National standard for Food Safety of the P. R. China

GB 5413.35—2010

National food safety standard

Determination of β-carotene in foods for infants and young children, milk and milk products

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Foreword

In this present National Standard, AOAC 2005.7, *β-carotene in Supplement and Raw material,* is referred to partially.

Annex A of this present National Standard is normative, and Annex B is informative.

This present National Standard is released for the first time.

National food safety standard
Determination of β-carotene in foods for infants and young children, milk and milk products

1. Scope

This present National Standard specifies the method for determination of β-carotene in foods and milk products for infant and young children.

This present National Standard is applicable to the determination of carotene level in foods for infant and young children and milk products.

2. Normative references:

The following standard contains provisions which, through reference in this text, constitute provisions of this present standard. As for the dated references, all the amendments or revisions after them except the corrigenda are not applicable to this present standard. As for the references that are not dated, their most recent editions are applicable to this present national standard.

GB/T 6682 Water for analytical laboratory use – Specification and test methods

3. Principle

After saponification, the β carotene in sample is turned to free form, which can be extracted by petroleum ether and determined by High Performance Liquid Chromatography; the external standard method is used for quantification.

4. Reagents and solvents

Unless otherwise specified, the reagents used are analytically pure or higher; water used in the analysis should comply with the requirement on Grade I water in GB/T 6682.

4.1 α- amylase: Enzyme activity ≥ 1.5 U/mg.

4.2 Anhydrous sodium sulfate.

4.3 Ascorbic acid.

4.4 Petroleum ether: Boiling range: 30 □ ~ 60 □.

4.5 Methyl alcohol: Chromatographically pure.

4.6 Acetonitrile: Chromatographically pure.

4.7 Trichloromethane: Chromatographically pure.

4.8 Normal hexane: Chromatographically pure.

4.9 Alcohol: 95% volume fraction

4.10 Water solution of potassium hydroxide: 250 g of solid f potassium hydroxide was weighed, dissolved with 200 mL of water. Prepared before use.

4.11 β carotene standard product.

4.12 β carotene standard solution.

4.12.1 β carotene standard stock solution（500 μg/mL）:

Weigh 50.0 mg of standard substance （4.11）of β carotene accurately, dissolve it in a 100mL brown volumetric flask with normal hexane (4.8), and dilute to volume.

Note: It should be stored by refrigeration below the temperature of-10 ℃, and the shelf life should not be over 3 months. Before use, the standard stock solution should be calibrated, of which the operation is listed in Annex A.

4.12.2 β carotene standard intermediate solution（100 μg/mL）:

Pipette 10mL of the β carotene standard stock solution (4.12.1) to a 50mL brown volumetric flask, and dilute to volume with normal hexane (4.8).

4.12.3 β carotene standard working solution:

Pipette 0.50, 1.00, 2.00, 3.00, and 4.00（mL）of the β carotene standard intermediate solution (4.12.2) to 5 of 100mL brown volumetric flasks, and dilute to volume with normal hexane (4.8) respectively. Thus, a series of standard working solutions are obtained, of which the concentrations are 0.5, 1.0, 2.0, 3.0, and 4.0 (μg/mL) respectively.

5. Apparatus

5.1 High Performance Liquid Chromatography (HPLC), with a UV detector.

5.2 Rotation evaporator.

5.3 Constant Temperature Magnetic Stirrer: 20 □ ~ 80 □.

5.4 Centrifuge: Speed ≥ 5000 r / min.

5.5 Analytical Balance: Accuracy 0.1 mg.

5.6 Nitrogen evaporator.

5.7 Incubator: 60 □ ± 2 □.

6. Operation steps

6.1 Pretreatment of sample

6.1.1 Starchy sample:

Weigh about 5g of solid sample that has been mixed well or 50g of liquid sample (accurate to 0.1mg) into a 250mL conical flask, and add 1.0g of ascorbic acid (4.3);dissolve the solid sample with 50mL of water at 45 ~ 50□, and add 1g of α-amylase (4.1). Lid the conical flask after filling nitrogen gas into it. Let stand in an incubator （5.7）at 60 ℃±2 ℃ for 30 min for enzymolysis.

6.1.2 Non-starchy sample:

Weigh about 10g of solid sample that has been mixed well or 50g of liquid sample (accurate to 0.1 mg) into a 250mL conical flask; add 1.0g of ascorbic acid; dissolve the solid sample with 50mL of water at 45 ~ 50□, and mix well.

6.2 Preparation of test solution:

6.2.1 Saponification: Add 100mL 95% of the ethyl alcohol solution of ascorbic acid (4.9) into the solution of test sample mentioned above. Mix well. Add 25mL of the potassium hydroxide solution (4.10); mix well; put in magnetic rod, fill in nitrogen to remove air, and lid it with a rubber lid. Add about 300mL of water in a 1000mL beaker, put the beaker in a constant temperature magnetic stirrer (5.3); when the water temperature is controlled at 53□±2□, put the triangular flask in the beaker, after magnetic stirrer (5.3) for 45 min for saponification. Take out, and cool to room temperature immediately.

6.2.2 Extraction: Transfer all the saponified solution into a 500mL separating funnel, add 100mL of the petroleum ether (4.4), shake gently to exhaust and decompress, and lid. Oscillate at room temperature for 10 min; let stand for demixing; transfer the water phase into another separating funnel, and carry out the second extraction in accordance with the method mentioned above. Merge the organic phases, rinse the ether solution to neutral with distilled water, and filter it through the anhydrous sodium sulfate (4.2) for dehydration. Collect the filtrate into a 500mL flat-bottom flask, and evaporate it in a rotation evaporator（5.2）filled with nitrogen gas at 40□±2□ to almost dry(steam dried is not permitted); dissolve the residue with the petroleum ether (4.4), and transfer it into a 10mL volumetric flask; dilute to volume.

Pipette 2.0mL of the petroleum ether solution from the above-mentioned volumetric flask to a 10mL tube with a screwed lid. Blow it dry with a 40□±2□ nitrogen evaporator （5.6）.Dissolve it with 1mL of normal hexane (4.8); shake to dissolve the residues,

centrifuge at a speed not less than 5000 r/min for 10 min（5.4）. The supernatant is the test solution.

Note: The operation should be carried out in dark place.

6.3 Chromatographic determination

6.3.1 Reference condition for chromatographic determination

Chromatographic column: C_{18} column, 250mm× 4.6mm, 5μ, or other chromatographic columns with the same performance.

Mobile phase: Chloroform - nitrile - methanol = 3 +12 +85, Add 0.4 g of ascorbic acid into 1 L of Solution A; and filter it through a 0.45μm membrane before use.

Flow rate: 2.0mL/min;

Wavelength: 450 nm.

Column temperature: 35□±1□。

Injection volume: 20μL.

6.3.2 Drawing of standard curve:

Inject the β carotene standard working solutions (4.12.3) into the liquid chromatograph (see the chromatogram map in Annex B) to get the peak area. Draw the standard curve, of which the y-axis is the peak area, and the x-axis is the concentration of β carotene standard working solutions.

6.3.3 Determination of test sample:

Inject the test solution (6.2.2) into the liquid chromatograph to get the peak area. Consult the standard curve to get the concentration of β carotene in the test solution.

7. Expression of analytic result

The content of β-carotene in test sample, *X*, should be calculated in accordance with formula (1):

$$X = \frac{c \times 10 \times 100}{m \times 2} \quad \text{..... (1)}$$

In which:

X is the content of β-carotene in test sample, of which the unit is μg/100g;

c is the concentration of β carotene in test solution, consulted from the standard curve, of which the unit is micro-gram per milliliter (μg/mL);

m is the mass of sample, of which the unit is gram (g).

10-volume of sample solution

Note: The result is sum of cis and trans β carotene.

The calculated result should be expressed as an arithmetic mean of results of two individual measurements, which should be accurate to three decimal places.

8. Precision

The absolute difference of results of two individual detections under repeated condition should not be over 10% of the arithmetic mean.

9. Others

The detection limit of the present standard is 2 μg/100g.

Annex A

(Informative annex)

Calibration method for concentration of standard solution

β carotene standard stock solution should be calibrated after preparation, of which the detailed operations are as follows:

Pipet 10μL of the β carotene standard stock solution (of which the concentration is 500μg/mL), add 3.0mL of normal hexane, and mix well. Measure the light absorbance; normal hexane acts as the blank, the wavelength of incident light is 450nm, and the thickness of cuvette is 1cm; carry out the measurement for 3 times, and record the average.

The concentration of solution should be calculated according to the following formula:

$$X = \frac{A}{E} \times \frac{3.01}{0.01} \quad \text{.....(2)}$$

In which:

X is the concentration of β carotene solution, of which the unit is microgram per milliliter (μg/ml);

A is ultraviolet absorbance of β carotene;

E is the absorption coefficient （0.2638）of β carotene in normal hexane solution, when the wavelength of incident light is 450 nm, the thickness of cuvette is 1cm, and the concentration of solution is 1 mg/L.

$\frac{3.01}{0.01}$ is the conversion factor of dilution times during measurement

Annex B

(Informative annex)

Liquid chromatogram of standard test sample

B.1 liquid chromatogram of β-carotene standard substance

The liquid chromatogram of β-carotene standard substance sees Fig. B.1

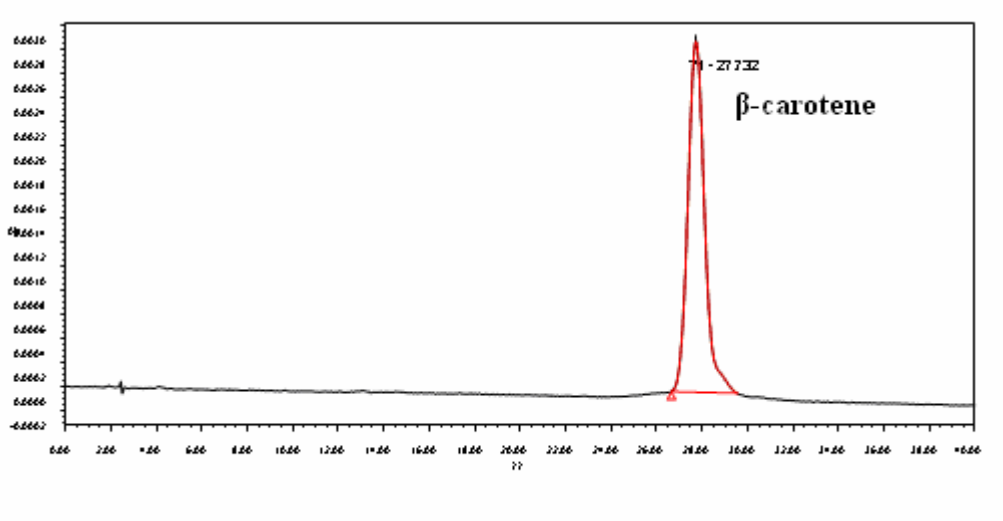

Figure B.1 Chromatogram of β-carotene standard substance