



GB 5009.259-2023

National Food Safety Standard Determination of Biotin in Food

食品安全国家标准 食品中生物素的测定

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

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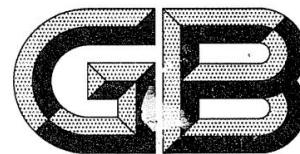
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National Standard of the People's Republic of China

GB 5009.254-2023
In Replacement of GB 5009.254-2016

National Food Safety Standard Determination of Biotin in Food

食品安全国家标准

食品中生物素的测定

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Foreword

This standard replaces GB 5009.259-2016 “National Food Safety Standard - Determination of Biotin in Foods”.

Compared with GB 5009.259-2016, the main changes of this standard are as follows:

——The first method, liquid chromatography-tandem mass spectrometry, was added, and the microbiological method was adjusted to the second method;

——The second method of microbiological method has added microplate culture method;

——The applicable scope, sample categories and pre-treatment methods of the standard have been modified;

—— Modified the description of standard curve and sample extract preparation;

——The detection limit, quantitation limit and linear range were modified.

National Food Safety Standard

Determination of Biotin in Food

1 Scope

This standard specifies the determination method of biotin in food.

The first method, liquid chromatography-tandem mass spectrometry, is applicable to the determination of biotin in formula milk powder and special dietary foods.

The second method, microbiological method, is applicable to the determination of biotin in food.

Method 1: Liquid chromatography-tandem mass spectrometry

2 Principle

The samples are dissolved and extracted, and the starch-containing samples are hydrolyzed with amylase, protein precipitated, centrifuged and filtered, separated on a C_{18} reverse phase column, detected by liquid chromatography-tandem mass spectrometry multiple ion reaction monitoring, and quantified by the isotope dilution internal standard method.

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 and materials

3.1.1 Formic acid (HCOOH): chromatographically pure.

3.1.2 Acetonitrile (CH_3CN): chromatographically pure.

3.1.3 Ethanol ($\text{CH}_3\text{CH}_2\text{OH}$): chromatographically pure.

3.1.4 Ammonium formate (HCOONH_4): chromatographically pure.

3.1.5 Perchloric acid (HClO_4): 70%~72%.

GB 5009.259-2023

3.1.6 Sodium hydroxide (NaOH): purity $\geq 99.9\%$.

3.1.7 Amylase: Taka-amylase, CAS: 9001-19-8, enzyme activity ≥ 100 U/mg.

3.2 Reagent preparation

3.2.1 0.1% formic acid-10mmol/L ammonium formate aqueous solution: Weigh 0.63g of ammonium formate, dissolve it in 100mL of water, transfer it to a 1000mL reagent bottle, add 1mL of formic acid, dilute to 1000mL with water, shake well and set aside.

3.2.2 Sodium hydroxide solution (2 mol/L): Weigh 8.00 g of sodium hydroxide into a beaker, add 100 mL of water to dissolve, shake well and set aside.

3.2.3 Ethanol solution (50%): Accurately measure 500 mL of ethanol into a 1000 mL reagent bottle, dilute to 1000 mL with water, shake well and set aside.

3.3 Standards

3.3.1 Biotin Standard ($C_{10}H_{16}N_2O_3S$):CAS:58-85-5, purity $\geq 98\%$, or standard substances certified by the state and awarded with standard substance certificates.

3.3.2 Biotin- D_4 ($C_{10}D_4H_{12}N_2O_3S$):CAS:1217850-77-5,purity $\geq 98\%$.

3.4 Preparation of standard solution

3.4.1 Biotin standard stock solution (100 $\mu\text{g/mL}$): According to the purity conversion, accurately weigh 10.00 mg of biotin standard (accurate to 0.01 mg), dissolve it in ethanol solution (50%) and make up to 100 mL. The solution is transferred to a brown glass bottle and stored in a sealed container at -20°C . The shelf life is 3 months.

3.4.2 Biotin standard intermediate solution (10 $\mu\text{g/mL}$): Accurately pipette 5.00 mL of biotin standard stock solution (100 $\mu\text{g/mL}$) into a 50 mL volumetric flask and dilute to 50 mL with ethanol solution (50%).The solution is transferred to a brown glass bottle and stored in a sealed container at -20°C . The shelf life is 3 months.

3.4.3 Biotin standard intermediate solution (1 $\mu\text{g/mL}$): Accurately pipette 1.00 mL of biotin standard intermediate solution (10 $\mu\text{g/mL}$) into a 10 mL volumetric flask and dilute to 10 mL with ethanol solution (50%). The solution is transferred to a brown glass bottle and stored in a sealed container at -20°C . The shelf life is 3 months.



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