



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

**GB 5009.24-2016**

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**National food safety standard - Determination of M Aflatoxins in  
Foods**

食品安全国家标准 食品中黄曲霉毒素M族的测定  
**(English Translation)**

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### Foreword

This Standard serves as a replacement of GB 5413.37-2010 National Food Safety Standard - Determination of Aflatoxin M1 in Milk and Milk Products; GB 5009.24-2010 National Food Safety Standard - Determination of Aflatoxin M1 and B1 in Foods; GB/T 23212-2008 Determination of Aflatoxin B1, B2, G1, G2, M1, M2 Content in Milk and Milk Powder - HPLC-fluorescence Detection Method; SN/T 1664-2005 Determination of Aflatoxin M1, B1, B2, G1, G2 Content in Milk and Milk Powder.

In comparison with GB 5413.37-2010, the main changes are as follows.

- The name of the standard is modified into "National Food Safety Standard - Determination of M Aflatoxins in Foods";
  - The scope of application of the methods is extended;
  - The detection of aflatoxins M2 is added;
  - Enzyme-linked immunosorbent assay is modified; the name of the third method is modified into enzyme-linked immunosorbent screening assay;
  - Liquid chromatography-mass spectrometry is modified;
  - The pretreatment method of liquid chromatography is modified;
  - Immunochromatography purification fluorescence spectrophotometry is deleted.
- National Food Safety Standard -  
Determination of M Aflatoxins in Foods

### 1 Scope

This Standard specifies the methods for the determination of aflatoxins M1 and aflatoxins M2 (hereinafter referred to as AFT M1 and AFT M2) in foods.

Method I is isotope dilution liquid chromatography - tandem mass spectrometry, which is applicable to the determination of AFT M1 and AFT M2 in milk, milk products and milk-containing special dietary food.

Method II is high-performance liquid chromatography; its scope of application is the same as Method I.

Method III is enzyme-linked immunosorbent screening assay, which is applicable to the screening determination of AFT M1 in milk, milk products and milk-containing special dietary food.

Method I -- Isotope Dilution Liquid Chromatography -  
Tandem Mass Spectrometry

## 2 Principle

Use methanol - water solution to extract aflatoxins M1 and aflatoxins M2 in sample. Use water or phosphate buffer solution to dilute the supernatant, then, purify and enrich it through immunoaffinity column. After concentration, constant-volume and filtering of the purified liquid, separate it through liquid chromatography. Detect it through tandem mass spectrometry, then, quantify it through isotope internal standard method.

## 3 Reagents and Materials

Unless it is otherwise stipulated, all reagents adopted in this Method shall be analytical pure; water shall be Grade-1 water stipulated in GB/T 6682.

### 3.2.1 Ammonium acetate solution (5 mmol/L). weigh-take 0.39 g of ammonium acetate;

dilute it in 1,000 mL of water, then, mix it up.

### 3.2.2 Acetonitrile - water solution (25 + 75). measure-take 250 mL of acetonitrile; add

it to 750 mL of water, then, mix it up.

**3.3.1 AFT M1 standard (C<sub>17</sub>H<sub>12</sub>O<sub>7</sub>, CAS. 6795-23-9). purity  $\geq$  98%, or nationally certified**

standard substance with the standard substance certificate.

**3.3.2 AFT M2 standard (C<sub>17</sub>H<sub>14</sub>O<sub>7</sub>, CAS. 6885-57-0). purity  $\geq$  98%, or nationally certified**

standard substance with the standard substance certificate.

3.3.3 <sup>13</sup>C<sub>17</sub>-AFT M1 isotope solution (C<sub>17</sub>H<sub>14</sub>O<sub>7</sub>). 0.5 µg/mL.

**0.01 mg) of AFT M1 and AFT M2; respectively use acetonitrile to dissolve it, then, dilute**

to the constant volume of 100 mL.

**1.00 mL of 10 µg/mL AFT M1 and AFT M2 standard stock solution, then, place them in**

the same 10 mL volumetric flask.

3.4.5 5 ng/mL isotope internal standard working solution 2(<sup>13</sup>C<sub>17</sub>-AFT M1). take 100 µL of AFT M1 isotope internal standard (0.5 µg/mL), then, use acetonitrile to dilute to 10 mL. Preserve it at -20 °C; it shall be used for the determination of solid sample. It shall remain valid for 3 months.

**4.9 Liquid chromatography - tandem mass spectrometer. equipped with electrospray**

ion source.

**4.12 Disposable microporous filter head. equipped with 0.22 µm microporous** membrane (before use, selected filter membrane shall adopt standard solution to confirm that there is no absorption phenomenon).

## **5 Analytical Procedure**

The application of immunoaffinity columns provided by different manufacturers might be slightly different in sample operation, such as sample loading, leaching and elution. Thus, operation shall comply with the requirements in the operating instructions

provided by the manufacturers.

#### **5.1.1 Liquid milk and yogurt**

Weigh-take 4 g (accurate to 0.001 g) of evenly mixed sample, then, place it in a 50 mL centrifuge tube. Add 100  $\mu$ L of 13C17-AFT M1 internal standard solution (5 ng/mL); start oscillation blending, then, evenly place it for 30 min. Add 10 mL of methanol, then, start vortex for 3 min. Place it at 4 °C; start centrifugation at 6,000 r/min for 10 min, or, filter it through glass fiber filter paper. Transfer an appropriate amount of the supernatant or filtrate to a beaker, then, add 40 mL of water or PBS to dilute it; reserve it for later use.

#### **5.1.3 Cream**

Weigh-take 1 g (accurate to 0.001 g) of sample, then, place it in a 50 mL centrifuge tube. Add 100  $\mu$ L of 13C17-AFT M1 internal standard solution (5 ng/mL); start oscillation blending, then, evenly place it for 30 min. Add 8 mL of petroleum ether; wait till cream dissolves, then, add 9 mL of water and 11 mL of methanol. Start oscillation for 30 min. Transfer all the liquid to separating funnel. Add 0.3 g of sodium chloride, then, thoroughly shake and dissolve it. Evenly place it and wait for layering, then, transfer the lower layer to a round-bottomed flask. Start rotary evaporation, till it is below 10 mL, then, use PBS to dilute it to 30 mL.

#### **5.2.1 Preparation of immunoaffinity column**

Restore immunoaffinity column, which is preserved at low temperature, to room temperature.

#### **5.2.2 Purification**

After abandoning the liquid in the immunoaffinity column, transfer the above-mentioned sample solution to a 50 mL syringe;

#### **5.3 Liquid Chromatography Reference Conditions**

Liquid chromatography reference conditions are listed below.