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Method of Test for Mycotoxins in Foods- Multimycotoxin Analysis

1. Scope

This method is applicable to the determination of 11 mycotoxins (aflatoxin B_1, etc. listed in the attached table) in cereals and their processed products.

2. Method

After extraction, mycotoxins are determined by liquid chromatography/tandem mass spectrometry (LC-MS/MS).

2.1. Equipment

2.1.1. Liquid chromatograph/tandem mass spectrometer.

2.1.1.1. Ion source: positive ion electrospray ionization, ESI^+.

2.1.1.2. Column: ACQUITY BEH C18, 1.7 μm, 2.1 mm i.d. × 10 cm, or an equivalent product.

2.1.2. Vortex mixer.

2.1.3. Centrifuge: centrifugal force ≥ 4300 ×g.

2.1.4. Nitrogen evaporator.

2.1.5. Shaker.

2.2. Chemicals

Potassium chloride, reagent grade;
Potassium dihydrogen phosphate, reagent grade;
Disodium hydrogen phosphate, reagent grade;
Sodium chloride, reagent grade;
Ammonium formate, reagent grade;
Sodium hydroxide, reagent grade;
Hydrochloric acid, reagent grade;
Formic acid, reagent grade;
Acetic acid, reagent grade;
Methanol, HPLC grade;
Acetonitrile, HPLC grade;
Deionized water, resistivity ≥ 18 MΩ • cm (at 25°C);
Aflatoxin B_1 and other mycotoxins listed in the attached table, reference standards.

2.3. Apparatus

2.3.1. Centrifuge tube: 50 mL, PP.

2.3.2. Volumetric flask: 10 mL and 100 mL.

2.3.3. Membrane filter: 0.22 μm, PTFE.

2.4. Reagents preparation

2.4.1. 0.1 N hydrochloric acid

Add 9 mL of hydrochloric acid slowly into 500 mL of deionized water, and dilute with deionized water to 1000 mL.

2.4.2. 0.1 N sodium hydroxide

Dissolve and dilute 4 g of sodium hydroxide with deionized water to 1000 mL.

2.4.3. Phosphate buffer

Dissolve 0.2 g of potassium chloride, 0.2 g of potassium dihydrogen phosphate, 2.92 g of disodium hydrogen phosphate and 8 g of sodium chloride with 900 mL of deionized water, adjust pH to 7.4 with 0.1 N hydrochloric acid or 0.1 N sodium hydroxide, and dilute with deionized water to 1000 mL.

2.4.4. 50% acetonitrile

Dilute 50 mL of acetonitrile with deionized water to 100 mL.

2.4.5. 20% acetonitrile

Dilute 20 mL of acetonitrile with deionized water to 100 mL.

2.4.6. Methanol containing 70% acetonitrile

Dilute 70 mL of acetonitrile with methanol to 100 mL.

2.5. Mobile phase

2.5.1. Solvent A

Dissolve and dilute 0.315 g of ammonium formate and 1 mL of formic acid with deionized water to 1000 mL, and filter with a membrane filter.

2.5.2. Solvent B

Dissolve and dilute 0.315 g of ammonium formate and 1 mL of formic acid with methanol to 1000 mL, and filter with a membrane filter.

2.6. Standard solution preparation

Transfer about 1 mg of aflatoxin B_1, aflatoxin B_2, aflatoxin G_1, aflatoxin G_2, deoxynivalenol, zearalenone, ochratoxin A, T-2 toxin and HT-2 toxin reference standards accurately weighed to each 10-mL volumetric flask, dissolve and dilute to volume with acetonitrile; transfer about 1 mg of fumonisin B_1 and fumonisin 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, and then store at -20°C. When to use, mix

appropriate volume of each standard stock solution, and dilute with acetonitrile to 0.005-0.3 μg/mL of aflatoxin B_1, aflatoxin B_2, aflatoxin G_1, aflatoxin G_2 and ochratoxin A, 0.01-0.6 μg/mL of T-2 toxin and HT-2 toxin, 0.05-3 μg/mL of deoxynivalenol and zearalenone, and 0.2-12 μg/mL of fumonisin B_1 and fumonisin B_2 as the standard solutions.

2.7. Sample solution preparation

Transfer about 5 g of the homogenized sample accurately weighed into a 50-mL centrifuge tube. Add 5 mL of phosphate buffer, and mix well. Add 20 mL of methanol containing 70% acetonitrile, shake for 30 min, and centrifuge at 4300 ×g for 5 min. Take 5 mL of the supernatant, and evaporate to dryness by gently flushing with a stream of nitrogen at 50°C. Dissolve the residue with 1 mL of 20% acetonitrile, and filter with a membrane filter. Take the filtrate as the sample solution.

2.8. Calibration standard curve preparation

Take a blank sample, add 500 μL of the standard solutions and 5 mL of phosphate buffer, and mix well. Add 19.5 mL of methanol containing 70% acetonitrile, and follow the procedure described in section 2.7 to obtain the calibration standard solutions. Operate LC-MS/MS according to the following conditions. Establish the calibration standard curve of each mycotoxin by peak areas vs. the added concentrations in the range as follows:

Analyte	Concentration range (ng/mL)
Aflatoxin B_1 Aflatoxin B_2 Aflatoxin G_1 Aflatoxin G_2 Ochratoxin A	0.5-30
T-2 toxin HT-2 toxin	1-60
Zearalenone Deoxynivalenol	5-300
Fumonisin B_1 Fumonisin B_2	20-1200

LC-MS/MS operating conditions(note)

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

Time (min)	A (%)	B (%)
0.0 → 5.5	95 → 15	5 → 85
5.5 → 5.8	15 → 0	85 → 100
5.8 → 6.9	0 → 0	100 → 100
6.9 → 7.0	0 → 95	100 → 5
7.0 → 9.0	95 → 95	5 → 5

Flow rate: 0.3 mL/min.

Injection volume: 10 μL.

Capillary voltage: 2.0 KV.

Ion source temperature: 150°C.

Ionization mode: ESI^+.

Desolvation temperature: 500°C.

Desolvation flow rate: 1000 L/hr.

Detection mode: multiple reaction monitoring (MRM). Detection ion pair, cone voltage and collision energy are shown in the attached table.

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

2.9. Identification and quantification

Accurately inject 10 μL of the sample solution and the calibration standard solutions into LC-MS/MS separately, and operate according to the conditions in section 2.8. Identify each mycotoxin based on the retention time and the relative ion intensities(note). Calculate the amount of each mycotoxin in the sample by the following formula:

$$\text{The amount of each mycotoxin in the sample (ppb)} = \frac{C \times V \times 5}{M}$$

Where,

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

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

M: the weight of the sample (g)

Note: Relative ion intensities are calculated by peak areas of qualitative ions divided by peak areas of quantitative ions (≤ 100%). Maximum

permitted tolerances for relative ion intensities are as follows:

Relative ion intensity (%)	Tolerance (%)
> 50	± 20
> 20-50	± 25
> 10-20	± 30
≤ 10	± 50

Remark

1. Limit of quantification (LOQ) for each mycotoxin is listed in the attached table.
2. Further validation should be performed when interfering compounds appear in samples.
3. This method is a multiple analysis for mycotoxins. If there is any doubt about the testing results, it should be subject to the prescribed testing method of individual mycotoxin.

Reference

Liao, C. D., Wong, J. W., Zhang, K., Hayward, D. G., Lee, N. S. and Trucksess, M. W. 2013. Multi-mycotoxin analysis of finished grain and nut products using high- performance liquid chromatography-triple-quadrupole mass spectrometry. J. Agric. Food Chem. 61: 4771-4782.

Table. MRM parameters and LOQs of 11 mycotoxins

No.	Analyte	Ion pair Precursor ion (*m/z*) > product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)	LOQ (ppb)
1	Aflatoxin B_1	313 > 241* 313 > 285	42 42	14 38	0.5
2	Aflatoxin B_2	315 > 287* 315 > 259	40 40	24 26	
3	Aflatoxin G_1	329 > 243* 329 > 200	42 42	28 28	
4	Aflatoxin G_2	331 > 245* 331 > 189	18 18	42 32	
5	Ochratoxin A	404 > 239* 404 > 102	24 24	26 72	
6	T-2 toxin	489 > 245* 489 > 327	40 40	26 24	1
7	HT-2 toxin	447 > 345* 447 > 285	32 32	20 20	
8	Deoxynivalenol	297 > 249* 297 > 203	20 20	12 14	5
9	Zearalenone	319 > 283* 319 > 185	18 18	19 24	
10	Fumonisin B_1	722 > 334* 722 > 352	48 48	44 40	20
11	Fumonisin B_2	706 > 336* 706 > 318	42 42	36 40	

* The quantitative ion.