NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5009.308-2025

National food safety standard - Determination of ascorbyl palmitate in food

食品安全国家标准 食品中抗坏血酸棕榈酸酯的测定

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1 Scope

GB 5009.308-2025 is the Chinese national food safety standard for determining ascorbyl palmitate in food by liquid chromatography. Ascorbyl palmitate is the fat-soluble form of vitamin C, used as an antioxidant in oils, fried products, infant formula and cosmetics, and it is a permitted additive with a maximum use level, which is what makes a reference method necessary: it must be distinguished from ascorbic acid itself, which behaves quite differently on the column, and recovered from a fatty matrix without hydrolysing it during extraction. The standard sets the principle, the reagents and materials, the instruments and equipment, the analytical procedure, the expression of results, the precision and the additional notes, with an annex giving the chromatogram of the standard solution. It takes effect on 2 March 2026.

This standard specifies a liquid chromatography method for the determination of ascorbyl palmitate in food. This standard applies to the determination of ascorbyl palmitate in food.

2 Principle

The ascorbyl palmitate in the sample is extracted with citric acid-isoascorbic acid methanol solution and then separated by reversed-phase liquid chromatography. Detection is performed using a diode array detector or ultraviolet detector, and quantification is performed using the external standard method.

3 Reagents and materials

Unless otherwise stated, all reagents used in this method are of analytical grade, and the water is Grade I water as specified in GB/T 6682.

3.1 Reagents

3.1.1 Methanol (CH4O). chromatographically pure.

3.1.2 Acetonitrile (C2H3N). chromatographically pure.

3.1.3 Phosphoric acid (H3PO4).

3.1.4 Citric acid (C6H8O7).

3.1.5 Isoascorbic acid (C6H8O6).

3.2.1 Citric acid-isoascorbic acid methanol solution. Weigh

1.0 g of citric acid and

1.0 g of isoascorbic acid, dissolve them in methanol and dilute to 1000 mL.

3.2.2 Phosphoric acid solution (0.1%). Measure

1.0 mL of phosphoric acid, dilute with water to 1000 mL, mix well and set aside.

3.2.3 Methanol-acetonitrile solution (50+50). Mix methanol and acetonitrile at a volume ratio of

50.50 and set aside.

3.3 Standard substances

3.3.1 Ascorbyl palmitate standard substance (C22H38O7, CAS No.. 137-66-6). with a purity of >=98%, or a standard substance that has obtained the national certification and been granted a standard substance certificate.

3.4.1 Ascorbyl palmitate standard solution (

1.00 mg/mL). Accurately weigh 25 mg (accurate to

0.1 mg) of the standard substance, dissolve it in citric acid-isoascorbic acid methanol solution and dilute to 25 mL, then mix well. Prepare fresh before use.

3.4.2 Ascorbyl palmitate standard intermediate solution (

0.100 mg/mL). Accurately pipette

1.00 mg/mL standard solution, dilute with citric acid-isoascorbic acid methanol solution and bring the volume to 10 mL, then mix well. Prepare fresh before use.

3.4.3 Ascorbyl palmitate standard working solution. Accurately pipette

0.10 mL,

0.20 mL,

0.50 mL,

5.0 mL of the intermediate standard solution, dilute with citric acid-isoascorbic acid methanol solution, and bring the volume to 10 mL. Mix well to obtain standard working solutions with mass concentrations of 1.00 μg/mL, 2.00 μg/mL, 5.00 μg/mL, 10.0 μg/mL, and 50.0 μg/mL, respectively. Prepare fresh before use. NOTE. The concentration points of the standard working solution can be adjusted appropriately within the linear range.

3.5 Materials Organic microporous filter membrane. 0.22 μm.

4 Instruments and equipment

4.1 Liquid chromatograph. It is equipped with a diode array detector or an ultraviolet detector.

4.2 Electronic balance. The sensitivity is

0.01 mg and

0.1 mg, respectively.

4.3 Tissue grinder.

4.4 Horizontal oscillator.

4.5 Centrifuge. with a speed of not less than 8000 r/min.

5 Analysis steps

5.1 Sample preparation Liquid samples such as peanut oil and fruit and vegetable juice

beverages shall be shaken well before testing; powdered or paste-like samples with uniform texture, such as milk powder and fruit puree, shall be thoroughly mixed before testing; other solid or semi-solid samples, such as canned goods, bread, oatmeal, and candy, shall be pulverized (for gum-based candies, chopping or freeze-pulverizing may be necessary), sieved to ensure a particle size of less than 2 mm, and then mixed evenly. Samples shall be stored in a light-proof, airtight container under cold conditions.

5.2.1 Sample extraction Weigh 5 g of the sample (accurate to

0.001

g) and place it in a 50 mL centrifuge tube. Add 30 mL of citric acid-isoascorbic acid methanol solution, tighten the cap, place the tube on a horizontal oscillator, and extract for 10 min by oscillating at a rate of not less than 250 times/min. Transfer the entire sample to a 50 mL volumetric flask, and dilute to the mark with citric acid-isoascorbic acid methanol solution. Mix well.

5.2.2 Purification The above sample extract is filtered through filter paper (discarding the initial filtrate) or centrifuged at 8000 r/min for 1 min to obtain the supernatant, and then filtered through a 0.22 μm organic microporous membrane into a brown sample vial for analysis by a high-performance liquid chromatograph. NOTE. If necessary, the sample solution can be diluted with citric acid-isoascorbic acid methanol solution to ensure that the concentration of ascorbyl palmitate in the sample solution is within the range of the standard working solution concentration; the sample solution shall be tested within 12 hours.

5.3 Blank test Except for the absence of a sample, all other steps are performed simultaneously with the sample processing as per 5.2.

5.4.1 Chromatographic column. C18 column (

4.6 mmx150 mm, 5 μm) or a column with equivalent performance.

5.4.2 Detection wavelength. 245 nm.