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Application News

No. C165

LC-MS/MS

Multi-residue analysis of 18 regulated mycotoxins by LC-MS/MS (2)

Fusarium mycotoxins are a structurally diverse group of secondary metabolites known to contaminate a diverse array of food and feed resulting in a risk for human and animal health. European guidance legislation has set maximum levels for mycotoxins in food and feed to minimize the impact to human and animal health. The most toxicologically important Fusarium mycotoxins are trichothecenes (including deoxynivalenol (DON) and T-2 toxin (T-2)), zearalenone (ZON) and fumonisin B1 (FB1).

In this work, 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 (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON), deoxynivalenol (DON), diasteoxyscripanol (DAS), fusarenon-X (FUS X), HT-2, neosolaninol (NEO), nivalenol (NIV), T2, zeareleonone (ZON)) with an analysis cycle time of 12.5 minutes.

Materials and Methods

Solvent extracts were provided by Concept Life Sciences following validated extraction protocols. Samples were measured using a Nexera UHPLC and the LCMS-8060 triple quadrupole detector (Table 1). To separate out the three pairs of regioisomers (3-AcDON/15-AcDON, FB2/FB3, and FA2/FA3) a pentafluorophenyl (PFP) column was used and compared against a C18 material. To enhance signal response a series of mobile phase additives were considered including ammonium acetate, ammonium fluoride, ammonium formate and acetic acid solutions.

In this work, ammonium fluoride solution and ammonium fluoride with acetic acid solution was the preferred solvent system as it resulted in a considerable enhancement of signal intensity in positive ion mode for all mycotoxins. Calibration was performed using ^{13}C internal standards spiked during sample extraction. All solvents used during analysis were LCMS quality from Sigma-Aldrich.

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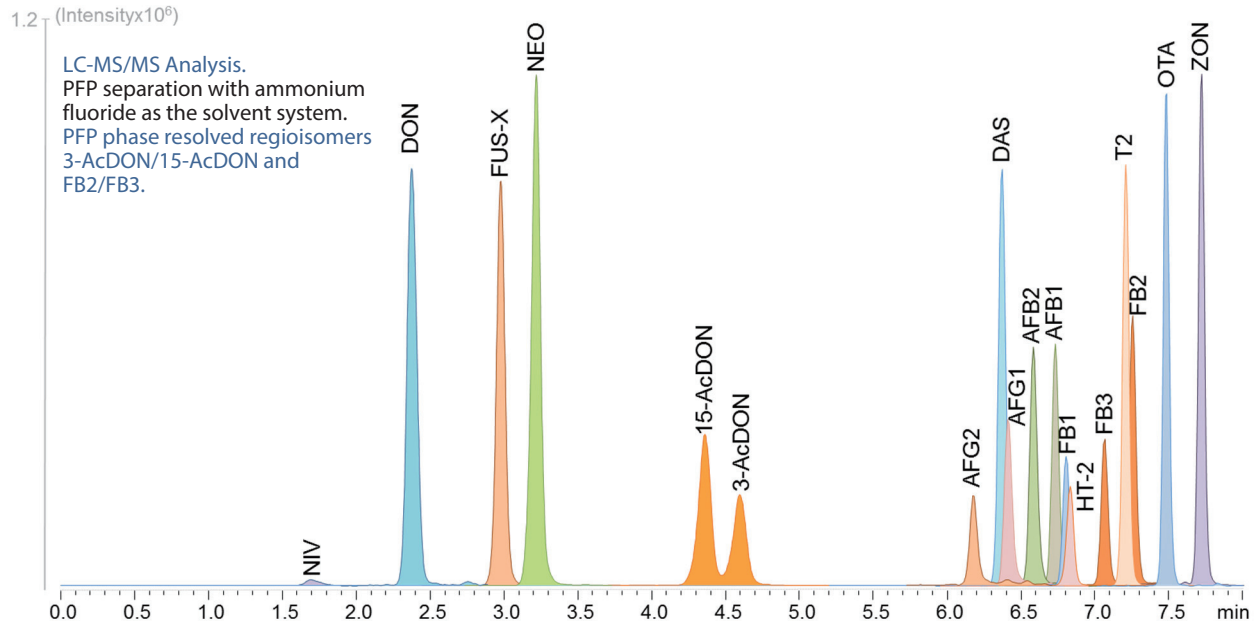

Fig. 1 MRM chromatograms of 18 mycotoxins using a PFP bonded phase.
AFB1 (aflatoxin B1; 1 µg/kg; rescaled x3), AFB2 (aflatoxin B2; 1 µg/kg; rescaled x3), AFG1 (aflatoxin G1; 1 µg/kg; rescaled x3), AFG2 (aflatoxin G2; 1 µg/kg; rescaled x3), OTA (ochratoxin A; 4 µg/kg), FB1 (fumonisin B1; 100 µg/kg; rescaled x2), FB2 (fumonisin B2; 100 µg/kg; rescaled x2), FB3 (fumonisin B3; 100 µg/kg; rescaled x2), 15-AcDON (15-acetyldeoxynivalenol; 100 µg/kg), 3-AcDON (3-acetyldeoxynivalenol; 100 µg/kg), DON (deoxynivalenol; 100 µg/kg), DAS (diasteoxyscripanol; 100 µg/kg), FUS-X (fusarenon-X; 100 µg/kg), HT-2 (100 µg/kg), T-2 (100 µg/kg; rescaled x0.3), NEO (neosolaniol; 100 µg/kg; rescaled x0.3), NIV (nivalenol; 100 µg/kg), ZON (zearalenone; 100 µg/kg)

Influence of ammonium fluoride on ion signal intensity

Ammonium fluoride solution has a high gas-phase basicity and known to be effective in improving sensitivity for small molecules in negative mode LC-MS. However, ammonium fluoride has also been shown to enhance sensitivity in positive ion mode. Compared to standard mobile phases used for mycotoxin analysis the addition of ammonium fluoride has a positive impact on ion signal intensity.

Fig. 2 indicates that ammonium fluoride markedly increases ion signal intensity compared to other solvent systems. All chromatograms are normalized to the same signal intensity. Ammonium fluoride delivered higher ion signal response for mycotoxins in positive ion mode compared to other mobile phase solvent system (Fig. 2a).

Table 1 Analytical Conditions

UHPLC	Nexera X2 LC system
Analytical column	Mastro PFP (100 mmL. × 2.1 mm I.D., 3 µm)
Column temperature	40 °C
Flow rate	0.4 mL/min
Solvent A	0.15 mmol/L ammonium fluoride aqueous solution
Solvent B	0.15 mM ammonium fluoride methanol solution with 2 % acetic acid
Binary Gradient	B conc. 15 % (0 min) - 25 % (1 min) - 40 % (2 min) - 41 % (4.5 min) - 100 % (7.5 - 10 min) - 15 % (10.1 min) – Stop (12.5 min)

Mass spectrometer	Shimadzu LCMS-8060
Pause time/Dwell time	1 msec/10-40 msec
Polarity switching time	Pos/neg switching time set to 5 msec
Source temperatures (interface; heat block; DL)	300 °C; 400 °C; 250 °C
Gas flows (nebulising; heating; drying)	3 L/min; 10 L/min; 10 L/min

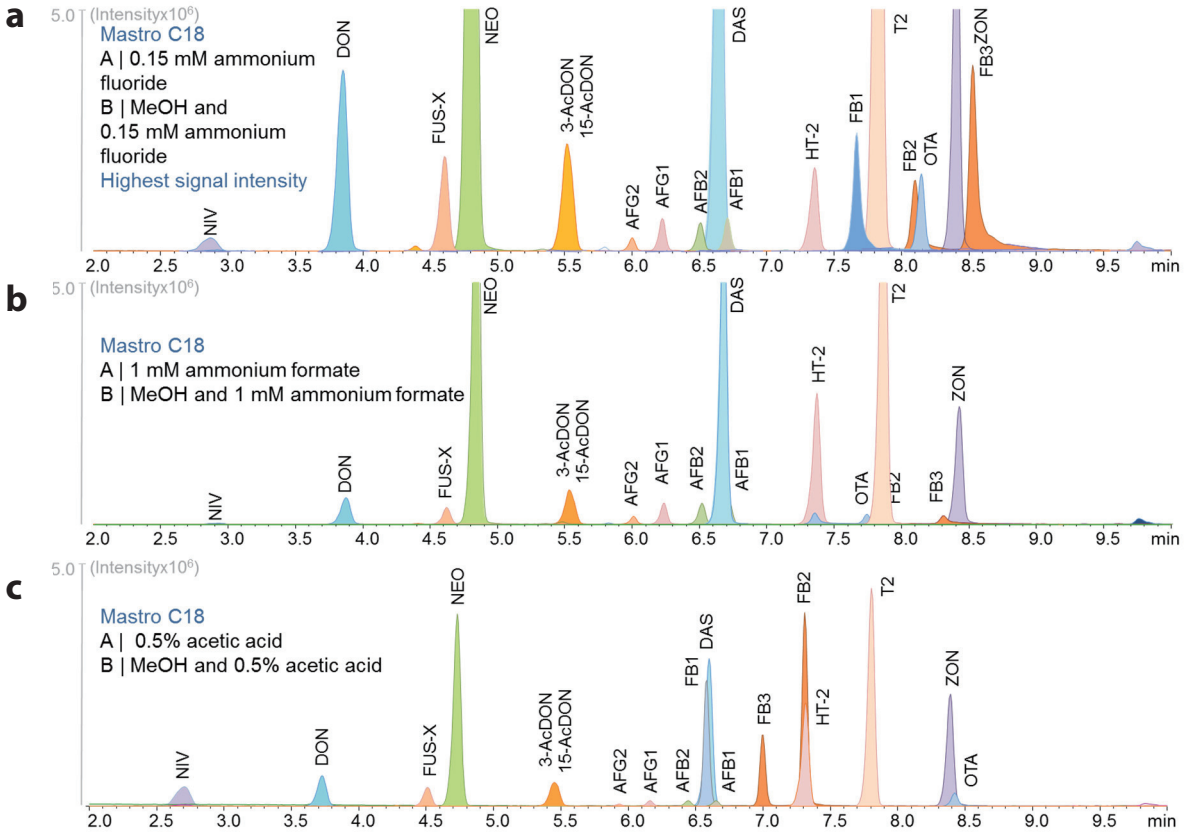

Fig. 2 Comparison of MRM Chromatograms of 18 Mycotoxins under the Different Mobile Phase Conditions (Mastro C18 Column)
a: Mobile Phase A = 0.15 mM Ammonium Fluoride Aqueous Solution, Mobile Phase B = 0.15 mM Ammonium Fluoride Methanol Solution
b: Mobile Phase A = 1 mM Ammonium Formate Aqueous Solution, Mobile Phase B = 1 mM Ammonium Formate Methanol Solution
c: Mobile Phase A = 0.5 % Acetic Acid Aqueous Solution, Mobile Phase B = 0.5 % Acetic Acid Methanol Solution

Fig. 3 shows 18 mycotoxins separated on a PFP phase compared to a C18 bonded material using ammonium fluoride as the mobile phase. PFP phases delivered near baseline resolution of 3- and 15-acetyldeoxynivalenol which is not possible on a C18 phase (C18 material can still be used due to preferential ionisation of 3-AcDON in negative ion mode).

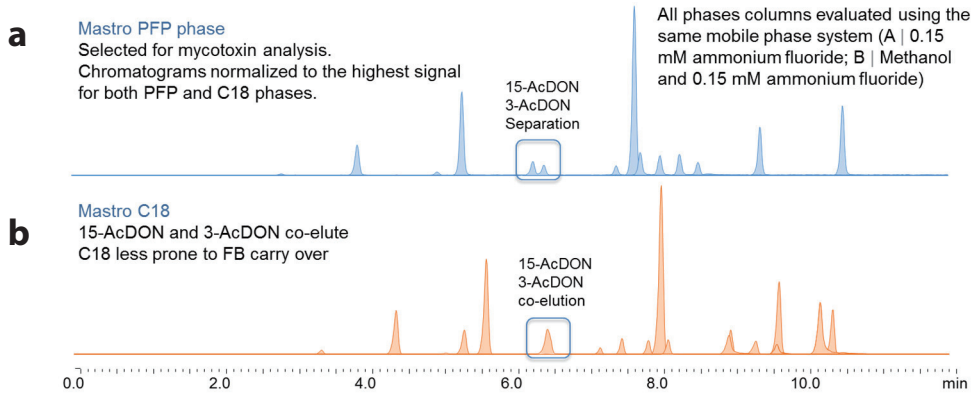

Fig. 3 Comparison of MRM Chromatograms of 18 Mycotoxins Using Different Columns
Mobile Phase A = 0.15 mM Ammonium Fluoride Aqueous Solution,
Mobile Phase B = 0.15 mM Ammonium Fluoride Methanol Solution (for Both Columns)
a: Mastro PFP Column, b: Mastro C18 Column

Analysis of sample matrices

To separate the regioisomers 3-AcDON/15-AcDON and FB2/FB3 several PFP phases were evaluated including Mastro PFP, Kinetix PFP, Discovery HS F5 PFP and ACE PFP. Compared to a C18 bonded phase, the PFP phases delivered near baseline resolution of the regioisomers 3-AcDON/15-AcDON and FB2/FB3 but required a modification of the mobile phase to reduce FB carry over (2 % acetic acid was added to the mobile phase to negate the effects of FB's carry over).

Fig. 4 shows the analysis of a mixed spice extract and a pepper extract spiked with Aflatoxins B1, B2, G1, G2 (2.5 µg/kg) and Ochratoxin A (10 µg/kg) using ammonium fluoride solution in the mobile phase. Repeatedly injecting the extracts resulted in a %RSD typically below 10 % (n=12) for Aflatoxins B1, B2, G1, G2 (2.5 µg/kg) and Ochratoxin A (10 µg/kg).

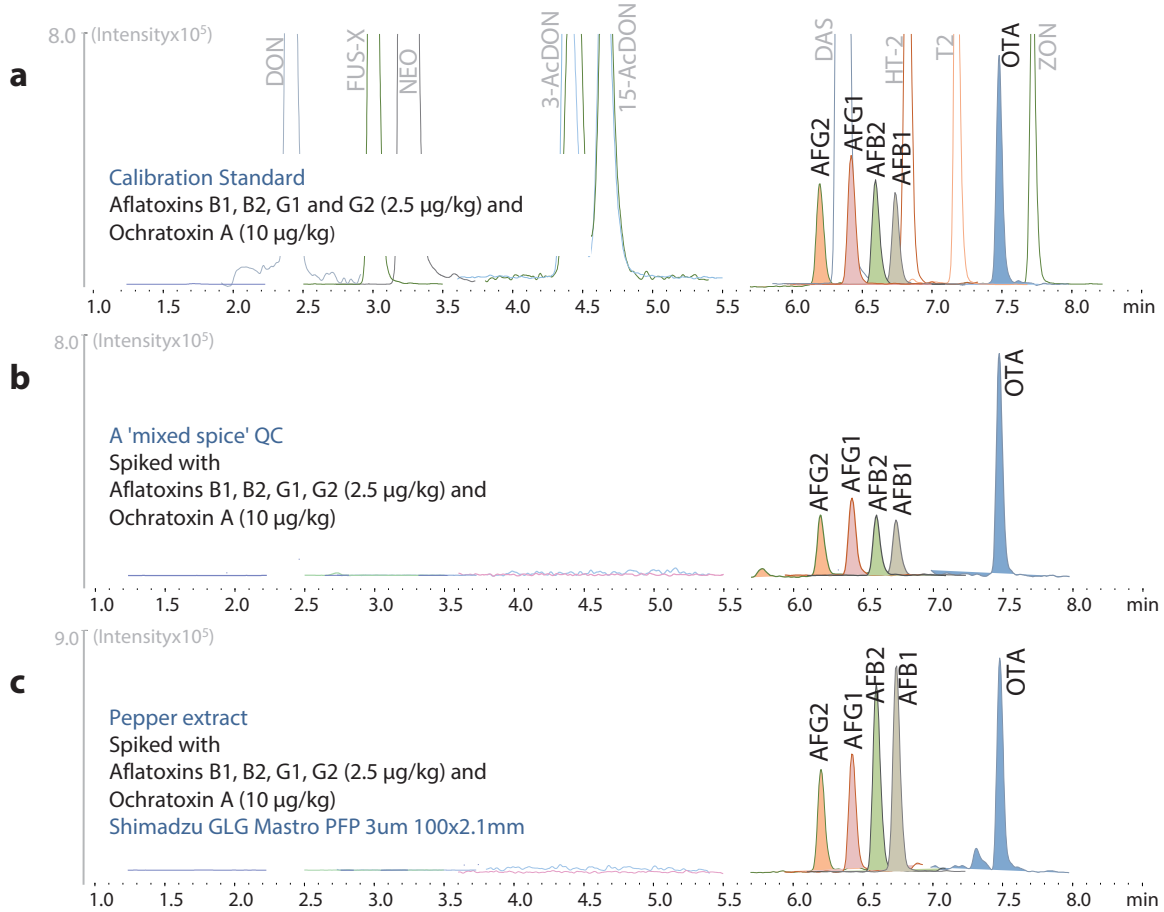

Fig. 4 Chromatograms of the Mycotoxin Standard Solution, Mixed Spice Extract, and Pepper Extract Spiked with Aflatoxins B1, B2, G1, G2 (2.5 µg/kg) and Ochratoxin A (10 µg/kg)
a: Mycotoxin Standard Solution, b: Mixed Spice Extract, c: Pepper Extract

Table 2 MRM's of mycotoxins in positive and negative mode ionisation.

Compound name	Parent ion	RT	MRM 1	MRM 2	Internal Standard	Calibration range (µg/kg)	R^2
Aflatoxin B1	$[M+H]^+$	6.773	313 > 241	313 > 285	^{13}C Aflatoxin B1	0.1 - 10	0.9988
Aflatoxin B2	$[M+H]^+$	6.621	315 > 259	315 > 287	^{13}C Aflatoxin B2	0.1 - 10	0.9995
Aflatoxin G1	$[M+H]^+$	6.453	329 > 243	329 > 200	^{13}C Aflatoxin G1	0.1 - 10	0.9998
Aflatoxin G2	$[M+H]^+$	6.219	331 > 245	331 > 285	^{13}C Aflatoxin G2	0.1 - 10	0.9965
Ochratoxin A	$[M+H]^+$	7.509	404 > 239	404 > 221	^{13}C Ochratoxin A	0.4 - 40	0.9969
Fumonisin B1	$[M+H]^+$	6.811	722 > 352	722 > 334	^{13}C Aflatoxin B2	10 - 1000	0.9937
Fumonisin B2	$[M+H]^+$	7.26	706 > 318	706 > 354	^{13}C Aflatoxin B2	10 - 1000	0.9998
Fumonisin B3	$[M+H]^+$	7.073	706 > 318	706 > 354	^{13}C Aflatoxin B2	10 - 1000	0.9991
Deoxynivalenol	$[M+H]^+$	2.372	297 > 279	297 > 249	^{13}C Deoxynivalenol	10 - 1000	0.9992
Diacetoxyscirpenol	$[M+NH_4]^+$	6.349	384 > 229	384 > 307	^{13}C T-2 Toxin	10 - 1000	0.9994
T-2	$[M+NH_4]^+$	7.206	484 > 185	484 > 215	^{13}C T-2 Toxin	10 - 1000	0.9989
HT-2	$[M+Na]^+$	6.822	447 > 345	447 > 285	^{13}C T-2 Toxin	10 - 1000	1.0000
Nivalenol	$[M+CH_3COO]^-$	1.684	371 > 281	371 > 311	^{13}C HT-2	10 - 1000	0.9991
Neosolaniol	$[M+NH_4]^+$	3.227	400 > 215	400 > 305	^{13}C Deoxynivalenol	10 - 1000	0.9995
Fusarenon X	$[M+H]^+$	2.986	355 > 247	355 > 277	^{13}C Deoxynivalenol	10 - 1000	0.9987
Zearalenone	$[M-H]^-$	7.711	317 > 175	317 > 131	^{13}C T2 Toxin	10 - 1000	0.9985
15-Acetyldeoxynivalenol	$[M+H]^+$	4.406	339 > 261	339 > 297	^{13}C Deoxynivalenol	10 - 1000	1.0000
3-Acetyldeoxynivalenol	$[M+H]^+$	4.618	339 > 261	339 > 297	^{13}C Deoxynivalenol	10 - 1000	0.9986
^{13}C HT-2	$[M+NH_4]^+$	6.844	464 > 278				
^{13}C T-2	$[M+NH_4]^+$	7.228	508 > 322				
^{13}C Aflatoxin B1	$[M+H]^+$	6.754	330 > 301				
^{13}C Aflatoxin B2	$[M+H]^+$	6.614	332 > 303				
^{13}C Aflatoxin G1	$[M+H]^+$	6.435	346 > 212				
^{13}C Aflatoxin G2	$[M+H]^+$	6.219	348 > 259				
^{13}C Ochratoxin A	$[M+H]^+$	7.516	424 > 250				

Conclusions

Ammonium fluoride as a solvent system results in a higher signal response for mycotoxins in positive ion detection.

To negate any possible carry over effects with fumonisin's 2 % acetic acid was added to the mobile phase.

PFP bonded phases deliver a separation of mycotoxin regioisomers which can be applied routinely.

This method results in higher sensitivity for mycotoxins and can be applied to both PFP and C18 phases in routine quantitation with a cycle time of 12.5 minutes.

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