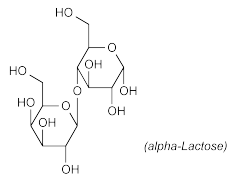
# Anhydrous Lactose

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.



(alpha-Lactose)

## 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. INFRARED ABSORPTION** ⟨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*.✦

***Delete the following:***

■• **C. PROCEDURE**
**Analysis:** Dissolve 250 mg in 5 mL of water. Add 3 mL of ammonium hydroxide, and heat in a water bath at 80° for 10 min.
**Acceptance criteria:** A red color develops.■2S *(NF30)*

## OTHER COMPONENTS

***Change to read:***

- ✦**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

[1] Restek Guard column is suitable.
[2] Varian CP-Sil 8 CB is suitable.

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

✦■2S (*NF30*)

**IMPURITIES**

- ✦**HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 5 ppm✦

***Change to read:***

- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.1% ■■2S (*NF30*)

**SPECIFIC TESTS**

***Change to read:***

- **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*.■2S (*NF30*)
- **LOSS ON DRYING** ⟨**731**⟩
  **Analysis:** Dry a sample at 80° for 2 h.
  **Acceptance criteria:** NMT 0.5%
- **WATER DETERMINATION,** *Method I* ⟨**921**⟩
  **Sample solution:** Anhydrous Lactose in a mixture of methanol and formamide (2:1)
  **Acceptance criteria:** NMT 1.0%
- **MICROBIAL ENUMERATION TESTS** ⟨**61**⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨**62**⟩**:** The total aerobic microbial count is NMT $10^2$ cfu/g and ✦the total combined molds and yeasts count is NMT 50 cfu/g✦. It meets the requirements of the test for absence of *Escherichia coli*.
- **PROTEIN AND LIGHT-ABSORBING IMPURITIES** (See *Spectrophotometry and Light-Scattering* ⟨851⟩.)
  **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 is required to produce a ■pink or■2S (*NF30*) red color.
- **OPTICAL ROTATION,** *Specific Rotation* ⟨**781S**⟩
  **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**

- ✦**PACKAGING AND STORAGE:** Preserve in tight containers.
- **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.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Dextrose RS
  USP Fructose RS
  USP Anhydrous Lactose RS
  USP Sucrose RS✦