PHARMACOPOEIAL DISCUSSION GROUP

CODE: E-64
NAME: ISOMALT

CORRECTION 1

Harmonized Attributes

Attribute	EP	JP	USP
Definition	+	+	+
Identification	+	+	+
Conductivity	+	+	+
Reducing sugars	+	+	+
Related substances	+	+	+
Nickel	+	+	+
Water	+	+	+
Assay	+	+	+
Labelling	+	+	+

Legend
+ will adopt and implement ; — will not stipulate

Non-harmonized attributes
Description (incl. test for optical rotation)/Characters, ~~Heavy metals,~~ Packaging and storage

Local requirements

EP	JP	USP
Identification (TLC, colour reaction)	Identification (colour reaction); Heavy metals	Identification by TLC; assay (RSD NMT 2.0%)

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

Date: Nov. 29, 2016.

Signatures:

European Pharmacopoeia

Japanese Pharmacopoeia
Harukiro Okuda
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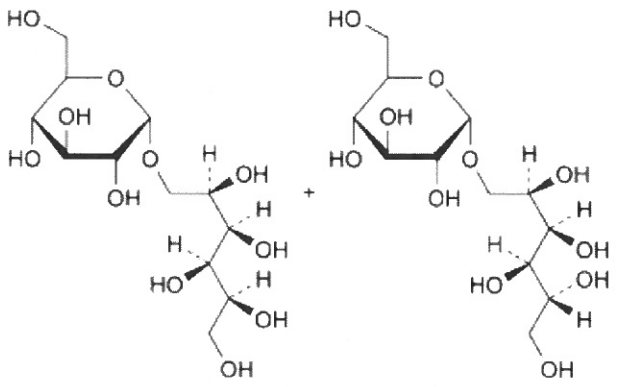
United States Pharmacopeia
CATHERINE SHEEHAN

E-64 ISOMALT



, 2 H_2O

$C_{12}H_{24}O_{11}$ M_r 344.3

$C_{12}H_{24}O_{11}$, $2H_2O$ M_r 380.3

DEFINITION

Mixture of 6-*O*-α-D-glucopyranosyl-D-glucitol (6-*O*-α-D-glucopyranosyl-D-sorbitol; 1,6-GPS) and 1-*O*-α-D-glucopyranosyl-D-mannitol (1,1-GPM).

Content: 98.0 per cent to 102.0 per cent for the mixture of 1,6-GPS and 1,1-GPM and neither of the 2 components is less than 3.0 per cent (anhydrous substance).

IDENTIFICATION

Liquid chromatography

Examine the chromatograms obtained in the assay.

Results: the 2 principal peaks in the chromatogram obtained with the test solution are similar in retention time to the 2 principal peaks in the chromatogram obtained with reference solution (a).

TESTS

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

Dissolve with gentle heating (40-50 °C) 20.0 g in *carbon dioxide-free water*, cool and dilute to 100.0 mL with the same solvent. Measure the conductivity of the solution while gently stirring with a magnetic stirrer.

Reducing sugars: maximum 0.3 per cent (expressed as glucose).

Dissolve 3.3 g in 10 mL of *water* with the aid of gentle heat. Cool and add 20 mL of *cupri-citric solution* and a few glass beads. Heat so that the boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 mL of a 2.4 per cent *V/V* solution of *glacial acetic acid* and 20.0 mL of *0.025 M iodine*. With continuous shaking, add 25 mL of a mixture of 6 volumes of *hydrochloric acid* and 94 volumes of *water*. When the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulfate* using 1 mL of *starch solution* as indicator, added towards the end of the titration. Not less than 12.8 mL of *0.05 M sodium thiosulfate* is required.

Related substances. Liquid chromatography.

Test solution. Dissolve 0.200 g of the substance to be examined in 4 mL of *water* and dilute to 10.0 mL with the same solvent.

Reference solution (a). Dissolve 0.200 g of *isomalt CRS* in 4 mL of *water* and dilute to 10.0 mL with the same solvent.

Reference solution (b). Dissolve 10.0 mg of *sorbitol CRS* (impurity C) and 10.0 mg of *mannitol CRS* (impurity B) in 20 mL of *water R* and dilute to 100.0 mL with the same solvent.

Precolumn:

— *size*: *l* = 30 mm, Ø = 4.6 mm;

— *stationary phase*: *strong cation-exchange resin (calcium form)* (9 µm);

— *temperature*: 80 ± 3 °C.

Column:

— *size*: *l* = 300 mm, Ø = 7.8 mm;

— *stationary phase*: *strong cation-exchange resin (calcium form)* (9 µm)[1];

— *temperature*: 80 ± 3 °C.

Mobile phase: degassed *water*.

Flow rate: 0.5 mL/min.

[1] (Information for the PDG only. Not to be published in the regional monograph)
Aminex® HPX-87C (Bio Rad), Repro-Gel® (Dr. Maisch), Nucleogel® Sugar 810 Ca (Macherey-Nagel), Rezex® RCM Monosaccharide Ca2+ (Phenomenex®) are suitable.

CXS
adf.

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

Injection: 20 µL.

Run time: 2.5 times the retention time of 1,1-GPM.

Relative retention with reference to 1,1-GPM (retention time = about 12 min): 1,6-GPS = about 1.2; impurity B = about 1.6; impurity C = about 2.0.

System suitability: reference solution (a)

— *resolution:* minimum 2.0 between the peaks due to 1,1-GPM and 1,6 GPS.

Limits:

— *impurities B, C*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *any other impurity*: for each impurity, not more than the area of the peak due to impurity C in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *total*: not more than 4 times the area of the peak due to impurity C in the chromatogram obtained with reference solution (b) (2.0 per cent);

— *disregard limit*: 0.2 times the area of the peak due to impurity C in the chromatogram obtained with reference solution (b) (0.1 per cent).

Nickel: maximum 1 ppm.

Determine the nickel by atomic absorption spectrometry – standard additions.

Test solution. Dissolve an amount of the substance to be examined corresponding to 10.0 g of anhydrous substance in 30 ml of *dilute acetic acid* (115 g/l to 125 g/l of $C_2H_4O_2$) and dilute to 100.0 ml with water. Add 2.0 ml of a solution of *ammonium pyrrolidinedithiocarbamate* ($C_5H_{12}N_2S_2$) at about 10g/l and 10.0 ml of *water-saturated methyl isobutyl ketone* 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. Prepare the blank in the same manner as the test solution, but 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.

Water: maximum 7.0 per cent.

Determined on 0.3 g by semi-micro-determination. Use as solvent, a mixture of 20 mL of *anhydrous methanol* and 20 mL of *anhydrous formamide* at 50 ± 5 °C.

ASSAY

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

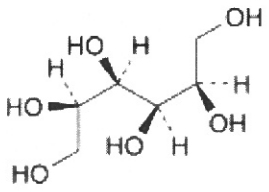
Injection: test solution and reference solution (a).

Calculate the percentage content of isomalt (1,1-GPM and 1,6-GPS) from the declared contents of 1,1-GPM and 1,6-GPS in *isomalt CRS*.

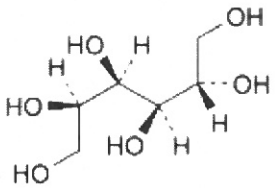
LABELLING

The label states the percentage contents of 1,6-GPS and of 1,1-GPM.

IMPURITIES



B. D-mannitol,



C. D-glucitol (D-sorbitol),

REAGENTS

Cation exchange resin (calcium form), strong.

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.

Cupri-citric solution.

Dissolve 25 g of *copper sulfate* ($CuSO_4,5H_2O$), 50 g of *citric acid* and 144 g of *anhydrous sodium carbonate* (Na_2CO_3)in *water* and dilute to 1000 mL with the same solvent.

Hydrochloric acid, dilute.

Contains 73 g/L of HCl. Dilute 20 g of *hydrochloric acid* to 100 mL with *water*.

0.5 M Iodine.

Dissolve 127 g of *iodine* and 200 g of *potassium iodide* in *water* and dilute to 1000.0 mL with the same solvent.

Methyl isobutyl ketone, water saturated.

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

Nickel standard solution (10 ppm Ni).

Immediately before use, dilute with *water* to 100 times its volume a solution containing *nickel sulfate* equivalent to 4.78 g of $NiSO_4,7H_2O$ in 1000.0 mL.

0.1 M Sodium thiosulfate.

Dissolve 25 g of *sodium thiosulfate* and 0.2 g of *sodium carbonate* in *carbon dioxide-free water* and dilute to 1000.0 mL with the same solvent.

Starch solution.

Triturate 1.0 g of *soluble starch* with 5 mL of *water* and whilst stirring pour the mixture into 100 mL of boiling *water R* containing 10 mg of *mercuric iodide* (HgI_2).

Water, carbon dioxide-free.

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

Anhydrous formamide. Complies with the requirements prescribed for *formamide* with the following additional requirement.

Water: maximum 0.1 per cent determined with an equal volume of *anhydrous methanol*.