A rapid and simple LC-MS/MS method for the quantification of the EU regulated mycotoxins in cereal-based products

Nicola Dreolin, Sara Stead, Janitha De-Alwis and Stuart Adams
Waters Corporation, Wilmslow, UK; nicola_dreolin@waters.com

INTRODUCTION

Many countries have maximum permitted levels for mycotoxins that pose the highest risk in different foodstuffs. Multi mycotoxins methods based upon LC-MS/MS offer an effective and efficient option to determine all regulated mycotoxins in a single method.

Here we describe the validation of a quantitative multi-mycotoxin method in wheat flour, which was further extended to a range of different cereal-based flours. The Xevo TQ-XS was chosen as it provides ultimate sensitivity, and it allows the analyst to implement a simple sample preparation process thus reducing the overall analysis time. The use of isotopically labelled internal standards was investigated in order to achieve better performance compared to the widely adopted external standard or standard addition methods. The analytical procedure was assessed according to criteria described by the European Commission Regulation No 401/2006 and SANTE guidelines.

INSTRUMENTAL CONDITIONS

UPLC System:	ACQUITY UPLC® I-Class-FL
MS:	XEVO TQ-XS
Column:	ACQUITY UPLC® BEH C_{18}, 1.7 µm; 2.1 x 100 mm (p/n 186002352)
Aqueous Mob. Phase:	1mM ammonium acetate in H_2O +0.5% AA +0.1% FA
Organic Mob. Phase:	MeOH +0.5% AA +0.1% FA
Column Temp:	40°C
Sample Temp:	15°C
Strong Needle Wash:	H_2O +20mM citric acid (20): MeOH (20) : MeCN (20): IPA (20): acetone (10): DMSO (10) + 1% FA
Weak Needle Wash:	H_2O:MeCN 1:1 +0.125mM EDTA
Injection volume:	15 µL

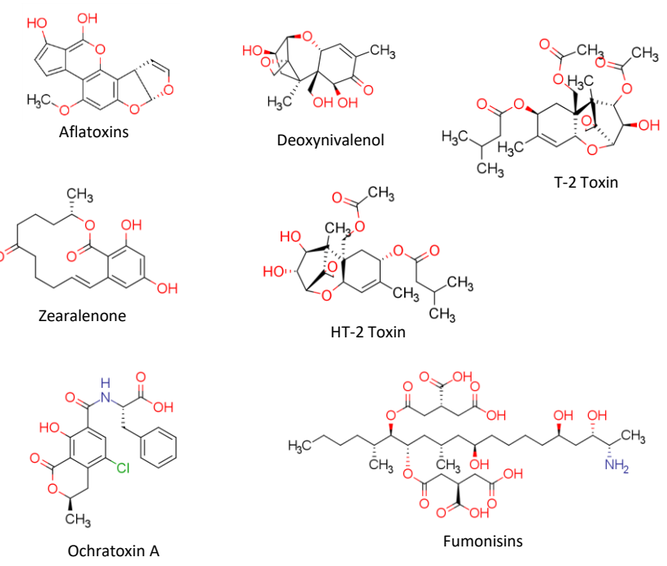


Figure 1. Chemical structure of some mycotoxins included in this method.

SAMPLE PREPARATION

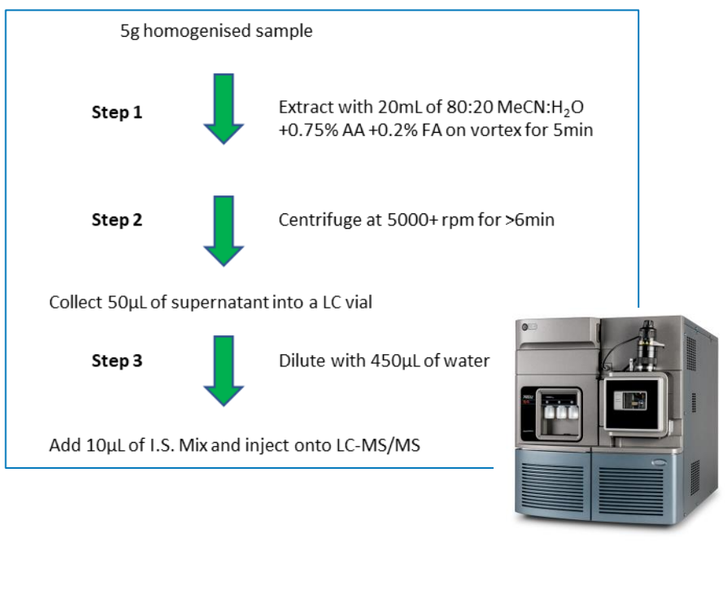
MATERIALS

Solvent calibration curves containing the 12 target mycotoxins were prepared by mixing and diluting the individual stock solutions, maintaining a solvent composition of H_2O:MeCN 95:5, to generate concentrations relevant to the appropriate MPLs. Eight calibration points (excluding the blank) were used for constructing the calibration series. An aliquot of the mixed Internal Standard solution (10µL) was added to 500µL of each level directly into LC vials.

SAMPLE EXTRACTION

Powdered and well-homogenized sample (5g) was weighed into a 50-mL plastic centrifuge tube and extracted with 20mL of MeCN:H_2O 80:20 +0.75% acetic acid +0.2% formic acid (shake vigorously or Vortex for 5min). After centrifugation at >5000*g*, 50µL of supernatant was transferred into an LC vial, followed by the addition of 450µL of water, resulting in a final dilution factor of 40 (i.e. concentration in sample [µg/kg] = concentration is vial [ng/mL] * 40). An aliquot of the mixed Internal Standard solution (10µL) was added to each LC vial prior to analysis.

Note. In the case of cloudy/viscous solutions post-dilution, the samples were filtered through 0.2µm glass fiber syringe filters.



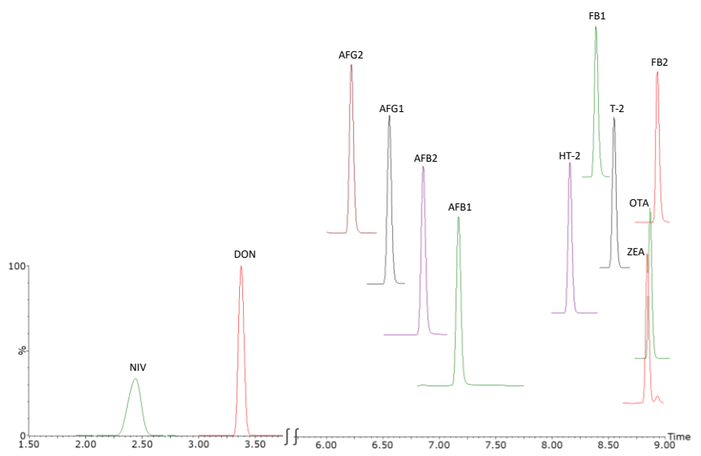


Figure 2. Chromatograms of 12 relevant mycotoxins in wheat samples.

RESULTS

LINEARITY, LIMITS OF DETECTION AND QUANTITATION (LOD & LOQ)

All regression equations showed coefficients of determination (R^2) between 0.9941 and 1.0000, and percentage residuals lower than 20%. The lowest spiking level, within the linear calibration range, was adopted as the method LOQ. Subsequently, the method LOD and LOQ were verified following Eurachem guidelines.[1]

Analyte	Instrumental LOD/LOQ (pg mL^{-1})	Method LOD/LOQ (µg kg^{-1})	Method linear range (µg kg^{-1})	Maximum permitted level in wheat (µg kg^{-1})[4]
AFB1	0.75/2.5	0.03/0.1	0.1 - 50	2.0
AFB2	0.93/3.1	0.04/0.1	0.1 - 50	4.0 (sum of B1, B2, G1 and G2)
AFG1	0.75/2.5	0.03/0.1	0.1 - 50	
AFG2	0.93/3.1	0.04/0.1	0.1 - 50	
FB1	75/250	3/10	10 - 2000	1000 (sum of B1 and B2 in maize-based food)
FB2	75/250	3/10	10 - 2000	
OTA	7.5/25	0.3/1.0	1.0 - 100	3.0
ZEA	37/123	1.5/5.0	5.0 - 500	75
HT-2	45/150	1.8/6.0	6.0 - 600	50 (sum of T-2 and HT-2, recommended value)[10]
T-2	45/150	1.8/6.0	6.0 - 600	
DON	90/300	3.6/12	12 - 2400	750
NIV	1500/5000	60/200	200 - 20000	-

EC Reg. No. 1881/2006

TUENESS AND INTRA-DAY REPEATABILITY

Percentage recovery was employed as an estimation of method trueness as determined using matrix fortified prior to extraction. A wheat flour sample was spiked with the 12 target mycotoxins at three different concentration levels, encompassing the method LOQs and bracketing the relevant MPLs within the linear range.

Recoveries (%) and Relative Standard Deviation (RSDr) were obtained under intra-day repeatability conditions from the analysis of seven independent replicates at each concentration level. Recoveries at three spiking levels ranged from 90 to 115%, whilst RSDr were below 10% in all cases.

MATRIX EFFECTS

Matrix effects ranged from >30% signal suppression for nivalenol, to >1000% signal enhancement for ochratoxin A. This finding justifies the use of the isotopically labelled internal standards to aid with quantitative accuracy, improve repeatability and to negate the effects of different matrices thus allowing the use of a calibration curve prepared using solvent standards.

REFERENCES

1. B. Magnusson and U. Ornemark (eds.) Eurachem Guide (2nd ed. 2014). ISBN 978-91-87461-59-0.

CARRYOVER MITIGATION

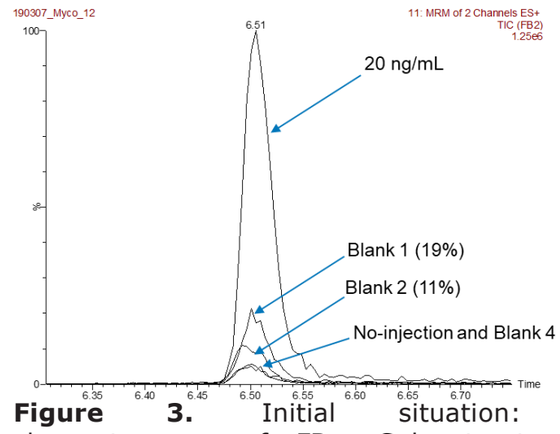


Figure 3. Initial situation: Overlaid chromatograms of FB_2: Solvent standard and subsequent solvent blank injections (carryover expressed as % area in blank compared to area in standard).

Dilution-adsorption carryover is due to chemical interactions between the analyte and the surface of materials in contact with the sample. The addition of citric acid or a different chelating agent was found to be beneficial in reducing adsorption on metal surfaces.

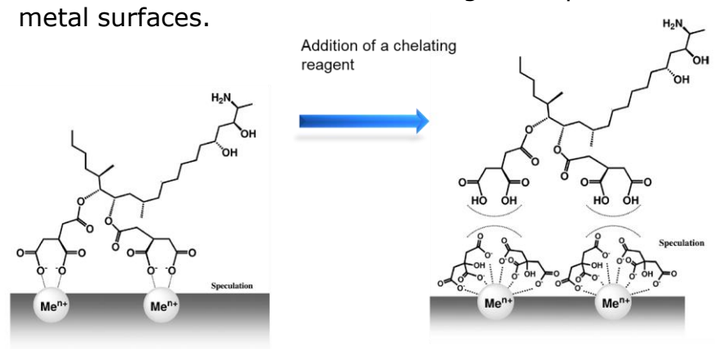


Figure 4. Metal chelation by FB_2 (right); shield effect by citric acid (left).

By performing a step-wise study, we found that the most likely sources of carryover in a FTN injector are the stainless-steel (SS) needle path and the needle seal. A significant reduction of carryover is achieved when replacing the FTN SS needle with the MP35N version (p/n 700011713).

In a FL system, the most likely sources of carryover can be attributed to the sample loop (made of stainless steel-based material) and to the Kalrez O-ring positioned at the bottom of the needle wash seal port (p/n 700002572) which can become contaminated over the time and may require replacing more often than the annual performance maintenance.

Both systems benefit from the use of an acidic washing solvent (below pH 3) to protonate the carboxylic acid moieties of fumonisins, and by adding a chelating agent. It is also possible to include a quick washing-step between batches or after a high-concentration standard/sample to control carryover to an acceptable level. A comprehensive study of the carryover is described in a white paper.

INTERLABORATORY STUDY

The performance of the method were evaluated via an interlaboratory study. Two cereal QC reference materials were sent to four laboratories in Europe and the USA. Each material was analyzed in triplicate by the four laboratories. 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%.

CONCLUSIONS

- **The ultimate sensitivity of Xevo TQ-XS allowed simplification of the sample treatment procedure.**
- **The incorporation of ^{13}C-labelled internal standards is recommended to correct for both matrix effects and losses during sample preparation.**
- **Method performance was assessed via an interlaboratory study with four laboratories.**
- **Carryover to an acceptable level by the careful selection of injector and washing solvents.**
- **The method can be applicable to different cereal-based matrices.**