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Method of Test for Isomaltooligosaccharides in Beverages

1. Scope

This method is applicable for the determination of isomaltose, panose and isomaltotriose in beverages.

2. Method

After filtration, analytes are determined by high performance liquid chromatography (HPLC).

2.1. Equipment

2.1.1. High performance liquid chromatograph

2.1.1.1. Detector: refractive index detector.

2.1.1.2. Column: ACQUITY UPLC BEH Amide, 1.7 μm, 2.1 mm i.d. × 15 cm, or an equivalent product.

2.1.2. Centrifuge: centrifugal force ≥ 5000 ×g.

2.1.3. Ultrasonicator.

2.1.4. Vortex mixer.

2.2. Chemicals

Acetonitrile, HPLC grade;
Triethylamine, reagent grade;
Deionized water, resistivity ≥ 18 MΩ·cm (at 25°C);
Isomaltose, panose and isomaltotriose, reference standards.

2.3. Apparatus

2.3.1. Volumetric flask: 5 mL.

2.3.2. Centrifuge tube: 15 mL, PP.

2.3.3. Membrane filter: 0.45 μm, PVDF.

2.4. Reagents

2.4.1. 77% acetonitrile

Dilute 770 mL of acetonitrile with deionized water to 1000 mL.

2.5. Mobile phase preparation

Dilute 2 mL of triethylamine with 77% acetonitrile to 1000 mL. Filter with a membrane filter, and the filtrate as the mobile phase.

2.6. Standard solution preparation

Transfer about 50 mg of isomaltose, panose and isomaltotriose reference standards accurately weighed to each 5-mL volumetric flask. Dissolve and dilute to volume with deionized water as the standard stock solutions. Store under refrigeration. When to use, mix appropriate volume of each standard

stock solution, and dilute with deionized water to 0.25~10 mg/mL as the standard solutions.

2.7. Sample solution preparation

Remove the carbon dioxide by ultrasonication for the sample containing carbon dioxide. Accurately transfer 10 mL of the sample into a 15-mL centrifuge tube, and centrifuge at 5000 ×g for 5 min. Collect the supernatant, and filter with a membrane filter. Take the filtrate as the sample solution.

2.8. Identification and quantification

Accurately inject 3 μL of the sample solution and the standard solutions into HPLC separately, and operate according to the following conditions. Identify each sugar based on the retention time. Calculate the amount of isomaltose, panose or isomaltotriose in the sample by the following formula:

The amount of isomaltose, panose or isomaltotriose in the sample (g/100 mL)

$$= \frac{C}{10}$$

C：the concentration of isomaltose, panose or isomaltotriose in the sample solution calculated by the standard curve (mg/mL)

HPLC operating condition(note)：

Detector: refractive index detector.
Column: ACQUITY UPLC BEH Amide, 1.7 μm, 2.1 mm i.d. × 15 cm.
Column temperature: 40°C.
Detector temperature: 40 °C.
Injection volume: 3 μL.
Mobile phase: prepared as section 2.5.
Flow rate: 0.25 mL/min.

Note: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

Remark

1. Limits of quantification (LOQs) are 0.025 g/100 mL for isomaltose, panose and isomaltotriose.
2. Further validation should be performed when interfering compounds appear in samples.

References

Nakanishi, T., Nomura, S. and Takeda, Y. 2006. An improved method for the quantitative analysis of commercial isomaltooligosaccharide products using the calibration curve of standard reagents. J. Appl. Glycosci. 53: 215-222.

Reference chromatogram

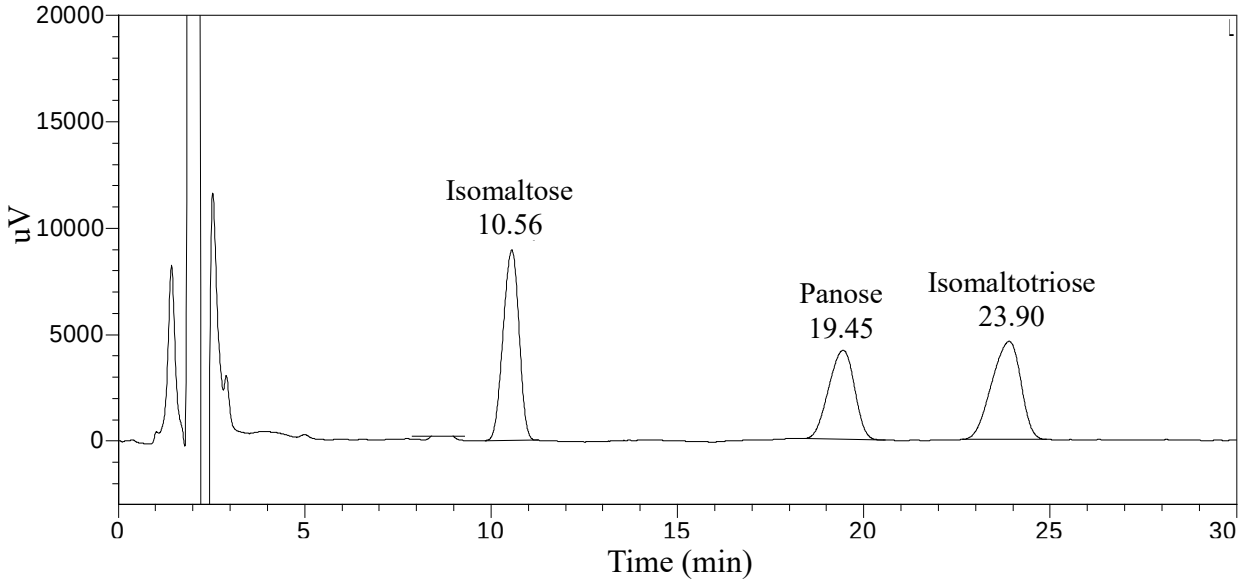

Figure. The HPLC chromatogram of isomaltose, panose and isomaltotriose standards.