

Note d'application

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 C₁₈ 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>](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>](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 ¹³C-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.

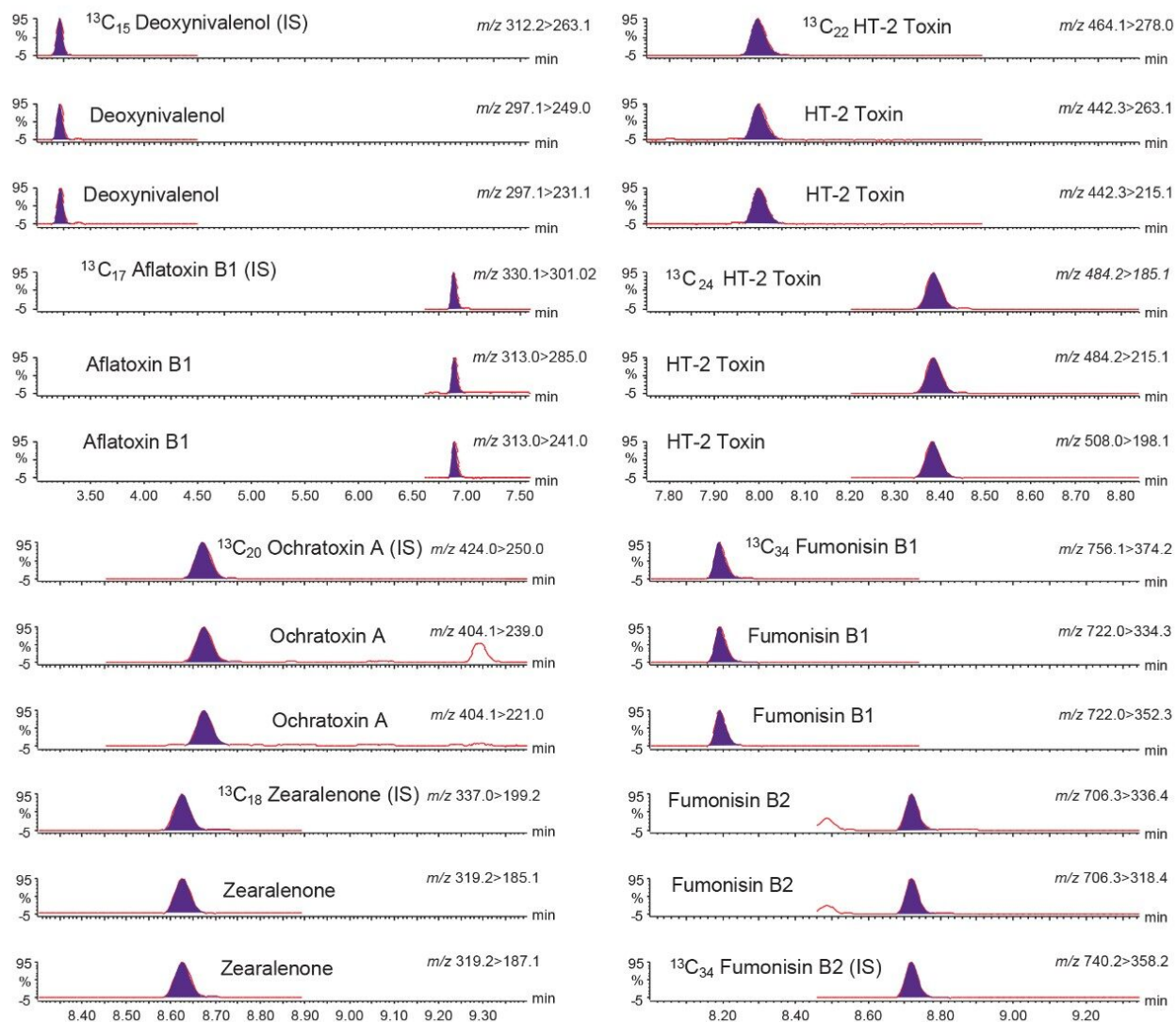
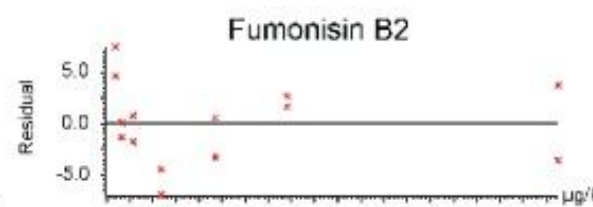
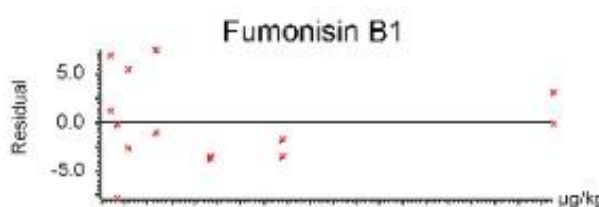
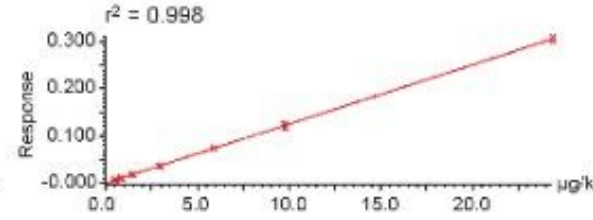
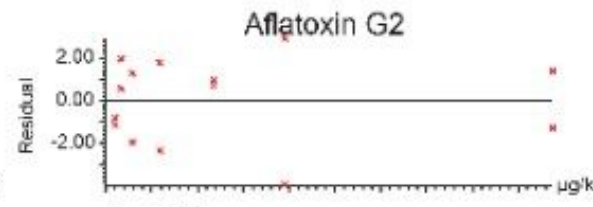
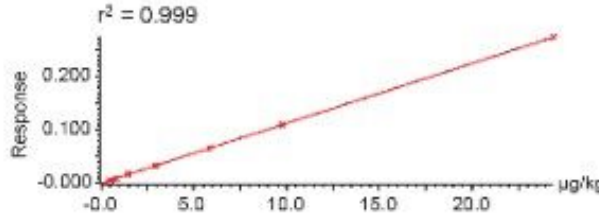
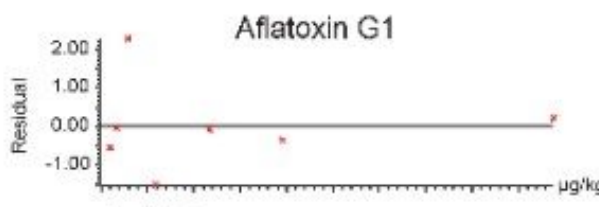
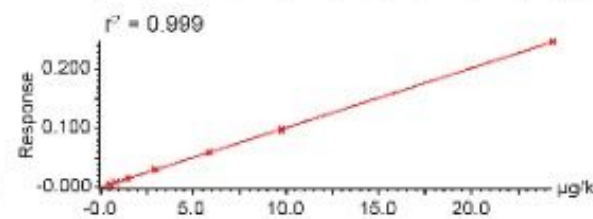
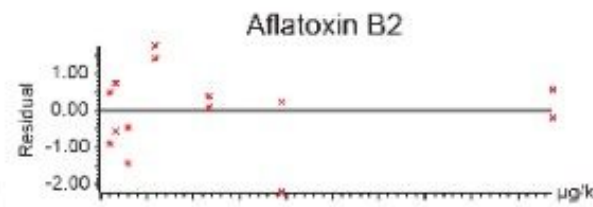
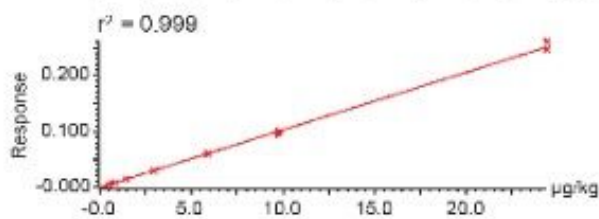
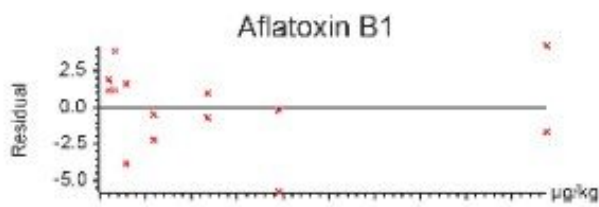
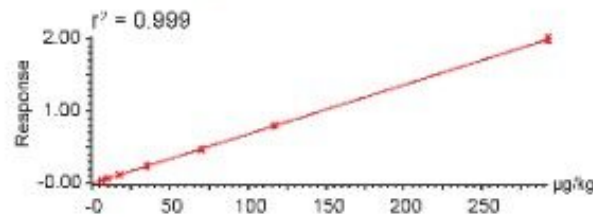
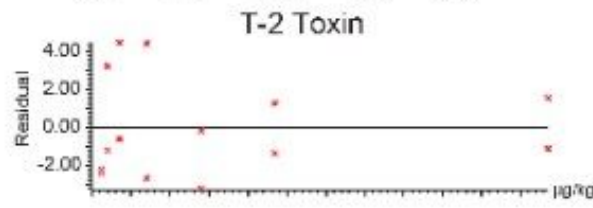
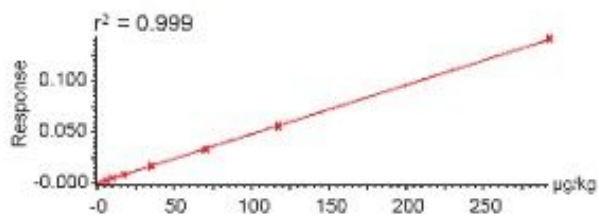
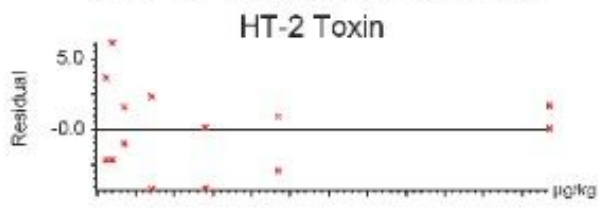
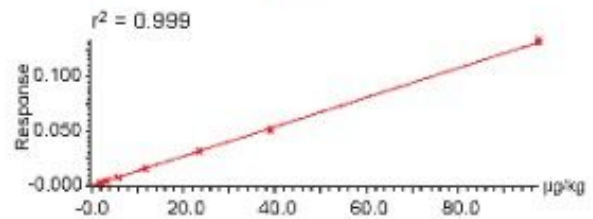
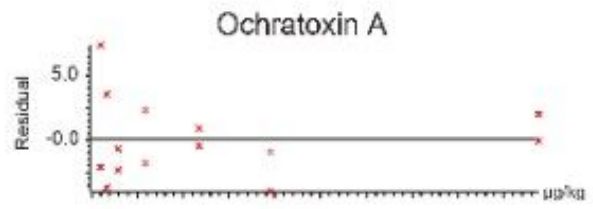
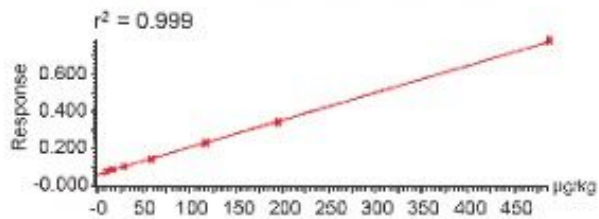
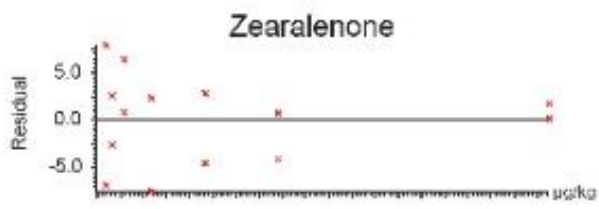


Figure 1. Typical chromatograms showing all eight mycotoxins detected from analysis of T04366QC and their labelled internal standards (for concentrations see Table 2).

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.



2. Commission Recommendation of 27 March 2013 on the Presence of T-2 and HT-2 Toxin in Cereals and Cereal Products (2013/165/EU). *Off. J. Eur. Union* 2013, L91, 12–15.
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) <
<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>> .
4. Document No. SANTE/12089/2016. Guidance Document on Identification of Mycotoxins in Food and Feed.
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.

Acknowledgements

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Featured Products

[ACQUITY UPLC I-Class PLUS System](https://www.waters.com/134613317) <<https://www.waters.com/134613317>>

[Xevo TQ-XS Triple Quadrupole Mass Spectrometry](https://www.waters.com/134889751) <<https://www.waters.com/134889751>>

[MassLynx MS Software](https://www.waters.com/513662) <<https://www.waters.com/513662>>

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