



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

GB 5009.292-2023

**National food safety standard - Determination of
8'-apo-beta,psi-caroten-8'-al in foods**

食品安全国家标准 食品中beta-阿朴-8'-胡萝卜素醛的测定

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Foreword

This document was issued on 6 September 2023 by the State Administration for Market Regulation; Standardization Administration of the PRC and takes effect on 6 March 2024.

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

1 Scope

GB 5009.292-2023 covers the liquid chromatographic determination of beta-apo-8'-carotenal in food, the carotenoid colorant written in full as 8'-apo-beta,psi-caroten-8'-al. The sample is saponified with potassium hydroxide solution and the freed colorant taken up in n-hexane; the extract is concentrated, made up again in acetonitrile, then separated by high-performance liquid chromatography and measured on a diode array or ultraviolet-visible detector. That opening step is what the matrix forces: the compound is fat soluble and sits in the fat of the food, so it has to be released from the fat before any chromatography is worth running. The standard gives the principle, the reagents and materials, the instruments and equipment, the analysis steps, the calculation and presentation of results, the precision and the detection figures, with Appendix A on calibrating the mass concentration of the standard solution and Appendix B showing the standard chromatogram. Repeat determinations are to agree within 15 percent of their arithmetic mean, and on a two gram test portion the detection limit is 0.0002 grams per kilogram against a quantification limit of 0.0005. A mandatory national food safety standard for inspection laboratories, regulators and manufacturers checking declared colorant levels.

This standard specifies the liquid chromatography method for the determination of beta-

Apo-8'-carotene aldehyde in food.

This standard is applicable to the determination of beta-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 beta-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

beta-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 beta-Apo-8'-carotene aldehyde standard stock solution (10 $\mu\text{g/mL}$): Accurately weigh 1 mg (accurate to 0.01 mg) of beta-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 degrees C, and the period of validity is 3 months.

NOTE: beta-Apo-8'-carotene aldehyde standard stock solution needs to be calibrated before use. See

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 mmx4.6 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 degrees C.

5.2.6 Injection volume: 20 μ L.

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 beta-Apo-8'-carotene aldehyde in the standard series working solutions as the abscissa, and the peak area of beta-Apo-8'-carotene aldehyde as the ordinate, draw a standard curve. See Appendix B for the chromatogram of the standard solution of beta-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 beta-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 $\pm 2.5\%$.

6 Calculation and presentation of results

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

where:

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

ρ -- the mass concentration of beta-Apo-8'-carotene aldehyde in the sample solution obtained from the standard curve, in micrograms per milliliter (μ g/mL);

ρ_0 -- the mass concentration of beta-Apo-8'-carotene aldehyde in the blank test obtained from the standard curve, in micrograms per milliliter (μ 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.