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Method of Test for Mycotoxins in Foods-Test of Aflatoxins

1. Scope

This method is applicable to the determination of aflatoxin B_1, B_2, G_1 and G_2 in spices, grains, dried fruit, edible fats and oils, tree nuts, oilseeds, soybeans and their processed products.

2. Method

After extraction and purification, aflatoxins 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: Cosmosil 5C18-AR, 5 μm, 4.6 mm i.d. x 25 cm, or an equivalent product.

2.1.1.3. Photochemical reactor: Knitted Reactor Coils (KRC) 25-25, or an equivalent product.

2.1.2. Homogenizer: rotary speed > 15000 rpm, suitable for organic solvents.

2.1.3. Grinder.

2.2. Chemicals

Sodium chloride, reagent grade;
Tween-20, reagent grade;
Methanol, HPLC grade;
Deionized water, resistivity ≥ 18 MΩ • cm (at 25°C);
Aflatoxin B_1 (1000 ng/mL), B_2 (300 ng/mL), G_1 (1000 ng/mL) and G_2 (300 ng/mL), mixed reference standard in methanol.

2.3. Apparatus

2.3.1. Centrifuge tube: 50 mL, PP.

2.3.2. Volumetric flask: 2 mL, 10 mL and 20 mL, brown.

2.3.3. Membrane filter: 0.22 μm, Nylon/PTFE.

2.3.4. Filter paper: Whatman No.1, diameter 11 cm, or an equivalent product.

2.3.5. Glass microfiber filter: diameter 9 cm.

2.3.6. Immunoaffinity column: an AflaTest-P column bound with specific antibodies for aflatoxin B_1, B_2, G_1 and G_2, or an equivalent product.

2.4. Reagents

2.4.1. 50% methanol

Mix methanol and deionized water at the ratio of 50:50 (v/v).

2.4.2. 60% methanol

Mix methanol and deionized water at the ratio of 60:40 (v/v).

2.4.3. 80% methanol

Mix methanol and deionized water at the ratio of 80:20 (v/v).

2.4.4. 10% Tween-20

Mix Tween-20 and deionized water at the ratio of 10:90 (v/v).

2.5. Mobile phase

Mix methanol and deionized water at the ratio of 45:55 (v/v), and filter with an Nylon membrane filter.

2.6. Standard solution preparation

Accurately 1 mL of aflatoxin B_1, B_2, G_1, and G_2 mixed reference standard to a 20-mL volumetric flask, and dilute to volume with 50% methanol as the standard stock solution. Stored in a refrigerator. When to use, dilute appropriate volume of the standard stock solution with 50% methanol to 0.1-50 ng/mL for aflatoxin B_1 and G_1, and 0.05-15 ng/mL for aflatoxin B_2 and G_2, as the standard solutions.

2.7. Sample solution preparation

2.7.1. Grains, dried fruit and their proceeds products

Transfer about 50 g of the homogenized sample accurately weighed into the homogenizer, and add 5 g of sodium chloride and 100 mL of 80% methanol. Homogenize at 15000 rpm for 2 min, and filter with a filter paper. Accurately take 10 mL of the filtrate, add 40 mL of deionized water, mix thoroughly, and filter with a glass microfiber filter. Accurately transfer 10 mL of the filtrate into the immunoaffinity column (flow rate controlled at 1 drop/second), and discard the eluent. Wash the column twice with 10 mL of deionized water (flow rate controlled at 1 drop/second), and discard the eluent. Dry the column by vacuum suction if necessary. Add 1 mL of methanol (flow rate controlled at 1 drop/second), collect the eluent, and dilute with deionized water to 2 mL. Filter with a PTFE membrane filter, and take the filtrate as the sample solution.

2.7.2. Edible fats and oils, tree nuts, oilseeds, soybeans and their proceeds

products

Transfer about 25 g of the homogenized sample accurately weighed into the homogenizer. Add 5 g of sodium chloride and 125 mL of 60% methanol, homogenize at 15000 rpm for 2 min, and filter with a filter paper. Accurately take 10 mL of the filtrate, add 30 mL of deionized water, mix thoroughly, and filter with a glass microfiber filter. Accurately transfer 20 mL of the filtrate into the immunoaffinity column (flow rate controlled at 1 drop/second), and discard the eluent. Wash the column twice with 10 mL of deionized water (flow rate controlled at 1 drop/second), and discard the eluent. Dry the column by vacuum suction if necessary. Add 1 mL of methanol (flow rate controlled at 1 drop/second), collect the eluent, and dilute with deionized water to 2 mL. Filter with a PTFE membrane filter, and take the filtrate as the sample solution.

2.7.3. Spices

Transfer about 25 g of the homogenized sample accurately weighed into the homogenizer. Add 5 g of sodium chloride and 100 mL of 80% methanol, homogenize at 15000 rpm for 2 min, and filter with a filter paper. Accurately take 5 mL of the filtrate, add 20 mL of 10% Tween-20, mix thoroughly, and filter with a glass microfiber filter. Accurately transfer 4 mL of the filtrate into the immunoaffinity column (flow rate controlled at 1 drop/second), and discard the eluent. Wash the column twice with 10 mL of deionized water (flow rate controlled at 1 drop/second), and discard the eluent. Dry the column by vacuum suction if necessary. Add 1 mL of methanol (flow rate controlled at 1 drop/second), collect the eluent, and dilute with deionized water to 2 mL. Filter with a PTFE membrane filter, and take the filtrate as the sample solution.

2.8. Identification and quantification

Accurately inject 50 μL of the sample solution and the standard solutions into HPLC separately, and operate according to the following conditions. Identify aflatoxins based on the retention time. Calculate the amount of each aflatoxin in the sample by the following formula(note 1):

$$\text{The amount of each aflatoxin in the sample (μg/kg)} = \frac{C \times V \times F}{M}$$

Where,

C: the concentration of each aflatoxin in the sample solution calculated by the standard curve (ng/mL)

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

F: as sampling according to section 2.7.1, F is 50
as sampling according to section 2.7.2, F is 25
as sampling according to section 2.7.3, F is 125

M: the weight of the sample (g)

HPLC operating conditions(note 2)

Column: Cosmosil 5C18-AR, 5 μm, 4.6 mm i.d. × 25 cm.

Photochemical reactor(note 3): Knitted Reactor Coils (KRC) 25-25.

Fluorescence detector: excitation wavelength, 360 nm; emission wavelength, 440 nm.

Mobile phase: prepared as section 2.5.

Flow rate: 1.0 mL/min.

Note: 1. The amount of total aflatoxin is the sum of aflatoxin B_1, B_2, G_1 and G_2.
2. All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.
3. If the sensitivity of the fluorescence detector can reach the LOQs, the photochemical reactor may not be used.

Remark

1. Limits of quantification (LOQs) are 0.2 μg/kg for aflatoxin B_1 and G_1 and 0.1 μg/kg for aflatoxin B_2 and G_2 in grains, dried fruit, edible fats and oils, tree nuts, oilseeds, soybeans and their processed products, and 1.0 μg/kg for aflatoxin B_1 and G_1 and 0.5 μg/kg for aflatoxin B_2 and G_2 in the spices.
2. Further validation should be performed when interfering compounds appear in samples
3. As confirm by LC-MS/MS, the multiple reaction monitoring (MRM) parameters(note) are shown as follows:

Analyte	Ion source	Ion pair	Declustering potential (V)	Collision energy (eV)
		Precursor ion (*m/z*) > product ion (*m/z*)		
Aflatoxin B_1	ESI^+	313 > 241*	48	36
		313 > 285	48	22
Aflatoxin B_2	ESI^+	315 > 259*	46	28
		315 > 287	46	26
Aflatoxin G_1	ESI^+	329 > 200*	46	42
		329 > 243	46	26
Aflatoxin G_2	ESI^+	331 > 189*	48	42
		331 > 313	48	24

*The quantitative ion.

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

References

1. VICAM LP. 1999. Aflatest® HPLC instruction manual. Milford, MA, USA.
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3. Wu, S. H., Chiu, J. Y., Yu, M. C., Lwo, C. H., Chang, T. P., Chen, J. H. and Shih, W. C. 2019. Development and validation of analytical methods for the determination of mycotoxins and PAHs in foods. Commissioned Research Report of Taiwan Food and Drug Administration.