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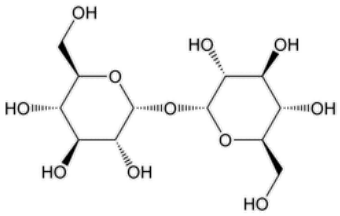
Trehalose

To view the Notice from the Expert Committee that posted in conjunction with this accelerated revision, please click https://www.uspnf.com/rb-trehalose-20241025.



• $[H_2O]_m$ m = 0 or 2

$C_{12}H_{22}O_{11}$ 342.30
$C_{12}H_{22}O_{11} \cdot 2H_2O$ 378.33

α-D-Glucopyranosyl α-D-glucopyranoside anhydrous CAS RN®: 99-20-7.
α-D-Glucopyranosyl α-D-glucopyranoside dihydrate CAS RN®: 6138-23-4.

DEFINITION

Trehalose is a stable, nonreducing disaccharide with two glucose molecules linked in an α,α-1,1 configuration. It is obtained through enzymatic conversion of food-grade starch. It contains NLT 97.0% and NMT 102.0% of trehalose ($C_{12}H_{22}O_{11}$), calculated on the anhydrous basis.

IDENTIFICATION

• **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197K

• **B.**

Sample solution: 400 mg/mL of Trehalose

Analysis: Add 0.4 mL of a solution containing 1-naphthol in 95% alcohol (1 in 20) to 1 mL of the *Sample solution*. Gently add 2 mL of sulfuric acid to the solution.

Acceptance criteria: A violet color develops at the interface between the two solutions.

• **C.**

Glycine solution: 40 mg/mL of glycine

Sample solution: 40 mg/mL of Trehalose

Analysis: Add 1 mL of diluted hydrochloric acid to 2 mL of the *Sample solution*. Allow to stand for 20 min at room temperature. Add 4 mL of sodium hydroxide TS and 2 mL of *Glycine solution* to the *Sample solution*. Heat the solution for 10 min in boiling water.

Acceptance criteria: A brown color does not develop.

ASSAY

Change to read:

• **Procedure**

Mobile phase: Water

Standard solution: ▲Dissolve an accurately weighed quantity of USP Trehalose RS in water to obtain a solution having a concentration of about 10 mg/mL of trehalose.▲ (RB 1-Nov-2024)

Sample solution: 10 mg/mL of Trehalose, calculated on the anhydrous basis

Chromatographic system

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

Mode: LC

Detector: Refractive index

Column: 8-mm × 30-cm; packing L58

Temperatures

Detector: 40°

Column: 80°

Flow rate: Adjust so that the retention time of trehalose is about 15 min.

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of trehalose ($C_{12}H_{22}O_{11}$) in the portion of Trehalose 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 USP Trehalose RS in the *Standard solution* (mg/mL)

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

Acceptance criteria: 97.0%–102.0% on the anhydrous basis

IMPURITIES

• **Residue on Ignition ⟨281⟩:** NMT 0.1%, determined on 2.0 g of Trehalose

• **Related Substances**

Mobile phase and Chromatographic system: Proceed as directed in the *Assay*.

Sample solution: 10 mg/mL of Trehalose

System suitability solution: Transfer 2.5 mL of the *Sample solution*, 25 mg of maltotriose, and 25 mg of glucose to a 10-mL volumetric flask, and dilute with water to volume.

Standard solution: 0.1 mg/mL of the *Sample solution*

System suitability

Sample: *System suitability solution*

[Note—The relative retention times for maltotriose, trehalose, and glucose are about 0.9, 1.0, and 1.2, respectively.]

Suitability requirements

Resolution: NLT 1.5 between trehalose and maltotriose

Relative standard deviation: NMT 2.0% for the trehalose peak

Analysis

Samples: *Sample solution* and *Standard solution*

Determine the peak areas for all peaks.

Acceptance criteria: For the *Sample solution*, the areas of any peaks corresponding to maltotriose and other polysaccharrides and eluting before trehalose are NMT half of the area of the peak corresponding to trehalose in the chromatogram of the *Standard solution* (0.5%). The areas of any peaks corresponding to glucose and eluting after trehalose are NMT half of the area of the peak corresponding to trehalose in the chromatogram of the *Standard solution* (0.5%).

SPECIFIC TESTS

• **Color and Clarity of Solution**

Sample solution: 33 g of Trehalose in 67 g of recently boiled water

Analysis: Using a suitable spectrophotometer (see *Ultraviolet-Visible Spectroscopy ⟨857⟩*), measure the absorbances of the *Sample solution* at 420 and 720 nm in a 10-cm cuvette. The absorbance of the *Sample solution* at 720 nm is NMT 0.050.

Determine the absorbance difference:

$$\text{Result} = A_{420} - A_{720}$$

A_{420} = absorbance of the *Sample solution* at 420 nm

A_{720} = absorbance of the *Sample solution* at 720 nm

Acceptance criteria: The absorbance difference is NMT 0.100.

• **Optical Rotation,** *Specific Rotation* ⟨781S⟩
Sample solution: 100 mg/mL
Acceptance criteria: +197° to +201° at 20°

• **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩**:** The total aerobic microbial count is NMT 100 cfu/g, and the total combined molds and yeasts count is NMT 100 cfu/g. It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

• **pH** ⟨791⟩
Sample solution: 100 mg/mL
Acceptance criteria: 4.5–6.5

• **Water Determination,** *Method I* ⟨921⟩
Sample: 0.1 g
Acceptance criteria
Anhydrous: NMT 1.0%
Dihydrate: 9.0%–11.0%

• **Bacterial Endotoxins Test** ⟨85⟩**:** If labeled for use in preparing parenteral dosage forms, it also meets the following requirements. The level of bacterial endotoxins is such that the requirement in the relevant dosage form monograph(s) in which Trehalose is used can be met. Where the label states that Trehalose must be subjected to further processing during the preparation of injectable dosage forms, the level of bacterial endotoxins is such that the requirement in the relevant dosage form monograph(s) in which Trehalose is used can be met.

• **Chloride and Sulfate,** *Chloride* ⟨221⟩
Sample: 2.0 g
Acceptance criteria: No more chloride than corresponds to 0.70 mL of 0.01 M hydrochloric acid (NMT 0.0125%)

• **Chloride and Sulfate,** *Sulfate* ⟨221⟩
Sample: 2.0 g
Acceptance criteria: No more sulfate than corresponds to 0.83 mL of 0.005 M sulfuric acid (NMT 0.0200%)

• **Nitrogen Determination,** *Method II* ⟨461⟩
Sample: 5.0 g
Analysis: Proceed as directed in *Method II*, except increase the volume of sulfuric acid for digestion to 30 mL and the volume of the sodium hydroxide solution (2 in 5) to 45 mL.
Acceptance criteria: NMT 0.005%

• **Soluble Starch**
Sample solution: 10% Trehalose (w/v)
Analysis: Add several drops of iodine TS to the *Sample solution*.
Acceptance criteria: No blue color develops.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers. No storage requirements specified.

• **Labeling:** Where Trehalose is intended for use in the manufacture of injectable dosage forms, it is so labeled. Where Trehalose must be subjected to further processing during the preparation of injectable dosage forms to ensure acceptable levels of bacterial endotoxins, it is so labeled.

• **USP Reference Standards** ⟨11⟩
USP Trehalose RS

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

Topic/Question	Contact	Expert Committee
TREHALOSE	Documentary Standards Support	SE2020 Simple Excipients
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SE2020 Simple Excipients

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
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