REGULATED MYCOTOXINS IN CEREAL GRAIN FLOURS: A SIMPLIFIED SAMPLE PREPARATION AND LC-MS/MS METHOD

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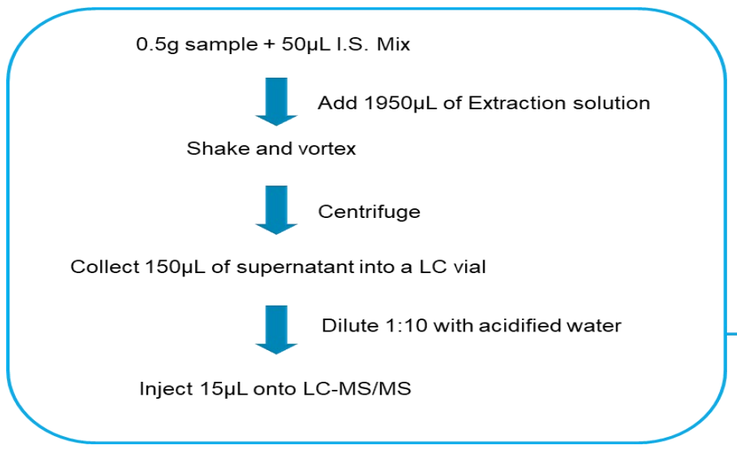
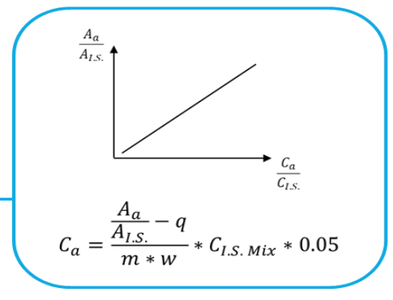
METHODS

- Powdered wheat spiked with I.S. mix and extracted with acidified MeCN:H_2O
- Centrifugation at ~5300*g* and collect supernatant
- Dilution 1:10 with acidified water and injection
- Analysis on ACQUITY I-Class FL coupled with Xevo TQ-XS
- Validation following Reg. EU 519/2014 [1] and SANTE/12089/2016 [2]
- Method applied to different cereal flours (oat, maize, rice, potato, tapioca)





RESULTS

- Washing solvents & mobile phase composition finely optimised to lower carryover of fumonisins whilst maintaining good separation and peak shape.

- The lowest detection capability was recorded for aflatoxins (instrumental LODs between 11 and 14 fg on column), while for others targeted analytes instrumental LOD were between 0.1 and 22.5 pg on column.

- The use of ^{13}C-labelled internal standards showed greater analytical performance compared to the external standardisation.

- Recoveries at three spiking levels ranged from 90 to 115%, whilst RSDr were below 10% in all cases (n=3).

- A mix of mycotoxins was spiked to oat and to a mixture of different flours at the LOQ level. Recoveries met the criteria set by the European Regulation (81-114%).

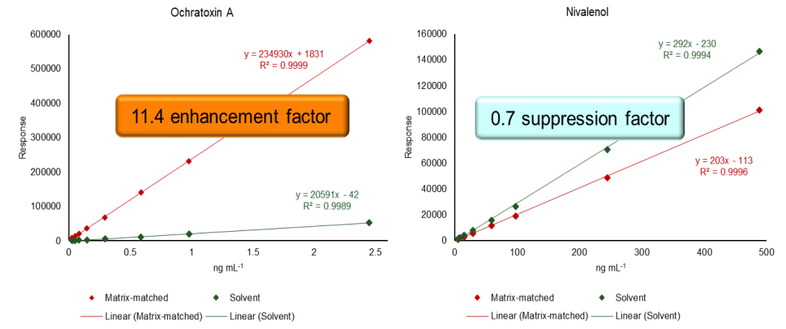
Simplified quantitative "**dilute-shoot**" method for the analysis of regulated mycotoxins in cereal-based food using LC-MS/MS

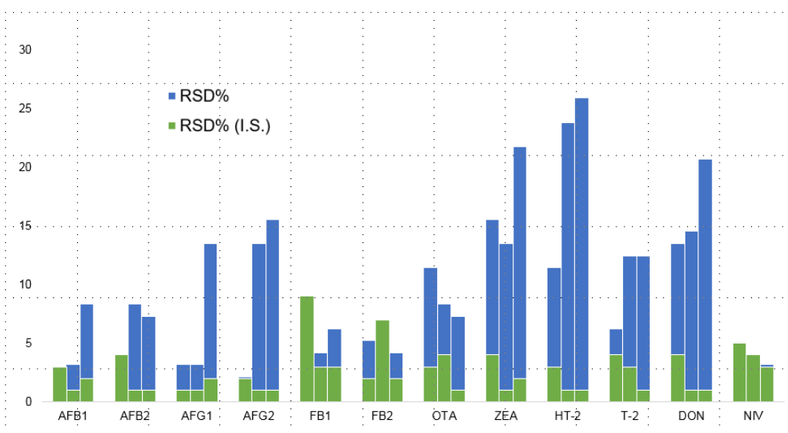
DISCUSSION

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. [3]

Matrix effects ranged from >30% signal suppression for nivalenol, to >1000% signal enhancement for ochratoxin A . This finding clearly 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.

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})
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)
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	-





CONCLUSIONS

- The method is "fit-for-purpose" for the quantitative analysis of EU regulated mycotoxins in dried cereal grain commodities such as wheat, oats, maize, rice, buckwheat-based food products.

- The excellent sensitivity of the TQ-XS and the selectivity of the MRM acquisition mode, made possible the extreme simplification of the sample treatment procedure.

- The incorporation of ^{13}C-labelled internal standards within the analytical workflow leads to enhanced method performance and is therefore recommended as an efficient approach to correct for both matrix effects and the inevitable analyte losses during the sample preparation.

- Internal standardisation allows the analyst to avoid the use of matrix-matched calibration and work with solvent calibration curves for accurate quantitation.

References

1. Commission Regulation (EU) 519/2014 on sampling and performance criteria for T2, HT2 and citrinin, 2014.
2. Guidance on identification of mycotoxins in food and feed, SANTE/12089/2016, 2017.
3. The Fitness for Purpose of Analytical Methods- A Laboratory Guide to Method Validation and Related Topics, Eurachem, 2014.

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