Analysis of Mycotoxins in Food Matrices using the Innovative Ultivo Triple Quad LC/MS

Theresa Sosienski[1]; Dan-Hui Dorothy Yang[1]; Joan Stevens[2]; Christian Hegmanns[3]

[1]Agilent Technologies Inc., Santa Clara, CA; [2]Agilent Technologies Inc., Wilmington, DE; [3]Agilent Technologies Sales & Services GmbH & Co. KG, Waldbronn, BW

AOAC 2017
P-T-026

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Introduction

Mycotoxins are compounds produced by fungi which grow on various crops ranging from grains to fruits, vegetables, nuts and spices. Mycotoxins can be harmful to humans and livestock through consumption of contaminated crops; therefore, mycotoxin levels are monitored in foods to minimize the risk of ingestion.[1] Regulatory agencies around the globe set Maximum Levels (ML), which range from <10 to >500ppb to ensure harmful levels of mycotoxins do not enter the food supply. It is important to have the ability to detect and accurately quantify the mycotoxin contents at low levels across various food matrices, as each matrix composition poses different challenges in detection.

In this study, we demonstrate the accurate and sensitive quantification of up to 12 regulated mycotoxin compounds in three commonly regulated foods using the novel Ultivo triple quad LC/MS. (Figure 1)

Agilent Ultivo Triple Quad LC/MS

Figure 1: Ultivo Integrated into LC Stack

Ultivo is designed to address many of the challenges faced by labs performing environmental and food safety analyses.

Innovative technologies housed within Ultivo allowed us to achieve a reduced overall footprint, while conserving the performance found in many larger MS systems.

Innovations such as VacShield, Cyclone Ion Guide, Vortex Collision Cell and the Hyperbolic Quads maximize quantitative performance in a small package, enhancing instrument reliability and robustness resulting in greater uptime.

Ultivo reduces the need for user intervention for system maintenance, making it attractive to the non-expert MS user to operate and maintain.

MassHunter simplifies data acquisition, method setup, data analysis and reporting. This results in the fastest acquisition-to-reporting time possible, increasing lab productivity and confidence in results

Experimental

Sample Preparation

Corn, peanut, and black pepper were chosen as commonly regulated food crops of diverse matrix components for mycotoxins. 12 mycotoxins in corn and peanut matrices, and 5 mycotoxins in black pepper matrix were quantified using dynamic MRM in a 9 minute LC/MS/MS method. Mycotoxin standards were spiked into matrix extracts for analysis.

5g corn flour, 5g peanuts, or 2g black pepper were extracted with 10 mL of ACN, 10 mL H_2O and EN Extraction Salts (5982-5650). Dispersive SPE for fruits and vegetables (5982-5058) was used on corn, and a universal dispersive SPE kit (5982-0029) was used for black pepper. A novel modified lipid removal sorbent in flow through cartridge format was used on each matrix as a final clean-up step. Spiked black pepper extracts were diluted 30:70 extract/water prior to analysis.

Instrument Parameters

1290 Infinity II LC Parameters		
Column	Eclipse Plus C18 3.0 x 150 mm, 1.8µm	
Column temp	45°C	
Injection volume	2 µL Corn,Peanut; 10 µL Black Pepper	
Mobile phase	A: Water, 0.5mM NH_4F +5 mM NH_4 formate+ 0.1% Formic Acid B: MeOH, 0.5mM NH_4F +5mM NH_4 formate+ 0.1% Formic Acid	
Flow rate	0.450 mL/min	
Gradient	Time	B%
	0	30
	0.5	30
	7.5	100
	9.0	100
	9.1	30

Ultivo Triple Quad MS Parameters	
Drying gas temp	250 °C
Drying gas flow	8 L/min
Sheath gas temp	350 °C
Sheath gas flow	12 L/min
Nebulizer pressure	30 psi
Capillary voltage	3300 V(+); 2800 V(-)
Nozzle voltage	0 V(+); 0 V(-)
Cycle Time	500 ms

Table 1: LC and MS Parameters

Results and Discussion

Mycotoxin Signal Response

Excellent precision and sensitivity was attained for mycotoxins in various food matrices due to a combination of sample preparation techniques, LC separation, and the innovative technology in the Ultivo triple quadrupole mass spectrometer.

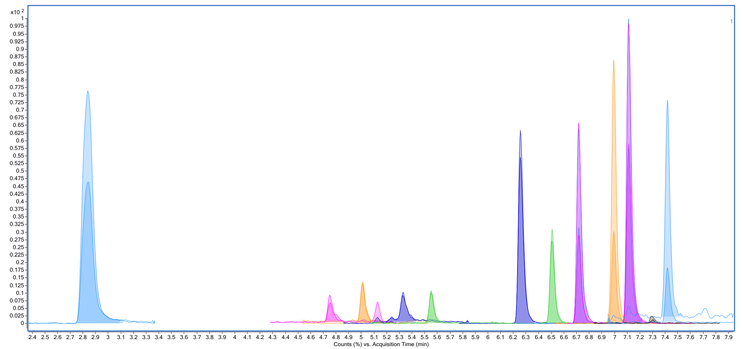

Figure 2: Excellent detection of mycotoxins in corn matrix at 1/10 assigned Maximum Levels (ML).

Mycotoxin Maximum Residue Limits and Sensitivity

Outstanding sensitivity was achieved, with the majority of the mycotoxin compounds in each matrix studied reaching a limit of quantitation (LOQ) at 1/20 the Assigned Maximum Levels (ML).

	European Union ML for Mycotoxins.[2,3]			Assigned ML used for this study.	
Mycotoxin	Corn	Peanut	Black Pepper	Corn and Peanut	Black Pepper
Aflatoxin B1	2 ppb	2 ppb	5 ppb[a]	2 ppb	5 ppb
Aflatoxin B2	Sum of Aflatoxins: 4 ppb	Sum of Aflatoxins: 4 ppb	Sum of Aflatoxins: 10 ppb	2 ppb	5 ppb
Aflatoxin G1				2 ppb	5 ppb
Aflatoxin G2				2 ppb	5 ppb
Ochratoxin A	3 ppb	n/a	15 ppb[b]	3 ppb	15 ppb
Fumonisin B1	Sum of B1 and B2: 1000ppb	n/a	n/a	500 ppb	Not included
Fumonisin B2			n/a	500 ppb	Not included
Fumonisin B3	n/a	n/a	n/a	500 ppb	Not included
Deoxynivalenol	750 ppb	n/a	n/a	75 ppb	Not included
Zearalenone	100 ppb	n/a	n/a	100 ppb	Not included
T-2 Toxin	n/a	n/a	n/a	100 ppb	Not included
HT-2 Toxin	n/a	n/a	n/a	500 ppb	Not included

Table 2: Maximum Levels (ML) for mycotoxins used in this study. EU reg No. 1881/2006 and 105/2010 used for reference. All assigned MLs in this study are equal to or lower than EU ML.

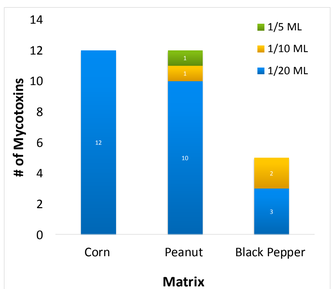

Figure 3: Quantitation limit for all mycotoxins studied in each matrix, defined as a fraction of the assigned ML.

Results and Discussion

Precision and Linearity of Mycotoxin Compounds

Great accuracy was achieved for all compounds studied in each matrix, even in the complex black pepper matrix. Excellent linearity was achieved over each calibration range.

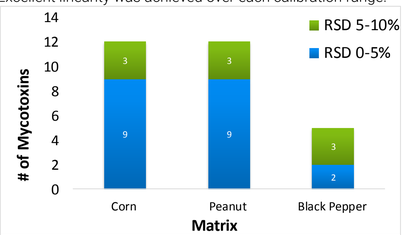

Figure 4: The mycotoxins show excellent precision in all three matrices, with all RSD<10% at LOQ.

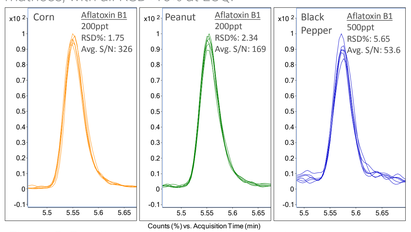

Figure 5: Excellent precision demonstrated for Aflatoxin B1 at 1/10 ML (200 ppt or 500ppt) in all matrices.

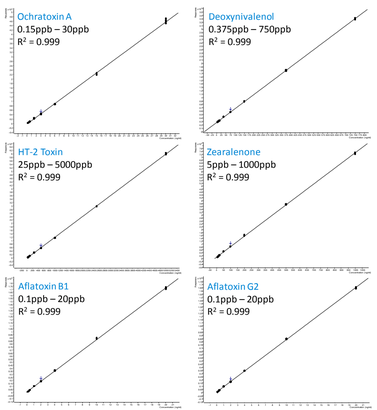

Figure 6: Exceptional linearity was demonstrated for all compounds, with R^2 values ≥0.99 for all compounds in all matrices. Pictured above, examples of excellent linearity for 6 selected compounds in corn matrix.

Conclusions

- Ultivo is an exceptionally innovative new mass spectrometer, which can minimize laboratory workspace needs, as well as reducing maintenance challenges, creating a productive work environment for high throughput laboratories.
- Ultivo is a small but powerful tool enabling the accurate and sensitive detection of commonly regulated mycotoxins in various food matrices well below set ML.
- Agilent MassHunter software provides an easy to use, all-inclusive tool for managing and reporting LC/MS data.

References

[1] Bennett JW, Klich M. Mycotoxins. Clinical Microbiology Reviews. 2003;16(3):497-516. 2003.
[2] Commission Regulation (EC) No 1881/2006. Setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union. L 364/5-24. 19 December 2006.
[3] Commission Regulation (EU) No 105/2010. Amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards ochratoxin A. L 35/7-8. Official Journal of the European Union. 5 February 2010