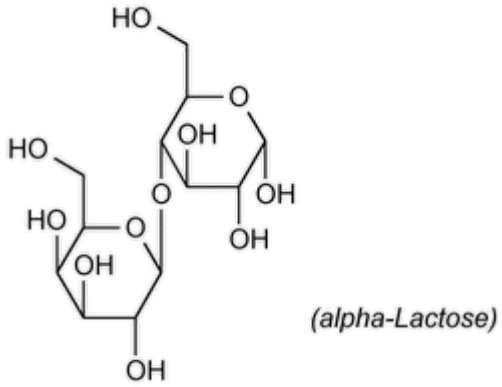
Stage 4 Harmonization
Official: December 1, 2024

Anhydrous Lactose

Portions of the monograph text that are national *USP* text, and are not part of the harmonized text, are marked with symbols (♦) to specify this fact.



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DEFINITION

Anhydrous Lactose is *O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranose (β-lactose), or a mixture of *O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranose and *O*-β-D-galactopyranosyl-(1→4)-α-D-glucopyranose (α-lactose).

IDENTIFICATION

- **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197K
- ♦**B. Thin-Layer Chromatographic Identification Test ⟨201⟩**

  Adsorbent: 0.25-mm layer of chromatographic silica gel

  Diluent: Methanol and water (3:2)

  Standard solution A: 0.5 mg/mL of USP Anhydrous Lactose RS in *Diluent*

  Standard solution B: Contains 0.5 mg/mL of USP Dextrose RS, 0.5 mg/mL of USP Anhydrous Lactose RS, 0.5 mg/mL of USP Fructose RS, and 0.5 mg/mL of USP Sucrose RS in *Diluent*

  Sample solution: 0.5 mg/mL of Anhydrous Lactose in *Diluent*

  Application volume: 2 µL

  Developing solvent system: Ethylene dichloride, glacial acetic acid, methanol, and water (10:5:3:2)

  Spray reagent: 5 mg/mL of thymol in a mixture of alcohol and sulfuric acid (19:1)

  Analysis

  Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

  Allow the spots to dry, and develop the plate in a paper-lined chromatographic chamber equilibrated with the *Developing solvent system* for about 1 h prior to use. Allow the chromatogram to develop until the solvent front has moved about three-quarters of the length of the plate. Remove the plate from the chamber, dry in a current of warm air, and redevelop the plate in fresh *Developing solvent system*. Remove the plate from the chamber, mark the solvent front, and dry the plate in a current of warm air. Spray the plate evenly with *Spray reagent*. Heat the plate at 130° for 10 min.

System suitability: The test is not valid unless *Standard solution B* shows four clearly discernible spots, disregarding any spots at the origin.

Acceptance criteria: The principal spot from the *Sample solution* corresponds in appearance and R_F value to that from *Standard solution A*.

OTHER COMPONENTS

- **Content of Alpha and Beta Anomers**

Silylation reagent: Dimethyl sulfoxide, pyridine, and trimethylsilylimidazole (19.5: 58.5: 22)

Standard solution: Prepare a mixture of alpha-lactose monohydrate and beta-lactose having an anomeric ratio of about 1:1 based on the labeled anomeric contents of the alpha-lactose monohydrate and the beta-lactose. Introduce 10 mg of this mixture into a vial with a screw cap. Add 4 mL of *Silylation reagent*. Sonicate for 20 min at room temperature. Transfer 400 µL to an injection vial. Add 1 mL of pyridine. Close the vial, and mix well.

Sample solution: Introduce 10 mg of Anhydrous Lactose into a vial with a screw cap. Add 4 mL of *Silylation reagent*. Sonicate for 20 min at room temperature. Transfer 400 µL to an injection vial. Add 1 mL of pyridine. Close the vial, and mix well.

Chromatographic system

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

Mode: GC

Detector: Flame ionization

Columns

Precolumn:[1] 0.53-mm × 2-m intermediate polarity deactivated fused silica

Analytical:[2] 0.25-mm × 15-m G27 on fused silica; film thickness 0.25 µm

Temperatures

Detector: 325°

Injection port: 275° or use cold on-column injection

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
80	—	80	1
80	35	150	—
150	12	300	2

Carrier gas: Helium

Flow rate: 2.8 mL/min

Injection volume: 0.5 µL

Injection type: Splitless or by cold on-column injection

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 3.0 between the peaks due to alpha-lactose and beta-lactose

Analysis

Sample: *Sample solution*

[Note—The relative retention time with reference to beta-lactose is about 0.9 for alpha-lactose (retention time = about 12 min).]

Calculate the percentage content of alpha-lactose:

$$\text{Result} = S_a/(S_a + S_b) \times 100$$

S_a = area of the peak due to alpha-lactose

S_b = area of the peak due to beta-lactose

Calculate the percentage content of beta-lactose:

$$\text{Result} = S_b/(S_a + S_b) \times 100$$

S_a = area of the peak due to alpha-lactose

S_b = area of the peak due to beta-lactose

IMPURITIES

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

SPECIFIC TESTS

- **Clarity and Color of Solution**

Hydrazine sulfate solution: Dissolve 1.0 g of hydrazine sulfate in water, and dilute to 100.0 mL. Allow to stand for 4–6 h.

Hexamethylenetetramine solution: In a 100-mL ground-glass stoppered flask dissolve 2.5 g of hexamethylenetetramine in 25.0 mL of water.

Primary opalescent suspension: To the *Hexamethylenetetramine solution* in the flask add 25.0 mL of the *Hydrazine sulfate solution*. Mix and allow to stand for 24 h. This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.

Standard opalescence: Dilute 15.0 mL of the *Primary opalescent suspension* to 1000.0 mL with water. This suspension is freshly prepared and may be stored for up to 24 h.

Reference suspension: To 5.0 mL of the *Standard opalescence* add 95.0 mL of water. Mix and shake before use.

Reference solution: To 6.0 mL of ferric chloride CS, 2.5 mL of cobaltous chloride CS, and 1.0 mL of cupric sulfate CS add hydrochloric acid (10 g/L HCl) to make 1000 mL.

Sample solution: 1 g in 10 mL of boiling water. Allow to cool.

Instrumental conditions

Mode: Vis

Analytical wavelength: 400 nm

Acceptance criteria: NMT 0.04 for the absorbance divided by the path length in centimeters; and the clarity of the *Sample solution* is the same as that of water or its opalescence is not more pronounced

than that of the *Reference suspension*, and it is not more colored than the *Reference solution*.

- **Loss on Drying** ⟨731⟩

  Analysis: Dry a sample at 80° for 2 h.

  Acceptance criteria: NMT 0.5%

- **Water Determination** ⟨921⟩*, Method I*

  Sample solution: Anhydrous Lactose in a mixture of methanol and formamide (2:1)

  Acceptance criteria: NMT 1.0%

Change to read:

- **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩**:** The total aerobic microbial count is NMT 10^2 cfu/g and ▲ (NF 1-Dec-2024) the total combined molds and yeasts count is NMT 50 cfu/g. ▲ (NF 1-Dec-2024) It meets the requirements of the test for absence of *Escherichia coli*.

- **Protein and Light-Absorbing Impurities**

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Sample solution: 1% solution (w/v)

Instrumental conditions

Mode: UV

Wavelength range: 210–300 nm

Acceptance criteria: NMT 0.25 for the absorbance divided by the path length in centimeters at 210–220 nm; NMT 0.07 for the absorbance divided by the path length in centimeters at 270–300 nm

Change to read:

- **Acidity or Alkalinity**

Sample solution: Dissolve 6 g by heating in 25 mL of carbon dioxide-free water, cool, and add 0.3 mL of phenolphthalein TS.

Acceptance criteria: The solution is colorless, and NMT 0.4 mL of ▲0.1 N sodium hydroxide VS▲ (NF 1-Dec-2024) is required to produce a pink or red color.

- **Optical Rotation** ⟨781S⟩*, Procedures, Specific Rotation*

Sample solution: Dissolve 10 g by heating in 80 mL of water to 50°. Allow to cool, and add 0.2 mL of 6 N ammonium hydroxide. Allow to stand for 30 min, and dilute with water to 100 mL.

Acceptance criteria: +54.4° to +55.9°, calculated on the anhydrous basis, at 20°

ADDITIONAL REQUIREMENTS

Change to read:

- ▲ **Packaging and Storage:** Preserve in tight containers. ▲ (NF 1-Dec-2024)

Change to read:

- ▲ (NF 1-Dec-2024) **Labeling:** Where the labeling indicates the relative quantities of alpha- and beta-lactose, determine compliance using *Content of Alpha and Beta Anomers*. Where the labeling states the particle size distribution, it also indicates the d_{10}, d_{50}, and d_{90} values and the range for each. ▲ (NF 1-Dec-2024)

Change to read:

- **USP Reference Standards** ⟨11⟩
  USP Dextrose RS
  USP Fructose RS
  USP Anhydrous Lactose RS
  USP Sucrose RS

▲ ▲ (NF 1-Dec-2024)

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[1] Restek Guard column is suitable.

[2] Varian CP-Sil 8 CB is suitable.

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