



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

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**National food safety standard - Determination of vitamin B6 in
foods**

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1 Scope

This Standard specifies the method for the determination of vitamin B6 in foods.

The first method "liquid chromatography - tandem mass spectrometry" is applicable to the determination of vitamin B6 in foods.

The second method "liquid chromatography - mass spectrometry" is applicable to the determination of vitamin B6 (pyridoxamine, pyridoxal and pyridoxine) in formulated milk powder, special dietary foods, ready-to-eat cereals, baked goods and beverages, in which, vitamin B6 (pyridoxamine, pyridoxal and pyridoxine) is added as a nutritional fortifier.

The third method "high-performance liquid chromatography - fluorescence detection method" is applicable to the determination of vitamin B6 (pyridoxamine, pyridoxal and pyridoxine) in formulated milk powder, special dietary foods (except foods for special medical purposes), ready-to-eat cereals, baked goods and beverages, in which, vitamin B6 (pyridoxamine, pyridoxal and pyridoxine) is added as a nutritional fortifier.

The fourth method "microbiological method" is applicable to the determination of vitamin B6 in foods.

Method I - Liquid Chromatography - Tandem Mass Spectrometry

2 Principle

Vitamin B6 (pyridoxamine, pyridoxal and pyridoxine) in foods is firstly hydrolyzed by acid, then, enzymatically hydrolyzed into pyridoxamine, pyridoxal and pyridoxine. After dilution and filtration, reversed-phase liquid chromatography separation, tandem mass spectrometry detection, and isotope internal standard method for quantitative determination, the total vitamin B6 content is calculated in terms of pyridoxine.

3 Reagents and Materials

Unless it is otherwise specified, the reagents used in this Method are all analytically pure, and the water is Grade-1 water specified in GB/T 6682.

5.1 Specimen Preparation

For meat, vegetables and fruits, etc., take the edible parts, use water to wash them, and use dry gauze to wipe off the surface moisture, use a homogenizer to homogenize them and store in sample bottles for later use. For powdery samples (milk powder and rice noodles, etc.), evenly mix them and conduct direct sampling. For liquid samples, evenly shake them and reserve them for later use. For liquid samples with granules, for example, large-grained yogurt, use a homogenizer to homogenize them and reserve them for later use. For flaky and granular samples, use a high-speed pulverizer to grind them into powder and seal for later use.

5.4 Drawing of Standard Curve

Inject the vitamin B6 mixed standard series working solutions into the liquid chromatography-mass spectrometer from low to high concentrations. Take the concentrations of the working solutions of pyridoxine, pyridoxal and pyridoxamine as the x-coordinate, and the peak area ratio of pyridoxine, pyridoxal and pyridoxamine to their corresponding isotope internal standards as the y-coordinate. Respectively draw the standard curves of pyridoxamine, pyridoxal and pyridoxine.

5.5 Determination of Specimen Solution

Inject the solution to be tested. In accordance with the three standard curves drawn above, adopt the internal standard method to calculate the concentration of pyridoxine, pyridoxal and pyridoxamine in the specimen solution to be tested.

6.1 The content of each component of vitamin B6 in the specimen is calculated in accordance

with Formula (1).

6.2 Total content of vitamin B6 (calculated as pyridoxine)

The total content of vitamin B6 in the specimen is calculated in accordance with Formula (2).

7 Precision

The absolute difference between the results of two independent determinations obtained under repeatability conditions shall not exceed 10% of the arithmetic mean.

8.1 Solid specimens. when the sampling size is 1.00 g, the detection limit of the Method is. 0.03

mg/kg for pyridoxine, 0.03 mg/kg for pyridoxal and 0.03 mg/kg for pyridoxamine; the quantitation limit is 0.10 mg/kg for pyridoxine, 0.10 mg/kg for pyridoxal and 0.10 mg/kg for pyridoxamine.

8.2 Liquid specimens. when the sampling size is 5.00 g, the detection limit of the Method is.

0.01 mg/kg for pyridoxine, 0.01 mg/kg for pyridoxal and 0.01 mg/kg for pyridoxamine;

9 Principle

Vitamin B6 (pyridoxine, pyridoxal and pyridoxamine) added to foods is subject to extraction, dilution and filtration, and separation by reversed-phase liquid chromatographic column, nitrogen and single quadrupole mass spectrometry detection. Adopt the isotope dilution

internal standard method to respectively quantify pyridoxamine, pyridoxal and pyridoxine. The total vitamin B6 content is calculated in terms of pyridoxine.

10 Reagents and Materials

Unless it is otherwise specified, the reagents used in this Method are all analytically pure, and the water is Grade-1 water specified in GB/T 6682.

10.2.1 Hydrochloric acid solution (0.1 mol/L). accurately draw 9 mL of hydrochloric acid and

use water to dilute to 1,000 mL.

10.2.2 Sodium hydroxide solution (0.1 mol/L). accurately weigh-take 0.4 g of sodium

hydroxide, add 50 mL of water to dissolve it; after cooling, use water to dilute to 100 mL.

10.4.5 Vitamin B6 standard mixed working solution (5.00 ?g/mL). accurately draw 1.00 mL of

vitamin B6 standard mixed stock solution (50.0 ?g/mL) into a 10 mL brown volumetric flask, use mobile phase A to dilute to the scale. Prepare it right before use.

10.6 Preparation of Standard Series of Working Solutions

Respectively and accurately draw an appropriate amount of vitamin B6 standard mixed working solution into a 50 mL brown volumetric flask, and accurately add 100 ?L of vitamin B6 isotope internal standard mixed working solution (50.0 ?g/mL). Use mobile phase A to dilute to the scale. The mass concentration of the vitamin B6 (pyridoxamine, pyridoxal and pyridoxine)

standard series working solutions is respectively 10.0 ng/mL, 20.0 ng/mL, 40.0 ng/mL, 100 ng/mL, 200 ng/mL and 250 ng/mL. Their corresponding isotope internal standard concentrations are all 100 ng/mL. Prepare them right before use.

12.3.1 Reference conditions of chromatography

The reference conditions of chromatography are as follows.

12.3.2 Reference conditions of mass spectrometry

The reference conditions of mass spectrometry are as follows.

12.4 Drawing of Standard Curve