



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

GB 5009.82-2016

**National Food Safety Standard Determination of vitamin A, D, E
in food**

食品安全国家标准 食品中维生素A、D、E的测定

(English Translation)

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1 Scope

This standard specifies the determination of vitamin A, vitamin E and vitamin D in food.

The first method of this standard is applicable to the determination of vitamin A and vitamin E in food.

The second method of this standard is applicable to the determination of vitamin E in edible oil, nuts, beans, pepper powder and other foods.

The third method of this standard is applicable to the determination of vitamin D2 and vitamin D3 in food.

The fourth method of this standard is applicable to the determination of vitamin D2 or vitamin D3 in formulated foods.

Method 1 Determination of vitamin A and vitamin E in food - Reversed- phase high performance liquid chromatography

2 Principles

The vitamin A and vitamin E in the test specimen are saponified (digested by amylase if containing starch), extracted, purified and concentrated, then separated by C30 or PFP reversed-phase liquid chromatography column, detected by ultraviolet detector or fluorescence detector, AND quantitative by external standard method.

3 Reagents and materials

Unless otherwise stated, the reagents used in this method are of analytical grade pure AND water is the primary water as specified in GB/T 6682.

3.1 Reagents

3.1.1

Absolute ethanol (C_2H_5OH). it shall not contain aldehydes after inspection, AND the inspection method is as shown in A.1.

3.1.2

Ascorbic acid ($C_6H_8O_6$).

3.1.3

Potassium hydroxide (KOH).

3.1.4

Diethyl ether [(CH₃CH₂)₂O]. it shall not contain peroxides after inspection,
AND the inspection method is as shown in A.2.

3.2.1 Potassium hydroxide solution (50 g/100 g). WEIGH 50 g of potassium

hydroxide; ADD 50 mL of water to dissolve it; after cooling it down, STORE it in a polyethylene bottle.

3.2.2 Petroleum ether - ether solution (1 +1). MEASURE 200 mL of petroleum

ether; ADD 200 mL of ether; MIX it uniformly.

3.2.3 Organic filter head (pore size of 0.22 μm).

3.4.1 Vitamin A standard stock solution (0.500 mg/mL). accurately WEIGH 25.0

mg vitamin A standard substance; USE absolute ethanol to dissolve it;

TRANSFER it into a 50 mL volumetric flask; MAKE the volume reach to the mark; at this time the solution concentration is about 0.500 mg/mL. TRANSFER the solution into a brown reagent bottle; SEAL it and PRESERVE it at -20 °C in the dark; AND the valid period is 1 month. Before use, INCREASE the temperature of the solution back to 20 °C; and CONDUCT concentration correction (as for the correction method, SEE Appendix B).

4 Instruments and equipment

4.1

Analytical balance. the sensitivity is 0.01 mg.

4.2

Constant temperature water bath oscillator.

4.3

Rotary evaporator.

4.4

Nitrogen blowing instrument.

4.5

UV spectrophotometer.

4.7 High performance liquid chromatography, equipped with UV detector or diode array detector or fluorescence detector.

5.1 Preparation of test specimens

A certain amount of samples are divided and crushed homogeneously, stored in the sample bottle under refrigerated conditions in the dark; AND it shall be used for determination as soon as possible.

5.2 Sample treatment

Caution. All vessels used shall not contain oxidizing substances. The surface of the separation funnel piston glass shall not be coated with oil.

During the treatment, AVOID ultraviolet light and AVOID light as far as possible. The extraction process shall be conducted in the fume hood.

5.2.1.1 Starch-free samples

WEIGH 2 g ~ 5 g (accurate to 0.01 g) of homogenized solid sample OR 50 g (accurate to 0.01 g) of liquid sample into a 150 mL flat-bottomed flask; as for the solid test specimen, it is required to add about 20 mL of warm water; MIX it uniformly; then ADD 1.0 g of ascorbic acid and 0.1 g of BHT; MIX it uniformly;

ADD 30 mL of absolute ethanol; ADD 10 mL ~ 20 mL potassium hydroxide solution while shaking; after mixing it uniformly, PLACE it in the 80 °C constant temperature water bath for shaking and saponification for 30 min; after saponification, immediately USE cold water to cool it to room temperature.

5.2.2 Extraction

USE 30 mL of water to transfer the saponification solution into a 250 mL separation funnel; ADD 50 mL of petroleum ether-ether mixture; SHAKE it for extraction for 5 min; TRANSFER the lower solution into another 250 mL separation funnel. ADD 50 mL of petroleum ether-ether mixture and CONDUCT extraction again; COMBINE the ether layers.

5.2.3 Washing

USE about 100 mL of water to wash the ether layer; REPEAT washing for about 3 times, until washing the ether layer to neutral (it may use the pH test strips to test the pH value of the lower solution); REMOVE the lower aqueous phase.