# GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

# GB 5009.240-2023

# National Food Safety Standard - Determination of Fumonisin in Foods

# 食品安全国家标准　食品中伏马菌素的测定

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**State Administration for Market Regulation.**

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# National Food Safety Standard - Determination of Fumonisin in Foods

## 1 Scope

This Standard specifies the immunoaffinity column purification - post-column derivatization high-performance liquid chromatography, high-performance liquid chromatography - tandem mass spectrometry and immunoaffinity column purification - pre-column derivatization high-performance liquid chromatography for the determination of fumonisin in foods.

This Standard is applicable to the determination of fumonisin $B_1$, fumonisin $B_2$ and fumonisin $B_3$ (hereinafter referred to as $FB_1$, $FB_2$ and $FB_3$) in cereals and products, cereal supplementary foods for infants and young children, and vegetable oils.

# Method I - Immunoaffinity Column Purification - Post-column Derivatization High-performance Liquid Chromatography

## 2 Principle

The specimen is extracted, purified by an immunoaffinity column, separated by $C_{18}$ reversed-phase chromatography column, derivatized by o-phthalaldehyde, detected by fluorescence detector, and quantified by the external standard method.

## 3 Reagents and Materials

Unless it is otherwise specified, the reagents used in this Method are all analytically pure, and the water is Grade-1 water specified in GB/T 6682.

### 3.1 Reagents

**3.1.1** Methanol ($CH_3OH$): chromatographically pure.

**3.1.2** Acetonitrile ($CH_3CN$): chromatographically pure.

**3.1.3** Formic acid (HCOOH): chromatographically pure.

**3.1.4** Acetic acid ($CH_3COOH$).

**3.1.5** Sodium hydroxide (NaOH).

**3.1.6** Sodium chloride (NaCl).

**3.1.7** Disodium hydrogen phosphate ($Na_2HPO_4$).

**3.1.8** Potassium dihydrogen phosphate ($KH_2PO_4$).

**3.1.9** Potassium chloride (KCl).

**3.1.10** Borax ($Na_2B_4O_7 \bullet 10H_2O$).

**3.1.11** 2-mercaptoethanol ($C_2H_6OS$).

**3.1.12** o-phthalaldehyde ($C_8H_6O$, OPA).

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

**3.1.14** Hydrochloric acid (HCl).

### 3.2 Preparation of Reagents

**3.2.1** Formic acid - water solution (0.1%): accurately transfer-take 1 mL of formic acid, use water to dilute to 1,000 mL and evenly mix it.

**3.2.2** Acetonitrile - water solution (50 + 50): respectively measure-take 500 mL of acetonitrile and 500 mL of water, and evenly mix them.

**3.2.3** Acetonitrile - water solution (20 + 80): respectively measure-take 20 mL of acetonitrile and 80 mL of water, and evenly mix them.

**3.2.4** Methanol - acetic acid solution (98 + 2): accurately transfer-take 2 mL of acetic acid, use methanol to dilute to 100 mL and evenly mix it.

**3.2.5** Sodium hydroxide solution (2 mol/L): accurately weigh-take 8.0 g of sodium hydroxide, add 50 mL of water to dissolve it; after cooling, use water to dilute it to 100 mL and evenly mix it.

**3.2.6** Phosphate buffer solution (PBS): weigh-take 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; use 980 mL of water to dissolve it, use hydrochloric acid to adjust pH to 7.4, use water to dilute to 1,000 mL and evenly mix it.

**3.2.7** Tween-20/PBS solution (0.1%): weigh-take 1.0 g of Tween-20, add phosphate buffer solution, dilute to 1,000 mL and evenly mix it.

**3.2.8** Borax solution (0.05 mol/L, pH 10.5): weigh-take 19.1 g of borax, dissolve it in 980 mL of water, use sodium hydroxide solution to adjust pH to 10.5, use water to dilute to 1,000 mL and evenly mix it.

**3.2.9** Derivatization solution: weigh-take 0.5 g of o-phthalaldehyde, dissolve it in 20 mL of

methanol, use borax solution (0.05 mol/L, pH 10.5) to dilute to 500 mL, add 500 μL of 2-mercaptoethanol and evenly mix it. After filtration, put it into a brown bottle; store it at room temperature away from light. It shall remain valid for 1 week.

### 3.3 Reference Materials

**3.3.1** Fumonisin $B_1$ ($C_{34}H_{59}NO_{15}$, $FB_1$, CAS No.: 116355-83-0), purity ≥ 95%, or a standard substance certified by the state and awarded a standard substance certificate.

**3.3.2** Fumonisin $B_2$ ($C_{34}H_{59}NO_{14}$, $FB_2$, CAS No.: 116355-84-1), purity ≥ 95%, or a standard substance certified by the state and awarded a standard substance certificate.

**3.3.3** Fumonisin $B_3$ ($C_{34}H_{59}NO_{14}$, $FB_3$, CAS No.: 136379-59-4), purity ≥ 95%, or a standard substance certified by the state and awarded a standard substance certificate.

### 3.4 Preparation of Standard Solutions

**3.4.1** Standard stock solutions (100 μg/mL): respectively and accurately weigh-take 10 mg (accurate to 0.01 mg) of $FB_1$, $FB_2$ and $FB_3$ into small beakers, use acetonitrile - water solution (50 + 50) to dissolve them, and respectively transfer to 100 mL volumetric flasks. Use acetonitrile - water solution (50 + 50) to reach a constant volume to the scale. Store them at −18 °C away from light. They shall remain valid for 6 months.

**3.4.2** Mixed standard stock solution: accurately transfer-take 1.0 mL of $FB_1$ standard stock solution, 0.5 mL of $FB_2$ standard stock solution and 0.5 mL of $FB_3$ standard stock solution into the same 10 mL volumetric flask; add acetonitrile - water solution (50 + 50) to dilute to the scale. The mass concentration of $FB_1$ is 10 μg/mL, and the mass concentration of $FB_2$ and $FB_3$ is 5 μg/mL. Store it at −18 °C away from light. It shall remain valid for 6 months.

**3.4.3** Mixed standard working solution: accurately transfer-take 1.0 mL of the mixed standard stock solution into a 10.0 mL volumetric flask, add acetonitrile - water solution (50 + 50) to dilute it and reach a constant volume to the scale. The mass concentration of $FB_1$ is 1 μg/mL, and the mass concentration of $FB_2$ and $FB_3$ is 0.5 μg/mL. Store it at 4 °C away from light. It shall remain valid for 6 months.

**3.4.4** Mixed standard series of working solutions: accurately transfer-take the mixed standard working solution, use acetonitrile - water solution (20 + 80) to dilute it, and prepare mixed standard series of working solutions respectively with a $FB_1$ mass concentration of 20.0 ng/mL, 80.0 ng/mL, 160 ng/mL, 240 ng/mL, 320 ng/mL, 400 ng/mL and 480 ng/mL, and a $FB_2$ and $FB_3$ mass concentration of 10.0 ng/mL, 40.0 ng/mL, 80.0 ng/mL, 120 ng/mL, 160 ng/mL, 200 ng/mL and 240 ng/mL. Prepare them right before use.

### 3.5 Materials

**3.5.1** Immunoaffinity column (see Appendix D for column capacity and column recovery verification methods).

**NOTE:** before use, the column capacity and column recovery rate of each batch of affinity columns

#### 5.2.2 Vegetable oils

The extraction of vegetable oils shall be performed in accordance with the steps in 5.2.1. The extracting solution is the lower layer.

### 5.3 Specimen Purification

#### 5.3.1 Cereal supplementary foods for infants and young children

Accurately transfer-take 5.0 mL of the extracting solution, add 45.0 mL of Tween-20/PBS solution for dilution and evenly mix it. At 4,000 r/min, centrifuge it for 10 minutes, take the supernatant and pass it all through the immunoaffinity column, and control the flow rate to 1 mL/min ~ 2 mL/min. Then, use 10 mL of PBS buffer solution and 10 mL of water to successively rinse the immunoaffinity column. Then, use 3 mL of methanol - acetic acid solution to elute the immunoaffinity column in three times; collect and combine the eluent. At 55 °C, use nitrogen to blow-dry it; add 1 mL of acetonitrile - water solution (20 + 80) to dissolve the residue and vortex for 30 s. After passing it through a microporous filter membrane, collect it in a sampling bottle and reserve it for testing.

#### 5.3.2 Cereals and products, and vegetable oils

Accurately transfer-take 0.5 mL of the extracting solution, add 4.5 mL of Tween-20/PBS solution for dilution and evenly mix it. At 4,000 r/min, centrifuge it for 10 minutes, take the supernatant and pass it all through the immunoaffinity column, and control the flow rate to 1 mL/min ~ 2 mL/min. Then, use 10 mL of PBS buffer solution and 10 mL of water to successively rinse the immunoaffinity column. Then, use 3 mL of methanol - acetic acid solution to elute the immunoaffinity column in three times; collect and combine the eluent. At 55 °C, use nitrogen to blow-dry it; add 1 mL of acetonitrile - water solution (20 + 80) to dissolve the residue and vortex for 30 s. After passing it through a microporous filter membrane, collect it in a sampling bottle and reserve it for testing.

**NOTE:** since the immunoaffinity column operating procedures provided by different manufacturers may be different, during actual operation, please refer to the operating instructions and procedures provided by the manufacturer.

### 5.4 Reference Conditions of Instrument

**5.4.1** Chromatographic column: $C_{18}$, particle size 5 μm, 4.6 × 250 mm, or equivalent.

**5.4.2** Detection wavelength: excitation wavelength 335 nm; emission wavelength 440 nm.

**5.4.3** Mobile phase: A: formic acid - water solution (0.1%); B: methanol. Gradient elution. The elution procedure is shown in Table 1.

**5.4.4** Mobile phase flow rate: 0.8 mL/min.

**5.4.5** Derivative solution flow rate: 0.4 mL/min.

### 10.5 Preparation of Isotope Internal Standard Solution

**10.5.1** Mixed isotope standard stock solution: accurately transfer-take 1 mL of $^{13}C_{34}$-$FB_1$ (25 μg/mL), $^{13}C_{34}$-$FB_2$ (10 μg/mL) and $^{13}C_{34}$-$FB_3$ (10 μg/mL) into the same 10 mL volumetric flask. Add acetonitrile - water solution (50 + 50) to dilute it and reach a constant volume to the scale. The mass concentration of $^{13}C_{34}$-$FB_1$ is 2.5 μg/mL, and the mass concentration of $^{13}C_{34}$-$FB_2$ and $^{13}C_{34}$-$FB_3$ is respectively 1 μg/mL. Store it at −18 °C away from light. It shall remain valid for 6 months.

**10.5.2** Mixed isotope standard working solution: accurately transfer-take 1.0 mL of the mixed isotope standard stock solution into a 10 mL volumetric flask, add acetonitrile - water solution (50 + 50) to dilute it and reach a constant volume to the scale. The mass concentration of $^{13}C_{34}$-$FB_1$ is 250 ng/mL, and the mass concentration of $^{13}C_{34}$-$FB_2$ and $^{13}C_{34}$-$FB_3$ is respectively 100 ng/mL. Store it at 4 °C away from light. It shall remain valid for 6 months.

### 10.6 Preparation of Mixed Standard Series of Working Solutions

Accurately transfer-take the mixed standard working solution, use acetonitrile - water solution (20 + 80) to dilute it, and add the mixed isotope standard working solution. Thus, mixed standard series of working solutions with a $FB_1$ mass concentration of 20.0 ng/mL, 80.0 ng/mL, 160 ng/mL, 240 ng/mL, 320 ng/mL, 400 ng/mL and 480 ng/mL, and a $FB_2$ and $FB_3$ mass concentration of 10.0 ng/mL, 40.0 ng/mL, 80.0 ng/mL, 120 ng/mL, 160 ng/mL, 200 ng/mL and 240 ng/mL are prepared. In each standard working solution, the mass concentration of $^{13}C_{34}$-$FB_1$, $^{13}C_{34}$-$FB_2$ and $^{13}C_{34}$-$FB_3$ is respectively 50.0 ng/mL, 20.0 ng/mL and 20.0 ng/mL. Prepare them right before use.

### 10.7 Materials

**10.7.1** Immunoaffinity column (see Appendix D for column capacity and column recovery verification methods).

> **NOTE:** before use, the column capacity and column recovery rate of each batch of affinity columns need to be verified.

**10.7.2** Strong anion exchange solid-phase extraction column (6 mL, 500 mg).

**10.7.3** Microporous filter membrane: 0.22 μm, organic type.

## 11 Instruments and Equipment

**11.1** High-performance liquid chromatograph - tandem mass spectrometer: equipped with electrospray ion source.

**11.2** Balance: with a division value of 0.01 g and 0.01 mg.

**11.3** Homogenizer: with a rotation speed ≥ 2,000 r/min.

**11.4** Oscillator (with a rotation speed ≥ 1,000 r/min) or ultrasonic extraction instrument (with a power ≥ 500 W).

**11.5** Centrifuge: with a rotation speed ≥ 4,000 r/min.

**11.6** Nitrogen blower.

# 12 Analytical Procedures

## 12.1 Specimen Preparation

For cereals and products, cereal supplementary foods for infants and young children, the sampling size shall be not lower than 1 kg; when the mass of sample is less than 1 kg, all specimens shall be taken. Use a high-speed pulverizer to pulverize it, and the fineness of pulverization shall be less than 1 mm. After evenly mix it, store in a clean container, seal and store at 4 °C away from light.

For vegetable oils, the sampling size shall be not lower than 1 kg; when the mass of sample is less than 1 kg, all specimens shall be taken. After evenly mix it, store in a clean container, seal and store at 4 °C away from light.

During the specimen preparation, sample contamination or changes in fumonisin content shall be prevented.

## 12.2 Specimen Extraction

### 12.2.1 Cereals and products, cereal supplementary foods for infants and young children

Accurately weigh-take 20 g (accurate to 0.01 g) of specimen into a 250 mL conical flask, accurately add 100 mL of acetonitrile - water (50 + 50) extracting solution, conduct ultrasonic or oscillating extraction for 20 minutes, transfer 20 mL of the extracting solution into a 50 mL centrifuge tube. At 4,000 r/min, centrifuge it for 5 minutes and reserve it for purification.

### 12.2.2 Vegetable oils

The extraction of vegetable oils shall be the same as 12.2.1. The extracting solution is the lower layer.

## 12.3 Specimen Purification

### 12.3.1 Immunoaffinity column purification

#### 12.3.1.1 Cereal supplementary foods for infants and young children

Accurately transfer-take 5.0 mL of the extracting solution, add 200 μL of the mixed isotope standard working solution and 45.0 mL of Tween-20/PBS solution for dilution, evenly mix it. At 4,000 r/min, centrifuge it for 10 minutes, take all the supernatant and pass it through the

immunoaffinity column, and control the flow rate to 1 mL/min ~ 2 mL/min. Then, use 10 mL of PBS buffer solution and 10 mL of water to successively rinse the immunoaffinity column. Then, use 3 mL of methanol - acetic acid solution (98 + 2) to elute the immunoaffinity column in three times; combine the eluent. At 55 °C, use nitrogen to blow-dry it; add 1 mL of acetonitrile - water solution (20 + 80) to dissolve the residue and vortex for 30 s. After passing it through a microporous filter membrane, collect it in a sampling bottle and reserve it for testing.

**12.3.1.2 Cereals and products, and vegetable oils**

Accurately transfer-take 0.5 mL of the extracting solution, add 200 μL of the mixed isotope standard working solution and 4.5 mL of Tween-20/PBS solution for dilution and evenly mix it. At 4,000 r/min, centrifuge it for 10 minutes, take all the supernatant and pass it through the immunoaffinity column, and control the flow rate to 1 mL/min ~ 2 mL/min. Then, use 10 mL of PBS buffer solution and 10 mL of water to successively rinse the immunoaffinity column. Then, use 3 mL of methanol - acetic acid solution to elute the immunoaffinity column in three times; collect and combine the eluent. At 55 °C, use nitrogen to blow-dry it; add 1 mL of acetonitrile - water solution (20 + 80) to dissolve the residue and vortex for 30 s. After passing it through a microporous filter membrane, collect it in a sampling bottle and reserve it for testing.

**12.3.2 Strong anion exchange solid-phase extraction purification column**

**12.3.2.1 Cereal supplementary foods for infants and young children**

Accurately transfer-take 5.0 mL of the extracting solution, add 200 μL of the mixed isotope standard working solution and 15.0 mL of methanol - water solution (60 + 20) for dilution, and evenly mix it. At 4,000 r/min, centrifuge it for 10 minutes. Take all the supernatant and pass it through a strong anion exchange solid-phase extraction column (before use, successively use 6.0 mL of methanol and 6.0 mL of water to activate it), and control the flow rate to 1 mL/min ~ 2 mL/min. Use 8 mL of methanol - water solution (60 + 20) and 3 mL of methanol to successively rinse it, and use 10 mL of methanol - acetic acid solution (99 + 1) to elute it; collect the eluent. At 55 °C, use nitrogen to blow-dry it. Add 1 mL of acetonitrile - water solution (20 + 80) to dissolve the residue and vortex for 30 s. After passing it through a microporous filter membrane, collect it in a sampling bottle and reserve it for testing.

**12.3.2.2 Cereals and products, and vegetable oils**

Accurately transfer-take 0.5 mL of the extracting solution, add 200 μL of the mixed isotope standard working solution and 5.0 mL of methanol - water solution (60 + 20) for dilution, and evenly mix it. At 4,000 r/min, centrifuge it for 5 minutes. Take all the supernatant and pass it through a strong anion exchange solid-phase extraction column (before use, successively use 6.0 mL of methanol and 6.0 mL of water to activate it), and control the flow rate to 1 mL/min ~ 2 mL/min. Use 8 mL of methanol - water solution (60 + 20) and 3 mL of methanol to successively rinse it, and use 10 mL of methanol - acetic acid solution (99 + 1) to elute it; collect the eluent. At 55 °C, use nitrogen to blow-dry it. Add 1 mL of acetonitrile - water solution (20 + 80) to dissolve the residue and vortex for 30 s. After passing it through a microporous filter membrane, collect it in a sampling bottle and reserve it for testing.

hydrogen phosphate, 0.2 g of potassium dihydrogen phosphate and 0.2 g of potassium chloride; use 980 mL of water to dissolve it, then, use hydrochloric acid to adjust pH to 7.4. Finally, use water to dilute to 1,000 mL and evenly mix it.

**17.2.7** Tween-20/PBS solution (0.1%): weigh-take 1.0 g of Tween-20, add phosphate buffer solution, dilute to 1,000 mL and evenly mix it.

**17.2.8** Borax solution (0.1 mol/L): weigh-take 3.8 g of borax, use water to dissolve it and dilute to 100 mL, and evenly mix it.

**17.2.9** Derivatization solution: weigh-take 25 mg of o-phthalaldehyde, dissolve it in 1 mL of methanol, use borax solution (0.1 mol/L) to dilute to 50 mL, add 50 μL of 2-mercaptoethanol and evenly mix it. After filtration, put it into a brown bottle; store it at room temperature away from light. It shall remain valid for 1 week.

### 17.3 Reference Materials

**17.3.1** Fumonisin $B_1$ ($C_{34}H_{59}NO_{15}$, $FB_1$, CAS No.: 116355-83-0), purity ≥ 95%, or a standard substance certified by the state and awarded a standard substance certificate.

**17.3.2** Fumonisin $B_2$ ($C_{34}H_{59}NO_{14}$, $FB_2$, CAS No.: 116355-84-1), purity ≥ 95%, or a standard substance certified by the state and awarded a standard substance certificate.

**17.3.3** Fumonisin $B_3$ ($C_{34}H_{59}NO_{14}$, $FB_3$, CAS No.: 136379-59-4), purity ≥ 95%, or a standard substance certified by the state and awarded a standard substance certificate.

### 17.4 Preparation of Standard Solutions

**17.4.1** Standard stock solutions (100 μg/mL): respectively and accurately weigh-take 10 mg (accurate to 0.01 mg) of $FB_1$, $FB_2$ and $FB_3$ into small beakers, use acetonitrile - water solution (50 + 50) to dissolve them, and respectively transfer to 100 mL volumetric flasks. Use acetonitrile - water solution (50 + 50) to reach a constant volume to the scale. Store them at −18 °C away from light. They shall remain valid for 6 months.

**17.4.2** Mixed standard stock solution: accurately transfer-take 1.0 mL of $FB_1$ standard stock solution, 0.5 mL of $FB_2$ standard stock solution and 0.5 mL of $FB_3$ standard stock solution into the same 10 mL volumetric flask; add acetonitrile - water solution (50 + 50) to dilute to the scale. The mass concentration of $FB_1$ is 10 μg/mL, and the mass concentration of $FB_2$ and $FB_3$ is 5 μg/mL. Store it at −18 °C away from light. It shall remain valid for 6 months.

**17.4.3** Mixed standard working solution: accurately transfer-take 1.0 mL of the mixed standard stock solution into a 10.0 mL volumetric flask, add acetonitrile - water solution (50 + 50) to dilute it and reach a constant volume to the scale. The mass concentration of $FB_1$ is 1.0 μg/mL, and the mass concentration of $FB_2$ and $FB_3$ is 0.5 μg/mL. Store it at 4 °C away from light. It shall remain valid for 6 months.

**17.4.4** Mixed standard series of working solutions: accurately transfer-take the mixed standard

During the specimen preparation, sample contamination or changes in fumonisin content shall be prevented.

## 19.2 Specimen Extraction

### 19.2.1 Cereals and products, cereal supplementary foods for infants and young children

Accurately weigh-take 20 g (accurate to 0.01 g) of specimen into a 250 mL conical flask, accurately add 100 mL of acetonitrile - water (50 + 50) extracting solution, conduct ultrasonic or oscillating extraction for 20 minutes, transfer 20 mL of the extracting solution into a 50 mL centrifuge tube. At 4,000 r/min, centrifuge it for 10 minutes and reserve it for purification.

### 19.2.2 Vegetable oils

The extraction of vegetable oils shall be performed in accordance with the steps in 19.2.1. The extracting solution is the lower layer.

## 19.3 Specimen Purification

### 19.3.1 Cereal supplementary foods for infants and young children

Accurately transfer-take 5.0 mL of the extracting solution, add 45.0 mL of Tween-20/PBS solution for dilution and evenly mix it. At 4,000 r/min, centrifuge it for 10 minutes, take all the supernatant and pass it through the immunoaffinity column, and control the flow rate to 1 mL/min ~ 2 mL/min. Then, use 10 mL of PBS buffer solution and 10 mL of water to successively rinse the immunoaffinity column. Then, use 3 mL of methanol - acetic acid solution (98 + 2) to elute the immunoaffinity column in three times; collect the eluent. At 55 °C, use nitrogen to blow-dry it; add 1 mL of acetonitrile - water solution (20 + 80) to dissolve the residue and vortex for 30 s. After passing it through a microporous filter membrane, collect it in a sampling bottle and reserve it for testing.

### 19.3.2 Cereals and products, and vegetable oils

Accurately transfer-take 0.5 mL of the extracting solution, add 4.5 mL of Tween-20/PBS solution for dilution and evenly mix it. At 4,000 r/min, centrifuge it for 10 minutes, take all the supernatant and pass it through the immunoaffinity column, and control the flow rate to 1 mL/min ~ 2 mL/min. Then, use 10 mL of PBS buffer solution and 10 mL of water to successively rinse the immunoaffinity column. Then, use 3 mL of methanol - acetic acid solution (98 + 2) to elute the immunoaffinity column in three times; collect the eluent. At 55 °C, use nitrogen to blow-dry it; add 1 mL of acetonitrile - water solution (20 + 80) to dissolve the residue and vortex for 30 s. After passing it through a microporous filter membrane, collect it in a sampling bottle and reserve it for testing.

**NOTE:** since the immunoaffinity column operating procedures provided by different manufacturers may be different, during actual operation, please refer to the operating instructions and procedures provided by the manufacturer.

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