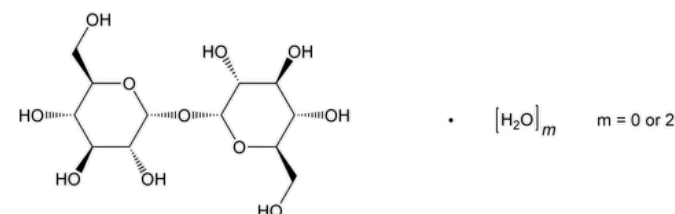


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Trehalose

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$C_{12}H_{22}O_{11}$ 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 polysaccharides 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