ANALYSIS OF MYCOTOXINS IN FOOD MATRICES

Agilent Ultivo Triple Quadrupole LC/MS System

Figure 1. Agilent Ultivo Triple Quadrupole LC/MS integrated into the Agilent 1260 Infinity II Prime LC.

Introduction

Mycotoxins are compounds produced by fungi that grow on 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 Residue Limits (MRLs), which range from less than 10 ng/g (ppb) to more than 500 ppb to ensure harmful levels of mycotoxins do not enter the food supply. It is important to detect and accurately quantify mycotoxin contents at low levels across various food matrices, as each matrix composition poses different detection challenges.

This study demonstrates the accurate quantification of up to 12 regulated mycotoxin compounds in three commonly regulated foods using the Agilent Ultivo Triple Quadrupole LC/MS. (Figure 1).

For more information, visit:
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Agilent Technologies

Agilent Ultivo Triple Quadrupole LC/MS

Ultivo is designed to address many of the challenges faced by labs performing environmental and food safety analyses. The 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 Hyperbolic Quads maximize quantitative performance in a small package, enhancing instrument reliability and robustness, resulting in greater uptime. Ultivo reduces user intervention for system maintenance, making it easy for the nonexpert MS user to operate and maintain.

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

Experimental

Sample preparation

Corn, peanut, and black pepper were chosen as commonly regulated food crops of diverse matrix components for mycotoxins analysis. Twelve mycotoxins in corn and peanut matrices, and five mycotoxins in black pepper matrix were quantified using dynamic MRM (dMRM) in a 9-minute LC/Triple Quad method. Mycotoxin standards were post-spiked into matrix extracts for analysis.

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

Instrument parameters

Table 1. LC and MS parameters.

Agilent 1290 Infinity II LC	
Column	Agilent Eclipse Plus C18, 3.0 × 150 mm, 1.8 µm
Column temperature	45 °C
Injection volume	2 µL (Corn and peanut extract); 10 µL (black pepper extract)
Mobile phase	A) Water, 0.5 mM ammonium fluoride + 5 mM ammonium formate + 0.1 % formic acid B) MeOH, 0.5 mM ammonium fluoride + 5 mM ammonium formate + 0.1 % formic acid
Flow rate	0.450 mL/min
Gradient	Time (min) %B 0 30 0.5 30 7.5 100 9.0 100 9.1 30
Agilent Ultivo	
Drying gas temperature	250 °C
Drying gas flow	8 L/min
Sheath gas temperature	350 °C
Sheath gas flow	12 L/min
Nebulizer pressure	30 psi
Capillary voltage	3,300 V(+), 2,800 V(–)
Nozzle voltage	0 V(+), 0 V(–)
Cycle time	500 ms

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 designed into the Ultivo triple quadrupole mass spectrometer.

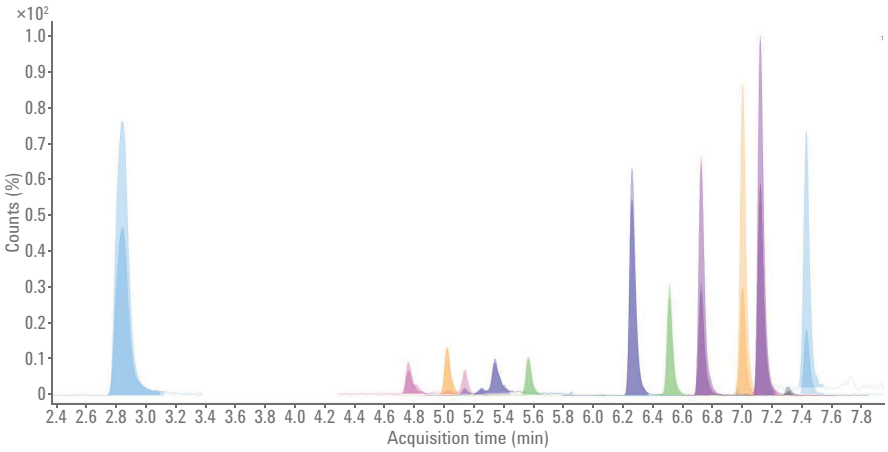

Figure 2. Detection of mycotoxins at 1/10 MRL in corn matrix.

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 MRL.

Table 2. Maximum residue limits for mycotoxins and specific matrices used in this study. EU reg No. 1881/2006 and 105/2010 used for reference. All assigned MRLs in this study are equal to or lower than SANTE MRLs.

	European Union MRL for mycotoxins[2,3]			Assigned MRL level 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	500 ppb	n/a	n/a	500 ppb	Not included
Fumonisin B2	Sum of Fumonisins: 1,000 ppb	n/a	n/a	500 ppb	Not included
Fumonisin B3		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

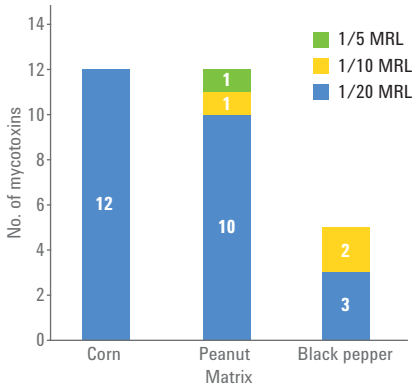

Figure 3. Quantitation limit for all mycotoxins studied in each matrix, defined as a fraction of the assigned MRL.

Precision and linearity of mycotoxins

Excellent linearities were obtained for all compounds in this study, with R^2 values >0.99 over the calibration ranges used. Precision values obtained for mycotoxins in the matrices investigated rendered %RSD values of <10 % at the LOQs.

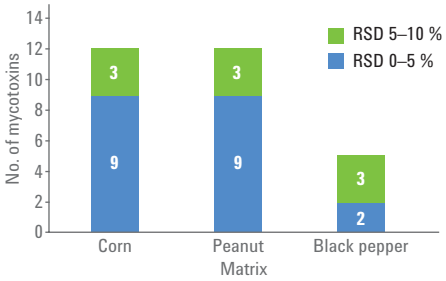

Figure 4. The mycotoxins show excellent precision in all three matrices, with all RSD <10 % at LOQ.

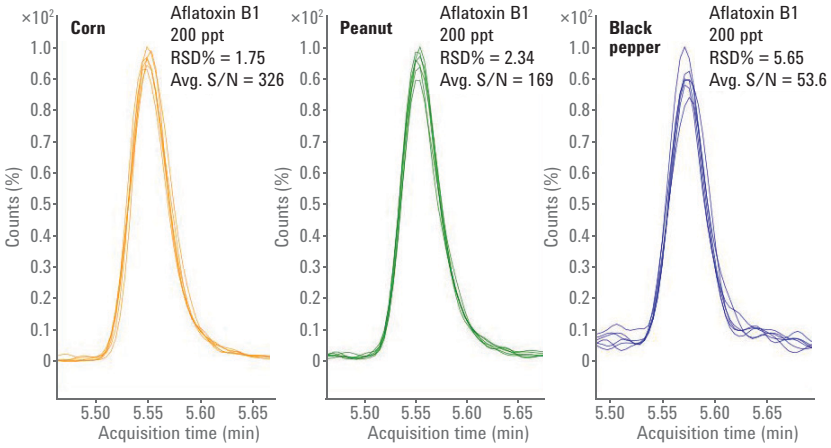

Figure 5. Excellent precision demonstrated for Aflatoxin B1 at 1/10 MRL (200 ppt or 500 ppt) in all matrices.

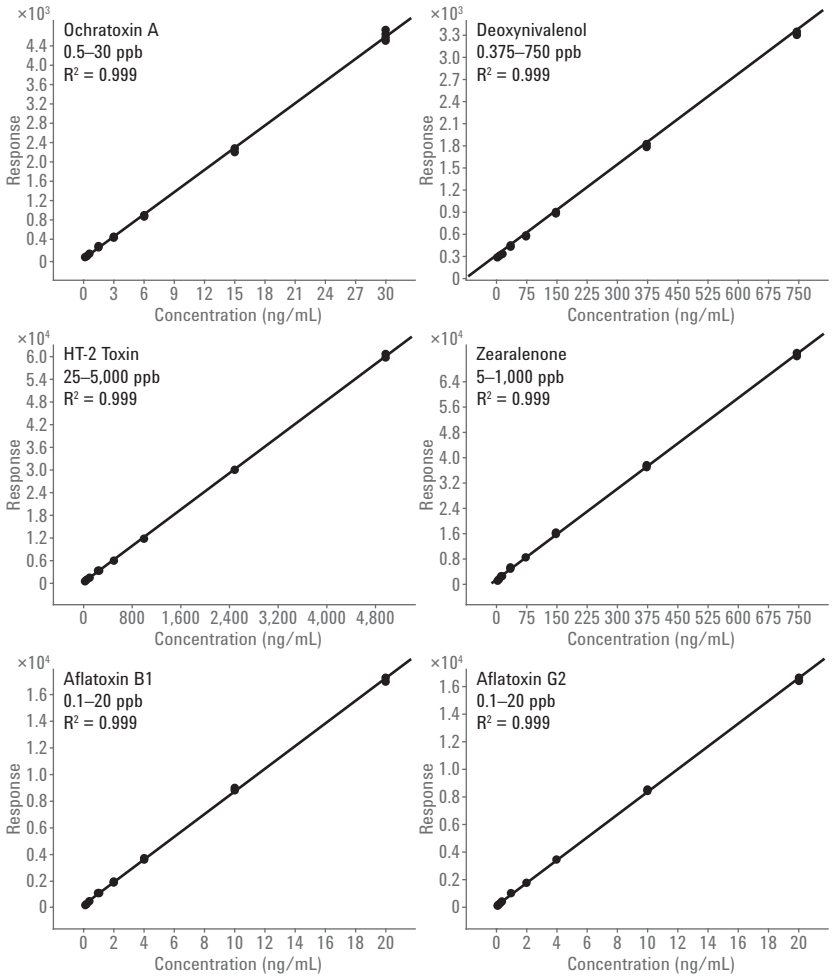

Figure 6. Exceptional linearity was demonstrated for all compounds, with R^2 values ≥0.99 for all compounds, in all matrices. Pictured above, examples of linearity for 6 mycotoxins in corn matrix.

Conclusions

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

References

1. Bennett, J. W.; Klich, M. Mycotoxins. Clinical Microbiology Reviews **2003**, *16(3)*, 497-516.
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**.
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