PHARMACOPOEIAL DISCUSSION GROUP
SIGN-OFF COVER SHEET
CODE: E-56
NAME: GLUCOSE ANHYDROUS/MONOHYDRATE

(Version 3 of the sign-off cover sheet of the Corr. 1 signed on January 20, 2015)

Amended Item: EP local requirement changed from “Pyrogens” to “Pyrogenicity”.

Harmonised attributes

Attribute	EP	JP	USP
Definition	+	+	+
Identification - A - B	 + +	 + +	 + -
Appearance of solution	+	+	+
Conductivity	+	+	+
Related substances	+	+	+
Dextrin	+	+	+
Soluble starch and sulfite	+	+	+
Water	+	+	+
Assay	+	+	+

Legend

\+ will adopt and implement; － will not stipulate

Non-harmonised attributes

Description/Characters, Packaging/Containers and storage, Labelling

Local requirements

EP	Identification (specific optical rotation), Second identification (TLC, colour reaction); Pyrogenicity
JP	Identification (colour reaction); Specific optical rotation
USP	Identification (IR)

Reagents and reference materials

Each pharmacopoeia will adapt the text to take account of local reference materials and reagent specifications.

European Pharmacopoeia

Signature	Name	Date
Signé par : Cathie VIELLE 5D202E6E19D1466...	C. Vielle	16 July 2025

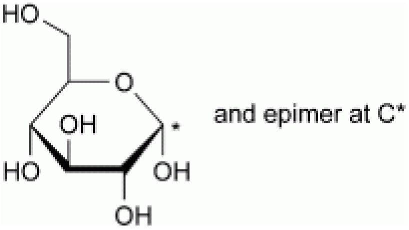
Japanese Pharmacopoeia

Signature	Name	Date
署名者: Yoshi Saito for T. Kihira 878995A356ED445...	Yoshiro Saito	Jul. 16, 2025

United States Pharmacopeia

Signature	Name	Date
Signed by: Kevin Moore A7467E52FCC94E9...	Kevin Moore	7/15/2025

E-56 GLUCOSE, ANHYDROUS



M_r 180.2

$C_6H_{12}O_6$

DEFINITION

Glucose anhydrous is (+)-D-glucopyranose and is derived from starch.
Content: 97.5 per cent to 102.0 per cent (anhydrous substance), determined by the LC described in the assay.

IDENTIFICATION

A. 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 reference solution (a).

B. Water (see tests).

TESTS

Appearance of solution. The test solution is clear (its clarity is the same as that of water or its opalescence is not more pronounced than that of reference suspension I) and not more intensely coloured than the reference solution.
Test solution: Dissolve 10.0 g in 15 mL of *water* using a bath of boiling water. Allow to cool.
Primary solutions:

- *Ferric chloride primary solution:* a 45.0 g/l solution of ferric chloride ($FeCl_3, 6H_2O$).
- *Cobalt chloride primary solution:* a 59.5 g/l solution of cobalt chloride ($CoCl_2, 6H_2O$).
- *Copper sulfate primary solution:* a 62.4 g/l solution of copper sulfate ($CuSO_4, 5H_2O$).

Reference solution: to 2.5 mL of cobalt chloride primary solution, 6.0 mL of ferric chloride primary solution and 1.0 mL of copper sulfate primary solution, add hydrochloric acid (10 g/l HCl) to make 1000.0 mL.

Conductivity: maximum 20 $\mu S \cdot cm^{-1}$ at 25°C.

Dissolve 20.0 g in *carbon dioxide-free water* prepared from *distilled water* and dilute to 100.0 mL with the same solvent. Measure the conductivity of the solution while gently stirring with a magnetic stirrer.

Related substances. Liquid chromatography.

Test solution. Dissolve 0.300 g of the substance to be examined in *water*, and dilute to 10.0 mL with the same solvent.

Reference solution (a). Dissolve 0.330 g of *glucose monohydrate CRS* in *water* and dilute to 10.0 mL with the same solvent.

Reference solution (b). Dilute 1.0 mL of the test solution to 250.0 mL with *water.*

Reference solution (c). Dilute 25.0 mL of reference solution (b) to 200.0 mL with *water.*

Reference solution (d). Dissolve 5 mg of *maltose* (impurity A), 5 mg of *maltotriose* (impurity C) and 5 mg of *fructose* (impurity D) in water and dilute to 50.0 mL with *water*.

Column:
- size: l = 0.3 m, Ø = 7.8 mm;
- stationary phase: strong cation-exchange resin (calcium form) (9µm)[1];
- temperature: 85 +/- 1 °C.

Mobile phase: degassed water.

Flow rate: 0.3 mL/min.

Detection: refractometer maintained at a constant temperature (40 °C for example).

Injection: 20 µl of the test solution and reference solutions (b), (c) and (d).

Run time: 1.5 times the retention time of glucose.

Relative retention with reference to glucose (retention time = about 21 min): impurity C = about 0.7; impurities A and B = about 0.8; impurity D = about 1.3.

System suitability : reference solution (d) :
- *resolution* : minimum 1.3 between the peaks due to impurities C and A.

Limits:
- *sum of impurities A and B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent),
- *impurity C*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent),

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[1] Agilent Metacarb 87C is suitable.

- *impurity D*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.15 per cent),
- *unspecified impurities*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent),
- *total*: not more than 1.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),
- *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Dextrin. Reflux 1 g of the substance to be examined finely powdered with 20 mL of ethanol (96 per cent): it dissolves completely.

Soluble starch and sulfites: maximum 15 ppm.
Dissolve 6.7 g in 15 mL of *water* using a bath of boiling water. Allow to cool and add 25 µl of 0.05 M iodine: the solution is yellow.

Water[2]: maximum 1.0 per cent, determined on 0.50 g by the semi-micro determination of water.

ASSAY

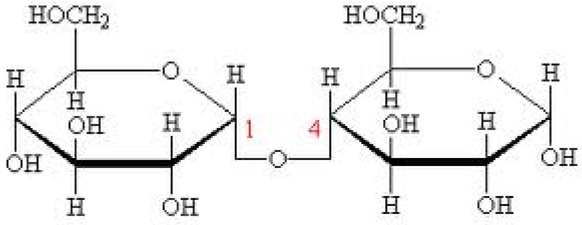
Liquid chromatography as described in the test for related substances with the following modification.

Injection: test solution and reference solution (a).

Calculate the percentage content of $C_6H_{12}O_6$ from the areas of the peaks and the assigned content of *glucose monohydrate CRS*.

IMPURITIES

A. Maltose



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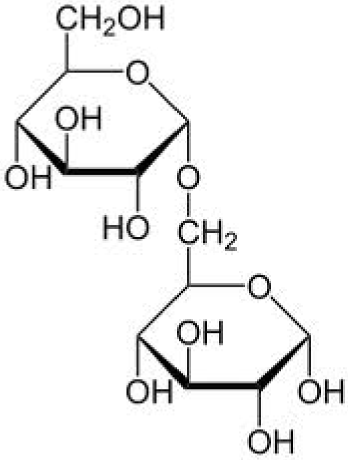
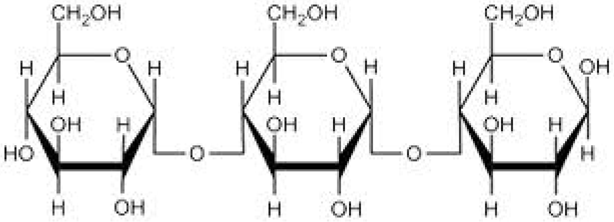
[2] Hydranal solvent and Hydranal 5 titrant are suitable

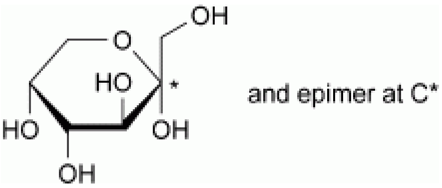
B. Isomaltose



C. Maltotriose



D. Fructose



REAGENTS

Hydrazine sulfate solution. Dissolve 1.0 g of hydrazine sulfate 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 sulfate 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.

Cation exchange resin (calcium form), strong.
A resin in calcium form with sulfonic acid groups attached to a polymer lattice consisting of polystyrene cross-linked with 8 per cent of divinylbenzene. The particle size is specified after the name of the reagent in the tests where it is used.

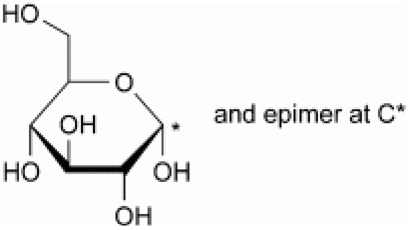
Fructose. $C_6H_{12}O_6$. (M_r 180.2). [57-48-7].

Maltose monohydrate. $C_{12}H_{22}O_{11}$, H_2O. (M_r 360.3). [6363-53-7].

Maltotriose. $C_{18}H_{32}O_{16}$. (M_r 504.4). [1109-28-0].

July 2025

E-56 GLUCOSE MONOHYDRATE



M_r 198.2

$C_6H_{12}O_6, H_2O$

DEFINITION

Glucose monohydrate is the monohydrate of (+)-D-glucopyranose and is derived from starch.
Content: 97.5 per cent to 102.0 per cent (anhydrous substance), determined by the LC described in the assay.

IDENTIFICATION

A. 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 reference solution (a).

B. Water (see tests).

TESTS

Appearance of solution. The test solution is clear (its clarity is the same as that of water or its opalescence is not more pronounced than that of reference suspension I) and not more intensely coloured than the reference solution.

Test solution: Dissolve 10.0 g in 15 mL of *water.*

Primary solutions:

- *Ferric chloride primary solution:* a 45.0 g/l solution of ferric chloride ($FeCl_3$, $6H_2O$).
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- *Copper sulfate primary solution:* a 62.4 g/l solution of copper sulfate ($CuSO_4$, $5H_2O$).

Reference solution:
To 2.5 mL of cobalt chloride primary solution, 6.0 mL of ferric chloride primary solution and 1.0 mL of copper sulfate primary solution, add hydrochloric acid (10 g/l HCl) to make 1000.0 mL.

Conductivity: maximum 20 µS·cm^{-1} at 25°C.
Dissolve 20.0 g in *carbon dioxide-free water* prepared from *distilled water* and dilute to 100.0 mL with the same solvent. Measure the conductivity of the solution while gently stirring with a magnetic stirrer.

Related substances. Liquid chromatography.

Test solution. Dissolve 0.330 g of the substance to be examined in *water*, and dilute to 10.0 mL with the same solvent.

Reference solution (a). Dissolve 0.330 g of *glucose monohydrate CRS* in *water* and dilute to 10.0 mL with the same solvent.

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Reference solution (c). Dilute 25.0 mL of reference solution (b) to 200.0 mL with *water.*

Reference solution (d). Dissolve 5 mg of *maltose* (impurity A), 5 mg of *maltotriose* (impurity C) and 5 mg of *fructose* (impurity D) in *water* and dilute to 50.0 mL with *water*.

Column:
- size: l = 0.3 m, Ø = 7.8 mm;
- stationary phase: strong cation-exchange resin (calcium form)[1] (9 µm);
- temperature: 85 +/- 1°C.

Mobile phase: degassed water.

Flow rate: 0.3 mL/min.

Detection: refractometer maintained at a constant temperature (40°C for example).

Injection: 20 µl of the test solution and reference solutions (b), (c) and (d).

Run time: 1.5 times the retention time of glucose.

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System suitability : reference solution (d) :
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[1] Agilent Metacarb 87C is suitable.

Limits:
- sum of impurities A and B: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent),
- impurity C: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent),
- impurity D: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.15 per cent),
- unspecified impurities for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent),
- total: not more than 1.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),
- disregard limit: area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Dextrin. Reflux 1 g of the substance to be examined finely powdered with 20 mL of ethanol (96 per cent): it dissolves completely.

Soluble starch and sulfites: maximum 15 ppm.
Dissolve 7.4 g of glucose monohydrate in 15 mL of *water* using a bath of boiling water. Allow to cool and add 25 µl of 0.05 M iodine: the solution is yellow.

Water[2]: 7.5 per cent to 9.5 per cent, determined on 0.25 g by the semi-micro determination of water.

ASSAY

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

Injection: test solution and reference solution (a).

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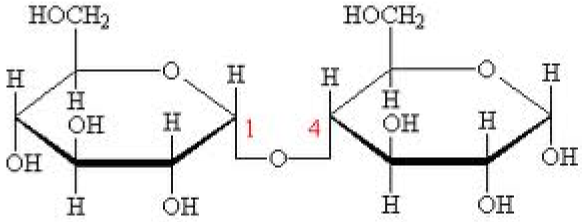

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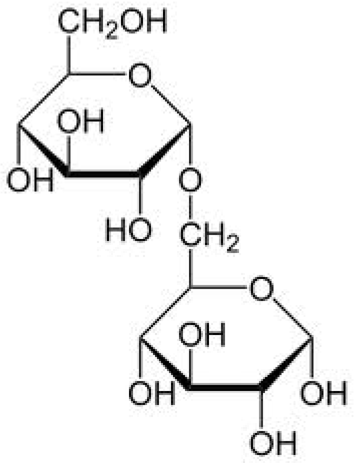
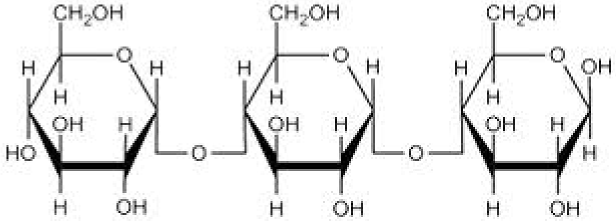
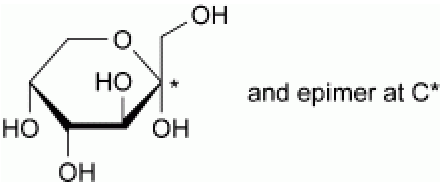
[2] Hydranal solvent and Hydranal 5 titrant are suitable



C. Maltotriose



D. Fructose



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Maltose monohydrate. $C_{12}H_{22}O_{11}$, H_2O. (M_r 360.3). [6363-53-7].

Maltotriose. $C_{18}H_{32}O_{16}$. (M_r 504.4). [1109-28-0].