PHARMACOPOEIAL DISCUSSION GROUP
SIGN-OFF DOCUMENT
CODE: E-58
NAME: MANNITOL

Harmonized Attributes

Attribute	EP	JP	USP
Definition	+	+	+
Identification by IR *	+	+	+
Appearance of solution	+	+	+
Conductivity	+	+	+
Melting point	+	+	+
Reducing sugars	+	+	+
Related substances	+	+	+
Nickel	+	+	+
Loss on drying	+	+	+
Microbial contamination	+	–	+
Bacterial endotoxins	+	–	+
Assay	+	+	+
Labelling	+	–	+

* EP and USP will use Mannitol Reference Standard; JP will use Reference Spectrum

Legend
+ will adopt and implement ; — will not stipulate

Non-harmonized attributes
Description/Characters, Heavy metals, Container/packaging and storage

Local requirements

EP	JP	USP
Identification (specific optical rotation, melting point, TLC) Absence of *Salmonella* Functionality-related characteristics		

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

Date: 06/06/2012

Signatures:

p.o.

European Pharmacopoeia

T. Kawanishi
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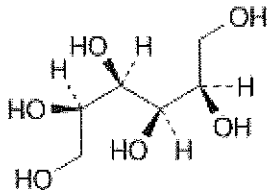
Japanese Pharmacopoeia

United States Pharmacopeia

E58 - MANNITOL

Stage 5B



$C_6H_{14}O_6$ M_r 182.2

DEFINITION

D-mannitol

Content: 97.0 per cent to 102.0 per cent (anhydrous substance).

IDENTIFICATION

Infrared absorption spectrophotometry. Record the infrared absorption spectrum of mannitol and compare with the Reference Spectrum or the spectrum obtained with the Reference Standard: the transmission minima correspond in position and relative size.

If the spectra obtained in the solid state shows differences, dissolve separately in 2 glass vials 25 mg each of the substance to be examined and of the reference substance in 0.25 mL of *distilled water* without heating. The solutions obtained are clear. Evaporate to dryness by one of the following methods: heat in a microwave oven with a power range of 600-700 W for 20 min or heat in an oven at 100 °C for 1 h then gradually apply vacuum until a dry residue is obtained. Non-sticky, white or slightly yellowish powders are obtained. Record new spectra using the residues.

TESTS

Appearance of solution. The test solution is clear and colourless: its clarity is the same as that of *water* or its opalescence is not more pronounced than that of *reference suspension I*, and it is not more intensely coloured than the reference solution.

Test solution. Dissolve 5.0 g in *water* and dilute to 50 mL with the same solvent.

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 sulphate primary solution:* a 62.4 g/L solution of *copper sulphate* ($CuSO_4$, $5H_2O$).

Reference solution:
To 3.0 mL of cobalt chloride primary solution, 3.0 mL of ferric chloride primary solution and 2.4 mL of copper sulphate 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 a *carbon dioxide-free water* prepared from *distilled water* by heating to 40-50°C and dilute to 100 mL with the same solvent. After cooling, measure the conductivity of the solution while gently stirring with a magnetic stirrer.

Melting point *(capillary method)*: 165°C to 170°C.

Reducing sugars: maximum 0.1% (expressed as glucose).

To 7.0 g of mannitol, add 13 mL of *water*. Boil gently with 40 mL of *cupri-tartaric solution* for 3 min, and allow to stand for about 2 min. A precipitate is formed. Filter through a sintered-glass filter (10-16 µm) coated with diatomaceous earth or a sintered-glass filter (5-10 µm). Wash the precipitate with hot *water* (at about 50-60°C) until the washing is no longer alkaline, and filter the washings through the filter described above. Discard all the filtrate at this step. Immediately, dissolve the precipitate in 20 mL of *ferric sulphate solution*, filter through the filter described above in a clean flask, and wash the filter with 15-20 mL of *water*. Combine the washings and the filtrate, heat to 80°C, and titrate with *0.02M potassium permanganate*. Not more than 3.2 mL is required to change the colour of the solution. The green colour turns to pink and the color persists at least 10 seconds.

Related substances: Liquid chromatography

Test solution. Dissolve 0.50 g of the substance to be examined in 2.5 mL of *water* and dilute to 10.0 mL with the same solvent.

Reference solution (a). Dissolve 0.50 g of *mannitol CRS* in 2.5 mL of water and dilute to 10.0 mL with the same solvent.

Reference solution (b). Dilute 2.0 mL of the test solution to 100.0 mL with *water*.

Reference solution (c). Dilute 0.5 mL of reference solution (b) to 20.0 mL with *water*.

Reference solution (d). Dissolve 0.25 g of *mannitol* and 0.25 g of *sorbitol* (impurity A) in 5 mL of water and dilute to 10.0 mL with the same solvent.

Reference solution (e). Dissolve 0.5 g of *maltitol* (impurity B) and 0.5 g of *isomalt* (impurity C) in 5 mL of *water* and dilute to 100 mL with the same solvent. Dilute 2 mL to 10 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 +/-2°C

Mobile phase: degassed *water*.

Flow rate: 0.5 mL/min.

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

[1] Aminex HPX-87C from Bio Rad is suitable

Injection: 20µL; inject the test solution and reference solutions (b), (c), (d) and (e).

Run time: 1.5 times the retention time of mannitol.

Relative retention with reference to mannitol (retention time = about 20 min): impurity C (first peak) = about 0.6; impurity B = about 0.69; impurity C (second peak) about 0.73, impurity A= about 1.2. Impurity C elutes in 2 peaks. Coelution of impurity B and the second peak of impurity C may be observed.

System suitability: reference solution (d):
- resolution: minimum 2.0 between the peaks due to mannitol and sorbitol (impurity A)

Limits:

- *impurity A :* not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);

- *sum of impurities B and C:* not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 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 the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);

- *disregard limit:* the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Nickel: maximum 1 ppm.

Determine the nickel by atomic absorption spectrometry - standard additions.

Test solution. Suspend 10.0 g of the substance to be examined in 30 mL of *dilute acetic acid* (115 g/L to 125 g/L of $C_2H_4O_2$), add *water* and shake to dissolve. Dilute to 100.0 mL with *water*. Add 2.0 mL of a saturated solution of *ammonium pyrrolidinedithiocarbamate* ($C_5H_{12}N_2S_2$) (about 10 g/L) and 10.0 mL of *water-saturated methyl isobutyl ketone* ($C_6H_{12}O$, 4-methyl-2-pentanone) and then shake for 30 s protected from bright light. Allow the layers to separate and use the methyl isobutyl ketone layer.

Reference solutions. Prepare 3 reference solutions in the same manner as the test solution but adding 0.5 mL, 1.0 mL and 1.5 mL respectively of *nickel standard solution (10 ppm Ni)* in addition to the 10.0 g of the substance to be examined.

Blank. Treat *water-saturated methyl isobutyl ketone* as described for preparation of the test solution omitting the substance to be examined.

Set the zero of the instrument using the blank. Measure the absorbance at 232.0 nm using a nickel hollow-cathode lamp as source of radiation and an air-acetylene flame. Between each measurement, rinse with water and ascertain that the readings return to zero with the blank.

Loss on drying: maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Microbial contamination (internationally harmonized methods):
TAMC: acceptance criterion 10^3 CFU/g.
TYMC: acceptance criterion 10^2 CFU/g.
Absence of Escherichia coli.
If intended for use in the manufacture of parenteral dosage forms: TAMC: acceptance criterion 10^2 CFU/g.

Bacterial endotoxins (internationally harmonized method):
If intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins, less than 4 UI/g for parenteral dosage forms having a concentration of 100g/L or less of mannitol, and less than 2.5 UI/g for parenteral dosage forms having concentration of more than 100 g/L of mannitol.

ASSAY

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

Injection: test solution and reference solution (a).

Calculate the percentage content of D-mannitol from the declared content of *mannitol CRS*.

LABELLING

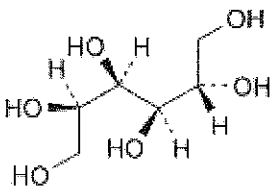
The label states:

- where applicable, the maximum concentration of bacterial endotoxins.

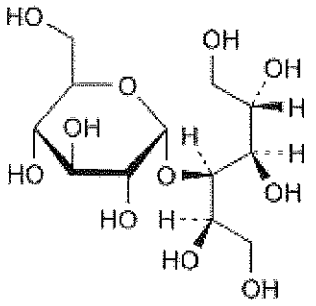
- where applicable, that the substance is suitable for use in the manufacture of parenteral dosage forms.

IMPURITIES

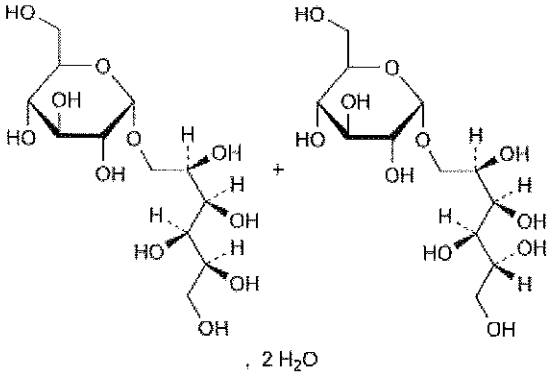
A. sorbitol



B. maltitol



C. isomalt



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.

Cation exchange resin (calcium form), strong. A resin in calcium form with sulphonic 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.

Sorbitol. $C_6H_{14}O_6$. (M_r 182.2). [50-70-4].

Maltitol. $C_{12}H_{24}O_{11}$. (M_r 344.3). [585-88-6].

Isomalt. $C_{12}H_{24}O_{11}$. (M_r 344.3). [64519-82-0].

Nickel standard solution (10 ppm Ni).

Immediately before use, dilute with *water* to 100 times its volume a solution containing *nickel sulphate* equivalent to 4.78 g of NiSO4,7H2O in 1000.0 mL.

Cupri-tartaric solution.

Solution I. Dissolve 34.6 g of *copper sulphate* ($CuSO_4,5H_2O$) in water and dilute to 500 mL with the same solvent.

Solution II. Dissolve 173 g of *sodium potassium tartrate* ($C_4H_4KNaO_6,4H_2O$) and 50 g of *sodium hydroxide* in 400 mL of *water*. Heat to boiling, allow to cool and dilute to 500 mL with *carbon dioxide-free water*.

Mix equal volumes of the 2 solutions immediately before use.

Water, carbon dioxide-free.

Water which has been boiled for a few minutes and protected from the atmosphere during cooling and storage.

Water-saturated methyl isobutyl ketone.

Shake *methyl isobutyl ketone* ($C_6H_{12}O$, 4-methyl-2-pentanone) with *water* prior to use.

Ferric sulphate solution. Dissolve 50 g of *iron (III) trisulphate hydrated* ($Fe_2(SO_4)_3,xH_2O$) in a excess of water, add 200 mL of *sulphuric acid* and dilute to 1000 mL with *water*.