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NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA

GB 5009.292-2023

**National food safety standard - Determination of β -Apo-8'-
carotene aldehyde in food**

食品安全国家标准 食品中 β -阿朴-8'-胡萝卜素醛的测定

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State Administration for Market Regulation.**

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National food safety standard - Determination of β -Apo-8'-carotene aldehyde in food

1 Scope

This standard specifies the liquid chromatography method for the determination of β -Apo-8'-carotene aldehyde in food.

This standard is applicable to the determination of β -Apo-8'-carotene aldehyde in flavored fermented milk, processed cheese, frozen drinks, candies, baked goods, semi-solid compound seasonings, and beverages.

2 Principle

The sample is saponified with potassium hydroxide solution, and the free β -Apo-8'-carotene aldehyde is extracted with n-hexane. After the extract is concentrated and reconstituted with acetonitrile, it is separated by high-performance liquid chromatography, detected with a UV-visible light detector or diode array detector, and quantified by an external standard method.

3 Reagents and materials

Unless otherwise stated, the reagents used in this method are of analytical grade and the water is first-grade water specified in GB/T 6682.

3.1 Reagents

3.1.1 Acetonitrile ($\text{C}_2\text{H}_3\text{N}$): chromatographically pure.

3.1.2 Absolute ethanol ($\text{C}_2\text{H}_6\text{O}$).

3.1.3 n-hexane (C_6H_{14}).

3.1.4 Potassium hydroxide (KOH).

3.1.5 2,6-Di-tert-butyl-p-cresol ($\text{C}_{15}\text{H}_{24}\text{O}$, butylated hydroxytoluene, referred to as BHT).

3.1.6 Formic acid (CH_2O_2): chromatographically pure.

3.2 Reagent preparation

3.2.1 Potassium hydroxide solution (200 g/L): Weigh 200 g of potassium hydroxide, add water to dissolve and dilute to 1000 mL.

3.2.2 0.1% BHT-acetonitrile solution: Weigh 0.1 g of BHT, dissolve it in 100 mL acetonitrile, and mix well.

3.2.3 0.1% formic acid (volume fraction) solution: Pipette 1 mL of formic acid and dilute to 1 L with water.

3.3 Standard product

β -Apo-8'-carotene aldehyde (C₃₀H₄₀O, CAS number: 1107-26-2): purity is $\geq 98\%$, or a reference material with national authentication and a Reference Material Certificate.

3.4 Preparation of standard solution

3.4.1 β -Apo-8'-carotene aldehyde standard stock solution (10 $\mu\text{g/mL}$): Accurately weigh 1 mg (accurate to 0.01 mg) of β -apo-8'-carotene aldehyde standard, dissolve it with 0.1% BHT-acetonitrile solution, and transfer it to a 100 mL brown volumetric flask; make the volume up to 100 mL with 0.1% BHT-acetonitrile solution, and mix well; store it at -18 °C, and the period of validity is 3 months.

NOTE: β -Apo-8'-carotene aldehyde standard stock solution needs to be calibrated before use. See Appendix A for specific operations.

3.4.2 β -Apo-8'-carotene aldehyde standard series working solution: Take appropriate amounts of β -Apo-8'-carotene aldehyde standard stock solution into 10 mL brown volumetric flasks, add acetonitrile to make the volume up to the mark, and prepare working solutions with mass concentrations of 0.0500 $\mu\text{g/mL}$, 0.100 $\mu\text{g/mL}$, 0.200 $\mu\text{g/mL}$, 0.500 $\mu\text{g/mL}$, and 1.00 $\mu\text{g/mL}$, respectively. Prepare solutions fresh just before use.

4 Instruments and equipment

4.1 Liquid chromatograph: equipped with a diode array detector or UV-visible light detector.

4.2 Balance: The sensitivity is 0.01 g and 0.01 mg, respectively.

4.3 Spectrophotometer.

4.4 Constant temperature oscillating water bath device.

4.5 Tissue masher.

4.6 Rotary evaporator.

5.1.3 Blank test

Except for adding no sample, conduct a blank test according to the measurement steps in 5.1.2.

5.2 Instrument reference conditions

5.2.1 Chromatographic column: C₁₈ column, 150 mm×4.6 mm (i.d.), the particle size of 5 μm, or a chromatographic column with equivalent performance.

5.2.2 Mobile phase: acetonitrile + 0.1% formic acid solution (95:5, volume ratio).

5.2.3 Flow rate: 1.0 mL/min.

5.2.4 Detection wavelength: 460 nm.

5.2.5 Column temperature: 40 °C.

5.2.6 Injection volume: 20 μL.

5.3 Preparation of standard curve

Inject the standard series working solutions into the high-performance liquid chromatograph respectively, and measure the corresponding peak areas. Taking the mass concentration of β-Apo-8'-carotene aldehyde in the standard series working solutions as the abscissa, and the peak area of β-Apo-8'-carotene aldehyde as the ordinate, draw a standard curve. See Appendix B for the chromatogram of the standard solution of β-Apo-8'-carotene aldehyde.

5.4 Determination of sample solution

The sample solution is injected into the high-performance liquid chromatograph to obtain the peak area of the object to be measured; it is put into the standard curve to calculate the mass concentration of β-Apo-8'-carotene aldehyde in the test solution.

Compared with the standard solution, the change range of the retention time of the compound chromatographic peak in the sample to be tested shall be within ±2.5%.

6 Calculation and presentation of results

The content of β-Apo-8'-carotene aldehyde in the sample is calculated according to formula (1).

$$X = \frac{(\rho - \rho_0) \times V}{m \times 1\,000} \dots\dots\dots (1)$$

where:

X -- the content of β -Apo-8'-carotene aldehyde in the sample, in grams per kilogram (g/kg);

ρ -- the mass concentration of β -Apo-8'-carotene aldehyde in the sample solution obtained from the standard curve, in micrograms per milliliter ($\mu\text{g/mL}$);

ρ_0 -- the mass concentration of β -Apo-8'-carotene aldehyde in the blank test obtained from the standard curve, in micrograms per milliliter ($\mu\text{g/mL}$);

V -- the reconstituted volume of the sample, in milliliters (mL);

m -- the sampling amount of the sample, in grams (g);

1000 -- unit conversion factor.

The calculation result is rounded to 2 significant figures.

7 Precision

The absolute difference between two independent determination results obtained under repeatability conditions shall not exceed 15% of the arithmetic mean.

8 Others

When the sample weight is 2 g, the detection limit is 0.0002 g/kg, and the quantification limit is 0.0005 g/kg.

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