SHIMADZU
Excellence in Science

Application News

No. AD-0188

Food Safety Analysis / LCMS-8060

Validation of a Low-Cost and Highly-Sensitive Method for Determination of Eighteen Mycotoxins in Food Matrices Using SPE and LC/MS/MS

Yin Ling Chew[1], Rui Bing Shannon Peck[2*], Muhammad Averus[3]; Suwiton[3]; Martin Uli Tua[3], Zhaoqi Zhan[1] and Jie Xing[1]
[1]Shimadzu (Asia Pacific), Singapore, [2] Department of Chemistry, Faculty of Science National University of Singapore,*Student, [3]PT Saraswanti Indo Genotech, Indonesia

❑ Introduction

Mycotoxins are metabolites produced by certain fungi in high humidity environment in food. Due to potentially mutagenic and carcinogenic, mycotoxins are monitored in food around the world strictly. Authorities such as European Union (EU) impose strict regulations on mycotoxins in food. To analyze trace levels of aflatoxins, immunoaffinity and SPE were developed and used for sample clean-up before analysis. However, there is not a single cartridge that can recover all the 18 monitoring mycotoxins and the cost of immunoaffinity cartridge is rather high. We developed and validated a SPE approach using ISOLUTE cartridge under two conditions to recover efficiently 6 polar and 12 less-polar mycotoxins for high sensitivity LC-MS/MS analysis to achieve the LOQs required by EU regulation.

❑ Experimental

Analytical conditions and sample preparation

The 18 mycotoxin standards were obtained from Supelco, Sigma Aldrich and Romer Labs. The SPE cartridges ISOLUTE® Myco – Biotage were purchased from Biotage. The 18 mycotoxin compounds were divided into two groups according to their polarities and were subjected to two different method preparations (Figure 1).

Method 1

1. Weigh 1 g of sample into centrifuge tube
2. Add 4 mL of 1% formic acid in water, vortex for 10 mins.
3. Centrifuge at 9000 rpm for 10 mins.
4. Transfer 1 mL of supernatant into 4 mL of water. Vortex for 10 mins. Repeat step
5. SPE
   - ➢Add 2 mL ACN
   - ➢Add 2 mL water
   - ➢Load 1 mL of supernatant
   - ➢Wash with 1 mL water
   - ➢Run dry
   - ➢Elute with 1 mL ACN Run dry
6. Blow dry with N2 gas and reconstitute with 1 mL 10% ACN

Method 2

1. Weigh 1 g of sample into centrifuge tube.
2. Add 4 mL of 0.1% formic acid in 1:1 water:ACN solution, vortex for 10 mins.
3. Centrifuge at 9000 rpm for 10 mins.
4. Transfer 1 mL of supernatant into 4 mL of water. Vortex for 10 mins. Repeat step 3.
5. SPE
   - ➢Add 2 mL ACN
   - ➢Add 2 mL water
   - ➢Load 4 mL of supernatant
   - ➢Wash with 2 mL water
   - ➢Wash with 2 mL 10% ACN
   - ➢Run dry
   - ➢Elute with 1 mL 1% FA in ACN
   - ➢Elute with 1 mL 1% FA in MeOH
   - ➢Run dry
6. Blow dry with N2 gas and reconstitute with 2 mL 10% ACN

Figure 1. Sample pre-treatment methods for 2 groups of mycotoxin compounds.

A LCMS-8060 triple quadrupole LC/MS/MS (Shimadzu Corporation, Japan) was used in this work. Shimadzu GLC Mastro™ PFP column (100 mm x 2.1 mm, 3µm) was used for fast separation of using a gradient elution program. The method development and performance evaluation were carried out using spiked mycotoxins in 5 different food matrices. Table 1 shows the analytical conditions on LCMS-8060.

Table 1. LC-MS/MS conditions

Column	Mastro PFP (100 mm x 2.1 mm; 3 µm)
Flow Rate	0.4 mL/min
Mobile Phase	A : 0.15mM ammonium fluoride in water B : 0.15mM ammonium fluoride in methanol with 2% acetic acid
Oven Temp.	40°C
Injection vol.	10 µL
Elution mode	**Gradient Elution**; B% : 15% (0.0 to 1.0 min) → 25% (1.0 min) → 40% (2.0 min) → 41% (4.5 min) → 100% (7.5 to 10.0 min) → 15% (10.1 to 12.5 min)

Interface	ESI
MS Mode	MRM, Positive & Negative
Block Temp.	400 °C
DL Temp.	250 °C
Interface Temp.	300 °C
CID gas	Ar (270 kPa)
Nebulizing Gas Flow	Nitrogen, 3.0 L/min
Drying Gas Flow	Nitrogen, 10 L/min
Heating Gas Flow	Zero air, 10 L/min

❑ Results & Discussion

Method Development

Automated MRM optimisation was carried out using LabSolutions workstation. Two transitions were obtained for each compound (see Table 2). 5 different food matrices (rice, barley, wheat flour, cashew and corn) were used for preparation of post-spiked calibrants. The MRM chromatogram of 18 mycotoxins spiked in barley matrices is shown in Figure 2.

Method Evaluation

Each calibrant was injected thrice and the average area was used to build the calibration curve in order to obtain reliable results. Good linearity with r2 greater than 0.998 over a concentration coverage of 0.01 – 500 ng/mL were achieved for all 18 mycotoxins in the agriculture product matrices. The LOD and LOQ of 18 mycotoxins were determined in the 5 matrices. Repeatability (n=6) was also performed for all 5 matrices with the mycotoxins at different concentrations (NIV, DON, FUS-X, NEO, 15-AcDON, and 3-AcDON at 2.5 ng/mL; AFB1 and AFG1 at 0.5 ng/mL; AFB2 and AFG2 at 0.15 ng/mL; DAS, FB1, FB2, FB3, HT-2, T-2, OA and ZON at 25 ng/mL). The % RSD ranges from 0.84 – 12.25%. The LOQ, LOD and % RSD results are reported in Table 3.

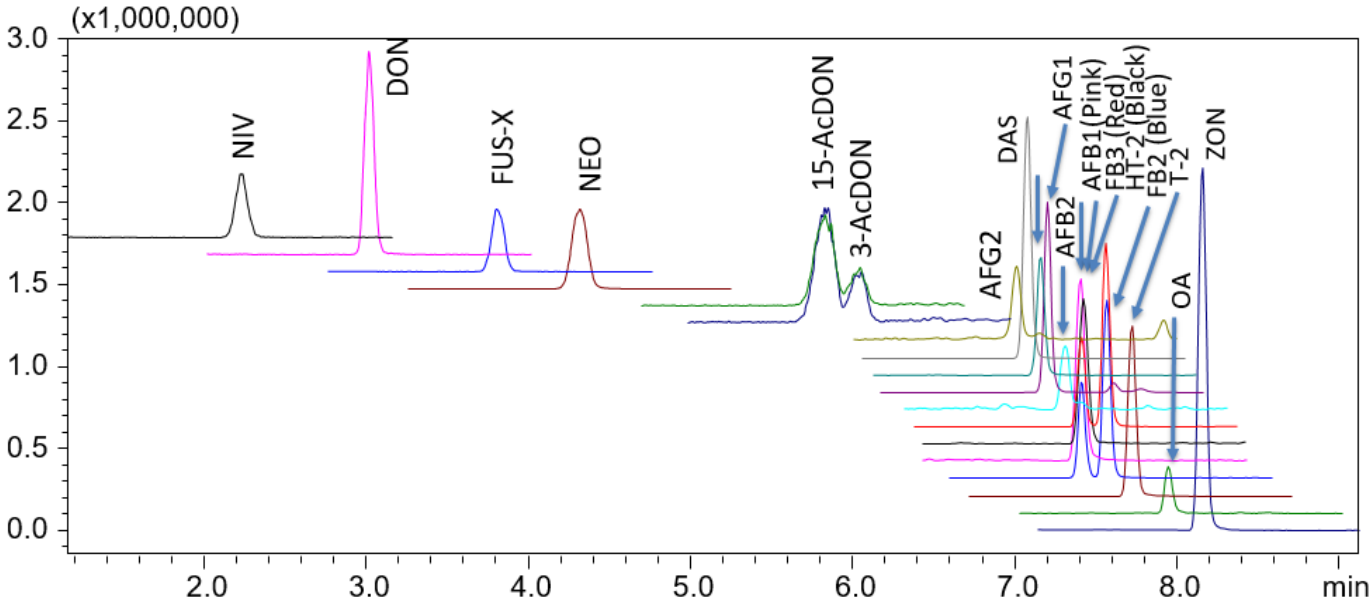

Figure 2. Chromatogram of 18 mycotoxins spiked in barley sample at different scale factors (NIV, DON, FUS-X, NEO, 15-AcDON, and 3-AcDON at 2.5 ng/mL; AFB1 and AFG1 at 0.5 ng/mL; AFB2 and AFG2 at 0.15 ng/mL; DAS, FB1, FB2, FB3, HT-2, T-2, OA and ZON at 25 ng/mL).

Table 2. MRM transitions of 18 mycotoxins

Compound	Retention Time (min)	Parent Ion	MRM 1	MRM 2
NIV	2.2	$[M\text{-}CH_3COO]^-$	371.1 > 281.2	371.1 > 311.2
DON	3.0	$[M+H]^+$	297.2 > 249.2	297.2 > 279.2
FUS-X	3.8	$[M+H]^+$	355.2 > 247.2	355.2 > 277.2
NEO	4.3	$[M+NH_4]^+$	400.2 > 305.2	400.2 > 215.2
15-AcDON	5.8	$[M+H]^+$	339.3 > 261.2	339.3 >297.2
3-AcDON	6.0	$[M+H]^+$	339.2 > 261.2	339.2 > 297.2
AFG2	7.0	$[M+H]^+$	331.2 > 245.1	331.2 > 285.1
DAS	7.1	$[M+NH_4]^+$	384.2 > 307.2	384.2 > 229.2
AFG1	7.2	$[M+H]^+$	329.1 > 243.1	329.1 > 200.1
FB1	7.2	$[M+H]^+$	722.4 >352.4	722.4 >334.3
AFB2	7.3	$[M+H]^+$	315.2 > 287.1	315.2 > 259.1
AFB1	7.4	$[M+H]^+$	313.1 > 285.1	313.1 > 241.1
FB3	7.4	$[M+H]^+$	706.4 > 318.3	706.40 > 354.4
HT-2	7.4	$[M+Na]^+$	447.3 > 345.2	447.3 > 285.1
FB2	7.6	$[M+H]^+$	706.4 > 318.3	706.4 > 354.4
T-2	7.7	$[M+NH_4]^+$	484.30 > 215.2	484.3 > 185.1
OA	7.9	$[M+H]^+$	404.2 > 221.0	404.2 > 239.1
ZON	8.2	$[M-H]^-$	317.1 > 175.1	317.1 > 131.2

Table 3. LOQ, LOD (both in ng/mL) and %RSD (n=6) of 18 mycotoxins spiked in different matrices at different concentrations (NIV, DON, FUS-X, NEO, 15-AcDON, and 3-AcDON at 2.5 ng/mL; AFB1 and AFG1 at 0.5 ng/mL; AFB2 and AFG2 at 0.15 ng/mL; DAS, FB1, FB2, FB3, HT-2, T-2, OA and ZON at 25 ng/mL.

Compound	Barley			Rice			Corn			Wheat Flour			Cashew		
	LOQ	LOD	%RSD	LOQ	LOD	%RSD	LOQ	LOD	%RSD	LOQ	LOD	%RSD	LOQ	LOD	%RSD
NIV	0.15	0.05	2.61	0.22	0.07	2.99	2.50	0.80	7.31	0.50	0.17	1.43	0.50	0.17	6.52
DON	0.25	0.08	1.64	0.05	0.02	3.69	0.50	0.17	6.18	0.25	0.08	3.74	2.50	0.80	0.84
FUS-X	0.06	0.02	2.43	0.10	0.03	2.38	0.09	0.03	7.49	0.25	0.08	1.66	1.57	0.52	5.96
NEO	0.01	<0.01	0.84	0.03	0.01	2.44	0.01	<0.01	7.46	0.01	<0.01	0.94	0.01	<0.01	5.43
15-AcDON	0.50	0.17	3.66	0.25	0.08	1.89	0.51	0.17	3.77	0.24	0.08	1.87	0.99	0.33	4.71
3-AcDON	0.44	0.15	3.25	0.50	0.17	1.29	0.63	0.21	3.56	0.27	0.09	3.74	2.50	0.80	3.09
AFG2	0.15	0.05	3.01	0.02	0.01	3.73	0.08	0.02	8.43	0.03	0.01	7.97	0.10	0.03	2.94
DAS	0.10	0.03	5.17	0.05	0.02	3.04	0.10	0.03	8.65	0.05	0.02	3.43	0.04	0.02	10.35
AFG1	0.01	<0.01	3.80	0.01	<0.01	2.40	0.04	0.01	5.27	0.01	<0.01	11.61	0.02	0.01	2.59
FB1	0.24	0.08	6.42	0.35	0.11	3.45	0.20	0.06	2.76	0.26	0.09	3.28	0.50	0.17	4.97
AFB2	0.03	0.01	5.77	0.01	<0.01	4.49	0.03	0.01	8.33	0.03	0.01	4.98	0.03	0.01	11.85
AFB1	0.01	<0.01	6.60	0.01	<0.01	3.85	0.02	0.07	5.31	0.02	0.01	12.25	0.03	0.01	7.37
HT-2	1.00	0.33	1.05	0.04	0.01	3.02	2.13	0.70	3.76	4.85	1.60	1.94	2.50	0.80	1.92
FB3	0.50	0.17	3.25	0.50	0.17	5.87	0.27	0.09	5.37	0.50	0.17	6.12	0.50	0.17	5.96
FB2	0.50	0.17	4.50	0.50	0.17	7.99	0.85	0.28	6.13	0.08	0.03	4.04	0.50	0.17	7.02
T-2	0.29	0.09	3.24	0.03	0.01	2.27	0.20	0.06	8.66	0.05	0.02	2.15	0.45	0.15	7.10
OA	0.05	0.02	6.79	0.08	0.03	2.43	0.31	0.10	10.12	0.05	0.02	11.65	0.05	0.02	3.41
ZON	0.10	0.03	3.25	0.05	0.02	4.26	0.22	0.07	8.27	0.10	0.03	2.66	0.10	0.03	3.09

Recovery

Recovery evaluation was performed on 5 matrices with different concentrations of the 18 mycotoxins (NIV, DON, FUS-X, NEO, 15-AcDON, 3-AcDON and HT-2 at 200 ng/g; AFB1, AFG1 and OA at 5 ng/g; AFB2 and AFG2 at 1.5 ng/g; FB1, FB2 and FB3 at 100 ng/g; DAS, T-2 and ZON at 20 ng/g). Each of the sample spiked with mycotoxins was injected three times and the average area was obtained to calculate the recovery results. Good recoveries of 65.9 – 143.0% except for NIV (59.7% for barley and 48.4% for wheat flour) and OA (254.6 – 312.7%) shown in Table 5.

Analysis of Real Samples

10 real samples were obtained from local supermarket and evaluated using the established method.

Out of the 10 samples, mycotoxins were detected in 2 samples, Barley J1 and Corn G9. The quantitation is tabulated in Table 4. MRM chromatograms of 2 samples displayed on Figure 3.

Table 4. Quantitation results of real samples

Compound	Concentration (ng/g)	
	Barley J1	Corn G9
FB1	46.4	178.0
FB2	44.6	85.0
FB3	-	17.4
AFB1	0.2	-
ZON	36.0	-

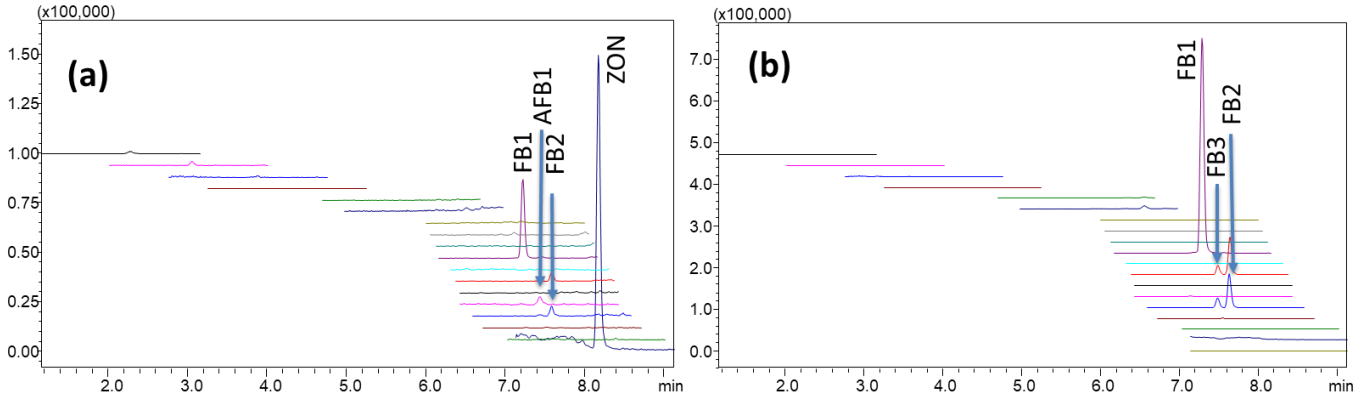

Figure 3. Chromatograms of real samples ; (a) Barley J1 and (b) Corn G9

Table 5. Recovery studies of 18 mycotoxins in 5 agricultural products

No	Compound	Recovery (%)				
		Barley	Rice	Corn	Wheat Flour	Cashew
1	**NIV**	59.7	71.2	70.3	48.4	66.6
2	**DON**	83.5	107.6	102.2	77.6	88.8
3	**FUS-X**	88.4	105.6	105.4	83.3	96.0
4	**NEO**	95.4	114.7	113.2	90.7	100.6
5	**15-AcDON**	86.2	117.7	103.5	86.1	88.9
6	**3-AcDON**	94.8	111.8	106.7	87.4	92.6
7	**AFG2**	75.7	70.2	80.8	70.1	82.0
8	**DAS**	67.1	78.6	102.6	77.0	92.0
9	**AFG1**	82.8	73.2	76.8	67.6	97.6
10	**FB1**	104.6	109.0	68.0	143.0	117.9
11	**AFB2**	84.9	78.3	80.3	71.2	70.0
12	**AFB1**	81.4	74.0	77.3	65.9	84.3
13	**HT-2**	101.5	114.5	101.3	93.7	112.7
14	**FB3**	93.7	111.1	86.7	125.2	90.9
15	**FB2**	100.7	84.0	88.9	125.5	77.2
16	**T-2**	82.7	97.7	118.9	85.9	109.0
17	**OA**	254.6	282.5	312.7	307.8	279.1
18	**ZON**	84.6	93.9	108.7	88.2	61.0

❑ Conclusion

A LCMS/MS method coupled with solid phase extraction (SPE) method has been established for 18 mycotoxins regulated under European Union EC 1881/2006. Good recoveries were obtained for the mycotoxins spiked in 5 different agriculture product matrices. Good linearity with r^2 greater than 0.998 over a concentration range of 0.01 – 500 ng/mL were achieved. The LOQ, LOD and repeatabilities of the 18 mycotoxins in different matrices were reported.

❑ References

1. COMMISSION REGULATION (EC) No 1881/2006 on setting maximum levels for certain contaminants in foodstuffs (2006) OJ L 364/5; http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R1881&from=EN
2. D. Baker, C. Titman, N. Loftus and J. Horner, Multi-residue analysis of 18 regulated mycotoxins by LC-MS/MS; ASMS 2017, Poster Session TP 185

SHIMADZU (Asia Pacific) Pte. Ltd
79 Science Park Drive, #02-01/08 Cintech IV, Singapore 118264,
www.shimadzu.com.sg; Tel: +65-6778 6280 Fax: +65-6778 2050