



**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB 5009.300-2025

**National food safety standard - Determination of L-carnitine in
foods**

食品安全国家标准 食品中左旋肉碱的测定

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1 Scope

GB 5009.300-2025 is the Chinese national food safety method for L-carnitine in food. L-carnitine is a permitted nutritional fortifier in China and it is added to infant formula, to sports and weight-management products and to a range of functional foods and drinks; because a claim is made for it on the label, the declared amount has to be verifiable, and because it is also sold as a slimming ingredient it is a substance where over-declaration and adulteration both occur. The standard describes the determination by liquid chromatography and by liquid chromatography-tandem mass spectrometry, and covers for each the principle, the reagents and materials, the apparatus, the preparation of the sample including the extraction from the matrix, the preparation of the standard solutions, the analytical procedure, the calculation of results, the precision and the limit of quantification. It is a new method, effective September 2025. For a formula maker, a supplement producer or a testing laboratory in China, this is the prescribed method behind the declared figure.

This standard specifies the determination method of L-carnitine in foods. Method I, the spectrophotometric method, is applicable to the determination of L- carnitine in infant foods (excluding partially hydrolyzed milk protein formula, deeply hydrolyzed milk protein formula and amino acid formula in infant formula foods for special medical purposes) and dairy products. Method II, the pre-column derivatization-high performance liquid chromatography, is suitable for the determination of L-carnitine in foods (excluding infant formula foods for

special medical purposes and formula foods for special medical purposes). Method III, the liquid chromatography-tandem mass spectrometry, is suitable for the determination of L-carnitine in infant formula foods and formula foods for special medical purposes. Method I. Spectrophotometric method

2 Principle

The test specimen is extracted with water and filtered after protein precipitation with perchloric acid. The bound L-carnitine in the filtrate is released by alkaline saponification and reacts with acetyl coenzyme A under the catalysis of acetylcarnitine transferase to generate acetylcarnitine and free coenzyme A. Free coenzyme A reacts with 5,5'-dithiobis(2-nitrobenzoic acid) to generate a yellow substance, the color depth of which is proportional to the content of free coenzyme A. Since free coenzyme A and L-carnitine are in an equimolar reaction relationship, the content of L-carnitine in the test specimen can be indirectly calculated.

3 Reagents and materials

Unless otherwise specified, all reagents used in this method are analytically pure, and the water is grade 3 water as specified in GB/T 6682.

3.1 Reagents

3.1.1 Perchloric acid (HClO_4).

3.1.2 Sodium hydroxide (NaOH).

3.1.3 Potassium hydroxide (KOH). 3.1.4 5,5'-Dithiobis(2-nitrobenzoic acid) ($\text{C}_{14}\text{H}_8\text{N}_2\text{O}_8\text{S}_2$).

3.1.5 N-2-Hydroxyethylpiperazine-N-2-ethanesulfonic acid ($\text{C}_8\text{H}_{18}\text{N}_2\text{O}_4\text{S}$).

3.1.6 Disodium ethylenediaminetetraacetate ($\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2 \cdot 2\text{H}_2\text{O}$).

3.1.7 Acetyl-CoA. With a purity of $\geq 85\%$, and stored at $2\text{ }^\circ\text{C} \sim 8\text{ }^\circ\text{C}$.

3.1.8 Acetylcarnitine transferase (CAT). Stored at $2\text{ }^\circ\text{C} \sim 8\text{ }^\circ\text{C}$.

3.2 Reagent preparation

3.2.1 Perchloric acid solution (13%). Pipette 13 mL of perchloric acid and dilute to 100 mL with water.

3.2.2 Perchloric acid solution (3%). Pipette 3 mL of perchloric acid and dilute to 100 mL with water.

3.2.3 Sodium hydroxide solution (10 mol/L). Weigh 40 g of sodium hydroxide and dissolve it

in water. After cooling, dilute to 100 mL.

3.2.4 Potassium hydroxide solution (4 mol/L). Weigh

22.4 g of potassium hydroxide and dissolve it in water. After cooling, dilute to 100 mL.

3.2.5 Color development stock solution. Weigh 50 mg of 5,5'-dithiobis(2-nitrobenzoic acid), 5.96 g of N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid, and 185 mg of disodium ethylenediaminetetraacetate respectively, and dissolve them in 30 mL of water. Adjust the pH to 7.4-

7.6 with 10 mol/L sodium hydroxide solution, and then dilute to 50 mL with water. Store at 4 °C, with a shelf life of 3 months.

3.2.6 Color development working solution. Take 5 mL of color development stock solution and dilute it to 25 mL with water.

3.2.7 Acetyl-CoA solution. Weigh 20 mg of acetyl CoA and dissolve it in 2 mL of water. Prepare it fresh just before use.

3.2.8 Acetylcarnitine transferase (CAT) solution. Pipette 100 μ L of acetylcarnitine transferase suspension, add water to 2 mL, and mix well. Prepare it fresh just before use.

3.3 Standards L-carnitine standard (C₇H₁₅NO₃, CAS No.. 541-15-1). With a purity of $\geq 98\%$, or a reference material certified by the nation and awarded with a reference material certificate. Dry in an electrothermal air blast drying oven at 102 ± 2 °C for 2 hours before use.

3.4 Preparation of standard solution

3.4.1 L-carnitine standard stock solution (1 mg/mL). Accurately weigh 10 mg (accurate to 0.01 mg) of L-carnitine and place it in a 10 mL beaker. Add 5 mL of water to dissolve it. Transfer all of it to a 10 mL volumetric flask, dilute to the mark with water, and mix well. Transfer the solution to a brown container and store it at 4 °C away from light. The shelf life is 3 months.

3.4.2 L-carnitine standard intermediate solution (80 μ g/mL). Pipette

2.00 mL of L- carnitine standard stock solution into a 25 mL volumetric flask, dilute to the mark with water, and mix well. Transfer the solution to a brown container and store at 4 °C away from light. The shelf life is 1 month.

3.4.3 L-carnitine series standard working solution. Pipette

0.125 mL,