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

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David Baker[1], Christopher Titman[1], Neil Loftus[1], Jonathan Horner[2]
[1]Shimadzu, Manchester, UK
[2]Concept Life Sciences, Cambridge, UK

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Introduction

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

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-ADON/15-ADON, 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 C13 internal standards spiked during sample extraction. All solvents used during analysis were LCMS quality from Sigma-Aldrich.

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Table 1. LC and MS/MS acquisition parameters used to create the LC-MS/MS method.

Liquid chromatography	
UHPLC	: Nexera LC system
Analytical column	: Mastro PFP (100 x 2.1, 3µm)
Column temperature / Flow rate	: 40°C ; 0.4mL/minute
Solvent A	: 0.15mM ammonium fluoride solution
Solvent B	: 0.15mM ammonium fluoride 2% acetic acid solution
Binary Gradient	:

Time (mins)	%B
0.00	15
1.00	25
2.00	40
4.50	41
7.50	100
10.00	100
10.10	15
12.50	Stop

Mass spectrometry	
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)	: 3L/min; 10 L/min; 10L/min

Results

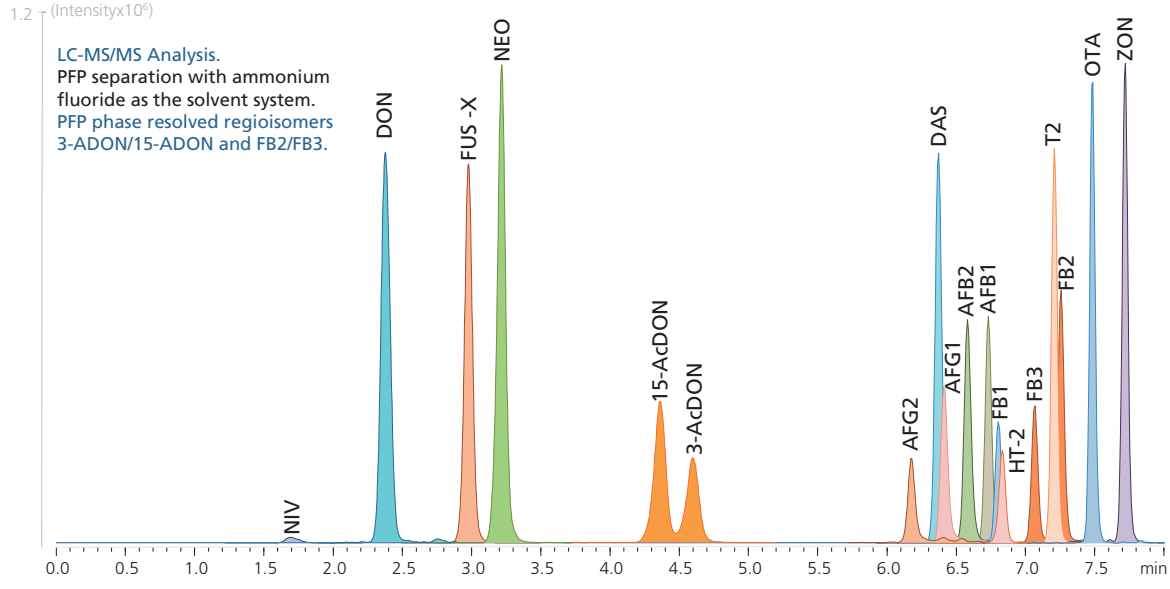

Figure 1. MRM chromatograms of 18 mycotoxins using a PFP bonded phase.

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AFB1 (aflatoxin B1; 1µg/kg; rescaled x3), AFB2 (aflatoxin B2; 1µg/kg; rescaled x3), AFG1 (aflatoxin; 1µg/kg; rescaled x3), AFG2 (aflatoxin G2; 1µg/kg; rescaled x3), OTA (ochratoxin A; 4µg/kg), FB1 (fumonisin B1; 100µg/kg; rescaled x2), FB2 (fumonisin B2; 100µg/kg; rescaled x2), FB3 (fumonisin B3; 100µg/kg; rescaled x2), 15-AcDON (15-acetyldeoxynivalenol; 100µg/kg), 3-AcDON (3-acetyldeoxynivalenol; 100µg/kg), DON (deoxynivalenol; 100µg/kg), DAS (diasteoxyscripanol; 100µg/kg), FUS-X (fusarenon-X; 100µg/kg), HT-2 (100µg/kg), T-2 (100µg/kg; rescaled x0.3), NEO (neosolaninol; 100µg/kg; rescaled x0.3), NIV (nivalenol; 100µg/kg), ZON (zearelenone; 100µg/kg).

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

Compound name	Parent ion	RT (mins)	MRM 1	MRM 2	Internal Standard	Calibration range µg/kg	R2
Aflatoxin B1	[M+H]+	6.773	313 > 241	313 > 285	C13 Aflatoxin B1	0.1 - 10	0.9988
Aflatoxin B2	[M+H]+	6.621	315 > 259	315 > 287	C13 Aflatoxin B2	0.1 - 10	0.9995
Aflatoxin G1	[M+H]+	6.453	329 > 243	329 > 200	C13 Aflatoxin G1	0.1 - 10	0.9998
Aflatoxin G2	[M+H]+	6.219	331 > 245	331 > 285	C13 Aflatoxin G2	0.1 - 10	0.9965
Ochratoxin A	[M+H]+	7.509	404 > 239	404 > 221	C13 Ochratoxin A	0.4 - 40	0.9969
Fumonisin B1	[M+H]+	6.811	722 > 352	722 > 334	C13 Aflatoxin B2	10 - 1000	0.9937
Fumonisin B2	[M+H]+	7.260	706 > 318	706 > 354	C13 Aflatoxin B2	10 - 1000	0.9998
Fumonisin B3	[M+H]+	7.073	706 > 318	706 > 354	C13 Aflatoxin B2	10 - 1000	0.9991
Deoxynivalenol	[M+H]+	2.372	297 > 279	297 > 249	C13 Deoxynivalenol	10 - 1000	0.9992
Diacetoxyscirpenol	[M+NH4]+	6.349	384 > 229	384 > 307	C13 T2 Toxin	10 - 1000	0.9994
T2	[M+NH4]+	7.206	484 > 185	484 > 215	C13 T2 Toxin	10 - 1000	0.9989
HT-2	[M+Na]+	6.822	447 > 345	447 > 285	C13 T2 Toxin	10 - 1000	1.0000
Nivalenol	[M-CH3COO]-	1.684	371 > 281	371 > 311	C13 HT-2	10 - 1000	0.9991
Neosolaniol	[M+NH4]+	3.227	400 > 215	400 > 305	C13 Deoxynivalenol	10 - 1000	0.9995
Fusarenon X	[M+H]+	2.986	355 > 247	355 > 277	C13 Deoxynivalenol	10 - 1000	0.9987
Zearelenone	[M-H]-	7.711	317 > 175	317 > 131	C13 T2 Toxin	10 - 1000	0.9985
15-Acetyldeoxynivalenol	[M+H]+	4.406	339 > 261	339 > 297	C13 Deoxynivalenol	10 - 1000	1.0000
3-Acetyldeoxynivalenol	[M+H]+	4.618	339 > 261	339 > 297	C13 Deoxynivalenol	10 - 1000	0.9986
C13 HT-2	[M+NH4]+	6.844	464 > 278				
C13 T2	[M+NH4]+	7.228	508 > 322				
C13 Aflatoxin B1	[M+H]+	6.754	330 > 301				
C13 Aflatoxin B2	[M+H]+	6.614	332 > 303				
C13 Aflatoxin G1	[M+H]+	6.435	346 > 212				
C13 Aflatoxin G2	[M+H]+	6.219	348 > 259				
C13 Ochratoxin A	[M+H]+	7.516	424 > 250				

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.

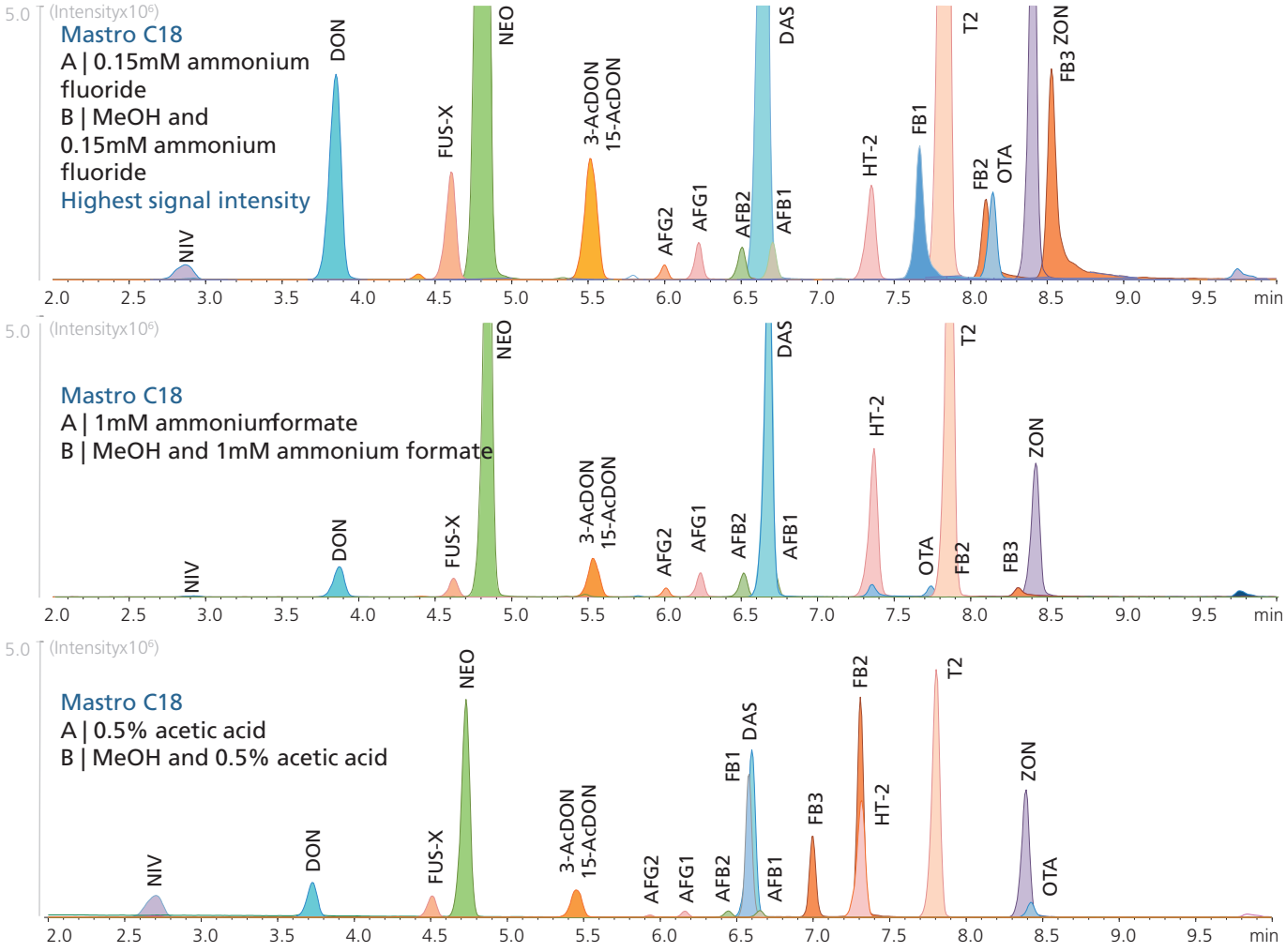

Figure 2. Ammonium fluoride markedly increases ion signal intensity compared to other solvent systems. All chromatograms are normalized to the same signal intensity. Ammonium fluoride delivered higher ion signal response for mycotoxins in positive ion mode compared to other mobile phase solvent systems.

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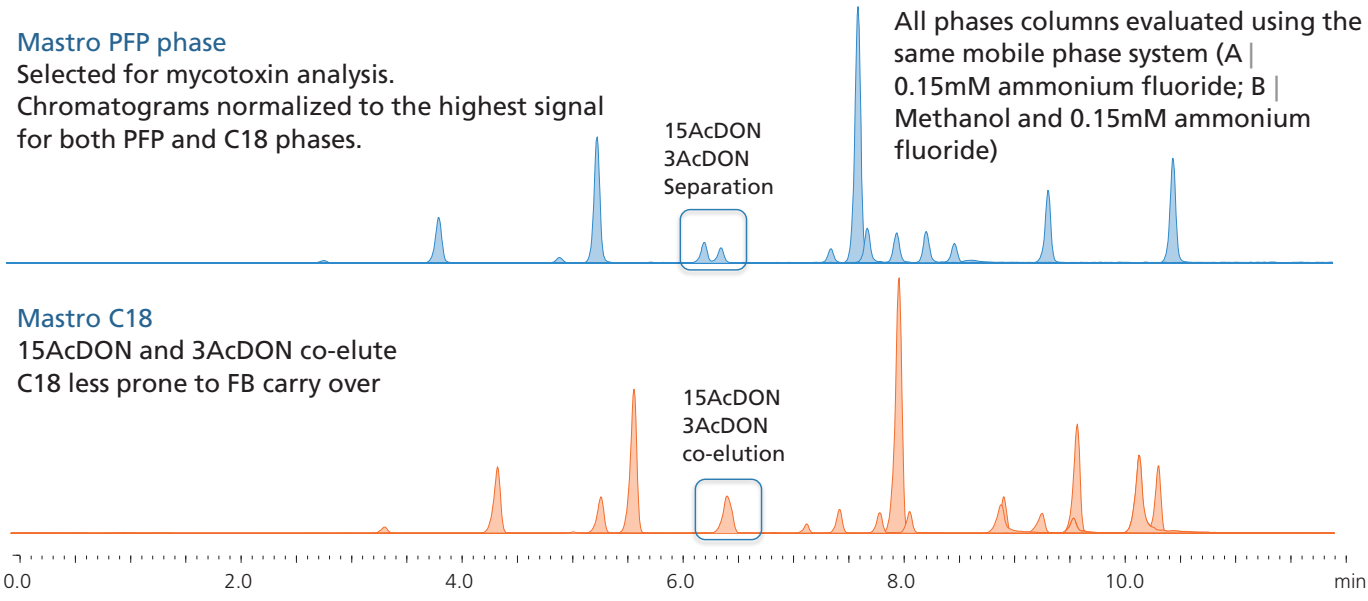

Figure 3. 18 Mycotoxins separated on a PFP phase compared to a C18 bonded material using ammonium fluoride as the mobile phase. PFP phases delivered near baseline resolution of 3- and 15 acetyldeoxynivalenol which is not possible on a C18 phase (C18 material can still be used due to preferential ionisation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

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Analysis of sample matrices

To separate the regioisomers 3-ADON/15-ADON 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-ADON/15-ADON 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).

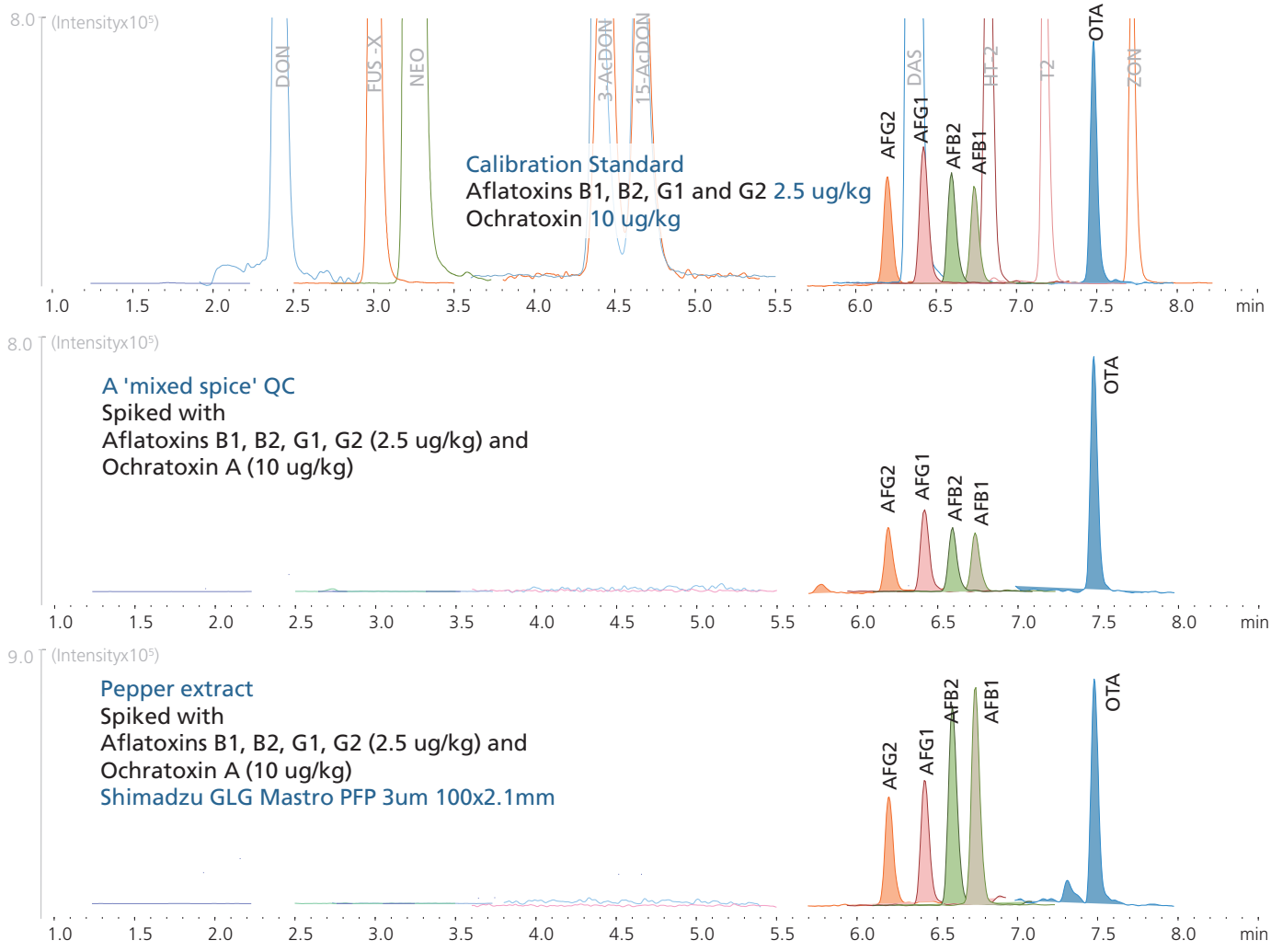

Figure 4. Analysis of a mixed spice extract and a pepper extract spiked with Aflatoxins B1, B2, G1, G2 (2.5 ug/kg) and Ochratoxin A (10 ug/kg) using ammonium fluoride solution in the mobile phase. Repeatedly injecting the extracts resulted in a %RSD typically below 10% (n=12) for Aflatoxins B1, B2, G1,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

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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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