NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 5009.86-2025

National food safety standard - Determination of ascorbic acid in food

食品安全国家标准 食品中抗坏血酸的测定

Issued on: September 2, 2025 **Implemented on: March 2, 2026**

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Standardization Administration of the PRC

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Foreword

This document was issued on 2 September 2025 by the State Administration for Market Regulation; Standardization Administration of the PRC and takes effect on 2 March 2026.

It is a GB standard without the /T suffix: compliance is mandatory in China.

1 Scope

GB 5009.86-2025 is the Chinese national food safety standard for determining ascorbic acid in food. Vitamin C is both a declared nutrient and an added antioxidant, so the figure is used to check a label claim and a permitted use level at the same time; the analytical difficulty is that ascorbic acid oxidises during extraction unless the sample is protected, and that its stereoisomer erythorbic acid, used as an antioxidant but with no vitamin activity, must not be counted as vitamin C. The standard gives two methods: liquid chromatography, which separates L-ascorbic acid from D-isoascorbic acid, and fluorescence spectrophotometry. Each is set out with its principle, reagents and materials, instruments and equipment, analytical procedure, expression of results, precision and notes. It takes effect on 2 March 2026.

This Standard specifies the methods for the determination of ascorbyl palmitate in food.

Method 1, liquid chromatography, is applicable to the determination of L-ascorbic acid and D-

isoascorbic acid in food.

Method 2, fluorescence spectrophotometry, is applicable to the determination of ascorbyl

palmitate in food for special dietary purposes, milk and dairy products, fruits and vegetables

and their products, as well as the determination of L-ascorbic acid in the above-mentioned food

without adding D-isoascorbic acid.

Method 3, 2,6-dichlorophenolindophenol titration, is applicable to the determination of reduced

ascorbyl palmitate in fruits and vegetables and their products.

Method 1 - Liquid Chromatography

2 Principle

After extraction, reduction and dilution, ascorbyl palmitate in the specimen is separated by

reversed-phase chromatography using a mixed solution containing ion-pairing reagents and

reducing agents as the mobile phase. Detection is performed using a UV detector or diode array

detector. Qualitative determination is based on the retention time of the chromatographic peaks,

and quantitative determination is performed using the external standard method.

3 Reagents and Materials

Unless otherwise specified, all reagents used in this Method are analytically pure, and the water

is Grade I water as specified in GB/T 6682.

3.1 Reagents

3.1.1 Glacial acetic acid ($C_2H_4O_2$).

3.1.2 Trichloroacetic acid ($C_2HCl_3O_2$, TCA).

3.1.3 Metaphosphoric acid (HPO_3).

3.1.4 40% tetrabutylammonium hydroxide aqueous solution (40% TBAH).

3.1.5 Ammonium acetate ($C_2H_7NO_2$).

3.1.6 L-cysteine ($C_3H_7NO_2S$).

3.1.7 Tris(2-carboxyethyl)phosphine hydrochloride ($C_9H_{15}O_6P$, TCEP).

3.1.8 Acetonitrile (C_2H_3N). chromatographically pure.

3.2 Reagent Preparation

3.2.1 Ammonium acetate solution (500 mmol/L, pH 5.4). weigh-take 19.27 g of ammonium

acetate, add water to dissolve it and dilute to 500 mL; use glacial acetic acid to adjust pH to 5.4.

3.2.2 TCA solution (150 g/L). weigh-take 75 g of TCA, add water to dissolve it, dilute to 500

mL, and mix it well.

3.2.3 Metaphosphoric acid solution (200 g/L). weigh-take 100 g of metaphosphoric acid, add

water to dissolve it, dilute to 500 mL, and mix it well.

3.2.4 TCEP solution (250 mg/L). weigh-take 125 mg of TCEP, add water to dissolve it, dilute

to 500 mL, and mix it well.

3.2.5 L-cysteine solution (40 g/L). weigh-take 40 g of L-cysteine, add an appropriate amount

of water and perform ultrasonic dissolution, then, add water to dilute to 1 L and mix it well.

Prepare it right before use.

3.2.6 Mobile phase. measure-take 900 mL of water into a beaker, pipette 3.25 mL of 40% TBAH

solution and weigh-take 3.85 g of ammonium acetate into the water, then, add 50 mg of TCEP

(or 20 mL of L-cysteine solution), followed by 10 mL of acetonitrile. Mix it well, then, use

glacial acetic acid (approximately 3.14 mL) to adjust the pH to 4.5 ~ 5.0.Finally, add water to

dilute to 1 L and mix it well; remove air bubbles through ultrasound.

3.3 Standard Substances

3.3.1 L-ascorbic acid standard (C6H8O6, CAS No.. 50-81-7). purity ? 99%, or a standard

substance certified by the state and granted a standard substance certificate.

3.3.2 D-isoascorbic acid standard (C6H8O6, CAS No.. 89-65-6). purity ? 99%, or a standard

substance certified by the state and granted a standard substance certificate.

3.4 Preparation of Standard Solutions

3.4.1 L-ascorbic acid standard solution (50 ?g/mL). weigh-take 10 mg of L-ascorbic acid

standard (accurate to 0.1 mg), use TCEP solution to dissolve it and reach a constant volume of

10 mL, mix it well. Accurately pipette 500 ?L of the above-mentioned standard solution into a

10 mL brown volumetric flask, use TCEP solution to reach a constant volume and mix it well.

Transfer the solution to a brown glass container and prepare it right before use.

3.4.2 D-isoascorbic acid standard solution (50 ?g/mL). weigh-take 10 mg of D-isoascorbic acid

standard (accurate to 0.1 mg), use TCEP solution to dissolve it and reach a constant volume of

10 mL, mix it well. Accurately pipette 500 ?L of the above-mentioned standard solution into a

10 mL brown volumetric flask, use TCEP solution to reach a constant volume, and mix it well.

Transfer the solution to a brown glass container and prepare it right before use.

3.4.3 L-ascorbic acid or D-isoascorbic acid standard series working solutions. respectively and