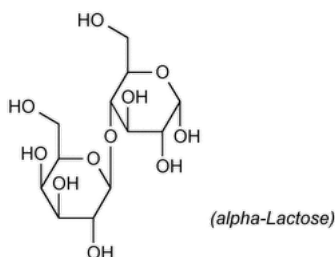


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Anhydrous Lactose

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.



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

• **†C. 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

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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.