample Blank* by ashing 5 mL of 25% sulfuric acid. Cool and cautiously wash down the inside of each evaporation dish with water.

Add 5 mL of 1 N hydrochloric acid. Place the dish on a steam bath, and evaporate to dryness. Add 1.0 mL of 3 N hydrochloric acid and approximately 5 mL of water, and heat briefly on a steam bath to dissolve any residue. Transfer each solution quantitatively to a 10-mL volumetric flask, dilute to volume, and mix.

**Procedure** Concomitantly determine the absorbances of the *Sample Blank*, the *Diluted Standard Lead Solutions*, and the *Sample Preparation* at the lead emission line of 283.3 nm, using a slit-width of 0.7 nm. Use a suitable atomic absorption spectrophotometer equipped with a lead electrodeless discharge lamp (EDL), an air–acetylene flame, and a 4-in burner head. Use water as the blank.

**Calculations** Determine the corrected absorbance values by subtracting the *Sample Blank* absorbance from each of the *Diluted Standard Lead Solutions* and from the *Sample Preparation* absorbances. Prepare a

standard curve by plotting the corrected *Diluted Standard Lead Solutions* absorbance values versus their corresponding concentrations expressed as micrograms per milliliter. Determine the lead concentration in the *Sample Preparation* by reference to the calibration curve. Calculate the quantity of lead, in milligrams per kilogram, in the sample taken:

$$\text{Result} = 10C/W_S$$

in which C is the concentration, in micrograms per milliliter, of lead from the standard curve; and  $W_S$  is the weight, in grams, of the sample taken.

## 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- $\mu\text{g}/\text{mL}$  lead stock to prepare (weekly) an intermediate 10- $\mu\text{g}/\text{mL}$  standard in 5% nitric acid. Prepare (daily) a *Lead Standard Solution* (1  $\mu\text{g}/\text{mL}$ ) by diluting the intermediate 10- $\mu\text{g}/\text{mL}$  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  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  of blanks, calibration standards, and sample solutions and 5  $\mu\text{L}$  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  $\times$  6-mm, end-heated furnace tube, correct the cause of insufficient sensitivity before proceeding. If the integrated absorbance is greater than 0.25 abs-sec, contamination is likely, and the source should be investigated. Calculate the characteristic mass ( $m_0$ ) (mass of Pb pg necessary to produce an integrated absorbance of 0.0044 abs-sec) as follows:

$$m_0 = (0.0044 \text{ abs-sec})(25 \text{ pg}/\mu\text{L})(20 \mu\text{L}) / (\text{measured } 25 \text{ pg}/\mu\text{L} \text{ abs-sec})$$

Record and track the integrated absorbance and  $m_0$  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  $\leq 25$  ng/mL.

Use the calibration algorithms provided in the instrument software. Recheck calibration periodically ( $\leq 15$  samples) by running a 25- or 50-ng/mL calibration standard interspersed with samples. If recheck differs from calibration by  $>10\%$ , recalibrate the instrument. The instrumental detection limit (DL) and quantitation limit (QL), in picograms, may be based on 7 to 10 replicates of the *Sample Preparation Blank* and

calculated as follows:

$$DL = (3)(s.d. \text{ blank abs-sec})(10 \text{ pg}/\mu\text{L})(20\mu\text{L}) / (\text{abs-sec } 10 \text{ ng/mL std})$$

$$QL = (10)(s.d. \text{ blank abs-sec})(10 \text{ pg}/\mu\text{L})(20 \mu\text{L}) / (\text{abs-sec } 10 \text{ ng/mL std})$$

During method development, detection limits were typically 10 to 14 pg, corresponding to 0.5 to 0.7 ng/mL for 20  $\mu\text{L}$ . 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:

$$\text{Pb (ng/g)} = (\text{blank-corrected Pb ng/mL})(\text{DF})[\text{sample vol (10 mL)}] / [\text{sample wt (approx. 1.5 g)}]^2$$

**Quality Assurance** To ensure analytical accuracy, NIST SRM 1643c acidified water or a similar material should be analyzed before the unknown samples are. The certified content of SRM 1643c is  $35.3 \pm 0.9$  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  $100\% \pm 20\%$ , and the precision for complete replicate digestions should be <5% RSD. Periodically, a sample digest should be checked using the method of standard additions to ensure that there are no multiplicative or chemical interferences. Spiking samples and checking recoveries is always a good practice.

## Method II

This method is primarily intended for the determination of lead at levels of less than 1 mg/kg in substances immiscible with water, such as edible oils.

**Apparatus** Use a suitable atomic absorption spectrophotometer (Perkin-Elmer Model 3100 or equivalent) fitted with a graphite furnace (Perkin-Elmer HGA 600 or equivalent). Use a lead hollow-cathode lamp (Perkin-Elmer or equivalent) with argon as the carrier gas. Follow the manufacturers' directions for setting the appropriate instrument parameters for lead determination.

[Note—For this test, use reagent-grade chemicals with as low a lead content as is practicable, as well as high-purity water and gases. Before use in this analysis, rinse all glassware and plasticware twice with 10% nitric acid and twice with 10% hydrochloric acid, and then rinse them thoroughly with high-purity water, preferably obtained from a mixed-bed strong-acid, strong-base ion-exchange cartridge capable of producing water with an electrical resistivity of 12 to 15 megohms.]

**Hydrogen Peroxide–Nitric Acid Solution** Dissolve equal volumes of 10% hydrogen peroxide and 10% nitric acid.

[Note—Use caution.]

**Lead Nitrate Stock Solution** Dissolve 159.8 mg of ACS Reagent-Grade Lead Nitrate (alternatively, use NIST Standard Reference Material, containing 10 mg of lead per kilogram, or equivalent) in 100 mL of *Hydrogen Peroxide–Nitric Acid Solution*. Dilute with *Hydrogen Peroxide–Nitric Acid Solution* to 1000.0 mL, and mix. Prepare and store this solution in glass containers that are free from lead salts. Each milliliter of this solution contains the equivalent of 100 µg of lead (Pb) ion.

**Standard Lead Solution** On the day of use, dilute 10.0 mL of *Lead Nitrate Stock Solution* with *Hydrogen Peroxide–Nitric Acid Solution* to 100.0 mL, and mix. Each milliliter of *Standard Lead Solution* contains the equivalent of 10 µg of lead (Pb) ion.

**Butanol–Nitric Acid Solution** Slowly add 50 mL of nitric acid to approximately 500 mL of butanol contained in a 1000-mL volumetric flask. Dilute with butanol to volume, and mix.

**Standard Solutions** Prepare a series of lead standard solutions serially diluted from the *Standard Lead Solution* in *Butanol–Nitric Acid Solution*. Pipet into separate 100-mL volumetric flasks 0.2, 0.5, 1, and 2 mL, respectively, of *Standard Lead Solution*, dilute with *Butanol–Nitric Acid Solution* to volume, and mix. The *Standard Solutions* contain, respectively, 0.02, 0.05, 0.1, and 0.2 µg of lead per milliliter. (For lead limits greater than 1 mg/kg, prepare a series of standard solutions in a range encompassing the expected lead concentration in the sample.)

**Sample Solution** [CAUTION— Perform this procedure in a fume hood, and wear safety glasses. ] Transfer 1 g of sample, accurately weighed, into a large test tube. Add 1 mL of nitric acid. Place the test tube in a rack in a boiling water bath. As soon as the rusty tint is gone, add 1 mL of 30% hydrogen peroxide dropwise to avoid a vigorous reaction, and wait for bubbles to form. Stir with an acid-washed plastic spatula if necessary. Remove the test tube from the water bath, and let it cool. Transfer the solution into a 10-mL volumetric flask, and dilute with *Butanol–Nitric Acid Solution* to volume, and mix. Use this solution for analysis.

## Procedure

*Tungsten Solution* Transfer 0.1 g of tungstic acid ( $\text{H}_2\text{WO}_4$ ) 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  $\mu\text{L}$  of the *Sample Solution* under identical conditions, and measure its corrected maximum absorbance. From the *Standard Curve*, determine the concentration,  $C$ , in micrograms per milliliter, of the *Sample Solution*. Calculate the quantity, in milligrams per kilogram, of lead in the sample by the formula:

$$\text{Result} = 10C/W$$

in which  $W$  is the weight, in grams, of the sample taken.

### APDC Extraction Method

Select reagents having as low a lead content as practicable, and store all solutions in high-density polyethylene containers. Rinse all plastic and glassware thoroughly with warm, 1:2 nitric acid followed by water.

**2% APDC Solution** Dissolve 2.0 g of ammonium pyrrolidinedithiocarbamate (APDC) in 100 mL of water. Filter any slight residue of insoluble APDC from the solution before use.

**Lead Nitrate Stock Solution (100  $\mu\text{g}/\text{mL}$ )** Dissolve 159.8 mg of reagent-grade lead nitrate [ $\text{Pb}(\text{NO}_3)_2$ ] in 100 mL of water containing 1 mL of nitric acid in a 1000-mL volumetric flask, and dilute with water to volume.

### Standard Lead Solutions

**2 mg/kg Lead Standard** On the day of use, transfer 2.0 mL of *Lead Nitrate Stock Solution* into a 100-mL volumetric flask, and dilute with water to volume. The resulting solution contains 2  $\mu\text{g}$  of lead per milliliter.

**3 mg/kg Lead Standard** On the day of use, transfer 3.0 mL of *Lead Nitrate Stock Solution* into a 100-mL volumetric flask, and dilute with water to volume. The resulting solution contains 3  $\mu\text{g}$  of lead per milliliter.

**4 mg/kg Lead Standard** On the day of use, transfer 4.0 mL of *Lead Nitrate Stock Solution* into a 100-mL volumetric flask, and dilute with water to volume. The resulting solution contains 4  $\mu\text{g}$  of lead per milliliter.

**10 mg/kg Lead Standard** On the day of use, transfer 10.0 mL of *Lead Nitrate Stock Solution* into a 100-mL volumetric flask, and dilute with water to volume. The resulting solution contains 10  $\mu\text{g}$  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 with deionized water to about 100 mL. Adjust the pH of the resulting solution to between 1.0 and 1.5 with 25% NaOH. Quantitatively transfer the pH-adjusted solution to a clean 250-mL separatory funnel, and dilute with water to about 200 mL. Add 2 mL of 2% APDC Solution, and mix. Extract with two 20-mL portions of chloroform, collecting the extracts in a clean 50-mL beaker. Evaporate to dryness on a steam bath. Add 3 mL of nitric acid to the residue, and heat to near dryness. Then add 0.5 mL of nitric acid and 10 mL of deionized water to the beaker, and heat until the volume is reduced to about 3 to 5 mL. Transfer the digested extract to a clean 10-mL volumetric flask, and dilute with water to volume.

**Procedure** Concomitantly determine the absorbances of the appropriate *Standard Lead Solution* and the *Sample Preparation* against the blank at the lead emission line of 283.3 nm, using a slit-width of 0.7 nm. Use a suitable atomic absorption spectrophotometer equipped with a lead electrode-less discharge lamp (EDL), or equivalent; an air-acetylene flame; and a 4-in burner head. Use water as the blank. The absorbance of the

*Sample Preparation* is not greater than that of the *Standard Lead Solution*.

## MANGANESE LIMIT TEST

**Manganese Detection Instrument** Use any suitable atomic absorption spectrophotometer equipped with a fast-response recorder or other readout device and capable of measuring the radiation absorbed by manganese atoms at the manganese resonance line of 279.5 nm.

**Standard Preparations** Transfer 1000 mg, accurately weighed, of manganese metal powder into a 1000-mL volumetric flask, dissolve by warming in a mixture of 10 mL of water and 10 mL of 0.5 N hydrochloric acid, cool, dilute with water to volume, and mix. Pipet 5.0 mL of this solution into a 50-mL volumetric flask, dilute with water to volume, and mix. Finally, pipet 5.0, 10.0, 15.0, and 25.0 mL of this solution into separate 1000-mL volumetric flasks, dilute each flask with water to volume, and mix. The final solutions contain 0.5, 1.0, 1.5, and 2.5 mg/kg of Mn, respectively.

**Sample Preparation** Transfer 10.000 g of the sample into a 200-mL Kohlrausch volumetric flask, previously rinsed with 0.5 N hydrochloric acid, add 140 mL of 0.5 N hydrochloric acid, and shake vigorously for 15 min, preferably with a mechanical shaker. Dilute with 0.5 N hydrochloric acid to volume, and shake. Centrifuge approximately 100 mL of the sample mixture in a heavy-walled centrifuge tube at 2000 rpm for 5 min, and use the clear supernatant liquid in the following *Procedure*.

**Procedure** Aspirate 0.5 N hydrochloric acid through the air-acetylene burner for 5 min, and obtain a baseline reading at 279.5 nm, following the manufacturer's instructions for operating the atomic absorption spectrophotometer being used for the analysis. Aspirate a portion of each *Standard Preparation* in the same manner, note the readings, then aspirate a portion of the *Sample Preparation*, and note the reading. Prepare a standard curve by plotting the mg/kg of Mn in each *Standard Preparation* against the respective readings. From the graph determine the mg/kg of Mn in the *Sample Preparation*, and multiply this value by 20 to obtain the mg/kg of Mn in the original sample taken for analysis.

## MERCURY LIMIT TEST

### Method I

**Mercury Detection Instrument** Use any suitable atomic absorption spectrophotometer equipped with a fast-response recorder and capable of measuring the radiation absorbed by mercury vapors at the mercury resonance line of 253.6 nm. A simple mercury vapor meter or detector equipped with a variable span recorder also is satisfactory.

[Note—Wash all glassware associated with the test with nitric acid, and rinse thoroughly with water before use.]

**Aeration Apparatus** The apparatus, shown in *Fig. 16*, consists of a flowmeter (*a*), capable of measuring flow rates from 500 to 1000 mL/min, connected via a three-way stopcock (*b*), with a Teflon plug, to 125-mL gas washing bottles (*c* and *d*), followed by a drying tube (*e*), and finally a suitable quartz liquid absorption cell (*f*), terminating with a vent (*g*) to a fume hood.

[Note—The absorption cell will vary in optical pathlength depending on the type of mercury detection instrument used.]

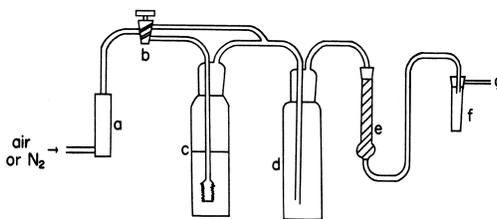


FIGURE 16 Aeration Apparatus for Mercury Limit Test.

Bottle *c* is fitted with an extra-coarse fritted bubbler (Coming 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 [ $\text{Hg}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$ ] into a 1000-mL volumetric flask, dissolve in a mixture of 100 mL of water and 2 mL of nitric acid, dilute with water to volume, and mix. Discard after 1 month. Transfer 10.0 mL of this solution into a second 1000-mL volumetric flask, acidify with 5 mL of a 1:5 sulfuric acid solution, dilute with water to volume, and mix. Discard after 1 week. On the day of use, transfer 10.0 mL of the second solution into a 100-mL volumetric flask, acidify with 5 mL of 1:5 sulfuric acid, dilute with water to volume, and mix. Each milliliter of this solution contains 1  $\mu\text{g}$  of mercury. Transfer 2.0 mL of this solution (2  $\mu\text{g}$  Hg) into a 50-mL beaker, and add 20 mL of water, 1 mL of a 1:5 sulfuric acid solution, and 1 mL of a 1:25 solution of potassium permanganate. Cover the beaker with a watch glass, boil for a few seconds, and cool.

**Sample Preparation** Prepare as directed in the individual monograph.

**Procedure** Assemble the aerating apparatus as shown in *Fig. 16*, with bottles *c* and *d* empty and stopcock *b* in the bypass position. Connect the apparatus to the absorption cell (*f*) in the instrument, and adjust the air or nitrogen flow rate so that in the following procedure, maximum absorption and reproducibility are obtained without excessive foaming in the test solution. Obtain a baseline reading at 253.6 nm, following the manufacturer's instructions for operating the instrument.

Treat the *Standard Preparation* as follows: destroy the excess permanganate by adding a 1:10 solution of hydroxylamine hydrochloride, dropwise, until the solution is colorless. Immediately wash the solution into bottle *c* with water, and dilute with water to the 60-mL mark. Add 2 mL of 10% stannous chloride solution (prepared fresh each week by dissolving 10 g of  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  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 with water to 100 mL, and mix.

**Mercury Stock Solution** Transfer 135.4 mg of mercuric chloride, accurately weighed, into a 100-mL volumetric flask, dissolve in and dilute with 1 N sulfuric acid to volume, and mix. Dilute 5.0 mL of this solution to 500.0 mL with 1 N sulfuric acid. Each milliliter contains the equivalent of 10 µg of mercury.

**Diluted Standard Mercury Solution** On the day of use, transfer 10.0 mL of *Mercury Stock Solution* into a 100-mL volumetric flask, dilute with 1 N sulfuric acid to volume, and mix. Each milliliter contains the equivalent of 1 µg of mercury.

**Sodium Citrate Solution** Dissolve 250 g of sodium citrate dihydrate in 1000 mL of water.

**Sample Solution** Dissolve 1 g of sample in 30 mL of 1.7 N nitric acid by heating on a steam bath. Cool to room temperature in an ice bath, stir, and pass through S and S No. 589, or equivalent, filter paper that has been previously washed with 1.7 N nitric acid, followed by water. Add 20 mL of *Sodium Citrate Solution* and 1 mL of *Hydroxylamine Hydrochloride Solution* to the filtrate.

**Procedure** [Note—Because mercuric dithizonate is light sensitive, perform this procedure in subdued light.] Prepare a control containing 3.0 mL of *Diluted Standard Mercury Solution* (3 µg Hg), 30 mL of 1.7 N nitric acid, 5 mL of *Sodium Citrate Solution*, and 1 mL of *Hydroxylamine Hydrochloride Solution*. Treat the control and the *Sample Solution* as follows: using a pH meter, adjust the pH of each solution to 1.8 with ammonium hydroxide, and transfer the solutions into different separators. Extract each with two 5-mL portions of *Dithizone Extraction Solution*, and then extract again with 5 mL of chloroform, discarding the aqueous solutions. Transfer the combined extracts from each separator into different separators, add 10 mL of 1:2 hydrochloric acid to each, shake well, and discard the chloroform layers. Extract the acid solutions with about 3 mL of chloroform, shake well, and discard the chloroform layers. Add 0.1 mL of 0.05 M disodium EDTA and 2 mL of 6 N acetic acid to each separator, mix, and then slowly add 5 mL of ammonium hydroxide. Stopper the separators, and cool under a stream of cold water, and dry the outside of the separators. To avoid loss, carefully pour the solutions through the tops of the separators into separate beakers, and using a pH meter, adjust the pH of both solutions to 1.8 with 6 N ammonium hydroxide. Return the sample and control solutions to their original separators, add 5.0 mL of *Diluted Dithizone Extraction Solution*, and shake vigorously. Any color developed in the *Sample Solution* does not exceed that in the control.

## NICKEL LIMIT TEST

[Note—Unless otherwise specified in the individual monograph, use *Method I*.]

### Method I

**Atomic Absorption System Apparatus** Use a suitable atomic absorption spectrometer equipped with a nickel hollow-cathode lamp and an air-acetylene flame to measure the absorbance of the *Blank Preparation*,

the *Standard Preparations*, and the *Test Preparation* as directed under *Procedure*.

**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*.

## 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<sup>3</sup> 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  $\mu\text{g}$ , of nickel in each g of test specimen taken:

$$\text{Result} = 100C/W$$

in which W is the weight, in g, of test specimen taken to prepare the *Test Solution*.

## PHOSPHORUS LIMIT TEST

### Reagents

*Ammonium Molybdate Solution (5%)* Dissolve 50 g of ammonium molybdate tetrahydrate,  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ , in 900 mL of warm water, cool to room temperature, dilute with water to 1000 mL, and mix.

*Ammonium Vanadate Solution (0.25%)* Dissolve 2.5 g of ammonium metavanadate,  $\text{NH}_4\text{VO}_3$ , in 600 mL of boiling water, cool to  $60^\circ$  to  $70^\circ$ , and add 20 mL of nitric acid. Cool to room temperature, dilute with water to 1000 mL, and mix.

*Zinc Acetate Solution (10%)* Dissolve 120 g of zinc acetate dihydrate,  $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2\cdot 2\text{H}_2\text{O}$ , in 880 mL of water, and pass through Whatman No. 2V or equivalent filter paper before use.

*Nitric Acid Solution (29%)* Add 300 mL of nitric acid (sp. gr. 1.42) to 600 mL of water, and mix.

*Standard Phosphorus Solution (100  $\mu\text{g}$  P in 1 mL)* Dissolve 438.7 mg of monobasic potassium phosphate,  $\text{KH}_2\text{PO}_4$ , in water in a 1000-mL volumetric flask, dilute with water to volume, and mix.

**Standard Curve** Pipet 5.0, 10.0, and 15.0 mL of the *Standard Phosphorus Solution* into separate 100-mL volumetric flasks. To each of these flasks, and to a fourth, blank flask, add in the order stated 10 mL of *Nitric Acid Solution*, 10 mL of *Ammonium Vanadate Solution*, and 10 mL of *Ammonium Molybdate Solution*, mixing thoroughly after each addition. Dilute with water to volume, mix, and allow to stand for 10 min. Determine the absorbance of each standard solution in a 1-cm cell at 460 nm, with a suitable spectrophotometer, using the blank to set the instrument to zero. Prepare a standard curve by plotting the absorbance of each solution versus its concentration, in mg of phosphorus (P) per 100 mL.

**Treated Sample** Place 20 to 25 g of the starch sample in a 250-mL beaker, add 200 mL of a 7:3 methanol:water mixture, disperse the sample, and agitate mechanically for 15 min. Recover the starch by vacuum filtration in a 150-mL medium-porosity fritted-glass or Büchner funnel, and wash the wet cake with 200 mL of the methanol:water mixture. Reslurry the wet cake in the solvent, and wash it a second time in the same manner. Dry the filter cake in an air oven at a temperature below  $50^\circ$ , 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  $120^\circ$  for 5 h.

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

**Sample Preparation** Transfer about 10 g of the *Treated Sample*, calculated on the dry-substance basis and accurately weighed, into a Vycor dish, and add 10 mL of *Zinc Acetate Solution* in a fine stream, distributing the solution uniformly in the sample. Carefully evaporate to dryness on a hot plate, then increase the heat, and carbonize the sample on the hot plate or over a gas flame. Ignite in a muffle furnace at 550° until the ash is free from carbon (about 1 to 2 h), and cool. Wet the ash with 15 mL of water, and slowly wash down the sides of the dish with 5 mL of *Nitric Acid Solution*. Heat to boiling, cool, and quantitatively transfer the mixture into a 200-mL volumetric flask, rinsing the dish with three 20-mL portions of water and adding the rinsings to the flask. Dilute with water to volume, and mix. Transfer an accurately measured aliquot (*V*, in mL) of this solution, containing not more than 1.5 mg of phosphorus, into a 100-mL volumetric flask, and add 50 mL of water to a second flask to serve as a blank. To each flask add in the order stated 10 mL of *Nitric Acid Solution*, 10 mL of *Ammonium Vanadate Solution*, and 10 mL of *Ammonium Molybdate Solution*, mixing thoroughly after each addition. Dilute with water to volume, mix, and allow to stand for 10 min.

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

$$\text{mg/kg P} = (a \times 200 \times 1000)/(V \times W)$$

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

## SELENIUM LIMIT TEST

### Reagents and Solutions

**2,3-Diaminonaphthalene Solution** On the day of use, dissolve 100 mg of 2,3-diaminonaphthalene ( $\text{C}_{10}\text{H}_{10}\text{N}_2$ ) and 500 mg of hydroxylamine hydrochloride ( $\text{NH}_2\text{OH}\cdot\text{HCl}$ ) in sufficient 0.1 N hydrochloric acid to make 100 mL.

**Selenium Stock Solution** Transfer 40.0 mg of powdered metallic selenium into a 1000-mL volumetric flask, and dissolve in 100 mL of 1:2 nitric acid, warming gently on a steam bath to effect solution. Cool, dilute with water to volume, and mix.

**Selenium Standard Solution** Pipet 5.0 mL of *Selenium Stock Solution* into a 200-mL volumetric flask, dilute with water to volume, and mix. Each milliliter of this solution contains the equivalent of 1  $\mu\text{g}$  of selenium (*Se*).

### Method I

**Standard Preparation** Pipet 6.0 mL of *Selenium Standard Solution* into a 150-mL beaker, add 50 mL of 0.25 N nitric acid, and mix.

**Sample Preparation** Using a 1000-mL combustion flask and 25 mL of 0.5 N nitric acid as the absorbing liquid, proceed as directed under *Oxygen Flask Combustion*, Appendix I, using the amount of sample specified in the individual monograph (and the magnesium oxide or other reagent, where specified).

[Note—If the sample contains water of hydration or more than 1% of moisture, dry it at 140° for 2 h before combustion, unless otherwise directed.]

Upon completion of combustion, place a few milliliters of water in the cup or lip of the combustion flask, loosen

the stopper of the flask, and rinse the stopper, sample holder, and sides of the flask with about 10 mL of water. Transfer the solution, with the aid of about 20 mL of water, into a 150-mL beaker, heat gently to boiling, boil for 10 min, and cool.

**Procedure** Treat the *Sample Preparation*, the *Standard Preparation*, and 50 mL of 0.25 N nitric acid, to serve as the blank, similarly and in parallel as follows: add a 1:2 solution of ammonium hydroxide to adjust the pH of the solution to  $2.0 \pm 0.2$ . 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*.

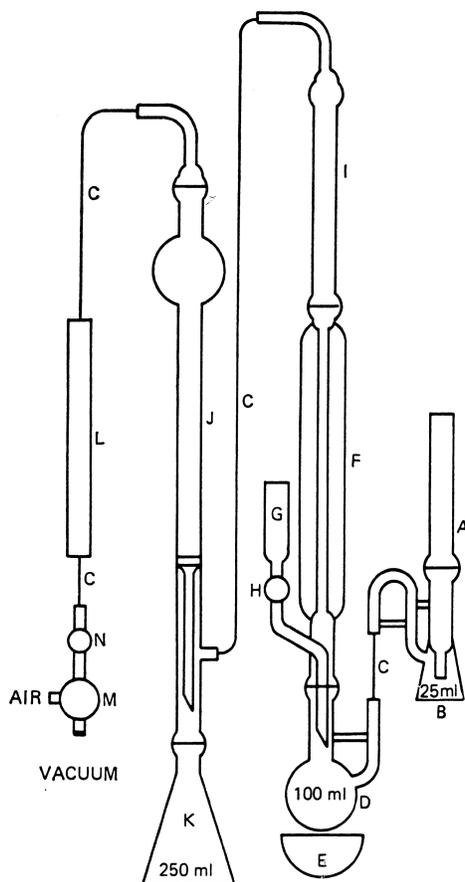


FIGURE 17 Apparatus for Alginates Assay.

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  $100.0 \pm 1.0\%$  ( $24.99 \pm 0.25\% CO_2$ ) 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 ( $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ ). 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 ( $\text{CO}_2$ ). Calculate the results on the dried basis.

## **$\alpha$ -AMINO NITROGEN (AN) DETERMINATION**

Transfer 7 to 25 g of sample, accurately weighed, into a 500-mL volumetric flask with the aid of several 50-mL portions of warm, ammonia-free water, dilute with water to volume, and mix. Neutralize 20.0 mL of the solution with 0.2 N barium hydroxide or 0.2 N sodium hydroxide, using phenolphthalein TS as the indicator, and add 10 mL of freshly prepared phenolphthalein-formol solution (50 mL of 40% formaldehyde containing 1 mL of 0.05% phenolphthalein in 50% alcohol neutralized exactly to pH 7 with 0.2 N barium hydroxide or 0.2 N sodium hydroxide). Titrate with 0.2 N barium hydroxide or 0.2 N sodium hydroxide to a distinct red color, add a small, but accurately measured, volume of 0.2 N barium hydroxide or 0.2 N sodium hydroxide in excess, and back titrate to neutrality with 0.2 N hydrochloric acid. Conduct a blank titration using the same reagents, with 20 mL of water in place of the test solution. Each milliliter of 0.2 N barium hydroxide or 0.2 N sodium hydroxide is equivalent to 2.8 mg of  $\alpha$ -amino nitrogen.

## **AMMONIA NITROGEN ( $\text{NH}_3\text{-N}$ ) DETERMINATION**

[CAUTION— Provide adequate ventilation. ]

[Note—Use nitrogen-free reagents, where available, or reagents very low in nitrogen content.]

Transfer between 700 mg and 2.2 g of sample into a 500- to 800-mL Kjeldahl digestion flask of hard, moderately thick, well-annealed glass. If desired, wrap the sample, if solid or semisolid, in nitrogen-free filter paper to facilitate the transfer.

Add about 200 mL of water, and mix. Add a few granules of zinc to prevent bumping, tilt the flask, and cautiously pour sodium hydroxide pellets, or a 2:5 sodium hydroxide solution, down the inside of the flask so that it forms a layer under the solution, using a sufficient amount (usually about 25 g of solid sodium hydroxide) to make the mixture strongly alkaline. Immediately connect the flask to a distillation apparatus consisting of a Kjeldahl connecting bulb and a condenser that has a delivery tube extending well beneath the surface of a

measured excess of 0.5 N hydrochloric or sulfuric acid contained in a 500-mL flask. Add 5 to 7 drops of methyl red indicator (1 g of methyl red in 200 mL of alcohol) to the receiver flask. Rotate the Kjeldahl flask to mix its contents thoroughly, and heat until all of the ammonia has distilled, collecting at least 150 mL of distillate. Wash the tip of the delivery tube, collecting the washings in the receiving flask, and titrate the excess acid with 0.5 N sodium hydroxide. Perform a blank determination (see *General Provisions*), substituting 2 g of sucrose for the sample, and make any necessary correction. Each milliliter of 0.5 N acid consumed is equivalent to 7.003 mg of ammonia nitrogen.

[Note—If it is known that the substance to be determined has a low nitrogen content, 0.1 N acid and alkali may be used, in which case each milliliter of 0.1 N acid consumed is equivalent to 1.401 mg of nitrogen.]

Calculate the percent ammonia nitrogen by the formula:

$$\text{Result} = (\text{NH}_3\text{-N/S}) \times 100$$

in which  $\text{NH}_3\text{-N}$  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*. Column materials and conditions that have been found suitable for this method are listed in the accompanying tables. See *Fig. 18* for a typical chromatogram obtained with column No. 5.

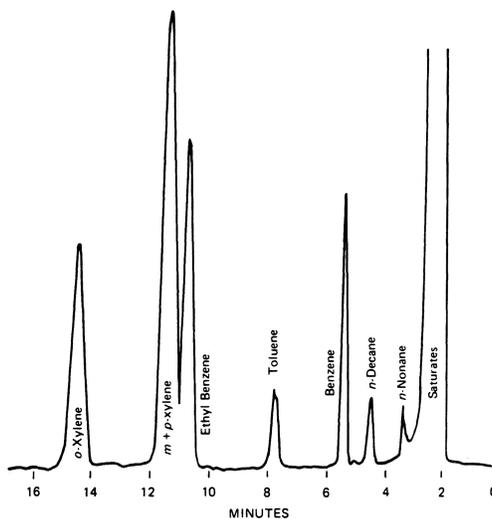


FIGURE 18 Typical Chromatogram for the Determination of Benzene in Hexanes Using Column No. 5.

## Reagents

*Isooctane* 99 mole percent minimum containing less than 0.05 mole percent aromatic material.

*Benzene* 99.5 mole percent minimum.

*Internal Standard* *n*-Decane and either *n*-undecane or *n*-dodecane according to the requirement of the *System Suitability Test*.

*Reference Solution A* Prepare a standard solution containing 0.5% by weight each of the *Internal Standard*

and of benzene in isooctane.

*Reference Solution B* Prepare a standard solution containing about 0.5% by weight each of *n*-decane, of *Internal Standard*, and of benzene in isooctane.

**Calibration** Select the instrument conditions necessary to give the desired sensitivity. Inject a known volume of *Reference Solution A*, and change the attenuation, if necessary, so that the benzene peak is measured with a chart deflection of not less than 25% or more than 95% of full scale. When choosing the attenuation, consider all unresolved peaks to represent a single compound. There may be tailing of the nonaromatic peak, but do not use any conditions that lead to a depth of the valley ahead of the benzene peak (A) less than 50% of the weight of the benzene peak (B) as depicted in *Fig. 19*.

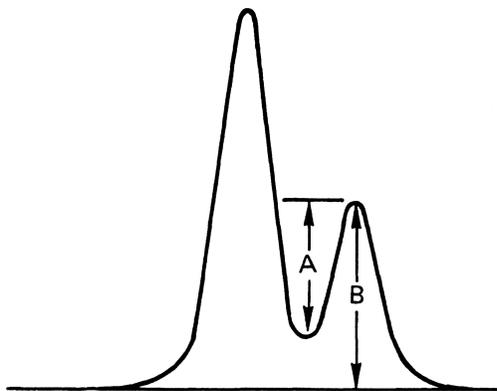


FIGURE 19 Illustration of A/B Ratio.

If there is tailing of the nonaromatic material, construct a baseline by drawing a line from the bottom of the valley ahead of the benzene peak to the point of tangency after the peak (see *Fig. 20*). Measure the areas of the benzene peak and the internal standard peak by any of the following means: triangulation, planimeter, paper cutout, or mechanical or electronic integrator. Do not use integrators on peaks without a constant baseline, unless the integrator has provision for making baseline corrections with accuracy at least as good as that of manual methods.

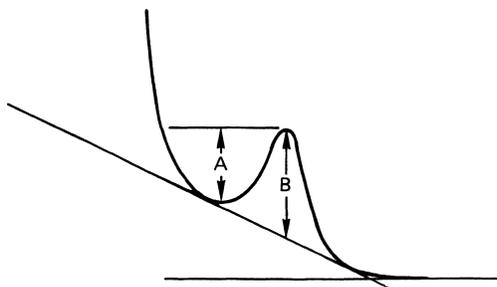


FIGURE 20 Illustration of A/B Ratio for a Small Component Peak on the Tail of a Large Peak.

Calculate a response factor for benzene ( $R_b$ ) relative to the *Internal Standard* by the formula:

$$\text{Result} = A_i/W_i \times W_b/A_b$$

in which  $A_i$  is the area of the *Internal Standard* peak in arbitrary units corrected for attenuation;  $W_i$  is the weight percent of *Internal Standard* in *Reference Solution A*;  $A_b$  is the area of the benzene peak in arbitrary units corrected for attenuation; and  $W_b$  is the weight percent of benzene in *Reference Solution A*.

**Procedure** Place approximately 0.1 mL of *Internal Standard* into a tared 25-mL volumetric flask, weigh on an analytical balance, dissolve in and dilute with the sample to be analyzed to volume.

Using the exact instrumental conditions that were used in the calibration, inject the same volume of sample containing the *Internal Standard*. Before measuring the area of the *Internal Standard* and benzene peaks, change the attenuation to ensure at least 25% chart deflection.

Measure the area of the *Internal Standard* and benzene peaks in the same manner as was used for the calibration. Calculate the weight percent of benzene in the sample ( $W_B$ ) by the formula:

$$\text{Result} = (A_b \times R_b \times W_i \times 100) / (A_i \times S)$$

in which  $A_b$  is the area of the benzene peak corrected for attenuation;  $R_b$  is the relative response factor for benzene;  $W_i$  is the weight, in grams, of *Internal Standard* added;  $A_i$  is the area of the *Internal Standard* peak corrected for attenuation; and  $S$  is the weight, in grams of the sample taken.

**System Suitability Test** Inject the same volume of *Reference Solution B* as in the *Calibration* and record the chromatogram. *n*-Decane must be eluted before benzene, and the ratio of A to B (*Fig. 19*) must be at least 0.5 where A is equal to the depth of the valley between the *n*-decane and benzene peaks and B is equal to the height of the benzene peak.

#### **Column Materials and Conditions for the Determination of Benzene in Hexanes**

Column No.	1	2	3	4	5	6	7
Liquid phase	CEF	PEF 200	CEF	DEGS	TCEPE	TCEPE	DEGS
Length, ft	15	6	16	10	15	100	12
m	—	4.5	2	5	3.1	—	313.7
Diameter, in (mm)							
Inside	0.07(1.8)	—	0.07	0.18(4.5)	0.06(1.5)	0.01(.254)	
Outside	1/8(3.2)	1/4(6.4)	1/8	—	—	—	1/8
Weight, percent	17	30	20	20	10	—	20
Solid support	Chromosorb P	Chromosorb P	Chromosorb P	Chromosorb P	Chromosorb P	Capillary	Chromosorb P
Mesh	60–80	60–80	60–80	80–100	60–80	—	80–100
Treatment	AW	AW	AW	none	AW	none	AW Sil
Inlet, deg	200	210	250	260	250	275	260
Detector, deg	200	155	250	200	175	250	240
Column, deg	115	95	90	100	115	95	65
Carrier gas	N <sub>2</sub>	He	He	He	N <sub>2</sub>	N <sub>2</sub>	He
Flow rate, cm <sup>3</sup> /min	30	60	60	60	1	3	52
Detector	FI	TC	FI	FI	FI	FI	FI
Recorder, mV	5	1	1	1	10	1	1
Sample, 1	5	10	1	2	5	0.8	5
Split	9 + 1	—	—	—	100 + 1	100 – 1	—
Area	Tri	EI	DI	Tri Plan	EI	EI	Tri

**Abbreviations Used in Table:**

AW—Acid washed; CEF—*N,N*-Bis(2-cyanoethyl)formamide; DEGS—Diethylene Glycol Succinate; DI—Disk integrator; EI—Electronic integrator; FI—Flame ionization; Sil—Silanized; TC—Thermal conductivity; TCEPE—Tetracyanoethylated Pentaerythritol; Tri—Triangulation.

**Retention Times in Minutes for Selected Hydrocarbons Under the Conditions for the Determination of Benzene in Hexanes**

Column No.	1	2	3	4	5	6	7
Benzene	3.4	2.0	6.5	6.7	5.4	6.1	6.7
Toluene	4.4	3.2	9.0	10.3	7.8	7.0	10.3
Ethylbenzene	5.4	5.2	11.5	14.8	10.8	8.0	14.8
<i>p-m</i> -Xylenes	5.8	—	12.5	—	11.4	8.5	—
<i>o</i> -Xylene	7.5	6.8	17.0	16.1	14.5	10.0	—
<i>n</i> -Undecane	3.0	2.8	3.5	—	—	—	—
<i>n</i> -Dodecane	—	—	—	12.8	8.5	6.5	—

## Chromium

### Standards

*Standard Chromium Solution* (1000 mg/kg) Transfer 2.829 g of  $K_2Cr_2O_7$ , accurately weighed (National Institute of Standards and Technology No. 136) into a 1-L volumetric flask; dissolve in and dilute with water to volume.

*Standard Colorant Solution* Transfer 62.5 g of colorant previously shown to be free of chromium to a 1-L volumetric flask; dissolve in and dilute with water to volume.

**Apparatus** Use any suitable atomic absorption spectrophotometer equipped with a fast response recorder and capable of measuring the radiation absorbed at 357.9 nm.

**Instrument Parameters** *Wavelength setting:* 357.9 nm; *optical passes:* 5; *lamp current:* 8 mA; *lamp voltage:* 500 v; *fuel:* hydrogen; *oxidant:* air; *recorder:* 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 with water to volume.

Transfer 5 g of the colorant to be analyzed to a 100-mL volumetric flask; dissolve in and dilute with water to volume. Prepare a calibration curve using the series of standards, and using this curve, determine the chromium content of the colorant samples.

## Ether Extracts

[**CAUTION**— Isopropyl ether forms explosive peroxides. To ensure the absence of peroxides, perform the following test: prepare a colorless solution of ferrous thiocyanate by mixing equal volumes of 0.1 N ferrous sulfate and 0.1 N ammonium thiocyanate. Using titanous chloride, carefully discharge any red coloration due to ferric ions. Add 10 mL of ether to 50 mL of the solution, and shake vigorously for 2 to 3 min. A red color indicates the presence of peroxides. If redistillation is necessary, the usual precautions against peroxide detonation should be observed. Immediately before use, pass the ether through a 30-cm column of chromatography-grade aluminum oxide to remove peroxides and inhibitors. ]

**Apparatus** Use an upward displacement-type liquid-liquid extractor, as shown in *Fig. 21*, with a sintered-glass diffuser and a working capacity of 200 mL. Suspend a piece of bright copper wire through the condenser, and place a small coil of copper wire (about 0.5 g) in the distillation flask.

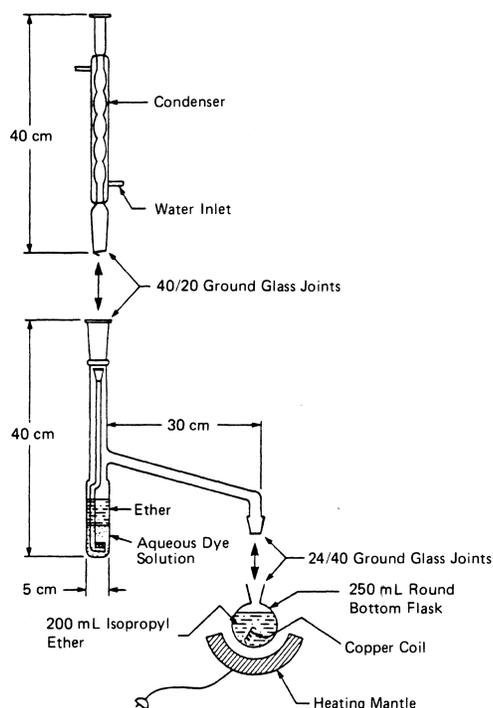


FIGURE 21 Upward Displacement-Type Liquid-Liquid Extractor with Sintered-Glass Diffuser.

**Alkaline Ether Extract** Transfer 5 g of the colorant to a beaker, and dissolve in 150 mL of water. Add 2 mL of 2.5 N NaOH solution, transfer the solution into the extractor; and dilute with water to approximately 200 mL. Add 200 mL of ether to the distillation flask, and extract for 2 h with a reflux rate of about 15 mL/min. Set the extracted colorant solution aside. Transfer the ether extract into a separatory funnel, and wash with two 25-mL portions of 0.1 N NaOH followed by two 25-mL portions of water. Reduce the volume of the ether extract to about 5 mL by distillation (in portions) from a tared flask containing a small piece of clean copper coil.

**Acid Ether Extract** Add 5 mL of 3 N hydrochloric acid to the extracted colorant solution set aside in the alkaline ether extract procedure above, mix, and extract with ether as directed above. Wash the ether extract with two 25-mL portions of 0.1 N hydrochloric acid and water. Transfer the washed ether in portions to the flask containing the evaporated alkaline extract, and carefully remove all the ether by distillation. Dry the residue in an oven at 85° for 20 min. Then allow the flask to cool in a desiccator for 30 min, and weigh. Repeat drying and cooling until a constant weight is obtained. The increase in weight of the tared flask, expressed as a percentage of the sample weight, is the combined ether extract.

## Leuco Base

### Reagents and Solutions

*Cupric Chloride Solution* Transfer 10.0 g of  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  to a 1-L volumetric flask; dissolve in and dilute with dimethylformamide (DMF) to volume.

*Sample Solution* Prepare as directed in the individual monograph.

### Procedure

*Solution 1* Pipet 50 mL of DMF into a 250-mL volumetric flask, cover, and place in the dark.

*Solution 2* Pipet 10 mL of the *Sample Solution* into a 250-mL volumetric flask, add 50 mL of DMF, and place in the dark.

*Solution 3* Pipet 50 mL of *Cupric Chloride Solution* into a 250-mL volumetric flask, and gently bubble air through the solution for 30 min.

*Solutions 4a and 4b* Pipet 10 mL of the *Sample Solution* into each of two 250-mL volumetric flasks, add 50 mL of *Cupric Chloride Solution* to each, and bubble air gently through the solutions for 30 min.

Dilute all of the solutions with water nearly to volume; incubate for 5 to 10 min, but no longer, in a water bath cooled with tap water; and dilute to volume. Record the spectrum for each solution between 500 nm and 700 nm using an absorbance range of 0 to 1 and a 1-cm pathlength cell; record all spectra on the same spectrogram.

Curve No.	Solution in Sample Cell	Solution in Reference Cell
I	1	1
II	1	2
III	3	3
IVa	3	4a
IVb	3	4b

*Calculation*

$$\% \text{Leuco Base} = \frac{[(IV - III) - (II - I)] \times 2500}{a \times W \times r}$$

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 (Lindburg Type 70T, or equivalent), and it is connected by clamped ball-joints at one end to a source of nitrogen and connected to a series of three traps at the other. The traps are constructed of a linear array of 18- × 2-mm Pyrex tubes connected by clamped ball-joints and extend from the connection at the combustion tube. Trap I contains anhydrous calcium sulfate packed between quartz-wool plugs, trap II contains Ascarite packed between cotton plugs, and trap III contains aluminum oxide packed between cotton plugs. The nitrogen flow forces the mercury through the combustion tube, the three traps, and a section of Tygon tube to a mercury vapor meter (Beckman model K-23, or equivalent). The mercury released from a sample during combustion is quantitated by comparing the recorder response with that given by a series of mercury standards.

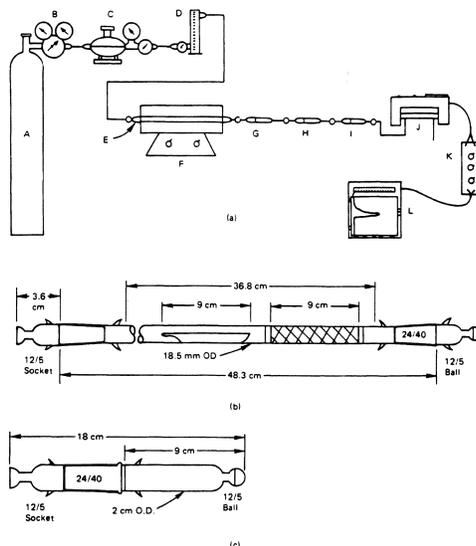


FIGURE 22 (a) Schematic Diagram of Apparatus for Photometric Mercury Vapor M Method:

A. Tank of nitrogen	G. Dehydrite trap
B. Two-stage pressure regulator	H. Ascarite trap
C. Low-pressure regulator	I. Aluminum oxide trap
D. Flowmeter	J. Mercury vapor meter
E. Combustion tube	K. Attenuator
F. Combustion-tube furnace	L. Recorder
(b) Quartz Combustion Tube with Boat and Copper Oxide Packing;	
(c) Schematic Diagram of Trap Used to Contain Ascarite, Dehydrite, and Aluminum Oxide.	

## Reagents and Equipment

*Absorbent Cotton*

*Aluminum Oxide* Anhydrous.

*Calcium Sulfate* Anhydrous, dehydrate, or equivalent.

*Asbestos Pads*, (1 × 0.5 × 1 cm) Preheated at 800° for 1 h.

*Ascarite* 20- to 30-mesh.

*Copper Oxide Wire* Preheated at 850° for 2 h.

*Nitrogen* Purified grade.

*Quartz Wool*

*Sodium Carbonate* Anhydrous, fine granular.

*Standard Solution* Transfer approximately 1.35 g of reagent-grade mercurous chloride, accurately weighed, into a 1-L volumetric flask. Dissolve in and dilute with water to volume. When diluted 100-fold, the solution contains 0.01 µg Hg per microliter (*Diluted Standard Solution*).

**Procedure** Preheat the furnace to 650°, and adjust the nitrogen flow to 1 L/min.

*Blank Analysis* Place a square piece of preheated asbestos pad in the combustion boat, and cover it with sodium carbonate. Stop the nitrogen flow, disconnect the ball-joint, quickly insert the boat into the combustion tube with large forceps, and reconnect the joint. Note the time, allow the boat to sit in the tube with no nitrogen flow for exactly 1 min, and then restart the flow of nitrogen. Mercury elutes almost immediately with the reinstated nitrogen flow; note the recorder response. Allow about 30 s between runs.

**Calibration** Determine the recorder response after the application to the asbestos pad of 1, 2, and 3  $\mu\text{L}$  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*, 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  $\mu\text{g}$  less than 0.5 cm), then the traps are packed too tightly. Remove or redistribute packing first in the aluminum oxide trap, then try the other traps.
3. The traps will need changing periodically as indicated by a change in the physical appearance of the trap material or by chart responses of different retention times or different symmetry from that of mercury standards.
4. If two or more standards are run in succession, a later sample might give an erroneous mercury response. Run blanks and then repeat the sample analysis to confirm the validity of the response.

## Sodium Chloride

Dissolve approximately 2 g of colorant, accurately weighed, in 100 mL of water, and add 10 g of activated carbon that is free of chloride and sulfate. Boil gently for 2 to 3 min. Cool to room temperature, add 1 mL of 6 N nitric acid, and stir. Dilute with water to volume in a 200-mL volumetric flask, and then filter through dry paper. Repeat the treatment with 2-g portions of carbon until no color is adsorbed onto filter paper dipped into the filtrate.

Transfer 50 mL of filtrate to a 250-mL flask. Add 2 mL of 6 N nitric acid, 5 mL of nitrobenzene, and 10 mL of standardized 0.1 N silver nitrate solution. Shake the flask until the silver chloride coagulates. Prepare a saturated solution of ferric ammonium sulfate, and add just enough concentrated nitric acid to discharge the red color; add 1 mL of this solution to the 250-mL flask to serve as the indicator. Titrate with 0.1 N ammonium thiocyanate solution that has been standardized against the silver nitrate solution until the color persists after shaking for 1 min. Calculate the weight percent of sodium chloride, P, by the equation:

$$P = [(V \times N)/W] \times 22.79$$

in which V is the net volume, in milliliters, of silver nitrate solution required; N is the normality of the silver nitrate solution; and W is the weight, in grams, of the sample taken. The factor 22.79 incorporates a total volume of 195 mL because 10 g of activated carbon occupies 5 mL.

## Sodium Sulfate

Place 25 mL of the decolorized filtrate obtained from the *Sodium Chloride* test into a 125-mL Erlenmeyer flask, and add 1 drop of a 0.5% phenolphthalein solution in 50% ethanol. Add 0.05 N sodium hydroxide, dropwise, until the solution is alkaline to pH paper, and then add 0.002 N hydrochloric acid until the indicator is decolorized. Add 25 mL of ethanol and about 0.2 g of tetrahydroquinone sulfate indicator. Titrate with 0.03 N barium chloride solution to a red endpoint. Make a blank determination.

Calculate the weight percent, P, of sodium sulfate by the equation:

$$P = [(V - B) \times N/W] \times 55.4$$

in which V is the volume, in milliliters, of barium chloride solution required to titrate the sample; B is the volume, in milliliters, of barium chloride solution required for the blank; N is the normality of the barium chloride solution; and W is the weight, in grams, of the sample taken. The factor 55.4 incorporates a total volume of 195 mL because 10 g of activated carbon occupies 5 mL.

**Change to read:**

## Total Color

### Method I (Spectrophotometric)

Pipet 10.0 mL of the dissolved colorant into a 250-mL Erlenmeyer flask containing 90 mL of 0.04 N ammonium acetate, and mix well. Determine the net absorbance of the solution relative to water at the wavelength maximum given for each color. Calculate the percentage of colorant present using the following equation, which presumes a 1-cm pathlength cell:

$$\% \text{total color} = (A \times 100)/(a \times W)$$

$$\% \text{total color} = A/(a \times C \times b) \times 100 \quad \text{■ 2S (FCC7)}$$

in which A is the absorbance; a is the absorptivity ■ specified in the individual monograph (L/(mg·cm)); ■ 2S (FCC7) and W is the weight, in grams, of the sample taken. ■ C is the concentration of sample in the final test solution (mg/L); and b is the cell pathlength (cm). ■ 2S (FCC7)

### Method II (Titration with Titanium Chloride)

**Apparatus** The apparatus for determining total color by titration with titanium chloride (TiCl<sub>3</sub>) is shown in *Fig. 23*. It consists of a storage bottle, A, of 0.1 N titanium chloride titrant maintained under hydrogen produced by a Kipp generator; an Erlenmeyer flask, B, equipped with a source of CO<sub>2</sub> or N<sub>2</sub> to maintain an inert atmosphere in which the reaction takes place; a stirrer; and the buret, C.

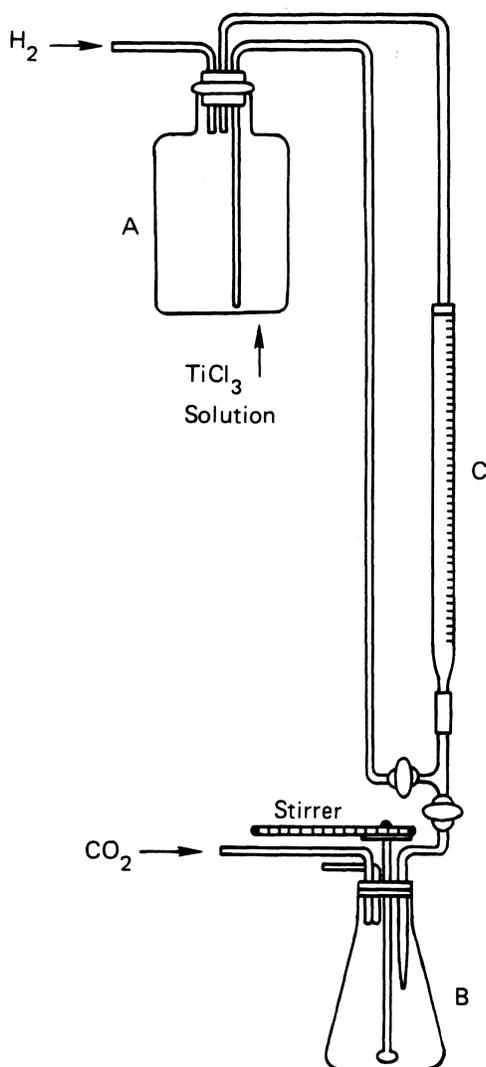


FIGURE 23 Titanous Chloride Titration Apparatus.

### Reagents and Solutions

**Titanium Chloride Solution (0.1 N)** Transfer 73 mL of commercially prepared 20%  $\text{TiCl}_3$  solution into a storage bottle, and carefully add 82 mL of concentrated HCl per L of final solution. Mix well, and bubble  $\text{CO}_2$  or  $\text{N}_2$  through the solution for 1 h. Before standardizing, maintain the solution under a hydrogen atmosphere for at least 16 h using a Kipp generator.

**Potassium Dichromate Solution (0.1 N, primary standard)** Transfer 4.9032 g of  $\text{K}_2\text{Cr}_2\text{O}_7$  (National Institute of Standards and Technology No. 136) to a 1-L volumetric flask; dissolve in and dilute with water to volume.

**Ammonium Thiocyanate (50%)** Transfer 500 g of  $\text{NH}_4\text{SCN}$ , 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**  $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ , ACS certified.

**Sodium Bitartrate**

**Standardization of the Titanium Chloride Solution** Drain any standing titanium chloride ( $\text{TiCl}_3$ ) from the feed lines and buret, and refill with fresh solution. Add 3.0 g of *Ferrous Ammonium Sulfate* to a wide-mouth Erlenmeyer flask followed by 200 mL of water, 25 mL of 50% sulfuric acid, 25 mL of 0.1 N *Potassium Dichromate Solution* (by pipet), and 2 or 3 boiling chips. Boil the solution vigorously on a hot plate for 30 s to

remove dissolved air, then quickly transfer the flask to the titration apparatus, securely connect the stopper assembly, and start the carbon dioxide flow and stirrer. Pass carbon dioxide over the solution for 1 min before beginning the titration.

Add the 0.1 N *Titanium Chloride Solution* at a fast, steady drip to within 1 mL of the estimated endpoint (about 20 mL). Reduce the carbon dioxide flow, remove the solid-glass rod from the stopper assembly, pipet 10 mL of *Ammonium Thiocyanate (50%)* into the flask, insert the glass rod, and increase the carbon dioxide flow. Continue titrating slowly until the endpoint: a color change from brown-red to light green is observed. Perform a blank determination using the same reagents and quantities, and calculate the normality, N, of the 0.1 N *Titanium Chloride Solution* on the basis of three titrations by the equation:

$$N = (V_r \times N_r / V_t - V_b)$$

in which  $V_r$  is the volume, in milliliters, of 0.1 N *Potassium Dichromate* used;  $N_r$  is the normality of the 0.1 N *Potassium Dichromate*;  $V_t$  is the volume, in milliliters, of 0.1 N *Titanium Chloride Solution* used; and  $V_b$  is the volume, in milliliters, of titanium dichloride used in the blank titration.

**Procedure** Transfer the quantity of colorant prescribed in the individual monograph into a 500-mL wide-mouth Erlenmeyer flask and add 21 to 22 g of *Sodium Bitartrate* (sodium citrate for Sunset Yellow), 275 mL of water, and two or three boiling chips. Boil the solution vigorously on a hot plate for 30 s to remove dissolved air, then quickly transfer the flask to the titration apparatus, securely connect the stopper assembly, and start the carbon dioxide flow and stirrer. Pass carbon dioxide over the solution for 1 min before beginning the titration.

Titrate the sample until the color lightens, wait 20 s, and then continue the addition with about 2 s between drops. When the color is almost completely bleached, wait 20 s, and then continue the addition with 5 s between drops. A complete color change indicates the endpoint. Perform a blank determination using the same reagents and quantities, and calculate the total color, T, in percent and on the basis of three titrations, by the equation:

$$T = [(V_t - V_b) / (W \times F_s)] \times 100 \times N$$

in which  $V_t$  is the volume of titrant used;  $V_b$  is the volume of titrant required to produce the endpoint in a blank; N is the normality of the titrant; W is the weight, in grams, of the sample taken, and  $F_s$  is a factor derived from the stoichiometry of the reaction characteristics of each colorant and is given in the individual monograph.

### Method III (Gravimetric)

Transfer approximately 0.5 g of colorant, accurately weighed, to a 400-mL beaker, add 100 mL of water, and heat to boiling. Add 25 mL of 1:50 hydrochloric acid, and bring to a boil. Wash down the sides of the beaker with water, cover, and keep on a steam bath for several hours or overnight. Cool to room temperature, and quantitatively transfer the precipitate into a tared filtering crucible with 1:100 hydrochloric acid. Wash the precipitate with two 15-mL portions of water, and dry the crucible for 3 h at 135°. Cool in a desiccator, and weigh. Calculate the total color, P, in weight percent, by the equation:

$$P = [(W_p \times F) / W_s] \times 100$$

in which  $W_p$  is the weight, in grams, of the precipitate; F is the gravimetric conversion factor given in the individual monograph; and  $W_s$  is the original weight, in grams, of the sample taken.

## Uncombined Intermediates and Products of Side Reactions

## UNCOMBINED INTERMEDIATES AND PRODUCTS OF SIDE REACTIONS

### Method I

**Sample Solution** Transfer approximately 2 g of colorant to a 100-mL volumetric flask; dissolve in and dilute with water to volume.

**Apparatus** Pack a 2.5- × 45-cm glass column with approximately 20 g of cellulose (Whatman CF-11 grade, or equivalent) that has been slurried in the eluant and from which the fines have been removed by decantation. Equilibrate the column thoroughly with the eluant, 35% ammonium sulfate.

**Procedure** Pipet 5 mL of *Sample Solution* into a beaker containing 5 g of cellulose that has been slurried in eluant and from which the fines have been removed by decantation. Stir the mixture thoroughly, add 10 g of ammonium sulfate, and stir until uniformly mixed. Mix the slurry with 15 mL of eluant, and apply it to the column. Allow the fluid to enter the column, and wash the beaker with eluant until the sample is quantitatively transferred. Elute the column with approximately 500 mL of 35% ammonium sulfate, and collect a total of eight 60-mL fractions. Divide each collected fraction in half and add 0.5 mL of NH<sub>4</sub>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:

$$C = A/(a \times b)$$

in which A is the absorbance at the wavelength of maximal absorption; b is the cell pathlength, in centimeters; and a is the absorptivity given in the individual monograph.

### Method II

**Apparatus** Use a suitable high-performance liquid chromatography system (see *Chromatography, Appendix IIA*) equipped with a dual wavelength detector system such that the effluent can be monitored serially at 254 nm and 325 to 385 nm (wide-band pass). Use a 1-m × 2.1-mm (id) column, or equivalent, packed with a strong anion-exchange resin (Dupont No. 830950405, or equivalent).

**Operating Conditions** The operating conditions required may vary depending on the system used. The following conditions have been shown to give suitable results for Allura Red, Tartrazine, and Sunset Yellow.

#### *Allura Red*

*Primary Eluant:* 0.01 M aqueous Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>.

*Secondary Eluant:* 0.20 M NaClO<sub>4</sub> in aqueous 0.01 M Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>.

*Sample Size:* 20 μL of a 0.25% solution.

*Flow Rate:* 0.60 mL/min.

*Gradient:* Linear, in two phases: 0% to 18% in 40 min, 18% to 62% in 8 min more, then hold for 18 min more at 62%.

*Temperature:* 50°.

*Pressure:* 1000 psi.

*Order of Elution:* (1) Cresidinesulfonic acid (CSA); (2) unknown; (3) Schaeffer's salt (SS); (4) unknown; (5) 4,4'-diazaminobis(5-methoxy-2-methylbenzenesulfonic acid) (DMMA); (6) unknown; (7) Allura Red; (8) 6,6'-oxybis(2-naphthalenesulfonic acid) (DONS).

#### *Tartrazine*

*Primary Eluant:* 0.01 M aqueous Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>.

*Secondary Eluant:* 0.10 M NaClO<sub>4</sub> in aqueous 0.01 M Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>.

*Sample Size:* 50 µL of a 0.15% solution, prepared within 13 min of injection.

*Flow Rate:* 1.00 mL/min.

*Gradient:* Exponential at 4%/min: 0.95%.

*Temperature:* 50°.

*Pressure:* 1000 psi.

*Order of Elution:* (1) Phenylhydrazine-*p*-sulfonic acid (PHSA); (2) sulfanilic acid (SA); (3) 1-(4-sulfophenyl)-3-ethylcarboxy-5-hydroxypyrazolone (PY-T); (4) 1-(4-sulfophenyl)-3-carboxy-5-hydroxypyrazolone (EEPT); (5) 4,4'-(diazamino)-dibenzenesulfonic acid (DAADBSA).

#### Sunset Yellow

*Primary Eluant:* 0.01 M aqueous Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>.

*Secondary Eluant:* 0.20 M NaClO<sub>4</sub> in aqueous 0.01 M Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>.

*Sample Size:* 5 µL of a 1% solution.

*Flow Rate:* 0.50 mL/min.

*Gradient:* Linear in four phases: 0% to 11% in 10 min; hold 25 min; 11% to 38% in 10 min; 38% to 42% in 10 min; 42% to 98% in 20 min; hold 20 min.

*Temperature:* 50°.

*Pressure:* 1000 psi.

*Order of Elution:* (1) Sulfanilic acid (SA); (2) Schaeffer's salt (SS); (3) 4,4'-(diazamino)-dibenzenesulfonic acid (DAADBSA); (4) *R*-salt dye; (5) Sunset Yellow; (6) 6,6'-oxybis(2-naphthalenesulfonic acid) (DONS).

#### Standard Solutions

*Allura Red* Prepare a solution containing 0.25 g of colorant, 0.5 mg of CSA, 0.75 mg of SS, 0.25 mg of DMMA, and 1.25 mg of DONS in a 100-mL volumetric flask. Dissolve in and dilute with 0.1 M Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> to volume.

*Tartrazine* Prepare a solution containing 0.15 g of colorant and 0.3 mg each of PHSA, SA, PY-T, EEPT, and DAADBSA in a 100-mL volumetric flask. Dissolve in and dilute with 0.1 M Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> to volume.

*Sunset Yellow* Prepare a solution containing 0.25 g of colorant, 0.5 mg of SA, 0.75 mg of SS, 0.25 mg of DAADBSA, and 1.25 mg of DONS in a 100-mL volumetric flask. Dissolve in and dilute with 0.1 M Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> to volume.

**Test Solutions** Prepare at least four test solutions, each containing the colorant, and one impurity, accurately weighed, dissolved in 0.1 M Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>, and diluted to volume in a 100-mL volumetric flask. The solutions should encompass the range of concentrations, evenly spaced, given below for each constituent:

*Allura Red* (250 mg) CSA (0.05 to 0.5 mg); SS (0.05 to 0.75 mg); DONS (0.5 to 2.5 mg); DMMA (0.025 to 0.25 mg). Inject 20 µL of each solution.

*Tartrazine* (150 mg) SA (7.5 to 300 µg); PY-T (7.5 to 300 µg); EEPT (7.5 to 300 µg); DAADBSA (7.5 to 300 µg). Inject 50 µL of each solution.

*Sunset Yellow* (250 mg) SA (0.05 to 0.5 mg); SS (0.05 to 0.75 mg); DONS (0.5 to 2.5 mg); DAADBSA (0.05 to 0.25 mg). Inject 20 µL of each solution.

#### System Suitability

*Resolution* Elute the column, or equivalent, with the gradient specified under *Operating Conditions* until a smooth baseline is obtained. Inject an aliquot of the *Standard Solution*. The resolution of the eluted

components matches or exceeds that shown for the corresponding colorant (see *Figs. 24, 25, and 26*). After determining that the column, or equivalent, will give the required resolution, allow it to rest for 2 weeks before use.

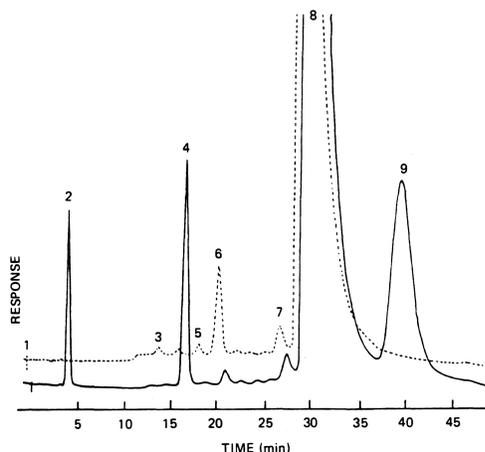


FIGURE 24 Allura Red–Top Trace: Eluant Monitored at 254 nm; Bottom Trace: Eluant Monitored at 375 to 385 nm.

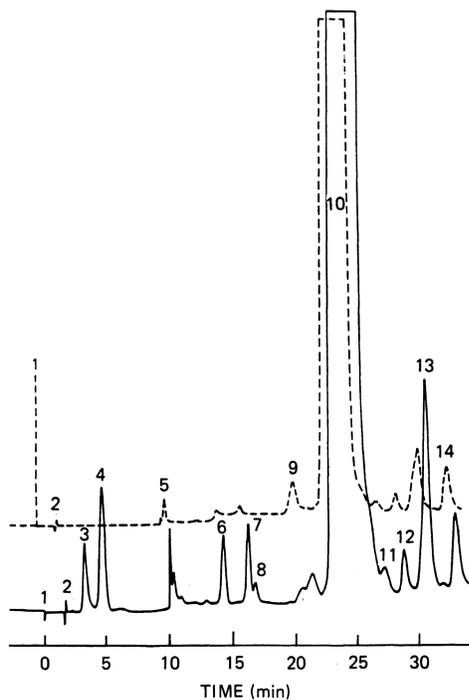


FIGURE 25 Tartrazine–Top Trace: Eluant Monitored at 254 nm; Bottom Trace: Eluant Monitored at 375 to 385 nm.

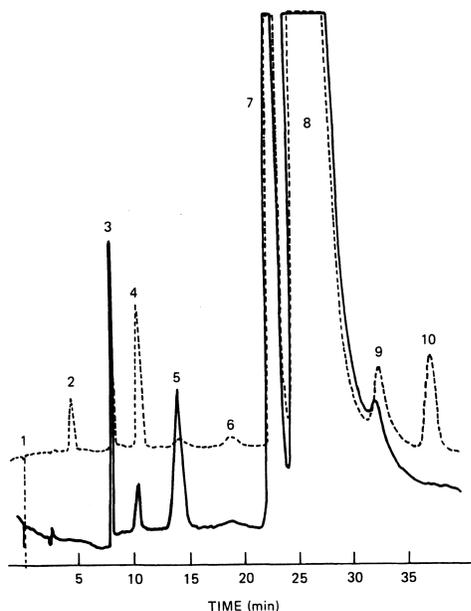


FIGURE 26 Sunset Yellow—Top Trace: Eluant Monitored at 254 nm; Bottom Trace: Eluant Monitored at 375 to 385 nm.

**Calibration** Inject the designated volume of each *Test Solution* onto a conditioned column, and prepare a standard curve corresponding to each unreacted intermediate and side reaction product. Determine the area,  $A$ , for each peak from the integrator if an automated system is used or by multiplying the peak height by the width at one-half the height. The peak height alone may be used for EEPT, PY-T, and DAADBSA. Calculate the concentration,  $C_i$ , of each intermediate or side product using the equation:

$$C_i = mA_i + b$$

in which  $A_i$  is the area of its corresponding chromatographic peak. Calculate the slope,  $m$ , and intercept,  $b$ , using the following linear regression equations:

$$m = \frac{[N\sum C_i A_i - \sum C_i \sum A_i]}{[N\sum A_i^2 - (\sum A_i)^2]}$$

$$b = [\bar{A}]_i - m[\bar{C}]_i$$

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:

$$r = \frac{[\sum (C_i - \bar{C})(A_i - \bar{A})]}{[\sum (C_i - \bar{C})^2 \times \sum (A_i - \bar{A})^2]}$$

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 10 determinations or 2 days, whichever occurs first.

**Sample Preparation** Prepare as directed in the individual monograph.

**Procedure** Inject the volume of *Sample Preparation* as designated in the monograph into the column.

Determine the concentration of intermediates and side reaction products from the peak areas using the slope,  $m$ , and intercept,  $b$ , calculated under *Calibration* by the equation:

$$C_S = mA_S + b$$

in which  $C_S$  is the concentration of the unknown in the *Sample Preparation* and  $A_S$  its corresponding peak area.

## Loss on Drying (Volatile Matter)

Transfer 1.5 to 2.5 g of colorant, accurately weighed, to a tared crucible. Heat in a vacuum oven at 135° for 12 to 15 h. Lower the pressure in the oven to – 125 mm Hg, and continue heating for an additional 2 h. Cover the crucible, and allow to cool in a desiccator. Reweigh the crucible when cool. The loss of weight is defined as the volatile matter.

## Water-Insoluble Matter

Transfer about 1 g of colorant, accurately weighed, to a 250-mL beaker, and add 200 mL of boiling water. Stir to facilitate dissolution of the color.

Tare a filtering crucible equipped with a glass fiber filter (Reeve Angel, No. 5270, or equivalent). Filter the solution with the aid of suction when it has cooled to ambient temperature. Rinse the beaker three times, pouring the rinsings through the crucible. Wash the filter with water until the filtrate is colorless.

Dry the crucible and filter in an oven at 135° for at least 3 h, cool them in a desiccator and reweigh to the nearest 0.1 mg. Calculate the percent water-insoluble matter,  $I$ , by the equation:

$$I = (W_C/W_S) \times 100$$

in which  $W_C$  is the difference in crucible weight and  $W_S$  is the sample weight.

**Add the following:**

## ■ ELEMENTAL IMPURITIES BY ICP

Before the initial use of either of the procedures below, the analyst should ensure that the procedure is appropriate for the instrument and sample used. *Method I* can be used for elemental impurities generally amenable to detection by inductively coupled plasma–atomic (optical) emission spectroscopy (ICP–OES). *Method II* can be used for elemental impurities generally amenable to detection by inductively coupled plasma–mass spectrometry (ICP–MS). If no method is specified in the individual monograph, analysts are instructed to use *Method II* (ICP–MS).

## Method I: ICP–OES

**Reagents** All reagents used for the preparation of sample and standard solutions should be free of elemental impurities. Reagents should be commercial elemental stock standards that are National Institute of Standards and Technology (NIST)-traceable, or equivalent, at a recommended concentration of 100 µg/mL or greater; or appropriate USP Reference Standards, as either single element or multielement.

*Aqua Regia* Ultra pure nitric acid/hydrochloric acid (1:3) prepared as needed. (A 1%–5% solution of aqua regia is used as a rinsing solution between analyses and as calibration blanks.)

**Sample Preparation** Use this sample preparation procedure unless otherwise specified in the individual monograph. [Note—Weights and volumes provided may be adjusted to meet the requirements of the microwave digestion apparatus used, if proportions remain constant.] Dehydrate and predigest 0.5 g of sample in 5 mL of freshly prepared *Aqua Regia*. Sulfuric acid may also be used as a last resort. [Note—Sulfuric acid should be used only when absolutely needed because addition of sulfuric acid may cause an extreme exothermic reaction and result in elements being lost and because the viscosity of sulfuric acid is higher than that of other acids, which affects the overall flow of solution.] Allow the sample to sit loosely covered for 30 min in a fume hood. Add an additional 10 mL of *Aqua Regia* and digest, using a closed vessel microwave technique. Microwave until digestion or extraction is complete. Repeat if necessary by adding an additional 5 mL of *Aqua Regia*. [Note—Follow the recommended procedures provided by the manufacturer of the closed vessel microwave digestion apparatus to ensure safe usage. In closed vessel microwave digestion, the use of concentrated hydrofluoric acid (HF) is not recommended; however, when its use is necessary, practice the utmost caution in the preparation of test articles, and review or establish local procedures for safe handling, safe disposal, and HF-tolerant instrumental configurations.]

**Sample Solution** Allow the digestion vessel containing the *Sample Preparation* to cool (for mercury measurements, add an appropriate stabilizer, such as gold at about 0.1 ppm), and dilute with water to 50.0 mL.

**Calibration Solution 1**  $2J$  of the element of interest in a matched matrix (acid concentrations similar to that of the *Sample Solution*), where  $J$  is the limit for the specific elemental impurity. [Note—Multiple elements of interest may be included in this solution at the same concentration ratio. For mercury analysis, add an appropriate stabilizer, such as gold at about 0.1 ppm.]

**Calibration Solution 2**  $0.1J$  of the element of interest in a matched matrix (acid concentrations similar to that of the *Sample Solution*), where  $J$  is the limit for the specific elemental impurity. [Note—Multiple elements of interest may be included in this solution at the same concentration ratio. For mercury analysis, add an appropriate stabilizer, such as gold at about 0.1 ppm.]

**Check Standard Solution** 1 ppm of the element of interest in a matched matrix (acid concentrations similar to that of the *Sample Solution*). [Note—Multiple elements of interest may be included in this solution at the same concentration ratio. For mercury analysis, add an appropriate stabilizer, such as gold at about 0.1 ppm.]

**Blank** Matched matrix (acid concentrations similar to that of the *Sample Solution*)

**Elemental Spectrometric System** (See *Plasma Spectrochemistry*, Appendix IIC)

*Mode:* ICP

*Detector:* Optical emission spectroscopy

*Rinse:* 5% *Aqua Regia*

*Calibration:* Two-point, using *Calibration Solution 1*, *Calibration Solution 2*, and *Blank*

### System Suitability

*Sample:* *Check Standard Solution*

*Suitability requirement:* The concentration determined from the resulting chromatogram differs from actual concentration by NMT 20%. [Note—If samples are high in mineral content, to minimize sample carryover, rinse system well (60 s) before introducing the *Check Standard Solution*.]

**Analysis** Analyze according to manufacturer's suggestions for program and wavelength. Calculate and report results on the basis of the original sample size.

**Calculation** Upon completion of the analysis, calculate the final concentration of a given element in the test article ( $\mu\text{g/g}$ ) from the solution element concentration ( $\mu\text{g/mL}$ ) as follows:

$$C = [(A \times V_1)/W] \times (V_2/V_3)$$

where C is the concentration of the analyte,  $\mu\text{g/g}$ ; A is the instrument reading,  $\mu\text{g/mL}$ ,  $V_1$  is the volume of the initial test article preparation, mL; W is the weight of the test article preparation, g;  $V_2$  is the total volume of any dilution performed, mL; and  $V_3$  is the volume of the aliquot of initial test article preparation used in any dilution performed, mL.

Similarly, calculate the final concentration of a given element in the test article ( $\mu\text{g/g}$ ) from the solution element concentration ( $\text{ng/mL}$ ) as follows:

$$C = [(A \times V_1)/W] \times (1 \mu\text{g}/1000 \text{ ng})(V_2/V_3)$$

where A is the instrument reading,  $\text{ng/mL}$ ; and the other factors are as defined above.

## Method II: ICP–MS

**Reagents** All reagents used for the preparation of sample and standard solutions should be free of elemental impurities. Reagents should be commercial elemental stock standards that are National Institute of Standards and Technology (NIST)-traceable, or equivalent, at a recommended concentration of 100  $\mu\text{g/mL}$  or greater; or appropriate USP Reference Standards, as either single element or multielement.

*Aqua Regia* Ultra pure nitric acid/hydrochloric acid (1:3) prepared as needed. (A 1%–5% solution of aqua regia is used as a rinsing solution between analyses and as calibration blanks.)

**Sample Preparation** Proceed as directed under *Method I*.

**Sample Solution** Allow the digestion vessel containing the *Sample Preparation* to cool, and add appropriate internal standards at appropriate concentrations (for mercury measurements, gold should be one of the internal standards). Dilute with water to 50.0 mL.

**Calibration Solution 1** Proceed as directed under *Method I*.

**Calibration Solution 2** Proceed as directed under *Method I*.

**Blank** Matched matrix (acid concentrations similar to that of the *Sample Solution*)

**Elemental Spectrometric System** (See *Plasma Spectrochemistry*, Appendix IIC)

*Mode:* ICP [Note—An instrument with a cooled spray chamber is recommended.]

*Detector:* Mass spectrometer

*Rinse:* 5% *Aqua Regia*

*Calibration:* *Calibration Solution 1*, *Calibration Solution 2*, and *Blank*

### System Suitability

*Sample:* *Calibration Solution 1*

*Suitability requirement:* The concentration determined from the resulting chromatogram differs from actual concentration by NMT 20%. [Note—If samples are high in mineral content, to minimize sample carryover, rinse system well (60 s) before introducing the *Check Standard Solution*.]

**Analysis** Analyze according to the manufacturer's suggestions for the program and m/z. Calculate and report results based on the original sample size. [Note—Arsenic is subject to interference from argon chloride. Appropriate measures, including a sample preparation without *Aqua Regia*, must be taken to correct for the interference, depending on instrumental capabilities.]

**Calculation** Upon completion of the analysis, calculate the final concentration of a given element in the test article ( $\mu\text{g/g}$ ) from the solution element concentration ( $\mu\text{g/mL}$ ) as follows:

$$C = [(A \times V_1)/W] \times (V_2/V_3)$$

where C is the concentration of the analyte,  $\mu\text{g/g}$ ; A is the instrument reading,  $\mu\text{g/mL}$ ;  $V_1$  is the volume of the initial test article preparation, mL; W is the weight of the test article preparation, g;  $V_2$  is the total volume of any dilution performed, mL; and  $V_3$  is the volume of the aliquot of initial test article preparation used in any dilution performed, mL.

Similarly, calculate the final concentration of a given element in the test article ( $\mu\text{g/g}$ ) from the solution element concentration ( $\text{ng/mL}$ ) as follows:

$$C = [(A \times V_1)/W] \times (1 \mu\text{g}/1000 \text{ ng})(V_2/V_3)$$

where A is the instrument reading,  $\text{ng/mL}$ ; and the other factors are as defined above.

■ 2S (FCC7)

## 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  $1250 \pm 2$  mg of reagent-grade glutamic acid, accurately weighed, into a 500-mL volumetric flask. Fill the flask half-full with water, add 5 mL of hydrochloric acid to help dissolve the amino acid, dilute with water to volume, and mix. Prepare the standard for analysis by diluting 1 mL of this solution with 4 mL of 0.2 N sodium citrate, pH 2.2, buffer. This *Standard Solution* contains 0.5 mg of glutamic acid per milliliter ( $C_S$ ).

**Sample Preparation** Dilute 5 mg of sample, accurately weighed, to exactly 5 mL with 0.2 N sodium citrate, pH 2.2, buffer. Remove any insoluble material by centrifugation or filtration.

**Procedure** Using 2-mL aliquots of the *Standard Solution* and *Sample Preparation*, proceed according to the apparatus manufacturer's instructions. From the chromatograms thus obtained, match the retention times produced by the *Standard Preparation* with those produced by the *Sample Solution*, and identify the peak produced by glutamic acid. Record the area of the glutamic acid peak from the sample as  $A_U$ , and that from the standards as  $A_S$ .

**Calculations** Calculate the concentration,  $C_A$ , in milligrams per milliliter, of glutamic acid in the *Sample Preparation* by the formula:

$$\text{Result} = A_U \times C_S/A_S$$

in which  $C_S$  is the concentration, in milligrams per milliliter, of glutamic acid in the *Standard Solution*.

Calculate the percent glutamic acid, on the basis of total protein, by the formula:

$$\text{Result} = (100 \times C_A) / (6.25 \times N_T)$$

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:

$$\text{Result} = 100 \times C_A / S_W$$

in which  $S_W$  is the weight, in milligrams, of the sample taken.

## HYDROXYPROPOXYL DETERMINATION

**Apparatus** The apparatus for hydroxypropoxyl group determination is shown in *Fig. 27*.

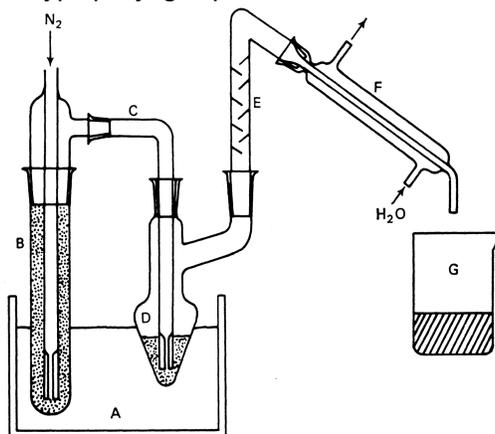


FIGURE 27 Apparatus for Hydroxypropoxyl Determination.

The boiling flask, *D*, is fitted with an aluminum foil-covered Vigreux 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 Vigreux column. The boiling flask and steam generator are immersed in an oil bath, *A*, equipped with a thermoregulator such that a temperature of  $155^\circ$  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  $105^\circ$  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  $155^\circ$  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 Vigreux 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:

$$\text{Result} = 75.0 \times [N_1(V_a - V_m) - kN_2(Y_a - Y_m)]$$

in which  $N_1$  is the exact normality of the 0.02 N sodium hydroxide solution,  $N_2$  is the exact normality of the 0.02 N sodium thiosulfate solution, and  $k = V_b N_1 / Y_b N_2$ .

The percentage of substitution, by weight, of hydroxypropoxyl groups, determined as directed above, may be converted to molecular substitution per glucose unit by reference to *Fig. 28*.

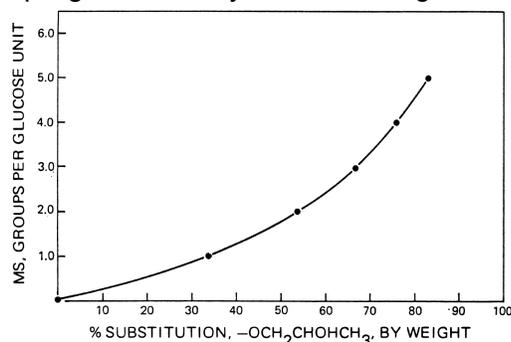


FIGURE 28 Chart for Converting Percentage of Substitution, by Weight, of Hydroxypropoxyl Groups to Molecular Substitution per Glucose Unit.

## METHOXYL DETERMINATION

**Apparatus** The apparatus for methoxyl determination, as shown in *Fig. 29*, consists of a boiling flask, *A*, fitted with a capillary side arm to provide an inlet for carbon dioxide and connected to a column, *B*, which separates aqueous hydriodic acid from the more volatile methyl iodide. After the methyl iodide passes through a suspension of aqueous red phosphorus in the scrubber trap, *C*, it is absorbed in the bromine-acetic acid absorption tube, *D*. The carbon dioxide is introduced from a device arranged to minimize pressure fluctuations and connected to the apparatus by a small capillary containing a small plug of cotton.



of methoxyl groups ( $-\text{OCH}_3$ ).

## NITROGEN DETERMINATION (Kjeldahl Method)

### *Change to read:*

[**CAUTION**— Provide adequate ventilation in the laboratory, and do not permit accumulation of exposed mercury.

■ 2S (FCC7)]

[Note—All reagents should be nitrogen free, where available, or otherwise very low in nitrogen content.]

### *Change to read:*

## 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~~  $\blacksquare$  1 g  $\blacksquare$  2S (FCC7) of sample into a 500- to 800-mL Kjeldahl digestion flask of hard  $\blacksquare$  borosilicate,  $\blacksquare$  2S (FCC7) 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.)~~  $\blacksquare$  Add 10 g of powdered potassium sulfate or anhydrous sodium sulfate, 500 mg of powdered cupric sulfate, and 20 mL of sulfuric acid.  $\blacksquare$  2S (FCC7) Place the flask in an inclined position  $\blacksquare$  (about  $45^\circ$ ),  $\blacksquare$  2S (FCC7) and heat gently  $\blacksquare$  keeping the temperature below the boiling point  $\blacksquare$  2S (FCC7) 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. ]

~~Beil~~  $\blacksquare$  Increase the heat until the acid boils  $\blacksquare$  2S (FCC7) briskly  $\blacksquare$  and continue the heating process  $\blacksquare$  2S (FCC7) 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  $\blacksquare$  150  $\blacksquare$  2S (FCC7) mL of water, mix, and then cool to below  $25^\circ$ . ~~Add 25 mL of sulfide or thiosulfate solution (40 g of  $\text{K}_2\text{S}$ , 40 g of  $\text{Na}_2\text{S}$ , or 80 g of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in 1000 mL of water), and mix to precipitate the mercury.~~  $\blacksquare$  Add cautiously 100 mL of a 2:5 sodium hydroxide solution, in such a manner as to cause the solution to flow down the inner side of the flask to form a layer under the acid solution, to make the mixture strongly alkaline.  $\blacksquare$  2S (FCC7) 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~~  $\blacksquare$  and immediately  $\blacksquare$  2S (FCC7) connect the flask to a distillation apparatus consisting of a Kjeldahl connecting bulb and a condenser, the delivery tube of which extends well beneath the surface of a measured excess of 0.5 N hydrochloric or sulfuric acid contained in a 500-mL flask. Add from 5 to 7 drops of methyl red indicator (1 g of methyl red in 200 mL of alcohol) to the receiver flask. ~~Retate~~  $\blacksquare$  100 mL of a 1:25 boric acid solution contained in a conical flask or a wide-mouth bottle of about 500-

mL capacity. Gently, rotate ~~■~~ <sup>2S (FCC7)</sup> 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 ~~■~~ (about 80% of the contents of the flask). ~~■~~ <sup>2S (FCC7)</sup> 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~~ ~~■~~ with 0.5 N sulfuric acid, determining the endpoint potentiometrically. ~~■~~ <sup>2S (FCC7)</sup> 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~~ ~~■~~ <sup>2S (FCC7)</sup> is equivalent to 7.003 mg of nitrogen.

■ [Note—An indicator solution can also be used to determine the titration endpoint. For example, dissolve 0.2 g methyl red in 100 mL 95% ethanol, 1 g bromocresol green in 500 mL 95% ethanol, then combine 1 part of the methyl red solution and 5 parts of the bromocresol green solution. Add 3 mL methyl red/bromocresol green indicator solution per L of boric acid solution. Then, titrate the sample to the first trace of pink.]

■ <sup>2S (FCC7)</sup>

[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.]

■ [Note—Nitrogen recovery verification can be run to check for accuracy of the procedure and the equipment.

1. *Nitrogen loss.* Use 0.12 g ammonium sulfate and 0.85 g sucrose. Add all other reagents, digest, and distill under the same conditions as for the sample. Recoveries should be NLT 99%.
2. *Digestion efficiency.* Use 0.16 g lysine hydrochloride or 0.18 g tryptophan, with 0.67 g sucrose per flask. Add all other reagents, digest, and distill under the same conditions as for the sample. Recoveries should be NLT 98%.] ~~■~~ <sup>2S (FCC7)</sup>

### 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~~ ~~■~~ a quantity of sample, accurately weighed, corresponding to about 150 mg of nitrogen ~~■~~ <sup>2S (FCC7)</sup> into a Kjeldahl flask, and add ~~40~~ ~~■~~ <sup>25</sup> ~~■~~ <sup>2S (FCC7)</sup> mL of 93% to 98% sulfuric acid containing ~~2~~ ~~■~~ <sup>1</sup> ~~■~~ <sup>2S (FCC7)</sup> g of salicylic acid. Mix thoroughly by shaking, and then allow to stand for 30 min or more, with ~~occasional~~ ~~■~~ <sup>frequent</sup> ~~■~~ <sup>2S (FCC7)</sup> shaking. Add 5 g of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ , ~~or 2 g of zinc dust~~ ~~■~~ <sup>2S (FCC7)</sup> (as an impalpable powder, not granules or filings), ~~shake, and allow to stand for 5 min.~~ Heat over a low flame until frothing ceases, then remove the heat, add 700 mg of mercuric oxide (or 650 mg of metallic mercury) and 15 g of powdered potassium sulfate (or anhydrous sodium sulfate), and boil briskly until the solution clears. Continue boiling for 30 min longer (or for 2 h for samples containing organic material), and then continue as directed under *Nitrates and Nitrates Absent*, beginning with “Cool, add about 200 mL of ~~water...~~” ~~■~~ mix, then add 500 mg of powdered cupric sulfate, and proceed as directed under *Nitrates and Nitrites Absent*, beginning with “Incline the flask at an angle of about  $45^\circ$ ”. When the nitrogen content of the substance is known to exceed 10%, 500 mg to 1 g of benzoic acid may be added, prior to digestion, to facilitate the decomposition of the substance. ~~■~~ <sup>2S (FCC7)</sup>

## 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  $\mu\text{g}$  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.

## 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  $\mu\text{m}$  to 15  $\mu\text{m}$  ( $3800\text{ cm}^{-1}$  to  $650\text{ cm}^{-1}$ ) unless otherwise specified in the individual monograph.

Designation	Specimen Preparation Technique
A	Intimately in contact with an internal reflection element for attenuated total reflectance (ATR) analysis
E	Pressed as a thin sample against a suitable plate for IR microscopic analysis
F	Suspended neat between suitable (for example sodium chloride or potassium bromide) plates
K	Mixed intimately with potassium bromide and compressed into a translucent pellet
M	Finely ground and dispersed in mineral oil
S	A solution of designated concentration, prepared as directed in the individual monograph, and examined in 0.1-mm cells (unless otherwise specified in the individual monograph)

[Note—A and E techniques can be used as alternative methods for K, M, F, and S where testing is performed qualitatively and the Reference Standard spectra are similarly obtained.]

**Ultraviolet Absorption** The test is used for comparison of the UV spectrum of a sample specimen with that of a physical USP Reference Standard.

Sample and USP Reference Standard specimens should be prepared using the solvents and concentrations specified in the individual monograph.

Record the spectra of the sample and USP Reference Standard solutions concomitantly using 1-cm cells over the spectral range of 200 to 400 nm, unless otherwise indicated in the individual monograph. Calculate absorptivities and/or absorbance ratios where these criteria are included in an individual monograph. Unless otherwise specified, absorbances indicated for these calculations are those measured at the maximum absorbance at the wavelength specified in the individual monograph. Where the absorbance is to be measured at a wavelength other than that of maximum absorbance, the abbreviations (min) and (sh) are used to indicate a minimum and shoulder, respectively, in an absorbance spectrum.

## SULFUR (by Oxidative Microcoulometry) (Based on ASTM D3120)<sup>5</sup>

[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 Dohmann Microcoulometric Titrating System (MCTS-30), or equivalent (shown in Fig. 30), unless otherwise specified in an individual monograph. It consists of a constant rate injector, A, a pyrolysis furnace, B, a quartz pyrolysis tube, C, a granular-tin scrubber, D, a titration cell, E, and a microcoulometer with a digital readout, F.

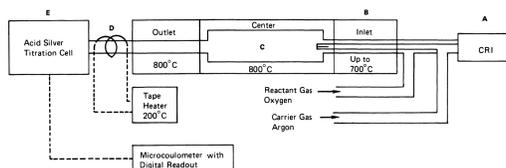


FIGURE 30 Microcoulometric Titrating System for the Determination of Sulfur in Hexanes.

**Granular-Tin Scrubber** Place 5 g of 20- to 30-mesh granular reagent-grade tin between quartz-wool plugs in an elongated 18/9-12/5 standard-taper adaptor that connects the pyrolysis tube and the titration cell.

**Microcoulometer** Must have variable attenuation; gain control; and be capable of measuring the potential of the sensing-reference electrode pair, and comparing this potential with a bias potential, amplifying the potential difference, and applying the amplified difference to the working-auxiliary electrode pair to generate a titrant.

Also the microcoulometer output voltage signal must be proportional to the generating current.

*Pyrolysis Furnace* The sample should be pyrolyzed in an electric furnace having at least two separate and independently controlled temperature zones, the first being an inlet section that can maintain a temperature sufficient to volatilize all the organic sample. The second zone is a pyrolysis section that can maintain a temperature sufficient to pyrolyze the organic matrix and oxidize all the organically bound sulfur. A third outlet temperature zone is optional.

*Pyrolysis Tube* Must be fabricated from quartz and constructed in such a way that a sample, which is vaporized completely in the inlet section, is swept into the pyrolysis zone by an inert gas where it mixes with oxygen and is burned. The inlet end of the tube shall hold a septum for syringe entry of the sample and side arms for the introduction of oxygen and inert gases. The center or pyrolysis section should be of sufficient volume to ensure complete pyrolysis of the sample.

*Sampling Syringe* A microlitre syringe of 10- $\mu$ L capacity capable of accurately delivering 1 to 10  $\mu$ L of sample into the pyrolysis tube. Three-inch  $\times$  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 <sup>3</sup> /min	200
Carrier-gas flow (Ar, He), cm <sup>3</sup> /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, $\mu$ L/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- $\mu$ L samples of this conditioning agent injected at a flow rate of 0.5  $\mu$ L/s produce a steadily increasing response, with final conditioning indicated by a constant reading from the offset during the second injection.

## Reagents

*Argon or Helium* (Argon preferred) High-purity grade, used as the carrier gas. Two-stage gas regulators must be used.

*Cell Electrolyte Solution* Dissolve 0.5 g of potassium iodide and 0.6 g of sodium azide in 500 mL of high-purity water, add 5 mL of glacial acetic acid and dilute to 1 L. Store in a dark bottle or in a dark place and prepare fresh at least every 3 months.

*Oxygen* High-purity grade, used as the reactant gas.

*Iodine* Resublimed, 20-mesh or less, for saturated reference electrode.

*Sulfur Standard* (approximately 100 mg/kg) Transfer 0.1569 g of *n*-butyl sulfide, accurately weighed, into a tared 500-mL volumetric flask. Dilute to the mark with isooctane, and reweigh. Calculate the sulfur concentration (S), in percent, by the formula:

$$S = W_b/W_s \times 2.192 \times 10^5$$

in which  $W_b$  is the weight of *n*-butyl sulfide and  $W_s$  is the weight of the solution.

**Calibration** Prepare a calibration standard (approximately 5 mg/kg) by pipetting 5 mL of *Sulfur Standard* into a 10-mL volumetric flask and diluting with isooctane to volume. Fill and clamp the syringe onto the constant-rate injector, push the sliding carriage forward to penetrate the septum with the needle, and zero the meter in case of long-term drift in the automatic baseline zero circuitry. Switch  $S_1$  automatically starts the stepper-motor syringe drive and initiates the analysis cycle. At 2.5 min (before the 3-min meter hold point) set the digital meter with the scan potentiometer to correspond to the sulfur content of the known standard to the nearest 0.01 mg/kg. At the 3-min point, the number displayed on the meter stops, the plunger drive block is retracted to its original position, as preset by switch  $S_2$ , and a baseline re-equilibration period equal to the injection period elapses before a ready light and a beeper indicate that a new sample may be injected. Repeat the *Calibration* step a total of at least four times.

**Procedure** Rinse the syringe several times with sample; then fill it, clamp it onto the constant-rate injector, push the sliding carriage forward to penetrate the septum with the needle, and zero the meter. Turn on switch  $S_1$  to start the stepper-motor syringe drive automatically and initiate the analysis cycle. After the 3-min hold point, the number displayed on the meter corresponds to the sulfur content of the injected sample.

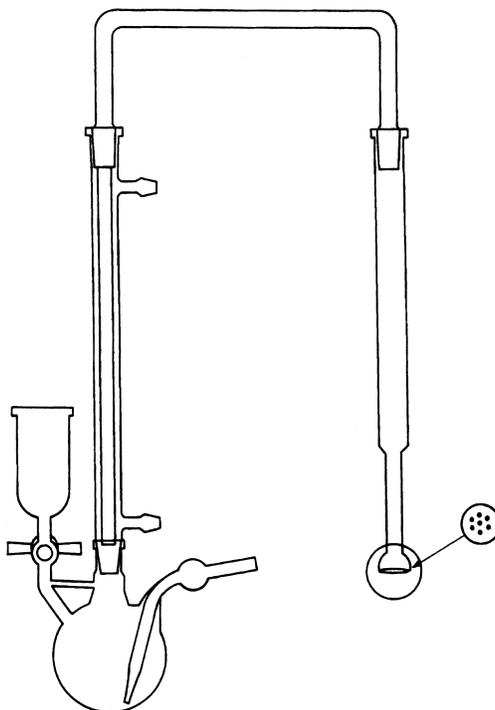


FIGURE 31 Raney Nickel Reduction Apparatus.

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<sup>1</sup> 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 Carbowax C, operating at 100 ° isothermal, with 20 mL/min of helium carrier flow. Under these conditions, the 1,4-dioxane elutes in about 4 min.

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<sup>2</sup> 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).

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<sup>3</sup> 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 RICOA Chemical Company, Arlington, TX (nickel standard, 1000 ppm Ni, for atomic absorption).

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<sup>4</sup> 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.

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<sup>5</sup> 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](mailto:service@astm.org), Website: [www.astm.org](http://www.astm.org).

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## BRIEFING

Appendix XIII: Adulterants and Contaminants in Food Ingredients. It is proposed to add a new Appendix to the FCC containing methods of analysis for adulterants and contaminants in food ingredients. Tests contained within this Appendix are not monograph requirements, but rather are provided as informational methods for FCC users. Two methods are proposed for inclusion in this Appendix, and it is anticipated that additional methods will be proposed as they become available. FCC stakeholders are encouraged to comment on these proposed methods and to submit methods for additional adulterants and contaminants.

1. On the basis of recommendation from USP's Advisory Panel on Food Ingredient Intentional Adulterants, it is proposed to add a GC method for the identification and quantification of diethylene glycol and ethylene glycol in glycerin. The method is based on a recently published method developed by USP. This test is not a requirement for the FCC *Glycerin* monograph, but rather is intended to aid FCC users interested in screening suspect food ingredient samples for these compounds. Comments on this proposed method can be submitted to Jeff Moore at JM@usp.org.
2. To support new monographs for botanical extracts such as *Maritime Pine Extract*, it is proposed to add methods for the analysis of pesticide residues. The methods are based on information contained in *Articles of Botanical Origin* 〈 561〉 in *USP 32-NF 27*—See *Briefing* under *Maritime Pine Extract*. Comments on this proposed method can be submitted to Kristie Laurick at kxb@usp.org.

(FIEC: J. Moore) RTS—C82098

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**Add the following:**

# APPENDIX XIII: ADULTERANTS AND CONTAMINANTS IN FOOD INGREDIENTS

Tests contained within this Appendix are not monograph requirements, but rather are provided as informational methods for FCC users.

## DIETHYLENE GLYCOL AND ETHYLENE GLYCOL IN GLYCERIN<sup>1</sup>

This method was developed for the identification and quantification of low levels of ethylene glycol and diethylene glycol in glycerin.

**Standard solution:** 0.025 mg/mL of USP Ethylene Glycol RS, 0.025 mg/mL of USP Diethylene Glycol RS, 50 mg/mL of USP Glycerin RS, and 0.05 mg/mL of 2,2,2-trichloroethanol (internal standard) in methanol

**Sample solution:** 50 mg/mL of sample and 0.05 mg/mL of 2,2,2-trichloroethanol (internal standard) in methanol

**Chromatographic system,** Appendix IIA

**Mode:**GC

**Detector:**Flame ionization

**Column:**0.53-mm × 30-m fused-silica analytical column coated with 3.0- $\mu$ m 6% cyanopropylphenyl–94% dimethylpolysiloxane stationary phase<sup>2</sup>

**Temperature**

**Injector:**220<sup>o</sup>

**Detector:**250<sup>o</sup>

**Column:**See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
100	—	100	4
100	50	120	10
100	50	220	6

**Carrier gas:**Helium

**Injection size:**1.0  $\mu$ L

**Flow rate:**4.5 mL/min

**Injection type:**Split flow ratio is about 10:1

**System suitability requirement:**The resolution, R, between diethylene glycol and glycerin from the *Standard solution* is NLT 1.5.

**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. Diethylene glycol and ethylene glycol can be identified in the *Sample solution* on the basis of peak retention times compared to those in the *Standard solution*. [Note—The relative retention times for ethylene glycol, 2,2,2-trichloroethanol, diethylene glycol, and glycerin are about 0.3, 0.6, 0.8, and 1.0, respectively. See *Figure 1* below for example chromatograms.]

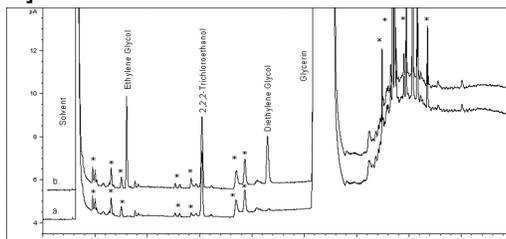


Figure 1. Overlay chromatogram of (a) *Sample solution* and (b) *Standard solution*

The percentages of diethylene glycol and ethylene glycol in the portion of sample taken are calculated using the following formula:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

in which  $R_U$  is the analyte relative response (analyte peak response/2,2,2-trichloroethanol peak response) from the *Sample solution*;  $R_S$  is the analyte relative response (analyte peak response/2,2,2-trichloroethanol peak response) from the *Standard solution*;  $C_S$  is the concentration of analyte in the *Standard solution* (mg/mL); and  $C_U$  is the concentration of sample in the *Sample solution* (mg/mL).

### Performance characteristics

**Limit of quantitation:** 0.025% (w/w) for ethylene glycol and diethylene glycol

**Range:** 0.013–0.031 mg/mL for ethylene glycol and 0.012–0.030 mg/mL for diethylene glycol.

[Note—Wider linear ranges may be achievable but were not investigated when developing this method.]

**Accuracy:** 99%–107% recovery from samples spiked with diethylene glycol and ethylene glycol at levels of 50%–120%

**Precision:** Instrument precision: Less than 3.0% RSD; repeatability less than 4.0% RSD for analysis in the 50%–120% range

## PESTICIDE RESIDUES

The methods and information contained in this section were designed to measure pesticide residues in food ingredients of botanical origin.

### General Method for Pesticide Residues Analysis

#### DEFINITION

Where used in this compendium, the designation *pesticide* applies to any substance or mixture of substances intended to prevent, destroy, or control any unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport, or marketing of pure articles. The designation includes substances intended for use as growth regulators, defoliants, or desiccants, and any substance applied to crops before or after harvest to protect the product from deterioration during storage and transport.

#### LIMITS

Limits for pesticides for foods are determined by the Environmental Protection Agency (EPA), and where no limit is set, the limit is zero. The limits contained in *Table 1*, therefore, may not be applicable in the United States and are provided for guidance purposes only, and not for the purpose of meeting a regulatory requirement in the United States. The limits may be applicable in other countries where the presence of pesticide residues is permitted. Unless otherwise specified in the individual monograph, the article under test contains NMT the amount of any pesticide indicated in *Table 1*.

#### Table 1

Substance	Limit (mg/kg)
Alachlor	0.02
Aldrin and Dieldrin (sum of)	0.05
Azinphos-methyl	1.0
Bromopropylate	3.0
Chlordane (sum of <i>cis</i> - and <i>trans</i> - isomers and oxychlordane)	0.05
Chlorfenvinphos	0.5
Chlorpyrifos	0.2
Chlorpyrifos-methyl	0.1
Cypermethrin (and isomers)	1.0
DDT (sum of <i>p,p'</i> -DDT, <i>o,p'</i> -DDT, <i>p,p'</i> -DDE, and <i>p,p'</i> -TDE)	1.0
Deltamethrin	0.5
Diazinon	0.5
Dichlorvos	1.0
Dithiocarbamates (as CS <sub>2</sub> )	2.0
Endosulfan (sum of endosulfan isomers and endosulfan sulfate)	3.0
Endrin	0.05
Ethion	2.0
Fenitrothion	0.5
Fenvalerate	1.5
Fonofos	0.05
Heptachlor (sum of heptachlor and heptachlor epoxide)	0.05
Hexachlorobenzene	0.1
Hexachlorocyclohexane isomers (other than $\gamma$ )	0.3
Lindane ( $\gamma$ -hexachlorocyclohexane)	0.6
Malathion	1.0
Methidathion	0.2
Parathion	0.5
Parathion-methyl	0.2
Permethrin	1.0
Phosalone	0.1
Piperonyl butoxide	3.0
Pirimiphos-methyl	4.0
Pyrethrins (sum of)	3.0
Quintozene (sum of quintozene, pentachloroaniline and methyl pentachlorophenyl sulfide)	1.0

#### SAMPLING

For articles in containers holding less than 1 kg, mix the contents, and withdraw a quantity sufficient for the tests. For articles in containers holding between 1 and 5 kg, withdraw equal portions from the upper, middle, and lower parts of the container, each of the samples being sufficient to carry out the tests.

Thoroughly mix the samples, and withdraw an amount sufficient to carry out the tests. For containers holding more than 5 kg, withdraw three samples, each weighing NLT 250 g, from the upper, middle, and lower parts of the container. Thoroughly mix the samples, and withdraw a portion sufficient to carry out the tests.

If the number of the containers,  $n$ , is three or fewer, withdraw samples from each container as indicated above. If the number of containers is more than three, take samples from

$$\sqrt{n + 1}$$

containers, rounding up to the nearest whole number if necessary.

[Note—Conduct tests without delay to avoid possible degradation of the residues. If this is not possible, store the samples in hermetic containers suitable for food contact, at a temperature below 0°, and protected from light.]

### REAGENTS

Use reagents and solvents that are free from any contaminants, especially pesticides, that might interfere with the analysis. It is often necessary to use special grade solvents suitable for pesticide residue analysis or solvents that have recently been redistilled in an apparatus made entirely of glass. In any case, suitable blank tests must be performed.

### PREPARATION OF APPARATUS

Clean all equipment, especially glassware, to ensure that it is free from pesticides. Soak all glassware for a minimum of 16 hours in a solution of phosphate-free detergent, rinse with copious quantities of distilled water, and then wash with acetone, followed by hexane or heptane.

### QUALITATIVE AND QUANTITATIVE ANALYSIS OF PESTICIDE RESIDUES

Use validated analytical procedures that satisfy the following criteria. The method, especially with respect to its purification steps, is suitable for the combination of pesticide residue and substance under test, and is not susceptible to interference from co-extractives. Measure the limits of detection and quantification for each pesticide matrix combination to be analyzed: the method is shown to recover between 70% and 110% of each pesticide; the repeatability and reproducibility of the method are NLT the appropriate values indicated in *Table 2*; and the concentrations of test and reference solutions and the setting of the apparatus are such that a linear response is obtained from the analytical detector.

**Table 2**

<b>Concentration of Pesticide (mg/kg)</b>	<b>Repeatability (difference, ± mg/kg)</b>	<b>Reproducibility (difference, ± mg/kg)</b>
0.010	0.005	0.01
0.100	0.025	0.05
1.000	0.125	0.25

### Test for Pesticides

Depending on the substance being examined, it may be necessary to modify, sometimes extensively, the procedure described hereafter. Additionally, it may be necessary to perform another method with another column having a different polarity, another detection method (e.g., mass spectrometry), or a different method (e.g., immunochemical method) to confirm the results.

## EXTRACTION

[Note—Use the following procedure for the analysis of samples and articles having a water content of less than 15%. Samples having a higher water content may be dried, provided that the drying procedure does not significantly affect the pesticide content.]

To 10 g of the coarsely powdered substance under test, add 100 mL of acetone, and allow to stand for 20 min. Add 1 mL of a solution in toluene containing 1.8 µg/mL of carbophenothion. Mix in a high-speed blender for 3 min. Filter this solution, and wash the residue with two 25-mL portions of acetone. Combine the filtrate and the washings, and heat, in a rotary evaporator, maintaining the temperature of the bath below 40° until the solvent has almost completely evaporated. To the residue add a few mL of toluene, and heat again until the acetone is completely removed. Dissolve the residue in 8 mL of toluene. Pass through a membrane filter having a 45-µm porosity, rinse the flask and the filter with toluene, and dilute with toluene to 10.0 mL. This is *Solution A*.

## PURIFICATION

**Organochlorine, organophosphorus, and pyrethroid insecticides:** The size-exclusion chromatograph is equipped with a 7.8-mm × 30-cm stainless steel column containing 5-µm packing of styrene-divinylbenzene copolymer. Toluene is used as the mobile phase at a flow rate of 1 mL/min.

**Performance of the column:** Inject 100 µL of a solution in toluene containing, in each mL, 0.5 mg of methyl red and 0.5 mg of oracet blue. The column is not suitable unless the color of the eluate changes from orange to blue at an elution volume of about 10.3 mL. If necessary, calibrate the column, using a solution in toluene containing suitable concentrations of the pesticide of interest having the lowest molecular weight (for example, dichlorvos) and that having the highest molecular weight (for example, deltamethrin). Determine which fraction of the eluate contains both pesticides.

**Purification of the sample solution:** Inject a suitable volume (100 to 500 µL) of *Solution A* into the chromatograph. Collect the fraction (*Solution B*) as determined above under *Performance of the column*. Organophosphorus pesticides elute between 8.8 and 10.9 mL. Organochlorine and pyrethroid pesticides elute between 8.5 and 10.3 mL.

**Organochlorine and pyrethroid insecticides:** Into a 5-mm × 10-cm chromatographic column, introduce a piece of fat-free cotton and 0.5 g of silica gel treated as follows. Heat chromatographic silica gel in an oven at 150° for NLT 4 h. Allow to cool, and add dropwise a quantity of water corresponding to 1.5% of the weight of the silica gel used. Shake vigorously until agglomerates have disappeared, and continue shaking by mechanical means for 2 h. Condition the column with 1.5 mL of hexane.

[Note—Prepacked columns containing about 0.50 g of a suitable silica gel may also be used, provided they have been previously validated.] Concentrate *Solution B* almost to dryness, with the aid of a stream of helium or oxygen-free nitrogen, and dilute with toluene to a suitable volume (200 µL to 1 mL, according to the volume injected in the preparation of *Solution B*). Quantitatively transfer this solution to the column, and proceed with the chromatography, using 1.8 mL of toluene as the mobile phase. Collect the eluate (*Solution C*).

## QUANTITATIVE ANALYSIS OF ORGANOPHOSPHORUS INSECTICIDES

**Sample solution:** Concentrate *Solution B* almost to dryness, with the aid of a stream of helium, and

dilute with toluene to 100  $\mu\text{L}$ .

**Standard solution:** Prepare at least three solutions in toluene containing each of the pesticides of interest and carbophenothion at concentrations suitable for plotting a calibration curve.

**Chromatographic system,** Appendix IIA

**Mode:**GC

**Detector:**Flame-ionization detector (alkali) or flame-photometric detector

**Column:**0.32-mm  $\times$  30-m fused silica column coated with a 0.25- $\mu\text{m}$  layer of dimethylpolysiloxane

**Carrier gas:**Hydrogen (may also use helium or nitrogen)

**Temperature**

**Injector:**250 $^{\circ}$

**Detector:**275 $^{\circ}$

**Column:**See the temperature program table below.

Initial Temperature ( $^{\circ}$ )	Temperature Ramp ( $^{\circ}/\text{min}$ )	Final Temperature ( $^{\circ}$ )	Hold Time at Final Temperature (min)
80	—	80	1
80	30	150	3
150	4	280	1

**Analysis:**Use carbophenothion as the internal standard. [Note—If necessary, use a second internal standard to identify any possible interference with the peak corresponding to carbophenothion.] Inject the chosen volume of each solution, record the chromatograms, and measure the peak responses. [Note—The approximate relative retention times are listed in *Table 3*.] Calculate the content of each pesticide from the peak areas and the concentrations of the solution.

**Table 3**

Substance	Relative Retention Time
Dichlorvos	0.20
Fonofos	0.50
Diazinon	0.52
Parathion-methyl	0.59
Chlorpyrifos-methyl	0.60
Pirimiphos-methyl	0.66
Malathion	0.67
Parathion	0.69
Chlorpyrifos	0.70
Methidathion	0.78
Ethion	0.96
Carbophenothion	1.00
Azinphos-methyl	1.17
Phosalone	1.18

#### QUANTITATIVE ANALYSIS OF ORGANOCHLORINE AND PYRETHROID INSECTICIDES

**Sample solution:** Concentrate *Solution C* almost to dryness, with the aid of a stream of helium or oxygen-free nitrogen, and dilute with toluene to 500  $\mu\text{L}$ .

**Standard solution:** Prepare at least three solutions in toluene containing each of the pesticides of interest and carbophenothion at concentrations suitable for plotting a calibration curve.

**Chromatographic system,** Appendix IIA

**Mode:**GC

**Detector:**Electron capture

**Column:**0.32-mm  $\times$  30-m fused silica column coated with a 0.25- $\mu\text{m}$  layer of dimethylpolysiloxane

**Carrier gas:**Hydrogen (may also use helium or nitrogen)

**Temperature**

**Injector:**275 $^{\circ}$

**Detector:**300 $^{\circ}$

**Column:**See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
80	—	80	1
80	30	150	3
150	4	280	1

**Analysis:** Use carbophenothion as the internal standard. [Note—If necessary, use a second internal standard to identify any possible interference with the peak corresponding to carbophenothion.] Inject the chosen volume of each solution, record the chromatograms, and measure the peak responses.

[Note—The approximate relative retention times are listed in *Table 4*.] Calculate the content of each pesticide from the peak areas and the concentrations of the solution.

**Table 4**

Substance	Relative Retention Time
$\alpha$ -Hexachlorocyclohexane	0.44
Hexachlorobenzene	0.45
$\beta$ -Hexachlorocyclohexane	0.49
Lindane	0.49
$\delta$ -Hexachlorocyclohexane	0.54
$\epsilon$ -Hexachlorocyclohexane	0.56
Heptachlor	0.61
Aldrin	0.68
<i>cis</i> -Heptachlor epoxide	0.76
<i>o,p'</i> -DDE	0.81
$\alpha$ -Endosulfan	0.82
Dieldrin	0.87
<i>p,p'</i> -DDE	0.87
<i>o,p'</i> -DDD	0.89
Endrin	0.91
$\beta$ -Endosulfan	0.92
<i>o,p'</i> -DDT	0.95
Carbophenothion	1.00
<i>p,p'</i> -DDT	1.02
<i>cis</i> -Permethrin	1.29
<i>trans</i> -Permethrin	1.31
Cypermethrin*	1.40
Fenvalerate*	1.47
	1.49
Deltamethrin	1.54
* The substance shows several peaks.	

■ 1S (FCC7)

<sup>1</sup> Based on the following published method: Holloway G., Maheswaran R., Bradby, S., and Wahab, S. 2010. Screening method for ethylene glycol and diethylene glycol in glycerin-containing products. *J Pharm Biomed Anal.* 51:507-511.

<sup>2</sup> DB-624 (J & W Scientific), or equivalent.

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

Topic/Question	Contact	Expert Committee
Monograph	Jeffrey Moore, Ph.D. Scientific Liaison 1-301-816-8288	(F12010) Monographs - Food Ingredients



**Volumetric Solutions**, FCC 7 page 1263. On the basis of comments received, a revision is proposed to replace the current instructions for the preparation of *Ceric Sulfate, 0.1 N* with those appearing under *Ceric Sulfate, Tenth Normal (0.1 N)* in *Volumetric Solutions* in *USP 32-NF 27*. The revised instructions eliminate the use of two hazardous substances in the standardization of the solution. FI07K. Laurick C90448

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## VOLUMETRIC SOLUTIONS

**Normal Solutions** A normal solution contains 1 g equivalent weight of the solute per L of solution. The normalities of solutions used in volumetric determinations are designated as 1 N, 0.1 N, 0.05 N, etc., in this Codex.

**Molar Solutions** A molar solution contains 1 g molecular weight of the solute per L of solution. The molarities of such solutions are designated as 1 M, 0.1 M, 0.05 M, etc., in this Codex.

**Preparation and Methods of Standardization** The details for the preparation and standardization of solutions used in several normalities are usually given only for the one most frequently required. Solutions of other normalities are prepared and standardized in the same general manner as described. Solutions of lower normalities may be prepared accurately by making an exact dilution of a stronger solution, but solutions prepared in this way should be restandardized before use.

Dilute solutions that are not stable, such as 0.01 N potassium permanganate and sodium thiosulfate, are preferably prepared by diluting exactly the higher normality with thoroughly boiled and cooled water on the same day they are to be used.

All volumetric solutions should be prepared, standardized, and used at the standard temperature of 25°, if practicable. When a titration must be carried out at a markedly different temperature, the volumetric solution should be standardized at that same temperature, or a suitable temperature correction should be made. Because the strength of a standard solution may change upon standing, the normality or molarity factor should be redetermined frequently.

Although the directions provide only one method of standardization, other methods of equal or greater accuracy may be used. For substances available as certified primary standards, or of comparable quality, the final standard solution may be prepared by weighing accurately a suitable quantity of the substance and dissolving it to produce a specific volume solution of known concentration. Hydrochloric and sulfuric acids may be standardized against a certified primary standard.

In volumetric assays described in this Codex, the number of mg of the test substance equivalent to 1 mL of the primary volumetric solution is given. In general, these equivalents may be derived by simple calculation (see also *Solutions*, in the *General Provisions*).

**Ammonium Thiocyanate, 0.1 N** (7.612 g NH<sub>4</sub>SCN per 1000 mL) Dissolve about 8 g of ammonium thiocyanate (NH<sub>4</sub>SCN) in 1000 mL of water, and standardize by titrating the solution against *0.1 N Silver Nitrate* as follows: Transfer about 30 mL of *0.1 N Silver Nitrate*, accurately measured, into a glass-stoppered flask. Dilute with 50 mL of water, then add 2 mL of *Ferric Ammonium Sulfate TS* and 2 mL of nitric acid, and titrate with the ammonium thiocyanate solution to the first appearance of a red-brown color. Calculate the normality, and, if desired, adjust the solution to exactly 0.1 N. If desired, *0.1 N Ammonium Thiocyanate* may be replaced by 0.1 N potassium thiocyanate where the former is directed in various tests and assays.

**Barium Hydroxide, 0.2 N** [17.14 g Ba(OH)<sub>2</sub> per 1000 mL] Dissolve about 36 g of barium hydroxide

[Ba(OH)<sub>2</sub>·8H<sub>2</sub>O] in 1 L of recently boiled and cooled water, and quickly filter the solution. Keep this solution in bottles with well-fitted rubber stoppers with a soda–lime tube attached to each bottle to protect the solution from carbon dioxide in the air. Standardize as follows: Transfer quantitatively about 60 mL of 0.1 N hydrochloric acid, accurately measured, to a flask; add 2 drops of *Phenolphthalein TS*; and slowly titrate with the barium hydroxide solution, with constant stirring, until a permanent pink color is produced. Calculate the normality of the barium hydroxide solution and, if desired, adjust to exactly 0.2 N with freshly boiled and cooled water.

[Note—Solutions of alkali hydroxides absorb carbon dioxide when exposed to air. Connect the buret used for titrations with barium hydroxide solution directly to the storage bottle, and provide the bottle with a soda–lime tube so that air entering must pass through this tube, which will absorb carbon dioxide. Frequently restandardize standard solutions of barium hydroxide.]

**Bromine, 0.1 N** (7.990 g Br per 1000 mL) Dissolve 3 g of potassium bromate (KBrO<sub>3</sub>) and 15 g of potassium bromide (KBr) in sufficient water to make 1000 mL, and standardize the solution as follows: Transfer about 25 mL of the solution, accurately measured, into a 500-mL iodine flask, and dilute with 120 mL of water. Add 5 mL of hydrochloric acid, stopper the flask, and shake it gently. Then add 5 mL of *Potassium Iodide TS*, restopper, shake the mixture, allow it to stand for 5 min, and titrate the liberated iodine with *0.1 N Sodium Thiosulfate*, adding *Starch TS* near the end of the titration. Calculate the normality. Store this solution in dark, amber-colored, glass-stoppered bottles.

**Change to read:**

**Ceric Sulfate, 0.1 N** [33.22 g Ce(SO<sub>4</sub>)<sub>2</sub> per 1000 mL] Transfer 59 g of ceric ammonium nitrate

[Ce(NO<sub>3</sub>)<sub>4</sub>·2NH<sub>4</sub>NO<sub>3</sub>·2H<sub>2</sub>O] to a beaker, add 31 mL of sulfuric acid, mix, and cautiously add water, in 20-mL portions, until solution is complete. Cover the beaker, let stand overnight, pass through a sintered-glass crucible of fine porosity, add water to make 1000 mL, and mix. Standardize the solution as follows: Weigh accurately 200 mg of primary standard arsenic trioxide (As<sub>2</sub>O<sub>3</sub>) previously dried at 100° for 1 h, and transfer to a 500-mL Erlenmeyer flask. Wash down the inner walls of the flask with 25 mL of a 2:25 solution of sodium hydroxide, swirl to dissolve the sample, and when solution is complete, add 100 mL of water, and mix. Add 10 mL of 1:3 sulfuric acid and 2 drops each of *Orthophenanthroline TS* and a solution of osmium tetroxide in 0.1 N sulfuric acid (1:400), and slowly titrate with the ceric sulfate solution until the pink color is changed to a very pale blue. Calculate the normality. Each 4.946 mg of As<sub>2</sub>O<sub>3</sub> is equivalent to 1 mL of 0.1 N Ceric Sulfate. ■

Alternatively, use commercially available volumetric standard solution.

Standardize *Ceric Sulfate, 0.1 N* as follows: Weigh 0.2 g of sodium oxalate, primary standard, dried according to the instructions on its label, and dissolve in 75 mL of water. Add, with stirring, 2 mL of sulfuric acid that has previously been mixed with 5 mL of water, mix well, add 10 mL of hydrochloric acid, and heat to 70°–75°. Titrate with *Ceric Sulfate, 0.1 N* to a permanent slight yellow color. Each 6.700 mg of sodium oxalate is equivalent to 1 mL of 0.1 N ceric sulfate.

$$N = (\text{mg Na}_2\text{C}_2\text{O}_4) / [67.00 \times \text{mL Ce(SO}_4)_2 \text{ solution}]$$

■ 2S (FCC7)

**Ceric Sulfate, 0.01 N** [3.322 g Ce(SO<sub>4</sub>)<sub>2</sub> per 1000 mL] Dissolve 4.2 g of ceric sulfate [Ce(SO<sub>4</sub>)<sub>2</sub>·4H<sub>2</sub>O] or 5.5 g of the acid sulfate [Ce(HSO<sub>4</sub>)<sub>4</sub>] in about 500 mL of water containing 28 mL of sulfuric acid, and dilute to 1000 mL. Allow the solution to stand overnight, and filter. Standardize this solution daily as follows: Weigh accurately about 275 mg of hydroquinone (C<sub>6</sub>H<sub>6</sub>O<sub>2</sub>), dissolve it in sufficient *0.5 N Alcoholic Sulfuric Acid* to

make 500.0 mL, and mix. To 25.0 mL of this solution add 75 mL of 0.5 N sulfuric acid, 20 mL of water, and 2 drops of *Diphenylamine TS*. Titrate with the ceric sulfate solution at a rate of about 25 drops per 10 s until an endpoint is reached that persists for 10 s. Perform a blank determination using 100 mL of 0.5 N *Alcoholic Sulfuric Acid*, 20 mL of water, and 2 drops of *Diphenylamine TS*, and make any necessary correction. Calculate the normality of the ceric sulfate solution by the formula

$$\text{Result} = 0.05W/55.057V,$$

in which *W* is the weight, in mg, of the hydroquinone sample taken, and *V* is the volume, in mL, of the ceric sulfate solution consumed in the titration.

**Disodium EDTA, 0.05 M** (16.81 g  $C_{10}H_{14}N_2Na_2O_8$  per 1000 mL) Dissolve 18.6 g of disodium ethylenediaminetetraacetate ( $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ ) in sufficient water to make 1000 mL, and standardize the solution as follows: Weigh accurately about 200 mg of chelometric standard calcium carbonate ( $CaCO_3$ ), transfer to a 400-mL beaker, add 10 mL of water, and swirl to form a slurry. Cover the beaker with a watch glass, and introduce 2 mL of 2.7 N hydrochloric acid from a pipet inserted between the lip of the beaker and the edge of the watch glass. Swirl the contents of the beaker to dissolve the calcium carbonate. Wash down the sides of the beaker, the outer surface of the pipet, and the watch glass, and dilute with water to about 100 mL. While stirring, preferably with a magnetic stirrer, add about 30 mL of the disodium EDTA solution from a 50-mL buret, then add 15 mL of 1 N *Sodium Hydroxide* and 300 mg of *Hydroxy Naphthol Blue Indicator*, and continue the titration to a blue endpoint. Calculate the molarity by the formula

$$\text{Result} = W/100.09V,$$

in which *W* is the weight, in mg, of  $CaCO_3$  in the sample of calcium carbonate taken, and *V* is the volume, in mL, of disodium EDTA solution consumed. Each 5.004 mg of  $CaCO_3$  is equivalent to 1 mL of 0.05 M *Disodium EDTA*.

For the determination of aluminum in its salts, use 0.05 M *Disodium EDTA* standardized as follows: Transfer 2 g, accurately weighed, of aluminum wire to a 1000-mL volumetric flask, and add 50 mL of a 1:1 hydrochloric acid–water mixture. Swirl the flask to ensure complete wetting of the wire, and allow the reaction to proceed. When dissolution is complete, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 250-mL beaker, add 25.0 mL of the disodium EDTA solution, boil gently for 5 min, and cool. Add in the order given, and with continuous stirring, 20 mL of pH 4.5 buffer solution (77.1 g of ammonium acetate and 57 mL of glacial acetic acid in 1000 mL of solution), 50 mL of alcohol, and 2 mL of *Dithizone TS*. Titrate with 0.05 M *Zinc Sulfate* to a bright rose pink color, and perform a blank determination, substituting 10 mL of water for the 10.0 mL of aluminum solution. Each mL of disodium EDTA solution is equivalent to 1.349 mg of aluminum (Al).

**Ferrous Ammonium Sulfate, 0.1 N** [39.21 g  $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$  per 1000 mL] Dissolve 40 g of ferrous ammonium sulfate hexahydrate in a previously cooled mixture of 40 mL of sulfuric acid and 200 mL of water, dilute with water to 1000 mL, and mix. On the day of use, standardize the solution as follows: Transfer from 25 to 30 mL of the solution, accurately measured, into a flask, add 2 drops of *Orthophenanthroline TS*, and titrate with 0.1 N *Ceric Sulfate* until the red color is changed to pale blue. From the volume of 0.1 N *Ceric Sulfate* consumed, calculate the normality.

**Hydrochloric Acid, 1 N** (36.46 g HCl per 1000 mL) Dilute 85 mL of hydrochloric acid with water to make 1000 mL, and standardize the solution as follows: Accurately weigh about 1.5 g of primary standard anhydrous sodium carbonate ( $Na_2CO_3$ ) that has been heated at a temperature of about 270° for 1 h. Dissolve it in 100 mL of water, and add 2 drops of *Methyl Red TS*. Add the acid slowly from a buret, with constant stirring, until the solution becomes faintly pink. Heat the solution to boiling, and continue the titration until the faint pink color is

no longer affected by continued boiling. Calculate the normality. Each 52.99 mg of  $\text{Na}_2\text{CO}_3$  is equivalent to 1 mL of 1 N Hydrochloric Acid.

**Hydroxylamine Hydrochloride, 0.5 N** (35 g  $\text{NH}_2\text{OH}\cdot\text{HCl}$  per 1000 mL) Dissolve 35 g of hydroxylamine hydrochloride in 150 mL of water, and dilute with anhydrous methanol to 1000 mL. To 500 mL of this solution add 15 mL of a 0.04% solution of bromophenol blue in alcohol, and titrate with 0.5 N Triethanolamine until the solution appears green-blue by transmitted light. *Prepare this solution fresh before each series of analyses.*

**Iodine, 0.1 N** (12.69 g I per 1000 mL) Dissolve about 14 g of iodine (I) in a solution of 36 g of potassium iodide (KI) in 100 mL of water, add 3 drops of hydrochloric acid, dilute with water to 1000 mL, and standardize as follows: Weigh accurately about 150 mg of primary standard arsenic trioxide ( $\text{As}_2\text{O}_3$ ) previously dried at  $105^\circ$  for 1 h, and dissolve it in 20 mL of 1 N Sodium Hydroxide by warming if necessary. Dilute with 40 mL of water, add 2 drops of Methyl Orange TS, and follow with 2.7 N hydrochloric acid until the yellow color is changed to pink. Then add 2 g of sodium bicarbonate ( $\text{NaHCO}_3$ ), dilute with 50 mL of water, add 3 mL of Starch TS, and slowly add the iodine solution from a buret until a permanent blue color is produced. Calculate the normality. Each 4.946 mg of  $\text{As}_2\text{O}_3$  is equivalent to 1 mL of 0.1 N Iodine. Store this solution in glass-stoppered bottles.

**Lithium Methoxide, 0.1 N** (3.797 g  $\text{CH}_3\text{OLi}$  per 1000 mL) Dissolve 600 mg of freshly cut lithium metal in a mixture of 150 mL of anhydrous methanol and 850 mL of benzene. Filter the resulting solution if it is cloudy, and standardize it as follows: Dissolve about 80 mg of benzoic acid (National Institute of Standards and Technology primary standard), accurately weighed, in 35 mL of dimethylformamide, add 5 drops of Thymol Blue TS, and titrate with the lithium methoxide solution to a dark blue endpoint.

[CAUTION— Protect the solution from absorption of carbon dioxide and moisture by covering the titration vessel with aluminum foil while dissolving the benzoic acid sample and during the titration. ]

Each mL of 0.1 N Lithium Methoxide is equivalent to 12.21 mg of benzoic acid.

**Mercuric Nitrate, 0.1 M** [32.46 g  $\text{Hg}(\text{NO}_3)_2$  per 1000 mL] Dissolve about 35 g of mercuric nitrate [ $\text{Hg}(\text{NO}_3)_2\cdot\text{H}_2\text{O}$ ] in a mixture of 5 mL of nitric acid and 500 mL of water, and dilute with water to 1000 mL. Standardize the solution as follows: Transfer an accurately measured volume of about 20 mL of the solution into an Erlenmeyer flask, and add 2 mL of nitric acid and 2 mL of Ferric Ammonium Sulfate TS. Cool to below  $20^\circ$ , and titrate with 0.1 N Ammonium Thiocyanate to the first appearance of a permanent brown color. Calculate the molarity.

**Oxalic Acid, 0.1 N** (4.502 g  $\text{H}_2\text{C}_2\text{O}_4$  per 1000 mL) Dissolve 6.45 g of oxalic acid ( $\text{H}_2\text{C}_2\text{O}_4\cdot 2\text{H}_2\text{O}$ ) in sufficient water to make 1000 mL. Standardize by titration against freshly standardized 0.1 N Potassium Permanganate as directed under Potassium Permanganate, 0.1 N. Store this solution in glass-stoppered bottles, protected from light.

**Perchloric Acid, 0.1 N** (10.046 g  $\text{HClO}_4$  per 1000 mL) Mix 8.5 mL of perchloric acid (70%) with 500 mL of glacial acetic acid and 30 mL of acetic anhydride.

[CAUTION— Handle perchloric acid in an appropriate fume hood. ]

Cool, and add glacial acetic acid to make 1000 mL. Allow the prepared solution to stand for 1 day for the excess acetic anhydride to be combined, and determine the water content by the Karl Fischer Titrimetric Method, Appendix IIB. If the water content exceeds 0.05%, add more acetic anhydride, but if the solution contains no titratable water, add sufficient water to make the content between 0.02% and 0.05%. Allow to stand for 1 day, and again determine the water content by titration. Standardize the solution as follows: Weigh

accurately about 700 mg of primary standard potassium biphthalate [ $\text{KHC}_6\text{H}_4(\text{COO})_2$ ], previously dried at  $120^\circ$  for 2 h, and dissolve it in 50 mL of glacial acetic acid in a 250-mL flask. Add 2 drops of *Crystal Violet TS*, and titrate with the perchloric acid solution until the violet color changes to emerald green. Deduct the volume of the perchloric acid consumed by 50 mL of the glacial acetic acid, and calculate the normality. Each 20.42 mg of  $\text{KHC}_6\text{H}_4(\text{COO})_2$  is equivalent to 1 mL of *0.1 N Perchloric Acid*.

**Perchloric Acid, 0.1 N, in Dioxane** Mix 8.5 mL of perchloric acid (70%) with sufficient dioxane, which has been especially purified by adsorption, to make 1000 mL.

[CAUTION— Handle perchloric acid in an appropriate fume hood. ]

Standardize the solution as follows: Weigh accurately about 700 mg of primary standard potassium biphthalate [ $\text{KHC}_6\text{H}_4(\text{COO})_2$ ], previously dried at  $105^\circ$  for 2 h, and dissolve in 50 mL of glacial acetic acid in a 250-mL flask. Add 2 drops of *Crystal Violet TS*, and titrate with the perchloric acid solution until the violet color changes to blue-green. Deduct the volume of the perchloric acid consumed by 50 mL of the glacial acetic acid, and calculate the normality. Each 20.42 mg of  $\text{KHC}_6\text{H}_4(\text{COO})_2$  is equivalent to 1 mL of *0.1 N Perchloric Acid*.

**Potassium Acid Phthalate, 0.1 N** [20.42 g  $\text{KHC}_6\text{H}_4(\text{COO})_2$  per 1000 mL] Dissolve 20.42 g of primary standard potassium biphthalate [ $\text{KHC}_6\text{H}_4(\text{COO})_2$ ], previously dried at  $105^\circ$  for 2 h, in glacial acetic acid in a 1000-mL volumetric flask, warming on a steam bath if necessary to effect solution and protecting the solution from contamination by moisture. Cool to room temperature, dilute with glacial acetic acid to volume, and mix.

**Potassium Dichromate, 0.1 N** (4.903 g  $\text{K}_2\text{Cr}_2\text{O}_7$  per 1000 mL) Dissolve about 5 g of potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ) in 1000 mL of water, transfer quantitatively 25 mL of this solution to a 500-mL glass-stoppered flask, add 2 g of potassium iodide (free from iodate) (KI), dilute with 200 mL of water, add 5 mL of hydrochloric acid, and mix. Allow to stand for 10 min in a dark place, and titrate the liberated iodine with *0.1 N Sodium Thiosulfate*, adding *Starch TS* as the endpoint is approached. Correct for a blank run on the same quantities of the same reagents, and calculate the normality.

**Potassium Hydroxide, 1 N** (56.11 g KOH per 1000 mL) Prepare and standardize 1 N potassium hydroxide by the procedure set forth for *1 N Sodium Hydroxide*, using 74 g of the potassium hydroxide (KOH) to prepare the solution. Each 204.2 mg of  $\text{KHC}_6\text{H}_4(\text{COO})_2$  is equivalent to 1 mL of *1 N Potassium Hydroxide*.

## Potassium Hydroxide, 0.5 N, Alcoholic

[CAUTION— The solution may become very hot. Allow it to cool before adding the aldehyde-free alcohol. ]

Dissolve about 35 g of potassium hydroxide (KOH) in 20 mL of water, and add sufficient aldehyde-free alcohol to make 1000 mL. Allow the solution to stand in a tightly stoppered bottle for 24 h. Then quickly decant the clear supernatant liquid into a suitable, tight container, and standardize as follows: Transfer quantitatively 25 mL of 0.5 N hydrochloric acid into a flask, dilute with 50 mL of water, add 2 drops of *Phenolphthalein TS*, and titrate with the alcoholic potassium hydroxide solution until a permanent, pale pink color is produced. Calculate the normality. Store this solution in tightly stoppered bottles protected from light.

**Potassium Iodate, 0.05 M** (10.70 g  $\text{KIO}_3$  per 1000 mL) Dissolve 10.700 g of potassium iodate of primary standard quality ( $\text{KIO}_3$ ), previously dried at  $110^\circ$  to constant weight, in sufficient water to make 1000.0 mL.

**Potassium Permanganate, 0.1 N** (3.161 g  $\text{KMnO}_4$  per 1000 mL) Dissolve about 3.3 g of potassium permanganate ( $\text{KMnO}_4$ ) in 1000 mL of water in a flask, and boil the solution for about 15 min. Stopper the

flask, allow it to stand for at least 2 days, and pass through a fine-porosity, sintered-glass crucible. If necessary, the bottom of the crucible may be lined with a pledget of glass wool. Standardize the solution as follows: Weigh accurately about 200 mg of sodium oxalate of primary standard quality ( $\text{Na}_2\text{C}_2\text{O}_4$ ), previously dried at  $100^\circ$  to constant weight, and dissolve it in 250 mL of water. Add 7 mL of sulfuric acid, heat to about  $70^\circ$ , and then slowly add the permanganate solution from a buret, with constant stirring, until a pale pink color that persists for 15 s is produced. The temperature at the conclusion of the titration should be not less than  $60^\circ$ . Calculate the normality. Each 6.700 mg of  $\text{Na}_2\text{C}_2\text{O}_4$  is equivalent to 1 mL of 0.1 N Potassium Permanganate. Potassium permanganate is reduced on contact with organic substances such as rubber; therefore, the solution must be handled in apparatus made entirely of glass or other suitably inert material. Store it in glass-stoppered, amber-colored bottles, and restandardize frequently.

**Silver Nitrate, 0.1 N** (16.99 g  $\text{AgNO}_3$  per 1000 mL) Dissolve about 17.5 g of silver nitrate ( $\text{AgNO}_3$ ) in 1000 mL of water, and standardize the solution as follows: Weigh accurately 100 mg of primary standard sodium chloride, previously dried at  $120^\circ$  for 16 h, into a 150-mL beaker, and dissolve it in 5 mL of water. Add 5 mL of acetic acid, 50 mL of methanol, and 2 or 3 drops of *Eosin Y TS*, and titrate with the silver nitrate solution to the endpoint. Calculate the normality.

**Sodium Acetate, 0.1 N** (8.203 g  $\text{CH}_3\text{COONa}$  per 1000 mL) Dissolve 8.20 g of anhydrous sodium acetate in glacial acetic acid to make 1000 mL, and standardize the solution as follows: To 25.0 mL of the prepared sodium acetate solution, add 50 mL of glacial acetic acid and 1 mL of  $\alpha$ -Naphtholbenzein *TS*. Titrate with 0.1 N Perchloric Acid until a yellow-brown color changes through yellow to green.  
[CAUTION— Handle perchloric acid in an appropriate fume hood. ]

Perform a blank determination, and make any necessary correction. Calculate the normality factor.

**Sodium Arsenite, 0.05 N** (3.248 g  $\text{NaAsO}_2$  per 1000 mL) Transfer 2.4725 g of arsenic trioxide, which has been pulverized and dried at  $100^\circ$  to constant weight, to a 1000-mL volumetric flask, dissolve it in 20 mL of 1 N Sodium Hydroxide, and add 1 N Sulfuric Acid or 1 N Hydrochloric Acid until the solution is neutral or only slightly acid to litmus. Add 15 g of sodium bicarbonate, dilute with water to volume, and mix.

**Sodium Hydroxide, 1 N** (40.00 g  $\text{NaOH}$  per 1000 mL) Dissolve about 40 g of sodium hydroxide ( $\text{NaOH}$ ) in about 1000 mL of carbon dioxide-free water. Shake the mixture thoroughly, and allow it to stand overnight in a stoppered bottle. Standardize the clear liquid as follows: Transfer about 5 g of primary standard potassium biphthalate [ $\text{KHC}_6\text{H}_4(\text{COO})_2$ ], previously dried at  $105^\circ$  for 2 h and accurately weighed, to a flask, and dissolve it in 75 mL of carbon dioxide-free water. If the potassium biphthalate is in the form of large crystals, crush it before drying. To the flask add 2 drops of *Phenolphthalein TS*, and titrate with the sodium hydroxide solution to a permanent pink color. Calculate the normality. Each 204.2 mg of potassium biphthalate is equivalent to 1 mL of 1 N Sodium Hydroxide.

[Note—Solutions of alkali hydroxides absorb carbon dioxide when exposed to air. Therefore, store them in bottles with well-fitted, suitable stoppers provided with a tube filled with a mixture of sodium hydroxide and lime so that air entering the container must pass through this tube, which will absorb the carbon dioxide. Frequently restandardize standard solutions of sodium hydroxide.]

**Sodium Hydroxide, 0.5 N, Alcoholic** (22.5 g  $\text{NaOH}$  per 1000 mL)  
[CAUTION— The following solution may become very hot. Allow it to cool before adding the aldehyde-free alcohol.) ]

Dissolve about 22.5 g of sodium hydroxide (NaOH) in 20 mL of water, and add sufficient aldehyde-free alcohol to make 1000 mL. Allow the solution to stand in a tightly stoppered bottle for 24 h. Then quickly decant the clear supernatant liquid into a suitable, tight container, and standardize as follows:

Quantitatively transfer 25 mL of 0.5 N hydrochloric acid into a flask, dilute with 50 mL of water, add 2 drops of *Phenolphthalein TS*, and titrate with the alcoholic sodium hydroxide solution until a permanent, pale pink color appears. Calculate the normality. Store this solution in tightly stoppered bottles protected from light.

**Sodium Methoxide, 0.1 N, in Pyridine** (5.40 g CH<sub>3</sub>ONa per 1000 mL) Weigh 14 g of freshly cut sodium metal, and cut into small cubes. Place about 0.5 mL of anhydrous methanol in a round-bottom 120-mL flask equipped with a ground-glass joint, add 1 cube of the sodium metal, and when the reaction subsides, add the remaining sodium metal to the flask. Connect a water-cooled condenser to the flask, and slowly add 100 mL of anhydrous methanol, in small portions, through the top of the condenser. Regulate the addition of the methanol so that the vapors are condensed and do not escape through the top of the condenser. After addition of the methanol is complete, connect a drying tube to the top of the condenser, and allow the solution to cool. Transfer 17.5 mL of this solution (approximately 6 N) into a 1000-mL volumetric flask containing 70 mL of anhydrous methanol, and dilute with freshly distilled pyridine to volume. Store preferably in the reservoir of an automatic buret suitably protected from carbon dioxide and moisture. Standardize the solution as follows: Weigh accurately about 400 mg of primary standard benzoic acid, transfer it into a 250-mL wide-mouth Erlenmeyer flask, and dissolve it in 50 mL of freshly distilled pyridine. Add a few drops of *Thymolphthalein TS*, and titrate immediately with the sodium methoxide solution to a blue endpoint. During the titration, direct a gentle stream of nitrogen into the flask through a short piece of 6-mm glass tubing fastened near the tip of the buret. Perform a blank determination (see the *General Provisions*), correct for the volume of sodium methoxide solution consumed by the blank, and calculate the normality. Each 12.21 mg of benzoic acid is equivalent to 1 mL of 0.1 N Sodium Methoxide in Pyridine.

**Sodium Methoxide, 0.02 N, in Toluene** (1.08 g CH<sub>3</sub>ONa per 1000 mL) Weigh 2.5 g of freshly cut sodium metal, and cut into small cubes. Place about 200 mL of anhydrous methanol in a 1000-mL volumetric flask, chill in an ice bath, and add the cubes one at a time to the methanol. When the last cube is dissolved, dilute with toluene to the mark, and mix. Standardize the solution as follows: Weigh accurately about 20 mg of primary standard benzoic acid, transfer it into a 50-mL conical flask, and dissolve it in 25 mL of dimethylformamide. Add 2 drops of a solution of 100 mg of thymol blue in 10 mL of dimethylformamide, and titrate immediately with the sodium methoxide solution to a blue endpoint. Titrate a blank solution of dimethylformamide in the same manner, correct the volume of sodium methoxide solution consumed by the blank, and calculate the normality. Each 2.442 mg of benzoic acid is equivalent to 1 mL of 0.02 N Sodium Methoxide in Toluene.

**Sodium Thiosulfate, 0.1 N** (15.81 g Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> per 1000 mL) Dissolve about 26 g of sodium thiosulfate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O) and 200 mg of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) in 1000 mL of recently boiled and cooled water. Standardize the solution as follows: Weigh accurately about 210 mg of primary standard potassium dichromate, previously pulverized and dried at 120° for 4 h, and dissolve in 100 mL of water in a 500-mL glass-stoppered flask. Swirl to dissolve the sample, remove the stopper, and quickly add 2 g of sodium bicarbonate, 3 g of potassium iodide, and 5 mL of hydrochloric acid. Stopper the flask, swirl to mix, and let stand in the dark for 10 min. Rinse the stopper and inner walls of the flask with water, and titrate the liberated iodine with the sodium thiosulfate solution until the solution is only faint yellow. Add *Starch TS*, and continue the titration to the discharge of the blue color. Calculate the normality.

**Sulfuric Acid, 1 N** (49.04 g H<sub>2</sub>SO<sub>4</sub> per 1000 mL) Add slowly, with stirring, 30 mL of sulfuric acid to about 1020

mL of water, allow to cool to 25<sup>o</sup>, and standardize by titration against primary standard sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) as directed under *1 N Hydrochloric Acid*. Each 52.99 mg of Na<sub>2</sub>CO<sub>3</sub> is equivalent to 1 mL of *1 N Sulfuric Acid*.

**Sulfuric Acid, Alcoholic, 5 N** (245.2 g H<sub>2</sub>SO<sub>4</sub> per 1000 mL) Add cautiously, with stirring, 139 mL of sulfuric acid to a sufficient quantity of absolute alcohol to make 1000.0 mL.

**Sulfuric Acid, Alcoholic, 0.5 N** Add cautiously, with stirring, 13.9 mL of sulfuric acid to a sufficient quantity of absolute alcohol to make 1000.0 mL. Alternatively, prepare this solution by diluting 100.0 mL of *5 N Sulfuric Acid* with absolute alcohol to make 1000.0 mL.

**Thorium Nitrate, 0.1 M** [48.01 g Th(NO<sub>3</sub>)<sub>4</sub> per 1000 mL] Weigh accurately 55.21 g of thorium nitrate [Th(NO<sub>3</sub>)<sub>4</sub>·4H<sub>2</sub>O], dissolve it in water, dilute to 1000.0 mL, and mix. Standardize the solution as follows: Transfer 50.0 mL into a 500-mL volumetric flask, dilute with water to volume, and mix. Transfer 50.0 mL of the diluted solution into a 400-mL beaker, add 150 mL of water and 5 mL of hydrochloric acid, and heat to boiling. While stirring, add 25 mL of a saturated solution of oxalic acid, then digest the mixture for 1 h just below the boiling point, and allow to stand overnight. Decant through Whatman No. 42, or equivalent, filter paper, and transfer the precipitate to the filter using about 100 mL of a wash solution consisting of 70 mL of the saturated oxalic acid solution, 430 mL of water, and 5 mL of hydrochloric acid. Transfer the precipitate and filter paper to a tared tall-form porcelain crucible, dry, char the paper, and ignite at 950<sup>o</sup> for 1.5 h or to constant weight. Cool in a desiccator, weigh, and calculate the molarity of the solution by the formula

$$\text{Result} = 200W/264.04,$$

in which W is the weight, in g, of thorium oxide obtained.

**Triethanolamine, 0.5 N** [74 g N(CH<sub>2</sub>CH<sub>2</sub>OH)<sub>3</sub> per 1000 mL] Transfer 65 mL (74 g) of 98% triethanolamine into a 1000-mL volumetric flask, dilute with water to volume, stopper the flask, and mix thoroughly.

**Zinc Sulfate, 0.05 M** (8.072 g ZnSO<sub>4</sub> per 1000 mL) Dissolve about 15 g of zinc sulfate (ZnSO<sub>4</sub>·7H<sub>2</sub>O) in sufficient water to make 1000 mL, and standardize the solution as follows: Dilute about 35 mL, accurately measured, with 75 mL of water, add 5 mL of *Ammonia–Ammonium Chloride Buffer TS* and 0.1 mL of *Eriochrome Black TS*, and titrate with *0.05 M Disodium EDTA* until the solution is deep blue. Calculate the molarity.