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Report Highlights:

On September 22, 2026, China's NHC and SAMR jointly released the National Food Safety Standard for Determination of Aflatoxins B and G in Foods (GB5009.22-2026) and National Food Safety Standard for Determination of Aflatoxins M Group in Foods (GB5009.24-2026). This report provides an unofficial translation of the final two standards. Stakeholders should conduct their own review of the regulations to assess any market or regulatory impacts on their business.

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On September 22, 2026, China's National Health Commission (NHC) and the State Administration for Market Regulation (SAMR) announced (link in Chinese) the National Food Safety Standard for Standard for Determination of Aflatoxins B and G in Foods (GB5009.22-2026) and the National Food Safety Standard for Standard for Determination of Aflatoxins M Group in Foods (GB5009.24-2026). The standards specifically regulate Determination of Aflatoxins B, G and, M in Foods, and it will enter into force on February 18, 2027.

GB5009.22-2026 specifies methods for the determination of aflatoxins B1, B2, G1, and G2 in foods. It describes four analytical methods: liquid chromatography with post-column derivatization, liquid chromatography with pre-column derivatization, liquid chromatography-tandem mass spectrometry (LC-MS/MS), and enzyme-linked immunosorbent assay (ELISA) for screening aflatoxin B1 in specified food categories. It outlines the scope and principles of each method, required reagents and materials, apparatus and equipment, sample preparation, extraction and cleanup procedures, reference instrumental conditions, qualitative identification and quantitative determination, expression of analytical results, precision requirements, and limits of detection and quantification.

GB5009.24-2026 specifies methods for the determination of aflatoxins M in milk, dairy products, and foods for special dietary uses. It describes three analytical methods: liquid chromatography with fluorescence detection, liquid chromatography-tandem mass spectrometry (LC-MS/MS) with isotope internal standard quantification, and enzyme-linked immunosorbent assay (ELISA) for screening aflatoxin M in specified food categories. It outlines the scope and principles of each method, required reagents and materials, apparatus and equipment, sample preparation and storage, extraction and cleanup procedures, reference instrumental conditions, qualitative identification and quantitative determination, expression of analytical results, precision requirements, and limits of detection and quantification.

The report provides an unofficial translation of the final standard. Stakeholders should conduct their own review of the regulations to assess any market or regulatory impacts on their business.

BEGIN UNOFFICIAL TRANSLATION

National Food Safety Standard

Determination of Aflatoxins B and G in Foods (GB 5009.22—2026)

Foreword

This standard replaces GB 5009.22—2016, National Food Safety Standard—Determination of Aflatoxins B and G in Foods.

The principal technical changes compared with GB 5009.22—2016 are as follows:

- the scope of application has been revised;

- the order of the liquid chromatography–post-column derivatization method and the liquid chromatography–tandem mass spectrometry method, as well as sample preparation and extraction, have been revised;
- the limits of detection and limits of quantification for the liquid chromatography–post-column derivatization, liquid chromatography–pre-column derivatization, and liquid chromatography–tandem mass spectrometry methods have been revised;
- the thin-layer chromatography method has been deleted.

1 Scope

This standard specifies methods for determining aflatoxin B1, aflatoxin B2, aflatoxin G1, and aflatoxin G2 (hereinafter AFB_1, AFB_2, AFG_1, and AFG_2, respectively) in foods.

Method 1, liquid chromatography with post-column derivatization, and Method 3, liquid chromatography–tandem mass spectrometry, apply to determination of AFB_1, AFB_2, AFG_1, and AFG_2 in foods.

Method 2, liquid chromatography with pre-column derivatization, applies to determination of AFB_1, AFB_2, AFG_1, and AFG_2 in oils and fats and their products.

Method 4, enzyme-linked immunosorbent assay (ELISA), applies to screening for AFB_1 in cereals and cereal products, legumes and legume products, nuts and seeds, oils and fats and their products, seasonings, infant formula, and complementary foods for infants and young children.

Method 1 — Liquid Chromatography with Post-Column Derivatization

2 Principle

The sample is extracted with an acetonitrile–water solution, purified using an immunoaffinity column, detected by liquid chromatography with a fluorescence detector, and quantified by the external-standard method.

3 Reagents and Materials

Unless otherwise specified, reagents used in this method are of analytical grade, and water is Grade 1 water as specified in GB/T 6682.

3.1 Reagents

3.1.1 Acetonitrile (CH_3CN).

3.1.2 Sodium chloride (NaCl).

3.1.3 Disodium hydrogen phosphate (Na_2HPO_4) or disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$).

3.1.4 Potassium dihydrogen phosphate (KH_2PO_4).

3.1.5 Potassium chloride (KCl).

3.1.6 Hydrochloric acid (HCl).

3.1.7 Tween-20 ($C_{58}H_{114}O_{26}$).

3.1.8 Methanol (CH_3OH), chromatographic grade.

3.1.9 Glycerol ($C_3H_8O_3$).

3.1.10 Acetonitrile (CH_3CN), chromatographic grade.

3.1.11 Iodine derivatization reagent: iodine (I_2).

3.1.12 Bromine derivatization reagent: pyridinium tribromide ($C_5H_6Br_3N_2$).

3.1.13 Electrochemical derivatization reagents: potassium bromide (KBr) and concentrated nitric acid (HNO_3).

3.2 **Preparation of Reagents**

3.2.1 Acetonitrile–water solution A (84+16): add 16 mL water to 84 mL acetonitrile and mix.

3.2.2 Acetonitrile–water solution B (70+30): add 30 mL water to 70 mL acetonitrile and mix.

3.2.3 Phosphate buffer: dissolve 8.00 g sodium chloride, 1.20 g disodium hydrogen phosphate (or 3.02 g disodium hydrogen phosphate dodecahydrate), 0.20 g potassium dihydrogen phosphate, and 0.20 g potassium chloride in 900 mL water. Measure the pH and, if necessary, adjust it with 1 mol/L hydrochloric acid to match the immunoaffinity column. Dilute to 1,000 mL with water and mix.

3.2.4 1 mol/L hydrochloric acid: add 8.6 mL hydrochloric acid to 80 mL water. After cooling, make up to 100 mL.

3.2.5 1% Tween-20 phosphate-buffered saline (PBST): dilute 10 mL Tween-20 with phosphate buffer to 1,000 mL and mix.

3.2.6 10% glycerol–methanol solution: dilute 1 mL glycerol with methanol to 10 mL and mix.

3.2.7 Acetonitrile–methanol solution: mix 50 mL chromatographic-grade acetonitrile with 50 mL chromatographic-grade methanol.

3.2.8 5 g/L iodine solution: dissolve 0.1 g iodine in 20 mL chromatographic-grade methanol, dilute with water to 200 mL, mix, and filter through a 0.45 μm aqueous-phase membrane. Prepare immediately before use; for the iodine post-column derivatization method only.

3.2.9 5 mg/L pyridinium tribromide solution: dissolve 5 mg pyridinium tribromide in 1 L water, mix, and filter through a 0.45 μm aqueous-phase membrane. Prepare immediately before use; for the bromine post-column derivatization method only.

3.3 Reference Standards

3.3.1 AFB_1 standard ($C_{17}H_{12}O_6$, CAS No. 1162-65-8): standard or solution with purity ≥98%

3.3.2 AFB_2 standard ($C_{17}H_{14}O_6$, CAS No. 7220-81-7): standard or solution with purity ≥98%.

3.3.3 AFG_1 standard ($C_{17}H_{12}O_7$, CAS No. 1165-39-5): standard or solution with purity ≥98%.

3.3.4 AFG_2 standard ($C_{17}H_{14}O_7$, CAS No. 7241-98-7): standard or solution with purity ≥98%.

3.4 Preparation of Standard Solutions

Warning: All analytical operations shall be performed in a designated area. The area shall be protected from light (direct sunlight), have a relatively separate workbench and waste-storage facilities. Throughout the test, operators shall take protective measures appropriate for handling highly toxic substances.

3.4.1 Standard stock solutions (10.0 μg/mL): accurately weigh 1 mg each of AFB_1, AFB_2, AFG_1, and AFG_2 (to 0.01 mg), dissolve each in acetonitrile, and transfer to separate 100 mL volumetric flasks; make up to volume. Store protected from light at −18 °C or below for up to 12 months. Calibrate concentrations before use; see Appendix A.

3.4.2 Mixed intermediate standard solution (1.00 μg/mL): accurately transfer 1.00 mL of each standard stock solution to a 10 mL volumetric flask and make up to volume with acetonitrile. Store protected from light at −18 °C or below for up to 12 months.

3.4.3 Mixed working standard solution (100 ng/mL): accurately transfer 10.00 mL of the mixed intermediate solution to a 100 mL volumetric flask and make up to volume with acetonitrile. Prepare immediately before use.

3.4.4 Standard working series: dilute appropriate amounts of the mixed working and mixed intermediate standard solutions with the mobile phase to obtain 0.200, 1.00, 5.00, 10.0, and 50.0 ng/ml. Prepare immediately before use.

3.5 Materials

3.5.1 Glass-fiber filter paper, pore size 1.6μm, or equivalent.

3.5.2 Immunoaffinity column: capacity ≥200 ng and recovery ≥85%.

3.5.3 Composite solid-phase extraction (SPE) column, 3 g/5 mL, packed with carbon–alumina and silica composite material, or equivalent.

3.5.4 Syringe membrane filter, 0.45μm, organic-compatible, or equivalent.

4 Apparatus and Equipment

4.1 Liquid chromatograph equipped with a fluorescence detector.

4.2 Photochemical post-column reactor (for photochemical post-column derivatization).

4.3 Solvent-based post-column derivatization system (for iodine or bromine derivatization).

4.4 Electrochemical post-column derivatization reactor.

4.5 Balances with readability of 0.01 mg and 0.01 g, respectively.

4.6 Homogenizer.

4.7 high-speed grinder.

4.8 tissue homogenizer.

4.9 Vortex shaker, speed ≥1,000 r/min

4.10 vortex mixer.

4.11 High-speed homogenizer, 6,500–24,000 r/min.

4.12 Centrifuge, speed ≥6,000 r/min.

4.13 Nitrogen evaporator.

4.14 pH meter.

5 Analytical Procedure

5.1 Sample Preparation and Storage

5.1.1 Sample Preparation

The sample quantity for testing shall exceed 1 kg. If less than 1 kg is available, take at least three packages from the same batch and, after subdivision, obtain no less than 0.5 kg. Grind solid samples with a high-speed grinder, homogenize semi-solid samples with a tissue homogenizer, and homogenize liquid samples with a homogenizer.

Note 1: For special samples such as high-fat granular samples (e.g., peanuts), elastic/high-viscosity samples (e.g., dumpling wrappers), or high-sugar samples (e.g., raisins), use freezing or addition of water during homogenization as appropriate. For peanuts, sample: water = 1:1.4; for other samples, use 1:1 or add water as needed to achieve a uniform sample.

Note 2: Carbonated beverages and alcoholic beverages may be degassed by sonication, stirring, vacuum treatment, or similar means before mixing.

Note 3: Sample preparation may be adjusted to the purpose of the test.

5.1.2 Sample Storage

Divide the thoroughly mixed prepared sample into multiple portions of approximately 100 g in clean containers. Seal and store protected from light at 2 °C–6 °C or below −18 °C, depending on the sample.

5.2 Sample Extraction

5.2.1Solid and semi-solid samples

Weigh 5 g (to 0.01 g; 10 g for corn) into a centrifuge tube. Add 20.0 mL acetonitrile–water solution A (40 mL for corn; 25.0 mL for highly water-absorbing samples such as infant rice cereal) or solution B for high-protein samples. Shake on a vortex shaker for 20 min (or homogenize for 3 min), centrifuge at 6,000 r/min for 10 min, or filter through glass-fiber filter paper, and retain the supernatant.

Note: For water-added preparations of high-fat granular, elastic/high-viscosity, or high-sugar samples, weigh 10 g (12 g for peanuts) into a centrifuge tube and add 25.0 mL acetonitrile (33.0 mL for peanuts). Perform the remaining steps as above.

5.2.2 Liquid samples

For oil samples (e.g., vegetable oil), weigh 5 g (to 0.01 g) into a centrifuge tube, add 20.0 mL acetonitrile–water solution A, shake for 20 min (or homogenize for 3 min), centrifuge at 6,000 r/min for 10 min (or filter through glass-fiber filter paper), and retain the supernatant. For other liquid samples, weigh 5 g, add acetonitrile to 30.0 mL, mix as above, and retain the supernatant.

5.3 Sample Clean-up

Take 4.0 mL supernatant and dilute to 50 mL with PBST; mix. If cloudy, centrifuge at 8,000 r/min for 10 min.

Allow the immunoaffinity column to reach room temperature. After the liquid initially in the column has drained, pass all diluted sample through the column at approximately 1 drop/s. After the sample has passed through, wash the column successively with 10 mL PBST and water and discard all effluent. Remove residual water from the column. Elute with 2 mL methanol at 1 drop/s and collect the eluate. Add 100 μL of 10% glycerol–methanol solution and gently evaporate under nitrogen at 50 °C to near dryness. Add 1.0 mL mobile phase, vortex for 30 s to dissolve the residue, filter through a syringe membrane filter, and collect the filtrate in an autosampler vial.

Note 1: For water-added high-fat granular, elastic/high-viscosity, or high-sugar samples and other liquid samples, take 6.0 mL supernatant (8.0 mL for peanuts). Proceed as above.

Note 2: If necessary, measure and adjust the pH of the diluted solution to ensure compatibility with the immunoaffinity column.

Note 3: For certain complex matrices, pre-clean the supernatant with an SPE column before the immunoaffinity column, following the manufacturer's recommendations and laboratory validation. Place an appropriate amount of supernatant in the SPE column's collection tube, slowly press the column into the tube, collect the purified liquid, and further purify it with the immunoaffinity column.

Note 4: Follow the instructions for the particular immunoaffinity and SPE columns used.

Note 5: Fully automated (online) or semi-automated (offline) SPE instruments may be used after optimization of operating parameters.

5.4 Reference Liquid Chromatography Conditions

Select a method according to the hardware configuration of the liquid chromatograph.

5.4.1 Direct Determination

The reference liquid chromatography conditions are as follows:

a) Mobile phase: Phase A, water; Phase B, 50% acetonitrile–methanol solution;

b) Isocratic elution conditions: 65% Phase A and 35% Phase B;

c) Chromatographic column: C18 column (100 mm in length, 2.1 mm in internal diameter, with a particle size of 1.7 μm), or equivalent;

d) Flow rate: 0.3 mL/min;

e) Column temperature: 40 °C;

f) Injection volume: 10 μL;

g) Detector: Fluorescence detector with a large-volume flow cell;

h) Excitation wavelength: 365 nm; emission wavelengths: 436 nm for AFB1 and AFB2, and 463 nm for AFG1 and AFG2.

5.4.2 Post-Column Photochemical Derivatization

The reference liquid chromatography conditions are as follows:

a) Mobile phase: Phase A, water; Phase B, 50% acetonitrile–methanol solution;

b) Isocratic elution conditions: 68% Phase A and 32% Phase B;

c) Chromatographic column: C18 column (150 mm or 250 mm in length, 4.6 mm in internal diameter, with a particle size of 5 μm), or equivalent;

d) Flow rate: 1.0 mL/min;

e) Column temperature: 40 °C;

f) Injection volume: 50 μL;

g) Post-column photochemical derivatization unit;

h) Excitation wavelength: 360 nm; emission wavelength: 440 nm.

5.4.3 Post-Column Iodine or Bromine Derivatization

5.4.3.1 Post-column Iodine Derivatization Method

The reference liquid chromatography conditions are as follows:

a) Mobile phase: Phase A, water; Phase B, 50% acetonitrile–methanol solution;

b) Isocratic elution conditions: 68% Phase A and 32% Phase B;

c) Chromatographic column: C18 column (150 mm or 250 mm in length, 4.6 mm in internal diameter, with a particle size of 5 μm), or equivalent;

d) Flow rate: 1.0 mL/min;

e) Column temperature: 40 °C;

f) Injection volume: 50 μL;

g) Post-column derivatization system;

h) Derivatization solution: 0.05% iodine solution;

i) Derivatization solution flow rate: 0.2 mL/min;

j) Derivatization reaction tube temperature: 70 °C;

k) Excitation wavelength: 360 nm; emission wavelength: 440 nm.

5.4.3.2 Post-column Bromine Derivatization Method

The reference liquid chromatography conditions are as follows:

a) Mobile phase: Phase A, water; Phase B, 50% acetonitrile–methanol solution;

b) Isocratic elution conditions: 68% Phase A and 32% Phase B;

c) Chromatographic column: C18 column (150 mm or 250 mm in length, 4.6 mm in internal diameter, with a particle size of 5 μm), or equivalent;

d) Flow rate: 1.0 mL/min;

e) Column temperature: 40 °C;

f) Injection volume: 50 μL;

g) Post-column derivatization system;

h) Derivatization solution: 5 mg/L aqueous solution of pyridinium tribromide;

i) Derivatization solution flow rate: 0.2 mL/min;

j) Derivatization reaction tube temperature: 70 °C;

k) Excitation wavelength: 360 nm; emission wavelength: 440 nm.

5.4.4 Post-column Electrochemical Derivatization Method

The reference liquid chromatography conditions are as follows:

a) Mobile phase: Phase A, water containing 119 mg of potassium bromide and 350 μL of 4 mol/L nitric acid per 1 L of water; Phase B, chromatographic-grade methanol;

b) Isocratic elution conditions: 60% Phase A and 40% Phase B;

c) Chromatographic column: C18 column (150 mm or 250 mm in length, 4.6 mm in internal diameter, with a particle size of 5 μm), or equivalent;

d) Column temperature: 40 °C;

e) Flow rate: 1.0 mL/min;

f) Injection volume: 50 μL;

g) Post-column electrochemical derivatization unit: Reaction cell operating current, 100 μA; one PEEK reaction tube (50 cm in length, 0.5 mm in internal diameter);

h) Excitation wavelength: 360 nm; emission wavelength: 440 nm.

5.5 Preparation of Calibration Curve

Inject each standard working solution into the liquid chromatograph and measure the corresponding peak area. Plot standard concentration on the x-axis and peak area on the y-axis to obtain the calibration curve. Chromatograms of standard solutions are shown in Figures B.1–B.6 of Appendix B.

5.6 Qualitative Identification

Under identical test conditions, the relative deviation between the retention time of each target compound in the sample solution and that in the standard working series shall be within ±2.5%.

5.7 Measurement of Sample Solution

Inject the sample solution into the liquid chromatograph and measure the relevant peak areas. Determine the concentrations of the target compounds in the sample solution from the calibration curve.

Responses shall fall within the linear range of the calibration curve. If a response exceeds that range, reduce the amount of extract used, then repeat clean-up and injection analysis.

5.8 Blank Test

Perform all the above analytical steps without weighing a sample.

6 Expression of Analytical Results

The content of AFB_1, AFB_2, AFG_1, and AFG_2 in the sample is calculated using Equation (1):

$$X = \frac{\rho \times V_1 \times V_3 \times 1000}{V_2 \times m \times 1000} \quad \text{---------- (1)}$$

Where: X is the content of the analyte in the sample (μg/kg);

ρ is the mass concentration of the analyte in the sample solution calculated from the calibration curve (ng/mL);

V_1 is the sample-extract volume (mL);

V_3 is the final volume after clean-up and elution (mL);

1000 is the conversion factor;

V_2 is the volume of extract used for clean-up (mL);

and m is the sample mass (g).

Note: For water-added preparations of high-fat granular, elastic/high-viscosity, or high-sugar samples, m is the mass of sample tested excluding the added water.

Report results to three significant figures.

7 Precision

Under repeatability conditions, the absolute difference between two independent results shall not exceed 20% of their arithmetic mean.

8 Other

When 5 g of sample is taken, the limit of detection for AFB_1, AFB_2, AFG_1, and AFG_2 is 0.1 μg/kg for each; the limit of quantification is 0.2 μg/kg for each.

Method 2 — Liquid Chromatography with Pre-Column Derivatization

9 Principle

The sample is extracted with acetonitrile–water solution, cleaned up with a solid-phase extraction column, derivatized with trifluoroacetic acid, detected by liquid chromatography with fluorescence detection, and quantified by the external-standard method.

10 Reagents and Materials

Unless otherwise specified, reagents are analytical grade and water is Grade 1 water as specified in GB/T 6682.

10.1 Reagents

10.1.1 Acetonitrile (CH_3CN).

10.1.2 n-Hexane (C_6H_{14}).

10.1.3 Trifluoroacetic acid (CF_3COOH).

10.1.4 Acetonitrile (CH_3CN), chromatographic grade.

10.1.5 Methanol (CH_3OH), chromatographic grade.

10.2 Reagent Preparation

10.2.1Acetonitrile–water solution A (84+16): add 16 mL water to 84 mL acetonitrile and mix.

10.2.2 Acetonitrile–methanol solution (50+50): mix 50 mL chromatographic-grade acetonitrile with 50 mL chromatographic-grade methanol.

10.3 Reference Standards

Same as 3.3.

10.4 Standard Solutions

Use the warning and standard-solution preparation requirements specified in 3.4.

10.5 Materials

10.5.1 Glass-fiber filter paper, 1.6 μm, or equivalent.

10.5.2 Composite SPE column, 3 g/5 mL, packed with carbon–alumina and silica composite material, or equivalent.

10.5.3 Syringe membrane filter, 0.45 μm, organic-compatible, or equivalent.

11 Apparatus and Equipment

11.1 Liquid chromatograph with fluorescence detector.

11.2 Balances with readability of 0.01 mg and 0.01 g.

11.3 Homogenizer;

11.4vortex shaker, speed ≥1,000 r/min;

11.5 vortex mixer;

11.6 high-speed homogenizer, 6,500–24,000 r/min;

11.7 centrifuge, speed ≥6,000 r/min;

11.8 nitrogen evaporator;

11.9 constant-temperature oven.

12 Analytical Procedure

12.1 Sample Preparation and Storage

12.1.1 Sample Preparation

The sample quantity for testing shall exceed 1 kg. If less than 1 kg is available, take at least three packages from the same batch and obtain at least 0.5 kg after subdivision.

Note: If an oil sample is solid, heat it appropriately to melt it before mixing with a homogenizer.

12.1.2 Sample Storage

Divide the thoroughly mixed sample into multiple portions of approximately 100 g in clean containers. Seal and store protected from light at 2 °C–6 °C or at −18 °C or below, according to sample characteristics.

12.2 Sample Extraction

Weigh 5 g sample (to 0.01 g) into a centrifuge tube, add 20 mL acetonitrile–water solution A, and shake on a vortex shaker for 20 min (or homogenize for 3 min). Centrifuge at 6,000 r/min for 10 min and retain the supernatant.

12.3 Sample Clean-up

Transfer an appropriate amount of supernatant into the collection tube supplied with the SPE column. Slowly press the SPE column into the tube so the purified liquid flows out. Collect the purified liquid.

12.4 Derivatization

Separately transfer 1.00 mL of each standard working solution and 4.0 mL of sample-clean-up solution into centrifuge tubes. Gently evaporate under nitrogen below 50 °C to near dryness. Add 200 μL n-hexane and 100 μL trifluoroacetic acid to each; vortex for 30 s and derivatize in a constant-temperature oven at (40 ± 1) °C for 15 min. After derivatization, gently evaporate under nitrogen below 50 °C to near dryness. Add 1.0 mL initial mobile phase, mix with a vortex mixer for 30 s to dissolve the residue, and filter through a syringe membrane filter before analysis.

12.5 Reference Liquid Chromatography Conditions

1.1 Reference Liquid Chromatography Conditions

a) Mobile phase: Phase A, water; Phase B, 50% acetonitrile–methanol solution;

b) Gradient elution program: See Table 1;

c) Chromatographic column: C18 column (150 mm or 250 mm in length, 4.6 mm in internal diameter, with a particle size of 5.0 μm), or equivalent;

d) Flow rate: 1.0 mL/min;

e) Column temperature: 40 °C;

f) Injection volume: 50 μL;

g) Detection wavelengths: Excitation wavelength, 360 nm; emission wavelength, 440 nm.

Table 1 — Gradient Elution Program

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	76	24
6.0	76	24
8.0	65	35
10.0	65	35
10.2	0	100
11.2	0	100
11.5	76	24
15.0	76	24

12.6 Calibration Curve

Inject each standard working solution into the liquid chromatograph and measure peak areas. Plot standard concentration on the x-axis and corresponding peak area on the y-axis. Standard chromatograms are shown in Appendix B, Figure B.6.

12.7 Qualitative Identification

Under the same test conditions, the relative deviation between retention times for target compounds in sample and standard solutions shall be within ±2.5%.

12.8 Measurement of Sample Solution

Inject the sample solution and measure peak areas. Use the calibration curve to determine the concentration of each target compound.

Responses shall be within the linear range; if not, reduce the amount of extract used and repeat clean-up and injection analysis.

12.9 Blank Test

Perform all the above steps without weighing a sample.

13 Expression of Analytical Results

Calculate AFB_1, AFB_2, AFG_1, and AFG_2 using Equation (2):

$$X = \frac{\rho \times V_1 \times V_3 \times 1000}{V_2 \times m \times 1000} \text{ ----------(2)}$$

Where:

X is analyte content (μg/kg);

ρ is analyte mass concentration determined from the calibration curve (ng/mL);

V_1 is sample-extract volume (mL);

V_3 is final volume after derivatization (mL);

1000 is the conversion factor;

V_2 is the extract volume used for derivatization (mL);

and m is sample mass (g).

Report results to three significant figures.

14 Precision

Under repeatability conditions, the absolute difference between two independent results shall not exceed 20% of their arithmetic mean.

15 Other

When 5 g of sample is taken, the limits of detection for AFB_1, AFB_2, AFG_1, and AFG_2 are each 0.1 μg/kg; the limits of quantification are each 0.2 μg/kg.

Method 3 — Liquid Chromatography–Tandem Mass Spectrometry

16 Principle

After isotope-labelled internal standards are added, the sample is extracted with acetonitrile–water solution, purified using an immunoaffinity column, analyzed by liquid chromatography–tandem mass spectrometry, and quantified using isotope-labelled internal standards.

17 Reagents and Materials

Unless otherwise specified, reagents are analytical grade and water is Grade 1 water as specified in GB/T 6682.

17.1 Reagents

17.1.1 Acetonitrile (CH_3CN);

17.1.2 sodium chloride (NaCl);

17.1.3 disodium hydrogen phosphate (Na_2HPO_4) or its dodecahydrate;

17.1.4 potassium dihydrogen phosphate (KH_2PO_4);

17.1.5 potassium chloride (KCl);

17.1.6 hydrochloric acid (HCl);

17.1.7 Tween-20 ($C_{58}H_{114}O_{26}$);

17.1.8 methanol (CH_3OH), chromatographic grade;

17.1.9 glycerol ($C_3H_8O_3$);

17.1.10 acetonitrile, chromatographic grade;

17.1.11 ammonium acetate (CH_3COONH_4).

17.2 Reagent Preparation

17.2.1 Acetonitrile–water solution A (84+16): add 16 mL water to 84 mL acetonitrile and mix.

17.2.2 Acetonitrile–water solution B (70+30): add 30 mL water to 70 mL acetonitrile and mix.

17.2.3 Phosphate buffer: dissolve 8.00 g sodium chloride, 1.20 g disodium hydrogen phosphate (or 3.02 g disodium hydrogen phosphate dodecahydrate), 0.20 g potassium dihydrogen phosphate, and 0.20 g potassium chloride in 900 mL water. If necessary, adjust pH with 1 mol/L hydrochloric acid to match the immunoaffinity column; dilute to 1,000 mL and mix.

17.2.4 1 mol/L hydrochloric acid: add 8.6 mL hydrochloric acid to 80 mL water; cool and make up to 100 mL.

17.2.5 1% Tween-20 phosphate buffer (PBST): dilute 10 mL Tween-20 with phosphate buffer to 1,000 mL and mix.

17.2.6 5 mmol/L ammonium acetate: dissolve 0.39 g ammonium acetate in 1,000 mL water and mix.

17.2.7 50% acetonitrile–methanol solution: mix 50 mL chromatographic-grade acetonitrile with chromatographic-grade methanol to 100 mL.

17.2.8 10% glycerol–methanol solution: dilute 1 mL glycerol with methanol to 10 mL and mix.

17.3 Reference Standards

17.3.1: Same as 3.3.1

17.3.2: Same as 3.3.2

17.3.3: Same as 3.3.3

17.3.4: Same as 3.3.4

17.3.5: $^{13}C_{17}$-AFB$_1$ ($^{13}C_{17}H_{12}O_6$, CAS No. 1217449-45-0): Standard solution at 0.5 μg/mL or reference standard with purity ≥ 98%.

17.3.6: $^{13}C_{17}$-AFB$_2$ ($^{13}C_{17}H_{14}O_6$, CAS No. 1217470-98-8): Standard solution at 0.5 μg/mL or reference standard with purity ≥ 98%.

17.3.7: $^{13}C_{17}$-AFG$_1$ ($^{13}C_{17}H_{12}O_7$, CAS No. 1217444-07-9): Standard solution at 0.5 μg/mL or reference standard with purity ≥ 98%.

17.3.8: $^{13}C_{17}$-AFG$_2$ ($^{13}C_{17}H_{14}O_7$, CAS No. 1217462-49-1): Standard solution at 0.5 μg/mL or reference standard with purity ≥ 98%.

17.4 Standard Solutions

Apply the warning and preparation requirements in 3.4.

17.4.1: Same as 3.4.1

17.4.2: Same as 3.4.2

17.4.3: Same as 3.4.3

17.4.4 Prepare the mixed isotope-labelled internal-standard working solution (100 ng/mL) by transferring 2.00 mL each of the 0.5 μg/mL $^{13}C_{17}$-AFB$_1$, $^{13}C_{17}$-AFB$_2$, $^{13}C_{17}$-AFG$_1$, and $^{13}C_{17}$-AFG$_2$ solutions into a 10 mL volumetric flask, diluting with acetonitrile to volume, and mixing. Store sealed and protected from light at −18 °C or below for up to six months.

17.4.5 Prepare the standard working series from appropriate amounts of the mixed standard working solution, intermediate solution, and mixed isotope-labelled internal-standard working solution using the initial mobile phase. Target analyte concentrations: 0.100, 0.500, 2.00, 10.0, and 50.0 ng/mL; isotope-labelled internal-standard concentration: 2.00 ng/mL. Prepare immediately before use.

17.5 Materials

17.5.1 Glass-fiber filter paper, 1.6 μm, or equivalent.

17.5.2 Immunoaffinity column, capacity ≥200 ng and recovery ≥85%.

17.5.3 Composite SPE column, 3 g/5 mL, carbon–alumina and silica composite material, or equivalent.

17.5.3 Syringe membrane filter, 0.22 μm, organic-compatible, or equivalent.

18 Apparatus and Equipment

18.1 Liquid chromatography–tandem mass spectrometer equipped with an electrospray ion source.

18.2 Balances with readability of 0.01 mg and 0.01 g

18.3 Homogenizer

18.4 high-speed grinder

18.5 tissue homogenizer

18.6 vortex shaker (≥1,000 r/min)

18.7 vortex mixer

18.8 high-speed homogenizer (6,500–24,000 r/min)

18.9 centrifuge (≥6,000 r/min)

18.10 nitrogen evaporator

18.11 pH meter.

19 Analytical Procedure

19.1 Sample Preparation and Storage

Use the sample preparation and storage procedure in 5.1.

19.2 Sample Extraction

19.2.1 Solid and semi-solid samples

Weigh 5 g (to 0.01 g; 10 g for corn) into a centrifuge tube. Add 100 μL mixed isotope-labelled internal-standard solution (200 μL for corn; 125 μL for highly water-absorbing samples such as infant rice cereal), mix by shaking, and let stand for 30 min. Add 20.0 mL acetonitrile–water solution A (40 mL for corn; 25.0 mL for infant rice cereal and similar samples) or solution B for high-protein samples. Shake for 20 min (or homogenize for 3 min), centrifuge at 6,000 r/min for 10 min or filter through glass-fiber paper, and retain the supernatant.

Note: For water-added high-fat granular, elastic/high-viscosity, or high-sugar samples, weigh 10 g (12 g for peanuts), add 150 μL mixed isotope-labelled internal-standard solution (200 μL for peanuts), mix and stand for 30 min, then add 25.0 mL acetonitrile (33.0 mL for peanuts). Perform the remaining steps as above.

19.2.2 Liquid samples

Oil samples (vegetable oil for example): weigh 5 g, add 100 μL mixed isotope-labelled internal-standard solution, mix and stand for 30 min, then add 20.0 mL acetonitrile–water solution A. Shake for 20 min (or homogenize for 3 min), centrifuge at 6,000 r/min for 10 min or filter, and retain the supernatant.

For other liquid samples, weigh 5 g, add 100 μL internal-standard solution (25 μL for liquid special-dietary-use foods), mix and stand for 30 min, add acetonitrile to 30.0 mL, vortex, shake for 20 min (or homogenize for 3 min), centrifuge at 6,000 r/min for 10 min or filter, and retain the supernatant.

19.3 Sample Clean-up

Take 4.0 mL supernatant and dilute to 50 mL with PBST; mix. If cloudy, centrifuge at 8,000 r/min for 10 min. Allow the immunoaffinity column to reach room temperature, drain the initial liquid, and pass the diluted sample through at approximately 1 drop/s. Wash successively with 10 mL PBST and water; discard effluent and remove residual water. Elute with 2 mL methanol at 1 drop/s and collect. Add 100 μL of 10% glycerol–methanol solution and gently evaporate under nitrogen at 50 °C to near dryness. Add 1.0 mL mobile phase, vortex for 30 s, filter through a syringe membrane filter, and collect in an autosampler vial.

Note 1: For liquid samples of foods for special medical purposes, take 12 mL of the supernatant and dilute to 100 mL with PBST. After purification and nitrogen blowdown, reconstitute the residue in 0.5 mL of mobile phase. For samples with high fat content in particulate form, flexible or high-viscosity samples, high-sugar samples, and other samples prepared by adding water, as well as other liquid samples, take 6.0 mL of the supernatant (8.0 mL for peanut samples) and dilute to 50 mL with PBST. After purification and nitrogen blowdown, reconstitute the residue in 1.0 mL of mobile phase. All other steps are the same as described above.

Note 2: If necessary, measure and adjust the pH of the dilution solution to ensure compatibility with the immunoaffinity column.

Note 3: For certain complex matrices, a solid-phase extraction (SPE) column should be used for preliminary purification before the immunoaffinity column, in accordance with the manufacturer's recommendations and laboratory validation results. Transfer an appropriate volume of the sample supernatant into the tube supplied with the SPE column. Place the SPE column in the tube and press it down slowly to allow the purified liquid to flow out. Collect the purified liquid and further purify it using an immunoaffinity column.

Note 4: When using immunoaffinity columns and SPE columns from different manufacturers, follow the instructions provided by the respective manufacturers.

Note 5: Fully automated (online) or semi-automated (offline) SPE instruments may be used after the operating parameters have been optimized.

19.4 Instrument Reference Conditions

19.4.1 Liquid Chromatography

Reference Liquid Chromatography Conditions

The reference liquid chromatography conditions are as follows:

a) Mobile phase: Phase A, 5 mmol/L ammonium acetate solution; Phase B, 50% acetonitrile–methanol solution;

b) Gradient elution program: See Table 2;

c) Chromatographic column: C18 column (100 mm in length, 2.1 mm in internal diameter, with a particle size of 1.7 μm), or equivalent;

d) Flow rate: 0.3 mL/min;

e) Column temperature: 40 °C;

f) Injection volume: 10 μL.

Table 2: Gradient Elution Program

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	68	32
0.5	68	32
3.0	55	45
4.0	55	45
4.2	0	100
4.8	0	100
5.0	68	32
7.0	68	32

19.4.2 Mass Spectrometry

Mass spectrometry reference conditions are provided in Appendix C.

19.5 Calibration Curve

Inject each standard working solution into the LC–MS/MS instrument and measure peak areas. Plot the concentration ratio of each target compound to its corresponding isotope-labelled

internal standard on the x-axis and the ratio of their chromatographic peak areas on the y-axis. See Figures D.1–D.4 in Appendix D.

19.6 Qualitative Identification

Under identical test conditions, the relative deviation between retention times of AFB1, AFB2, AFG1, and AFG2 in sample and standard solutions shall be within ±2.5%. All qualitative ions shall be detected (S/N ≥3). The relative abundance of qualitative and quantitative ions shall agree with that in a standard working solution of similar concentration; allowable deviations are specified in Table 3.

Table 3: Maximum Permissible Deviation in Relative Ion Abundance

Relative ion abundance k (%)	k ≥ 50	20 ≤ k < 50	10 ≤ k < 20	k < 10
Maximum allowable deviation (%)	±20	±25	±30	±50

19.7 Measurement of Sample Solution

Inject the sample solution into the LC–MS/MS instrument and determine the concentrations of the target compounds from the calibration curve using the analyte/internal-standard response ratios. If responses exceed the linear range, reduce the extract volume used and repeat clean-up and analysis.

19.8 Blank Test

Perform all analytical steps without weighing a sample.

20 Expression of Analytical Results

The contents of AFB_1, AFB_2, AFG_1, and AFG_2 in the test sample shall be calculated using Equation (3):

$$X = \frac{\rho \times V_1 \times V_3 \times 1000}{V_2 \times m \times 1000} \quad \text{---------- (3)}$$

where:

- X is the content of the analyte in the test sample, expressed in micrograms per kilogram (μg/kg);
- ρ is the mass concentration of the analyte in the test solution, calculated from the calibration curve, expressed in nanograms per milliliter (ng/mL);
- V1is the volume of the sample extract, expressed in milliliters (mL);

- V3 is the final volume of the sample solution after purification and elution, expressed in milliliters (mL);
- 1000 is the conversion factor;
- V2 is the volume of the extract used for purification, expressed in milliliters (mL);
- mis the mass of the test sample, expressed in grams (g).

Note: For samples prepared by adding water, m refers to the mass of the test sample excluding the added water.

The calculated result shall be reported to three significant figures.

21 Precision

Under repeatability conditions, the absolute difference between two independent results shall not exceed 20% of their arithmetic mean.

22 Other

When a 5 g test sample is weighed, the limits of detection for AFB_1, AFB_2, AFG_1, and AFG_2 in liquid foods for special medical purposes are all 0.01 μg/kg, and the limits of quantification are all 0.03 μg/kg. For other foods, the limits of detection for AFB_1, AFB_2, AFG_1, and AFG_2 are all 0.03 μg/kg, and the limits of quantification are all 0.1 μg/kg.

Method 4 — Enzyme-Linked Immunosorbent Assay (ELISA)

23 Principle

The test sample is extracted using the extraction solution specified in the test kit and competitively binds to specific antibodies with horseradish peroxidase (HRP)-labeled aflatoxin B1 or antibodies immobilized in the reaction wells. The absorbance is measured using a microplate reader, and quantification is performed using the external standard method.

24 Reagents and Materials

The reagents used for solution preparation shall be of analytical grade, and the water shall be Grade 2 water as specified in GB/T 6682.

Prepare the required solutions in accordance with the test kit instructions.

Commercially available test kits shall be used only after passing validation in accordance with the method described in Appendix E.

25 Apparatus and Equipment

25..1 Microplate Reader: Equipped with 450 nm and 630 nm (optional) filters.

25.2 Grinder.

25.3 Shaker.

25.4 Electronic Balance: Readability of 0.01 g.

25.5 Centrifuge: Rotational speed ≥ 6,000 r/min.

26.6 Rapid quantitative filter paper.

25.7 Other instruments required by the test kit.

26 Analytical Procedure

Warning: All analytical procedures shall be performed in a designated area. The area shall be protected from direct sunlight and equipped with a relatively independent workbench and a waste storage facility. Throughout the test, the operator shall take appropriate protective measures in accordance with the requirements for handling highly toxic substances.

26.1 Sample Preparation

Same as 5.1.

26.2 Sample Extraction

Weigh 5.0 g to 10 g of the sample into a centrifuge tube. Add the extraction solution specified in the test kit and perform the test according to the method described in the kit instructions.

26.3 Sample Determination

Quantitatively determine the test solution using the operating procedure specified in the enzyme-linked immunosorbent assay (ELISA) kit.

27 Calculation and Expression of Results

27.1 Preparation of the Standard Working Curve for Quantitative Determination Using an Enzyme-Linked Immunosorbent Assay (ELISA) Kit

Prepare the standard working curve based on the relationship between the standard concentrations and the corresponding changes in absorbance, using the calculation method or computer software provided in the kit instructions.

27.2 Calculation of the Concentration in the Test Solution

Using the calculation method and computer software provided in the kit instructions, substitute the absorbance of the test solution into the equation obtained in 27.1 to calculate the mass concentration (ρ) of the analyte in the test solution.

27.3 Calculation of Results

The content of AFB_1 in the sample shall be calculated according to Formula (4):

$$X = \frac{\rho \times V \times f \times 1000}{m \times 1000} \quad (4)$$

where:

- X is the content of the analyte in the sample, expressed in micrograms per kilogram $(\mathrm{ug/kg})$
- ρ is the mass concentration of the analyte in the sample solution, calculated from the standard curve, expressed in nanograms per milliliter (ng/mL);
- V is the volume of the extraction solution (for 5.2.2, the final volume after volume adjustment), expressed in milliliters (mL);
- f is the dilution factor during sample pretreatment;
- 1000 is the conversion factor;
- m is the mass of the sample taken for analysis, expressed in grams (g).

Note: For samples to which water is added during sample preparation, the sample mass (m) shall be adjusted to account for the amount of water added.

The result shall be reported to three decimal places.

28 Precision

Under repeatability conditions, the absolute difference between the results of two independent determinations shall not exceed 20% of their arithmetic mean.

29 Others

When 5 g of samples of cereals and cereal products, legumes and legume products, nuts and seeds, oils and fats and their products, condiments, and similar foods are taken for analysis, the limit of detection of the method is 1 μg/kg, and the limit of quantification is 3 μg/kg.

When 5 g of samples of infant formula and complementary foods for infants and young children are taken for analysis, the limit of detection of the method is 0.1 μg/kg, and the limit of quantification is 0.3 μg/kg.

Note: The formula as displayed in your message appears to contain a formatting issue. I have rendered it as $X = \frac{\rho \times V \times f \times 1000}{m \times 1000}$based on the visible text, but the numerator and denominator conversion factors cancel out. Please check the original PDF to confirm the formula before using this translation in a formal standard.

Appendix A — Calibration of Standard Concentrations of AFB1, AFB2, AFG1, and AFG2

Prepare standard solutions of AFB1, AFB2, AFG1, and AFG2, each at a concentration of 8 μg/mL to 10 μg/mL, using benzene–acetonitrile (98 + 2), toluene–acetonitrile (9 + 1), methanol, or acetonitrile as the solvent. Determine the actual concentrations of AFB_1, AFB_2, AFG_1, and AFG_2, respectively, by measuring the absorbance of each solution at its wavelength of maximum absorption in accordance with the method described below.

Measure the absorbance of the solution at 340 nm to 370 nm using a spectrophotometer. After subtracting the solvent blank background and correcting for systematic errors in the cuvette system, record the absorbance value (A) at the wavelength of maximum absorption (λ_{max}) of the standard solution. Calculate the actual concentration (ρ) of the test solution according to Formula (A.1):

$$\rho = \frac{A \times M \times 1000}{\varepsilon} \text{---------- (A.1)}$$

where:

- ρ is the actual concentration of AFB_1, AFB_2, AFG_1, and AFG_2 determined, expressed in micrograms per milliliter (μg/mL);
- A is the absorbance measured at λ_{max};
- M is the molar mass of AFB_1, AFB_2, AFG_1, and AFG_2, expressed in grams per mole (g/mol);
- 1 000 is the conversion factor;
- ε is the molar absorption coefficient of AFB_1, AFB_2, AFG_1, and AFG_2 in solution, expressed in square meters per mole (m^2/mol).

The molar masses and molar absorption coefficients of AFB_1, AFB_2, AFG_1, and AFG_2 are provided in Table A.1.

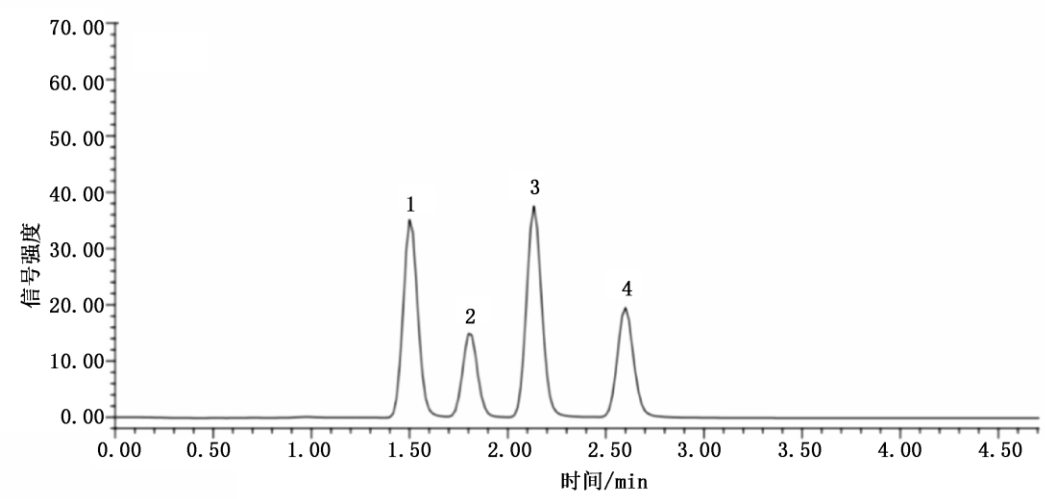
Table A.1 Molar Masses and Molar Absorptivity of AFB_1, AFB_2, AFG_1, and AFG_2

Aflatoxin Name	Molar Mass (g/mol)	Solvent	Molar Absorptivity (m^2/mol)
AFB_1	312	Benzene-acetonitrile (98+2)	19,800
		Toluene-acetonitrile (9+1)	19,300
		Methanol	21,500
		Acetonitrile	20,700
AFB_2	314	Benzene-acetonitrile (98+2)	20,900
		Toluene-acetonitrile (9+1)	21,000
		Methanol	21,400
		Acetonitrile	22,100
AFG_1	328	Benzene-acetonitrile (98+2)	17,100
		Toluene-acetonitrile (9+1)	16,400
		Methanol	17,700
		Acetonitrile	17,600

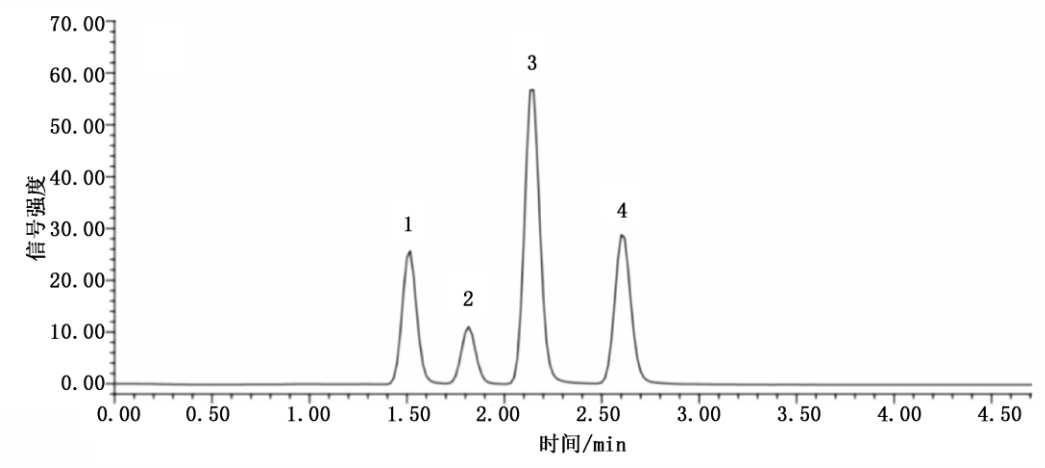
AFG_2	330	Benzene-acetonitrile (98+2)	18,200
		Toluene-acetonitrile (9+1)	18,300
		Methanol	19,200
		Acetonitrile	18,900

Annex B Liquid Chromatograms

B.1 The liquid chromatograms for the direct determination method of four aflatoxins are shown in Figure B.1.



a) Chromatogram 1 (Excitation wavelength: 365 nm; Emission wavelength: 463 nm)



b) Chromatogram 2 (Excitation wavelength: 365 nm; Emission wavelength: 436 nm)

Legend index description:

1 — AFG_2;

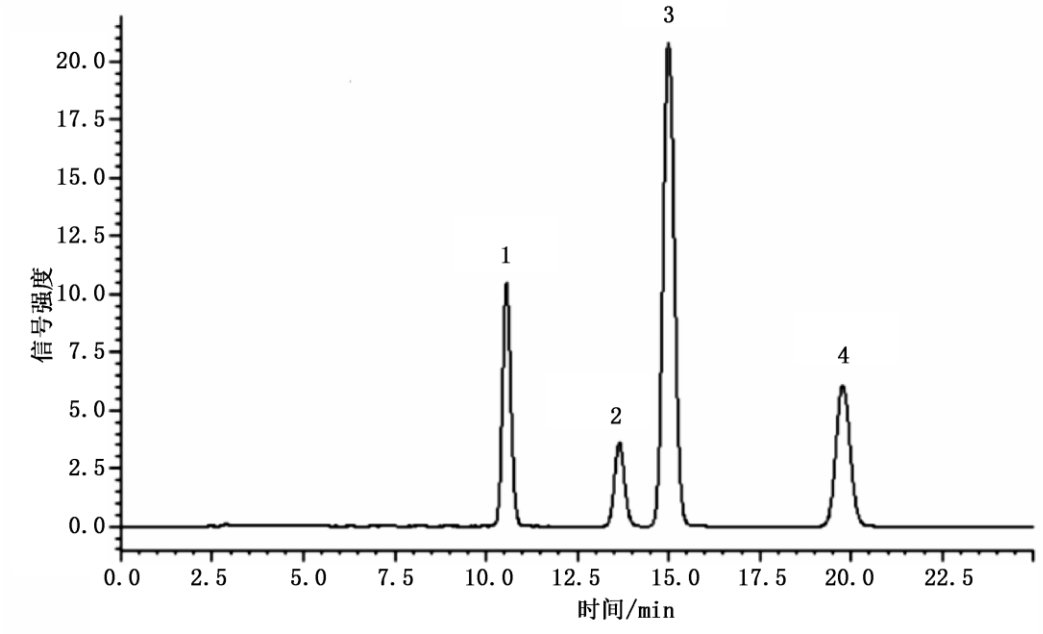
2 — AFG_1;

3 — AFB_2;

4 — AFB_1.

Figure B.1 Liquid Chromatograms for Direct Determination Method of Four Aflatoxins (Dual Wavelength Detection) (2 ng/mL Standard Solution)

B.2 The liquid chromatogram for the post-column photochemical derivatization method of four aflatoxins is shown in Figure B.2.



Legend index description:

1 — AFG_2;

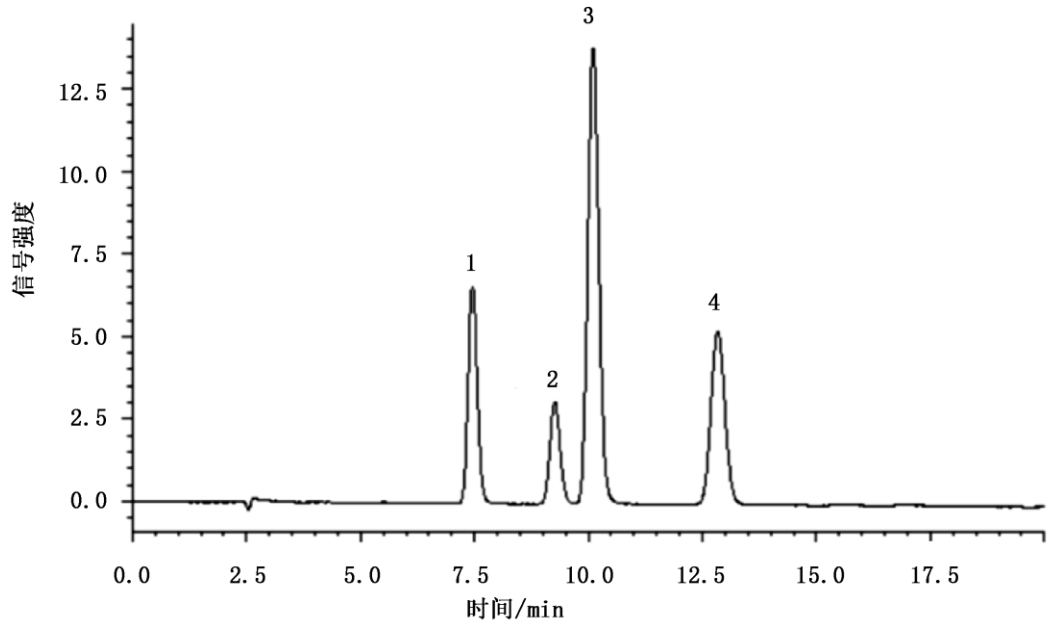
2 — AFG_1;

3 — AFB_2;

4 — AFB_1.

Figure B.2 Liquid Chromatogram for Post-Column Photochemical Derivatization Method of Four Aflatoxins (5 ng/mL Standard Solution)

B.3 The liquid chromatogram for the post-column iodine derivatization method of four aflatoxins is shown in Figure B.3.



Legend index description:

1 — AFG_2;

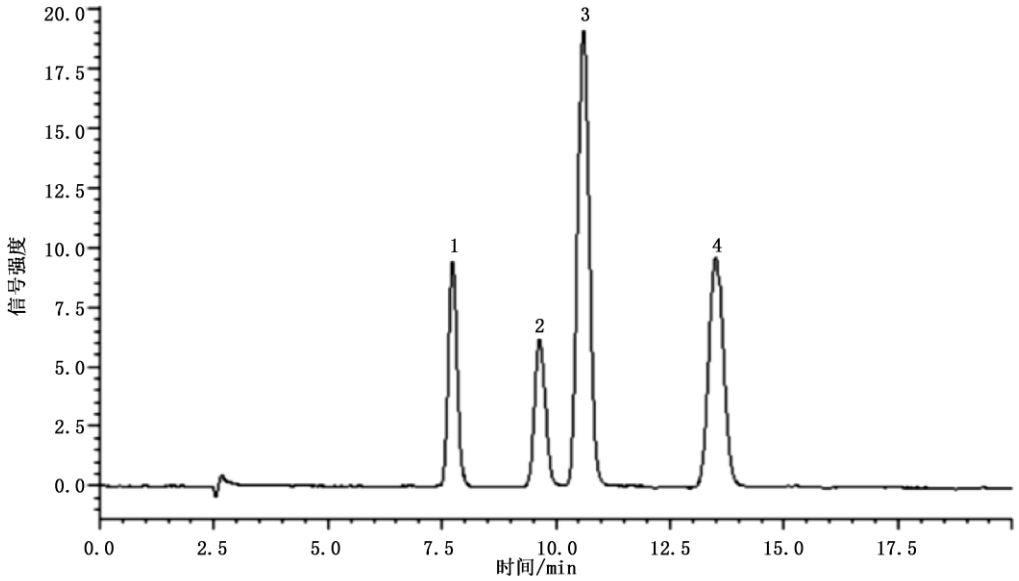
2 — AFG_1;

3 — AFB_2;

4 — AFB_1.

Figure B.3 Liquid Chromatogram for Post-Column Iodine Derivatization Method of Four Aflatoxins (5 ng/mL Standard Solution)

B.4 The liquid chromatogram for the post-column bromine derivatization method of four aflatoxins is shown in Figure B.4.



Legend index description:

1 — AFG_2;

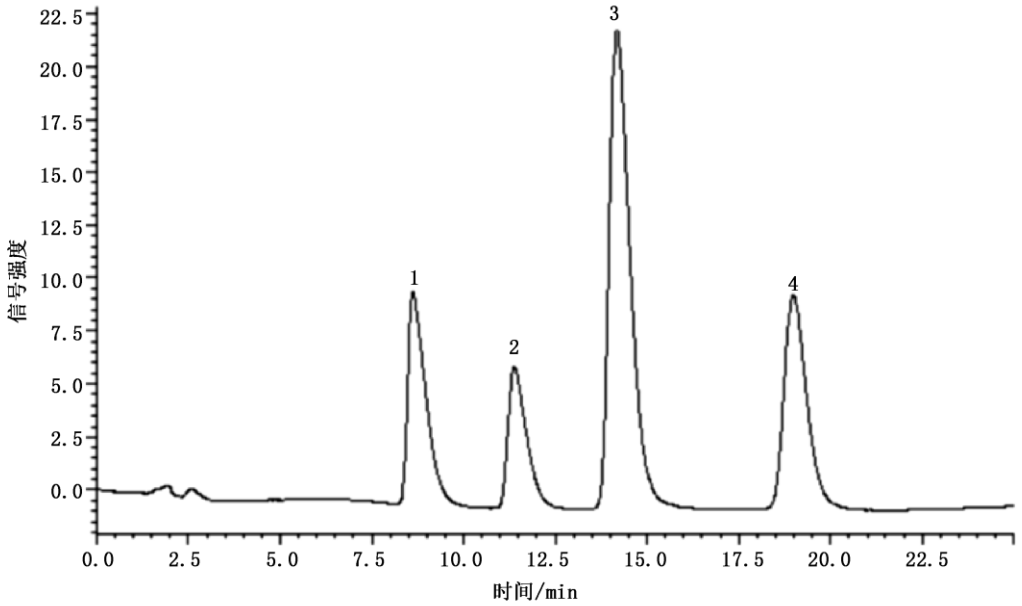
2 — AFG_1;

3 — AFB_2;

4 — AFB_1.

Figure B.4 Liquid Chromatogram for Post-Column Bromine Derivatization Method of Four Aflatoxins (5 ng/mL Standard Solution)

B.5 The liquid chromatogram for the post-column electrochemical derivatization method of four aflatoxins is shown in Figure B.5.



Legend index description:

1 — AFG_2;

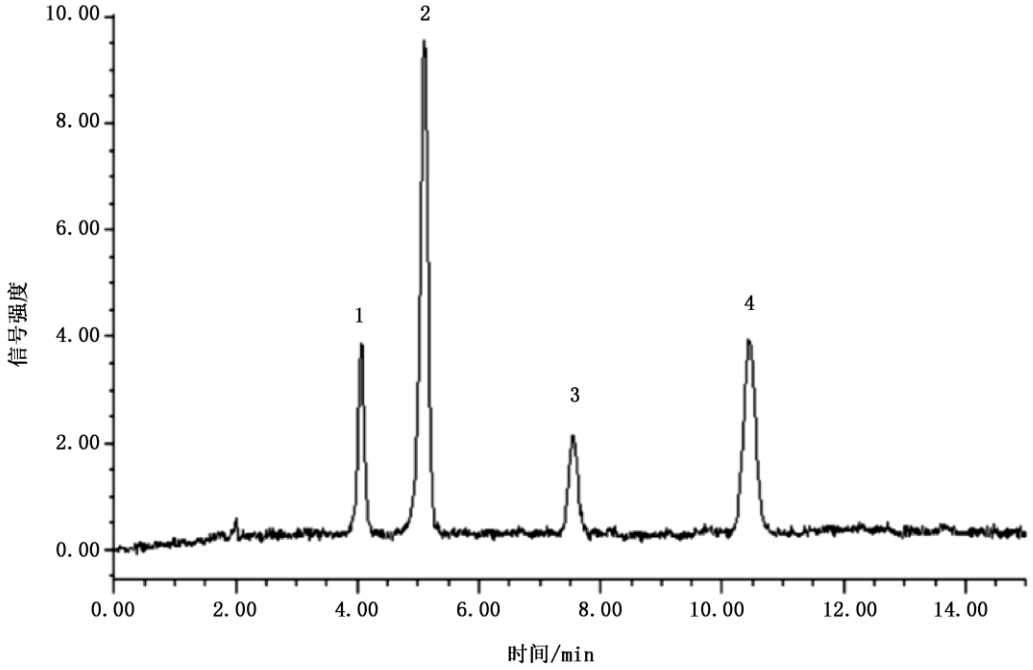
2 — AFG_1;

3 — AFB_2;

4 — AFB_1.

Figure B.5 Liquid Chromatogram for Post-Column Electrochemical Derivatization Method of Four Aflatoxins (5 ng/mL Standard Solution)

B.6 The liquid chromatogram for the pre-column derivatization method of four aflatoxins is shown in Figure B.6.



Legend index description:

1 — AFG_2;

2 — AFG_1;

3 — AFB_2;

4 — AFB_1.

Figure B.6 Liquid Chromatogram for Pre-Column Derivatization Method of Four Aflatoxins (0.5 ng/mL Standard Solution)

Annex C

Mass Spectrometry Reference Conditions for Liquid Chromatography-Tandem Mass Spectrometry Method

Mass spectrometry reference conditions are as follows:

a) Multiple Reaction Monitoring (MRM), monitored ion parameters are listed in Table C.1;

b) Ionization mode: Electrospray Ionization in Positive Ion Mode (ESI+);

c) Capillary voltage: 3.5 kV;

d) Cone voltage: 30 V;

e) Ion source / interface temperature: 150 °C;

f) Cone gas flow rate: 50 L/h;

g) Desolvation gas temperature: 500 °C;

h) Desolvation gas flow rate: 800 L/h.

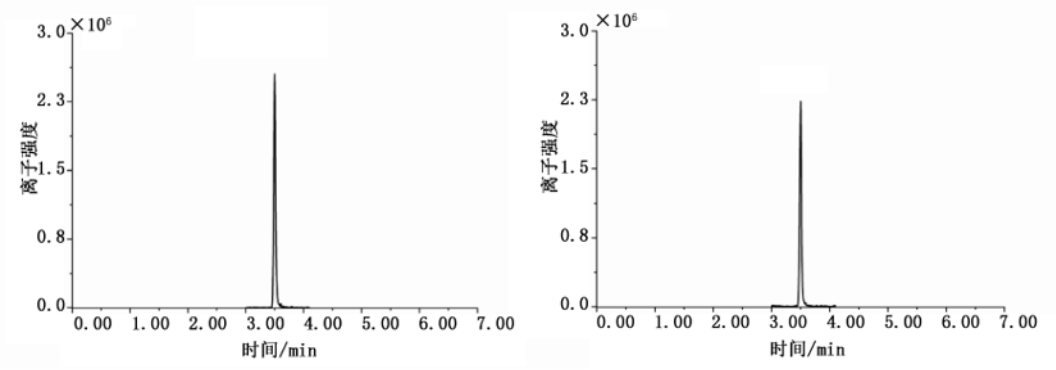
Table C.1 Monitored Ions for Aflatoxins

Compound	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (eV)
AFB_1	313.1	285.1*	22
		241.0	38
$^{13}C_{17}$-AFB_1	330.1	301.1*	23
AFB2	315.1	287.1*	25
		259.1	28
$^{13}C_{17}$- -AFB_2	332.1	303.1*	25
AFG_1	329.1	243.1*	25
		283.1	25
$^{13}C_{17}$-AFG_1	346.1	257.1*	25
AFG_2	331.1	245.1*	30
		285.1	27
$^{13}C_{17}$-AFG_2	348.1	259.1*	30
* Quantitative ion.			

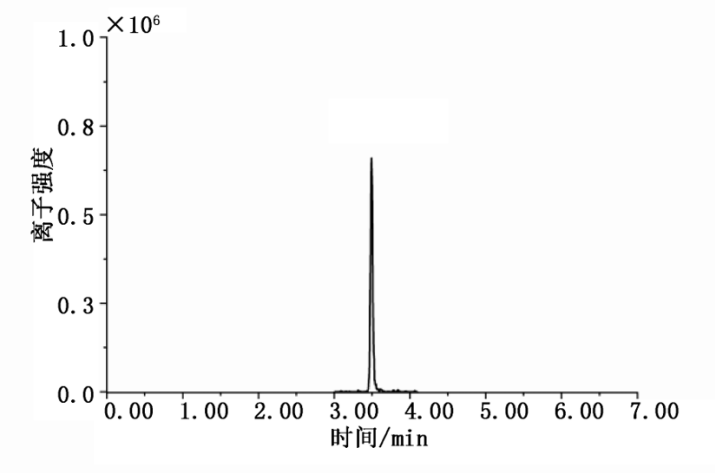
Annex D Chromatograms for Liquid Chromatography-Tandem Mass Spectrometry Method

The liquid chromatography-tandem mass spectrometry chromatograms for four aflatoxins and their isotopic internal standards are shown in Figure D.1 to Figure D.4.

Figure D.1 LC-MS/MS Chromatograms of AFB1 and Its Isotopic Internal Standard

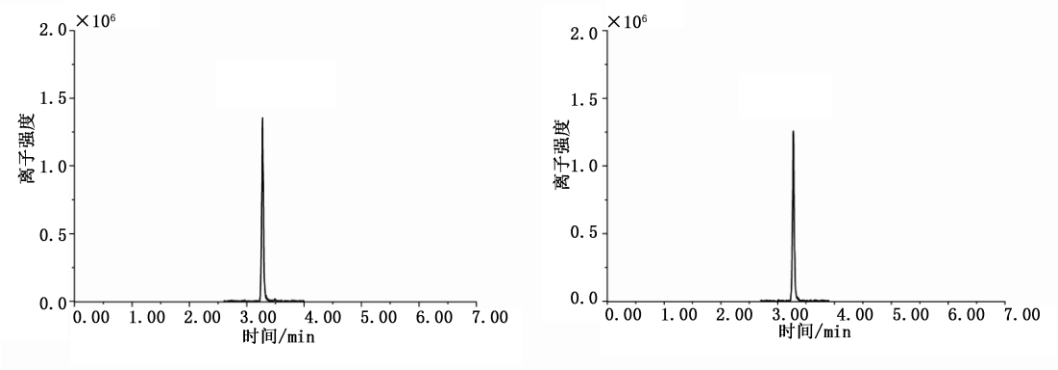


a) Ion channel chromatogram of AFB1 quantitative ion (AFB1 313.1/285.1) b) Ion channel chromatogram of AFB1 qualitative ion (AFB1 313.1/241.0)

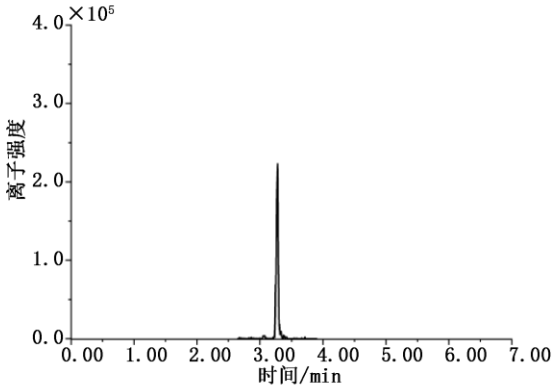


c) Ion channel chromatogram of AFB1 isotopic internal standard quantitative ion ($^{13}C_7$-AFB_1 330.1/301.1)

Figure D.1 LC-MS/MS Chromatograms of AFB_1 and Its Isotopic Internal Standard

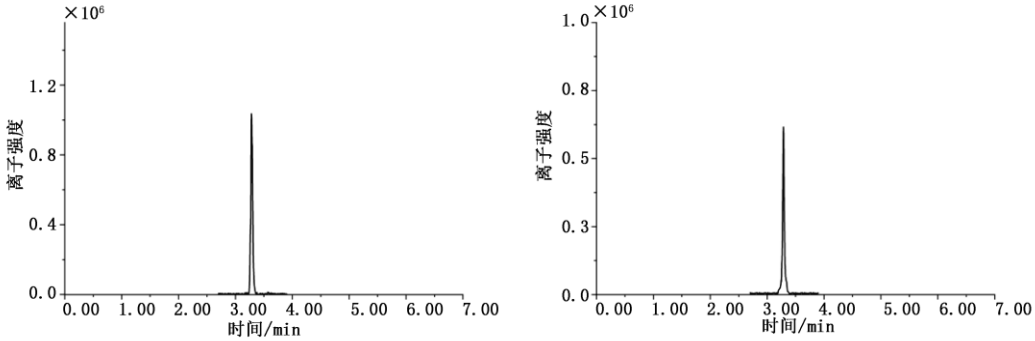


a) Ion channel chromatogram of AFB2 quantitative ion (AFB2 315.1/287.1) b) Ion channel chromatogram of AFB2 qualitative ion (AFB2 315.1/289.1)

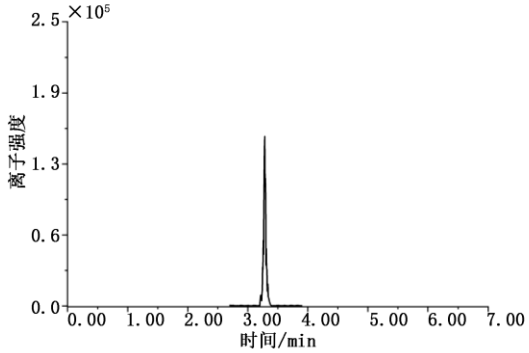


c) Ion channel chromatogram of AFB2 isotopic internal standard quantitative ion (13C7-AFB2 332.1/303.1)

Figure D.2 LC-MS/MS Chromatograms of AFB2 and Its Isotopic Internal Standard

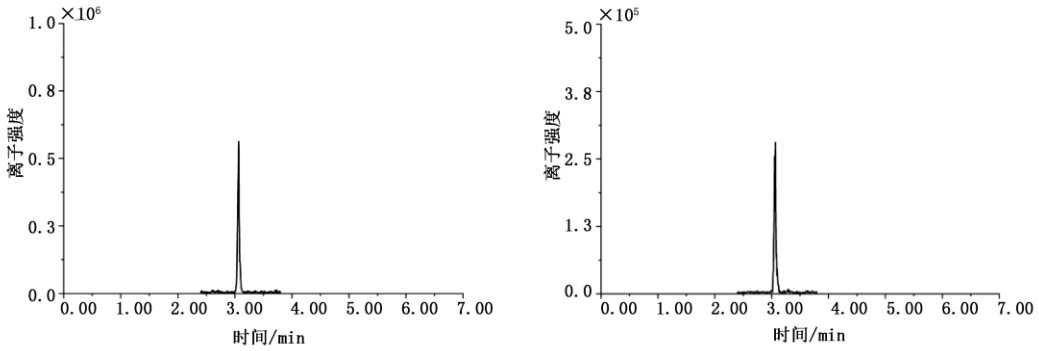


a) Ion channel chromatogram of AFG1 quantitative ion (AFG1 329.1/243.1) b) Ion channel chromatogram of AFG1 qualitative ion (AFG1 329.1/283.1

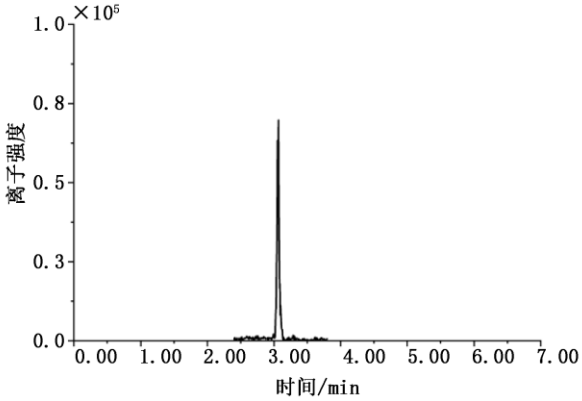


c) Ion channel chromatogram of AFG1 isotopic internal standard quantitative ion (13C7-AFG1 346.1/257.1)

Figure D.3 LC-MS/MS Chromatograms of AFG1 and Its Isotopic Internal Standard



a) Ion channel chromatogram of AFG2 quantitative ion (AFG2 331.1/245.1) b) Ion channel chromatogram of AFG2 qualitative ion (AFG2 331.1/285.1)



c) Ion channel chromatogram of AFG2 isotopic internal standard quantitative ion (13C17-AFG2 348.1/259.1)

Figure D.4 LC-MS/MS Chromatograms of AFG2 and Its Isotopic Internal Standard

Annex E

Quality Acceptance Method for Enzyme-Linked Immunosorbent Assay (ELISA) Kits

Select wheat flour or other negative samples. According to the limit of detection and linear range of the standard curve of the ELISA kit, add an appropriate amount of AFB1 standard solution to the negative matrix to prepare spiked samples at three concentration levels, corresponding to the low concentration point (or limit of quantitation), medium concentration point, and high concentration point of the standard curve, respectively. Perform determination according to the instruction manual operating procedures. The product batch can only be used if the recovery rate of the measured AFB1 value X meets the following requirements: when $X \leq 1$ μg/kg, the recovery rate is 50% to 120%; when 1 μg/kg < X < 10 μg/kg, the recovery rate is 60% to 120%; when 10 μg/kg < X < 100 μg/kg, the recovery rate is 70% to 120%.

Note: When the ELISA kit is used for testing special dietary food matrices, the AFB1 concentration levels of spiked samples include 0.2 μg/kg.

National Food Safety Standard

Determination of Aflatoxins M Group in Foods (GB 5009.24—2026)

Foreword

This standard replaces GB 5009.24-2016 "National Food Safety Standard - Determination of Aflatoxins M Group in Foods".

Compared with GB 5009.24-2016, the main changes are as follows:
— Modified the sample pretreatment method for Method I High Performance Liquid Chromatography and Method II Liquid Chromatography-Tandem Mass Spectrometry;
— Adjusted the order of Method I and Method II;
— Modified the title of Method I to Liquid Chromatography;
— Modified the title of Method II to Liquid Chromatography-Tandem Mass Spectrometry;
— Modified the title of Method III to Enzyme-Linked Immunosorbent Assay.

1 Scope

This standard specifies the methods for the determination of aflatoxin M_1 and aflatoxin M_2 (hereinafter referred to as AFT M_1 and AFT M_2) in foods.

Method I (Liquid Chromatography) and Method II (Liquid Chromatography-Tandem Mass Spectrometry) of this standard are applicable to the determination of AFT M_1 and AFT M_2 in milk, milk products, and foods for special dietary uses.

Method III (Enzyme-Linked Immunosorbent Assay) of this standard is applicable to the screening determination of AFT M_1 in milk, milk products, and foods for special dietary uses.

Method I Liquid Chromatography

2 Principle

The test sample is extracted with methanol or methanol-water, purified by an immunoaffinity column, detected by liquid chromatography with a fluorescence detector, and quantified by external standard method.

3 Reagents and Materials

Unless otherwise specified, all reagents used in this method are of analytical grade, and water is Grade 1 water as specified in GB/T 6682.

3.1 Reagents

3.1.1 Acetonitrile (CH_3CN): Chromatographic grade.
3.1.2 Acetonitrile (CH_3CN).
3.1.3 Glycerol ($C_3H_8O_3$).
3.1.4 Methanol (CH_3OH).
3.1.5 Sodium chloride (NaCl).
3.1.6 Disodium hydrogen phosphate (Na_2HPO_4).
3.1.7 Potassium dihydrogen phosphate (KH_2PO_4).
3.1.8 Potassium chloride (KCl).
3.1.9 Hydrochloric acid (HCl).
3.1.10 Petroleum ether (C_nH_{2n+2}): Boiling range 30 °C to 60 °C.
3.1.11 Tween-20 ($C_{58}H_{114}O_{26}$).

3.2 Preparation of Reagents

3.2.1 Phosphate buffered saline (hereinafter referred to as PBS): Weigh 8.0 g of sodium chloride, 1.2 g of disodium hydrogen phosphate, 0.2 g of potassium dihydrogen phosphate, and 0.2 g of potassium chloride. Dissolve in 980 mL of water, adjust pH to 7.2–7.6 with hydrochloric acid, add water to 1000 mL, and mix well.

3.2.2 Acetonitrile-water solution (15+85): Measure 85 mL of water and 15 mL of acetonitrile, and mix well.

3.2.3 Methanol-water solution (50+50): Measure 500 mL of water and 500 mL of methanol, and mix well.

3.2.4 10% Glycerol-methanol solution: Weigh 1.0 g of glycerol, dilute to 10 mL with methanol, and mix well.

3.2.5 Phosphate buffered saline containing 0.5% Tween-20 (PBS-T): Weigh 5.0 g of Tween-20, dilute to 1000 mL with PBS, and mix well.

3.3 Standards

3.3.1 AFT M1 (C17H12O7, CAS: 6795-23-9): Standard material with purity ≥ 98% or its standard solution.

3.3.2 AFT M2 (C17H14O7, CAS: 6885-57-0): Standard material with purity ≥ 98% or its standard solution.

3.4 Preparation of Standard Solutions

WARNING: The entire analytical procedure should be performed in a designated area. This area should be protected from light (direct sunlight) and equipped with a relatively independent workbench and waste disposal facility. Throughout the test, operators should take appropriate protective measures in accordance with the requirements for handling highly toxic substances.

3.4.1 Standard Stock Solution (10.0 mg/L): Weigh 10.0 mg (accurate to 0.1 mg) of AFT M1 and AFT M2 respectively into small beakers, add acetonitrile to dissolve, transfer to a 1000 mL volumetric flask, bring to volume with acetonitrile, and mix well. Calibrate the concentration using a spectrophotometer (refer to Appendix A) to obtain a standard stock solution with a concentration of 10.0 mg/L. Transfer the solution to brown reagent bottles and store protected from light at -18 °C or below for up to 12 months.

3.4.2 Mixed Standard Intermediate Solution (1.00 mg/L): Pipette 1.00 mL of AFT M1 and AFT M2 standard stock solutions respectively into the same 10.0 mL volumetric flask, dilute to volume with acetonitrile, and mix well to obtain a 1.00 mg/L mixed standard intermediate solution. Transfer the solution to brown reagent bottles and store protected from light at -18 °C or below for up to 12 months.

3.4.3 Mixed Standard Working Solution (100 μg/L): Accurately pipette 1.00 mL of the mixed standard intermediate solution into a 10.0 mL volumetric flask, dilute to volume with acetonitrile, and mix well to obtain a 100 μg/L mixed standard working solution. Transfer the solution to brown reagent bottles and store protected from light at 2 °C to 6 °C for up to 3 months.

3.4.4 Standard Working Series Solutions: Accurately transfer appropriate amounts of the mixed standard working solution and dilute with acetonitrile-water (15+85) to prepare standard working series solutions with mass concentrations of both AFT M1 and AFT M2 at 0.100 μg/L, 0.200 μg/L, 0.500 μg/L, 1.00 μg/L, and 5.00 μg/L, respectively. Prepare fresh before use.

3.5 Materials

3.5.1 Glass fiber filter paper.
3.5.2 Microfiltration membrane: 0.45 μm, organic phase.
3.5.3 Immunoaffinity column: Column capacity ≥ 100 ng, column recovery for AFT M1 and M2 ≥ 85%.

4 Instruments and Equipment

4.1 Liquid chromatograph: Equipped with a fluorescence detector.
4.2 Electronic balance: Sensitivities of 0.01 g, 0.001 g, and 0.1 mg.
4.3 Water bath: Temperature controlled at 50 °C ± 2 °C.
4.4 Vortex mixer.
4.5 Refrigerated centrifuge: Speed not less than 6000 r/min.
4.6 Solid phase extraction manifold (with vacuum pump).
4.7 Nitrogen evaporator.
4.8 High-speed grinder.
4.9 High-speed homogenizer.

5 Analytical Procedures

5.1 Sample Preparation and Storage

5.1.1 Sample Preparation

The sample amount used for testing should be greater than 1 kg; when the sample amount is less than 1 kg, at least 3 packages from the same batch should be taken; after reduction, take not less

than 0.5 kg. Solid samples are ground with a high-speed grinder, semi-solid samples are homogenized with a tissue homogenizer, and liquid samples are homogenized with a homogenizer.

Note 1: For special samples such as high-fat particle samples, flexible/pliable samples, high-viscosity samples, and high-sugar samples, freezing or other measures can be taken for sample preparation.
Note 2: For carbonated beverages or alcoholic beverages, degassing by ultrasound, stirring, or vacuuming followed by mixing can be performed.
Note 3: Samples can be prepared according to actual situations based on the purpose of inspection.

5.1.2 Sample Storage

The prepared homogeneous sample is divided equally into multiple packages of about 100 g each, stored in clean containers, sealed, and kept protected from light at 2 °C to 6 °C or -18 °C and below depending on sample properties.

5.2 Sample Extraction

5.2.1 Liquid Samples (except butter) and Semi-solid Samples

Weigh 4 g of sample (accurate to 0.01 g) into a 50 mL centrifuge tube, add 10 mL of analytical grade methanol, and vortex or shake for 3 min. Place the mixed sample in a 2 °C to 6 °C refrigerator for 1 h to 2 h. When the sample temperature stabilizes, centrifuge at 2 °C to 6 °C at 6000 r/min or higher speed for 5 min, filter to remove the top lipid layer, then transfer all supernatant to a 50 mL centrifuge tube, dilute to 50 mL with PBS-T (if turbid, filter through glass fiber filter paper or centrifuge at high speed before loading onto column), mix well, and await column purification.

5.2.2 Solid Samples (except butter)

Weigh 1 g of sample (accurate to 0.001 g) into a 50 mL centrifuge tube, add 4 mL of water, incubate in a 50 °C water bath for 10 min, and vortex mix after dissolution. After cooling to room temperature, add 10 mL of methanol, and vortex or shake for 3 min. Place the mixed sample in a 2 °C to 6 °C refrigerator for 1 h to 2 h. When the sample temperature stabilizes, centrifuge at 2 °C to 6 °C at 6000 r/min or higher speed for 5 min, filter to remove the top lipid layer, then transfer all supernatant to a 50 mL centrifuge tube, dilute to 50 mL with PBS-T (if turbid, filter through glass fiber filter paper or centrifuge at high speed before loading onto column), mix well, and await column purification.

5.2.3 Butter

Weigh 1 g of sample (accurate to 0.001 g) into a 50 mL centrifuge tube, add 5 mL of petroleum ether, and after the butter dissolves, add 20 mL of methanol-water (50+50), and vortex or shake for 30 min. Add 0.3 g of sodium chloride, shake thoroughly to dissolve, place the mixed sample in a 2 °C to 6 °C refrigerator for 1 h to 2 h. When the sample temperature stabilizes, centrifuge at 2 °C to 6 °C at 6000 r/min or higher speed for 5 min, discard the upper petroleum ether layer,

dilute with PBS-T to 50 mL (if turbid, filter through glass fiber filter paper or centrifuge at high speed before loading onto column), mix well, and await column purification.

5.3 Sample Purification

Allow the immunoaffinity column to return to room temperature. After the original liquid drains out completely, pass all the diluted solution to be purified through the immunoaffinity column at a flow rate of approximately 1 drop per second. After the diluted solution drains out, rinse the immunoaffinity column sequentially with 10 mL of PBS-T and 10 mL of water. After water drops finish, drain the immunoaffinity column dry. Add 1 mL of methanol for elution, controlling the drop flow rate at 1 mL/min to 2 mL/min. After methanol drains out, add another 1 mL of methanol to repeat elution once, drain dry, and collect all eluate. Add 50 μL of 10% glycerol-methanol solution into the eluate, blow dry with nitrogen at 50 °C, redissolve in 0.5 mL of acetonitrile-water (15+85), vortex for 30 s, pass through a microfiltration membrane, and collect the filtrate into an autosampler vial with an insert tube for testing.

Note 1: Operating procedures for immunoaffinity columns provided by different manufacturers may vary; in actual operation, please refer to the operating instructions and procedures provided by the manufacturer.

Note 2: Fully automated (online) or semi-automated (offline) solid-phase extraction instruments may be used after optimizing operational parameters.

5.4 Liquid Chromatography Reference Conditions

Liquid chromatography reference conditions are as follows:

a) Chromatographic column: C18 column (length 150 mm, internal diameter 4.6 mm, particle size 5 μm), or equivalent;

b) Flow rate: 1.0 mL/min;

c) Column temperature: 40 °C;

d) Mobile phase: Phase A: water, Phase B: chromatographic grade acetonitrile; gradient elution program is shown in Table 1;

e) Injection volume: 100 μL;

f) Detection wavelength: Excitation wavelength: 360 nm; Emission wavelength: 430 nm.

Table 1 Gradient Elution Program

Time (min)	Phase A (%)	Phase B (%)
0.0	85	15
5.0	80	20
11.5	65	35
12.0	0	100
13.5	0	100
14.0	85	15
20.0	85	15

5.5 Construction of Standard Curve

Inject the standard series working solutions into the liquid chromatograph respectively, measure the corresponding peak areas, and plot the standard curve with the mass concentration of the standard series working solutions as the abscissa and the corresponding chromatographic peak area as the ordinate. Chromatograms of the standard solution are shown in Figure B.1 in Appendix B.

5.6 Qualitative Determination

Under the same testing conditions, the relative deviation of the retention time of each target compound in the sample solution compared with that in the standard solution shall be within ±2.5%.

5.7 Determination of Sample Solution

Inject the sample solution into the liquid chromatograph, measure the corresponding peak area, and obtain the mass concentration of each target compound in the sample solution according to the standard curve.

The mass concentration of each target compound in the sample solution should be within the linear range of the standard curve. If it exceeds the linear range, the sample extract volume should be appropriately reduced before purification and injection analysis.

5.8 Blank Test

Except for not adding the sample, perform all other operations in the same manner as the sample operation.

6 Expression of Analytical Results

The content of AFT M1 or AFT M2 in the sample is calculated according to Formula (1):

$$X = (\rho \times V \times f \times 1000) / (m \times 1000) \qquad \text{..... (1)}$$

Where:

X — Content of AFT M1 or AFT M2 in the sample, in micrograms per kilogram (μg/kg);
ρ — Mass concentration of AFT M1 or AFT M2 in the sample solution, in micrograms per liter (μg/L);
V — Constant volume, in milliliters (mL);
f — Dilution factor;
m — Sample weight, in grams (g);
1000 — Unit conversion factor.

Calculation results are retained to three significant figures.

7 Precision

The absolute difference between two independent determination results obtained under repeatability conditions shall not exceed 20% of the arithmetic mean.

8 Others

For liquid samples (except butter) and semi-solid samples, when 4 g of sample is weighed, the limit of detection (LOD) of this method for AFT M_1 is 0.005 μg/kg, the LOD for AFT M_2 is 0.003 μg/kg, the limit of quantification (LOQ) for AFT M_1 is 0.02 μg/kg, and the LOQ for AFT M_2 is 0.01 μg/kg.

For solid samples and butter samples, when 1 g of sample is weighed, the LOD of this method for AFT M_1 is 0.02 μg/kg, the LOD for AFT M_2 is 0.01 μg/kg, the LOQ for AFT M_1 is 0.05 μg/kg, and the LOQ for AFT M_2 is 0.03 μg/kg.

Method II Liquid Chromatography-Tandem Mass Spectrometry

9 Principle

After adding an isotopic internal standard, the test sample is extracted with methanol or methanol-water, purified by an immunoaffinity column, detected by liquid chromatography-tandem mass spectrometry, and quantified by isotopic internal standard method.

10 Reagents and Materials

Unless otherwise specified, all reagents used in this method are of analytical grade, and water is Grade 1 water as specified in GB/T 6682.

10.1 Reagents

10.1.1 Acetonitrile (CH_3CN): Chromatographic grade.
10.1.2 Formic acid (HCOOH): Chromatographic grade.
10.1.3 Acetonitrile (CH_3CN).
10.1.4 Glycerol ($C_3H_8O_3$).
10.1.5 Methanol (CH_3OH).
10.1.6 Sodium chloride (NaCl).
10.1.7 Disodium hydrogen phosphate (Na_2HPO_4).
10.1.8 Potassium dihydrogen phosphate (KH_2PO_4).
10.1.9 Potassium chloride (KCl).
10.1.10 Hydrochloric acid (HCl).
10.1.11 Petroleum ether (C_nH_{2n+2}): Boiling range 30 °C to 60 °C.
10.1.12 Tween-20 ($C_{58}H_{114}O_{26}$).

10.2 Preparation of Reagents

10.2.1 Phosphate buffered saline (PBS): Same as 3.2.1.
10.2.2 Acetonitrile-water (15+85): Same as 3.2.2.
10.2.3 Methanol-water (50+50): Same as 3.2.3.
10.2.4 10% Glycerol-methanol solution: Same as 3.2.4.
10.2.5 Phosphate buffered saline containing 0.5% Tween-20 (PBS-T): Same as 3.2.5.
10.2.6 0.1% Formic acid solution: Pipette 1.0 mL of formic acid, dilute to 1000 mL with water, and mix well.

10.3 Standards

10.3.1 AFT M_1 ($C_{17}H_{12}O_7$, CAS: 6795-23-9): Standard material with purity ≥ 98% or its standard solution.
10.3.2 AFT M_2 ($C_{17}H_{14}O_7$, CAS: 6885-57-0): Standard material with purity ≥ 98% or its standard solution.

10.3.3 $^{13}C_{17}$-AFT M_1 ($^{13}C_{17}H_{12}O_7$): Standard solution with mass concentration of 0.5 mg/L, or standard material with purity ≥ 98%.

10.4 Preparation of Standard Solutions

WARNING: The entire analytical procedure should be performed in a designated area. This area should be protected from light (direct sunlight) and equipped with a relatively independent workbench and waste disposal facility. Throughout the test, operators should take appropriate protective measures in accordance with the requirements for handling highly toxic substances.

10.4.1 Standard Stock Solution (10.0 mg/L): Same as 3.4.1.
10.4.2 Mixed Standard Intermediate Solution (1.00 mg/L): Same as 3.4.2.
10.4.3 Mixed Standard Working Solution (100 μg/L): Same as 3.4.3.

10.4.4 Isotopic Internal Standard Intermediate Solution (50.0 μg/L): Pipette 1.00 mL of $^{13}C_{17}$-AFT M_1 isotopic internal standard solution into a 10.0 mL volumetric flask, dilute to volume with acetonitrile, and mix well to obtain a 50.0 μg/L isotopic internal standard intermediate solution. Transfer to brown reagent bottles and store protected from light at -18 °C or below for up to 12 months.

10.4.5 Isotopic Internal Standard Working Solution (5.00 μg/L): Pipette 1.00 mL of isotopic internal standard intermediate solution into a 10.0 mL volumetric flask, dilute to volume with acetonitrile, and mix well to obtain a 5.00 μg/L isotopic internal standard working solution. Transfer to brown reagent bottles and store protected from light at 2 °C to 6 °C for up to 3 months.

10.4.6 Standard Series Working Solutions: Accurately transfer appropriate amounts of mixed standard working solution and isotopic internal standard working solution respectively, and dilute with acetonitrile-water (15+85) to prepare standard series working solutions with mass concentrations of both AFT M_1 and AFT M_2 at 0.100 μg/L, 0.200 μg/L, 0.500 μg/L, 1.00 μg/L, and 5.00 μg/L, respectively. The concentration of $^{13}C_{17}$-AFT M_1 isotopic internal standard in each concentration point is 0.500 μg/L. Prepare fresh before use.

10.5 Materials

10.5.1 Glass fiber filter paper.
10.5.2 Microfiltration membrane: 0.22 μm, organic phase.
10.5.3 Immunoaffinity column: Column capacity ≥ 100 ng, column recovery for AFT M_1 and AFT M_2 ≥ 85%.

11 Instruments and Equipment

11.1 Liquid chromatography-tandem mass spectrometer: Equipped with electrospray ionization (ESI) source.
11.2 Electronic balance: Sensitivities of 0.01 g, 0.001 g, and 0.1 mg.
11.3 Water bath: Temperature controlled at 50 °C ± 2 °C.
11.4 Vortex mixer.
11.5 Refrigerated centrifuge: Speed ≥ 6000 r/min.
11.6 Solid phase extraction manifold (with vacuum pump).
11.7 Nitrogen evaporator.

11.8 High-speed grinder.
11.9 High-speed homogenizer.

12 Analytical Procedures

12.1 Sample Preparation and Storage

Same as 5.1.

12.2 Sample Extraction

12.2.1 Liquid Samples (except butter) and Semi-solid Samples

Weigh 4 g of sample (accurate to 0.01 g) into a 50 mL centrifuge tube, add 50 μL of isotopic internal standard working solution, shake to mix well, and let stand for 30 min. Remaining operations are the same as 5.2.1.

12.2.2 Solid Samples (except butter)

Weigh 1 g of sample (accurate to 0.001 g) into a 50 mL centrifuge tube, add 50 μL of isotopic internal standard working solution, shake to mix well, and let stand for 30 min. Remaining operations are the same as 5.2.2.

12.2.3 Butter

Weigh 1 g of sample (accurate to 0.001 g) into a 50 mL centrifuge tube, add 50 μL of isotopic internal standard working solution, shake to mix well, and let stand for 30 min. Remaining operations are the same as 5.2.3.

12.3 Sample Purification

Same as 5.3.

12.4 Instrument Reference Conditions

12.4.1 Liquid Chromatography Reference Conditions

a) Chromatographic column: C18 column (length 100 mm, internal diameter 2.1 mm, particle size 1.8 μm), or equivalent;
b) Flow rate: 0.3 mL/min;
c) Column temperature: 40 °C;
d) Injection volume: 10 μL;
e) Mobile phase A: 0.1% formic acid solution; Mobile phase B: chromatographic grade acetonitrile; gradient elution program is shown in Table 2.

Table 2 Gradient Elution Program

Time (min)	Phase A (%)	Phase B (%)
0.0	85	15
2.0	85	15
5.0	70	30
5.1	0	100
7.0	0	100
7.1	85	15
10.0	85	15

12.4.2 Mass Spectrometry Reference Conditions

Mass spectrometry reference conditions are as follows:
a) Ionization mode: Electrospray ionization positive ion mode (ESI+);
b) Multiple reaction monitoring (MRM), monitored ion information is shown in Table 3;
c) Capillary voltage: 3.0 kV;
d) Source temperature: 150 °C;
e) Cone gas flow rate: 50 L/h;
f) Cone voltage: 30 V;
g) Desolvation temperature: 350 °C;
h) Desolvation gas flow rate: 1000 L/h.

Table 3 Monitored Ions for Aflatoxins M

Compound	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (eV)
AFT M1	329.1	273.1*	23
		259.1	23
AFT M2	331.1	273.1*	23
		259.1	23
13C17-AFT M1	346.1	288.1*	23

Note: * indicates quantitative ion.

12.5 Construction of Standard Curve

Inject the standard series working solutions into the liquid chromatography-tandem mass spectrometer respectively, measure the corresponding peak areas, and plot the standard curve with the mass concentration ratio of each target compound to its corresponding isotopic internal standard in the standard series working solution as the abscissa, and the peak area ratio of the mass chromatogram of each target compound to its corresponding isotopic internal standard as the ordinate. Liquid chromatography-tandem mass chromatograms of the standard solution are shown in Figure B.2 in Appendix B.

12.6 Qualitative Determination

Under the same testing conditions, the relative deviation of the retention time of aflatoxins M in the sample solution compared with that in the standard solution shall be within ±2.5%. All qualitative ions are detected (S/N ≥ 3), and the relative ion abundance of qualitative ions and quantitative ions should be consistent with that of standard working solutions of similar concentration. The relative ion abundance deviation shall meet the requirements in Table 4.

Table 4 Maximum Permissible Tolerances for Relative Ion Abundance

Relative Ion Abundance k (%)	**≥ 50**	**20 < k < 50**	**10 < k ≤ 20**	**k ≤ 10**
Maximum Permissible Tolerance (%)	± 20	± 25	± 30	± 50

12.7 Determination of Sample Solution

Inject the sample solution into the liquid chromatography-tandem mass spectrometer to obtain the peak area ratio of each target compound to its corresponding isotopic internal standard, and obtain the mass concentration of aflatoxins M in the sample solution according to the standard curve.

The peak area ratio of the mass chromatogram between the analyte and the internal standard in the sample solution should be within the linear range of the standard curve. If it exceeds the linear range, re-weigh the sample, appropriately increase the amount of isotopic internal standard working solution added, reduce the purification loading volume proportionally, and re-determine.

12.8 Blank Test

Except for not adding the sample, perform all other operations in the same manner as the sample operation.

13 Expression of Analytical Results

The content of AFT M_1 or AFT M_2 in the sample is calculated according to Formula (2):

$$\mathbf{X = (\rho \times V \times f \times 1000) / (m \times 1000)} \qquad \mathbf{..... (2)}$$

Where:

X — Content of AFT M_1 or AFT M_2 in the sample, in micrograms per kilogram (μg/kg);
ρ — Mass concentration of AFT M_1 or AFT M_2 in the sample solution, in micrograms per liter (μg/L);
V — Constant volume of the sample solution, in milliliters (mL);
f — Dilution factor;
m — Sample weight, in grams (g);
1000 — Unit conversion factor.

Calculation results are retained to three significant figures.

14 Precision

The absolute difference between two independent determination results obtained under repeatability conditions shall not exceed 20% of the arithmetic mean.

15 Others

For liquid samples (except butter) and semi-solid samples, when 4 g of sample is weighed, the LOD of this method for AFT M1 is 0.005 μg/kg, the LOD for AFT M_2 is 0.005 μg/kg, the LOQ for AFT M_1 is 0.02 μg/kg, and the LOQ for AFT M2 is 0.02 μg/kg.
For solid samples and butter samples, when 1 g of sample is weighed, the LOD of this method for AFT M_1 is 0.02 μg/kg, the LOD for AFT M_2 is 0.02 μg/kg, the LOQ for AFT M_1 is 0.05 μg/kg, and the LOQ for AFT M_2 is 0.05 μg/kg.

Method III Enzyme-Linked Immunosorbent Assay

16 Principle

The test sample is extracted with the extraction reagent provided in the kit, competitively binds to specific antibodies with aflatoxin M labeled with horseradish peroxidase or immobilized in the reaction wells, detected by a microplate reader, and quantified by external standard method.

17 Reagents and Materials

Reagents required for solution preparation are all of analytical grade, and water is Grade 2 water as specified in GB/T 6682.

Prepare required solutions according to the kit instruction manual.

18 Instruments and Equipment

18.1 Microplate reader: Equipped with 450 nm and 630 nm (optional) filters.

18.2 Electronic balance: Sensitivity of 0.01 g.

18.3 Refrigerated centrifuge: Speed ≥ 6000 r/min.

18.4 Vortex mixer.

18.5 High-speed grinder.

18.6 High-speed homogenizer.

19 Analytical Procedures

WARNING: The entire analytical procedure should be performed in a designated area. This area should be protected from light (direct sunlight) and equipped with a relatively independent workbench and waste disposal facility. Throughout the test, operators should take appropriate protective measures in accordance with the requirements for handling highly toxic substances.

19.1 Sample Preparation and Storage

Same as 5.1.

19.2 Sample Extraction

19.2.1 Liquid Samples and Semi-solid Samples

Weigh 10 g of sample (accurate to 0.01 g) into a 50 mL centrifuge tube, place the mixed sample in a 2 °C to 6 °C refrigerator for 1 h to 2 h. When the sample temperature stabilizes, centrifuge at 2 °C to 6 °C at 6000 r/min or higher speed for 10 min. Remove the surface lipid layer, take about 1 g of lower-layer liquid into another test tube. This solution can be measured directly, or measured after dilution using the method provided by the kit.

19.2.2 Solid Samples (except cheese)

Weigh 10 g of sample (accurate to 0.01 g) into a small beaker, add water to dissolve, transfer to a 1000 mL volumetric flask, and bring to volume with water. Remaining steps are same as 19.2.1.

19.2.3 Cheese

Weigh 5 g of homogeneous sample (accurate to 0.01 g), add the extraction solution provided by the kit, extract according to the kit manual, and the extract solution is the sample solution.

19.3 Quantitative Detection

Perform quantitative detection on the sample solution according to the operating steps described in the ELISA kit.

20 Calculation and Expression of Analytical Results

Plot the standard working curve according to the relationship between the mass concentration of the standard solution and absorbance changes.
Calculate the mass concentration of aflatoxin M in the sample solution (ρ) from the absorbance of the sample solution based on the plotted standard curve.
The content of aflatoxins M in the sample is calculated according to Formula (3):

$$X = (\rho \times V \times f \times 1000) / (m \times 1000) \quad \text{..... (3)}$$

Where:

X — Content of aflatoxins M in the sample, in micrograms per kilogram (μg/kg);
ρ — Mass concentration of aflatoxins M in the sample solution, in nanograms per milliliter (ng/mL);
V — Constant volume (liquid samples, semi-solid samples, solid samples except cheese) or extraction volume (cheese), in milliliters (mL);
f — Dilution factor;
m — Sample weight, in grams (g);
1000 — Unit conversion factor.

Calculation results are retained to three significant figures.

21 Precision

The absolute difference between two independent determination results obtained under repeatability conditions shall not exceed 20% of the arithmetic mean.

22 Others

For liquid samples and semi-solid samples, when 10 g of sample is weighed, the LOD of this method is 0.01 μg/kg, and the LOQ is 0.03 μg/kg.
For solid samples (except cheese), when 10 g of sample is weighed, the LOD of this method is 0.1 μg/kg, and the LOQ is 0.3 μg/kg.
For cheese samples, when 5 g of sample is weighed, the LOD of this method is 0.02 μg/kg, and the LOQ is 0.06 μg/kg.

Appendix A Concentration Calibration Method for AFT M_1 and AFT M_2 Standard Solutions

Prepare AFT M_1 and AFT M_2 standard solutions with mass concentrations of 8 μg/mL to 10 μg/mL using acetonitrile. Measure the absorbance of the solution at maximum absorption wavelength according to the following method to determine actual concentrations of AFT M_1 and AFT M_2.

Measure using a spectrophotometer at 340 nm to 370 nm. After subtracting the solvent blank background and correcting cuvette system errors, read the absorbance value A at the maximum

absorption wavelength (λ) of the standard solution. The actual mass concentration ρ of the calibrated solution is calculated according to Formula (A.1):

$$\rho = (A \times M \times 1000) / \varepsilon \qquad \text{..... (A.1)}$$

Where:

ρ — Actual mass concentration of AFT M1 and AFT M2 determined by calibration, in micrograms per milliliter (μg/mL);

A — Absorbance value measured at λ;

M — Molar mass of AFT M_1 and AFT M_2, in grams per mole (g/mol);

ε — Molar absorption coefficient of AFT M_1 and AFT M_2 (see Table A.1), in square meters per mole (m^2/mol).

Table A.1 Molar Mass and Molar Absorption Coefficient of AFT M1 and AFT M2

Toxin Name	Molar Mass (g/mol)	Solvent	Molar Absorption Coefficient (m^2/mol)
AFT M_1	328	Acetonitrile	19,000
AFT M_2	330	Acetonitrile	21,400

Appendix B Chromatograms of Aflatoxin M Standard Solutions

The liquid chromatogram of aflatoxin M is shown in Figure B.1, and the liquid chromatography-tandem mass chromatogram is shown in Figure B.2.

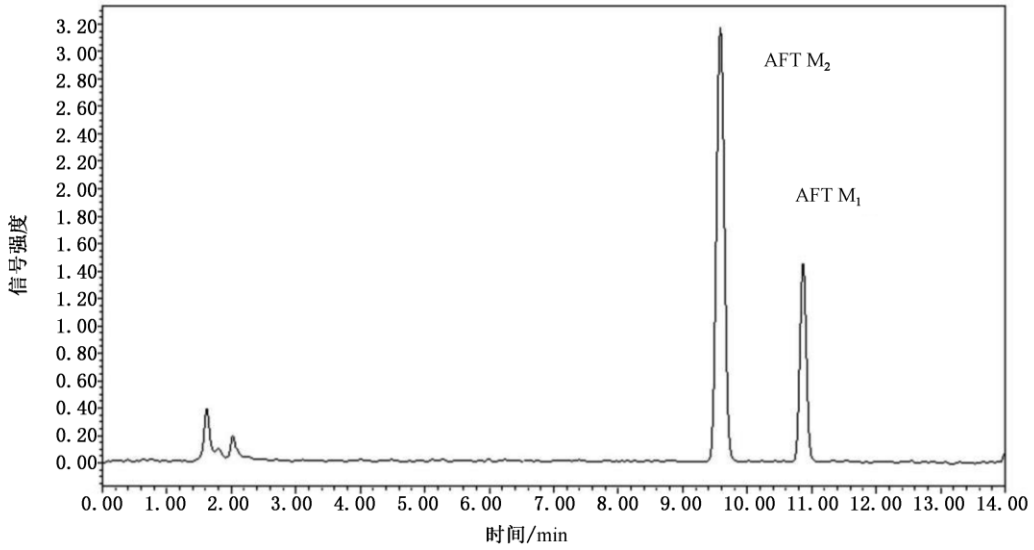
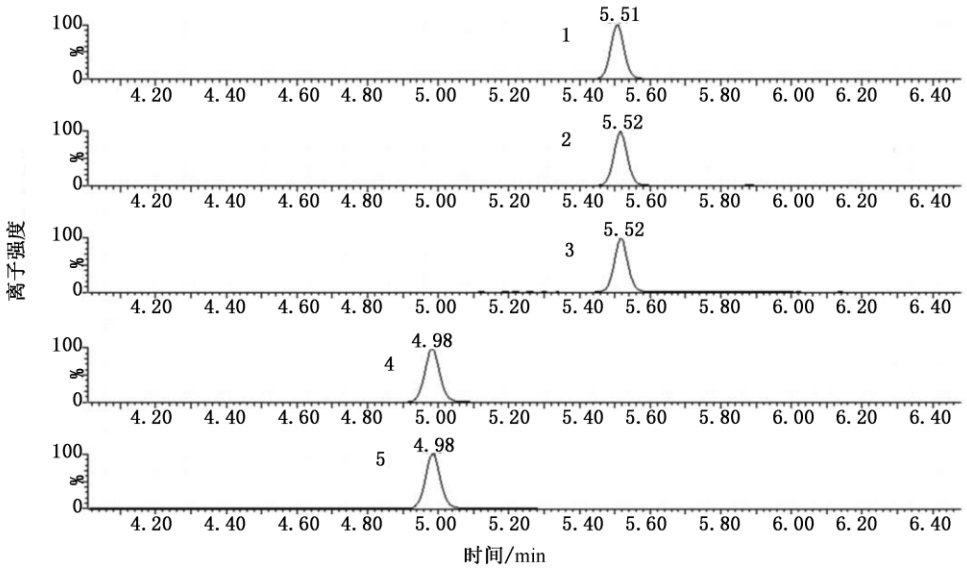


Figure B.1 Liquid Chromatogram of AFT M_1 and AFT M_2 Standard Solutions (1.0 μg/L)



Legend Description:
1 — AFT M_1, 329.1 > 273.1;
2 — AFT M_1, 329.1 > 259.1;
3 — $^{13}C_{17}$-AFT M_1, 346.1 > 288.1;
4 — AFT M_2, 331.1 > 273.1;
5 — AFT M_2, 331.1 > 259.1.

Figure B.2 Liquid Chromatography-Tandem Mass Chromatograms of AFT M_1, AFT M_2 and $^{13}C_{17}$-AFT M_1, Standard Solutions (0.5 µg/L)

END UNOFFICIAL TRANSLATION

Attachments:

No Attachments.