Application Note

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Analysis of mycotoxins in food matrices using the Agilent Ultivo Triple Quadrupole LC/MS

Figure 1. Agilent Ultivo Integrated into LC stack.

Authors

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Abstract

This Application Note demonstrates a sensitive and precise method for analyzing 12 mycotoxins in corn and peanut matrices and five mycotoxins in black pepper matrix on the Agilent Ultivo triple quadrupole mass spectrometer. The Ultivo LC/MS was designed to save laboratory space, while maintaining the performance required for high-throughput analyses. All mycotoxins could be quantified below the maximum levels defined by the European Union Commission Regulations (EC) No. 1881/2006 and No. 105/2010 in each matrix. Excellent method precision was achieved on the Ultivo system, with relative standard deviations (%RSD) of <10 % at the lowest level of quantitation. The combination of the cleanup of matrix interferences, chromatography, and the newly developed triple quadrupole allows for sensitive and precise detection of mycotoxins.

Introduction

Mycotoxins are produced by fungi that can grow on various crops. At certain levels and combinations, mycotoxins can be harmful to humans and livestock through consumption of contaminated crops; therefore, mycotoxin levels are regulated in foods to minimize the risk of ingestion[1]. To ensure that harmful levels of mycotoxins do not enter the food supply, regulatory agencies have established maximum levels (MLs), ranging from 0.1 ppb in some baby foods to ppm levels in some animal feeds. It is important to accurately quantify the mycotoxin contents below the ML across various food matrices, as each matrix composition poses different challenges.

The Agilent Ultivo Triple Quadrupole LC/MS is designed to address many of the challenges faced by labs performing environmental and food safety analyses. Innovative technologies within the Ultivo allowed us to achieve a reduced overall footprint, while conserving the performance found in traditional systems (Figure 1). Innovations such as the Cyclone Ion Guide, Vortex Collision Cell, and the Hyperbolic Quads maximize quantitative performance in a small package. These innovations enhance instrument reliability and robustness, resulting in greater uptime. VacShield and easy change of detector assembly reduce the time and expertise required for system maintenance, making it easier for the nonexpert MS user to operate and maintain. The Agilent MassHunter software suite simplifies data acquisition, method setup, data analysis, and reporting. This software leads to faster acquisition-to-reporting time, increasing lab productivity and confidence in results.

This Application Note demonstrates the sensitive and precise quantification of up to 12 regulated mycotoxin compounds in three commonly regulated food commodities using the novel Ultivo triple quad LC/MS.

Experimental

Reagents and chemicals

All reagents used in this application were HPLC or LC/MS grade. Acetonitrile was purchased from Honeywell (Morristown, NJ, USA), and ultrapure water was sourced from a Milli-Q Integral system with a LC-Pak Polisher and a 0.22-µm point-of-use membrane filter cartridge (EMD Millipore, Billerica, MA, USA). Formic acid (FA) and ammonium formate were purchased from Fluka (Sigma-Aldrich Corp., St. Louis, MO, USA), and ammonium fluoride was purchased from Aldrich (Sigma-Aldrich Corp., St. Louis, MO, USA). Chemical standards were purchased from Sigma-Aldrich or Cayman Chemical.

Sample preparation

Corn flour, peanuts, and ground black pepper were obtained from local grocery stores. Five grams of corn flour, 5 g of ground peanuts (ground in a blender, and frozen until analysis), or 2 g of ground black pepper were weighed into 50-mL polypropylene tubes and extracted using 10 mL of acetonitrile and 10 mL of ultrapure H_2O with 0.2 % FA and shaken using a rotary shaker (Heidolph Hei-MIX Multi reax, 545-10000-00) for 30 minutes. Agilent QuEChERS EN Extraction Salts (p/n 5982-5650) were added to the extract, and the tubes were shaken for a further 2 minutes, then centrifuged for 5 minutes at 4,500 rpm (Sorvall Heraeus, Labofuge 400K). Black pepper extract was subjected to an additional cleanup step using an Agilent universal dispersive SPE kit (p/n 5982-0029). Then, 1.6 mL of all extracts were diluted with 0.4 mL of ultrapure water, and cleaned using Agilent Captiva EMR—Lipid cartridges (p/n 5190-1003). Spiked black pepper extracts were diluted 30:70 extract/ultrapure water before analysis. Figure 2 presents the flowchart of sample preparation.

Instrumentation

Agilent 1290 Infinity II UHPLC

- Agilent 1290 Infinity high speed pump (G7120A)
- Agilent 1290 Infinity II multisampler with cooler (G7167B)
- Agilent 1290 Infinity II multicolumn thermostat (G7116B)

Agilent Ultivo Triple Quadrupole LC/MS system

- Agilent Jet Stream electrospray ionization source

Method

Table 1 summarizes the Agilent 1290 Infinity II UHPLC conditions. Table 2 summarizes the Ultivo Triple Quadrupole parameters and Agilent Jet Stream ESI source parameters. Analysis was carried out with positive ionization and dynamic multiple reaction monitoring (dMRM). Data were evaluated using the Agilent MassHunter Quantitative Analysis Software B.09 with the Quant-My-Way feature.

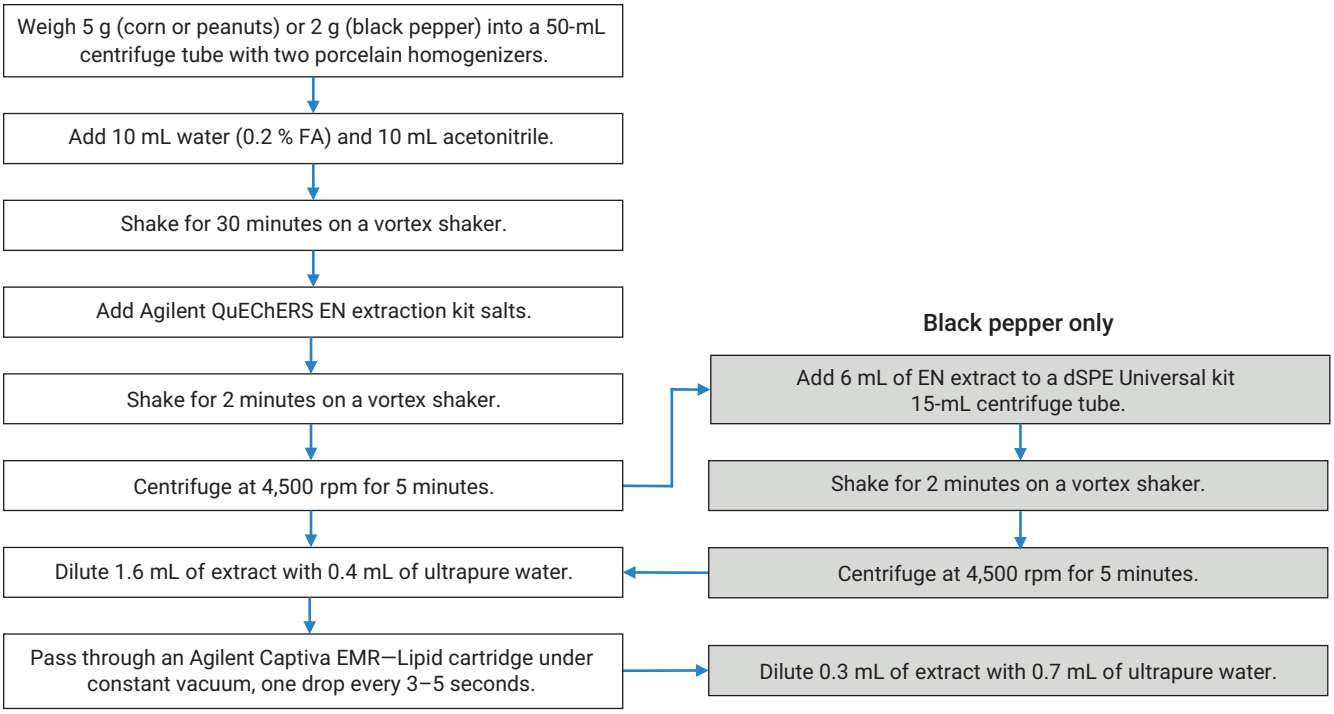

Figure 2. Sample preparation procedure using Agilent Captiva EMR—Lipid for the analysis of mycotoxins in corn, peanuts, and black pepper.

Table 1. Agilent 1290 Infinity II UHPLC parameters.

Parameter	Value
Column	Agilent Eclipse Plus C18 3.0 × 150 mm, 1.8 µm (p/n 959759-302)
Column temperature	45 °C
Injection volume	2 µL Corn, peanut; 10 µL black pepper
Mobile phase	A) 0.5 mM Ammonium fluoride + 5 mM ammonium formate + 0.1 % formic acid in water B) 0.5 mM Ammonium fluoride + 5 mM ammonium formate + 0.1 % formic acid in methanol
Flow rate	0.45 mL/min
Gradient	Time %B 0 30 0.5 30 7.5 100 9.0 100 9.1 30
Stop time	9.1 minutes
Post time	1.9 minutes

Table 2. Agilent Ultivo Triple Quadrupole and Agilent Jet Stream source parameters.

Parameter	Value
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 (+)
Nozzle voltage	0 V (+)
Cycle time	500 ms

Results and Discussion

Mycotoxin regulatory maximum levels

Mycotoxins are regulated at many different concentrations depending on the specific mycotoxin, the matrix being analyzed, the regulatory agency, and the targeted consumer. This study used the European Union regulations No. 1881/2006 and No. 105/2010 as a benchmark. This procedure was done because the EU generally had the lowest maximum level (ML) for mycotoxins compared to other regional regulatory agencies. The nonregulated compounds were either not included in the matrix, or estimated based on current levels of concern established in Table 4.

Table 3. Transitions for mycotoxin detection in dMRM mode.

Compound	Precursor (*m/z*)	Product (*m/z*)	RT (min)	RT Window (min)	Fragmentor (V)	CE (V)	Polarity
Aflatoxin B1 (AB1)	313.3	285.1	5.9	1	190	20	Positive
Aflatoxin B1 (AB1)	313.3	241.1	5.9	1	190	40	Positive
Aflatoxin B2 (AB2)	315.1	287.1	5.7	1	190	24	Positive
Aflatoxin B2 (AB2)	315.1	259.1	5.7	1	190	28	Positive
Aflatoxin G1 (AG1)	329.1	311.1	5.3	1	180	20	Positive
Aflatoxin G1 (AG1)	329.1	243.1	5.3	1	180	28	Positive
Aflatoxin G2 (AG2)	331.1	313.1	5.1	1	190	24	Positive
Aflatoxin G2 (AG2)	331.1	115	5.1	1	190	80	Positive
Deoxynivalenol (DON)	297.1	249.2	3.0	1	120	4	Positive
Deoxynivalenol (DON)	297.1	77	3.0	1	120	80	Positive
Fumonisin B1 (FB1)	722.4	352.3	6.6	1	240	36	Positive
Fumonisin B1 (FB1)	722.4	334.3	6.6	1	240	40	Positive
Fumonisin B2 (FB2)	706.4	336.3	7.4	1	240	36	Positive
Fumonisin B2 (FB2)	706.4	318.4	7.4	1	240	40	Positive
Fumonisin B3 (FB3)	706.4	336.3	7.0	1	240	36	Positive
Fumonisin B3 (FB3)	706.4	318.4	7.0	1	240	36	Positive
HT-2 Toxin (HT-2)	442.2	263.2	6.8	1	120	4	Positive
HT-2 Toxin (HT-2)	442.2	215.1	6.8	1	120	4	Positive
Ochratoxin A (OTA)	404.1	239	7.6	1	140	20	Positive
Ochratoxin A (OTA)	404.1	221	7.6	1	140	36	Positive
T-2 Toxin (T-2)	484.2	215.2	7.3	1	140	12	Positive
T-2 Toxin (T-2)	484.2	185.1	7.3	1	140	4	Positive
Zearalenone (ZEA)	319.2	301.2	7.7	1	120	4	Positive
Zearalenone (ZEA)	319.2	185.1	7.7	1	120	24	Positive

Table 4. MLs for mycotoxins in this study. EU reg No. 1881/2006 and No. 105/2010 are used for reference. All assigned MLs in this study are equal to or lower than the EU ML.

Mycotoxin	European Union ML for mycotoxins[2,3]			Assigned ML used for this study	
	Corn (ppb)	Peanut (ppb)	Black pepper (ppb)	Corn and peanut (ppb)	Black pepper (ppb)
Aflatoxin B1	2	2	5	2	5
Aflatoxin B2	Sum of aflatoxins: 4 ppb	Sum of aflatoxins: 4 ppb	Sum of aflatoxins: 10 ppb	2	5
Aflatoxin G1				2	5
Aflatoxin G2				2	5
Ochratoxin A	3	n/a	15	3	15
Fumonisin B1	Sum of B1 and B2: 1,000 ppb	n/a	n/a	500	Not included
Fumonisin B2			n/a	500	Not included
Fumonisin B3	n/a	n/a	n/a	500	Not included
Deoxynivalenol	750	n/a	n/a	75	Not included
Zearalenone	100	n/a	n/a	100	Not included
T-2 Toxin	n/a	n/a	n/a	100	Not included
HT-2 Toxin	n/a	n/a	n/a	500	Not included

Method recovery

The extraction procedure for this method was simple and effective. The Agilent Captiva EMR—Lipid Kit was a quick step that adequately separated matrix components from compounds of interest. This method demonstrated good recovery for all compounds in each matrix, with recoveries ranging 60–110 % at ML for each compound (Table 5).

Method sensitivity

Figure 3 shows the excellent signal response of the 12 mycotoxins analyzed in this method, displaying the chromatogram at ML in corn matrix. Mycotoxin standards at levels ranging from 1/20 of the assigned ML to 10 times the assigned ML were evaluated. Eight concentration levels were spiked into matrix extracts for limit of quantitation (LOQ) and %RSD analysis.

Enhanced signal response allowed most of the mycotoxins in each matrix to have a quantitation limit at 1/20 the assigned ML. Figure 4 shows that all the mycotoxins had LOQs at least 1/5 the assigned ML. LOQs were defined as having four out of six replicate injections with accuracy of 80–120 %, and a signal-to-noise ratio (S/N) greater than 10.

Table 5. Recovery (%) of each mycotoxin studied in corn, peanut, and black pepper matrix at ML. Fumonisin compounds were evaluated at ½ ML.

Mycotoxin	Mycotoxin recovery (%)		
	Corn	Peanut	Black pepper
Aflatoxin B1	107	105	90
Aflatoxin B2	110	109	97
Aflatoxin G1	109	108	102
Aflatoxin G2	110	96	104
Ochratoxin A	83	83	109
Fumonisin B1	60	65	–
Fumonisin B2	67	77	–
Fumonisin B3	90	61	–
Deoxynivalenol	111	72	–
Zearalenone	98	90	–
T-2 Toxin	105	104	–
HT-2 Toxin	108	102	–

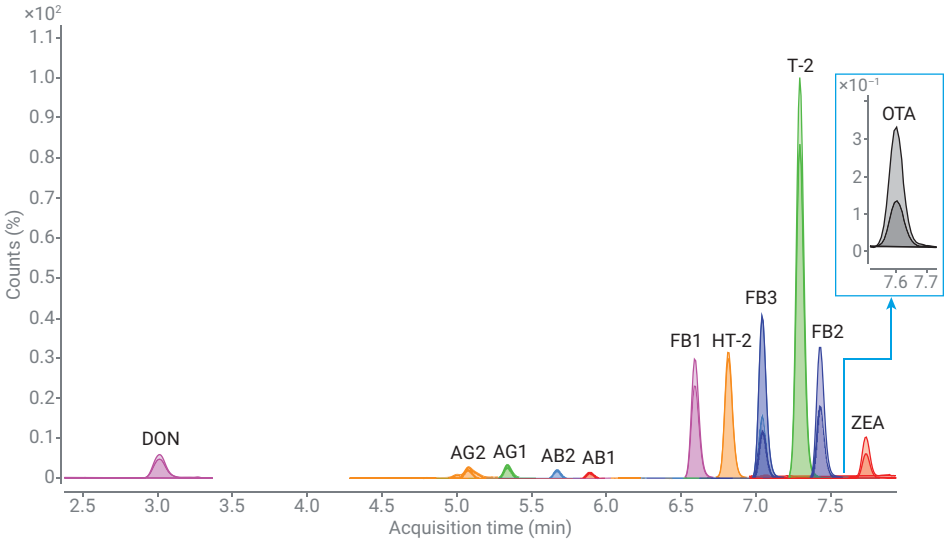

Figure 3. Composite dMRM chromatogram of mycotoxins in corn matrix at assigned ML.

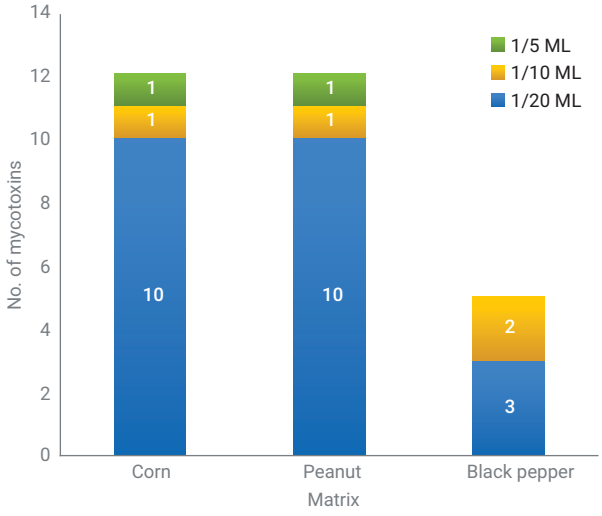

Figure 4. LOQ for all mycotoxins studied in each matrix. All could be accurately quantified at a fraction of the assigned ML.

Method precision and linearity

Excellent precision was achieved for all compounds studied in each matrix. Figure 5 shows the %RSD of six replicate injections of mycotoxins at their LOQ in each matrix. All compounds have a %RSD less than 10 % in each matrix, with most of the compounds having a %RSD less than 5 %. The exceptional precision in this method is highlighted in the close-up view of the six replicate injections of aflatoxin B1 at 200 ppt in corn and peanut matrix, and 500 ppt in black pepper matrix, corresponding to 10x lower than the ML (Figure 6).

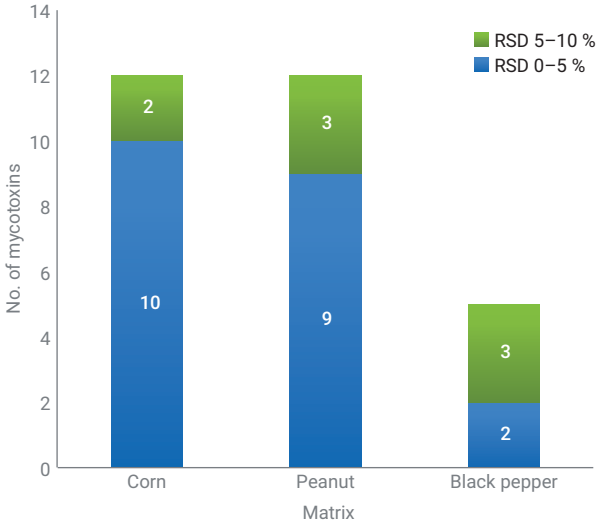

Figure 5. The mycotoxins show excellent precision in all three matrices, with all %RSDs <10 % at LOQ.

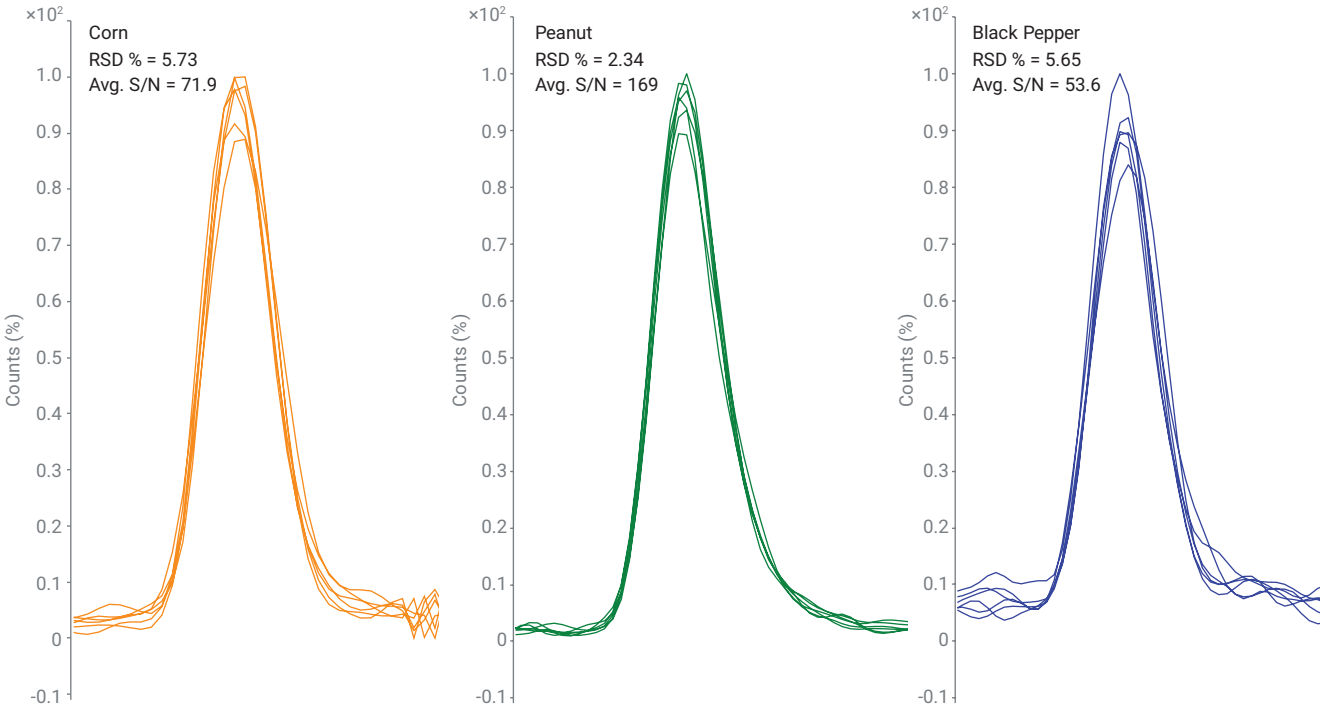

Figure 6. Excellent precision demonstrated for six replicate injections of aflatoxin B1 at 1/10 ML (200 ppt or 500 ppt) in all matrices.

All compounds demonstrated outstanding linearity. Six to eight calibration levels were analyzed for each compound, ranging from 1/20 to 10x the ML, resulting in R^2 values ≥0.99 for all compounds in all matrices. Figure 7 shows examples of excellent linearity for six selected compounds in peanut matrix.

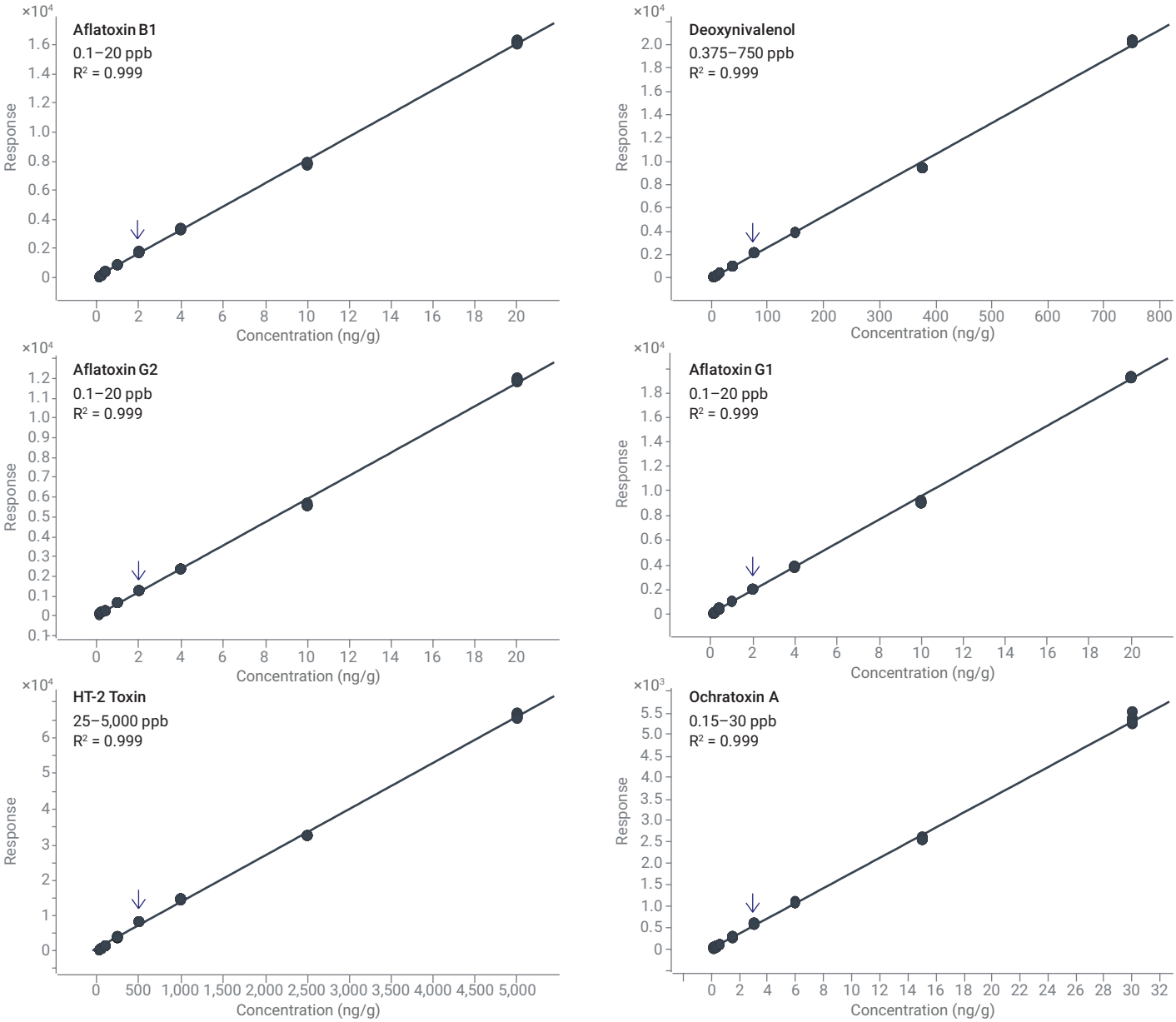

Figure 7. Calibration curves for selected compounds in peanut matrix. Linear fit and no weighting used. Arrows indicate ML.

Conclusions

The Agilent Ultivo Triple Quadrupole LC/MS is an exceptionally innovative mass spectrometer that minimizes laboratory workspace needs. It also reduces 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 established MLs. Agilent MassHunter software provides an easy-to-use, all-inclusive tool for acquiring and reporting LC/MS data.

References

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