NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5009.296-2023

National food safety standard - Determination of Vitamin D in foods

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Foreword

This Standard replaces Method 3 "Determination of vitamin D in foods - Liquid chromatography - tandem mass spectrometry" and Method 4 "Determination of vitamin D in foods - High performance liquid chromatography" in GB 5009.82-2016 National food safety standard - Determination of vitamins A, D and E in foods.

Compared with GB 5009.82-2016, the major changes of this Standard are as follows.

-- Change the standard name to National food safety standard - Determination of vitamin D in foods;

-- Add online column switching - reversed-phase liquid chromatography;

-- Add the sample preparation method;

-- Modify the linear range and apparatus reference conditions of the liquid chromatography-tandem mass spectrometry;

-- Modify the preparation method of the standard calibration solution in the appendix.

National food safety standard - Determination of Vitamin D in foods

1 Scope

This Standard specifies methods for the determination of vitamin D in foods.

Method 1 - Normal phase chromatography purification - reversed-phase liquid chromatography applies to the determination of vitamin D2 and vitamin D3 in foods containing ergocalciferol or cholecalciferol.

Method 2 - Online column switching - reversed-phase liquid chromatography applies to the determination of vitamin D2 and vitamin D3 in foods.

Method 3 - Liquid chromatography - tandem mass spectrometry applies to the determination of vitamin D2 and vitamin D3 in foods.

Method 1 - Normal phase chromatography purification - reversed-phase liquid chromatography.

2 Principle

After the sample is saponified with an ethanol solution of potassium hydroxide, purified and concentrated by the liquid-liquid extraction method, use a normal-phase high-performance liquid chromatograph to separate vitamin D from other impurities through a silica gel column. After concentrating the collected fractions, separate vitamin D2 and vitamin D3 through a reverse-phase chromatography column. Detect with a UV detector and use the internal standard method (or external standard method) for quantification. When the sample does not contain vitamin D2, use vitamin D2 as the internal standard to determine vitamin D3; when the sample does not contain vitamin D3, use vitamin D3 as the internal standard to determine vitamin D2.Otherwise, use the external standard method for determination.

3 Reagents and materials

Unless otherwise specified, all the reagents in this method are analytical reagents, the water is grade-I water specified by GB/T 6682.

3.3.1 Vitamin D2 standard substance. ergocalciferol (C28H44O, CAS number. 50-14-6),

purity >=98%, or a standard substance certified by the state and awarded a standard substance certificate.

3.3.2 Vitamin D3 standard substance. cholecalciferol (C27H44O, CAS number. 67-97-0),

purity >=98%, or a standard substance certified by the state and awarded a standard substance certificate.

3.4.1 Vitamin D2 standard stock solution (1 000 mg/L). Accurately weigh 50 mg (accurate to 0.1 mg) of vitamin D2 standard substance in a small beaker; dissolve it in absolute ethanol and transfer it to a 50 mL volumetric flask; adjust the volume to the mark; mix well. Calibrate the concentration of the stock solution according to Appendix A. Put the stock solution into a brown reagent bottle; seal it and store it in the dark at -

3.4.4 Vitamin D3 standard working solution (10.0 mg/L). Accurately draw 1.00 mL

of

vitamin D3 standard stock solution (1 000 mg/L) into a 100 mL volumetric flask; use absolute ethanol to dilute to the mark; mix well. Store in the dark at -18 °C, with a use-by day of 3 months.

4.1 Normal-phase high-performance liquid chromatograph. equipped with a UV detector; the injector is equipped with a 500 μL quantitative loop.

4.2 Reversed-phase high-performance liquid chromatograph. equipped with a UV detector; the injector is equipped with a 100 μL quantitative loop.

5.1 Sample pretreatment

UV irradiation shall be avoided during processing.

5.1.1 Sample preparation

After a certain number of samples are divided, crushed and homogenized as required, store them in sample bags, protected from light and refrigerated, and measure as soon as possible.

5.1.1.3 Other foods

Weigh 5 g ~ 20 g (m, accurate to 0.01 g) of the homogenized solid sample or 20 g ~ 50 g (m, accurate to 0.01 g) of the liquid sample into a 150 mL flat-bottomed flask. For solid samples, add 20 mL ~ 30 mL of warm water (40 °C ~ 45 °C); mix well.

5.1.3.1 Sample extraction

Use 30 mL of water to transfer the above saponification solution into a 250 mL separatory funnel; add 50 mL of petroleum ether; shake and extract for 5 minutes; transfer the lower solution to another 250 mL separatory funnel; add 50 mL of petroleum ether and repeat the extraction 1 to 2 times; combine the petroleum ether layer. Use about 150 mL of water to wash the petroleum ether layer; remove the lower water phase; repeat washing at least 3 times until the petroleum ether layer is washed to neutral (a pH test paper universal can be used to detect the lower solution).