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應用手冊

Rapid Multi-Mycotoxin Analysis using ACQUITY UPLC and Quattro Premier XE

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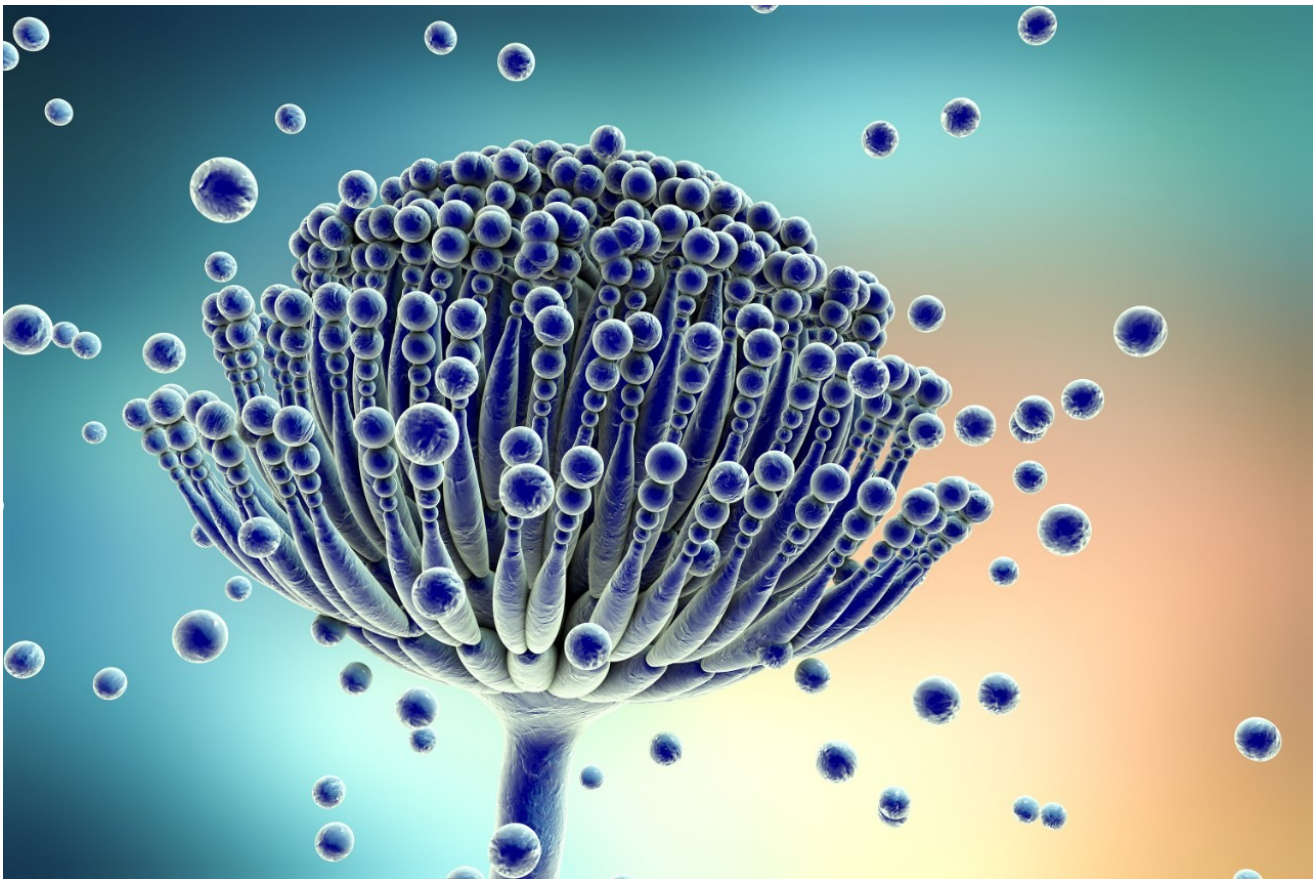

Abstract

This application note describes an extended multi-mycotoxin method for 25 contaminants in a variety of sample

types which not only meets the requirements for analysis of regulated compounds, but also includes a range of other compounds of concern.

Introduction

Many agricultural crops are susceptible to colonization by molds 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, odor, 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.

There are various classes of mycotoxins, produced by several species of mold, and some of the most important in terms of food safety are the aflatoxins, tricothecenes, ochratoxins and fumonisins. The aflatoxins, for example, first rose to notoriety in 1960, when they caused the deaths of thousands of turkeys on farms in the UK. The bird feed had been made with peanut meal, imported from Brazil, which had been contaminated with the mold *Aspergillus flavus*. This incident highlighted the dangers posed by these compounds, dangers exacerbated by the global nature of modern agricultural trade.[1,2]

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. The aflatoxins, ochratoxin A, the fumonisins and tricothecenes such as deoxynivalenol are legislated against in many countries. Rapid, sensitive, and accurate analysis may be carried out for these compounds using immunoaffinity test kits. Immunoaffinity sample preparation is also appropriate for chromatography based analysis where the maximum sensitivity and selectivity is required.[3] In addition, a single analytical method able to target a variety of mycotoxin classes in a range of agricultural produce is desirable in order to obtain more comprehensive information on the range of contaminants that are present in human food. Such a multi-mycotoxin method is appropriate for laboratories testing food for consumption in the European Union, where the range of contaminants legislated against is the most extensive in the world.

The use of HPLC, coupled to a Waters Quattro Ultima Tandem Quadrupole Mass Spectrometer has been reported previously for multi-mycotoxin analysis.[4,5] Using UltraPerformance Liquid Chromatography (UPLC), it is possible to expand the method while significantly reducing the analysis time and increasing sensitivity.

This note describes an extended multi-mycotoxin method for 25 contaminants in a variety of sample types which

not only meets the requirements for analysis of regulated compounds, but also includes a range of other compounds of concern. The method uses a simple, generic sample preparation method followed by Waters ACQUITY UPLC separation and detection with a Waters Quattro Premier XE Tandem Quadrupole Mass Spectrometer.

Waters ACQUITY UPLC System with Quattro Premier XE Mass Spectrometer.

Experimental

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.

LC Conditions

Column:	ACQUITY UPLC BEH C_{18} 1.7 µm; 2.1 x 100 mm

	Column
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile
Flow rate:	0.4 mL/min

Mobile phase gradient 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. Mobile phase gradient.

MS Conditions

The eluent from the column was directed into the electrospray source of a Quattro Premier XE Tandem Quadrupole Mass Spectrometer operated in positive ionization, multiple reaction monitoring (MRM) mode. Tables 2a and 2b show the two MRM transitions monitored for each compound. The monitoring of two transitions allows the presence of a mycotoxin contaminant to be confirmed.

Acquisition and Processing Methods

The data were acquired and processed using Waters MassLynx Software.

	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
	722	352	50	40
Fumonisin B2	706	336	50	40
	706	318	50	40
Nivalenol	313	295	13	8
	313	175	13	20
Diacetoxyscirpenol	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

Table 2a. Two MRM transitions monitored for each compound.

	Parent Ion (m/z)	Product Ion (m/z)	Cone Voltage (V)	Collision Voltage (V)
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	10
	319	185	20	23
Penicillic acid	171	125	18	12
	171	153	18	7
Fusarenon X	355	247	15	13
	355	268	30	27
Ergotamine	582	208	30	42
	582	208	30	28
Roquefortin	390	193	30	19
	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	24
	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 2b.

Two MRM transitions monitored for each compound.

Results and Discussion

Figure 1 shows the chromatogram obtained from this multimycotoxin 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 (eluting during the isocratic portion of the chromatographic method) to approximately 4.5 seconds wide at base for some that were better retained. Figure 2 shows chromatograms for the four aflatoxin compounds. In this study the method was validated for the matrix pistachio nut and Figures 3-8 show calibration curves obtained for the aflatoxins, ochratoxin A and DON. 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.

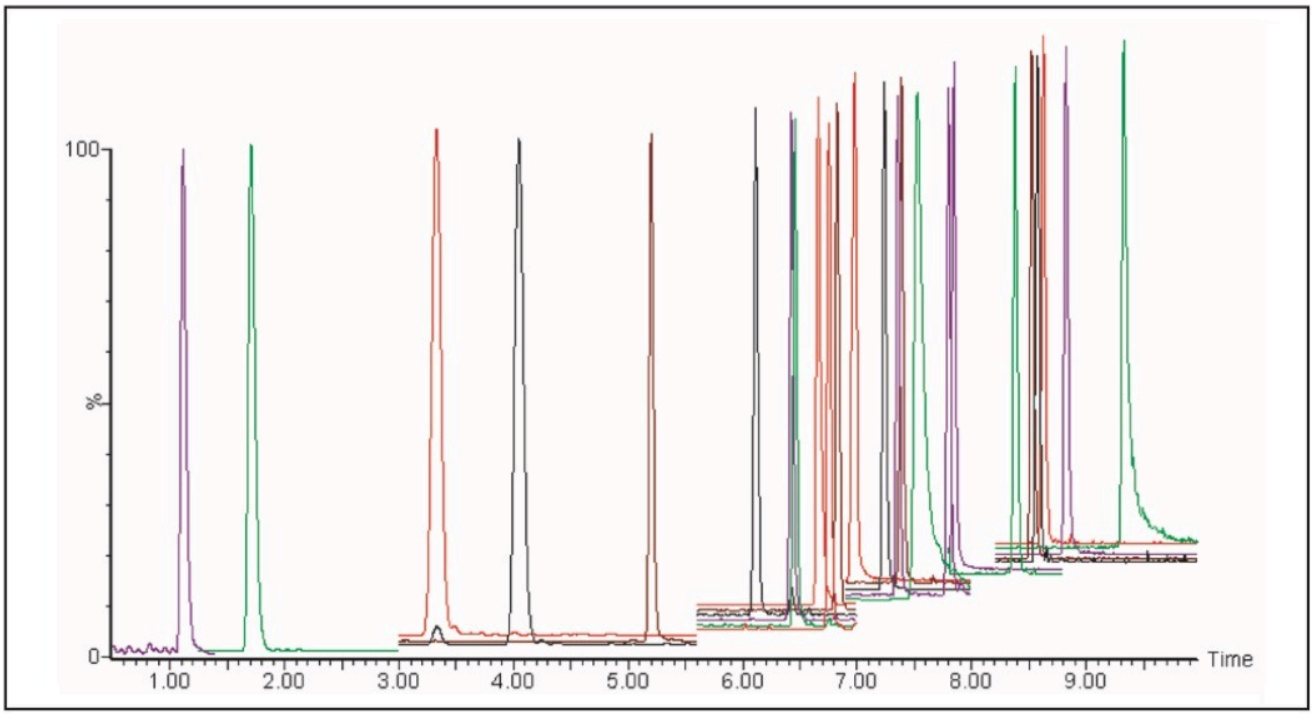

Figure 1. Chromatograms for all 25 mycotoxins.

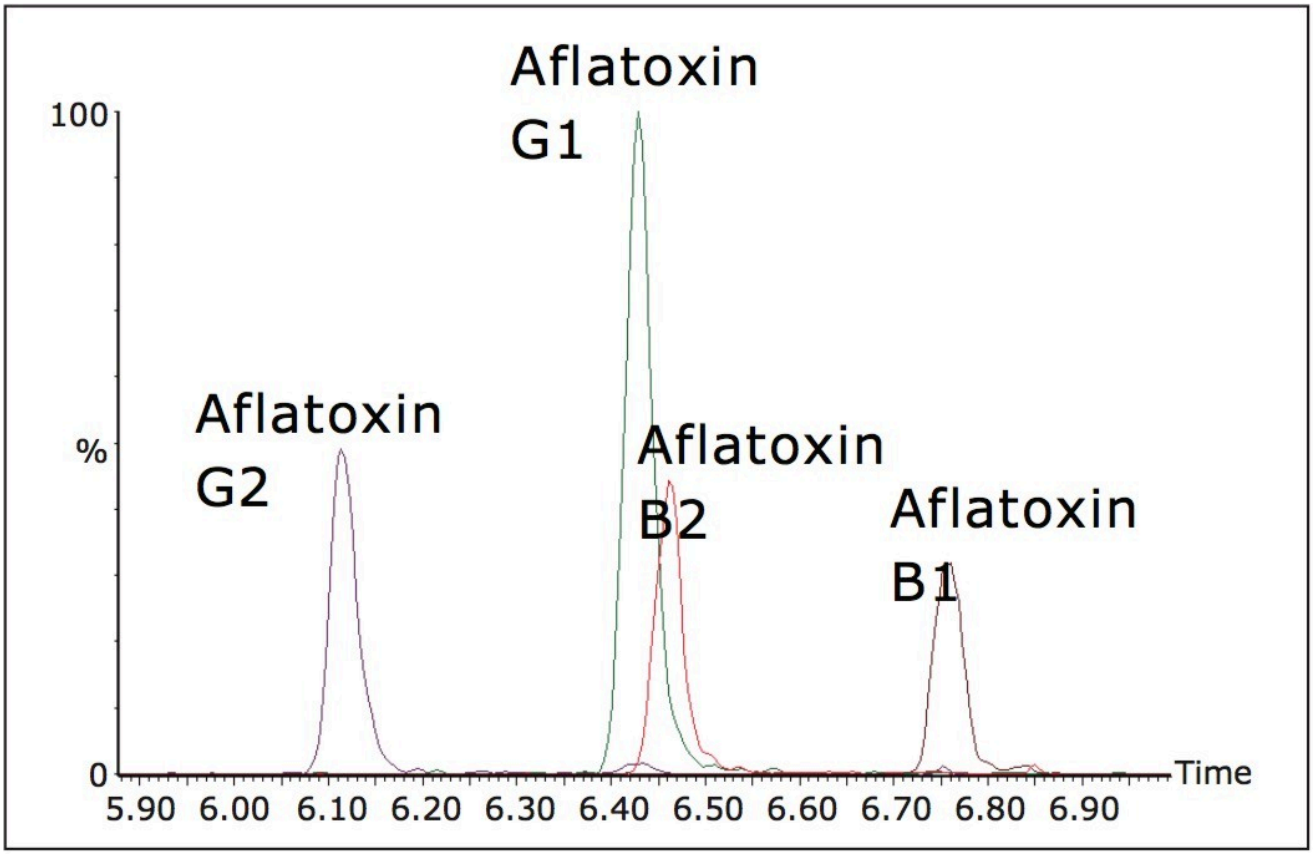

Figure 2. Chromatograms for the 4 aflatoxins.

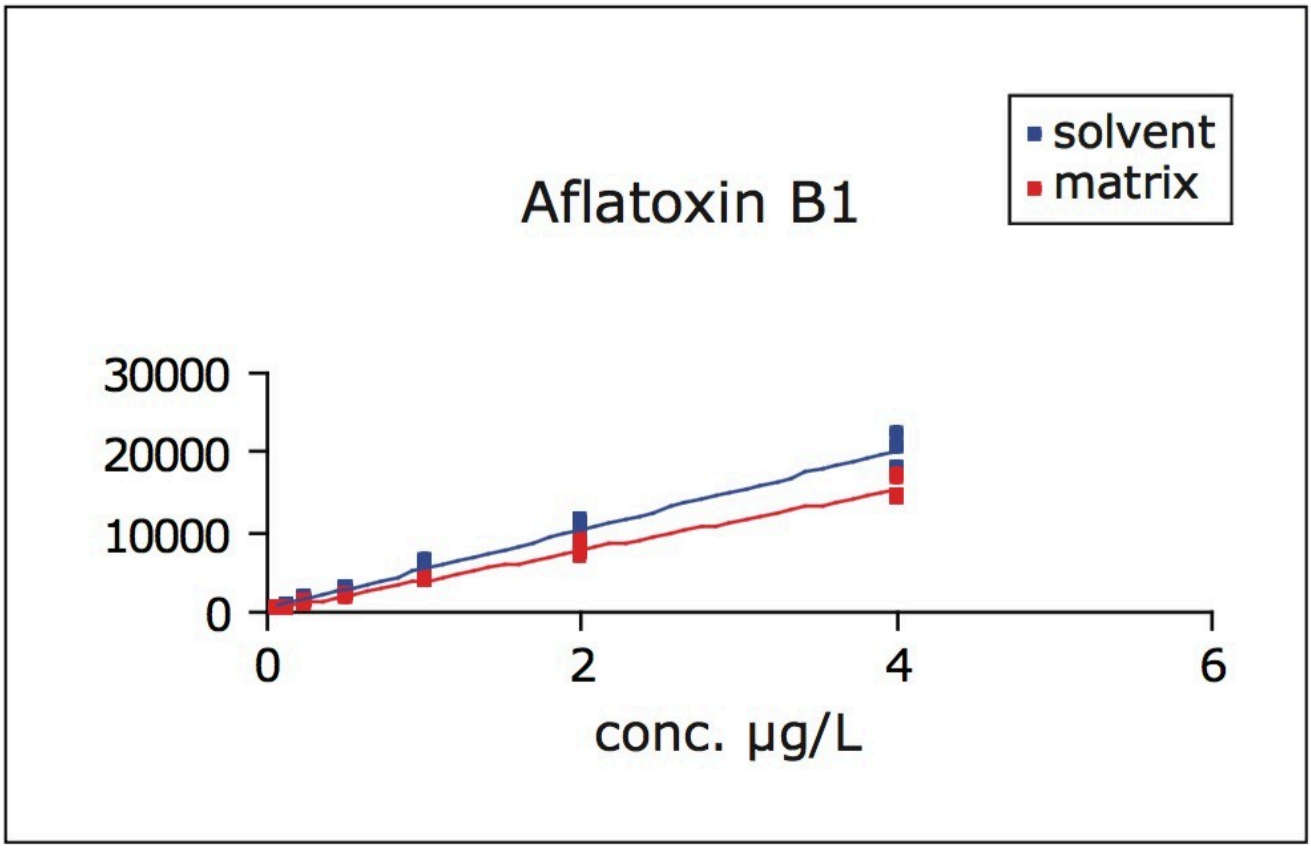

Figure 3. Matrix-effect aflatoxin B1.

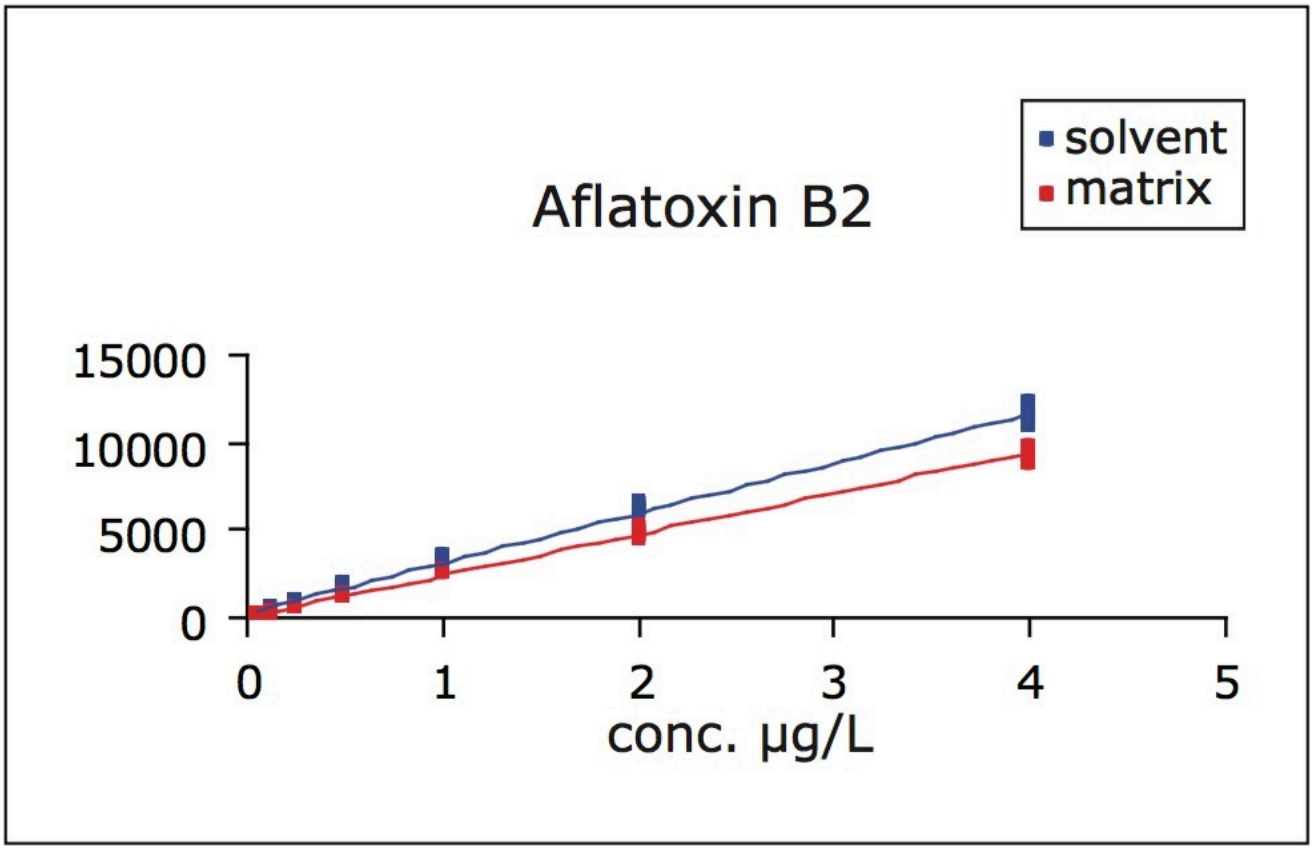

Figure 4. Matrix-effect aflatoxin B2.

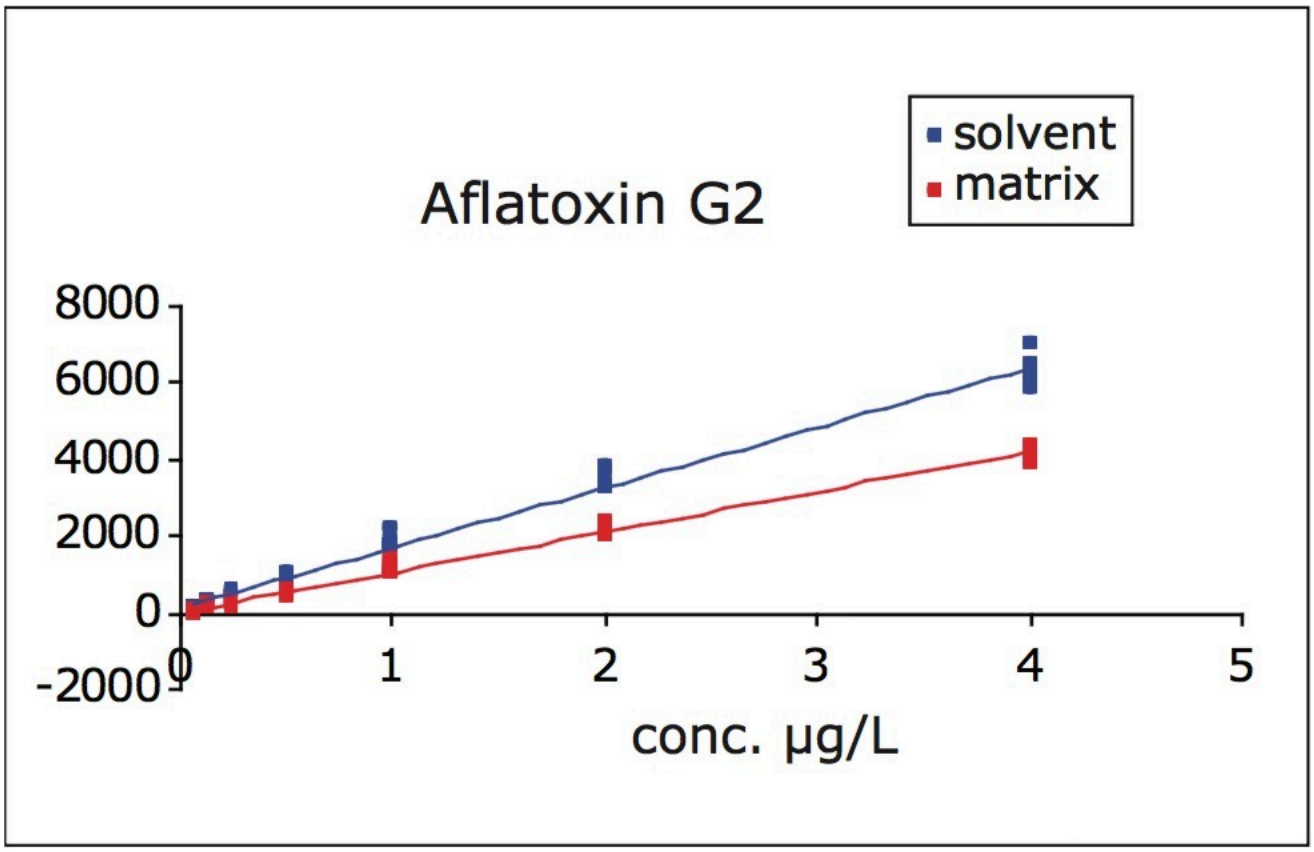

Figure 5. Matrix-effect aflatoxin G1.

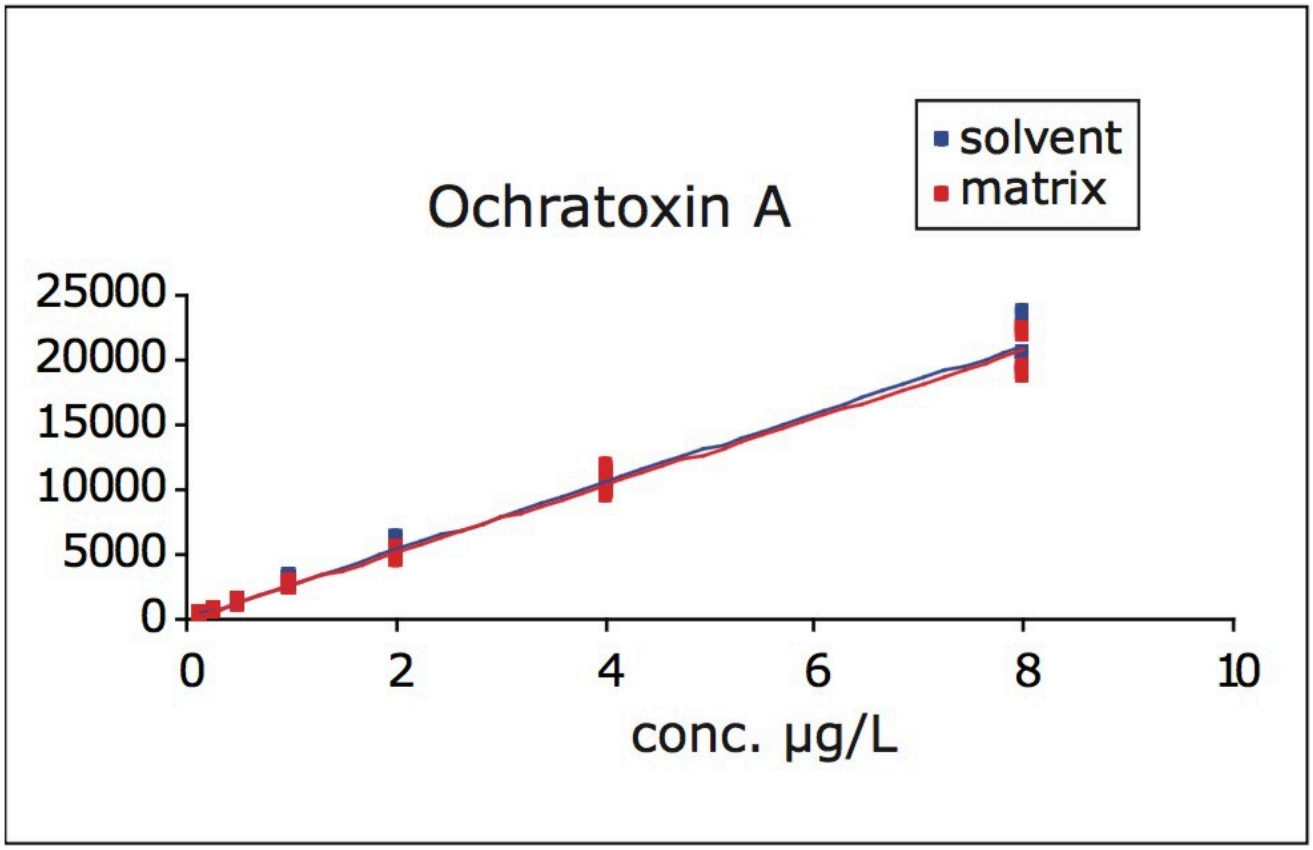

Figure 6. Matrix-effect aflatoxin G2.

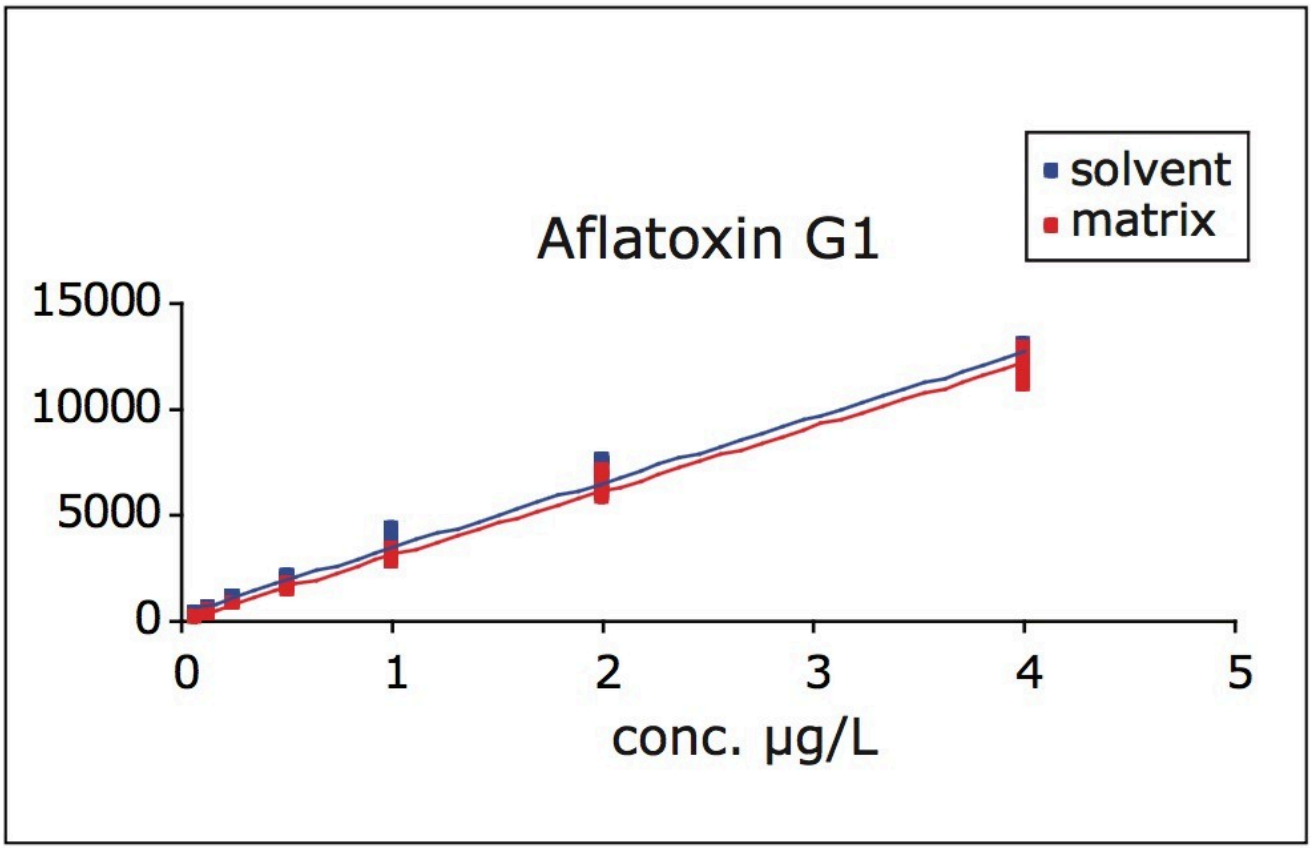

Figure 7. Matrix-effect ochratoxin A.

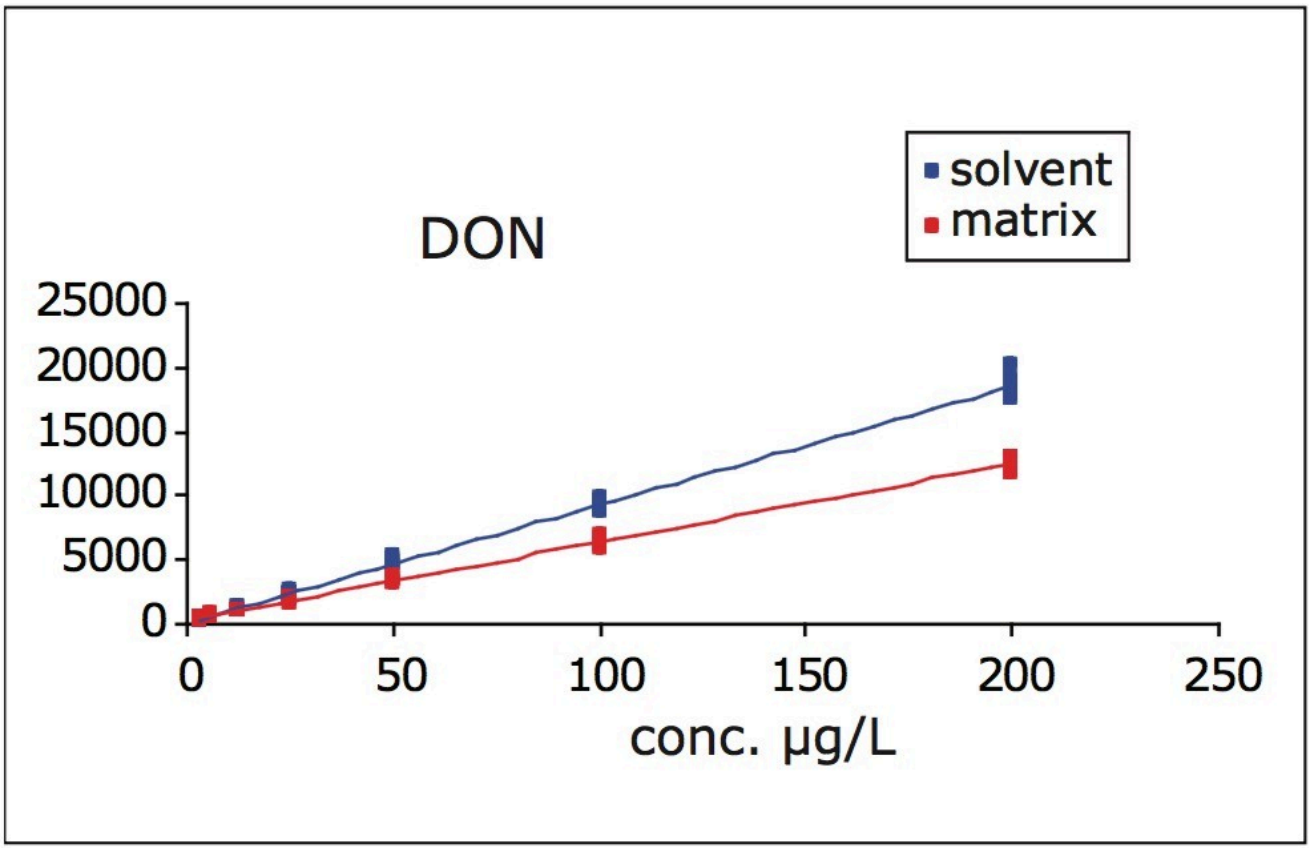

Figure 8. Matrix-effect DON.

Figure 8 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. From these figures, it is clear that ion suppression in this matrix (for the mycotoxins tested) varies from almost absent to clearly present. Such matrix effects can be reduced or eliminated by the use of SPE sample cleanup and this may be investigated in future work. These six mycotoxins were chosen because they are presently subject to EU law; nevertheless, the presence of DON in a pistachio sample would not normally be expected. Ochratoxin A, however, can be found in pistachio nuts. The figures presented clearly indicate that the matrix effect depends on the analyte, which makes it obligatory to determine ion suppression for every single separate matrix-mycotoxin combination. Validation in peanut and cornflake matrix has been published before.

Conclusion

The method described 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

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