Japan Customs Analysis Methods

No. 304

Quantitative Analysis of Sorbitol

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1. Scope

This analysis method is applied to sorbitol of subheading 2905.44 or 3824.60 of Customs Tariff Law (Appendix Table – Customs Tariff Schedule) which requires the determination of its purity.

2. Outline of Test Method

In this method, sorbitol content is determined either by an n-butane boronic acid derivatization-gas chromatography (GC) with internal standard method using methyl nonadecanate as an internal standard or by high-performance liquid chromatography (HPLC) with internal standard method using propylene glycol as an internal standard.

3. Apparatus

(1) For GC method

・ Gas chromatograph (GC), equipped with a flame ionization detector.

(a) Column

2 m × 3 mm (i.d.) glass column, packed with a solid phase carrier (e.g., Chromosorb WAW, DMCS, 100 – 120 meshes) coated with silicone OV-225 as the liquid phase, or its equivalent.

(b) Operating parameters

Column temperature, 220 °C; Injection port temperature, 250 °C; Carrier gas, nitrogen or helium. Flow rates of carrier gas, hydrogen gas and air should be adjusted to be the optimum conditions for a gas chromatograph used.

(2) For HPLC method

・ High-performance liquid chromatograph (HPLC), equipped with a refractive index detector (RID).

(a) Column

Calcium type cation-exchange resin gel column, 7.9 mm i.d. × 300 mm, or its equivalent.

(b) Operating parameters

Column temperature, 75 °C; Mobile phase, Water (HPLC grade); Flow rate, 0.8 ml/min; Injection volume, 20 µl.

4. Reagents

All chemicals must be JIS special reagent grade or equivalent, unless otherwise specified.

(a) n-butane boronic acid (available as a derivatization reagent in gas chromatography.)

Due to its hydroscopic property, once its package is opened, it should tightly be sealed again and kept in a desiccator.

(b) Methyl nonadecanate (internal standard substance)

(c) Propylene glycol (internal standard substance)

(d) Sorbitol

5. Procedure

5.1. Determination of Moisture Content

For liquid samples, accurately weigh about 2 g of the sample into a weighing bottle dried to a constant weight after placing sea sand in. Place the weighing bottle in an oven and dry the content for 1 hour at a constant temperature of 105 °C. Remove it from the oven, cool it to room temperature in a desiccator and weigh. Repeat until the loss in weight does not exceed 2

mg per 1 hour drying period. For solid samples, accurately weigh about 2 g of the sample in a weighing bottle previously dried, and dry the content in the same manner as with liquid samples. Constant weight is attained, when the loss in weight does not exceed 2 mg per 1 hour drying period.

Calculate the moisture content from the following formula. Round off the numerical value to one decimal place.

$$\%, \text{moisture} = \frac{W_0 - W_1}{W_0} \times 100$$

Where- W_0: Amount (g) of test sample collected

W_1: Weight (g) of test sample after drying

5.2. Determination of Sorbitol Content

A. GC Method

A.1. Preparation of Sorbitol Standard Solution

Accurately weigh about 150 mg of sorbitol in a beaker, and dissolve it in a small amount of water. Transfer the solution to a 100 ml volumetric flask, and dilute to volume with methanol.

A.2. Preparation of n-Butane Boronic Acid-Methyl Nonadecanate (Internal Standard) Solution

Accurately weigh about 200 mg of n-butane boronic acid and about 40 mg of methyl nonadecanate in a beaker, and dissolve them in pyridine. Transfer the solution into a 20 ml volumetric flask, and dilute to volume with pyridine.

A.3. Preparation of Sample Solution

Accurately weigh the amount of sample corresponding to 150 mg of sorbitol in a beaker, and dissolve it in a small amount of water. Transfer the solution into a 100 ml volumetric flask, and dilute to volume with methanol.

A.4. Preparation of Derivatization Solution and Gas Chromatography

Accurately take 1 ml each of the sorbitol standard solution and sample solution using whole pipets, and place them into separate 5 ml vials. Place the vials on a hot water bath maintained at around 70 °C to evaporate the solvent (methanol). Then, evaporate the residues to dryness in a vacuum drying oven at 70 °C.

Accurately add 1ml of the n-butane boronic acid-methyl nonadecanate (internal standard) solution to each vial using whole pipets. Shake the vials gently. After the content of the solution is completely dissolved, leave the vials to stand for 20 min. Inject the solutions into GC.

A.5. Calculation of Sorbitol Content

Based on peak areas of sorbitol and the internal standard substance in the obtained gas chromatograms, calculate sorbitol content (on the dry matter) in the sample according to the following formula. Round to the first decimal place.

$$\%, \text{sorbitol} = \frac{A \times D}{B \times C} \times \frac{100}{(100 - M)} \times 100$$

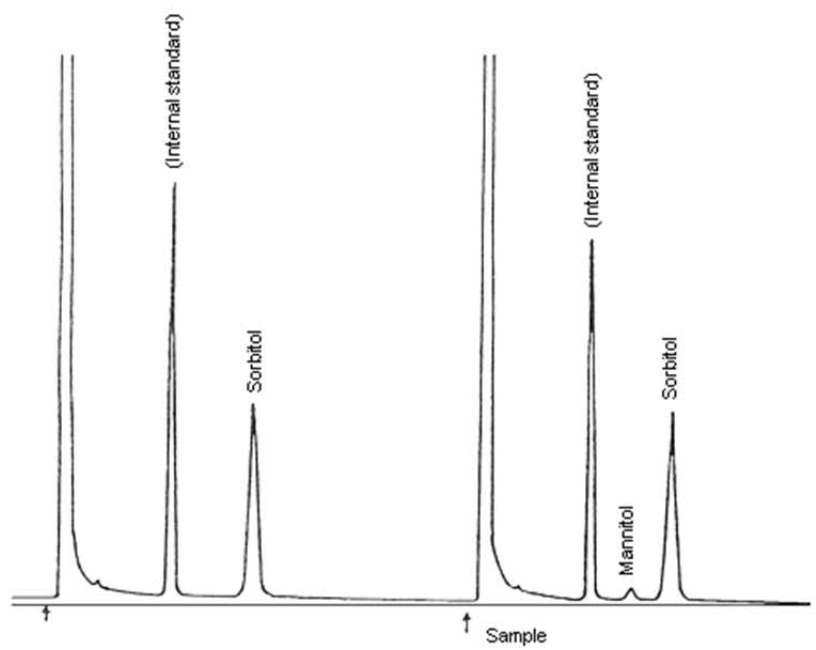
Where- A: Amount (mg) of standard sorbitol collected

B: Amount (mg) of test sample collected

C: The peak area ratio of sorbitol derivative to methyl nonadecanate, based on a gas chromatogram for standard sorbitol (i.e. the peak area of sorbitol derivative / the peak area of methyl nonadecanate)

D: The peak area ratio of sorbitol derivative to methyl nonadecanate, based on a gas chromatogram for the test sample (i.e., the peak area of sorbitol derivative / the peak area of methyl nonadecanate)

M: Mositure content (%) in the test sample



Reference 1 Examples of gas chromatograms

B. HPLC Method

B.1. Preparation of Propylene Glycol (Internal Standard) Solution

Accurately weigh 10 g of propylene glycol in a 100 ml volumetric flask, and dilute to volume with water.

B.2. Preparation of Sorbitol Standard Solution

Accurately weigh 0.5, 1 and 2 g of sorbitol in separate 100 ml volumetric flasks. Accurately add 10 ml of the propylene glycol (internal standard) solution prepared in B.1. to each of the flasks, using a whole pipet. Dilute to volume with water.

B.3. Preparation of Sample Solution

Accurately weigh sample of an amount corresponding to 1 g of sorbitol into a 100 ml volumetric flask. Accurately add 10 ml of the propylene glycol (internal standard) solution prepared in B.1. to the flask, using a whole pipet. Dilute to volume with water.

B.4. High Performance Liquid Chromatography

Filter the sorbitol standard solutions prepared in B.2. and the sample solution prepared in B.3. through a membrane filter, and place the respective filtrates into 1 ml vials,. Inject the filtrates into HPLC.

B.5. Preparation of Calibration Curve

Measure the peak areas of sorbitol and propylene glycol (internal standard) in the HPLC chromatograms of the sorbitol standard solutions prepared in B.4. Calculate the peak area ratio (Ax/As) of sorbitol (Ax) to propylene glycol (internal standard) (As). Construct a calibration curve by plotting the calculated peak area ratio (Ax/As) against the weight ratio (Wx/Ws) of sorbitol to propylene glycol (internal standard).

B.6. Calculation of Sorbitol Content

Measure the peak areas of sorbitol and propylene glycol (internal standard) in the HPLC chromatogram of the test sample obtained in B.4. Calculate the area ratio of sorbitol to propylene glycol. Calculate the weight ratio (Wx/Ws) of sorbitol (Wx) to propylene glycol (internal standard) (Ws) from the calibration curve constructed in B.5.

Calculate the sorbitol content in the test sample using the following formula (Round to the

first decimal place.).

$$\%, \text{sorbitol (on the dry matter)} = \frac{(W_X/W_S) \times Ms}{S \times 1000} \times \frac{100}{100 - W} \times 100$$

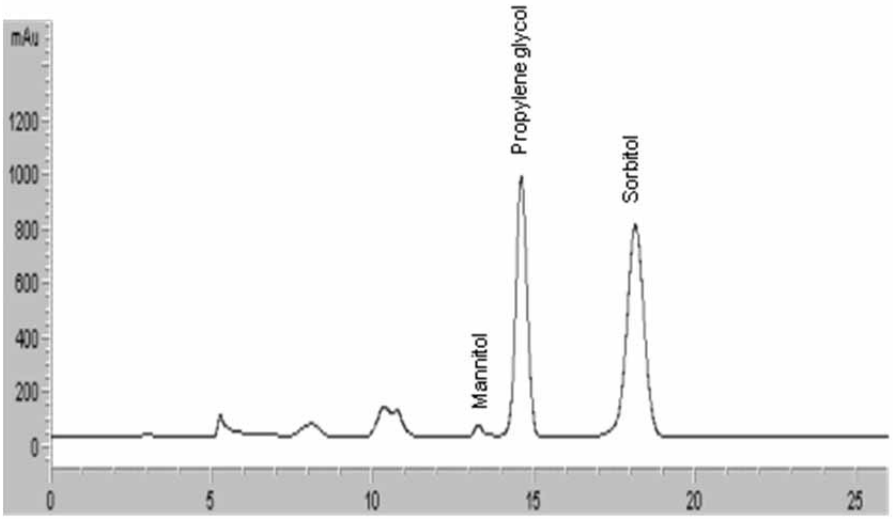
Where -

Wx/Ws: Weight ratio of sorbitol to propylene glycol, calculated based on the calibration curve

Ms: Amount (mg) of propylene glycol in the 100 ml of test solution prepared

S: Amount (g) of test sample collected

W: Moisture content (%) in test sample



Reference 2 Example of HPLC chromatogram

Measurement conditions: Separation Column, Shim-Pack SCR 101C 7.9 mm i.d. × 300 mm; Column temperature, 75 °C; Mobile phase, Water; Flow rate, 0.8 ml/min; Detector, RID.

6. References

(1) The United State Pharmacopeia 18ed.(1970)

(2) JAPAN'S SPECIFICATIONS AND STANDARDS FOR FOOD ADDITIVES, the Seventh Edition