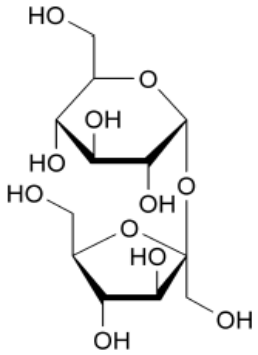
Rev. 1, Stage 2

SUCROSE



$C_{12}H_{22}O_{11}$ Mr 342.30

β-D-Fructofuranosyl α-D-glucopyranoside [57-50-1]

DEFINITION

Sucrose is a sugar obtained from *Saccharum officinarum Linné* (Fam. Gramineae), *Beta vulgaris Linné* (Fam. Chenopodiaceae), and other sources. It contains no added substances.

IDENTIFICATION

A. Infrared absorption spectrophotometry – KBr or ATR

Record the infrared absorption spectrum of Sucrose and compare with the reference spectrum or the spectrum obtained with the reference standard: the transmission minima or absorption maxima correspond in position and relative size.

B. Examine the chromatograms obtained in the assay.

The principal peak in the chromatogram obtained with the test solution is similar in

retention time and size to the principal peak in the chromatogram obtained with Sucrose standard solution.

Solution S. Dissolve 50.0 g in *water* and dilute to 100 ml with the same solvent.

Appearance of solution. Solution S is clear.

Its clarity is the same as that of water or its opalescence is not more pronounced than that of reference suspension I.

Conductivity: maximum 35 $\mu S \cdot cm^{-1}$ at 20°C.

Dissolve 31.3 g in *carbon dioxide-free water* prepared from *distilled water* and dilute to 100 ml with the same solvent. Measure the conductivity of the solution (C_1), while gently stirring with a magnetic stirrer, and that of the water used for preparing the solution (C_2). The readings must be stable within 1 per cent over a period of 30 s. Calculate the conductivity of the solution of the substance to be examined from the expression: $C_1 - 0.35\ C_2$

Specific optical rotation: +66.3 to +67.0.

Dissolve 26.0 g in *water* and dilute to 100.0 ml with the same solvent.

Related substances

Diluent: Water

System suitability solution: 10 mg/mL of Sucrose reference standard (RS) and 0.05 mg/mL each of Raffinose RS, Glucose RS and Fructose RS in *Diluent*

Impurity Standard solution: 0.05mg/mL each of Sucrose RS, Raffinose RS, Glucose RS and Fructose RS in *Diluent*

Sample solution: 10 mg/mL of Sucrose in *Diluent*

Mobile phase: Degassed water

Chromatographic system

- Mode: LC
- Detector: Refracted Index, maintained at a constant temperature (40°C)
- Column:

  - Size: 7.8-mm × 30-cm

  - Strong cation-exchange resin consisting of sulfonated cross-linked

styrenedivinylbenzene copolymer in the calcium form (9-µm) *

[*Note: Aminex HPX-87C is suitable.]

- Temperature: 80 ± 1°C

- Flow rate: 0.5 mL/min
- Injection volume: 10 µL
- Run time: 30 min

System suitability requirements:

- Peak-to-valley ratio:
  - NLT 2.5 between Raffinose and Sucorse

  where Hp = height above the baseline of the raffinose, and Hv = height above the baseline of the lowest point of the curve separating the impurity peak from the sucrose peak in the *System suitability solution.*

- Resolution:
  - NLT 1.5 between Sucrose and Glucose

  [Notes:

  1) The relative retention time with reference to sucrose for raffinose/theanderose, glucose and fructose are 0.9, 1.2, and 1.6, respectively. The retention time for Sucrose is about 10 min.
  2) Raffinose is present in sucrose obtained from sugar beet and Theanderose is present in sucrose obtained from sugar cane.]

- Relative standard deviation (%RSD): NMT 5.0%, for 6 replicate injections of sucrose and each known impurity in the *Impurity Standard Solution*.

Injection: *Impurity Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of sucrose taken:

$$\%\text{Impurity} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of each impurity from the *Sample* solution

r_S = peak response of each impurity from the *Impurity Standard solution*

C_S = concentration of each corresponding impurity RS in the *Impurity Standard solution* (mg/mL);

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

[Note: Unknown impurities are calculated by comparing the unknown impurity peak area to the sucrose peak area in the *Impurity Standard*

solution.]

Acceptance criteria:

- Individual known impurity (Raffinose/Theanderose, Glucose, Fructose): NMT 0.2%
- Unknown impurities: NMT 0.10%
- Total impurities: NMT 1.0%
- Reporting threshold: 0.05%

Dextrins. If intended for use in the preparation of large-volume infusions, it complies with the test for dextrins. To 2 ml of solution S add 8 ml of *water,* 0.05 ml of *dilute hydrochloric acid* (73 g/l of HCl) and 0.05 ml of *0.05 M iodine.* The solution remains yellow.

Reducing sugars. To 5 ml of solution S in a test-tube about 150 mm long and 16 mm in diameter add 5 ml of *water,* 1.0 ml of *1 M sodium hydroxide* and 1.0 ml of a 1 g/l solution of *methylene blue.* Mix and place in a water-bath. After exactly 2 min, take the tube out of the bath and examine the solution immediately. The blue colour does not disappear completely. Ignore any blue colour at the air/solution interface.

Sulphite: maximum 10 ppm calculated as SO_2.

Determine the sulphite content by a suitable enzymatic method based on the following reactions. Sulphite is oxidised by sulphite oxidase to sulphate and hydrogen peroxide which in turn is reduced by nicotinamide-adenine dinucleotide-peroxidase in the presence of reduced nicotinamide-adenine dinucleotide (NADH). The amount of NADH oxidised is proportional to the amount of sulphite.

Test solution. Dissolve 4.0 g of the substance to be examined in freshly prepared *distilled water* and dilute to 10.0 ml with the same solvent.

Reference solution. Dissolve 4.0 g of the substance to be examined in freshly prepared *distilled water,* add 0.5 ml of *sulphite standard solution (80 ppm SO_2)* and dilute to 10.0 ml with freshly prepared *distilled water.* Use freshly prepared *distilled water* as a blank.

Separately introduce 2.0 ml each of the test solution, the reference solution and the blank in 10 mm cuvettes and add the reagents as described in the kit instructions. Measure the absorbance at the maximum at about 340 nm before and at the end of the reaction time and subtract the value obtained with the blank.

The absorbance difference of the test solution is not greater than half the absorbance difference of the reference solution.

Loss on drying : maximum 0.1 per cent, determined on 2.000 g, by heating in an oven at 105 °C for 3 h.

Bacterial endotoxins : less than 0.25 IU/mg, if intended for use in the preparation of large-volume infusions.

ASSAY

Liquid chromatography as described in the test for related substances with the following modifications.

Standard solution: 10 mg/mL of Sucrose RS in water

System suitability requirements:

- Peak-to-valley ratio and resolution: the same as those in the test for related substances
- Relative standard deviation (%RSD): NMT 0.73%, for 5 replicate injections of *Standard solution*.

Injection: *Standard solution* and *Sample solution*

Calculate the percentage of Sucrose ($C_{12}H_{22}O_{11}$) in the portion of sucrose taken:

$$\%\text{Assay} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of Sucrose from the *Sample* solution

r_S = peak response of Sucrose from *Standard solution*

C_S = concentration of Sucrose RS in *Standard solution* (mg/mL)

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

Acceptance criteria: 98.0%–102.0%

LABELLING

The label states, where applicable, that the substance is suitable for use in the manufacture of large-volume parenteral dosage forms.

Reagents

Hydrazine sulphate solution. Dissolve 1.0 g of hydrazine sulphate in water and dilute to 100.0 ml with the same solvent. 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 (formazin suspension). To the hexamethylenetetramine solution in the flask add 25.0 ml of the hydrazine sulphate 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 of 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 I. To 5.0 ml of standard of opalescence add 95.0 ml of water. Mix and shake before use.

Sulphite standard solution (80 ppm SO_2).
Dissolve 3.150 g of *anhydrous sodium sulphite* in freshly prepared *distilled water* and dilute to 100.0 ml with the same solvent. Dilute 0.5 ml to 100.0 ml with freshly prepared *distilled water.*