AUTOMATED QUECHERS EXTRACTION AND LC/MS/MS ANALYSIS OF MYCOTOXINS

APPLICATION NOTE: AS-242

Authors

Colin Hastie
Sean O'Connor

Anatune Limited
Unit 4, Wellbrook Court, Girton Road,
Cambridge, CB3 0NA

+44 (0)1223 279210
enquiries@anatune.co.uk

Abstract

Mycotoxins are toxic secondary metabolism products of fungi that can cause illness and potentially death if consumed. To protect consumers, food and livestock feed producers are required to ensure that mycotoxins do not enter the food chain at levels unsuitable for consumption. Crops such as wheat, barley, corn or dried fruits can be susceptible to moulds in poor growing seasons or with poor or inadequate storage conditions once harvested.

Mycotoxins testing typically uses enzyme-linked immunosorbent assay (ELISA) or liquid chromatography with mass spectrometric detection, the latter being seen as the gold standard. Both methods require labour intensive extraction procedures which are limited to laboratory working hours and ELISA requires a different test for each analyte.

Anatune have developed an automated method for the extraction and analysis of mycotoxins, using QuEChERS extraction of ground crop, followed by LC-MS/MS analysis. This method was evaluated in wholemeal wheat flour for six common mycotoxins, all of which showed reproducible extraction and allowed low levels of analyte detection.

This automated method has many advantages over traditional approaches, including; higher throughput, reduced analyst time, reduced analyst exposure to harmful mycotoxins and a reduced environmental impact due to lower solvent usage.

INTRODUCTION

Mycotoxins are toxic secondary metabolism products of fungi that can cause illness and potentially death if consumed. To ensure mycotoxins are within safe thresholds, food manufactures are required to test crops such as wheat, barley, nuts, grapes, rapeseed, and other ground-based crops to ensure they are below maximum permitted concentrations. A mycotoxin result which exceeds the threshold would result in rejection of raw materials and product loss. This analysis is carried out either using ELISA methods or extraction followed by LC/MS analysis. Although these methods are not complicated, they require significant amounts of analyst time for sample preparation, that can be performed by robotics in a more timely and reproducible fashion. The safety implications of the highly toxic mycotoxins also make automation appealing due to minimised handling requirements.

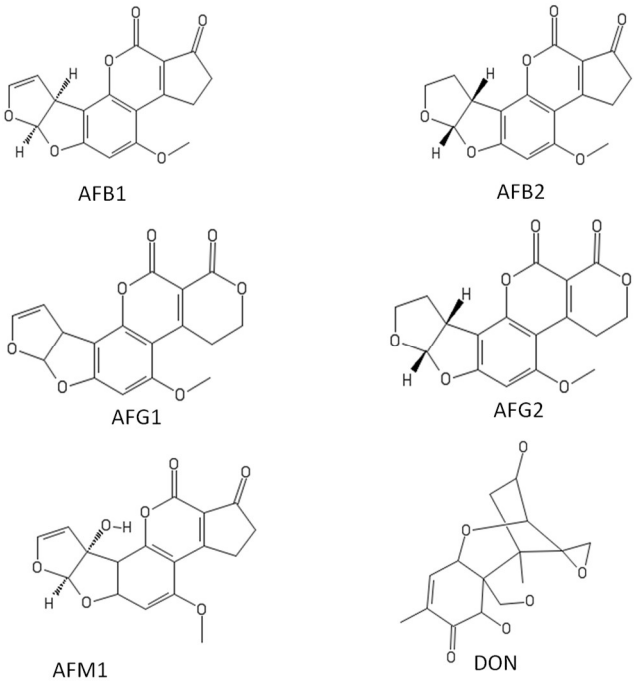

Figure 1: Chemical structure of target mycotoxins

EXPERIMENTAL

Standards

The mycotoxin standard contained Aflatoxin B1 (AF B1), Aflatoxin B2 (AF B2), Aflatoxin M1 (AF M1), Aflatoxin G1 (AF G1), Aflatoxin G2 (AF G2) and Deoxynivalenol (DON). The structures of which can be seen in Figure 1.

METHOD

Instrumentation

The system used for this method is shown in Figure 2.

Extraction

Into 10 mL vials, 1 g of finely ground sample matrix, magnesium sulphate and sodium acetate (QuEChERS salts) are weighed. The vials are sealed and placed on the GERSTEL Multipurpose sampler (MPS). The MPS automatically spikes the samples with internal standard and, where applicable, native mycotoxin spike solutions. 5 mL of 50:50 acetonitrile: 0.2 % formic acid aq. (v/v) extraction solution is added to the vials. Samples are then mixed on the GERSTEL QuickMix wrist action shaker at 2000 rpm for

Figure 2: Picture of the GERSTEL MPS Robotic and Agilent LC/MS/MS

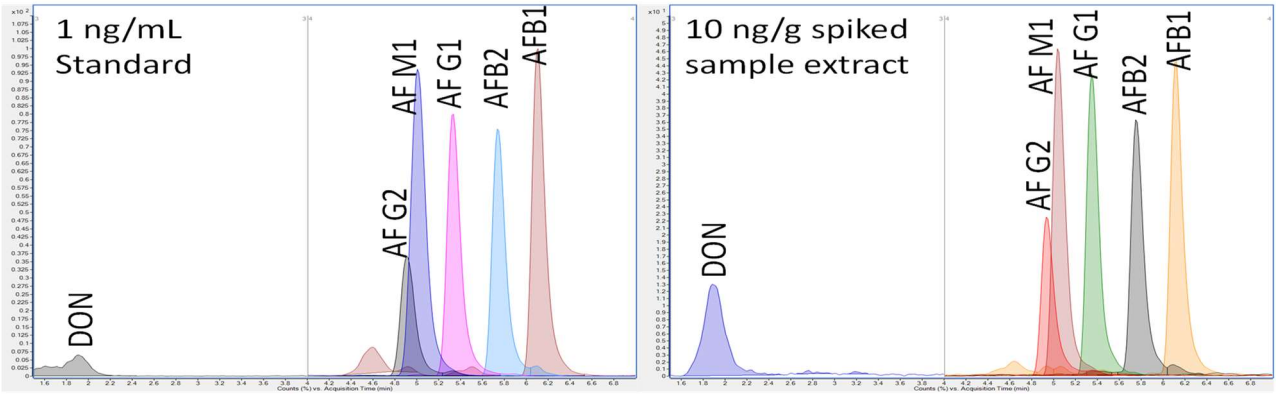

Figure 3: Extracted ion chromatogram of target analytes in a 1 ng/mL standard and 10 ng/g spiked sample

10 minutes. Sample vials are then centrifuged at 4500 rpm for 5 minutes to separate the acetonitrile extract from the sample matrix and aqueous layer (see Figure 4). An aliquot of the acetonitrile extract is diluted in water (HPLC Grade) and injected onto Agilent 1260 HPLC.

Figure 4: Image of sample extract post centrifugation

Analysis method

Solvent A:	0.1% formic acid (aq) containing 5 mM ammonium formate
Solvent B:	95:5 acetonitrile: water (v/v)
Column:	Poroshell 120 BonusRP 2.7 µm 2.1 x 100 mm
Flow rate:	0.3 mL/min.

Table 1: HPLC gradient

Time (min)	*% A*	*%B*
0	95	5
1	95	5
11	0	100
12	0	100
12.01	95	5
15	95	5

Detector: Agilent 6470 Triple Quadrupole LC/MS System using a JetStream electrospray ionisation (ESI) source operating in multiple reaction monitoring mode (MRM).

Table 2: MRM conditions

Analyte	Precursor ion (m/z)	Product ion (m/z)	Fragmentor Voltage (V)	Collision Energy (V)	Polarity
DON	297.0	249.0	125	14	Positive
	297.0	203.0	125	10	Positive
AFB1	313.1	285.0	180	24	Positive
	313.1	241.0	180	44	Positive
AFB2	315.1	287.0	180	30	Positive
	315.1	258.9	180	35	Positive
AFM1	329.1	273.1	143	21	Positive
	329.1	229.0	143	45	Positive
AFG1	329.1	243.0	170	30	Positive
	329.1	214.1	170	40	Positive
AFG2	331.1	313.0	170	29	Positive
	331.1	245.0	170	35	Positive

Method performance evaluation

To evaluate method effectiveness, linearity and reproducibility were measured using blank wholemeal flour matrix. Flour samples were spiked at 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 25, 50 and 100 ng/g with additional spiking in triplicate at 50 ng/g. Spiked solutions were left to contact the samples for 3 days prior to extraction.

RESULTS AND DISCUSSION

Linearity

Due to the variation in regulatory maximum limit requirements for the mycotoxins, standards were prepared between 0.001 and 50 ng/mL. The evaluated analytes all showed good linearity, with all r^2 values greater than 0.9998. All evaluated Mycotoxins also showed good linearity in extracted sample matrix. These all had r^2 values of greater than 0.995, demonstrating that the method has good intrinsic extraction repeatability. See Table 3 for results in both standards and sample.

Table 3: Linearity in standards (0.001- 50 ng/mL) and samples (0.01-100 ng/g)

Analyte	Standards r^2	Sample r^2
DON	0.9998	0.9959
AFG2	1.0000	0.9991
AFM1	0.9998	0.9991
AFG1	0.9999	0.9986
AFB2	1.0000	0.9984
AFB1	0.9998	0.9980

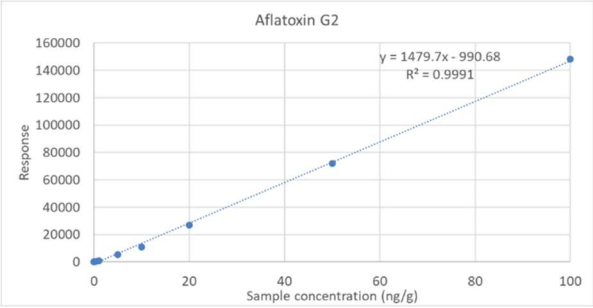

Figure 5: Example of linearity in spiked matrix

Reproducibility

Flour samples spiked with 50 ng/g of mycotoxins were extracted (in triplicate) and Figure 6 shows reproducible recoveries of all target mycotoxins (78-109%). It was noted that the flour samples showed a native level of DON of around 7 ng/g as determined by standard addition, this is significantly below the maximum permitted levels in food stuffs and highlights the excellent inherent sensitivity of the method. Sample concentration data for DON in Figure 6 has been blank subtracted.

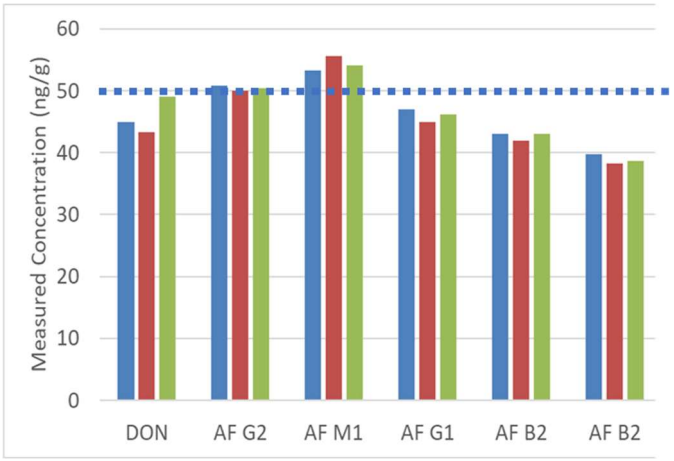

Figure 6: Reproducibility of extraction of Mycotoxins in triplicate wholemeal flour samples spiked 50 ng/g

CONCLUSIONS

A QuEChERS extraction method was developed for analysis of mycotoxins followed by LC/MS/MS analysis. This method showed good linearity from spiked samples and reproducible recoveries from wholemeal flour.

As well as the benefits of good reproducibility and linearity there are a number of other benefits.

- Robotic sample preparation and analysis can be integrated into a simple sequence which runs unattended by the analyst leaving time for more productive tasks.
- Once the standards and solvents have been added to the robot, there is no need for further analyst contact with the solvents or highly toxic standards. The MPS is an entirely closed system, thus ensuring a safer working environment for the analyst.
- By using approximately an order of magnitude less solvent than conventional extraction methods, the automated method also has a decreased environmental impact and reduced disposal costs.