



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

GB 29989-2013

Determination of L-carnitine in infant foods and dairy products

婴幼儿食品和乳品中左旋肉碱的测定

(English Translation)

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1 Scope

This Standard specifies the method for determination of levocarnitine in foods and milk products for infants and young children.

This Standard is applicable to the determination of levocarnitine in foods and milk products for infants and young children.

2 Principle

Extract the specimen with water; use perchloric acid to precipitate the protein; then filtrate it. After the filtrate is gone through alkaline saponification, the combined-state levocarnitine in solution is dissociated. Under the catalysis of carnitine acetyl transferase, levocarnitine reacts with acetyl coenzyme A to generate acetylcarnitine and free coenzyme A. Free coenzyme A reacts with 2-nitrobenzoic acid to generate yellow substance; the color of which is proportional to the content of free coenzyme A.

As the free coenzyme A and levocarnitine are of same mole reaction, the content of levocarnitine in specimen may be calculated indirectly.

3 Reagents and Materials

Note: Unless otherwise specified, all reagents adopted in this method are analytical reagents and the water is Grade 3 specified in GB/T 6682.

3.1 Reagents

3.1.1

Perchloric acid (HClO_4).

3.1.6

Ethylenediamine tetraacetic acid disodium ($\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\cdot 2\text{H}_2\text{O}$).

3.1.7

Acetyl coenzyme A (AcetylCoA). kept under $2\sim 8^\circ\text{C}$.

3.1.8

Carnitine acetyl transferase (CAT). kept under $2\sim 8^\circ\text{C}$.

3.2 Reagent preparation

3.2.1

Perchloric acid solution (13%). dilute 13mL of perchloric acid to 100mL.

3.2.2 Sodium hydroxide solution (10mol/L). dissolve 40g of sodium hydroxide with

water; dilute to 100mL after cooling down.

3.2.3 Potassium hydroxide solution (4.0mol/L). dissolve 22.4g with water; dilute to 100mL after cooling down.

3.2.6 Acetyl coenzyme A (AcetylCoA) solution. dissolve 20.0mg of acetyl coenzyme

A into 2.0mL water. Prepare it just before use.

3.2.7 Carnitine acetyl transferase (CAT) solution. pipet 100 μL of carnitine acetyl

transferase suspension; centrifuge it for 10min with rotation speed of 1500r/min;

discard the supernatant liquid and dissolve the sediment into 2mL of water. Prepare it just before use.

3.3 Standard product

Standard levocarnitine product ($\text{C}_7\text{H}_{15}\text{NO}_3$). purity $\geq 98\%$.

4 Apparatuses and devices

4.1

Analytical balance. with a sensitivity of 0.1mg.

4.2

pH meter. with a precision of 0.01.

4.5

Spectrophotometer.

5.1 Specimen treatment

Accurately weigh 5g (accurate to 0.0001g) of well-mixed specimen; place it into a beaker; dissolve it with 30mL of 40°C warm water; transfer it into 100mL volumetric flask. Add 10mL of 13% perchloric acid solution into it; keep static for 20min after mixed uniformly. Fix the volume to the scale with distilled water; filter it with quantitative filter paper.

5.2 Drawing of standard curve

Pipet 2.0mL of standard levocarnitine working solution into a 1cm cuvette; add 0.8mL of color rendering working solution and 100μL of acetyl coenzyme A solution into it;

then cover it; after mixing it uniformly, put it in spectrophotometer;

5.3 Specimen determination

Take 2.0mL of specimen treatment solution; determine its absorbance according to the procedures specified in Section 5.2. Obtain the concentration of specimen solution to be tested through standard curve.

6 Expression of analytical result

The content X of levocarnitine in specimen is expressed by mass fraction mg/100g and calculated according to Formula (1).

7 Precision

The absolute deviation of two independent determination results under repeatability conditions shall not exceed 10% of arithmetic mean.

8 Other

Method detection limit is 0.6mg/100g; quantitation limit is 2mg/100g.

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