



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

GB 5009.158-2016

**National food safety standard - Determination of Vitamin K1 in
Foods**

食品安全国家标准 食品中维生素K1的测定

(English Translation)

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Foreword

This Standard is drafted as a replacement of GB/T 5009.158-2003 "Determination of Vitamin K1 in Vegetables" and GB 5413.10-2010 "National Food Safety Standard - Determination of Vitamin K1 in Foods for Infants and Young Children, Milk and Milk Products".

In comparison with GB/T 5009.158-2003, there are several main modifications as follows.

- The name of this Standard is modified into "National Food Safety Standard - Determination of Vitamin K1 in Foods";
- The method of high-performance liquid phase chromatography - fluorescence detection is added;
- The method of liquid phase chromatography - tandem mass spectrometry is added;
- The method of high-performance liquid phase chromatography - UV detection is deleted.

National Food Safety Standard -
Determination of Vitamin K1 in Foods

1 Scope

This Standard specifies the method of determining vitamin K1 in foods.

In this Standard, Method I is high-performance liquid phase chromatography - fluorescence detection, Method II is liquid phase chromatography - tandem mass spectrometry, which are both applicable to the determination of vitamin K1 in all kinds

of formula food, vegetable oil, fruit and vegetable.

Method I -- High-performance Liquid Phase

Chromatography - Fluorescence Detection

2 Principle

Through lipase and amylase enzymolysis of samples like foods for infants and young children, dairy products and vegetable oil, adopt N-hexane to extract vitamin K1 from the sample. Adopt C18 liquid phase chromatographic column to separate vitamin K1 from other impurities. Adopt zinc column for reduction, adopt fluorescence detector for detection; quantify with the external standard method.

3 Reagents and Materials

Unless otherwise indicated, the reagents adopted under this method are of analytical purity. The water is first-grade water as specified in GB/T 6682.

3.2 Preparation of Reagents

3.2.1 40% potassium hydroxide solution. weigh-take 20 g of potassium hydroxide and place it in 100 mL beaker; add 20 mL of water to dissolve it; cool it down, then, add water to 50 mL; store in polyethylene bottle.

3.3 Standards

Vitamin K1 (C₃₁H₄₆O₂, CAS No..84-80-0). purity≥99%, or standard substance with a national-level certificate and a certificate of standard substance.

3.4.1 Standard stock solution of vitamin K1 (1 mg/mL). accurately weigh-take 50 mg

(accurate to 0.1 mg) of standard vitamin K1 and place it in 50 mL volumetric flask; use methanol to dissolve and dilute to the constant volume. Transfer the solution to a brown glass container, store under -20 °C and keep away from light.

3.4.2 Standard intermediate fluid of vitamin K1 (100 µg/mL). accurately absorb 10.00

mL of standard stock solution of vitamin K1 and place it in 100 mL volumetric flask; add

methanol to the constant volume, mix it up. Transfer the solution to a brown glass container, store under -20 °C and keep away from light. It can remain valid for 2 months.

3.5.2 Neutral alumina column. 2 g/6 mL, 10% of water is contained in the filling.

Commodity column can be purchased directly, or it can be replaced by filling.

3.5.3 Zinc column. length 50 mm, internal diameter 4.6 mm; zinc column can be purchased directly, or it can be replaced by filling.

5.1 Preparation of Samples

After mixing powdered samples like rice flour and flour, directly take the sample; use sample grinder to grind flaky and granular samples into powder, store it in a sample bag for later usage;

5.2 Processing of Samples

Note: during the processing, avoid direct UV light irradiation, keep away from light.

5.2.2.1 Extraction

Accurately weigh-take 1 g~5 g (accurate to 0.01 g, the content of vitamin K1 shall be $\geq 0.05 \mu\text{g}$) of homogenized sample, place it in 50 mL centrifuge tube. Add 5 mL of isopropanol, start vortex for 1 min and ultrasonic for 5 min. Add 10 mL of N-hexane, start vortex or oscillation extraction for 3 min, start centrifugation at 6,000 r/min for 5 min. Remove-take the supernatant in 25 mL brown volumetric flask.

5.2.2.2 Purification and concentration

Use a small amount of N-hexane to transfer 1 mL of the above-mentioned extract to neutral alumina column that's previously activated with 5 mL of N-hexane. Wait till the extract is almost drained, use 5 mL of N-hexane to rinse it; use 6 mL of N-hexane - ethyl acetate mixed solution to elute to 10 mL tube.

5.4 Draw a Standard Curve Line

Adopt the external standard curve line to quantify. Respectively inject the standard series of working fluid of vitamin K1 into high-performance liquid phase chromatograph, determine the corresponding peak area.

5.5 Determination of Sample Solution

Under the same chromatographic conditions, respectively inject the prepared blank solution and sample solution for high-performance liquid phase chromatographic analysis.

6 Expression of Analysis Results

The content of vitamin K1 in the sample shall be calculated in accordance with Formula (1).

7 Precision

The absolute difference between the two independent determination results obtained under repeatability conditions shall not exceed 10% of the arithmetic mean value.

8 Others

Foods for infants and young children, dairy products and vegetable oil. when the amount of sampling is 1 g and the constant volume is 5 mL, the detection limit is 1.5 µg/100 g and the quantitation limit is 5 µg/100 g;

9 Principle

Through lipase and amylase enzymolysis of samples like foods for infants and young children, dairy products and vegetable oil, adopt N-hexane to extract vitamin K1 from the sample. Adopt C18 liquid phase chromatographic column to separate vitamin K1 from other impurities. Adopt tandem mass spectrometry for detection; quantify with the isotope-labeled internal standard method.