Method of Test for Preservatives in Foods- Test of Propionic Acid

1. Scope

This method is applicable to the determination of propionic acid in foods.

2. Method

After extraction or distillation, propionic acid is determined by high performance liquid chromatograph (HPLC).

2.1. Equipments

2.1.1. High performance liquid chromatograph

2.1.1.1. Detector: photodiode array detector.

2.1.1.2. Column: ZORBAX SB-Aq, 5 μm, 4.6 mm i.d. × 25 cm, or an equivalent product.

2.1.2. Blender

2.1.3. Ultrasonicator.

2.1.4. Centrifuge: > 3500 xg.

2.1.5. Distillation apparatus.

2.1.6. pH meter.

2.2. Chemicals

Phosphoric acid, 85%, AR grade;

Diammonium hydrogen phosphate, AR grade;

Deionized water, resistivity ≥ 18 MΩ · cm (at 25℃);

Propionic acid, reference standard.

2.3. Apparatus

2.3.1. Centrifuge tube: 50 mL, PP.

2.3.2. Volumetric flask: 50 mL, 100 mL and 500 mL.

2.3.3. Distillation flask: 1000 mL.

2.3.4. Membrane filter: 0.45 μm, Nylon.

2.4. Reagents

2.4.1. 1 M phosphoric acid

Dilute 67.4 mL of phosphoric acid with deionized water to 1000 mL.

2.5. Mobile phase

Dissolve 1.5 g of diammonium hydrogen phosphate with deionized water to 1000 mL. Adjust pH to about 3.0 with 1 M

phosphoric acid, and filter with a membrane filter.

2.6. Standard solution preparation

Transfer about 1 g of propionic acid reference standard accurately weighed into a 100-mL volumetric flask, dissolve and dilute with deionized water to volume as the standard stock solution, and then keep refrigerated. When to use, dilute 5.0 mL of the standard stock solution with deionized water to 50 mL, then transfer 0.25-5.0 mL of the solution into each 10-mL volumetric flask, add 0.2 mL of 1 M phosphoric acid, and dilute with deionized water to 0.025-0.5 mg/mL as the standard solutions.

2.7. Sample solution preparation

2.7.1. Direct extraction method

Transfer about 5 g of the fine-cut and homogenized sample accurately weighed into a 50-mL centrifuge tube, add 0.5 mL of 1 M phosphoric acid and 40 mL of deionized water, and ultrasonicate for 10 min. Adjust pH to about 3.0 with 1 M phosphoric acid, and then dilute with deionized water to 100 mL. Centrifuge at 3,500 xg for 10 min, and filter the supernatant with a membrane filter. Take the filtrate as the sample solution.

2.7.2. Steam distillation method (note)

Transfer about 25 g of the fine-cut and homogenized sample accurately weighed into a 1000-mL distillation flask, and add 150 mL of deionized water and 20 mL of 1 M phosphoric acid. The end of condenser tube must be soaked in about 10 mL of deionized water in a 500-mL volumetric flask. Distill and collect about 480 mL of distillate. Adjust pH of the distillate to about 3.0 with 1 M phosphoric acid, and dilute to volume with deionized water. Filter with a membrane filter, and take the filtrate as the sample solution.

Note: This method is applicable to samples with high fat or those may be interfered by the direct extraction method.

2.8. Identification and quantification

Inject 25 μL of the sample solution and the standard solutions into HPLC separately, and analyze according to the following conditions. Identify propionic acid based on the retention time and the absorption spectrum. Calculate the amount of propionic acid in the sample by the following formula:

$$\text{The amount of propionic acid in the sample (g/kg)} = \frac{C \times V}{M}$$

Where,

C: the concentration of propionic acid in the sample solution (mg/mL)

V: the make up volume of sample (mL)

M: the weight of the sample (g)

HPLC operating conditions (note):

Photodiode array detector: the quantitative wavelength of 214 nm.

Column: ZORBAX SB-Aq, 5 μm, 4.6 mm i.d. × 25 cm.

Mobile phase: as section 2.5.

Flow rate: 1.2 mL/min.

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

Remark

1. The limit of quantitation (LOQ) for propionic acid is 0.5 g/kg.
2. Further validation should be done when interference compounds appear in samples.

Reference

The Pharmaceutical Society of Japan. 2015. Methods of Analysis in Health Science. 336-337. KANEHARA & Co., LTD. Tokyo, Japan.