ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related proteins with the following modification.

Injection: test solution and reference solution.

Calculate the content of human glucagon ($C_{153}H_{225}N_{43}O_{49}S$) from the declared content of $C_{153}H_{225}N_{43}O_{49}S$ in *human glucagon CRS*.

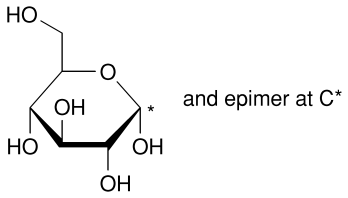
STORAGE

In an airtight container, protected from light, at a temperature lower than – 15 °C.

01/2008:0177
corrected 6.0

GLUCOSE, ANHYDROUS

Glucosum anhydricum



$C_6H_{12}O_6$ M_r 180.2
[50-99-7]

DEFINITION

(+)-D-Glucopyranose.

CHARACTERS

Appearance: white or almost white, crystalline powder.

It has a sweet taste.

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

IDENTIFICATION

A. Specific optical rotation (see Tests).

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

Solvent mixture: *water R, methanol R* (2:3 *V/V*).

Test solution. Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (a). Dissolve 10 mg of *glucose CRS* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Reference solution (b). Dissolve 10 mg each of *fructose CRS, glucose CRS, lactose CRS* and *sucrose CRS* in the solvent mixture and dilute to 20 ml with the solvent mixture.

Plate: *TLC silica gel G plate R*.

Mobile phase: *water R, methanol R, anhydrous acetic acid R, ethylene chloride R* (10:15:25:50 *V/V/V/V*); measure the volumes accurately since a slight excess of water produces cloudiness.

Application: 2 µl; thoroughly dry the starting points.

Development A: over a path of 15 cm.

Drying A: in a current of warm air.

Development B: immediately, over a path of 15 cm, after renewing the mobile phase.

Drying B: 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 *sulphuric acid R* and 95 ml of *ethanol (96 per cent) R*. Heat at 130 °C for 10 min.

System suitability: test solution (b):

– the chromatogram 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. Dissolve 0.1 g in 10 ml of *water R*. Add 3 ml of *cupri-tartaric solution R* and heat. A red precipitate is formed.

TESTS

Solution S. Dissolve 10.0 g in *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_7 (*2.2.2, Method II*).

Dissolve 10.0 g in 15 ml of *water R*.

Acidity or alkalinity. Dissolve 6.0 g in 25 ml of *carbon dioxide-free water R* and add 0.3 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 0.15 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

Specific optical rotation (*2.2.7*): + 52.5 to + 53.3 (anhydrous substance).

Dissolve 10.0 g in 80 ml of *water R*, add 0.2 ml of *dilute ammonia R1*, allow to stand for 30 min and dilute to 100.0 ml with *water R*.

Foreign sugars, soluble starch, dextrins. Dissolve 1.0 g by boiling in 30 ml of *ethanol (90 per cent V/V) R*. Cool; the appearance of the solution shows no change.

Sulphites: maximum 15 ppm, expressed as SO_2.

Test solution. Dissolve 5.0 g in 40 ml of *water R*, add 2.0 ml of *0.1 M sodium hydroxide* and dilute to 50.0 ml with *water R*. To 10.0 ml of the solution, add 1 ml of a 310 g/l solution of *hydrochloric acid R*, 2.0 ml of *decolorised fuchsin solution R1* and 2.0 ml of a 0.5 per cent *V/V* solution of *formaldehyde R*. Allow to stand for 30 min.

Reference solution. Dissolve 76 mg of *sodium metabisulphite R* in *water R* and dilute to 50.0 ml with the same solvent. Dilute 5.0 ml of this solution to 100.0 ml with *water R*. To 3.0 ml of this solution add 4.0 ml of *0.1 M sodium hydroxide* and dilute to 100.0 ml with *water R*. Immediately add to 10.0 ml of this solution 1 ml of a 310 g/l solution of *hydrochloric acid R*, 2.0 ml of *decolorised fuchsin solution R1* and 2.0 ml of a 0.5 per cent *V/V* solution of *formaldehyde R*. Allow to stand for 30 min.

Measure the absorbance (*2.2.25*) of the 2 solutions at the absorption maximum at 583 nm using for both measurements a solution prepared in the same manner using 10.0 ml of *water R* as the compensation liquid. The absorbance of the test solution is not greater than that of the reference solution.

Chlorides (*2.4.4*): maximum 125 ppm.

Dilute 4 ml of solution S with 15 ml of *water R*.

Sulphates (*2.4.13*): maximum 200 ppm.

Dilute 7.5 ml of solution S with 15 ml of *distilled water R*.

Arsenic (*2.4.2, Method A*): maximum 1 ppm, determined on 1.0 g.

Barium. To 10 ml of solution S add 1 ml of *dilute sulphuric acid R*. When examined immediately and after 1 h, any opalescence in the solution is not more intense than that in a mixture of 1 ml of *distilled water R* and 10 ml of solution S.

Calcium (*2.4.3*): maximum 200 ppm.

Dilute 5 ml of solution S with 15 ml of *distilled water R*.

Lead (*2.4.10*): maximum 0,5 ppm.

Water (*2.5.12*): maximum 1.0 per cent, determined on 0.50 g.

Sulphated ash: maximum 0.1 per cent.

Dissolve 5.0 g in 5 ml of *water R*, add 2 ml of *sulphuric acid R*, evaporate to dryness on a water-bath and ignite to constant mass. If necessary, repeat the heating with *sulphuric acid R*.

Pyrogens (*2.6.8*). If intended for use in the manufacture of large-volume parental dosage forms without a further appropriate procedure for the removal of pyrogens, the competent authority may require that it comply with the test for pyrogens. Inject per kilogram of the rabbit's mass 10 ml of a solution in *water for injections R* containing 50 mg of the substance to be examined per millilitre.

01/2008:1330

GLUCOSE, LIQUID

Glucosum liquidum

DEFINITION

Aqueous solution containing a mixture of glucose, oligosaccharides and polysaccharides obtained by hydrolysis of starch.

It contains a minimum of 70.0 per cent dry matter.

The degree of hydrolysis, expressed as dextrose equivalent (DE), is not less than 20 (nominal value).

CHARACTERS

Appearance: clear, colourless or brown, viscous liquid.

Solubility: miscible with water.

It may partly or totally solidify at room temperature and liquefies again when heated to 50 °C.

IDENTIFICATION

A. Dissolve 0.1 g in 2.5 ml of *water R* and heat with 2.5 ml of *cupri-tartaric solution R*. A red precipitate is formed.

B. Dip, for 1 s, a suitable stick with a reactive pad containing glucose-oxidase, peroxidase and a hydrogen-donating substance, such as tetramethylbenzidine, in a 5 g/l solution of the substance to be examined. Observe the colour of the reactive pad; within 60 s the colour changes from yellow to green or blue.

C. It is a clear, colourless or brown, viscous liquid, miscible with water. The substance may partly or totally solidify at room temperature and liquefies again when heated to 50 °C.

D. Dextrose equivalent (see Tests).

TESTS

Solution S. Dissolve 25.0 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

pH (*2.2.3*): 4.0 to 6.0.

Mix 1 ml of a 223.6 g/l solution of *potassium chloride R* and 30 ml of solution S.

Sulphur dioxide (*2.5.29*): maximum 20 ppm; maximum 400 ppm if intended for the production of lozenges or pastilles obtained by high boiling techniques, provided that the final product contains maximum 50 ppm of sulphur dioxide.

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

Dilute 2 ml of solution S to 30 ml with *water R*. The solution complies with test E. Prepare the reference solution using 10 ml of *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*): maximum 30.0 per cent, determined on 1.000 g. Triturate the sample with 3.000 g of *kieselguhr G R*, previously dried at 80 °C under reduced pressure for 2 h, and dry at 80 °C under reduced pressure for 2 h.

Sulphated ash (*2.4.14*): maximum 0.5 per cent, determined on 1.0 g.

Dextrose equivalent (DE): within 10 per cent of the nominal value.

Weigh an amount of the substance to be examined equivalent to 2.85-3.15 g of reducing carbohydrates, calculated as dextrose equivalent, into a 500 ml volumetric flask. Dissolve in *water R* and dilute to 500.0 ml with the same solvent. Transfer the solution to a 50 ml burette.

Pipette 25.0 ml of *cupri-tartaric solution R* into a 250 ml flask and add 18.5 ml of the test solution from the burette, mix and add a few glass beads. Place the flask on a hot plate, previously adjusted so that the solution begins to boil after 2 min ± 15 s. Allow to boil for exactly 120 s, add 1 ml of a 1 g/l solution of *methylene blue R* and titrate with the test solution (V_1) until the blue colour disappears. Maintain the solution at boiling throughout the titration.

Standardise the cupri-tartaric solution using a 6.00 g/l solution of *glucose R* (V_0).

Calculate the dextrose equivalent using the following expression:

$$\frac{300 \times V_0 \times 100}{V_1 \times M \times D}$$

V_0 = total volume of glucose standard solution, in millilitres,

V_1 = total volume of test solution, in millilitres,

M = mass of the sample, in grams,

D = percentage content of dry matter in the substance.

LABELLING

The label states the dextrose equivalent (DE) (= nominal value).

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corrected 6.0

GLUCOSE, LIQUID, SPRAY-DRIED

Glucosum liquidum dispersione desiccatum

DEFINITION

Mixture of glucose, oligosaccharides and polysaccharides, obtained by the partial hydrolysis of starch.

The degree of hydrolysis, expressed as dextrose equivalent (DE), is not less than 20 (nominal value).

CHARACTERS

Appearance: white or almost white, slightly hygroscopic powder or granules.

Solubility: freely soluble in water.