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Application News

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Liquid Chromatography Mass Spectrometry

Multi-Residue Analysis of 18 Regulated Mycotoxins by LC/MS/MS

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Mycotoxins are one of the most important contaminants in food and feed due to their widespread distribution in the environment and toxic effects on humans and animals.[1)] Structurally, mycotoxins are a very diverse group with a wide range of physicochemical properties and low molecular weights.[2)] They are produced by fungi (mould) frequently found on agricultural produce, and are often not visible to the naked eye.[3)] Some of the most commonly contaminated food stuffs include wheat, oats, rye, corn, barley, rice, nuts and milk.[4)]

Due to the risks posed by mycotoxins in food they are regulated globally, including, the EU, US, China, Singapore and Brazil.[5)] In the EU, reporting limits are harmonised in Regulation (EC) No 1886/2006 (amended by (EC) No 1126/2007) and sampling and analysis in Regulation (EC) No 401/2006.

LC/MS/MS is the technique most commonly employed for mycotoxin quantitation in order to achieve the necessary low reporting limits in complex food and feed matrices.

Experimental

Solvent extracts were provided by Scientific Analysis Laboratories (SAL, UK) following validated extraction protocols. Samples were analysed using the Nexera UHPLC and the LCMS-8060 triple quadrupole detector (Table 1) . Calibration was performed using ^{13}C internal standards spiked during sample extraction. All MRM transitions and associated internal standards for each compound are listed in Table 2. All solvents used during analysis were LCMS quality from Sigma-Aldrich.

Due to the wide range of physicaland chemical properties of mycotoxins, different LC/MS/MS methods are typically developed for small groups of compounds with similar properties.

In this application paper a single LC/MS/MS method has been developed for the determination of 18 mycotoxins in food safety. Limits of quantification were at or below the maximum levels set in the EC/1886/2006 document. The scope of the method included Aflatoxins (B1, B2, G1, G2), Fumonisins (B1, B2, B3), Ochratoxin A (OTA) and Trichothecenes (3-acetyldeoxynivalenol (3AcDON), 15-acetyldeoxynivalenol (15AcDON), Deoxynivalenol (DON), Diaceteoxyscripanol (DAS), Fusarenon-X (FUS X), HT-2, Neosolaninol (NEO), Nivalenol (NIV), T2, Zearalenone (ZON)) with an analysis cycle time of 12.5 minutes.

Table 1 Analytical Conditions

UHPLC	: Nexera LC System
Mobile Phase	: A; Water with additives B; Methanol with additives
Column	: Reversed phase column (100 mm L.× 2.1 mm I.D.)
Column Temperature	: 40 °C
Flowrate	: 0.4 mL/minute
Gradient	: B. Conc 15 % (0 min) → 25 % (1 min) → 40 % (2 min) → 41 % (4.5 min) → 100 % (7.5 - 10.0 min) → 15 % (10.10 min) → Stop (12.5 min)
LC-MS/MS	: LCMS-8060
Dwell Time	: 10 to 40 msec.
Pause Time	: 1 msec.
Ionisation Mode	: ESI +/-
Polarity Switching	: 5 msec.

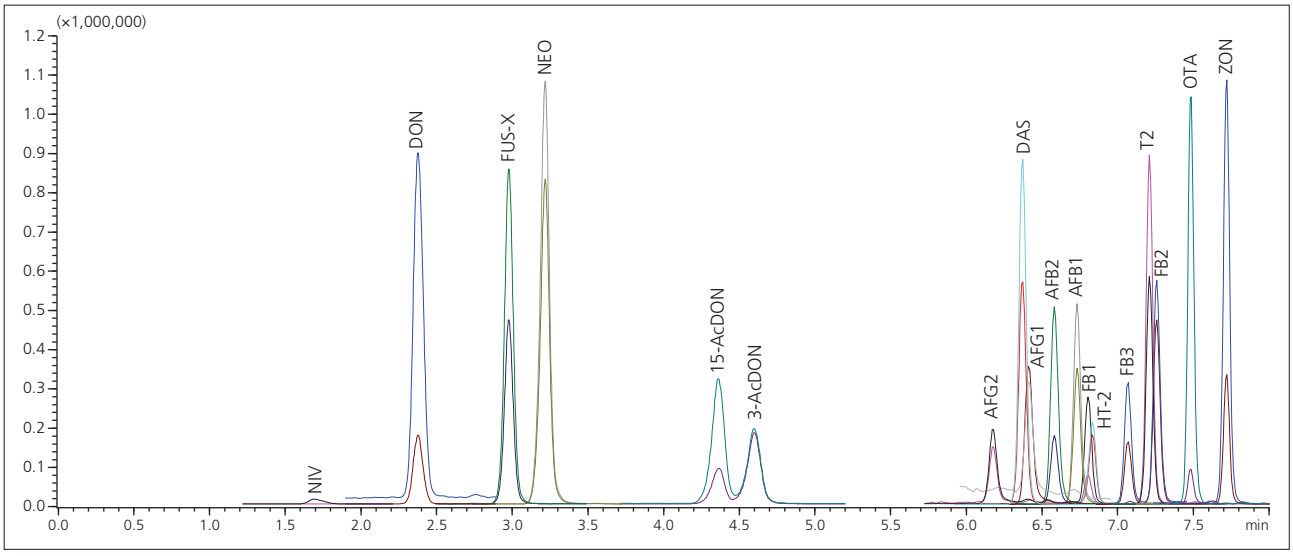

Fig. 1 MRM Chromatograms of 18 Mycotoxins

AFB1 (aflatoxin B1; 1 µg/kg), AFB2 (aflatoxin B2; 1 µg/kg), AFG1 (aflatoxin G1; 1 µg/kg), AFG2 (aflatoxin G2; 1 µg/kg), OTA (ochratoxin A; 4 µg/kg), FB1 (fumonisin B1; 100 µg/kg), FB2 (fumonisin B2; 100 µg/kg), FB3 (fumonisin B3; 100 µg/kg), 15-AcDON (15-acetyldeoxynivalenol; 100 µg/kg), 3-AcDON (3-acetyldeoxynivalenol; 100 µg/kg), DON (deoxynivalenol; 100 µg/kg), DAS (diaceteoxyscripanol; 100 µg/kg), FUS-X (fusarenon-X; 100 µg/kg), HT-2 (100 µg/kg), T-2 (100 µg/kg), NEO (neosolaninol; 100 µg/kg), NIV (nivalenol; 100 µg/kg), ZON (zearalenone; 100 µg/kg).
For clarity only 2 MRM transitions are displayed per compound and the following MRM chromatograms were changed; neosolaniol (x0.3), T2 (x0.3), aflatoxins (x3), fumonisins (x2).

Table 2 All MRM's Measured in the Mycotoxin Method and Corresponding Calibration Range and R^2 Result

	Compound name	Parent ion	Ret. Time (mins)	MRM 1	MRM 2	MRM 3	ISTD	Calibration range µg/kg	R^2
1	Aflatoxin B1	$[M+H]^+$	6.773	313 > 241	313 > 285	313 > 269	^{13}C Aflatoxin B1	0.1 - 10	0.9988
2	Aflatoxin B2	$[M+H]^+$	6.621	315 > 259	315 > 287	315 > 243	^{13}C Aflatoxin B2	0.1 - 10	0.9995
3	Aflatoxin G1	$[M+H]^+$	6.453	329 > 243	329 > 200		^{13}C Aflatoxin G1	0.1 - 10	0.9998
4	Aflatoxin G2	$[M+H]^+$	6.219	331 > 245	331 > 285		^{13}C Aflatoxin G2	0.1 - 10	0.9965
5	Ochratoxin A	$[M+H]^+$	7.509	404 > 239	404 > 221	404 > 358	^{13}C Ochratoxin A	0.4 - 40	0.9969
6	Fumonisin B1	$[M+H]^+$	6.811	722 > 352	722 > 334	722 > 704	^{13}C Aflatoxin B2	10 - 1000	0.9937
7	Fumonisin B2	$[M+H]^+$	7.260	706 > 318	706 > 354	706 > 688	^{13}C Aflatoxin B2	10 - 1000	0.9998
8	Fumonisin B3	$[M+H]^+$	7.073	706 > 318	706 > 354	706 > 688	^{13}C Aflatoxin B2	10 - 1000	0.9991
9	Deoxynivalenol	$[M+H]^+$	2.372	297 > 279	297 > 249		^{13}C Deoxynivalenol	10 - 1000	0.9992
10	Diacetoxyscirpenol	$[M+NH_4]^+$	6.349	384 > 229	384 > 307	384 > 247	^{13}C T2 Toxin	10 - 1000	0.9994
11	T2	$[M+NH_4]^+$	7.206	484 > 185	484 > 215	484 > 245	^{13}C T2 Toxin	10 - 1000	0.9989
12	HT-2	$[M+Na]^+$	6.822	447 > 345	447 > 285		^{13}C T2 Toxin	10 - 1000	1.0000
13	Nivalenol	$[M-CH_3COO]^-$	1.684	371 > 281	371 > 311		^{13}C HT-2	10 - 1000	0.9991
14	Neosolaniol	$[M+NH_4]^+$	3.227	400 > 215	400 > 305	400 > 185	^{13}C Deoxynivalenol	10 - 1000	0.9995
15	Fusarenon X	$[M+H]^+$	2.986	355 > 247	355 > 277		^{13}C Deoxynivalenol	10 - 1000	0.9987
16	Zearalenone	$[M-H]^-$	7.711	317 > 175	317 > 131	317 > 273	^{13}C T2 Toxin	10 - 1000	0.9985
17	15-Acetyldeoxynivalenol	$[M+H]^+$	4.406	339 > 261	339 > 297		^{13}C Deoxynivalenol	10 - 1000	1.0000
18	3-Acetyldeoxynivalenol	$[M+H]^+$	4.618	339 > 261	339 > 297		^{13}C Deoxynivalenol	10 - 1000	0.9986
19	^{13}C HT-2	$[M+NH_4]^+$	6.844	464 > 278					
20	^{13}C T2	$[M+NH_4]^+$	7.228	508 > 322					
21	^{13}C Aflatoxin B1	$[M+H]^+$	6.754	330 > 301					
22	^{13}C Aflatoxin B2	$[M+H]^+$	6.614	332 > 303					
23	^{13}C Aflatoxin G1	$[M+H]^+$	6.435	346 > 212					
24	^{13}C Aflatoxin G2	$[M+H]^+$	6.219	348 > 259					
25	^{13}C Ochratoxin A	$[M+H]^+$	7.516	424 > 250					

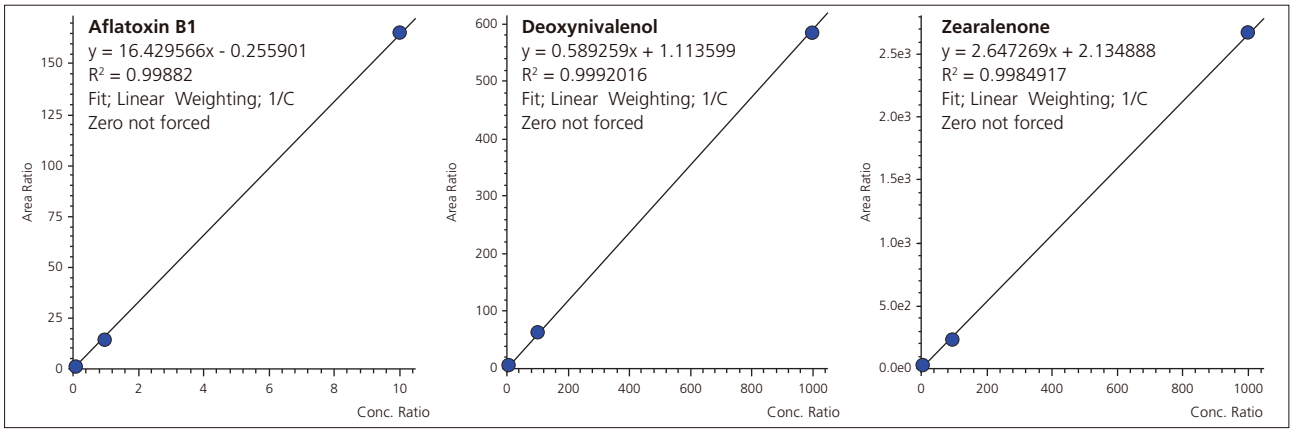

Fig. 2 Calibration Curves for Selected Compounds
Calibration Curves for Aflatoxin (0.1 – 10 µg/kg), Deoxynivalenol (10 – 1000 µg/kg), and Zearalenone (10 – 1000 µg/kg).

Conclusions

In this study a single method has been developed for the analysis of 18 regulated mycotoxins with an injection to injection cycle time of 12.5 minutes. This method achieves the required EU reporting limits (between 0.1 -10 µg/kg) with linear regression coefficients R^2 typically greater than 0.998 (Fig. 2 and Table 1). The LC mobile phase, column and gradient were all optimised and provided chromatographic resolution of 15-acetyldeoxynivalenol and 3-acetyldeoxynivalenol.

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