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

GB 5413.10—2010

National food safety standard

Determination of vitamin K_1 in foods for infants and young children, milk and milk products

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Foreword

This present National Standard is identical to AOAC Official Method 999.15, *Vitamin K in Milk and Infant Formulas Liquid Chromatographic Method*.

This present National Standard replaces GB 5413.10 -1997, *Milk powder and formula foods for infant and young children — Determination of Vitamin K_1 content.*

When compared with GB 5413.10 -1997, the following modifications have been carried out in this present National Standard:

— The standard name is modified as Determination of vitamin K_1 in foods for infants and young children, milk and milk products

— The treatment of sample is modified as: after enzyme hydrolysis, the sample should be saponified by NaOH and extracted by normal hexane";

— in this present National Standard, it has been modified as that, after HPLC column, reduction fluorescence method is used for quantitative determination of Vitamin K_1;

— In the apparatus, the original "liquid chromatograph with UV detector" is modified as "HPLC with fluorescence detector".

Annexes A, B, and C of this present National Standard are all informative annexes.

The original editions replaced by this present National Standard include:

— GB/T 5413-1985 and GB/T 5413.10 -1997.

National food safety standard

Determination of vitamin K_1 in foods for infants and young children, milk and milk products

1. Scope

This present National Standard specifies the method for determination of Vitamin K_1 in foods for infants and young children, milk and milk products.

This present National Standard is applicable to the determination of Vitamin K_1 in foods for infants and young children, milk and milk products.

2. Normative references

The following standards contain 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.

3. Principle

The fats and unsaturated fatty acids in test sample are degraded by lipase first; then, for the starch of the samples requiring degradation with starch amylase, after saponification by alkali, Vitamin K_1 is extracted by normal hexane. After separation by liquid chromatography, post-column reduction of Vitamin K_1 is carried out; the fluorescence detector is used for detection, and external standard method is used for quantification.

4. Reagents and materials

Unless otherwise specified, purity of all reagents used in this present method is analytically pure, and that of water used in the test is the third-graded specified in GB/T 6682.

4.1 Sodium hydroxide solution (10 mol/L): prepare before use.

4.2 95% ethyl alcohol.

4.3 Saturated sodium chloride solution.

4.4 Normal hexane: Chromatographically pure.

4.5 Anhydrous sodium sulfate.

4.6 Methanol: Chromatographically pure.

4.7 Dichloromethane: Chromatographically pure.

4.8 Glacial acetic acid.

4.9 Zinc chloride.

4.10 Anhydrous sodium acetate.

4.11 Mobile phase: 900 mL of methanol (4.6), 100 mL of dichloromethane (4.7), 0.3 mL of glacial acetic acid (4.8), 1.5 g of zinc chloride (4.9), and 0.5 g of anhydrous sodium acetate (4.10); after dissolution, it is filtered through a 0.45 μm membrane.

4.12 Amylase: Enzyme activity ≥ 1.5 U/mg.

4.13 Lipase: Enzyme activity ≥ 700 U/mg.

4.14 Zinc powder: particle size: 50μm ~ 70 μm.

4.15 Standard solution of Vitamin K_1: the calibration method of standard solution concentration sees Annex A.

4.15.1 Standard stock solution of Vitamin K_1 (2 mg/mL): Weigh 0.05 g of standard substance of Vitamin K_1, accurate to 0.1 mg, in a 25 ml volumetric flask. Dissolve and dilute to volume with normal hexane.

4.15.2 Standard intermediate solution of Vitamin K_1: The concentration is 20 μg/ml. Dilute 1 ml of the standard stock solution (4.15.1) with normal hexane to 100 mL.

5. Apparatus

5.1 High-pressure liquid chromatograph: Equipped with fluorescence detector.

5.2 Balance: accurate to 0.1 mg.

5.3 Separating funnel, 250 ml capacity.

5.4 Rotatory evaporator.

5.5 Constant-temperature air bath shaker.

5.6 Centrifuge: rotary speed≥3000 rpm

5.7 Nitrogen evaporator.

6. Analytical procedure

6.1 Pretreatment of sample

6.1.1 Starch-contained sample

Weigh about 2.5 g of solid sample or 10 g of liquid sample that has been mixed well before in a conical flask, accurate to 0.1 mg. Add 0.5 g of amylase (4.12), and dissolve it with 30 mL of warm water.

6.1.2 Starch-free sample

Weigh about 2.5 g of solid sample or 10 g of liquid sample that has been mixed well before in a conical flask, accurate to 0.1 mg. Dissolve it with 30 mL of warm water.

6.2 Preparation of test solution

Add 1 g of lipase (4.13) in the above-mentioned pretreated sample solution. Keep shaking in the constant temperature air bath shaker at 37□±5□overnight to achieve complete enzymolysis. Then take out the solution; add 2mL of the sodium hydroxide solution (4.1); transfer this solution into a 250 mL separating funnel; add 50 mL of ethyl alcohol (4.2), and mix well (when necessary, add a small amount of saturated sodium chloride solution (4.3)). Add 50 ml of normal hexane (4.4), shake for 2 minutes, and let stand for demixing. Separate the aqueous phase from the bottom into another 250 ml separating funnel, and retain the organic phase. Extract the aqueous phase with another 50 ml of normal hexane again. Merge the

organic phase. Wash the organic phase twice with a proper amount of distilled water, and then filter the organic phase to a 500 mL flat-bottom flask through anhydrous sodium sulfate (4.5). Evaporate it in the rotatory evaporator at 40 □ ±2 □until it is almost dry. Transfer the residue with normal hexane (4.4) to a 10 mL test tube. Puff with nitrogen evaporator until it is dry at 40 □±2□. Add 1 mL of normal hexane (4.4) accurately. Shake thoroughly to dissolve the residue. The tube should be frozen in refrigerator for 1 h before use at the temperature below 0 □ .

6.3 Determination

6.3.1 Reference condition for chromatographic analysis:

Chromatographic column: C_{18} column, 150 × 4.6 mm, 5 μm, or chromatographic column with equivalent performance.

Zinc reduction column: 4.6 × 50 mm

Wavelength of detection: Excitation wavelength = 243 nm; emission wavelength = 430 nm

Mobile phase: 900 mL methanol (4.6), 100 mL dichloromethane (4.7), 0.3 mL glacial acetic acid (4.8) and 1.5 g, zinc chloride (4.9), 0.5 g anhydrous sodium acetate (4.10), were dissolved, and filtered with 0.45 μm filter membrane.

Flow rate: 1 mL/min.

Injection volume: 10 μL.

6.3.2 Drawing of standard curve of Vitamin K_1:

Pipette 0.0, 0.5, 1.0, 1.5, 2.0, and 2.5mL of the standard intermediate solution (4.15.2) respectively, and dilute them to 25mL with normal hexane separately. The concentrations of this series of standard working solutions of Vitamin K_1 are 0.0, 0.40, 0.80, 1.20, 1.60, and 2.00 μg/mL respectively.

Inject the series of standard working solutions of Vitamin K_1 into the HPLC to get the peak heights or peak areas. Draw the standard curve, in which the y-axis is peak height or peak area, and the x-axis is the concentration of standard working solutions of Vitamin.

6.3.3 Determination of test solution:

Let the test solution (6.2) that has been prepared stand in dark place until it reaches room temperature; centrifuge for 5 min at a rate of 3000 rpm, pipette the supernatant and inject it into the HPLC to determine corresponding peak area or peak height(standard chromatogram map sees Annex C). The concentration of Vitamin K_1 in test solution can be consulted from the standard curve, or calculated by the regression equation (μg/mL).

7. Expression of result

The content of Vitamin K_1 in test sample, should be calculated as formula (1):

$$X = \frac{C_i \times V_i \times n}{m_i} \times 100 \quad \text{……(1)}$$

in which:

X is the content of vitamin K_1, of which the unit is microgram per hundred grams (μg/100 g),

C_i is the concentration of test sample solution, of which the unit is microgram per milliliter (μg/mL);

V_i is the volume of test sample solution, of which the unit is milliliter (mL);

m_i is mass of sample, of which the unit is gram (g);

n is the dilution times of test sample solution.

The calculation result should be expressed as the arithmetic mean of two individual determinations, and should be accurate to one decimal place.

8. Precision

The absolute difference of results of two individual determinations under repeated conditions shall not exceed 10% of the arithmetic mean value.

9. Others

9.1 As Vitamin K_1 is easy to decompose under light, the operation should be carried out in dark place.

9.2 The detection limit under this standard is 1 μg/100 g.

Annex A

(Informative Annex)

Calibration method for concentration of standard solution

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

Calibration of concentration of Vitamin K_1 standard solution: Determine the light absorbance of Vitamin K_1 standard solution at a given wavelength, and calculate the concentration of Vitamin K_1. The determination condition is as follows:

Table A.1 Determination Conditions of Absorbance of Vitamin K1

Standard	Specific absorbance $E_{cm}^{1\%}$	Wavelength λ (nm)
Vitamin K_1	422	248

The concentration should be calculated according to the formula (2):

$$C_1 = \frac{A}{E} \times \frac{1}{100} \quad \text{.....(2)}$$

In which:

C_1 is the concentration of Vitamin K_1, of which the unit is gram per milliliter (g/mL);

A is the average ultraviolet absorbance of Vitamin K_1

E is the 1% specific light coefficient of Vitamin K_1.

Annex B

(Informative annex)

Filling method of zinc reduction column

Fill the zinc powder (4.14) densely in the reduction column (4.6 mm × 50 mm, stainless steel material). During filling, the zinc powder should be filled with slapping to make it dense.

Annex C

(Informative annex)

Liquid chromatogram of Vitamin K_1

C.1 Liquid chromatogram of Vitamin K_1

The liquid chromatogram of Vitamin K_1 sees FigC.1.

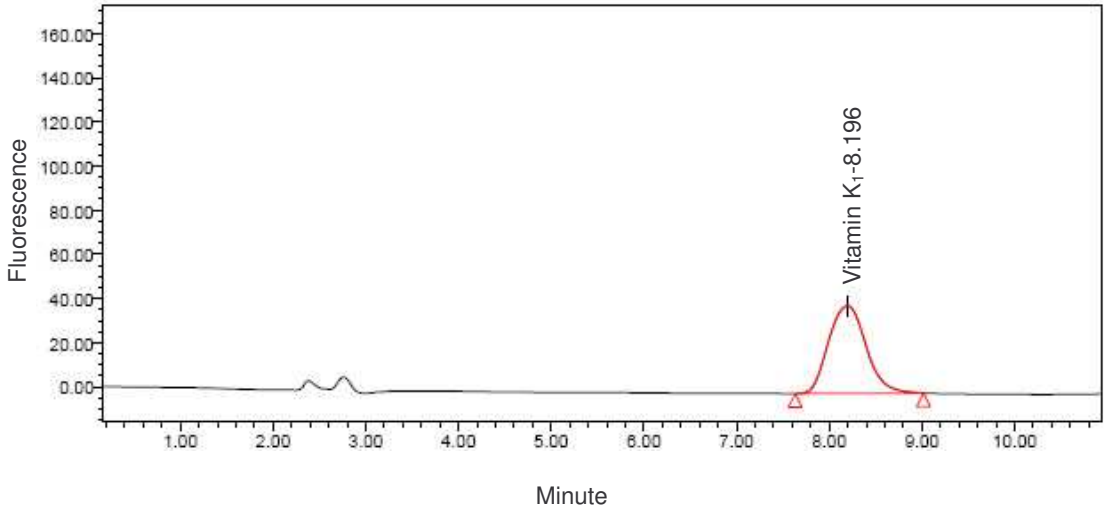

Figure C.1 Liquid chromatogram of Vitamin K_1