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 $<10$ to $>500$ 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 and sensitive 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:
www.agilent.com/chem/Ultivo
**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 in results.

**Experimental**

**Sample preparation**

Corn, peanut, and black pepper were chosen as commonly regulated food crops of diverse matrix components for mycotoxins. 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 spiked into matrix extracts for analysis.

A 5 g sample of corn flour, 5 g of peanuts, or 2 g of black pepper were extracted with 10 mL of ACN, 10 mL H₂O, 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 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.
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.
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 1/20th the assigned MRL.

Table 2. Maximum residue limits for mycotoxins 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.

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>EU MRL for mycotoxins</th>
<th>Assigned MRL level used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin B1</td>
<td>2 ppb</td>
<td>2 ppb</td>
</tr>
<tr>
<td>Aflatoxin B2</td>
<td>Sum of Aflatoxins: 4 ppb</td>
<td>Sum of Aflatoxins: 2 ppb</td>
</tr>
<tr>
<td>Aflatoxin G1</td>
<td>2 ppb</td>
<td></td>
</tr>
<tr>
<td>Aflatoxin G2</td>
<td>2 ppb</td>
<td></td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>3 ppb</td>
<td>3 ppb</td>
</tr>
<tr>
<td>Fumonisin B1</td>
<td>500 ppb</td>
<td>500 ppb</td>
</tr>
<tr>
<td>Fumonisin B2</td>
<td>Sum of Fumonisins: 1,000 ppb</td>
<td>500 ppb</td>
</tr>
<tr>
<td>Fumonisin B3</td>
<td>N/A</td>
<td>500 ppb</td>
</tr>
<tr>
<td>Deoxynivalenol</td>
<td>750 ppb</td>
<td>75 ppb</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>100 ppb</td>
<td>100 ppb</td>
</tr>
<tr>
<td>T-2 Toxin</td>
<td>N/A</td>
<td>100 ppb</td>
</tr>
<tr>
<td>HT-2 Toxin</td>
<td>N/A</td>
<td>500 ppb</td>
</tr>
</tbody>
</table>

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 >0.999 over the calibration ranges used. Precision values obtained for mycotoxins in the matrices investigated rendered %RSD values of <10 % at the LOQs.

Figure 4. Calibration curves for PFOS, PFOA, N-EtFOSAA, and PFBS.

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


Figure 6. Exceptional linearity was demonstrated for all compounds, with r2 values ≥0.99 for all compounds, in all matrices. Pictured above, examples of linearity for 6 mycotoxins in corn matrix.