



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

GB 5413.10-2010

**National food safety standard -- Determination of vitamin K1 in
foods for infants and young children, milk and milk products
(English Translation)**

Issued on: March 26, 2010

Implemented on: June 1, 2010

Issued by: Ministry of Health of the People's Republic of China Foreword

.....

Table of Contents

Foreword	3
1 Scope	4
2 Normative references	4
3 Principle	4
4 Reagents and materials	4
5 Instruments and apparatuses	5
6 Analytical procedure	5
7 Expression of result	7
8 Precision	7
9 Others	7
Annex A	8
Annex B	9
Annex C	10

1 Scope

This Standard specifies the method for determination of Vitamin K1 in foods for infants and young children, milk and milk products.

This 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 the reference in this text, constitute provisions of this Standard. For the dated references, all the amendments or revisions after them, except the corrigenda, are not applicable to this Standard. For the references that are not dated, their most recent editions are applicable to this Standard.

3 Principle

Use lipase to degrade the fats and unsaturated fatty acids in test sample. For the sample containing starch, it needs to use starch amylase to degrade the starch in sample; after saponification by alkali, use normal hexane to extract Vitamin K1 . After separation by liquid chromatography, post-column reduction of Vitamin K1 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, all reagents used in this method are analytically pure. Water is the Grade 1 specified in GB/T 6682.

4.1

Sodium hydroxide solution (10 mol/L)

Prepare before use.

4.2 95% ethanol.

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, use 0.45 µm membrane to filter.

4.12

Amylase

Enzyme activity ≥ 1.5 U/mg.

4.13

Lipase

Enzyme activity ≥ 700 U/mg.

4.14

Zinc powder

Particle size: $50\mu\text{m} \sim 70\mu\text{m}$.

4.15

Standard solution of Vitamin K 1

The calibration method of standard solution concentration is shown in Annex A.

4.15.1

Standard stock solution of Vitamin K1 (2 mg/mL)

Weigh 0.05 g of standard substance of Vitamin K 1 (accurate to 0.1 mg). Put into a 25 ml volumetric flask.

Use normal hexane to dissolve and fix the volume.

4.15.2

Standard intermediate solution of Vitamin K ($20\mu\text{g/ml}$). Take 1 mL of standard stock solution (4.15.1). Add normal hexane to fix the volume to 100 mL.

5 Instruments and apparatuses

5.1

High-pressure liquid chromatograph

Equipped with fluorescence detector.

5.2

Balance

Sensitivity is 0.1 mg.

5.3

Separating funnel

250 ml.

5.4

Rotatory evaporator.

5.5

Constant-temperature air bath shaker.

5.6

Centrifuge

Rotary speed ≥ 3000 rpm.

5.7

Nitrogen evaporator.

6.1.1 Starch-contained sample

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

6.1.2 Starch-free sample

Annex B (Informative)

Filling method of zinc reduction column

Fill the zinc powder (4.14) densely into the reduction column (4.6 mm x 50 mm, stainless steel material). During the filling, the zinc powder shall be filled in small amount and multiple times; gently slap it while filling; so as to fill in the zinc powder densely.