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MOHWT0011.04

# Method of Test for Mycotoxins in Foods- Test of Fumonisin $B_1$ and Fumonisin $B_2$

## 1. Scope

This method is applicable to the determination of fumonisin $B_1$ and $B_2$ in maize and maize products, processed maize-based foods for infant and young child and baby foods.

## 2. Method

After extraction, purification, and derivatization, fumonisin $B_1$ and $B_2$ are determined by high performance liquid chromatography (HPLC).

**2.1.** Equipment

**2.1.1.** High performance liquid chromatograph.

**2.1.1.1.** Detector: fluorescence detector.

**2.1.1.2.** Column: RP-18, 5 μm, 4.6 mm i.d. x 25 cm, or an equivalent product.

**2.1.2.** Grinder.

**2.1.3.** Blender.

**2.1.4.** Shaker.

**2.1.5.** Vortex mixer.

**2.1.6.** Centrifuge: centrifugal force > 2500 ×g.

**2.1.7.** Nitrogen evaporator.

**2.1.8.** Vacuum freeze-drying device: temperature control ≤ -40°C and degree of vacuum ≤ 133 mBar.

**2.1.9.** pH meter.

**2.1.10.** Oven: with an automatic temperature adjustment, capable of controlling temperature at ± 2°C.

**2.1.11.** Desiccator.

**2.2.** Chemicals

Methanol, HPLC grade;

Acetonitrile, HPLC grade;

Sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$), reagent grade;

*o*-Phthaldialdehyde, reagent grade;

2-Mercaptoethanol, reagent grade;

Sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$), reagent grade;

Disodium hydrogen phosphate ($Na_2HPO_4$), reagent grade;

Potassium dihydrogen phosphate ($KH_2PO_4$), reagent grade;

Hydrochloric acid, reagent grade;

Phosphoric acid, 85%, reagent grade;
Sodium chloride, reagent grade;
Potassium chloride, reagent grade;
Deionized water, resistivity ≥ 18 MΩ•cm (at 25°C);
Fumonisin $B_1$ and fumonisin $B_2$, reference standards.

**2.3.** Apparatus

**2.3.1.** Centrifuge tube: 50 mL, PP.

**2.3.2.** Volumetric flask: 10 mL and 50 mL, amber.

**2.3.3.** Filter paper: ADVANTEC No. 5A, diameter 12.5 cm, or an equivalent product.

**2.3.4.** Glass microfiber filter: diameter 9 cm.

**2.3.5.** Immunoaffinity column: an FumoniTest column containing the monoclonal antibodies specific for fumonisins, or an equivalent product.

**2.3.6.** Membrane filter: 0.45 μm, Nylon.

**2.3.7.** Sample vial: 2 mL, amber, glass.

**2.3.8.** Weighing bottle: with cap.

**2.4.** Reagents preparation

**2.4.1.** 0.1 M sodium dihydrogen phosphate

Dissolve 15.6 g of sodium dihydrogen phosphate dihydrate with 900 mL of deionized water, adjust pH to 3.3 with phosphoric acid, and dilute with deionized water to 1000 mL.

**2.4.2.** 0.1 M sodium tetraborate

Dissolve and dilute 3.8 g of sodium tetraborate decahydrate with deionized water to 100 mL.

**2.4.3.** Extraction solution

Mix acetonitrile, methanol and deionized water at the ratio of 1:1:2 (v/v/v).

**2.4.4.** 2 N hydrochloric acid

Add 16.7 mL of hydrochloric acid slowly into 80 mL of deionized water, and mix well. After cooling, dilute with deionized water to 100 mL.

**2.4.5.** Phosphate buffer

Dissolve 8 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 with 990 mL of deionized water, adjust pH to 7.0 with 2 N hydrochloric acid, and dilute with deionized water to 1000 mL.

**2.4.6.** *o*- Phthaldialdehyde solution

Dissolve 40 mg of *o*-phthaldialdehyde with 1 mL of methanol, add 5 mL of 0.1 M sodium tetraborate and 50 μL of 2-mercaptoethanol, and mix well. Store in the dark at room temperature for 1 week.

**2.4.7.** 50% acetonitrile

Mix acetonitrile and deionized water at the ratio of 1:1 (v/v).

**2.5.** Mobile phase

**2.5.1.** Mobile phase A

Mix methanol and 0.1 M sodium dihydrogen phosphate at the ratio of 1:1 (v/v), and filter with a membrane filter.

**2.5.2.** Mobile phase B: methanol

**2.6.** Standard solution preparation

Transfer about 1 mg of fumonisin $B_1$ and $B_2$ reference standards accurately weighed to each 10-mL volumetric flask, dissolve and dilute to volume with 50% acetonitrile as the standard stock solutions. Store under freezing. When to use, mix appropriate volume of each standard stock solution and dilute with 50% acetonitrile to 0.6-12 μg/mL for fumonisin $B_1$ and 1.4-28 μg/mL for fumonisin $B_2$ as the standard solutions.

**2.7.** Sample solution preparation

**2.7.1.** Extraction

**2.7.1.1.** Maize and maize products

Transfer about 10 g of the homogenized sample accurately weighed into a centrifuge tube. Accurately add 25 mL of extraction solution, shake for 2 min, centrifuge at 2500 ×g for 10 min, and collect the supernatant. Accurately add 25 mL of extraction solution to the precipitate, and repeat the procedure described above once. Combine the supernatants, dilute to 50-mL with extraction solution, and filter with a filter paper. Accurately take 10 mL of the filtrate into a centrifuge tube, add 40 mL of phosphate buffer, mix thoroughly, and filter with a glass microfiber filter. Accurately take 10 mL of the filtrate for purification.

**2.7.1.2.** Processed maize-based foods for infant and young child and baby foods

Freeze-dry the sample until the moisture content less than 10%, and grind thoroughly. Transfer about 5 g of the homogenized freeze-dried

sample accurately weighed into a centrifuge tube. Accurately add 25 mL of extraction solution, shake for 2 min, centrifuge at 2500 ×g for 10 min, and collect the supernatant. Accurately add 25 mL of extraction solution to the precipitate, and repeat the procedure described above once. Combine the supernatants, dilute to 50-mL with extraction solution, and filter with a filter paper. Accurately take 10 mL of the filtrate into a centrifuge tube, add 40 mL of phosphate buffer, mix thoroughly, and filter with a glass microfiber filter. Accurately take 40 mL of the filtrate for purification.

**2.7.2.** Purification

Transfer the filtrate from section 2.7.1 into the immunoaffinity column (flow rate controlled at 1 drop/second), discard the eluent, and wash the column with 10 mL of phosphate buffer (flow rate controlled at 1 drop/second). After draining off the phosphate buffer in the column, discard the eluent. Add 1.5 mL of methanol (flow rate controlled at 1 drop/second), collect the eluent, and evaporate to dryness by gently flushing with a stream of nitrogen. Dissolve the residue with 200 μL of 50% acetonitrile, filter with a membrane filter, and take the filtrate for derivatization.

**2.7.3.** Derivatization

Accurately transfer 50 μL of the filtrate from section 2.7.2 into a sample vial, add 50 μL of *o*-phthaldialdehyde solution, vortex-mix for 30 sec, and incubate the reaction for 3 min as the sample solution(note).

Note: Because the fluorescence of fumonisin-*o*-phthaldialdehyde derivative will gradually decreased after 3 min of reaction, the sample solution must be injected immediately into the HPLC system.

**2.8.** Calibration standard curve

Take a blank sample, accurately add the standard solutions in section 2.6 (0.5 mL for maize and maize products, and 0.125 mL for processed maize-based foods for infant and young child and baby foods), and follow the procedures described in section 2.7 to obtain the calibration standard solutions. Operate HPLC according to the following conditions. Establish the calibration standard curve of each fumonisin by the peak areas of each fumonisin vs. the added concentrations in the range of 60-1200 ng/mL for fumonisin $B_1$, and 140-2800

ng/mL for fumonisin $B_2$.

HPLC operating conditions:

Fluorescence detector: excitation wavelength, 335 nm; emission wavelength, 440 nm.

Column: RP-18, 5 μm, 4.6 mm i.d. x 25 cm.

Column temperature: 35°C.

Injection volume: 20 μL.

Mobile phase: a gradient program of solvent A and solvent B is as follows:

| Time (min) | A (%) | B (%) |
|---|---|---|
| 0.0 → 8.0 | 50 → 50 | 50 → 50 |
| 8.0 → 8.5 | 50 → 40 | 50 → 60 |
| 8.5 → 15.0 | 40 → 40 | 60 → 60 |
| 15.0 → 15.1 | 40 → 50 | 60 → 40 |
| 15.1 → 20.0 | 50 → 50 | 50 → 50 |

Flow rate: 1.0 mL/min.

**2.9.** Moisture content determination

Transfer about 2 g of the pre-freeze-dried homogenized processed maize-based foods for infant and young child and baby food sample accurately weighed into a weighing bottle predried to constant weight ($m_0$), and weigh accurately ($m_1$). Place the weighing bottle in an oven, and dry at 105ºC for 2 hr. Remove the weighing bottle from the oven, cap the weighing bottle, place in a desiccator to cool to room temperature (about 30 min), and weigh the weighing bottle. Replace the weighing bottle in the oven, dry for 1 hr, and weigh following the above procedure until constant weight ($m_2$). Calculate the moisture content of the sample by the following formula:

$$\text{The moisture content of the sample (\%)} = \frac{m_1 - m_2}{m_1 - m_0} \times 100\%$$

Where,

$m_0$: the weight of the weighing bottle with cap (g)

$m_1$: the weight of the weighing bottle with cap and the sample (g)

$m_2$: the weight of the weighing bottle with cap and the sample after drying to constant weight (g)

**2.10.** Identification and quantification

Accurately inject 20 μL of the sample solution and the calibration standard solutions into HPLC separately, and operate according to the conditions described in section 2.8. Identify fumonisin $B_1$ and $B_2$ based on the retention time of standard solution, and calculate the total amount of fumonisin $B_1$ and $B_2$ in the sample using the following formula (μg/kg):

**2.10.1.** Maize and maize products

The total amount of fumonisin $B_1$ and $B_2$ in the sample (μg/kg)

$$= \frac{\sum C \times V \times F}{M}$$

Where,

C: the concentration of fumonisin $B_1$ or $B_2$ in sample solution calculated by the calibration standard curve (ng/mL)

V: the final make-up volume of the sample (0.2 mL)

M: the weight of the sample (g)

F: dilution factor (25)

**2.10.2.** Processed maize-based foods for infant and young child and baby foods

The total amount of fumonisin $B_1$ and $B_2$ in the sample (μg/kg)

$$= \frac{\sum C \times V \times F}{M \times (1 - W/100)}$$

Where,

C: the concentration of fumonisin $B_1$ or $B_2$ in the sample solution calculated by the calibration standard curve (ng/mL)

V: the final make-up volume of the sample (0.2 mL)

M: the weight of the sample (g)

F: dilution factor (6.25)

W: the moisture content of the sample (%)

**Remark**

1. Limits of quantitation (LOQs) are 30 μg/kg for fumonisin $B_1$ and 70 μg/kg for fumonisin $B_2$ in maize and maize products, and 15 μg/kg for fumonisin $B_1$ and 35 μg/kg for fumonisin $B_2$ in processed maize-based foods for infant and young child and baby foods (on the dried basis).
2. Further validation should be performed when interfering compounds appear in the samples.

3. As confirm by LC-MS/MS, the multiple reaction monitoring (MRM) parameters(note) are shown as follows:

| Analyte | Ionization mode | Ion pair<br>Precursor ion (*m/z*) > product ion (*m/z*) | Declustering potential (V) | Collision energy (eV) |
|---|---|---|---|---|
| Fumonisin $B_1$ | $ESI^+$ | 722.4 > 334* | 48 | 44 |
| | | 722.4 > 352 | 48 | 40 |
| Fumonisin $B_2$ | $ESI^+$ | 706.4 > 336* | 42 | 36 |
| | | 706.4 > 318 | 42 | 40 |

* The quantitative ion.

Note: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

**References**

1. AOAC. 2001. Fumonisins $B_1$ and $B_2$ in corn and corn flakes. AOAC Official Method 2001.04.
2. Wu, S. H., Chiu, J. Y., Yu, M. C., Lwo, C. H., Chang, T. P., Chen, J. H. and Shih, W. C. 2019. The development of analytical methods for natural toxins and contaminants. Commissioned Research Report of Taiwan Food and Drug Administration.