Development and validation of a UHPLC-ToF-MS for determination of multi-mycotoxins in maize

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INTRODUCTION

The main goal of this study was to develop and validate a multi-mycotoxin UHPLC-ToF-MS method to determine aflatoxins (AFB1, AFB2, AFG1 and AFG2), ochratoxin A (OTA), zearalenone (ZEA), toxin T2 (T2) and fumonisins (FB1 and FB2) in maize (Fig. 1).

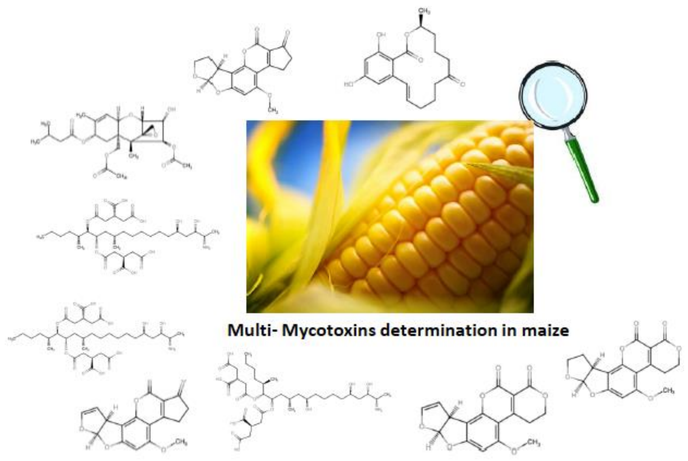

Figure 1. Mycotoxins determined in the present study.

MATERIAL AND METHODS

Extraction

About 2 g of maize flour was extracted with 2 x 10 mL of acetonitrile 80% (v/v) for 1 h at 110 rpm using a shaker. After centrifugation at 3000 rpm for 10 min, the supernatants were collected.

For analysis of fumonisins (Method 1- M1): 1 mL of the extract was diluted with ultra-pure water, filtered and injected into the UHPLC-ToF-MS system.

For the analysis of the other mycotoxins (Method 2- M2): 8 mL of the extract was evaporated to dryness under a gentle stream of nitrogen at 40 °C. The residue was redissolved with 1 mL of acetonitrile 40% (v/v), vortexed for 30 s, filtered and injected into the UHPLC-Tof-MS system.

UHPLC-Tof-MS

Equipment: Nexera X2 Shimadzu UHPLC coupled with a 5600+ ToF-MS detector (SCIEX, Foster City, CA) equipped with a Turbo Ion Spray electrospray ionization source working in positive mode (ESI+); column: Zorbax Eclipse Plus C18 (2.1 x 50 mm, 1.8 µm) at 30 °C; injection volume: 20 µL; mobile phase: 0.1% formic acid and acetonitrile with a flow rate of 0.5 mL/min and with a gradient program; acquisition was performed in full-scan from 100 to 750 Da; software: Analyst® TF (SCIEX, Foster City, CA); ion source voltage: 5500 V; source temperature: 575 °C; curtain gas (CUR): 30 psi; Gas 1 and Gas 2: 55 psi; declustering potential (DP): 100 V.

RESULTS AND DISCUSSION

Table 1. Results of recovery, repeatability and reproducibility at different spiking levels in blank maize samples (n=6, at each spiking level).

Mycotoxin	Ion	Retention time (min)	Spiked level (µg/kg)	Rec. (%)	RSD_r (%)	RSD_R (%)
AFB1	313.07066 [M+H]+	5.01	1.0	92.4	9.33	9.85
			1.5	95.4	2.50	
			2.0	96.9	6.34	5.94
			3.0	103.5	2.08	
			4.0	105.1	3.71	4.73
			8.0	98.7	1.14	
AFB2	315.08631 [M+H]+	4.53	1.0	85.2	6.97	
			2.0	97.1	8.74	9.25
			3.0	95.9	2.57	
			4.0	97.0	5.06	5.06
			6.0	105.3	2.81	
			8.0	104.6	2.85	2.49
			16.0	98.5	2.36	
AFG1	329.06558 [M+H]+	4.53	1.0	110.4	1.54	
			2.0	103.4	8.80	6.10
			3.0	98.4	3.64	
			4.0	93.7	3.13	4.23
			6.0	102.4	3.13	
			8.0	100.6	1.17	3.61
			16.0	79.8	1.84	
AFG2	331.08123 [M+H]+	4.05	2.0	73.4	3.38	15.87
			3.0	86.2	9.85	
			4.0	96.4	8.01	10.28
			6.0	108.8	7.04	
			8.0	106.3	6.16	8.74
			16.0	98.2	5.92	
FB1	722.39575 [M+H]+	5.34	125.0	95.8	4.0	6.84
			250.0	104.2	3.3	
			500.0	100.5	2.1	
			750.0	97.9	2.4	
			1000.0	99.8	1.1	5.16
			1500.0	101.3	1.9	
			2000.0	99.6	3.5	7.21
			4000.0	82.3	2.0	
FB2	706.40081 [M+H]+	6.47	125.0	87.1	6.3	14.0
			250.0	104.8	3.7	
			500.0	100.0	2.3	
			750.0	98.4	3.3	
			1000.0	101.8	1.2	9.2
			1500.0	100.9	1.3	
			2000.0	99.3	1.6	11.5
			4000.0	94.1	4.5	
OTA	404.08954 [M+H]+	7.95	1.50	82.9	15.4	14.5
			2.25	91.2	9.3	
			3.00	92.4	6.9	9.59
			4.50	109.6	2.0	
			6.00	109.5	3.1	5.03
			12.0	97.3	4.4	
T2	489.2095 $[M+Na]^+$	7.20	25.0	99.3	5.33	
			50.0	100.5	15.4	
			100.0	105.5	8.67	15.1
			150.0	101.0	7.02	
			200.0	98.3	9.13	8.77
			300.0	95.1	12.3	
			400.0	102.7	8.55	14.3
ZEA	319.154 [M+H]+	7.82	50.0	101.6	7.99	
			100.0	103.7	5.51	8.5
			150.0	98.5	6.56	
			200.0	100.5	5.23	3.8

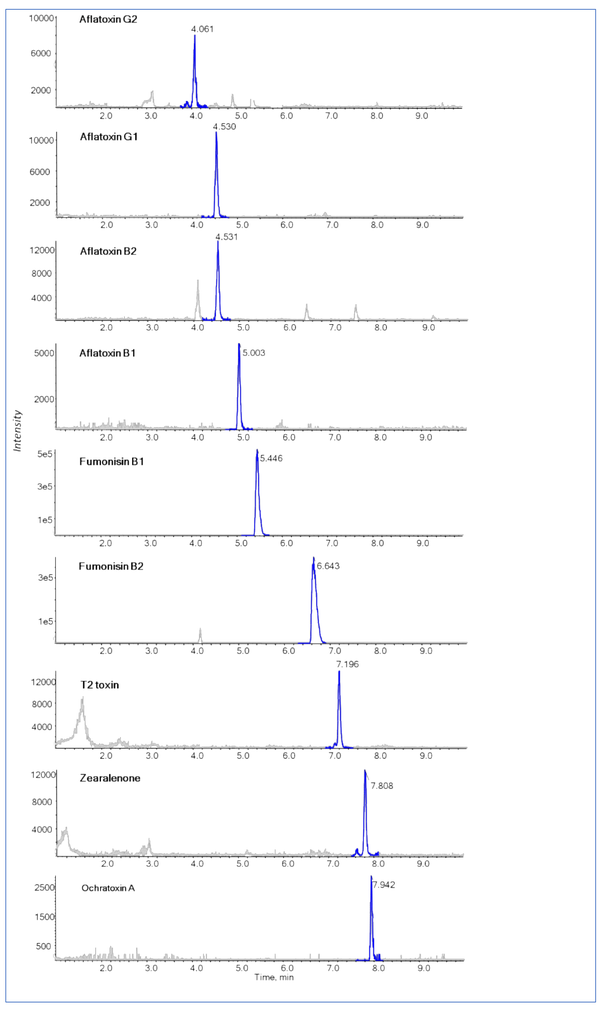

Figure 2. Chromatogram of a blank maize sample spiked with 2 µg/kg of AFB1, 4 µg/kg of AFB2, AFG1 and AFG2, 3 µg/kg of OTA, 1000 µg/kg of FB1 and FB2, 200 µg/kg of ZEA and T2.

Table 2. Linearity and sensitivity of UHPLC-ToF-MS method for the simultaneous determination of nine mycotoxins.

Mycotoxin	Linear range (µg/kg)	Calibration curve parameters			LoD (µg/kg)	LoQ (µg/kg)
		a	b	r^2		
AFB1	1.0-8.0	9093.4	579.45	0.9961	0.5	1
AFB2	1.0-16	10665	2747.5	0.9962	0.5	1
AFG1	1.0-16	10402	- 3099.2	0.9947	0.5	1
AFG2	1.0-16	2543.4	2967	0.9789	1	2
FB1	125-2000	279.72	- 4748.2	0.9978	62.5	125
		216.56	72007	0.9834		
FB2	125-4000	252.78	- 3297.7	0.9988	62.5	125
OTA	1.5-12	2405.6	1393.4	0.9851	0.75	1.5
T2	25-400	256.5	-426.2	0.9786	10	25
ZEA	50-400	207.42	-579.11	0.9928	25	50
	100-800	173.79	6426.6	0.9876		

The identification and data processing was made through the PeakView™ and MultiQuant™ (SCIEX, Foster City, CA) softwares (Figure 2).

In terms of identification criteria three parameters were used: maximum relative retention time deviation (ΔRRT) of 2.5%; difference in the isotope pattern with a tolerance of 10% and exact mass deviation (Δm) with a tolerance of 5 ppm.

Validation of the method revealed it meets the criteria of performance of analytical methods established by Commission Regulation EC no. 401/2006[1] (Tables 1-2).

FB1 and FB2 were detected in maize samples collected in September-October 2018 but any of the samples exceeded EU maximum permitted levels for maize[2]. All the samples were negative for the other mycotoxins under study.

CONCLUSIONS

The analytical UHPLC-ToF-MS method developed and validated in maize is an excellent tool to monitor the levels of mycotoxins in this cereal and its application was demonstrated in real samples

References

1. Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Off J Eur Union. 2006; L70:12–34.
2. EC. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off J Eur Union. 2006; L 364:5–24.

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