NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5009.120-2025

Replacing GB 5009.120-2016

National food safety standard - Determination of propionic acid and its salts in foods

食品安全国家标准 食品中丙酸及其盐的测定

Issued on: March 16, 2025 **Implemented on: September 16, 2025**

Issued by: State Administration for Market Regulation;
Standardization Administration of the PRC

Table of Contents

0 Foreword

The standard replaces GB 5009.120-2016 National food safety standard - Determination of sodium propionate and calcium propionate in foods.

Compared with GB 5009.120-2016 the main changes are: the title was changed to National food safety standard - Determination of propionic acid and its salts in foods; the scope of application of the method was widened; the drying step in the preparation of bread samples was deleted; a clean-up step and a calculation formula were added for the extraction procedure of the first method, high performance liquid chromatography; the limit of detection and the limit of quantification of the extraction procedure were supplied; and the preparation of the calibration curve for the gas chromatographic method was modified.

1 Scope

The standard lays down liquid chromatographic and gas chromatographic methods for determining propionic acid and its salts in foods.

It applies to the determination of propionic acid and its salts in grain and grain products, baked foods, fried foods, meat products, condiments, bean products and canned foods.

2 Principle (first method, high performance liquid chromatography)

Propionate in the test sample is converted to propionic acid by acidification. The sample is steam distilled, the distillate is collected, the pH adjusted and the volume made up, and the solution measured on a high performance liquid chromatograph. Alternatively the sample is extracted with ultrasound, the extract has its pH adjusted and its volume made up, it is cleaned up on a solid phase extraction cartridge, and it is measured on a high performance liquid chromatograph. Identification is by chromatographic peak retention time and quantification is by the external standard method. Propionic acid and its salts in the sample are expressed as propionic acid.

3 Reagents and materials (first method)

Unless otherwise stated the reagents are analytical grade and the water is grade one water as laid down in GB/T 6682.

3.1 Reagents: phosphoric acid (H_3PO_4); diammonium hydrogen phosphate; silicone oil; methanol (CH_3OH) of chromatographic grade.

3.2 Reagent preparation: phosphoric acid solution (1 mol/L), made by adding 53.5 mL of phosphoric acid to 50 mL of water, mixing and making up to 1 000 mL with water; diammonium hydrogen phosphate solution (1.5 g/L), made by weighing 1.5 g of diammonium hydrogen phosphate, dissolving in water and making up to 1 000 mL; methanol-water solution (10+90), made by mixing methanol and water in the volume ratio 10 to 90; methanol-water solution (40+60), made by mixing methanol and water in the volume ratio 40 to 60.

3.3 Standard substance: propionic acid standard, CAS number 79-09-4, purity 99.0 % or higher, or a standard certified by the State and issued with a certificate of reference material.

3.4 Standard solutions: propionic acid stock standard solution (10.0 mg/mL), made by weighing 250.0 mg of the propionic acid standard to the nearest 0.1 mg, dissolving in water, transferring to a 25 mL volumetric flask and making up to the mark with water, stored in a refrigerator at 4 °C with a shelf life of 6 months; propionic acid intermediate standard solution (1.00 mg/mL), made by taking 5.00 mL of the stock solution accurately into a 50

mL volumetric flask and diluting to the mark with water, stored in a refrigerator at 4 °C with a shelf life of 3 months.

3.5 Materials: solid phase extraction cartridge, 500 mg/6 mL, packed with a hydrophilic-lipophilic balanced polystyrene-divinylbenzene sorbent or an equivalent cartridge; aqueous and organic microporous filter membranes of 0.45 μm.

4 Instruments and equipment (first method)

4.1 High performance liquid chromatograph fitted with an ultraviolet detector or a diode array detector.

4.2 Balance with a readability of 0.0001 g and of 0.01 g.

4.3 Ultrasonic generator.

4.4 Centrifuge with a speed of at least 4 000 r/min.

4.5 Tissue homogenizer.

4.6 Steam distillation apparatus, 500 mL.

4.7 pH meter with a precision of 0.01.

4.8 Solid phase extraction manifold.

5 Analytical procedure (first method)

5.1.1 Sample preparation. Solid and semi-solid samples are chopped and mixed in a tissue homogenizer before use; liquid samples are shaken before use.

5.1.2.1 Distillation procedure. Weigh 25 g of the sample accurately to the nearest 0.01 g into a 500 mL distillation flask, add 100 mL of water, rinse the container with a further 50 mL of water and transfer that to the flask, add 20 mL of 1 mol/L phosphoric acid solution and 2 to 3 drops of silicone oil, and steam distil. A 250 mL volumetric flask standing in an ice bath serves as the receiver. When about 240 mL has distilled over, remove the receiver, let it stand for 30 min at room temperature, adjust the pH to about 3 with 1 mol/L phosphoric acid solution, make up to the mark with water and mix. Filter through a 0.45 μm aqueous microporous membrane before the liquid chromatographic measurement.

5.1.2.2 Extraction procedure, for bread and pastry. Weigh 5 g of the sample accurately to the nearest 0.01 g into a 100 mL beaker, add about 25 mL of water and 0.50 mL of 1 mol/L phosphoric acid solution, mix, extract with ultrasound for 10 min and adjust the pH to about 3 with 1 mol/L phosphoric acid solution. Transfer all of the sample to a 50 mL volumetric flask, make up to the mark with water and mix. Transfer the solution to a 50 mL centrifuge tube and centrifuge for 10 min at not less than 4 000 r/min, then clean up. Condition the solid phase extraction cartridge with 5 mL of methanol followed by 5 mL of water, keeping

the bed wet. Load 5.0 mL of the solution to be cleaned up onto the cartridge at once. Wash with 5.0 mL of methanol-water solution (10+90) and draw the bed nearly dry under vacuum. Elute with 5.0 mL of methanol-water solution (40+60), collect the eluate, make it up to 5.0 mL and mix, and filter through a 0.45 μm organic microporous membrane before the liquid chromatographic measurement.

5.2 Reference instrument conditions. Column: water-tolerant C18 column, 4.6 mm x 250 mm, 5 μm, or a column of equivalent performance. Mobile phase: 1.5 g/L diammonium hydrogen phosphate solution adjusted to pH 2.7 to 3.5 with 1 mol/L phosphoric acid solution. Flow rate 1.0 mL/min. Column temperature 25 °C. Injection volume 20 μL. Wavelength 214 nm.

5.3.1 Calibration curve, distillation procedure. Take accurately 0.250 mL, 0.500 mL, 1.00 mL, 2.50 mL, 5.00 mL and 12.5 mL of the stock standard solution into 500 mL distillation flasks and treat them as in 5.1.2.1; the final concentrations of the propionic acid standard solutions are 0.0100 mg/mL, 0.0200 mg/mL, 0.0400 mg/mL, 0.100 mg/mL, 0.200 mg/mL and 0.500 mg/mL. Filter through a 0.45 μm aqueous microporous membrane, inject in order of increasing concentration and plot the calibration curve with concentration as abscissa and peak area as ordinate. The chromatogram of the propionic acid standard solution is shown in Figure A.1.

5.3.2 Calibration curve, extraction procedure. Take accurately 0.100 mL, 0.200 mL, 0.400 mL, 1.00 mL, 2.00 mL and 5.00 mL of the intermediate standard solution into 10 mL volumetric flasks, add 0.20 mL of 1 mol/L phosphoric acid to each, make up to 10 mL with water and mix; the final concentrations are 0.0100 mg/mL, 0.0200 mg/mL, 0.0400 mg/mL, 0.100 mg/mL, 0.200 mg/mL and 0.500 mg/mL. Filter through a 0.45 μm aqueous microporous membrane, inject in order of increasing concentration and plot the calibration curve with concentration as abscissa and peak area as ordinate. The chromatogram is shown in Figure A.2.

5.4 Measurement of the sample solution. The treated sample solution is injected into the liquid chromatograph in the same way as the standard series and the propionic acid concentration in the sample is calculated from the calibration curve. The response of propionic acid in the solution measured is to fall inside the linear range of the calibration curve; where it exceeds the linear range the solution is diluted and injected again.

6 Expression of the analytical result (first method)

6.1 For the distillation procedure the content of propionic acid and its salts in the sample, expressed as propionic acid, is calculated by formula (1). The symbols are X, the content of propionic acid and its salts in the sample expressed as propionic acid, in grams per kilogram (g/kg); rho, the mass concentration of propionic acid in the sample solution read from the calibration curve, in milligrams per millilitre (mg/mL); V, the final volume to which the sample solution was made up, in millilitres (mL); 1000, a conversion factor; m, the mass