ISOMALT


**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)
  - 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\(_2\)O

- **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-glucopyranosyl-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<sub>f</sub> values and colours of the spots on the TLC-plate of the substances specified above are described as "Compound / Colour / Solvent A(R<sub>f</sub>) / Solvent B(R<sub>f</sub>)". 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<sub>f</sub> 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<sub>f</sub> 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-β-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:

\[
\frac{w_I}{100} = \frac{a_i x m_s}{a_s x m_{ISOMALT}} \times \frac{F_i}{f_s} \times x \frac{100}{\% \text{ purity}}
\]

where
\( a_i = \) peak area of component I (\( \mu \text{V} \cdot \text{s} \))
\( a_s = \) peak area of internal standard (\( \mu \text{V} \cdot \text{s} \))
\( m_s = \) 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: \( f_i = (a_i/m_i) x (100/\% \text{ purity}) \)
\( f_s = \) response factor of internal standard: \( f_s = (a_s/m_s) x (100/\% \text{ purity}) \)
\( m_i, m_s = \) 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-β-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\%. 