GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5009.158-2016

National Food Safety Standard -

Determination of Vitamin K_1 in Foods

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Issued by: National Health and Family Planning Commission of the People's Republic of China;

State Food and Drug Administration of the People's Republic of China.

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Foreword

This Standard is drafted as a replacement of GB/T 5009.158-2003 “Determination of Vitamin K_1 in Vegetables” and GB 5413.10-2010 “National Food Safety Standard - Determination of Vitamin K_1 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 K_1 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 K_1 in Foods

1 Scope

This Standard specifies the method of determining vitamin K_1 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 K_1 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 K_1 from the sample. Adopt C_{18} liquid phase chromatographic column to separate vitamin K_1 from other impurities. Adopt zinc column for reduction, adopt fluorescence detector for detection; quantify with the external standard method.

Adopt isopropanol and N-hexane to extract vitamin K_1 from low-fat plant samples like fruit and vegetable; adopt neutral alumina column for purification, remove interfering substances like chlorophyll. Adopt C_{18} liquid phase chromatographic column to separate vitamin K_1 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.

3.1 Reagents

3.1.1 Anhydrous ethanol (CH_3CH_2OH).

sample grinder to grind flaky and granular samples into powder, store it in a sample bag for later usage; shake liquid samples like liquid milk and vegetable oil, directly take the ample; take edible part from fruit and vegetable, use water to rinse it, then, use gauze to remove water on the surface; homogenize with the homogenizer, store in a sample bottle for later usage. Determine the sample as soon as possible once the sample is prepared.

5.2 Processing of Samples

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

5.2.1 Foods for infants and young children, dairy products and vegetable oil

5.2.1.1 Enzymolysis

Accurately weigh-take 1 g ~5 g (accurate to 0.01 g, the content of vitamin K_1 shall be ≥0.05 μg) of homogenized sample, place it in 50 mL centrifuge tube. Add 5 mL of warm water to dissolve it (directly absorb 5 mL of liquid sample; no water shall be added to dilute vegetable oil), add 5 mL of phosphate buffer solution (pH8.0), mix it up. Add 0.2 g of lipase and 0.2 g of amylase (no amylase shall be added if the sample doesn't contain starch); put on the lid, start vortex for 2 min~3 min. Mix it up, place it in 37°C±2°C constant-temperature water bath oscillator and start oscillation for over 2 h, so that it can go through thorough enzymolysis.

5.2.1.2 Extraction

After enzymolysis, take out the sample, respectively add 10 mL of ethanol and 1 g of potassium carbonate, then, mix it up; add 10 mL of N-hexane and 10 mL of water, start vortex or oscillation extraction for 10 min, start centrifugation at 6,000 r/min for 5 min; or transfer enzymolysis solution to 150 mL separating funnel for extraction, start static stratification (if emulsification emerges, more N-hexane or water can be properly added to eliminate emulsification); transfer the supernatant to 100 mL rotary evaporation bottle, add 10 mL of N-hexane to the underlayer fluid; repeat the above operation once, then, combine the supernatant into the above-mentioned rotary evaporation bottle.

5.2.1.3 Concentration

Start rotary evaporation of the above-mentioned N-hexane extract, till it reaches dryness (if there's any residual solution, slightly blow with nitrogen till it reaches dryness); use methanol to transfer and dilute to the constant volume in 5 mL volumetric flask, mix it up. Use 0.22 μm membrane to filter it, reserve the filtrate for inlet.
Add no sample, comply with the same method of operation and start a blank test.

5.2.2 Fruit and vegetable

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; fruit and vegetable: when the amount of sampling is 5 g, the volume of dispensed extract is 5 mL 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.

Method II -- Liquid Phase Chromatography - Tandem Mass Spectrometry

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 K_1 from the sample. Adopt C_{18} liquid phase chromatographic column to separate vitamin K_1 from other impurities. Adopt tandem mass spectrometry for detection; quantify with the isotope-labeled internal standard method.

Adopt isopropanol and N-hexane to extract vitamin K_1 from low-fat plant samples like fruit and vegetable; adopt neutral alumina column for purification, remove interfering substances like chlorophyll. Adopt C_{18} liquid phase chromatographic column to separate vitamin K_1 from other impurities. Adopt tandem mass spectrometry for detection; quantify with the isotope-labeled internal standard method.

10 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.

10.1 Reagents

10.1.1 Anhydrous ethanol (CH_3CH_2OH).

10.1.2 Potassium carbonate (K_2CO_3).

10.1.3 Potassium hydroxide (KOH).

10.1.4 Formic acid (HCOOH): chromatographic purity.

10.1.5 Ammonium formate ($HCOONH_4$): chromatographic purity.

10.1.6 Isopropanol [$(CH_3)_2CHOH$].

standard intermediate fluid of vitamin K_1 and place it in 100 mL volumetric flask; add methanol to the constant volume, mix it up.

10.4.4 Isotope-labeled internal standard stock solution of vitamin K_1-D_7 (100 μg/mL): accurately weigh-take 1 mg (accurate to 0.01 mg) of isotope-labeled internal standard of vitamin K_1-D_7; use methanol to dissolve and dilute to the constant volume of 10 mL.

10.4.5 Isotope-labeled internal standard working fluid of vitamin K_1-D_7 (1 μg/mL): absorb 1.00 mL of isotope-labeled internal standard stock solution of vitamin K_1-D_7, then, place it in 100 mL volumetric flask; add methanol to the constant volume, mix it up.

10.4.6 Standard series of working fluid: respectively and accurately absorb 0.10 mL, 0.20 mL, 0.50 mL, 1.00 mL, 2.00 mL and 4.00 mL of standard working fluid of vitamin K_1, place it in 10 mL volumetric flask; respectively add 0.50 mL of isotope-labeled internal standard working fluid; add methanol to dilute to the constant volume. The concentration of the standard series of working fluid of vitamin K_1 is 10 ng/mL, 20 ng/mL, 50 ng/mL, 100 ng/mL, 200 ng/mL and 400 ng/mL respectively.

10.5 Materials

10.5.1 Neutral alumina: particle size 50 μm~150μm.

10.5.2 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.

Note: the method of filling neutral alumina column:

a) Filling processing: take 200 g of neutral alumina and place it in 500 mL wide-mouth bottle; bake for 2 h in a drying oven under 150 °C; put on the lid, then, cool it down in the drying oven, till it reaches the room temperature. Slowly add 20 mL of pure water, shake it while adding the water; put on the lid, place it in the oven for 3 min~5 min under 80 °C; take it out, violently shake it, till alumina in the bottle can flow freely without showing any agglomeration; place it in the drying oven and cool it down for 30 min, reserve for later usage.

b) Filling of chromatographic column: take 6 mL syringe-shaped column sleeve, add sieve plate; weigh-take 2.00 of the above-mentioned filling that's deactivated; add another sieve plate, then, compress it with filling instrument.

10.5.3 Microporous filter head: equipped with 0.22 μm microporous membrane.

11 Instruments and Equipment

11.1 Liquid phase chromatography - tandem mass spectrometer: equipped with electrospray ionization (ESI).

starch); put on the lid, start vortex for 2 min~3 min. Mix it up, place it in 37°C±2°C constant-temperature water bath oscillator and start oscillation for over 2 h, so that it can go through thorough enzymolysis.

12.2.1.2 Extraction

After enzymolysis, take out the sample, respectively add 10 mL of ethanol and 1 g of potassium carbonate, then, mix it up; add 10 mL of N-hexane, start vortex extraction for 10 min, start centrifugation at 6,000 r/min for 3 min. Transfer the supernatant to another 50 mL centrifuge tube, add 10 mL of N-hexane to the underlayer fluid. Start vortex for 5 min and centrifugation at 6,000 r/min for 3 min. Combine the supernatant, add N-hexane solution to dilute to the constant volume of 25 mL, reserve for purification.

12.2.1.3 Purification

Add 20 mL of water to the above-mentioned extract, shake for 0.5 min. After static stratification, respectively take 5 mL of the supernatant and place it in 10 mL glass tube. Use nitrogen to blow it, till it reaches dryness. Add 1 mL of methanol to dissolve it. Use 0.22 μm membrane to filter it, reserve the filtrate for inlet.
Add no sample, comply with the same method of operation and start a blank test.

12.2.2 Fruit and vegetable

12.2.2.1 Extraction

Accurately weigh-take 1 g~5 g (accurate to 0.01 g, the content of vitamin K_1 shall be ≥0.02 μg) of homogenized sample, place it in 50 mL centrifuge tube. Add 0.25 mL of isotope-labeled internal standard working fluid (1 μg/mL); 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. Add 10 mL of N-hexane to the underlayer fluid; repeat extraction once, then, combine the supernatant into the above-mentioned volumetric flask. Use N-hexane to dilute to the constant volume; use pipette to accurately dispense 5 mL of the supernatant to 10 mL tube. Use nitrogen to slightly blow it, till it reaches dryness. Add 1 mL of N-hexane to dissolve it, reserve for purification.

12.2.2.2 Purification

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. Use nitrogen to blow it, till it reaches dryness; add 1 mL of methanol. Adopt 0.22 μm membrane to filter it; reserve the filtrate for analysis and determination.
Add no sample, comply with the same method of operation and start a blank test.

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