ISOMALT

Prepared at the 46th JECFA (1996), published in FNP 52 Add 4 (1996) superseding specifications prepared at the 39th JECFA (1992), published in FNP 52 Add 1 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). An ADI 'not specified' was established at the 29th JECFA (1985)

SYNONYMS Hydrogenated isomaltulose; INS No. 953

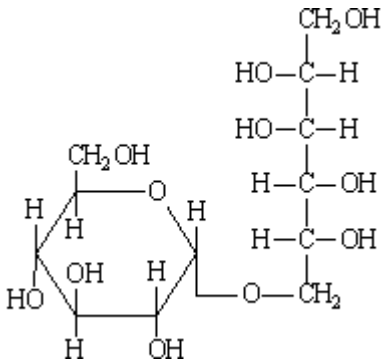
DEFINITION A mixture of hydrogenated mono- and disaccharides whose principal components are the disaccharides:

Chemical names
6-O-alpha-D-Glucopyranosyl-D-sorbitol (1,6-GPS) and
1-O-alpha-D-Glucopyranosyl-D-mannitol dihydrate (1,1-GPM)

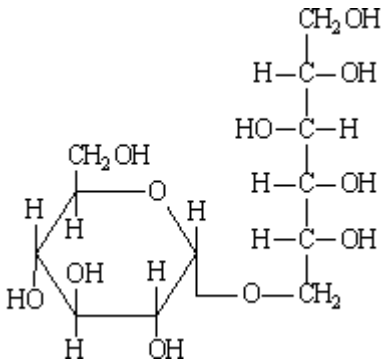
C.A.S. number
64519-82-0

Chemical formula
6-O-alpha-D-Glucopyranosyl-D-sorbitol: $C_{12}H_{24}O_{11}$
1-O-alpha-D-Glucopyranosyl-D-mannitol dihydrate: $C_{12}H_{24}O_{11} \cdot 2H_2O$

Structural formula



6-O-alpha-D-Glucopyranosyl-D-sorbitol



1-O-alpha-D-Glucopyranosyl-D-mannitol (without molecules of crystal water)

Formula weight
6-O-alpha-D-Glucopyranosyl-D-sorbitol: 344.32
1-O-alpha-D-Glucopyranosyl-D-mannitol dihydrate: 380.32

Assay
Not less than 98% of hydrogenated mono- and disaccharides and not less than 86% of the mixture of 6-O-alpha-D-glucopyranosyl-D-sorbitol

and 1-O-alpha-D-glucopyranosyl-D-mannitol on the anhydrous basis

DESCRIPTION Odourless, white, crystalline slightly hygroscopic substance

FUNCTIONAL USES Sweetener, bulking agent, anticaking agent, glazing agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water, very slightly soluble in ethanol
Thin layer chromatography (Vol. 4)	Passes test See description under TESTS

PURITY

Water (Vol. 4)	Not more than 7.0% (Karl Fischer Method)
Sulfated ash (Vol. 4)	Not more than 0.05% Test 5 g of the sample (Method I)
D-Mannitol	Not more than 3% See Method of Assay
D-Sorbitol	Not more than 6% See Method of Assay
Reducing sugars (Vol. 4)	Not more than 0.3% Proceed as directed under *Reducing Substances (as glucose),* Method II. The weight of cuprous oxide shall not exceed 50 mg.
Nickel (Vol. 4)	Not more than 2 mg/kg See description under TESTS
Lead (Vol. 4)	Not more than 1 mg/kg Prepare a sample solution as directed in the Limit Test for organic compounds and determine the lead content by *atomic absorption spectrometry*

TESTS

IDENTIFICATION TESTS

Thin layer chromatography	TLC plates TLC aluminium foils or plates of approx. 12 cm length and coated with a layer of approx. 0.2 mm, Kieselgel 60 F_{254}, Art. 5554, Merck, or equivalent Reference solution Dissolve 500 mg of each of the following sugar alcohols in 100 ml of water: Sorbitol, mannitol, lactitol, maltitol, 1-O-alpha-D-gluco-pyranosyl-D-mannitol (1,1-GPM), and 6-O-alpha-D-glucopyranosyl-D-sorbitol (1,6-GPS)

Test solution
Dissolve 500 mg of sample in 100 ml of water

Solvent A
Isopropanol:n-butanol:aqueous boric acid solution (25 mg/ml):acetic acid:propionic acid (50:30:20:2:16;v/v)

Solvent B
Ethylacetate:pyridine:water:acetic acid:propionic acid (50:50:10:5:5;v/v)

Detecting solutions
I 0.1% Na-metaperiodate in water (w/w)
II ethanol:sulfuric acid:anisaldehyde:acetic acid (90:5:1:1;v/v)

Procedure
Apply approximately 0.3 µl each of the reference and test solution to the bottom of the TLC plate. Dry the spots in warm air. Develop the plate to a height of 10 cm in a developing chamber containing either solvent A or solvent B. Allow the plate to dry in warm air and dip the plate for up to 3 sec into Detecting solution I.

Dry the plate in hot air. Note: The plate should be completely dry on both sides. Dip the plate in Detecting solution II up to 3 sec and dry in hot air until coloured spots become visible. Optionally, the background colour may be brightened in warm steam.

The approximate R_f values and colours of the spots on the TLC-plate of the substances specified above are described as "Compound / Colour / Solvent A(R_f) / Solvent B(R_f)". See below.

mannitol / reddish (light) / 0.36 / 0.40
sorbitol / brown / 0.36 / 0.36
GPM / blue-grey / 0.28 / 0.16
GPS / blue-grey / 0.25 / 0.13
maltitol / green / 0.26 / 0.22
lactitol / olive-green / 0.23 / 0.14

The R_f values may vary slightly depending on the commercial source of the silica gel plates.

The principal spots in the chromatogram obtained from a test solution of isomalt are similar in R_f value and colour to GPM and GPS.

PURITY TESTS

Nickel

Test solution
Dissolve 20.0 g of the substance to be examined in a mixture of equal volumes of dilute acetic acid TS* and water and dilute to 100 ml with the same mixture of solvents. Add 2.0 ml of a 1% w/v solution of ammonium

pyrrolidinedithiocarbamate and 10 ml of methyl isobutyl ketone. Mix and allow the layers to separate and use the methyl isobutyl ketone layer.

Reference solution
Prepare three reference solutions in the same manner as the test solution but adding 0.5 ml, 1.0 ml, and 1.5 ml, respectively, of a standard nickel solution containing 10 mg/kg Ni, in addition to the 20.0 g of the substance to be tested.

Procedure
Set the instrument to zero using methyl isobutyl ketone as described for the preparation of the test solution but omitting the substance to be examined. Measure the absorbance at 232.0 nm using a nickel hollow-cathode lamp as source of radiation and an air-acetylene flame.

METHOD OF ASSAY

Internal standard solution
Dissolve suitable quantities of phenyl-ß-D-glucopyranoside and maltitol in water to obtain a solution of about 1 mg phenyl-ß-D-glucopyranoside and 50 mg maltitol per g water.

Standard solutions
Dissolve accurately weighed quantities of 1-O-alpha-D-glucopyranosyl-D-mannitol (1,1-GPM) and 6-O-alpha-D-glucopyranosyl-D-sorbitol (1,6-GPS), calculated as dry substance, in water to obtain two separate solutions having a concentration of about 50 mg per g each. Also prepare an aqueous standard solution containing approx. 1 mg mannitol and 1 mg sorbitol per g.

Sample solution
Dissolve an accurately weighed quantity of the sample (approx. 1 g) in water to obtain a concentration of about 10 g per 100 g.

Procedure
Pipet 100.0 mg of standard solution or sample solution into a glass tube fitted with a screw cap and add 100.0 mg of internal standard solution. Remove the water by lyophilization and dissolve the residue in 1.0 ml of pyridine. Add 4 mg O-benzyl-hydroxylamine hydrochloride, and cap the tube and set it aside for 12 h at room temperature. Then, add 1 ml of N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) and heat to 80º for 12 h shaking occasionally and allow to cool. Inject 1 µl portions of these solutions directly into a gas chromatograph under the following operating conditions with helium as carrier gas (initial flow rate: approx. 1 ml/min at 80º and 1 atm; split flow: 25 ml/min):
Column: Fused silica HT-8 (25 m x 0.22 mm x 0.25 µm), or equivalent
Injector: Programmed temperature vaporizer: 30º; 270º/min to 300º (49 min)
Detector: Flame ionization detector; 360º
Temperature program: 80º (3 min); 10º/min to 210º; 5º/min to 350º (6 min)

Approximate retention times
Hydrogenated monosaccharides:
Mannitol 19.5 min

Sorbitol 19.6 min
Internal standards:
Phenyl-ß-D-glucopyranoside 26.8 min
Maltitol 33.5 min
Hydrogenated disaccharides (32 - 36 min)
1,1-GPS 33.9 min
1,1-GPM 34.5 min
1,6-GPS 34.6 min

Calculate the percentages of the individual components, w_I, in the sample according to the following formula:

$$w_I\,(\%) = \frac{a_I \times m_S}{F_I \times a_S \times m_{ISOMALT}} \times 100$$

where
a_I = peak area of component I (µV·s)
a_S = peak area of internal standard (µV·s)
m_S = mass of internal standard used for derivatization (mg d.s.)
$m_{ISOMALT}$ = mass of sample used for derivatization (mg d.s.)
F_I = relative response factor f_I/f_S
f_I = response factor of component I: $f_I = (a_I/m_I) \times (100/\%\ \text{purity})$
f_S = response factor of internal standard: $f_S = (a_S/m_S) \times (100/\%\ \text{purity})$
m_I, m_S = mass of component I or internal standard used for derivatization of standard sample (mg d.s.)

Note: Use maltitol as internal standard for the calculation of hydrogenated disaccharides (e.g. 1,1-GPM, 1,6-GPS) and phenyl-ß-D-glucoside for the calculation of hydrogenated monosaccharides (mannitol, sorbitol). For the total of other saccharides (hydrogenated or not) subtract the sum of 1,1-GPM, 1,6-GPS, sorbitol and mannitol from 100%.