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Nota applicativa

Rapid Quantitative Analysis of 12 Mycotoxins in Processed Maize Using Myco6in1+ Immunoaffinity Clean-Up and the ACQUITY QDa Detector

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Abstract

Mycotoxins are naturally occurring secondary metabolites produced by fungi. A variety of mycotoxins can contaminate a wide range of fruits, cereals and grains. Chemically stable and resistant to different forms of decomposition, many of these compounds are known to have carcinogenic, estrogenic, and immunotoxic effects in vivo. Therefore, extensive global regulations are enforced laying down sampling and testing methods, along with permitted limits for specific mycotoxins. Due to the variety of complex food commodities naturally contaminated by fungal species multi-mycotoxin analysis can prove both challenging and time consuming. Therefore, the availability of rapid and sensitive screening tools that are capable of achieving the regulatory levels for the relevant mycotoxins and commodities is essential. The development of methods for the determination of multiple mycotoxins is highly desirable, where LC-MS plays an important role in this field. However due to the differences in chemical and physical properties of the major mycotoxins, sample preparation

can become the most challenging task.

Given the frequency of naturally contaminated food and feeds by various mycotoxins, the need exists for the simple and sensitive detection of regulated mycotoxins. In this application note, a simple, sensitive, and cost-effective method has been developed for the quantitative analysis of 12 regulated mycotoxins in processed maize extract. Using a single sample preparation procedure, all analytes were extracted and readily detected on the ACQUITY QDa Detector. No additional or time-consuming sample preparation was required, thus allowing for the rapid screening of multiple mycotoxins on a single detector.

Benefits

- Multiple mycotoxin detection in a single consolidated method.
- Sensitive detection of 12 mycotoxins at regulatory limits in complex cereal-based foods.

Introduction

Mycotoxins are naturally occurring secondary metabolites produced by fungi. A variety of mycotoxins can contaminate a wide range of fruits, cereals and grains. Chemically stable and resistant to different forms of decomposition, many of these compounds are known to have carcinogenic, estrogenic, and immunotoxic effects *in vivo*. Therefore, extensive global regulations are enforced laying down sampling and testing methods, along with permitted limits for specific mycotoxins.

Within the European Union, maximum permitted levels for deoxynivalenol (DON), fumonisins (B1 and B2), aflatoxins (B1, B2, G1, and G2), ochratoxin A, and zearalenone are regulated in EU Commission Regulation 1881/2006 and 1126/2007. More recently, recommended levels for the sum of T2 and HT2 toxins have been provided in Recommendation 2013/165. Additionally, rules for sampling and performance of analytical methods are specified in Regulation 401/2006. Although currently not regulated, attention is paid in this study to the occurrence of nivalenol (NIV), another *Fusarium* toxin that frequently contaminates cereals in combination with DON.

Due to the variety of complex food commodities naturally contaminated by fungal species multi-mycotoxin analysis can prove both challenging and time consuming. Therefore, the availability of rapid and sensitive

screening tools that are capable of achieving the regulatory levels for the relevant mycotoxins and commodities is essential. The development of methods for the determination of multiple mycotoxins is highly desirable, where LC-MS plays an important role in this field. However due to the differences in chemical and physical properties of the major mycotoxins, sample preparation can become the most challenging task.

Given the frequency of naturally contaminated food and feeds by various mycotoxins, the need exists for the simple and sensitive detection of regulated mycotoxins. This work aims to develop a simple and accurate method for the quantitative analysis of multiple mycotoxins in processed maize using an immuno-affinity clean-up method and mass detection.

Experimental

UPLC Conditions

LC system:	ACQUITY UPLC I-Class
Run time:	12 min
Column:	CORTECS $C_{18,}$ 1.6 µm, 2.1 x 100 mm
Mobile phase A:	2 mM ammonium acetate with 0.1% formic acid in water
Mobile phase B:	2 mM ammonium acetate with 0.1% formic acid in methanol

Flow rate:	0.4 mL.min-1
Injection volume:	10 µL

Gradient

Time (min)	%A	%B
Initial	99	1
7	50	50
10	1	99
11.5	1	99
11.6	99	1
14	99	1

MS Conditions

MS system:	ACQUITY QDa
Ionization mode:	ESI±
Desolvation temp.:	600 °C

Capillary voltage:	Default (0.8 kV)
Sampling rate:	Default (5 Hz)
SIR channels:	See Table 1

Sample Preparation

Ten grams of ground sample were extracted by high speed blending with 40 mL of water followed by 60 mL of methanol. The extract was filtered and a 5 mL aliquot was reduced to approximately 2 mL under nitrogen. Phosphate buffer (5 mL) was added and the resulting solution was loaded onto the VICAM Myco6in1+ Immunoaffinity Column (IAC). The column was washed with 10 mL water and the toxins were eluted, first with methanol (3 mL) followed by water (2 mL). The eluate was subsequently evaporated to dryness, under a gentle flow of nitrogen, and reconstituted to 0.2 mL in 10:90 *v/v* methanol:mobile phase A.

Preparation of Standards

Blank cereal food samples (unfortified) were prepared using the Myco6in1+ IACs. The resultant eluates were fortified with a mixed mycotoxin standard solution to allow for a 5-point calibration curve, where the midpoint range on all of the calibration curves were equal to the permitted level of each mycotoxin. The remaining points were equally distributed with two standards below and two standards above the permitted limit for each relevant mycotoxin. These standards were then evaporated to dryness and reconstituted in 10:90 *v/v* methanol:mobile phase A.

Results and Discussion

The default source conditions of the Waters ACQUITY QDa Mass Detector offered the optimum performance for all 12 mycotoxin analytes. The individual masses, cone voltages and electrospray ionization mode were identified and are shown in Table 1.

	Mycotoxin	Abbreviation	RT (min)	SIR (*m/z*)	Cone voltage (V)	Calibration range ($\mu g.kg^{-1}$)
1.	Nivalenol	[NIV-H_2O+H^+]	2.2	295.0	15	468.75 to 5625.00
2.	Deoxynivalenol	[DON+H^+]	2.9	297.0	10	468.75 to 5625.00
3.	Aflatoxin G2	[AFG2+H^+]	5.8	331.0	20	0.625 to 7.50
4.	Aflatoxin G1	[AFG1+H^+]	6.2	329.0	20	0.625 to 7.50
5.	Aflatoxin B2	[AFB2+H^+]	6.5	315.0	20	1.250 to 15.00
6.	Aflatoxin B1	[AFB1+H^+]	6.8	313.0	20	0.625 to 7.50
7.	HT2 toxin	[HT2+Na^+]	8.2	447.0	15	31.250 to 375.00
8.	Fumonisin B1	[FB1+H^+]	8.3	722.0	20	500.000 to 6000.00
9.	T2 toxin	[T-2+NH_4^+]	8.6	484.0	15	31.250 to 375.00
10.	Ochratoxin A	[OTA+H^+]	8.8	404.2	20	62.500 to 750.00
11.	Zearalenone (negative mode)	[ZEA-H]$^-$	8.8	317.0	20	1.875 to 22.50
12.	Fumonisin B2	[FB2+H^+]	9.0	706.0	20	125.000 to 1500.00

Table 1. The 12 mycotoxins with experimental parameters.

Good linearity was obtained for all 12 mycotoxins over the relevant calibration ranges. A minimum of 12 data points per chromatographic peak were obtained for each analyte. An example of the linearity is shown in Figure 1, where AFG1 and DON depict the difference in the regulatory limits and calibration ranges required. AFG1 runs over the range of 0.125 to 4.0 $\mu g.kg^{-1}$ due to the low regulatory limit assigned to aflatoxins; while the calibration required for DON is over the range of 94.0 to 1500 $\mu g.kg.^{-1}$ The linearity observed for all analytes over these varying calibration ranges shows the instrument's excellent robustness over the wide dynamic range in the presence of complex matrix.

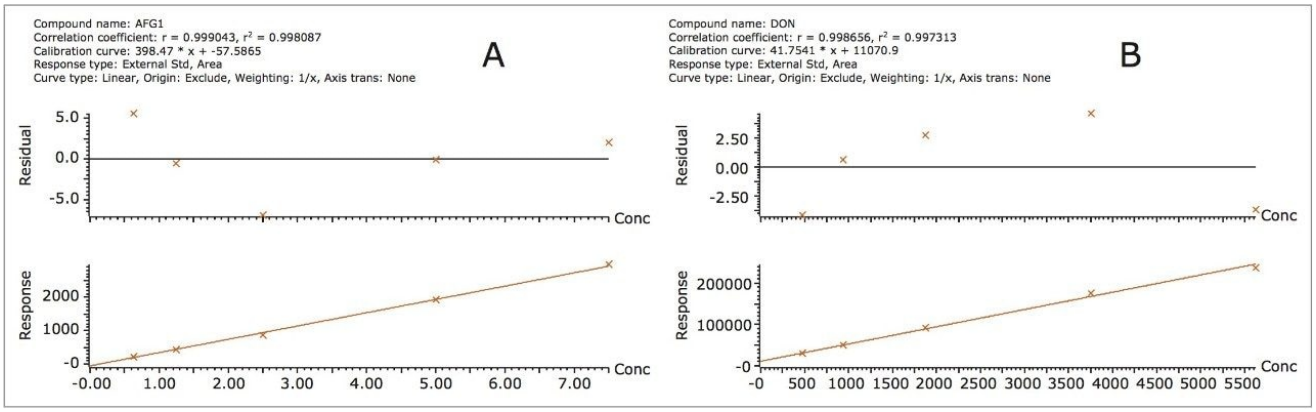
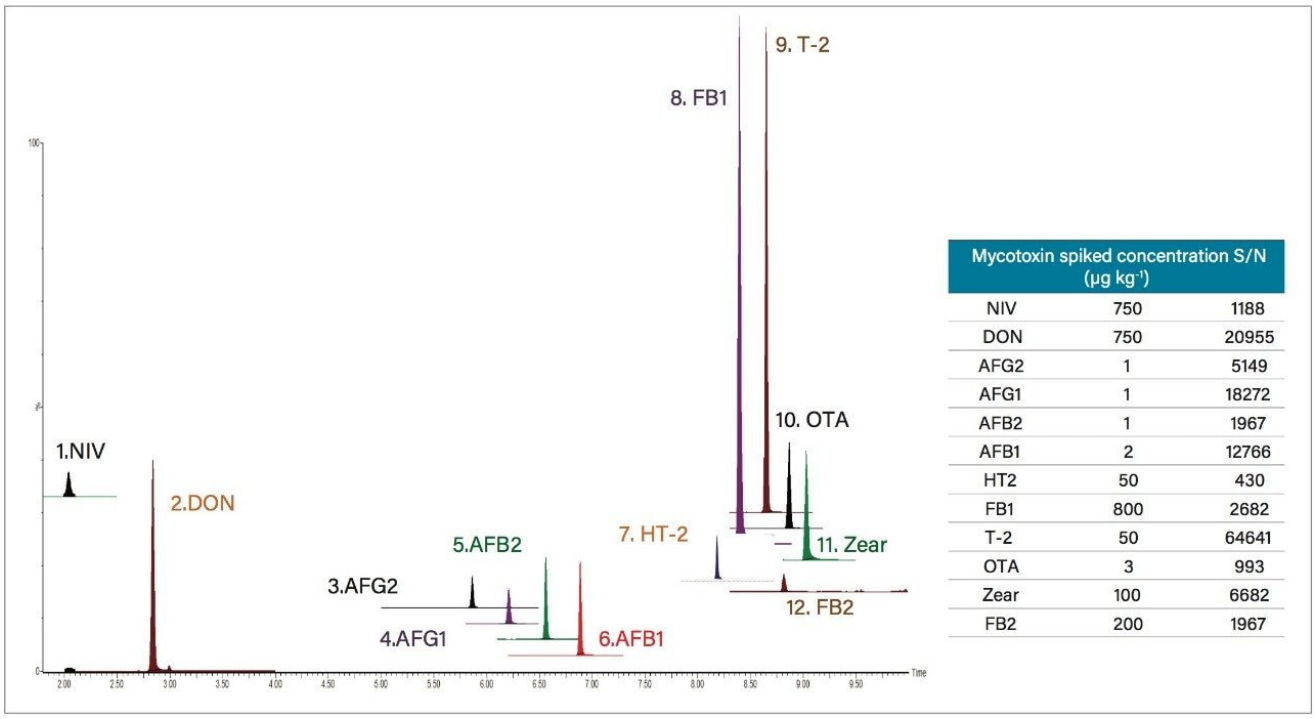


Figure 1. Examples of the matrix matched calibration curves for 1A. Aflatoxin G1 equating to the range of 0.125 to 4 µg.kg,$^{-1}$ and 1B. Deoxynivalenol equating to the range of 94.0 to 1500 µg.kg.$^{-1}$

An example of the chromatography achieved is shown in Figure 2, where the maize snack food was fortified to the regulatory limits. Satisfactory sensitivity was reported for each of the analytes, and excellent signal-to-noise (S/N) ratios were achieved. All four aflatoxins, plus the additional eight mycotoxins can readily be detected by LC coupled with mass detection.



Mycotoxin spiked concentration S/N (µg kg⁻¹)		
NIV	750	1188
DON	750	20955
AFG2	1	5149
AFG1	1	18272
AFB2	1	1967
AFB1	2	12766
HT2	50	430
FB1	800	2682
T-2	50	64641
OTA	3	993
Zear	100	6682
FB2	200	1967

Figure 2. Processed maize food sample fortified at the displayed concentrations (EU regulatory limits). Chromatographically resolved peaks (normalized) were detected with excellent S/N ratios at legally permitted levels.

Conclusion

A simple, sensitive, and cost-effective method has been developed for the quantitative analysis of 12 regulated mycotoxins in processed maize extract. Using a single sample preparation procedure, all analytes were extracted and readily detected on the ACQUITY QDa Mass Detector. No additional or time-consuming sample preparation was required, thus allowing for the rapid screening of multiple mycotoxins on a single detector.

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