



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

GB 5009.259-2023

National food safety standard - Determination of biotin in foods

Issued on: September 6, 2023

Implemented on: September 6, 2024

Issued by: NHC; SAMR

Table of Contents

Foreword...	3
1 Scope...	4
Method I - Liquid Chromatography - Tandem Mass Spectrometry...	4
2 Principle...	4
3 Reagents and Materials...	4
4 Instruments and Equipment...	6
5 Analytical Procedures...	6
6 Expression of Analysis Results...	9
7 Precision...	10
8 Others...	10
Method II - Microbiological Method...	10
9 Principle...	10
10 Reagents and Materials...	10
11 Instruments and Equipment...	12
12 Analytical Procedures...	13
13 Expression of Analysis Results...	18
14 Precision...	19
15 Others...	19

Foreword

This Standard serves as a replacement of GB 5009.259-2016 National Food Safety Standard -

Determination of Biotin in Foods.

In comparison with GB 5009.259-2016, the main changes are as follows.

---Method 1 "Liquid Chromatography - Tandem Mass Spectrometry" is added, and the "Microbiological Method" is adjusted to Method 2;

---The microplate culture method is added to Method 2 - "Microbiological Method";

---The scope of application, sample category and pre-treatment method of the Standard are modified;

---The expression of the drawing of standard curve and the preparation of sample extracting solution is modified;

---The detection limit, quantitation limit and linear range are modified.

National Food Safety Standard - Determination of Biotin in Foods

1 Scope

This Standard specifies the methods for the determination of biotin in foods.

Method 1 - liquid chromatography - tandem mass spectrometry is applicable to the determination of biotin in prepared milk powder and special dietary foods.

Method 2 - microbiological method is applicable to the determination of biotin in foods.

Method 1 - Liquid Chromatography - Tandem Mass Spectrometry

2 Principle

The specimen is dissolved and extracted, and the starch-containing specimen is subject to enzymatic hydrolysis by amylase, protein precipitation and centrifugal filtration, and separated

on a C18 reversed-phase chromatography column. Adopt the liquid chromatography - tandem

mass spectrometry multi-ion reaction monitoring mode for detection, and the isotope dilution

internal standard method for quantitative determination.

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.

3.6 Preparation of Standard Series of Working Solutions

Respectively and accurately draw-take an appropriate amount of biotin standard working solution into 10 mL volumetric flasks, accurately add 150 μ L of biotin-D4 isotope internal

standard working solution (1 ?g/mL), use mobile phase A to reach a constant volume to the scale, so that the mass concentration of the standard series of biotin is respectively. 1.0 ng/mL,

2.0 ng/mL, 5.0 ng/mL, 10 ng/mL, 15 ng/mL, 20 ng/mL and 25 ng/mL. The mass concentration

of isotope in each series of solution is 15 ng/mL. Prepare them right before use.

4.6 Vortex mixer. 600 r/min ~ 3,200 r/min.

4.8 2 mm aperture test sieve (optional).

5.1.1 Solid samples

The sampling size needs to be greater than 0.5 kg. Use a high-speed pulverizer to pulverize non-uniform samples, until all of them pass through a 2 mm aperture test sieve. After evenly mixing, divide the samples to 100 g and store in a wide-mouth bottle. Seal it and reserve it for

testing. For uniform samples, directly evenly mix them and reserve them for testing.

5.1.2 Semi-solid samples

The sampling size needs to be greater than 0.5 kg. At least 3 packages (from the same batch)

need to be collected. After all samples are homogenized and evenly mixed in a container, store

any 100 g of them in a wide-mouth bottle. Seal it and reserve it for testing.

5.1.3 Liquid samples

The sampling size needs to be greater than 0.5 L. At least 3 packages (from the same batch)

need to be collected. After all samples are evenly mixed in a container, store any 100 mL of them in a wide-mouth bottle. Seal it and reserve it for testing.

5.4 Qualitative Determination

The selected ions all appear, and in the same detection batch, compared with the relative abundance of the standard solution of an equivalent concentration, the relative abundance of

two daughter ions of biotin in the sample shall have an allowable deviation of not exceeding the range specified in Table 3.

5.5 Drawing of Standard Curve

In accordance with the concentration from low to high, successively inject the biotin standard

series of working solutions into the liquid chromatograph - tandem mass spectrometer, take the

ratio of biotin concentration to biotin internal standard concentration as the x-coordinate, and

the peak area ratio of biotin to biotin internal standard as the y-coordinate to draw a standard

curve of biotin.

6 Expression of Analysis Results

The content of biotin in the specimen is calculated in accordance with Formula (1).

7 Precision

The absolute difference between the results of two independent determinations obtained under

repeatability conditions shall not exceed 15% of the arithmetic mean.

8 Others

When the sampling size is 5.0 g and the constant volume is 50 mL, the detection limit of this

Method is 0.300 μ g/100 g, and the quantitation limit is 1.00 μ g/100 g.

9 Principle

Biotin is an essential nutrient for the growth of *Lactiplantibacillus plantarum*. In the biotin determination culture medium, the growth of *Lactiplantibacillus plantarum* is correlated with the biotin content.

10 Reagents and Materials

Unless it is otherwise specified, the reagents used in this Method are all analytically pure,