dilute hydrochloric acid R and boil for 1 min. Add 4 mL of *dilute sodium hydroxide solution R*; an orange precipitate is formed immediately.

C. Dissolve about 15 mg in a mixture of 0.5 mL of *dilute hydrochloric acid R* and 2 mL of *water R*. The solution gives the reaction of aluminium (*2.3.1*).

TESTS

Impurity A. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 450.0 mg of the substance to be examined in a mixture of equal volumes of an 88 g/L solution of *sodium hydroxide R* and a 196.2 g/L solution of *sulfuric acid R* and dilute to 10.0 mL with the same mixture of solvents. Without delay, while shaking at a moderate rate, add a volume (*V*), accurately measured in millilitres, of a 4 g/L solution of *sodium hydroxide R* to adjust the solution to approximately pH 2.3. Dilute the solution with (15.0 – *V*) mL of *water R*. Shake for 1 min. If the pH is not between 2.3 and 3.5, repeat the test using a different volume of a 4 g/L solution of *sodium hydroxide R*.

Reference solution (a). Dissolve 40.0 mg of *potassium sucrose octasulfate CRS* in the mobile phase and dilute to 5.0 mL with the mobile phase.

Reference solution (b). Dilute 1.0 mL of reference solution (a) to 10.0 mL with the mobile phase.

Column:

– *size*: *l* = 0.25 m, Ø = 4.0 mm;

– *stationary phase*: *aminopropylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: 70 g/L solution of *ammonium sulfate R*, adjusted to pH 3.5 with *concentrated phosphoric acid R*.

Flow rate: 1 mL/min.

Detection: differential refractometer.

Injection: 50 µL of the test solution and reference solution (b).

Relative retention with reference to sucrose octasulfate (retention time = about 6 min): impurity A = about 0.6.

System suitability: reference solution (b):

– *number of theoretical plates*: minimum 400;

– *symmetry factor*: maximum 4.0.

Limit:

– *impurity A*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5.0 per cent).

Neutralising capacity. Disperse 0.25 g in 100.0 mL of *0.1 M hydrochloric acid*, previously heated at 37 °C, stir continuously for 1 h in a water-bath at 37 °C and cool. Titrate 20.0 mL of this solution with *0.1 M sodium hydroxide* to pH 3.5; not more than 14.0 mL of *0.1 M sodium hydroxide* is required.

Chlorides (*2.4.4*): maximum 0.50 per cent.

Dissolve 0.10 g in 5 mL of *dilute nitric acid R* and dilute to 50 mL with *water R*. Dilute 5 mL of this solution to 15 mL with *water R*.

Arsenic (*2.4.2, Method A*): maximum 4 ppm.

Introduce 0.25 g of the substance to be examined and 5 mL of *sulfuric acid R* into a combustion flask. Carefully add a few millilitres of *strong hydrogen peroxide solution R* and heat to boiling until a clear, colourless solution is obtained. Continue heating to eliminate the water and as much sulfuric acid as possible and dilute to 25 mL with *water R*.

Heavy metals (*2.4.8*): maximum 10 ppm.

2.0 g complies with test F. Prepare the reference solution using 2 mL of *lead standard solution (10 ppm Pb) R*.

ASSAY

Aluminium. Disperse 1.0 g in 10 mL of *6 M hydrochloric acid R*. Heat with continuous stirring in a water-bath at 70 °C for 5 min. Cool to room temperature, transfer quantitatively to a volumetric flask, dilute to 250.0 mL with *water R*, and mix. Filter the solution, discarding the 1st portion of the filtrate. To 10.0 mL of the solution, add 10.0 mL of *0.1 M sodium edetate* and 30 mL of a mixture of equal volumes of *ammonium acetate solution R* and *dilute acetic acid R*. Heat in a water-bath at 70 °C for 5 min, then cool. Add 25 mL of *ethanol (96 per cent) R* and 1 mL of a freshly prepared 0.25 g/L solution of *dithizone R* in *ethanol (96 per cent) R*. Titrate the excess of sodium edetate with *0.1 M zinc sulfate* until the colour changes to pink.

1 mL of *0.1 M sodium edetate* is equivalent to 2.698 mg of Al.

Sucrose octasulfate. Liquid chromatography (*2.2.29*) as described in the test for impurity A with the following modifications.

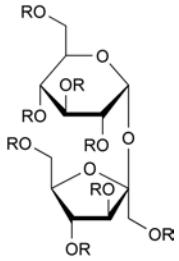
Mobile phase: 132 g/L solution of *ammonium sulfate R*, adjusted to pH 3.5 with *concentrated phosphoric acid R*.

Injection: test solution and reference solution (a).

Calculate the percentage content of $C_{12}H_{14}O_{35}S_8$ from the declared content of *potassium sucrose octasulfate CRS* and by multiplying the potassium sucrose octasulfate content by 0.757.

IMPURITIES

Specified impurities: A.



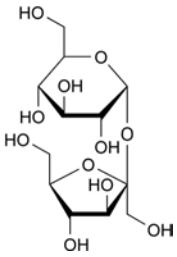
R = SO_3H
and H in a 7 to 1 ratio

A. β-D-fructofuranosyl-α-D-glucopyranoside heptakis (hydrogensulfate).

01/2009:0204

SUCROSE

Saccharum



$C_{12}H_{22}O_{11}$ M_r 342.3
[57-50-1]

DEFINITION

β-D-Fructofuranosyl α-D-glucopyranoside.

It contains no additives.

CHARACTERS

Appearance: white or almost white, crystalline powder, or lustrous, colourless or white or almost white crystals.

Solubility: very soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in anhydrous ethanol.

IDENTIFICATION

First identification: A.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *sucrose CRS*.

B. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 10 mg of the substance to be examined in a mixture of 2 volumes of *water R* and 3 volumes of *methanol R* and dilute to 20 mL with the same mixture of solvents.

Reference solution (a). Dissolve 10 mg of *sucrose CRS* in a mixture of 2 volumes of *water R* and 3 volumes of *methanol R* and dilute to 20 mL with the same mixture of solvents.

Reference solution (b). Dissolve 10 mg each of *fructose CRS*, *glucose CRS*, *lactose CRS* and *sucrose CRS* in a mixture of 2 volumes of *water R* and 3 volumes of *methanol R* and dilute to 20 mL with the same mixture of solvents.

Plate: *TLC silica gel G plate R*.

Mobile phase: *cold saturated boric acid solution R*, 60 per cent *V/V* solution of *glacial acetic acid R*, *ethanol R*, *acetone R*, *ethyl acetate R* (10:15:20:60:60 *V/V/V/V/V*).

Application: 2 µL.

Development: in an unsaturated tank over a path of 15 cm.

Drying: in a current of warm air.

Detection: spray with a solution of 0.5 g of *thymol R* in a mixture of 5 mL of *sulfuric acid R* and 95 mL of *alcohol R*. Heat the plate at 130 °C for 10 min.

System suitability: the chromatogram obtained with reference solution (b) shows 4 clearly separated spots.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

C. Dilute 1 mL of solution S (see Tests) to 100 mL with *water R*. To 5 mL of the solution add 0.15 mL of freshly prepared *copper sulfate solution R* and 2 mL of freshly prepared *dilute sodium hydroxide solution R*. The solution is blue and clear and remains so after boiling. To the hot solution add 4 mL of *dilute hydrochloric acid R* and boil for 1 min. Add 4 mL of *dilute sodium hydroxide solution R*. An orange precipitate is formed immediately.

TESTS

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

Appearance of solution. Solution S is clear (*2.2.1*).

Conductivity (*2.2.38*): maximum 35 µS·cm^{-1} at 20 °C.

Dissolve 31.3 g in *carbon dioxide-free water R* prepared from *distilled water R* 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 following expression:

$$C_1 - 0.35\,C_2$$

Specific optical rotation (*2.2.7*): + 66.3 to + 67.0.

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

Colour value: maximum 45.

Dissolve 50.0 g in 50.0 mL of *water R*. Mix, filter (diameter of pores 0.45 µm) and degas. Measure the absorbance (*2.2.25*) at 420 nm, using a cell of minimum 4 cm (a cell length of 10 cm or more is preferred).

Calculate the colour value using the following expression:

$$\frac{A \times 1000}{b \times c}$$

A = absorbance measured at 420 nm;

b = path length in centimetres;

c = concentration of the solution, in grams per millilitre, calculated from the refractive index (*2.2.6*) of the solution; use Table 0204.-1 and interpolate the values if necessary.

Table 0204.-1

n_D^{20}	c (g/mL)
1.4138	0.570
1.4159	0.585
1.4179	0.600
1.4200	0.615
1.4221	0.630
1.4243	0.645
1.4264	0.661

System suitability:

– *repeatability*: the absolute difference between 2 results is not greater than 3.

Dextrins. If intended for use in the manufacture of large-volume parenteral preparations, it complies with the test for dextrins. To 2 mL of solution S add 8 mL of *water R*, 0.05 mL of *dilute hydrochloric acid R* 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 R*, 1.0 mL of *1 M sodium hydroxide* and 1.0 mL of a 1 g/L solution of *methylene blue R*. 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.

Sulfites: maximum 10 ppm, calculated as SO_2.

Determine the sulfites content by a suitable enzymatic method based on the following reactions. Sulfite is oxidised by sulfite oxidase to sulfate 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 sulfite.

Test solution. Dissolve 4.0 g of the substance to be examined in freshly prepared *distilled water R* 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 R*, add 0.5 mL of *sulfite standard solution (80 ppm SO_2) R* and dilute to 10.0 mL with freshly prepared *distilled water R*.

Blank solution. Freshly prepared *distilled water R*.

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 instructions in the kit for sulfite determination. Measure the absorbance (*2.2.25*) at the absorption 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 (*2.2.32*): maximum 0.1 per cent, determined on 2.000 g by drying in an oven at 105 °C for 3 h.

Bacterial endotoxins (*2.6.14*): less than 0.25 IU/mg, if intended for use in the manufacture of large-volume parenteral preparations.

LABELLING

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

07/2009:2319

SUCROSE MONOPALMITATE

Sacchari monopalmitas

DEFINITION

Mixture of sucrose monoesters, mainly sucrose monopalmitate, obtained by transesterification of palmitic acid methyl esters of vegetable origin with *Sucrose (0204)*. The manufacture of the fatty acid methyl esters includes a distillation step.

It contains variable quantities of mono-, di-, tri- and polyesters.

Content:

– *monoesters*: minimum 55.0 per cent;
– *diesters*: maximum 40.0 per cent;
– *sum of triesters and polyesters*: maximum 20.0 per cent.

CHARACTERS

Appearance: white or almost white, unctuous powder.

Solubility: very slightly soluble in water, sparingly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Composition of fatty acids (see Tests).

B. It complies with the limits of the assay.

TESTS

Acid value (*2.5.1*): maximum 6.0, determined on 3.00 g.

Use a freshly neutralised mixture of 1 volume of *water R* and 2 volumes of *2-propanol R* as solvent and heat gently.

Composition of fatty acids (*2.4.22, Method C*). Use the mixture of calibrating substances in Table 2.4.22.-1.

Composition of the fatty-acid fraction of the substance:

– *lauric acid*: maximum 3.0 per cent;
– *myristic acid*: maximum 3.0 per cent;
– *palmitic acid*: 70.0 per cent to 85.0 per cent;
– *stearic acid*: 10.0 per cent to 25.0 per cent;
– *sum of the contents of palmitic acid and stearic acid*: minimum 90.0 per cent.

Free sucrose. Liquid chromatography (*2.2.29*).

Solvent mixture: *water for chromatography R, tetrahydrofuran for chromatography R* (12.5:87.5 *V/V*).

Test solution. Dissolve 0.200 g of the substance to be examined in the solvent mixture and dilute to 4.0 mL with the solvent mixture.

Reference solution (a). Dissolve 20.0 mg of *sucrose CRS* in the solvent mixture and dilute to 10.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b). In 4 volumetric flasks, introduce respectively 5.0 mg, 10.0 mg, 20.0 mg and 25.0 mg of *sucrose CRS*, dissolve in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;
– *stationary phase*: spherical *aminopropylsilyl silica gel for chromatography R* (4 µm).

Mobile phase:

– *mobile phase A*: 0.01 g/L solution of *ammonium acetate R* in *acetonitrile for chromatography R*;
– *mobile phase B*: 0.01 g/L solution of *ammonium acetate R* in a mixture of 10 volumes of *water for chromatography R* and 90 volumes of *tetrahydrofuran for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)	Flow rate (mL/min)
0 - 1	100	0	1.0
1 - 9	100 → 0	0 → 100	1.0
9 - 16	0	100	1.0
16 - 16.01	0	100	1.0 → 2.5
16.01 - 32	0	100	2.5
32 - 33	0 → 100	100 → 0	2.5
33 - 36	100	0	2.5 → 1.0

Detection: evaporative light-scattering detector; the following settings have been found to be suitable; if the detector has different setting parameters, adjust the detector settings so as to comply with the system suitability criterion:

– *carrier gas*: *nitrogen R*;
– *flow rate*: 1.0 mL/min;
– *evaporator temperature*: 45 °C;
– *nebuliser temperature*: 40 °C.

Injection: 20 µL.

Retention time: about 26 min.

System suitability: reference solution (a):

– *signal-to-noise ratio*: minimum 10.

Limit: maximum 4.0 per cent.

Water (*2.5.12*): maximum 4.0 per cent, determined on 0.20 g.

Total ash (*2.4.16*): maximum 1.5 per cent.

ASSAY

Size-exclusion chromatography (*2.2.30*): use the normalisation procedure.

Test solution. Dissolve 60.0 mg of the substance to be examined in *tetrahydrofuran R* and dilute to 4.0 mL with the same solvent.

Column:

– *size*: *l* = 0.6 m, Ø = 7 mm;
– *stationary phase*: *styrene-divinylbenzene copolymer R* (5 µm) with a pore size of 10 nm.

Mobile phase: *tetrahydrofuran R*.

Flow rate: 1.2 mL/min.

Detection: differential refractometer.

Injection: 20 µL.

Relative retention with reference to monoesters (retention time = about 10 min): diesters = about 0.92; triesters and polyesters = about 0.90.

Calculations:

– *disregard limit*: disregard the peaks having a signal-to-noise ratio less than 10;
– *free fatty acids (D)*: calculate the percentage content of free fatty acids, using the following expression:

$$\frac{I_A \times 256}{561.1}$$

I_A = acid value.

– *monoesters*: calculate the percentage content of monoesters using the following expression:

$$\frac{A \times (100 - D - S - E)}{100}$$