ACQUITY UPLC/MS/MS FOR MULTI-MYCOTOXIN ANALYSIS

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INTRODUCTION

Many agricultural crops are susceptible to colonisation by moulds and fungi. Stress during plant growth or poor post-harvest storage conditions allow fungal species to infect a variety of commodities, often leading to unacceptable taste, odour or appearance. It is also possible for some fungal infestations to produce toxic secondary metabolites that have the potential to contaminate both animal feed and food intended for human consumption. These secondary metabolites are known generally as mycotoxins.

It is possible for foodstuffs to be contaminated with a range of mycotoxins from more than one class. The consumption of mycotoxins can have long-term adverse effects on health, so both human foodstuffs and animal feed must be routinely monitored for their presence. These compounds are typically analysed by immunoaffinity sample preparation followed by single compound or class specific analytical techniques[1]. A single analytical method, able to target a variety of mycotoxin classes in a range of agricultural produce, would enable a more efficient use of laboratory resources.

The use of HPLC coupled to tandem quadrupole mass spectrometry for multi-mycotoxin analysis has been reported previously[2]. Using ultra performance liquid chromatography (UPLC) it is possible to expand the method whilst significantly reducing the analysis time.

This note describes an extended multi-mycotoxin method, for 25 contaminants in a variety of sample types, which is able to meet the requirements for analysis of regulated compounds but which also includes a range of other compounds of concern. The method uses a simple, generic sample preparation followed by ACQUITY UPLC separation and detection with a Quattro Premier XE tandem quadrupole mass spectrometer.

METHODS

Sample Preparation

- 25 g of ground sample is mixed with 100 ml 80:20 acetonitrile/water for 2 hours
- Extracts are filtered and diluted 4 fold with water
- 20 µL of extract is injected for LC/MS/MS analysis

Chromatographic Method

Column: Acquity UPLC BEH C_{18} 1.7 µm; 2,1 x 100 mm
Mobile Phase A: 0.1% formic acid in water
Mobile Phase B: 0.1% formic acid in acetonitrile
Flow Rate: 0.4 mL/min

The LC gradient program is shown in table 1.

Time (min)	%A	%B
Initial	90	10
3	90	10
10	30	70
10.1	10	90
12	10	90
12.1	90	10
15	90	10

Table 1.

Total runtime, including column regeneration time, was 15 minutes.

MS Method

The eluent from the UPLC column was directed into the electrospray source of a Quattro Premier XE tandem quadrupole mass spectrometer operated in positive ionisation, multiple reaction monitoring (MRM) mode. Table 2 shows the two MRM transitions monitored for each compound. The monitoring of two transitions allows the presence of a mycotoxin contaminant to be confirmed.

	Parent Ion (m/z)	Product Ion (m/z)	Cone Voltage (V)	Collision Voltage (V)
Aflatoxin B1	313	241	50	37
	313	285	50	23
Aflatoxin B2	315	259	50	30
	315	287	50	26
Aflatoxin G1	329	243	40	25
	329	283	40	25
Aflatoxin G2	331	245	50	30
	331	257	50	30
Ochratoxin A	404	239	25	22
	406	241	25	22
Deoxynivalenol	297	249	20	10
	297	231	20	13
Fumonisin B1	722	334	50	40
Fumonisin B2	706	336	50	40
	706	318	50	40
Nivalenol	313	295	13	8
	313	175	13	20
Diacetoxyscirpe-nol	367	307	15	10
	367	289	15	10
T2 Toxin	467	305	10	9
	467	245	10	9
HT2 Toxin	425	263	15	12
	425	105	15	40
3-acetyl-DON	339	231	20	12
	339	213	20	12
15-acetyl-DON	339	231	20	12
	339	279	20	10
Zearalenone	319	187	20	19
	319	185	20	23
Penicillic acid	171	125	18	12
	171	153	18	7
Fusarenon X	355	247	15	13
	355	229	15	15
Ergotamine	582	268	30	27
	582	208	30	42
Roquefortin	390	193	30	28
	390	322	30	19
β-Zearalanone	323	305	15	7
	323	277	15	15
α-Zearalanone	323	305	15	7
	323	277	15	15
Citrinin	251	205	28	28
	251	191	28	24
Zearalanone	321	303	18	13
	321	285	18	13
Cyclopiazonic acid	337	196	20	26
	337	182	20	20
Sterigmatocystin	325	281	50	36
	325	253	50	39

Table 2.

RESULTS

Figure 1 shows the chromatogram obtained from this multi-mycotoxin method, with nivalenol eluting first, at a retention time of 1.1 minutes, and cyclopiazonic acid eluting last, at a retention time of 9.3 minutes. Peak widths range from approximately 7 seconds wide at base for some early eluting components to approximately 4.5 seconds wide at base for some better retained ones.

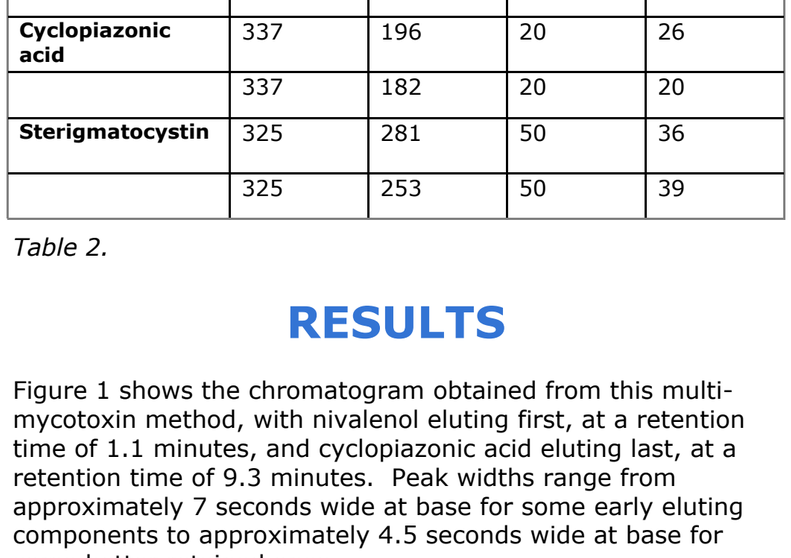

Figure 1.

The method was validated for pistachio nuts and figures 2, 3, 4 and 5 show calibration curves obtained for four of the analytes. The red lines are obtained from a matrix matched set of calibration standards and the blue lines are obtained from a set of solvent standards. Figure 5 shows the highest level of matrix suppression obtained for any of the analytes in this method; the signal for deoxynivalenol is suppressed by approximately 34% in the pistachio matrix.

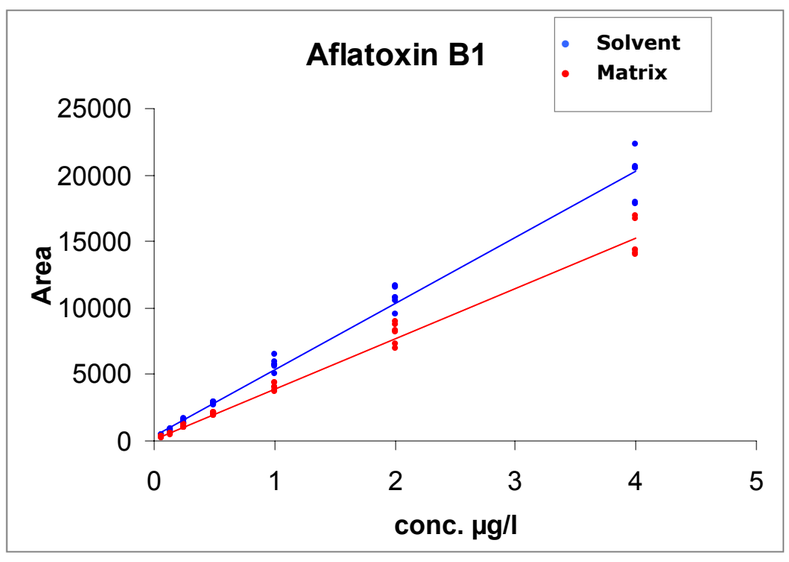

Figure 2.

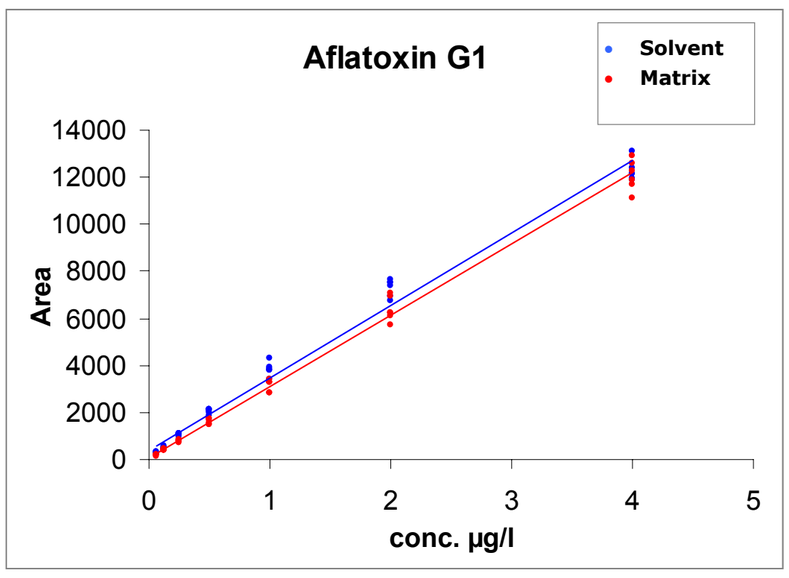

Figure 3.

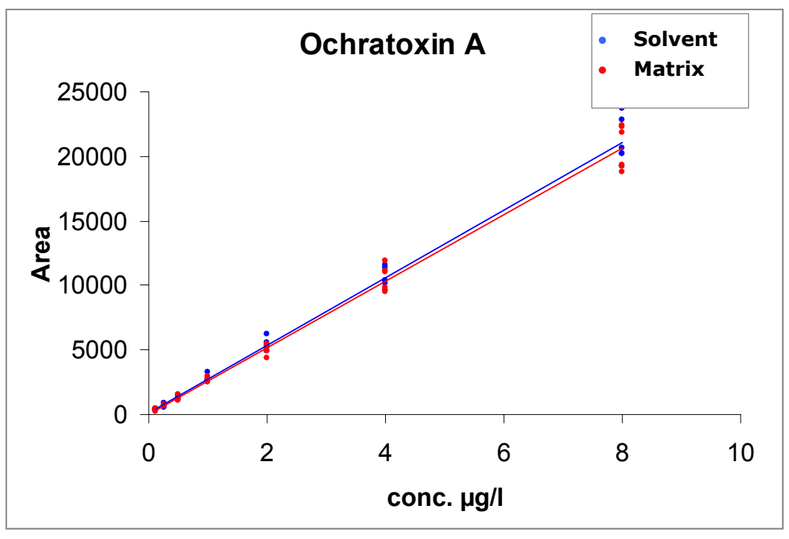

Figure 4.

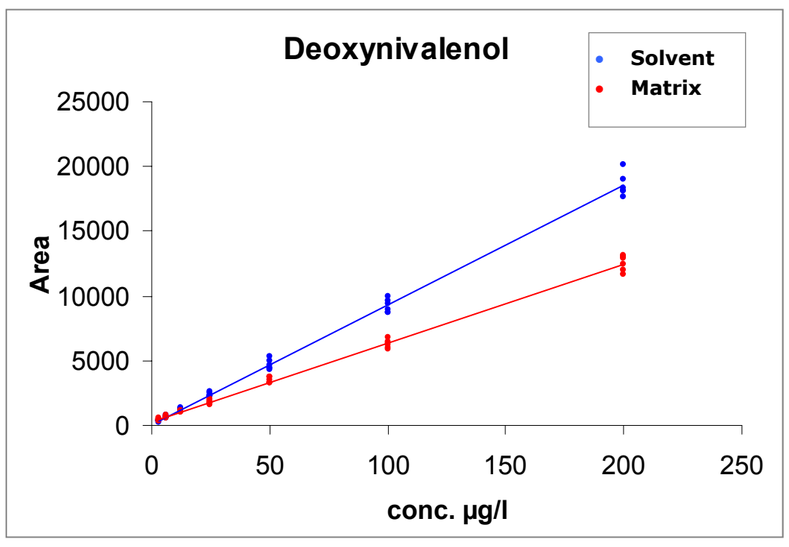

Figure 5.

CONCLUSION

The method shown above is applicable to the enforcement of action levels for regulated substances, such as the aflatoxins, in agricultural produce and foodstuffs. It is also applicable to the monitoring of various mycotoxin contaminants of emerging concern. It allows the determination of multiple contaminants per sample, which may ultimately enable a more strategic picture to be obtained of exposure to these compounds from the human diet.

References

1. European Mycotoxin Awareness Network, http://www.mycotoxins.org/
2. M.C. Spanjer, P.M. Rensen and J.M. Scholten, Multi-mycotoxin analysis: the LC/MS Approach, Proceedings of the Third Conference of the World Mycotoxin Forum, 10-11

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