



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

GB 5009.240-2023

**National food safety standard - Determination of fumonisin in
foods**

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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 B1, fumonisin B2 and fumonisin B3 (hereinafter referred to as FB1, FB2 and FB3) 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 C18 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.

3.1.10 Borax ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$).

3.1.11 2-mercaptoethanol ($\text{C}_2\text{H}_6\text{OS}$).

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 μ L 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.

3.3.1 Fumonisin B1 (C₃₄H₅₉NO₁₅, FB1, 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 B2 (C₃₄H₅₉NO₁₄, FB2, 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 B3 (C₃₄H₅₉NO₁₄, FB3, CAS No.. 136379-59-4), purity ≥ 95%, or a standard

substance certified by the state and awarded a standard substance certificate.

3.4.1 Standard stock solutions (100 µg/mL). respectively and accurately weigh-take 10 mg

(accurate to 0.01 mg) of FB1, FB2 and FB3 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 FB1 standard stock

solution, 0.5 mL of FB2 standard stock solution and 0.5 mL of FB3 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 FB1 is 10 µg/mL, and the mass concentration of FB2 and FB3 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 FB1 is 1 µg/mL, and the mass concentration of FB2 and FB3 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 FB1 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 FB2 and FB3 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.

3.5.1 Immunoaffinity column (see Appendix D for column capacity and column recovery

verification methods).