



GB 5009.154-2023

National Food Safety Standard Determination of Vitamin B₆ in Food

食品安全国家标准 食品中维生素B₆的测定

National Health Commission of the People's
Republic of China
State Administration for Market Regulation

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National Standard of the People's Republic of China

GB 5009.154-2023
In Replacement of GB 5009.154-2016

National Food Safety Standard Determination of Vitamin B₆ in Food

食品安全国家标准

食品中维生素 B₆ 的测定

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State Administration for Market Regulation

Foreword

This standard replaces GB 5009.154-2016 "National Food Safety Standard - Determination of Vitamin B₆ in Food".

Compared with GB5009.154-2016, the main changes of this standard are as follows:

——The first method, liquid chromatography-tandem mass spectrometry, and the second method, liquid chromatography-mass spectrometry, were added;

——The third method, high performance liquid chromatography-fluorescence detection method, and the fourth method, microbiological method, have been modified.

National Food Safety Standard

Determination of Vitamin B₆ in Food

1 Scope

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

The first method, liquid chromatography-tandem mass spectrometry, is suitable for the determination of vitamin B₆ in food.

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

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

The fourth method, microbiological method, is applicable to the determination of vitamin B₆ in food.

Method 1: Liquid chromatography-tandem mass spectrometry

2 Principle

Vitamin B₆ (pyridoxine, pyridoxal, pyridoxamine) in food is first treated with acid hydrolysis and then enzymatic hydrolysis into pyridoxamine, pyridoxal and pyridoxine. After dilution and filtration, they are separated by reversed-phase liquid chromatography, detected by tandem mass spectrometry, and quantified by the isotope internal standard method. The total vitamin B₆ content is calculated using the pyridoxine counter.

3 Reagents and materials

Unless otherwise specified, all reagents used in this method were of analytical grade and the water was Grade-1 water as specified in GB/T6682.

3.1 Reagents

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3.1.1 Methanol (CH_3OH): chromatographically pure.

3.1.2 Formic acid (HCOOH): chromatographically pure.

3.1.3 Ammonium formate (HCOONH_4): chromatographically pure.

3.1.4 Sodium hydroxide (NaOH).

3.1.5 Hydrochloric acid (HCl).

3.1.6 α -Amylase: enzyme activity $\geq 50\text{U/mg}$.

3.1.7 Acid phosphatase: enzyme activity $\geq 0.5\text{U/mg}$.

3.1.8 Papain: enzyme activity $\geq 800\text{U/mg}$.

3.2 Reagent preparation

3.2.1 Hydrochloric acid solution (0.1 mol/L): Accurately pipette 9 mL of hydrochloric acid and dilute to 1000 mL with water.

3.2.2 Sodium hydroxide solution (0.1 mol/L): Accurately weigh 0.4 g of sodium hydroxide, add 50 mL of water to dissolve it, cool it, and dilute it to 100 mL with water.

3.2.3 Mobile phase A (10mmol/L ammonium formate in 2% formic acid aqueous solution): Weigh 0.63g of ammonium formate, add about 950mL of water to dissolve, add 20mL of formic acid, dilute with water to 1000mL, mix well, and degas by ultrasonication for later use.

3.2.4 Mobile phase B (0.1% formic acid-methanol solution): Pipette 1 mL of formic acid, dilute to 1000 mL with methanol, and mix by ultrasonication.

3.3 Standards

3.3.1 Pyridoxine hydrochloride ($\text{C}_8\text{H}_{12}\text{ClNO}_3$, CAS No.: 58-56-0): Purity $\geq 98\%$, or standard substances certified by the state and awarded with standard substance certificates.

3.3.2 Pyridoxal hydrochloride ($\text{C}_8\text{H}_{10}\text{ClNO}_3$, CAS No.: 65-22-5): Purity $\geq 99\%$, or standard substances certified by the state and awarded with standard substance certificates.



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