Development of a Multi-Toxin UHPLC-MS/MS Method for 50 Mycotoxins and Tropane Alkaloids in Cereal Commodities

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INTRODUCTION

The demand for mycotoxin testing has significantly increased over the last decade, in part carried out by the food/feed industry for due diligence, but also as a response to calls for data on occurrence. In this work, we describe the performance of an extension to the scope of an earlier application note, to provide a multi-toxin quantitative method for analysis of cereal-based foods using the high sensitivity of the Xevo™ TQ-XS Mass Spectrometer.

This method allows for simultaneous determination of more than 50 mycotoxins and plant toxins in a single LC-MS/MS method, and demonstrates the *MS Quan* application with the new *waters_connect*™ *quantitation* software, which reduces the time taken to process data and review results.

STANDARD AND SAMPLE PREPARATION

A mixture of wheat, barley, rice, and maize flours was extracted using a procedure based on a previous work. Briefly, 5.0 g of homogenized sample were placed in a 50 mL plastic centrifuge tube and extracted with 20 mL of 79:20:1 MeCN:H_2O:acetic acid (v/v/v), then placed on an automated Vortex for 10 minutes. After centrifugation for 6 minutes at >5000 *g*, 150 µL of supernatant were transferred into a LC vial, followed by the addition of 1350 µL of water (1:10 dilution) and filtered (glass fiber syringe filter) prior to LCMS/MS. The overall dilution factor equals 40.

Solvent-based standards were prepared by serial dilutions of a Stock Mix solution, maintaining a solvent composition of H_2O:MeCN 95:5 (v/v) 1. Matrix-matched standards were prepared over the same range by serial dilutions of a Stock Mix solution with a blank sample extract.

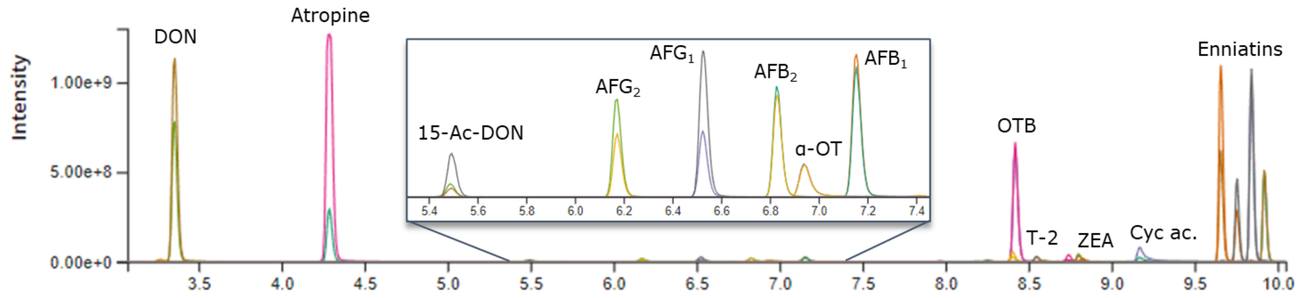

Figure 1. Chromatograms of the tested mycotoxins and plant toxins in two different spiked cereal samples. Each peak is characterized by a ***quan****tifier* and a ***qual****ifier* ion trace.

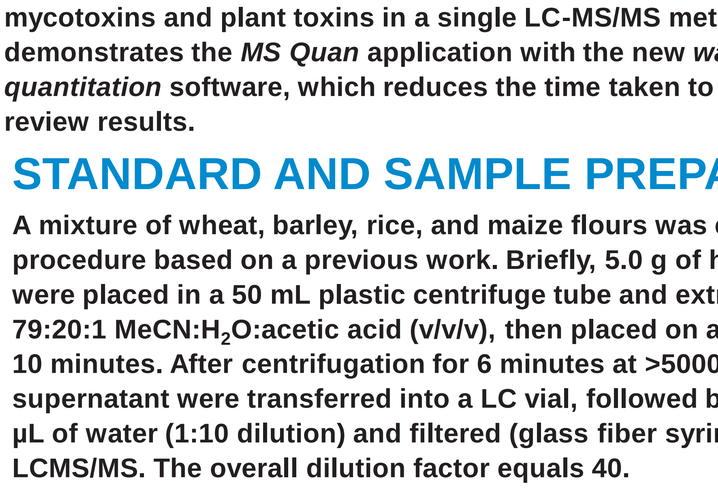

Figure 2. Distribution of method-limit of detection (m-LOD).

SENSITIVITY & QUANTIFICATION

From the analysis of solvent calibrants, extremely low instrument limits of detection (i-LOD) were achieved. For example, an i-LOD of 0.0003 ng/mL was determined for aflatoxins B1 , B2 , G1 , G2, and M1 . The method limits of quantification (m-LOQ), the lowest level of the matrix-matched standards with S/N >10 and which pass calibration acceptance criteria ($R^2 \geq 0.99$ and residuals ≤ 20%), were as low as 0.1 µg/kg for aflatoxins; ≤1 µg/kg for ochratoxins, sterigmatocystin, beauvericin, enniatins, atropine, scopolamine, and roquefortine C; while for the remaining compounds m-LOQs were ≤10 µg/kg, with the exception of nivalenol, zearalenone, and penitrem A.

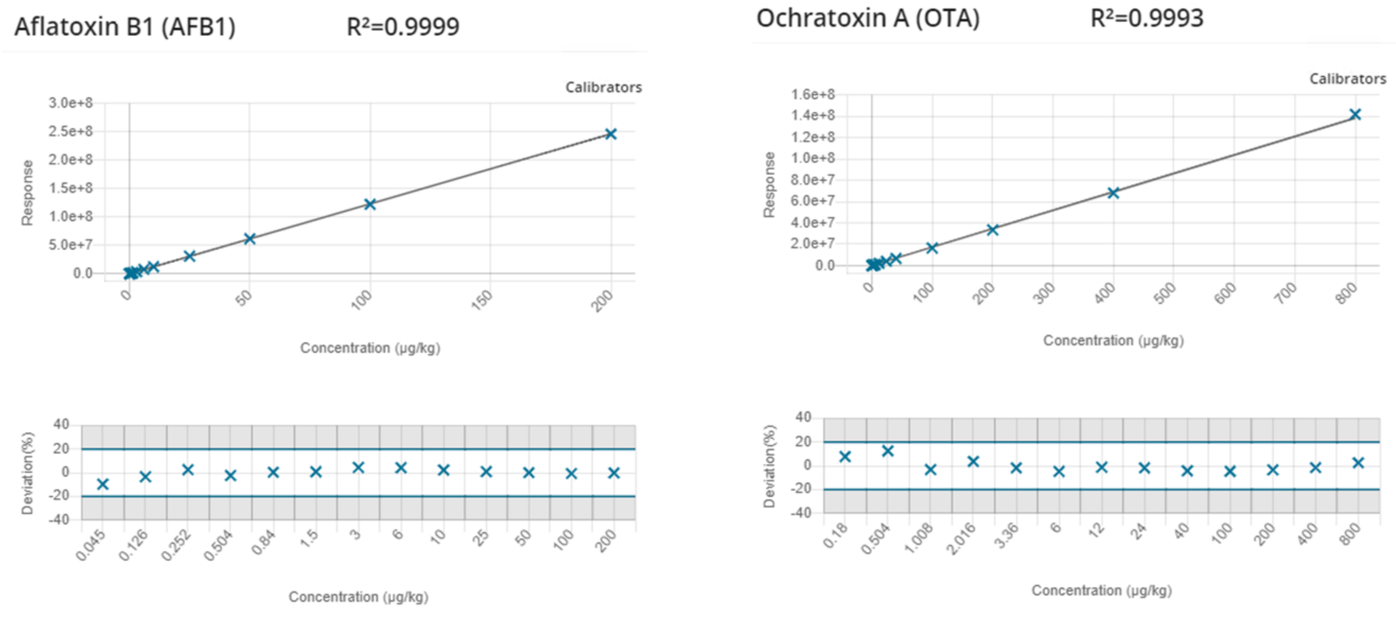

Figure 3. Matrix-matched calibration curves and residual plots of aflatoxin B_1 and ochratoxin A.

CHROMATOGRAPHY

Good chromatographic separation was achieved for almost all the targeted compounds using the previously developed UHPLC Metho d. With the exception of Moniliformin (0.71 minutes retention time), all compounds presented two MRM transitions and a retention time more than twice the retention time correspo nding to the void volume of the column (Vd ~ 0.55min), as per SANTE 12089/2016 guidelines. Moniliformin is a very unique mycotoxin given it is a small, highly polar, acidic molecule, so its determination using Hydrophilic Interaction Chromatography (HILIC) would be a better approach for any confirmation analysis.

The retention times of all the analytes were observed to be very stable with no variation greater than ±0.03 minutes within each sequence. The range of the calibration graphs was three orders of magnitude for most analytes. Coefficients of determination (R^2) of both solvent and matrix-matched calibration curves were almost all >0.99, and the calibration range was determined using only the standards with percentage residuals lower than 20%.

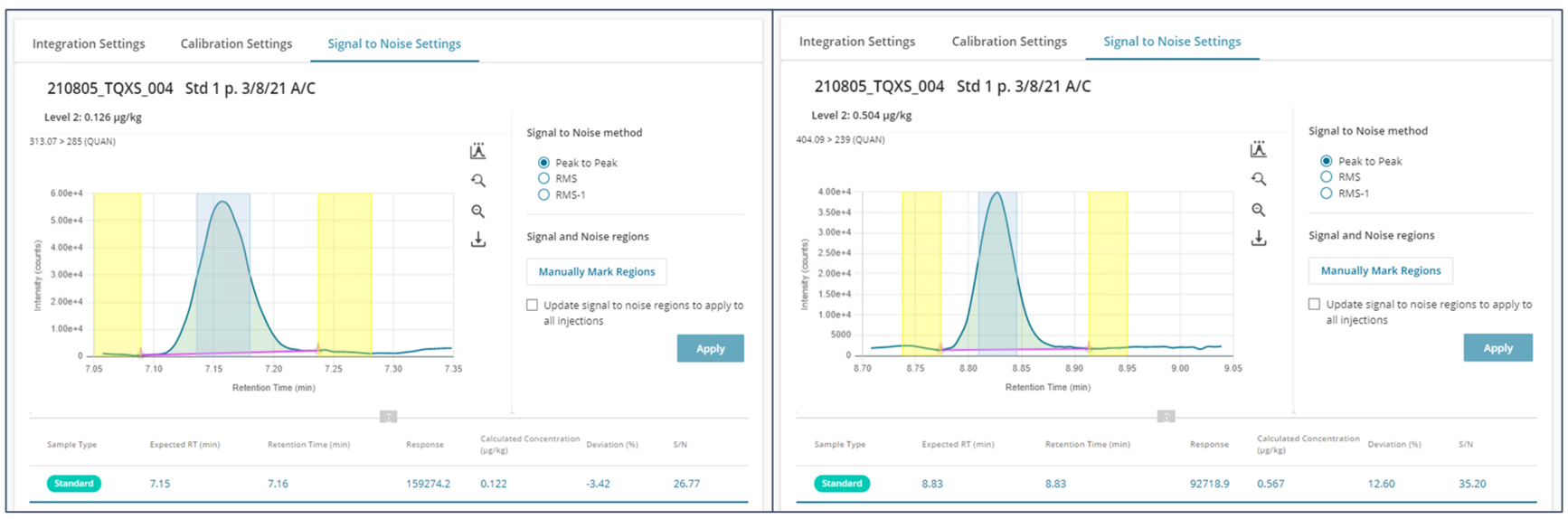

Figure 4. Chromatograms from the analysis of matrix-matched standards showing aflatoxin B_1 (0.1 µg/kg) and ochratoxin A (0.5 µg/kg) in the mixed cereal extract. For the calculation of signal-to-noise, the software algorithms automatically identified the signal (green) and noise regions (yellow).

CONCLUSION

The developed UPLC-MS/MS method is suitable for the quantitative determination of regulated mycotoxins, regulated tropane alkaloids, as well as a range of emerging and masked mycotoxins. Where available, the use of ^{13}C-labelled internal standards is recommended as an alternative to matrix-matched calibration.

The ultimate sensitivity of the Xevo TQ-XS Mass Spectrometer allows considerably dilution of the sample extract while still reaching extremely low limits of quantification. This in turn reduces the need for complicated sample preparation and as demonstrated herein, a simple and quick dilute-and-shoot approach can be applied. Nevertheless, the implementation of a clean-up step, such as a SPE pass-through using the Oasis™ PRiME HLB SPE cartridge, is an option to reduce the content of matrix co-extractives prior to injection, thus increasing method and instrument robustness.

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