Official Monographs for NF 33

Acacia

DEFINITION
Acacia is the dried gummy exudate from the stems and branches of *Acacia senegal* (L.) Willd. or of other related African species of *Acacia* (Fam. Leguminosae).

IDENTIFICATION
- **A.**
  Analysis: To 10 mL of a cold solution (1 in 50) add 0.2 mL of diluted lead subacetate TS.
  Acceptance criteria: A flocculent, or curdy, white precipitate is formed immediately.

IMPURITIES
- **Arsenic,** *Method II* ⟨211⟩: NMT 3 ppm
- **Lead** ⟨251⟩: NMT 10 ppm

Delete the following:

- •**Heavy Metals,** *Method II* ⟨231⟩: NMT 40 ppm• (Official 1-Dec-2015)

SPECIFIC TESTS
- **Botanic Characteristics**
  Acacia: Spheroidal tears up to 32 mm in diameter or in angular fragments of white to yellowish white color. It is translucent or somewhat opaque from the presence of numerous minute fissures; very brittle, the fractured surface glassy and occasionally iridescent. It is almost odorless and produces a mucilaginous sensation on the tongue.
  Flake Acacia: White to yellowish white, thin flakes, appearing under the microscope as colorless, striated fragments
  Powdered Acacia: White to yellowish white, angular microscopic fragments with only traces of starch or vegetable tissues present
  Granular Acacia: White to pale yellowish white, fine granules. Under the microscope it appears as colorless, glassy, irregularly angular fragments up to 100 µm in thickness, some of which exhibit parallel linear streaks.
  Spray-dried Acacia: White to off-white compacted microscopic fragments or whole spheres
- **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩: Meets the requirements of the tests for absence of *Salmonella* species
- **Water Determination,** *Method III (Gravimetric)* ⟨921⟩
  Analysis: For unground Acacia, crush in a mortar until it passes through a No. 40 sieve, and mix the ground material before weighing the test specimen. Dry a sample at 105° for 5 h.
  Acceptance criteria: NMT 15.0% of its weight
- **Articles of Botanical Origin,** *Acid-Insoluble Ash* ⟨561⟩: NMT 0.5%
- **Articles of Botanical Origin,** *Total Ash* ⟨561⟩: NMT 4.0%
- **Insoluble Residue**
  Sample solution: Dissolve 5.0 g of powdered or finely ground Acacia in 100 mL of water. Add 10 mL of 3 N hydrochloric acid.
  Analysis: Boil the *Sample solution* gently for 15 min. Pass by suction, while hot, through a tared filtering crucible. Wash thoroughly with hot water, dry at 105° for 1 h, and weigh.
  Acceptance criteria: The weight of the residue thus obtained does not exceed 50 mg.
- **Starch or Dextrin**
  Sample solution: A solution (1 in 50)
  Analysis: Boil the *Sample solution* cool, and add iodine TS.
  Acceptance criteria: No bluish or reddish color is produced.
- **Solubility and Reaction**
  Sample: 1 g
  Analysis: Dissolve the *Sample* in 2 mL of water.
  Acceptance criteria: The resulting solution flows readily and is acid to litmus.
- **Tannin-Bearing Gums**
  Sample solution: A solution (1 in 50)
  Analysis: To 10 mL of the *Sample solution* add 0.1 mL of ferric chloride TS.
  Acceptance criteria: No blackish coloration or blackish precipitate is produced.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in tight containers.

Acacia Syrup

DEFINITION
Prepare Acacia Syrup as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

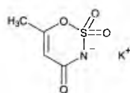
Acacia, granular or powdered	100 g
Sodium Benzoate	1 g
Vanilla Tincture	5 mL
Sucrose	800 g
Purified Water, a sufficient quantity to make	1000 mL

Mix *Acacia, Sodium Benzoate*, and *Sucrose*. Add 425 mL of *Purified Water*, and mix. Heat the mixture on a steam bath until dissolved. When cool, remove the scum, add *Vanilla Tincture* and sufficient *Purified Water* to make the product measure 1000 mL, and strain, if necessary.

SPECIFIC TESTS
- **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩: Meets the requirements of the test for absence of *Salmonella* species

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Package in tight containers, and prevent exposure to excessive heat.
- **Labeling:** The label states the Latin binomial name and, following the official name, the part of the plant source from which the article was derived.

Acesulfame Potassium



$C_4H_4NO_4SK$ 201.24

6-Methyl-1,2,3-oxathiazine-4(3*H*)-one-2,2-dioxide potassium salt;

3,4-Dihydro-6-methyl-1,2,3-oxathiazine-4-one-2,2-dioxide potassium salt [55589-62-3].

DEFINITION

Acesulfame Potassium contains NLT 99.0% and NMT 101.0% of $C_4H_4NO_4SK$, calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** ⟨197K⟩
- **B. Identification Tests—General,** *Potassium* ⟨191⟩
  Sample solution: 100 mg/mL
  Acceptance criteria: Meets the requirements

ASSAY

- **Procedure**
  Sample: 150 mg
  Titrimetric system
  (See *Titrimetry* ⟨541⟩)
  Mode: Direct titration
  Titrant: 0.1 N perchloric acid VS
  Blank: 50 mL of glacial acetic acid
  Endpoint detection: Potentiometric
  Analysis: Dissolve the *Sample* in 50 mL of glacial acetic acid. Titrate with 0.1 N perchloric acid VS. Perform a blank determination.
  Calculate the percentage of acesulfame potassium ($C_4H_4NO_4SK$) in the *Sample:*

$$\text{Result} = [(V - B) \times N \times F \times 100]/W$$

V = titrant volume consumed by the *Sample* (mL)
B = titrant volume consumed by the *Blank* (mL)
N = titrant actual normality (mEq/mL)
F = equivalency factor, 201.2 mg/mEq
W = weight of *Sample* (mg)

Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES

- **Limit of Fluoride**
  [Note—Use plasticware throughout this test.]
  Solution A: Dissolve 210 g of citric acid monohydrate in 400 mL of water. Adjust with concentrated ammonia to a pH of 7.0, and dilute with water to 1000 mL.
  Solution B: 132 mg/mL of dibasic ammonium phosphate
  Solution C: To a suspension of 292 g of edetic acid in 500 mL of water, add 200 mL of ammonium hydroxide, adjust with ammonium hydroxide to a pH between 6 and 7, and dilute with water to make 1000 mL.
  Buffer solution: Mix equal volumes of *Solution A, Solution B,* and *Solution C,* and adjust with ammonium hydroxide to a pH of 7.5.
  Standard stock solution: Weigh 0.442 g of sodium fluoride, previously dried at 300° for 12 h, into a 1-L volumetric flask, and dilute with water to volume. Store the solution in a closed plastic container. Immediately before use, pipet 5 mL of this solution into a 100-mL volumetric flask, and dilute with water to volume. Each mL of this solution contains 10 µg of fluoride ion.
  Standard solution A: Mix 0.5 mL of *Standard stock solution* and 15.0 mL of *Buffer solution,* and dilute with water to 50 mL.
  Standard solution B: Mix 1.0 mL of *Standard stock solution* and 15.0 mL of *Buffer solution,* and dilute with water to 50 mL.
  Standard solution C: Mix 1.5 mL of *Standard stock solution* and 15.0 mL of *Buffer solution,* and dilute with water to 50 mL.
  Standard solution D: Mix 3.0 mL of *Standard stock solution* and 15.0 mL of *Buffer solution,* and dilute with water to 50 mL.
  Sample solution: To a 50-mL volumetric flask add 3 g of Acesulfame Potassium. Dissolve in water, add 15.0 mL of *Buffer solution,* and dilute with water to volume.
  Analysis
  Samples: *Standard solution A, Standard solution B, Standard solution C, Standard solution D,* and *Sample solution*
  Concomitantly measure the potential (see *Titrimetry* ⟨541⟩), in mV, of the *Standard solutions* and the *Sample solution,* with a suitable pH meter equipped with a fluoride-specific ion electrode and a silver–silver chloride reference electrode. When taking the measurements, transfer the solution to a 25-mL beaker, and immerse the electrodes. Insert a polytef-coated stirring bar into the beaker, place the beaker on a magnetic stirrer having an insulated top, and allow to stir until equilibrium is attained (1–2 min). Rinse, and dry the electrodes between measurements, taking care not to scratch the crystal in the fluoride-specific ion electrode. Measure the potential of each *Standard solution,* and plot the fluoride concentration, in µg/mL, versus the potential, in mV, on semilogarithmic paper. Measure the potential of the *Sample solution,* and determine the fluoride concentration from the standard curve, in µg/mL.
  Calculate the content, in ppm, of fluoride in the portion of Acesulfame Potassium taken:

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

V = volume of the *Sample solution* (mL)
C = concentration of fluoride in the *Sample solution,* from the standard curve (µg/mL)
W = weight of Acesulfame Potassium taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 3 ppm

Delete the following:

- •**Heavy Metals,** *Method I* ⟨231⟩: NMT 10 ppm• (Official 1-Dec-2015)
- **Chromatographic Purity**
  Solution A: 3.3 mg/mL of tetrabutylammonium hydrogen sulfate
  Mobile phase: Acetonitrile and *Solution A* (2:3)
  System suitability solution: 2 µg/mL each of USP Acesulfame Potassium RS and ethylparaben
  Standard solution: 0.2 µg/mL of USP Acesulfame Potassium RS
  Sample solution: 10 mg/mL
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability.*)
  Mode: LC
  Detector: UV 227 nm
  Column: 4.6-mm × 25-cm; 5-µm packing L1
  Flow rate: 1 mL/min
  Injection size: 20 µL
  System suitability
  Sample: *System suitability solution*
  Suitability requirements
  Resolution: NLT 2 between acesulfame potassium and ethylparaben

Analysis
Samples: *Standard solution* and *Sample solution*
Record the chromatograms for a run time NLT 3 times the retention time of the acesulfame potassium peak, and measure the area responses of the peaks.
Acceptance criteria: The response of any peak at a retention time other than that of acesulfame potassium from the *Sample solution* does not exceed the response of the acesulfame potassium peak from the *Standard solution* (0.002%).

SPECIFIC TESTS
- **ACIDITY OR ALKALINITY**
Sample solution: 4.0 g in 20 mL of carbon dioxide-free water
Analysis: Add 0.1 mL of bromothymol blue TS. If the solution is yellow, titrate with 0.01 N sodium hydroxide to produce a blue color. If the solution is blue, titrate with 0.01 N hydrochloride acid to produce a yellow color.
Acceptance criteria: NMT 0.2 mL of 0.01 N sodium hydroxide or NMT 0.2 mL of 0.01 N hydrochloric acid is required.
- **LOSS ON DRYING** 〈731〉**:** Dry a sample at 105° for 3 h: it loses NMT 1.0% of its weight.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in a well-closed container, and protect from light. Store at room temperature.
- **USP REFERENCE STANDARDS** 〈11〉
USP Acesulfame Potassium RS

Acetic Acid

Acetic acid;
Acetic acid [64-19-7].

DEFINITION
Acetic Acid is a solution containing NLT 36.0% and NMT 37.0%, by weight, of $C_2H_4O_2$.

IDENTIFICATION
- **A. IDENTIFICATION TESTS—GENERAL,** *Acetate* 〈191〉**:** Meets the requirements

ASSAY
- **PROCEDURE**
Analysis: Place 6 mL in a tared, glass-stoppered flask, and weigh. Add 40 mL of water, then add phenolphthalein TS. Titrate with 1 N sodium hydroxide VS. Each mL of 1 N sodium hydroxide is equivalent to 60.05 mg of $C_2H_4O_2$.
Acceptance criteria: 36.0%–37.0%

IMPURITIES
- **NONVOLATILE RESIDUE**
Analysis: Evaporate 20 mL in a tared porcelain dish on a steam bath, and dry at 105° for 1 h.
Acceptance criteria: The weight of the residue does not exceed 1.0 mg (0.005%).
- **CHLORIDE**
Sample solution: Acetic acid (1 in 10) in water
Analysis: To 10 mL of the *Sample solution* add 5 drops of silver nitrate TS.
Acceptance criteria: No opalescence is produced.
- **SULFATE**
Sample solution: Acetic acid (1 in 10) in water
Analysis: To 10 mL of the *Sample solution* add 5 drops of barium chloride TS.
Acceptance criteria: No turbidity is produced.

Delete the following:

•• **HEAVY METALS** 〈231〉
Sample solution: To the residue obtained in the test for *Nonvolatile Residue* add 8 mL of 0.1 N hydrochloric acid, warm gently until completely dissolved, and dilute with water to 100 mL. Use 10 mL of this solution.
Acceptance criteria: NMT 10 ppm• (Official 1-Dec-2015)
- **READILY OXIDIZABLE SUBSTANCES**
Analysis: Dilute 4.0 mL in a glass-stoppered vessel with 20 mL of water, and add 0.30 mL of 0.10 N potassium permanganate.
Acceptance criteria: The pink color is not changed to brown at once, and the liquid does not become entirely brown or free from a pink tint in less than 30 s.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.

Diluted Acetic Acid

DEFINITION
Diluted Acetic Acid is a solution containing, in each 100 mL, NLT 5.7 g and NMT 6.3 g of acetic acid ($C_2H_4O_2$).
Prepare Diluted Acetic Acid as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* 〈795〉).

Acetic Acid	158 mL
Purified Water, a sufficient quantity to make	1000 mL

Mix the ingredients.

IDENTIFICATION
- **A. IDENTIFICATION TESTS—GENERAL,** *Acetate* 〈191〉**:** Meets the requirements

ASSAY
- **PROCEDURE**
Sample: 25 mL
Analysis: To the *Sample* add 15 mL of carbon dioxide-free water. Add phenolphthalein TS, and titrate with 1 N sodium hydroxide VS. Each mL of 1 N sodium hydroxide is equivalent to 60.05 mg of acetic acid ($C_2H_4O_2$).
Acceptance criteria: 5.7–6.3 g of acetic acid per 100 mL of Diluted Acetic Acid

IMPURITIES

Delete the following:

•• **HEAVY METALS,** *Method I* 〈231〉
Test preparation: Evaporate 5 mL in a porcelain dish on a steam bath to dryness. Warm the residue with 2 mL of 1 N acetic acid, and dilute with water to 50 mL. Dilute 20 mL of this solution with water to 25 mL.
Acceptance criteria: NMT 10 ppm• (Official 1-Dec-2015)
- **LIMIT OF CHLORIDE**
Sample solution: A solution of Diluted Acetic Acid in water (6 in 10)
Analysis: Add 5 drops of silver nitrate TS to 10 mL of the *Sample solution.*
Acceptance criteria: No opalescence is found.
- **LIMIT OF SULFATE**
Sample solution: A solution of Diluted Acetic Acid in water (6 in 10)

Analysis: Add 5 drops of barium chloride TS to 10 mL of the *Sample solution.*
Acceptance criteria: No turbidity is produced.

- **Limit of Nonvolatile Residue**
  Sample: 20 mL
  Analysis: Evaporate the *Sample* in a tared porcelain dish on a steam bath, and dry it at 105° for 1 h.
  Acceptance criteria: The weight of the residue does not exceed 1.0 mg (NMT 0.005%).

SPECIFIC TESTS

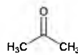
- **Readily Oxidizable Substances**
  Sample: 20 mL in a glass-stoppered flask
  Analysis: Add 0.30 mL of 0.10 N potassium permanganate to the *Sample.*
  Acceptance criteria: The pink color is not changed to brown immediately, and the liquid does not become entirely brown or free from a pink tint in less than 30 s.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.

Acetic Acid, Glacial—*see Glacial Acetic Acid General Monographs*

Acetone



C_3H_6O 58.08
2-Propanone;
Acetone [67-64-1].

DEFINITION
Acetone contains NLT 99.0% of C_3H_6O, calculated on the anhydrous basis.
[**Caution**—Acetone is very flammable. Do not use where it may be ignited.]

IDENTIFICATION

- **A. Infrared Absorption** ⟨197F⟩
- **B.** The retention time of the *Sample* corresponds to that of USP Acetone RS, as obtained in the *Assay.*

ASSAY

- **Procedure**
  Sample: Acetone
  System suitability solution: Dilute 1.0 mL of USP Methyl Alcohol RS and 1.0 mL of USP Acetone RS with tetrahydrofuran to 50 mL.
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability.*)
  Mode: GC
  Detector: Flame ionization
  Column: 0.32-mm × 30-m fused-silica capillary; 1.8-µm of phase G43
  Temperature
  Column: See *Table 1.*

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	5
40	20	240	—

Injector: 200°
Detector: 280°
Carrier gas: Helium
Flow rate: 35 cm/s (linear velocity)
Split ratio: 400:1
Injection volume: 1 µL
System suitability
Sample: *System suitability solution*
[Note—The relative retention times for methyl alcohol, acetone, and tetrahydrofuran are about 0.6, 1.0, and 1.9, respectively.]
Suitability requirements
Resolution: NLT 15 between the methyl alcohol and acetone peaks
Analysis
Sample: *Sample*
Calculate the percentage of acetone (C_3H_6O) in the portion of Acetone taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area due to the acetone peak in the *Sample*
r_T = sum of the areas of all the peaks in the *Sample*

[Note—No separate correction is applied for water content, because water does not respond to the flame-ionization detector.]
Acceptance criteria: NLT 99.0% on the anhydrous basis

SPECIFIC TESTS

- **Specific Gravity** ⟨841⟩: NMT 0.789
- **Nonvolatile Residue:** Evaporate 50 mL in a tared porcelain dish on a steam bath, and dry at 105° for 1 h.
  Acceptance criteria: The weight of the residue does not exceed 2 mg (0.004%).
- **Water**
  Sample: Acetone
  Standard solution: Transfer 0.50 mL of water to a dry 100-mL volumetric flask, dilute with dehydrated isopropyl alcohol to volume, and mix.
  Blank: Dehydrated isopropyl alcohol
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability.*)
  Mode: GC
  Detector: Thermal conductivity
  Column: 0.32-mm × 50-m capillary; 5.0-µm layer of support S2
  Temperature
  Column: See *Table 2.*

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
100	25	190	—

Injector: 250°
Detector: 250°
Carrier gas: Helium
Flow rate: 11 mL/min
Split ratio: 4.5:1
Injection size: 1.0 µL
Analysis
Samples: Acetone, *Standard solution,* and *Blank*
[Note—Identify the peaks based on their relative retention times, which are 1.0 for water and about 1.9 for isopropyl alcohol.]
Acceptance criteria: The area of the water peak for Acetone is NMT that from the *Standard solution,* corrected for the area of the water peak from the *Blank* (0.5%).

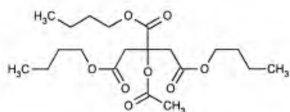
NF Monographs

- **READILY OXIDIZABLE SUBSTANCES**
  Analysis: Mix 20 mL with 0.10 mL of 0.10 N potassium permanganate in a glass-stoppered bottle.
  Acceptance criteria: The permanganate color of the mixture does not completely disappear within 15 min.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers, remote from fire.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Acetone RS
  USP Methyl Alcohol RS

Acetylcysteine—*see Acetylcysteine General Monographs*

Acetyltributyl Citrate



$C_{20}H_{34}O_8$ 402.48

DEFINITION
Acetyltributyl Citrate contains NLT 99.0% of $C_{20}H_{34}O_8$, calculated on the anhydrous basis.

IDENTIFICATION
- **A. INFRARED ABSORPTION** ⟨197F⟩
- **B.** The retention time of the *Sample solution* corresponds to that of a similar preparation of USP Acetyltributyl Citrate RS, as obtained in the *Assay*.

ASSAY
- **PROCEDURE**
  System suitability solution: 30 mg/mL each of USP Acetyltributyl Citrate RS and USP Tributyl Citrate RS in toluene
  Sample solution: 30 mg/mL of Acetyltributyl Citrate in toluene
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability*.)
  Mode: GC
  Detector: Flame ionization
  Column: 0.32-mm × 30-m, bonded with a 0.5-µm layer of phase G42
  Temperature
  Injector: 240°
  Detector: 280°
  Column: See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
80	—	80	0
80	20	230	15

  Flow rate: 1.9 mL/min
  Carrier gas: Helium
  Injection type: Split, 30:1
  Injection size: 1 µL
  System suitability
  Sample: *System suitability solution*
  [NOTE—The relative retention times for tributyl citrate and acetyl tributyl citrate are 0.9 and 1.0, respectively.]
  Suitability requirements
  Resolution: NLT 1.5 between tributyl citrate and acetyl tributyl citrate
  Relative standard deviation: NMT 2.0% determined from both the tributyl citrate and acetyl tributyl citrate peaks, based on area percent calculation
  Analysis
  Sample: *Sample solution*
  [NOTE—Measure all of the peak areas, excluding the solvent peak.]
  Calculate the percentage of $C_{20}H_{34}O_8$ in the portion of Acetyltributyl Citrate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of the *Sample solution*
r_T = sum of all the peak areas
  Acceptance criteria: NLT 99.0% on the anhydrous basis

IMPURITIES
Inorganic Impurities

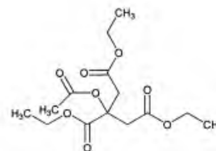
Delete the following:

- •**HEAVY METALS,** *Method II* ⟨231⟩: NMT 10 ppm• (Official 1-Dec-2015)

SPECIFIC TESTS
- **SPECIFIC GRAVITY** ⟨841⟩: 1.045–1.055
- **REFRACTIVE INDEX** ⟨831⟩: 1.4410–1.4425
- **ACIDITY**
  Neutralized isopropyl alcohol: To a suitable quantity of isopropyl alcohol add 2–3 drops of bromothymol blue TS and just sufficient 0.10 N sodium hydroxide dropwise to produce a faint blue color. [NOTE—Prepare *Neutralized isopropyl alcohol* just before use.]
  Sample solution: 32.0 g of Acetyltributyl Citrate in 30 mL of *Neutralized isopropyl alcohol*
  Analysis: Add bromothymol blue TS. Titrate with 0.10 N sodium hydroxide to a faint blue endpoint.
  Acceptance criteria: NMT 1.0 mL of 0.10 N sodium hydroxide is required.
- **WATER DETERMINATION,** *Method I* ⟨921⟩: NMT 0.25%

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Acetyltributyl Citrate RS
  USP Tributyl Citrate RS

Acetyltriethyl Citrate



$C_{14}H_{22}O_8$ 318.32

DEFINITION

Acetyltriethyl Citrate contains NLT 99.0% of acetyltriethyl citrate ($C_{14}H_{22}O_8$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** ⟨197F⟩
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *System suitability solution*, as obtained in the *Assay*.

ASSAY

- **Procedure**

System suitability solution: 30 mg/mL each of USP Acetyltriethyl Citrate RS and USP Triethyl Citrate RS in toluene

Sample solution: 30 mg/mL in toluene

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC, equipped with an on-column, temperature-programmable injector

Detector: Flame ionization

Column: 0.32-mm × 30-m, bonded with a 0.5-µm layer of phase G42

Temperatures

Injector: See *Table 1*.

Detector: 275°

Column: See *Table 2*.

Table 1

Start Temperature (°)	Ramp (°)	End Temperature (°)	Hold Time (min)
85	—	85	0.5
85	20	225	10

Table 2

Start Temperature (°)	Ramp (°)	End Temperature (°)	Hold Time (min)
80	—	80	0.5
80	20	220	10

Flow rate: 2.3 mL/min

Carrier gas: Helium

Injection volume: 1 µL

System suitability

Sample: *System suitability solution*

[Note—The relative retention times of triethyl citrate and acetyltriethyl citrate are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between triethyl citrate and acetyltriethyl citrate

Relative standard deviation: NMT 2.0% determined from both the triethyl citrate and acetyltriethyl citrate peaks

Analysis

Sample: *Sample solution*

Calculate the percentage of acetyltriethyl citrate ($C_{14}H_{22}O_8$) in the portion of sample taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of acetyltriethyl citrate from the *Sample solution*

r_T = sum of all the peaks excluding the solvent peak

Acceptance criteria: NLT 99.0% on the anhydrous basis

IMPURITIES

Delete the following:

•• **Heavy Metals,** *Method II* ⟨231⟩: NMT 10 µg/g• (Official 1-Dec-2015)

SPECIFIC TESTS

- **Specific Gravity** ⟨841⟩: 1.135–1.139
- **Refractive Index** ⟨831⟩: 1.432–1.441
- **Acidity**

Sample: 32.0 g

Analysis: Dissolve the *Sample* in 30 mL of isopropyl alcohol, previously neutralized to bromothymol blue. Add bromothymol blue TS, and titrate with 0.10 N sodium hydroxide to a faint blue endpoint.

Acceptance criteria: NMT 1.0 mL of 0.10 N sodium hydroxide is required.

- **Water Determination,** *Method I* ⟨921⟩: NMT 0.3%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers.
- **USP Reference Standards** ⟨11⟩

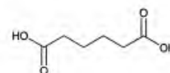
USP Acetyltriethyl Citrate RS

USP Triethyl Citrate RS

Activated Charcoal—*see Activated Charcoal General Monographs*

see Activated Charcoal General Monographs

Adipic Acid



$C_6H_{10}O_4$ 146.14

Hexanedioic acid;

1,4-Butanedicarboxylic acid [124-04-9].

DEFINITION

Adipic Acid contains NLT 99.0% and NMT 101.0% of $C_6H_{10}O_4$, calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** ⟨197K⟩

ASSAY

- **Procedure**

Sample: 60 mg

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 N sodium hydroxide VS

Blank: 50.0 mL of water

Endpoint detection: Colorimetric

Analysis: Dissolve the *Sample* in 50 mL of water. Add 0.2 mL of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS to a permanent pale pink endpoint. Perform a blank determination. Calculate the percentage of adipic acid ($C_6H_{10}O_4$) in the *Sample* taken:

$$\text{Result} = [(V - B) \times N \times F \times 100]/W$$

V = titrant volume consumed by the *Sample* (mL)

B = titrant volume consumed by the *Blank* (mL)

N = titrant actual normality (mEq/mL)

F = equivalency factor, 73.1 mg/mEq

W = weight of the *Sample* (mg)

Acceptance criteria: 99.0%–101.0% on the dried basis

IMPURITIES
- **Residue on Ignition** ⟨281⟩: NMT 0.1%

Change to read:

- **Limit of Nitrates**
  - **Standard stock solution:** 1.63 mg/mL of potassium nitrate
  - **Standard solution:** Dilute 1 mL of the *Standard stock solution* with water to 10 mL. Dilute 1 mL of this solution with water to 50 mL to obtain a solution containing 2 µg/mL of nitrate.
  - **Sample solution:** Transfer 5 g of Adipic Acid to a 50-mL volumetric flask. Dissolve in water, with heating, and dilute with water to volume. Allow to cool and crystallize, then pass through a sintered-glass filter. Wash the filter with water, and collect the filtrate and washings until a volume of 50 mL is obtained. [Note—This solution is also to be used for *Chloride, Sulfate,* and *Iron.* ●● (Official 1-Dec-2015)]
  - **Control:** 2 mg/L of potassium permanganate
  - **Analysis:** Transfer 1.0 mL of the *Sample solution,* 1.5 mL of the *Standard solution,* and 1 mL of water (blank) to three separate flasks. To each flask add 2 mL of concentrated ammonia, 0.5 mL of 10 mg/mL manganese sulfate, and 1 mL of 10 mg/mL sulfanilamide, and dilute each solution with water to 20 mL. Add 100 mg of zinc powder to each of the three flasks, and cool in an ice bath for 30 min, shaking the solutions periodically. Separately filter 10 mL of each solution, cool in an ice bath, and then add 2.5 mL of hydrochloric acid and 1 mL of 10 mg/mL of naphthylethylenediamine dihydrochloride. Allow the solutions to stand at room temperature for 15 min.
  - **System suitability:** The test is invalid if the concomitantly prepared blank solution is darker than the *Control.*
  - **Acceptance criteria:** The color of the solution containing the *Sample solution* is not darker than the concomitantly prepared solution containing the *Standard solution* (NMT 30 ppm).

Delete the following:

- ●**Heavy Metals,** *Method I* ⟨231⟩: NMT 10 ppm● (Official 1-Dec-2015)
- **Chloride and Sulfate,** *Chloride* ⟨221⟩
  - **Sample:** A 5-mL portion of the *Sample solution* from *Limit of Nitrates*
  - **Analysis:** Proceed as directed in the chapter.
  - **Acceptance criteria:** The *Sample* shows no more chloride than a corresponding 0.14-mL portion of 0.020 N hydrochloric acid (NMT 0.02%).
- **Chloride and Sulfate,** *Sulfate* ⟨221⟩
  - **Sample:** A 5-mL portion of the *Sample solution* from *Limit of Nitrates*
  - **Analysis:** Proceed as directed in the chapter.
  - **Acceptance criteria:** The *Sample* shows no more sulfate than a corresponding 0.26-mL portion of 0.020 N sulfuric acid (NMT 0.05%).
- **Iron** ⟨241⟩
  - **Sample:** A 10-mL portion of the *Sample solution* from *Limit of Nitrates*
  - **Analysis:** Proceed as directed in the chapter.
  - **Acceptance criteria:** NMT 10 ppm

SPECIFIC TESTS
- **Melting Range or Temperature** ⟨741⟩: 151°–154°
- **Loss on Drying** ⟨731⟩: Dry a sample at 105° to constant weight; it loses NMT 0.2% of its weight.

ADDITIONAL REQUIREMENTS
- **Packaging and Storage:** Preserve in a tight containers. No storage requirements specified.
- **USP Reference Standards** ⟨11⟩
  USP Adipic Acid RS

Agar

[9002-18-0].

DEFINITION

Agar is the dried, hydrophilic, colloidal substance consisting of the polysaccharides extracted from *Gelidium cartilagineum* (Linné) Gaillon (Fam. Gelidiaceae), *Gracilaria confervoides* (Linné) Greville (Fam. Sphaerococcaceae), and related red algae (Class Rhodophyceae).

IDENTIFICATION
- **A. Infrared Absorption** ⟨197K⟩
- **B.** Iodine TS colors some of the fragments of Agar bluish black, with some areas reddish to violet.
- **C.**
  - **Analysis:** Boil a sample with 65 times its weight of water for 10 min, with constant stirring, and subsequently adjust with hot water to a concentration of 1.5%, by weight.
  - **Acceptance criteria:** Agar forms a clear liquid that congeals at 30°–39° to form a firm resilient gel, which does not liquefy below 80°.

IMPURITIES

Inorganic Impurities
- **Arsenic,** *Method II* ⟨211⟩: NMT 3 ppm
- **Lead** ⟨251⟩: NMT 10 ppm

Delete the following:

- ●**Heavy Metals,** *Method II* ⟨231⟩: NMT 40 ppm● (Official 1-Dec-2015)
- **Articles of Botanical Origin,** *Acid-Insoluble Ash* ⟨561⟩: NMT 0.5%, on a dry-weight basis

Organic Impurities
- **Procedure 1: Limit of Gelatin**
  - **Sample solution:** Dissolve 1 g of sample in 100 mL of boiling water. Allow to cool to about 50°.
  - **Analysis:** To 5 mL of the *Sample solution* add 2–3 drops of a mixture of 0.2 M potassium dichromate solution and 3 N hydrochloric acid (4:1).
  - **Acceptance criteria:** No yellow precipitate is formed.
- **Procedure 2: Limit of Foreign Starch**
  - **Sample solution:** Boil 0.10 g in 100 mL of water.
  - **Acceptance criteria:** The *Sample solution* does not, upon cooling, produce a blue color upon the addition of iodine TS.
- **Procedure 3: Limit of Foreign Insoluble Matter**
  - **Sample dispersion:** Add sufficient water to 7.5 g of sample to make 500 g, boil for 15 min, and readjust to the original 500 g.
  - **Analysis:** To 100 g of the uniformly mixed *Sample dispersion* add hot water to make 200 mL. Heat almost to boiling, filter while hot through a tared filtering crucible. Rinse the container with several portions of hot water, and pass these rinsings through the crucible. Dry the crucible and its contents at 105° to a constant weight.
  - **Acceptance criteria:** NMT 15 mg (1.0%) remains in the crucible.
- **Procedure 4: Articles of Botanical Origin,** *Foreign Organic Matter* ⟨561⟩: NMT 1.0%

SPECIFIC TESTS

- **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩: The total aerobic microbial count does not exceed 10^3 cfu/g, and the total combined molds and yeasts count does not exceed 10^2 cfu/g. It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.
- **Water Determination,** *Method III* ⟨921⟩
  Analysis: If necessary, cut a sample into pieces from a 2- to 5-mm square, and dry at 105° for 5 h.
  Acceptance criteria: The sample loses NMT 20.0% of its weight.
- **Articles of Botanical Origin,** *Total Ash* ⟨561⟩: NMT 6.5%, on a dry-weight basis
- **Water Absorption**
  Sample: 5.0 g
  Analysis: Place the *Sample* in a 100-mL graduated cylinder, fill to the mark with water, mix, and allow to stand at 25° for 24 h. Pour the contents of the cylinder through moistened glass wool, allowing the water to drain into a second 100-mL graduated cylinder.
  Acceptance criteria: NMT 75 mL of water is obtained.
- **Botanic Characteristics**
  Agar: Usually occurs in bundles consisting of thin, membranous, agglutinated strips or in cut, flaked, or granulated forms. It may be colored weak yellowish orange, yellowish gray to pale yellow, or colorless. It is tough when damp, brittle when dry.
  Histology: When mounted in water, Agar appears granular and somewhat filamentous; a few fragments of the spicules of sponges and a few frustules of diatoms may be present. In Japanese Agar, the frustules of *Arachnoidiscus ehrenbergii* Baillon often occur, being disk-shaped and 100–300 µm in diameter.
  Powdered agar: White to yellowish white or pale yellow; in chloral hydrate TS, its fragments are transparent, more or less granular, striated, and angular, and occasionally they contain frustules of diatoms.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers. No storage requirements are specified.
- **USP Reference Standards** ⟨11⟩
  USP Agar RS

Albumin Human—*see Albumin Human General Monographs*

rAlbumin Human

```
DAHKSEVAHR FKDLGEENFK ALVLIAFAQY LQQCPFEDHV KLVNEVTEFA
KTCVADESAE NCDKSLHTLF GDKLCTVATL RETYGEMADC CAKQEPERNE
CFLQHKDDNP NLPRLVRPEV DVMCTAFHDN EETFLKKYLY EIARRHPYFY
APELLFFAKR YKAAFTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC
ASLQKFGERA FKAWAVARLS QRFPKAEFAE VSKLVTDLTK VHTECCHGDL
LECADDRADL AKYICENQDS ISSKLKECCE KPLLEKSHCI AEVENDEMPA
DLPSLAADFV ESKDVCKNYA EAKDVFLGMF LYEYARRHPD YSVVLLLRLA
KTYETTLEKC CAAADPHECY AKVFDEFKPL VEEPQNLIKQ NCELFEQLGE
YKFQNALLVR YTKKVPQVST PTLVEVSRNL GKVGSKCCKH PEAKRMPCAE
DYLSVVLNQL CVLHEKTPVS DRVTKCCTES LVNRRPCFSA LEVDETYVPK
EFNAETFTFH ADICTLSEKE RQIKKQTALV ELVKHKPKAT KEQLKAVMDD
FAAFVEKCCK ADDKETCFAE EGKKLVAASQ AALGL
```

$C_{2936}H_{4624}N_{786}O_{889}S_{41}$ 66,438 Da

DEFINITION

Recombinant Albumin Human (rAlbumin Human or rHA) is produced by recombinant DNA expression in *Saccharomyces cerevisiae*. Structural equivalence (primary, secondary, and tertiary) between rHA and human serum albumin (HSA) has been demonstrated. It consists of three domains composed of 585 amino acids containing a single tryptophan (Trp_{214}), one free thiol (Cys_{34}), and 17 disulfide bridges. It is presented as a sterile and nonpyrogenic aqueous liquid consisting of a 10% (w/v) or 20% (w/v) solution in Water for Injection. No human- or animal-derived raw material is involved in its manufacture. It contains NLT 95% and NMT 105% of the labeled amount and NLT 99% of its total protein is albumin. It contains no added antimicrobial agents, but it may contain appropriate stabilizing agents. The presence of process-related impurities, host cell DNA, and host cell proteins is process specific; suitable limits should be determined by appropriately validated methods. However, the limit for host cell proteins should be NMT 0.15 µg/g.

IDENTIFICATION

- **A. Peptide Mapping**
  [Note—See *Biotechnology-Derived Articles—Peptide Mapping* ⟨1055⟩ for guidance.]
  Tris buffer: 0.1 M tris(hydroxymethyl)aminomethane. Adjust with hydrochloric acid to a pH of 8.0.
  Dilute Tris buffer: *Tris buffer* and water (50:50)
  Solution A: Trifluoroacetic acid and water (1:1000)
  Solution B: To 350 mL of acetonitrile add 150 mL of water and 425 µL of trifluoroacetic acid.
  Dithiothreitol solution: 0.1 M dithiothreitol
  Iodoacetamide solution: 0.1 M iodoacetamide in *Tris buffer*
  Trypsin solution: 1 mg/mL of trypsin in 10 mM hydrochloric acid
  EDTA solution: 0.1 M ethylenediaminetetraacetic acid (EDTA) in water
  Diluent: To 5.76 g of guanidine hydrochloride add 5 mL of *Dilute Tris buffer* and 200 µL of *EDTA solution*. Dilute with *Dilute Tris buffer* to a final volume of 10 mL.
  Standard solution: Add 20 µL of USP rAlbumin Human RS to 80 µL of *Diluent*. Add 5 µL of *Dithiothreitol solution*, and incubate at 37° for 75 min. Add 10 µL of *Iodoacetamide solution*, and incubate for an additional 75 min at 37° in the dark. Add 100 µL of *Dilute Tris buffer*, 400 µL of water, and 10 µL of *Trypsin solution*, and incubate at 37° with shaking for 24 h. Centrifuge, and dilute a portion of the supernatant in *Solution A* (50:50).
  Sample solution: 50 mg/mL of rAlbumin Human in water. To 20 µL of this solution add 80 µL of *Diluent*. Add 5 µL of *Dithiothreitol solution*, and incubate at 37° for 75 min. Add 10 µL of *Iodoacetamide solution*, and incubate for an additional 75 min at 37° in the dark. Add 100 µL of *Dilute Tris buffer*, 400 µL of water, and 10 µL of *Trypsin solution*, and incubate at 37° with shaking for 24 h. Pulse centrifuge, and dilute a portion of the supernatant in *Solution A* (50:50).
  Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)	Flow Rate (mL/min)
0	95	5	0.5
5	95	5	0.5
75	60	40	0.5
100	40	60	0.5
104	0	100	0.5
108	0	100	0.5
109	95	5	1.0
115	95	5	1.0
116	95	5	0.5
120	95	5	0.5

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 25-cm; 5-µm packing L1
Column temperature: 35°
Flow rate: See *Table 1*.
Injection volume: 100 µL
Analysis
Samples: *Standard solution* and *Sample solution*
Acceptance criteria: The peptide map chromatographic profiles of the *Sample solution* are similar to those of the *Standard solution*.

• **B. Electrospray Mass Spectrometry**
Solution A: Trifluoroacetic acid and water (1:1000)
Solution B: To 140 mL of acetonitrile add 60 mL of water and 180 µL of trifluoroacetic acid.
Solution C: Acetonitrile and water (50:50)
Solution D: To 5 mL of *Solution C* add 10 µL of formic acid.
System suitability solution: Dissolve 2 mg of horse heart myoglobin in 589 µL of Water for Injection. Dilute 25 µL of this solution with 475 µL of *Solution D*.
Sample solution: 10 mg/mL of rAlbumin Human in water
Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	95	5
5	95	5
10	0	100
15	0	100

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 2.1-mm × 3-cm; desalting cartridge, equilibrated with *Solution C*[1]
Flow rate: 0.2 mL/min
Injection volume: 20 µL of the *Sample solution*
Analysis: Desalt the *Sample solution*, and collect the eluate. Ensure that a single protein peak elutes.
Spectrometric system
(See *Mass Spectrometry* ⟨736⟩.)
Mode: LC/MS (using infusion pump)
[Note—The infusion system flow rate can be adjusted as needed. To assist in nebulization, the infusion system can contain a sheathing gas fluid.]
Mobile phase: *Solution C*
Detector: Electrospray in the positive ion mode
Injection volume: 50 µL of desalted *Sample solution*
System suitability
Sample: *System suitability solution*
Suitability requirements
Peak position: A single peak in the 16,949–16,953 Da range is found.
Analysis: Obtain and transform the spectrogram for the desalted *Sample solution*.
Acceptance criteria: The mass is within 20 Da of the theoretical mass.

ASSAY

• **Albumin Content**
Stock sample buffer:[2] Mix 4 mL of 0.5 M Tris hydrochloride pH 8.6, 0.5 mL of 0.1% bromophenol blue, 2.0 mL of glycerol, and dilute with water to 1000 mL.
Diluted sample buffer: *Stock sample buffer* and water (1:1)
Native stock running buffer:[3] 29 mg/mL of Tris base and 144 mg/mL of glycerol
Running buffer: *Native stock running buffer* and water (1:9)
Gel-staining solution: A suitable Coomassie G-250-based solution[4]
Native PAGE gel: Prepare a 14% Tris-Glycine gel.[5]
Sample solution: 4 mg/mL of rAlbumin Human in water. Dilute this solution with *Stock sample buffer* to 2 mg/mL.
Calibration solutions: Dilute the *Sample solution* quantitatively, and stepwise if necessary, with *Diluted sample buffer* to 100, 20, 15, 10, 5, 2, and 1 µg/mL of rAlbumin Human.
Electrophoretic system
(See *Electrophoresis* ⟨726⟩.)
Run buffer: *Running buffer*
Voltage: 125 V
Amperage: 35 mA
Wattage: 5.0 W
Run time: Approximately 2 h
Loading volume: 10 µL
Analysis
Samples: *Sample solution* and *Calibration solutions*
Gel loading scheme
Lane 1: 1 µg/mL *Calibration solution*
Lane 2: 2 µg/mL *Calibration solution*
Lane 3: 5 µg/mL *Calibration solution*
Lane 4: 10 µg/mL *Calibration solution*
Lane 5: 15 µg/mL *Calibration solution*
Lane 6: 20 µg/mL *Calibration solution*
Lane 7: *Diluted sample buffer*
Lane 8: *Sample solution*
Lane 9: *Sample solution*
Lane 10: *Diluted sample buffer*
Gel staining: Place the gel in 100 mL of water, and shake gently with circumgyration for about 30 min. Pour approximately 50 mL of *Gel-staining solution* into a staining container. Place the gel into the staining container, and allow the stain to completely cover the gel. Place the staining container on an orbital shaker, and stain the gel for 120 min with gentle shaking.
Destaining: Drain the *Gel-staining solution*, and add 100 mL of water to the container to cover the gel. Place the container on an orbital shaker, and shake at low speed for about 60 min. Change the water, and repeat for a total of two washes.
Gel scan procedure: Set up a gel scanner according to the manufacturer's instructions. Place the gel in the detector, and obtain a single image of all 10 lanes of the gel.
Data analysis: Perform image analysis of *Lanes 1–6* to generate a linear calibration curve. Determine the linear regression equation of the standards by the least-squares method, with standard concentrations, in ng, as the dependent variable (x), and the sample band intensity (optical density) as the independent variable (y). Record the linear regression equation and the correlation coefficient, r. A suitable system is one that yields a line having an r^2 of NLT 0.990.
Examine *Lanes 8* and *9* (the *Sample solution* lanes) for the presence of bands below the main albumin band. If bands are present below the main albumin band in either or both lanes, quantify the relative amount, in ng, of protein present in each band against the calibration curve. Convert the quantified value to a contaminant level in percentage by dividing the quantified value by a factor of 200.

[1] A suitable reverse-phase desalting column is available from Perkin Elmer (No. 0711-0056).
[2] Available from Invitrogen (No. LC2673).
[3] Available from Invitrogen (No. LC2672).
[4] A suitable Coomassie stain is available from Pierce (No. 24890 or No. 24592).
[5] Alternatively, a precast 14% Tris-Glycine gel is available from Invitrogen (No. EC6485).

NF Monographs

Calculate the purity of the *Sample solution*:

$$\text{Result} = 100 - C_I$$

C_I = mean of the percentages of contaminant levels found in *Lanes 8* and *9* (all the bands other than the albumin band), disregarding any band due to the *Diluted sample buffer*

Acceptance criteria: *Sample solution* purity is NLT 99.0%. [NOTE—The main albumin band is not quantitated. See the test for *Total Protein*.]

- **TOTAL PROTEIN**

Sodium chloride solution: 0.15 M sodium chloride in water

Copper sulfate solution: 60 mg/mL of copper sulfate pentahydrate and 600 mg/mL of potassium sulfate[6] in sulfuric acid low in nitrogen

Sample solution: Dilute 0.5 g of rAlbumin Human with 2.5 mL of *Sodium chloride solution* (equivalent to about 3.3 mg/mL of total protein).

Blank: 33.3 mg/mL of glycine in *Sodium chloride solution*

Analysis: To 3.0 mL of the *Sample solution* and the *Blank*, in suitable distillation tubes, add 5 mL of *Copper sulfate solution*. Incubate at 420° for a minimum of 2 h, or until the residues appear white. When the solutions are cool, transfer the residues quantitatively with a minimum quantity of water to a micro-Kjeldahl flask, and determine the residues, using *Nitrogen Determination* ⟨461⟩, *Method II*. Multiply the result, corrected for the *Blank* and for the specific gravity of the *Sample solution*, by 6.25 to calculate the quantity of protein.

Acceptance criteria: 95%–105% of the quantity of protein stated on the label

OTHER COMPONENTS

- **SODIUM CONTENT**

Diluent: 1.0 mg/mL of cesium chloride in water

Standard solutions: Prepare 0.5, 1.00, 1.50, and 2.00 mg/mL of sodium chloride in *Diluent*.

Sample solution: 80 µg/mL of rAlbumin Human in *Diluent*

Apparatus

Mode: Atomic absorption

Emission wavelength: 589 nm

Analysis: [NOTE—Use peak area measurements for quantitation.]

Samples: *Diluent* (as blank), *Standard solutions*, and *Sample solution*

Introduce a blank solution (*Diluent*) into the atomic generator, and adjust the instrument reading to zero. Determinations are made by comparison with the *Standard solutions* of known concentration. If the *Sample solution* emission exceeds that of the *Standard solutions* with the highest concentration, dilute the *Sample solution* with *Diluent*. Introduce the most concentrated *Standard solution* into the instrument, and adjust the sensitivity to obtain a suitable reading. Introduce the *Sample solution* and *Standard solutions* into the instrument at least three times, and record the steady reading. Rinse the apparatus with blank solution each time, and ascertain that the reading returns to its initial blank value. Plot the mean of the readings obtained for the *Standard solutions* against their respective sodium concentrations. From the standard curve, calculate the sodium concentration content in the *Sample solution*, and adjust for the specific gravity of the rAlbumin Human (see *Total Protein*).

Acceptance criteria: 120–160 mM sodium

IMPURITIES

- **LIMIT OF HIGH MOLECULAR WEIGHT PROTEINS**

Solution A: 200 mg/mL of sodium azide

Buffer: Dissolve 54.2 g of dibasic sodium phosphate dihydrate, 30.0 g of monobasic sodium phosphate dihydrate, and 284.0 g of anhydrous sodium sulfate in 1600 mL of water. Add 50 mL of *Solution A*, and dilute with water to 2000 mL.

Mobile phase: *Buffer* and water (10:90)

Sample solution: 40 mg/mL of rAlbumin Human

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 7.8-mm × 30-cm; 5-µm packing L59

Flow rate: 1.0 mL/min

Injection volume: 50 µL. [NOTE—The peak due to high molecular weight impurities, such as the polymer of albumin, appears in the void volume of the chromatogram.]

Analysis

Sample: *Sample solution*

Calculate the percentage of albumin polymer in the sample:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of albumin polymer

r_T = sum of all rAlbumin Human related peak responses

Acceptance criteria

Individual impurities: NMT 1.0%

SPECIFIC TESTS

- **pH** ⟨791⟩

Sample solution: 1% (w/v) protein solution diluted with 0.9% (w/v) sodium chloride

Acceptance criteria: 6.4–7.4

- **STERILITY TESTS** ⟨71⟩**:** Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 0.5 USP Endotoxin Unit/mL of rAlbumin Human

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight glass containers, and store at 2°–8°. Do not freeze.
- **LABELING:** Label to indicate that the material is of recombinant DNA origin.
- **USP REFERENCE STANDARDS** ⟨11⟩

USP rAlbumin Human RS

USP Endotoxin RS

Alcohol—*see Alcohol General Monographs*

Diluted Alcohol

DEFINITION

Diluted Alcohol is a mixture of Alcohol and water containing NLT 41.0% and NMT 42.0% by weight, corresponding to NLT 48.4% and NMT 49.5% by volume, at 15.56°, of C_2H_5OH.

Diluted Alcohol may be prepared as follows.

Alcohol	500 mL
Purified Water	500 mL

Measure the *Alcohol* and the *Purified Water* separately at the same temperature, and mix. If the water and the Alcohol

[6] Copper sulfate pentahydrate and potassium sulfate tablets (each tablet with 1.5 g of K_2SO_4 + 0.15 g of $CuSO_4 \cdot 5H_2O$) are available from Foss (No. 15270054).

and the resulting mixture are measured at 25°, the volume of the mixture will be 970 mL.

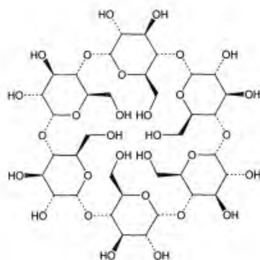
SPECIFIC TESTS

- **SPECIFIC GRAVITY** ⟨841⟩: 0.935–0.937 at 15.56°, indicating 41.0%–42.0% by weight, or between 48.4% and 49.5% by volume, of C_2H_5OH
- **OTHER REQUIREMENTS:** In other respects, it meets the requirements in *Alcohol,* allowance being made for the difference in alcohol concentration.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, remote from fire.

Alfadex



$(C_6H_{10}O_5)_6$ 972.84

Alpha cyclodextrin [10016-20-3].

DEFINITION

Alfadex is composed of six alpha-(1-4) linked D-glucopyranosyl units. It contains NLT 98.0% and NMT 101.0% of alfadex $(C_6H_{10}O_5)_6$, calculated on the anhydrous basis.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution,* as obtained in the *Assay.*
- **B.** It meets the requirements of the test for *Optical Rotation* ⟨781S⟩, *Specific Rotation.*
- **C.**
  Sample: 0.2 g
  Analysis: Mix the *Sample* with 2 mL of iodine TS, warm in a water bath to dissolve, and allow to stand at room temperature.
  Acceptance criteria: A yellow-brown precipitate is formed.

ASSAY

- **PROCEDURE**
  Mobile phase: Methanol and water (7:93)
  System suitability solution: 0.5 mg/mL of USP Alpha Cyclodextrin RS, 0.5 mg/mL of USP Beta Cyclodextrin RS, and 0.5 mg/mL of USP Gamma Cyclodextrin RS
  Standard solution: 1.0 mg/mL of USP Alpha Cyclodextrin RS
  Sample stock solution: Transfer 250 mg of Alfadex to a 25-mL volumetric flask, and dissolve in water with the aid of heat. Cool, and dilute with water to volume.
  Sample solution: 1.0 mg/mL of Alfadex, diluted from the *Sample stock solution*
  Chromatographic system
  (See *Chromatography* ⟨621⟩, *System Suitability.*)
  Mode: LC
  Detector: Refractive index
  Column: 4.6-mm × 15-cm; 5-µm packing L1
  Temperature
  Column: 30°
  Detector: 40°
  Flow rate: 1.5 mL/min
  Injection volume: 50 µL
  System suitability
  Sample: *System suitability solution*
  [NOTE—The relative retention times for gamma cyclodextrin, alpha cyclodextrin, and beta cyclodextrin are 0.8, 1.0, and 2.0, respectively.]
  Suitability requirements
  Resolution: NLT 1.5 between the gamma cyclodextrin and alpha cyclodextrin peaks
  Tailing factor: 0.8–2.0 for the three cyclodextrins
  Relative standard deviation: NMT 2.0%
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of alfadex $(C_6H_{10}O_5)_6$ in the portion of the sample taken:

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

r_U = peak response of alpha cyclodextrin from the *Sample solution*
r_S = peak response of alpha cyclodextrin from the *Standard solution*
C_S = concentration of alpha cyclodextrin in the *Standard solution* (mg/mL)
C_U = concentration of alpha cyclodextrin in the *Sample solution* (mg/mL)

  Acceptance criteria: 98.0%–101.0% on the anhydrous basis

IMPURITIES

- **RESIDUE ON IGNITION** ⟨281⟩
  Sample: 1.0 g
  Acceptance criteria: NMT 0.1%

Delete the following:

- •**HEAVY METALS,** *Method II* ⟨231⟩: NMT 10 µg/g• (Official 1-Dec-2015)
- **LIMIT OF LIGHT-ABSORBING SUBSTANCES**
  Sample solution: 10 mg/mL of Alfadex, calculated on the anhydrous basis, in water that has been previously boiled and cooled to room temperature. Pass through a filter of 0.2-µm pore size.
  Analysis: Determine the absorbance of the *Sample solution* in a 1-cm cell with a suitable spectrophotometer, after correcting for the blank.
  Acceptance criteria: Between 230 and 350 nm, the absorbance is NMT 0.10; and between 350 and 750 nm, the absorbance is NMT 0.05.
- **LIMIT OF BETADEX, GAMMA CYCLODEXTRIN, AND OTHER RELATED SUBSTANCES**
  System suitability solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay.*
  Standard solution: *System suitability solution* and water (1:9)
  Sample solution: Use the *Sample stock solution* prepared as directed in the *Assay.*
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Acceptance criteria
  Beta cyclodextrin: The area of any peak corresponding to beta cyclodextrin is NMT 0.5 times the area of the corresponding peak from the *Standard solution* (0.25%).