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Application Note

Evaluation of the Performance of a Simple Method for Regulated Mycotoxins in Cereals by LC-MS/MS Using an Interlaboratory Study

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

Waters previously reported the development and single laboratory validation of a method for the determination of the 12 mycotoxins regulated in the EU in various cereals based upon LC-MS/MS after a simple generic extraction method without any clean-up. This application brief shows the successful evaluation of the performance of this method by interlaboratory study. Two cereal FAPAS QC materials were sent to four laboratories in Europe and the USA. Each material was analyzed in triplicate by the four laboratories. The laboratories demonstrated good accuracy and precision for the determination of the 8 mycotoxins in the two FAPAS QC materials. Reported concentrations matched the assigned values provided by FAPAS. Trueness was within the range of 85 to 113%, the within-laboratory repeatability was between 3.0 to 13% and between laboratory reproducibility was between 3.1 and 23%. These results, which meet performance criteria set out by the European Commission, demonstrate the method is suitable for the monitoring of mycotoxins in cereals for both official control and testing conducted by food business operators.

Benefits

- Simple to implement analytical method covering extraction to determination
- Comparable method performance across multiple laboratories in different geographical locations

Introduction

Mycotoxins are secondary metabolites of filamentous fungi that can occur in food and agricultural products via many contamination pathways, including production, processing, transport, and storage. Mycotoxins have a significant negative impact on human and animal health and are responsible for significant losses in revenue and the potential erosion of brand and reputation. The most common mycotoxins are regulated in many countries of the world after thorough risk assessment, considering toxicity, occurrence, and consumption data as well as economic and political considerations. For example, European Commission Regulation No. 1881/2006 sets maximum limits for aflatoxins B1, B2, G1 and G2, fumonisins B1 and B2, deoxynivalenol, zearalenone, and ochratoxin A, in a range of different foodstuffs and indicative limits set for

T-2 and HT-2 toxins.[1,2]

Analytical testing is needed to check compliance with such regulations. Approaches for the determination of multiple mycotoxins in a single method require generic extraction conditions to ensure recovery of the different types of mycotoxin. Previously, we have shown that due to the sensitivity and selectivity of LC-MS/MS, simple dilution of the extract, typically without clean-up, is often preferred.[3] Here we report the results of an inter-laboratory study to further evaluate the performance of the method.

Results and Discussion

Four laboratories (one located in Austria, one in the UK and two in the USA) were supplied with:

- ACQUITY UPLC BEH C_{18} 1.7 µm, 2.1 x 100 mm (p/n: 186002352 < https://www.waters.com/nextgen/us/en/shop/columns/186002352-acquity-uplc-beh-c18-column-130a-17--m-21-mm-x-100-mm-1-pk.html>)
- Analytical protocol (720006685EN <https://www.waters.com/nextgen/us/en/library/application-notes/2019/lc-ms-ms-method-development-and-validation-for-the-quantitative-determination-of-regulated-mycotoxins-in-cereal-grain-flours-using-simplified-sample-preparation-conditions-on-xevo-tq-xs.html>), including the mycotoxins of interest, the details of the method to be used, and the analytical run sequence
- FAPAS QC materials: T04366QC (maize flour) and T04359QC (maize flour)
- Pre-prepared calibration standards as solutions
- Stock solution of ^{13}C-labelled analogues for use as internal standards

The four laboratories were instructed to analyze the two FAPAS QC materials for the 12 mycotoxins in the application, extracted in triplicate, using the method provided.[3] Calibration standards were prepared in solvent at Wilmslow (UK laboratory) before dispatch. The concentration of the mycotoxins in the FAPAS QC materials was determined using bracketed, internally standardized calibration. Each laboratory used ACQUITY UPLC Systems coupled with Xevo TQ-XS Tandem Quadrupole Mass Spectrometers.

Each laboratory successfully implemented the chromatographic method from the published method so that the peak shapes and retention times for each compound of interest were similar to that from the reference laboratory. The retention time for the earliest eluting peak, nivalenol, was more than twice the retention time

corresponding to the void volume of the column.[4] Figure 1 shows the typical chromatography and response for the analytes detected in one of the FAPAS QC materials.

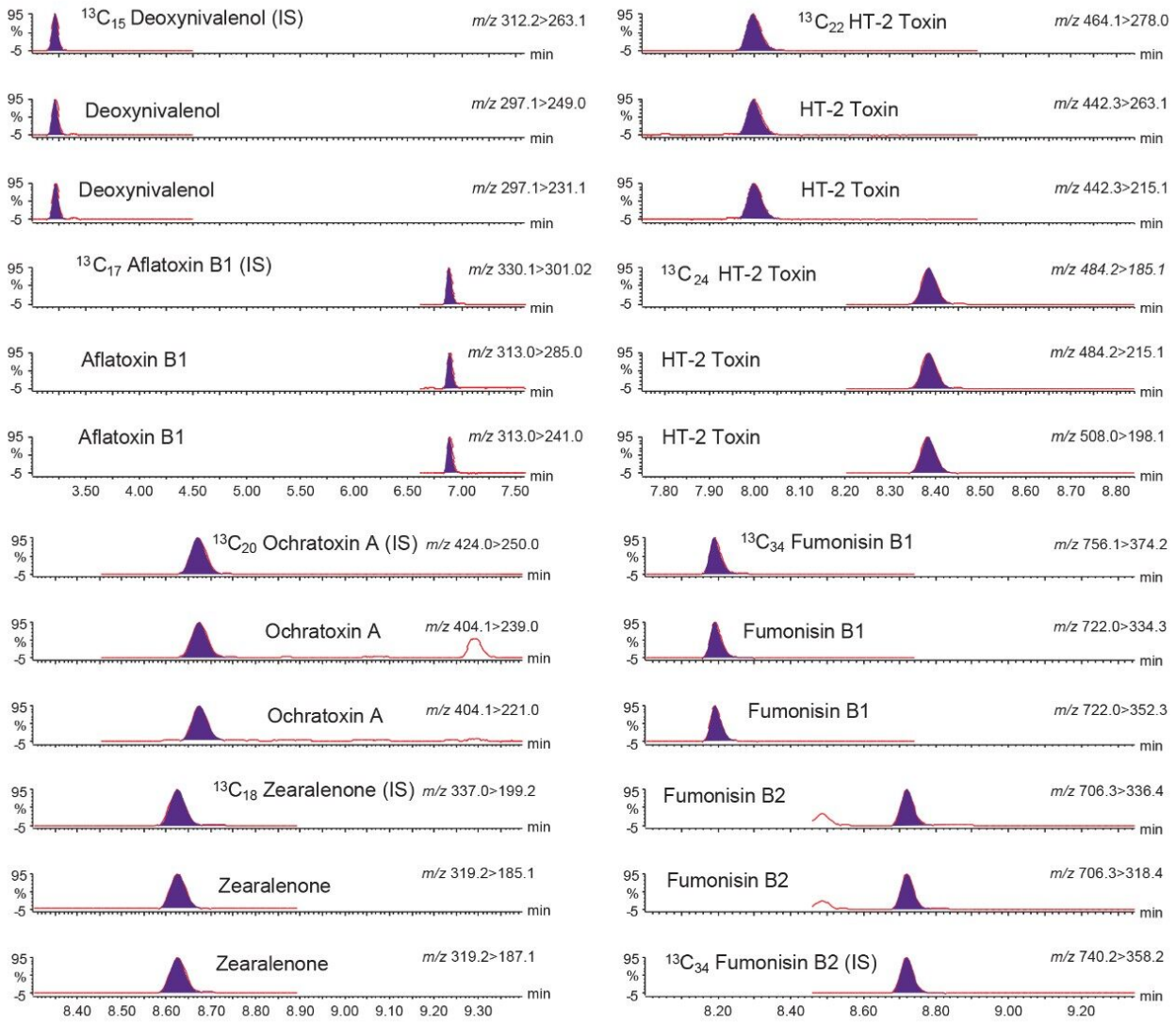
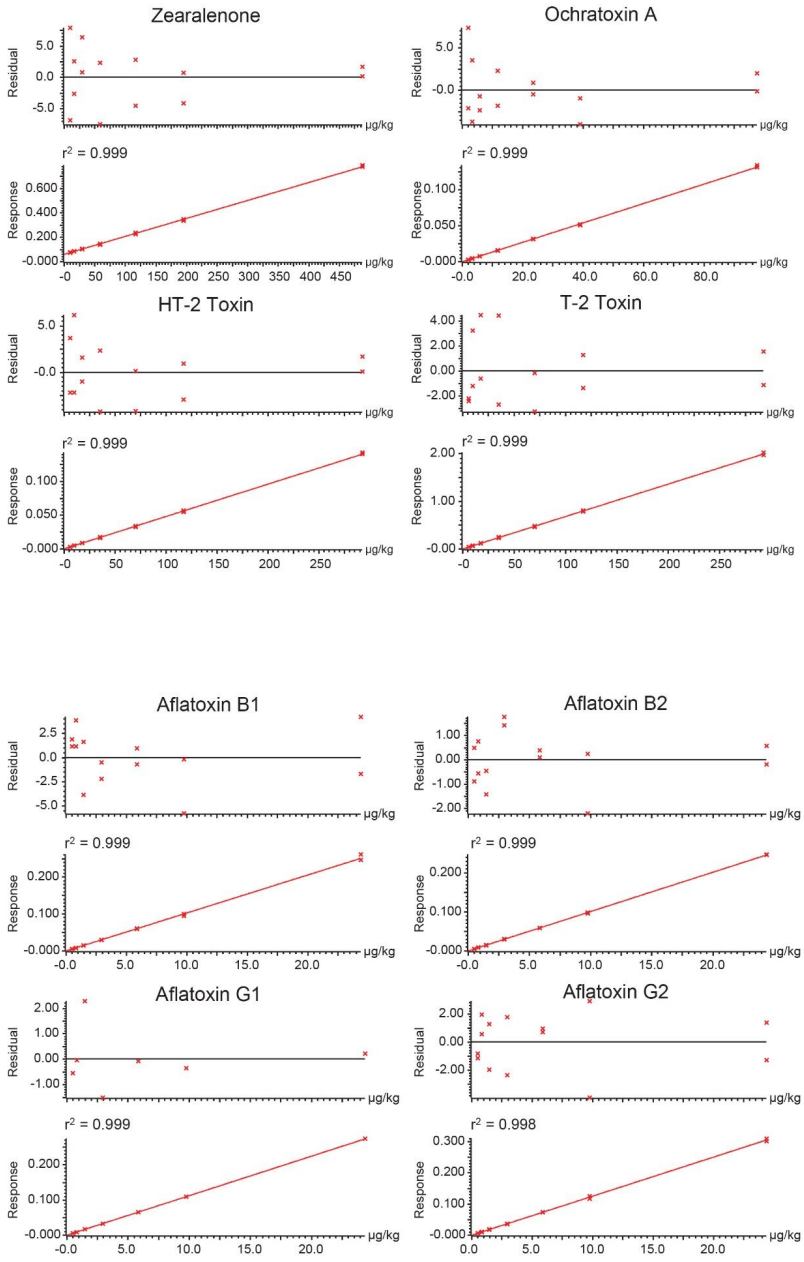


Figure 1. Typical chromatograms showing all eight mycotoxins detected from analysis of T04366QC and their labelled internal standards (for concentrations see Table 2).

All but one calibration graph exhibited coefficients of determination >0.95 and residuals <20%. One laboratory experienced an issue with background contamination with aflatoxin G2, but this compound was not present in either reference material. In two cases, extracts were diluted x10 to ensure response from the analyte was within the calibration range: fumonisin B1 in T04366QC and deoxynivalenol in T04395QC. Values agreed well with those from the undiluted extracts (data not shown). Figure 2 shows typical

calibration graphs for all 12 regulated mycotoxins covered by this method.



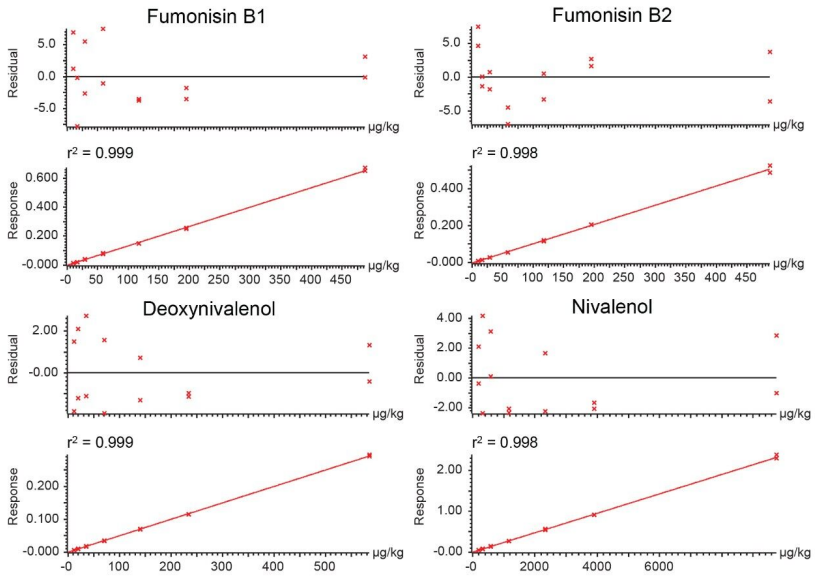


Figure 2. Typical calibration graphs for the 12 regulated mycotoxins (internally standardized).

The results from the analysis of the two FAPAS QC materials showed that the laboratories demonstrated good performance. There is close agreement between measured concentrations and the assigned values; all measured values are within the acceptable range supplied by FAPAS for z ≤2 for all but one set of measurements: fumonisin B2 in T04366QC. The values for trueness, calculated from comparing the measured concentrations with the assigned values, gave values of 85 to 113%. The within laboratory-repeatability (RSD_r) was between 3.0 to 13% and between laboratory reproducibility (RSD_{RL}) was between 3.1 and 23%. Fumonisins proved to be the most challenging of these compounds, but all results meet the method performance criteria set out by the European Commission.[5] There were no false positives reported for other mycotoxins covered by the method. A summary of the results from the interlaboratory study can be found in Tables 1 and 2.

T04395QC	Aflatoxin B1	Ochratoxin A	HT-2 Toxin	T-2 Toxin	Zearalenone	Deoxynivalenol	Fumonisin B1	Fumonisin B2
Internal standard	U-[$^{13}C_{17}$]-AFB1	U-[$^{13}C_{20}$]-OTA	U-[$^{13}C_{22}$]-HT-2	U-[$^{13}C_{24}$]-T-2	U-[$^{13}C_{18}$]-ZEA	U-[$^{13}C_{15}$]-DON	U-[$^{13}C_{34}$]-FB1	U-[$^{13}C_{34}$]-FB2
Assigned values (µg/kg)	9.22	2.69	215	209	95.8	948	287	273
Mean of the measured values (µg/kg)	9.08	2.38	199	186	94.4	878	246	309
Range for [z] ≤2 (µg/kg)	5.26–13.3	1.51–3.87	128–302	125–294	53.7-138	643–1254	176–397	167–370
Range of measured values (µg/kg)	8.28–10.5	2.19–2.60	185–229	163–231	86.5–97.7	813–1004	222–284	276–356
Trueness (%)	98.5	88.6	92.5	88.8	98.5	92.6	85.8	113
Within lab repeatability (% RSDr)	7.39	4.03	4.88	6.36	3.14	4.22	6.04	3.22
Between lab reproducibility (% RSD_{RL})	7.83	4.51	6.35	10.6	3.12	7.27	20.9	16.9

Table 1. Results of analysis of FAPAS QC materials TO4395QC by the four participating laboratories.

T04366QC	Aflatoxin B1	Ochratoxin A	HT-2 Toxin	T-2 Toxin	Zearalenone	Deoxynivalenol	Fumonisin B1	Fumonisin B2
Internal standard	U-[$^{13}C_{17}$]-AFB1	U-[$^{13}C_{20}$]-OTA	U-[$^{13}C_{22}$]-HT-2	U-[$^{13}C_{24}$]-T-2	U-[$^{13}C_{18}$]-ZEA	U-[$^{13}C_{15}$]-DON	U-[$^{13}C_{34}$]-FB1	U-[$^{13}C_{34}$]-FB2
Assigned values (µg/kg)	1.88	1.99	22.9	22.2	26.7	231	526	125
Mean of the measured values (µg/kg)	1.74	1.74	20.1	19.6	25.8	208	446	127
Range for [z] ≤2 (µg/kg)	1.05-2.70	1.11-2.86	12.8-33.0	12.4-31.9	14.9-38.4	139-323	341-711	70-172
Range of measured values (µg/kg)	1.58-1.89	1.45-1.93	17.5-24.2	17.0-23.1	22.2-28.8	183-246	391-510	108-175
Trueness (%)	92.4	87.5	87.7	88.4	96.6	90.2	84.7	102
Within lab repeatability (% RSDr)	2.99	3.51	7.78	5.02	4.68	6.88	7.99	12.7
Between lab reproducibility (% RSD_{RL})	6.44	11.3	9.49	10.1	6.99	10.2	22.9	13.1

Table 2. Results of analysis of FAPAC QC material TO4366QC by the four participating laboratories.

In each case, the relative retention times and ion ratios from the mycotoxins detected in the FAPAS QC materials matched those from analysis of the calibration standards within the prescribed acceptance criteria.[4] As ^{13}C-isotopically labelled analogues of the analytes were used as internal standards, the retention time of the analyte corresponded to that of its labelled internal standard within a tolerance of ±0.05 minutes.

Conclusion

The performance of the method was investigated using an interlaboratory study. Each laboratory successfully implemented the method as outlined in the published application note for the determination of 12 regulated mycotoxins. The laboratories demonstrated good accuracy and precision for the determination of the eight

mycotoxins in the two FAPAS QC materials. These results, which meet performance criteria set out by the European Commission, demonstrate the method is suitable for the monitoring mycotoxins in cereals for both official control and testing conducted by food business operators.

Scientists must validate the method in their own laboratories and demonstrate that the performance is fit for purpose and meets the needs of the relevant analytical control assurance system.

References

1. Commission Regulation (EC) No 1881/2006 of 19 December 2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs. *Off. J. Eur. Union* 2006, L364, 5–23.
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3. Dreolin N, Stead S (2019). LC-MS/MS Method Development and Validation for the Quantitative Determination of Regulated Mycotoxins in Cereal Grain Flours Using Simplified Sample Preparation Conditions on Xevo TQ-XS. Waters Application Note 720006685EN < https://www.waters.com/nextgen/us/en/library/application-notes/2019/lc-ms-ms-method-development-and-validation-for-the-quantitative-determination-of-regulated-mycotoxins-in-cereal-grain-flours-using-simplified-sample-preparation-conditions-on-xevo-tq-xs.html> .
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5. European Commission Regulation No. 519/2014 of May 16, 2014 amending Regulation (EC) No. 401/2006 as Regards Methods of Sampling of Large Lots, Spice and Food Supplements, Performance Criteria for T-2, HT-2 Toxin and Citrinin and Screening Methods of Analysis, *Off. J. Eur. Union* 2014 L147, 29–43.

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