



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

GB 5009.22-2016

**National food safety standard - B-group and G-group aflatoxins
in foods**

(English Translation)

Issued on: December 23, 2016

Implemented on: June 23, 2017

Issued by: National Health and Family Planning Commission of the PRC

Table of Contents

Foreword...	4
1 Scope...	5
2 Principle...	6
3 Reagents and materials...	6
4 Instruments and equipment...	8
5 Analytical procedures...	9
6 Expression of analytical results...	15
7 Precision...	15
8 Others...	16
9 Principle...	16
10 Reagents and materials...	16
11 Instruments and equipment...	18
12 Analytical procedures...	19
13 Expression of analytical results...	21
14 Precision...	22
15 Others...	22
16 Principle...	23
17 Reagents and materials...	23
18 Instruments and equipment...	25
19 Analytical procedures...	27
20 Expression of analytical results...	31
21 Precision...	32
22 Others...	32
23 Principle...	33
24 Reagents and materials...	33
25 Instruments and equipment...	33
26 Analytical procedures...	34
27 Expression of analytical results...	34
28 Precision...	35
29 Others...	35
30 Principle...	36
31 Reagents and materials...	36

32 Instruments and equipment...	38
33 Analytical steps...	38
34 Precision...	46
35 Others...	46

1 Scope

This standard specifies the method for determination of aflatoxin B1, aflatoxin

B2, aflatoxin G1, aflatoxin G2 (hereinafter referred to as AFT B1, AFT B2, AFT G1 and AFT G2) in food.

The first method of this standard is the isotope dilution liquid chromatography- tandem mass spectrometry, which is suitable for the determination of AFT B1,

AFT B2, AFT G1 and AFT G2 in cereals and their products, beans and their products, nuts and seeds, fats and their products, seasonings, infant formula and infant complementary foods.

The second method of this standard is the high-performance liquid chromatography-pre-column derivatization, which is suitable for the determination of AFT B1, AFT B2, AFT G1 and AFT G2 in cereals and their products, beans and their products, nuts and seeds, fats and their products, seasonings, infant formula and infant complementary foods.

The third method of this standard is the high-performance liquid chromatography-post-column derivatization, which is suitable for the determination of AFT B1, AFT B2, AFT G1 and AFT G2 in cereals and their products, beans and their products, nuts and seeds, fats and their products, seasonings, infant formula and infant complementary foods.

The fourth method of this standard is the enzyme-linked immunoadsorption screening method, which is suitable for the determination of AFT B1 in cereals and their products, beans and their products, nuts and seeds, fats and their products, seasonings, infant formula and infant complementary foods.

The fifth method of this standard is the thin-layer chromatography, which is suitable for the determination of AFT B1 in cereals and their products, beans and their products, nuts and seeds, fats and their products, and seasonings.

Method I. Isotope dilution liquid chromatography- tandem mass spectrometry

2 Principle

The aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2 in the specimen are extracted by acetonitrile-water solution or methanol-water solution. After the extract is diluted by the phosphate buffer solution which contains 1% Triton X-

100 (or Tween-20) (if necessary, it is initially purified by the aflatoxin solid-phase purification column), through purification and enrichment by immunoaffinity column, the purification liquid is concentrated, constant-volume, filtered, separated by liquid chromatography, tested by tandem mass spectrometry, then subjected to quantification by isotope internal standard method.

3 Reagents and materials

Unless otherwise stated, the reagents used in this method are of analytical grade, the water is the grade I water as specified in GB/T 6682.

4 Instruments and equipment

4.1

Homogenizer.

4.2

High-speed pulverizer.

4.3

Tissue masher.

4.4

Ultrasonic / vortex oscillator or shaker.

4.5

Balance. Sensitivity is 0.01 g and 0.00001 g.

4.6

Vortex mixer.

4.9 Glass-fiber filter paper. Fast, high-load, which retains 1.6 μm particles in liquid.

4.13 Liquid chromatography column.

4.14 Immunoaffinity column. Capacity of AFT B1 column $\geq$ 200 ng, recovery rate

of AFT B1 column $\geq$ 80%, cross-reaction rate of AFT G2 $\geq$ 80% (see Appendix B for verification method).

4.15 Aflatoxin-specific solid-phase extraction purification column or functionally

equivalent solid-phase extraction column (hereinafter referred to as purification column). which is used for the determination of samples of complex matrix.

4.18 pH meter.

5 Analytical procedures

The use of immunoaffinity columns from different manufacturers may slightly differ in sample loading, rinsing and elution operations, so it shall be performed in accordance with the operation instructions as provided by the manufacturer.

Caution.

5.1.1 Liquid samples (vegetable oil, soy sauce, vinegar, etc.)

The sampling amount shall be greater than 1 L. For packaging samples such as bags and bottles, it shall collect at least 3 packages (same batch or number).

All liquid samples shall be mixed by a homogenizer in a container, any 100 g (mL) of sample is taken for testing.

5.1.2 Solid samples (cereals and their products, nuts and seeds, cereal

supplements for infants, etc.)

The sampling amount shall be greater than 1 kg. It shall be crushed by high- speed pulverizer and sieved to make the particle size less than the 2 mm aperture test sieve. It is evenly mixed and then reduced to 100 g, stored in the sample bottle, sealed for preservation, to prepare for testing.

5.1.3 Semi-fluid (fermented bean curd, fermented soybean, etc.)

The sampling amount shall be greater than 1 kg (L). For packaging samples such as bags and bottles, it shall collect at least 3 packages (same batch or number). Then it is crushed and mixed uniformly by the tissue crusher, stored in sample bottle, sealed for preservation, to prepare for testing.