



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

GB 5413.9-2010

**National food safety standard -- Determination of vitamin A, D, E
in foods for infants and young children, milk and milk products**

婴幼儿食品和乳品中维生素A、D、E的测定

(English Translation)

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Foreword

This Standard replaces GB/T 5413.9 -1997 Milk powder and formula foods for infant and young children - Determination of vitamin A, D, E content.

This Standard, compared with GB/T 5413.9 -1997, has been modified as follows.

- The antioxidant reagent has been modified from original pyrogalllic acid to ascorbic acid.
- The heating and refluxing in the pretreatment method has been modified as constant temperature saponification.
- The correction of standard solution has been added.
- The previous single-point quantitative has been modified as the standard curve method.
- The determination of vitamin D recovery has been supplemented.
- The calculation formula has been modified.

Annex A of this Standard is normative, and Annex B is informative.

The previous edition replaced by this Standard is as follows.

- GB/T 5413.9 -1997.

National food safety standard -

Determination of vitamin A, D, E in foods for infants and young children, milk and milk products

1 Scope

This Standard specifies the method for determination of vitamin A, D, E in foods for infant and young children, milk and milk products.

This Standard is applicable to the determination of vitamin A, D, E content in foods for infant and young children, milk and milk products.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

3 Principle

After saponification and extraction with petroleum ether, vitamin A and E is separated by reverse phase chromatography method, of which the content is determined by external standard method; vitamin D is purified by normal phase chromatography method and separated by reverse phase chromatography method, of which the quantitative is determined by external standard method.

4 Reagents and materials

Unless otherwise specified, all reagents shall be analytical pure or higher graded, and all water used for the experiment shall be Grade 1 water specified in GB/T 6682.

4.5 Potassium hydroxide solution. weigh 250 g of solid potassium hydroxide, and dissolve it with 200 mL of water.

6.1.1 Sample containing starch

Weigh about 5 g of solid sample that has been mixed well or about 50 g of liquid sample (accurate to 0.1 mg), and put it into a 250 mL Erlenmeyer flask. Add 1g of alpha-amylase (4.1). For solid sample, about 50 mL of distilled water at 45 ~ 50°C shall be added in to dissolve it. After it is well mixed, use nitrogen gas to exhaust air from the flask. Insert the stopper. Place it in a 60°C±2°C incubator (5.6) for 30 min.

6.1.2 Sample free of starch

Weigh about 10 g of solid sample that has been mixed well or about 50 g of liquid sample (accurate to 0.1 mg), and put it into a 250 mL Erlenmeyer flask. For solid sample, about

6.2 Recovery test shall be carried out simultaneously for test of sample with vitamin D.

6.3.1 Saponification. Add about 100 mL of ethyl alcohol solution of vitamin C (4.10) in

the above-mentioned sample solution that has been pretreated. After it is well mixed, add 25 mL of the potassium hydroxide solution (4.5). Well mix. Then add magnetic rod and fill nitrogen gas for protection. Insert the rubber stopper. Add about 300 mL of water in a 1000 mL beaker, and place the beaker in a constant temperature magnetic stirrer (5.3); when the temperature is controlled at 53°C ±2°C, place the triangular flask in a beaker, after saponification under magnetic force stirring for about 45 min, immediately take it out and cool it down to room temperature.

6.3.2 Extraction. Transfer all the saponified solution into a 500 mL separating funnel.

Add 100 mL of petroleum ether (4.6) in. Shake it gently. Insert the stopper after exhaust. Severely vibrate it for about 10 min; let stand for demixing. Place the aqueous phase in another 500 mL separating funnel. Repeat the above extraction process. Merge the ether solution, and wash it with distilled water until it becomes neutral. Use anhydrous sodium sulfate for filter and dehydration. Collect the filtrate in a 500 mL flat-bottomed

flask. After it is evaporated under $40^{\circ}\text{C}\pm 2^{\circ}\text{C}$ nitrogen gas flow on rotator evaporator and becomes nearly dry (it is not allowed to make it completely dry), use petroleum ether to transfer it into a 10 mL volumetric flask, and dilute it to fixed-volume.

6.3.3 Take 2.0 mL of petroleum ether solution from the aforesaid volumetric flask and

put it into cuvette A. Then take 7.0 mL of petroleum ether solution and put it into cuvette B. Blow-dry the petroleum ether in both cuvettes A and B with nitrogen gas respectively in a $40^{\circ}\text{C}\pm 2^{\circ}\text{C}$ of nitrogen evaporator (5.4). Add 5.0 mL of methanol (4.7) in cuvette A to stir and dissolve the residues. Add 2.0 mL of normal hexane (4.8) in cuvette B to stir and dissolve the residues. Then centrifuge the cuvettes A and B at 5000 r/min for 10 min; take out, let stand until it cools to room temperature before determination. The cuvette A is used for determination of vitamin A and E, and the cuvette B is used for determination of vitamin D.

6.4.1.1 Reference condition for chromatography

Chromatographic column. C18 column, 250 mm x 4.6 mm, 5 μm , or chromatographic column with the same performance.

Mobile phase. methanol (4.7).

Flow rate. 1.0 mL/min.

Wavelength. Vitamin A, 325 nm; vitamin E, 294 nm.

Column temperature. $35^{\circ}\text{C}\pm 1^{\circ}\text{C}$.

Injection amount. 20 μL .

6.4.1.2 Drawing of standard curve of vitamin A and E

Draw 0.50 mL, 1.00 mL, 1.50 mL, 2.00 mL, and 2.50 mL of the vitamin A standard stock solution (4.11.1) in 50 mL brown volumetric flasks respectively; dilute them with ethanol to graduation; mix well. The concentrations of this series of standard working solutions are 1.00 $\mu\text{g/mL}$, 2.00 $\mu\text{g/mL}$, 3.00 $\mu\text{g/mL}$, 4.00 $\mu\text{g/mL}$, and 5.00 $\mu\text{g/mL}$ respectively.

Draw 1.0 mL, 2.0 mL, 3.0 mL, 4.0 mL and 5.0 mL of the vitamin E standard stock solution (4.11.2) in 50 mL brown volumetric flasks respectively; dilute them with ethanol to