Fast Data Acquisition Speed and High Quantitative Performance in the Simultaneous Determination of Mycotoxins, Illegal Dyes, and Pesticides in Spices

Application Note

Food

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Introduction

Spices have been used since antiquity for the flavoring and coloring of food. There have been many alerts from European countries on the safety of spices with regards to different groups of contaminants. This raised the need for the simultaneous screening and quantitation of pesticides, mycotoxins, and dyes using a single multiresidue method. Major challenges are the complex matrixes, the large number of analytes, the diversity of compound classes, and the concentration range that must be covered in the analysis.

This Application Note evaluates an ion-funnel triple quadrupole mass spectrometer operated with dynamic MRM and fast polarity switching for the accurate quantitation of an extended suite of analytes at trace-levels. We further evaluated a modified software and firmware to optimize inter-MRM delay times, enabling high quality data at dwell times as low as 0.5 ms.

The excellent sensitivity achieved using the Agilent 6495B Triple Quadrupole LC/MS enables precise and accurate quantitation of pesticides, mycotoxins, and dyes with high degrees of sample dilution, allowing reduction in matrix effects and improved method robustness.

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Experimental

Sample preparation

Ground black pepper and paprika were obtained from a local grocery store, and extracted according to the EN 15662 QuEChERS protocol using Agilent BondElut QuEChERS kits (p/n 5982-6650). The extracts were further cleaned with the QuEChERS dSPE Enhanced Matrix Removal—Lipid kit (p/n 5982-1010), followed by a polishing step (p/n 5982-0102). Final extracts were spiked at 2 ppb (10 µg/kg) with the Agilent comprehensive pesticide mixture (p/n 5190-0551), mycotoxins, and dye standards (Sigma). Spiked extracts were diluted 1:5, 1:10, 1:20, 1:50, and 1:100 with acetonitrile.

Method design

Agilent MassHunter Optimizer software was used to optimize MRM transitions for five mycotoxins and nine dyes. At least two MRM transitions per compound and the corresponding MS conditions were also taken from the Agilent Pesticide Triggered MRM (tMRM) LC/MS Application Kit. Analysis was carried out with positive and negative electrospray ionization in dynamic MRM (dMRM) or tMRM in a single analytical run. Major UHPLC and dMRM MS parameters are shown in the Instrument conditions table.

Results and Discussion

Evaluation of optimized inter-MRM delay times

An MRM method was developed targeting 100 transitions representing 53 pesticides to assess a modified software and firmware that optimizes inter-MRM delay times. Figure 1 shows the overlaid MRM chromatograms of two pesticides at four different dwell times, demonstrating the importance of using appropriate inter-MRM delay times for maintaining ion signal at short dwell times.

Instrument conditions

Agilent 1290 Infinity II UHPLC System	
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 150 mm, 1.8 µm (p/n 959759-902)
Column temperature	40 °C
Injection volume	2 µL
Mobile phase	A) 5 mM ammonium formate + 0.1 % formic acid B) 5 mM ammonium formate + 0.1 % formic acid in methanol
Flow rate	0.4 mL/min
Gradient	95 %A hold for 0.5 minutes, to 40 %B in 3.5 minutes, to 98 %B in 17 minutes, hold for 3 minutes, down to 5 %B in 0.1 minutes Hold for 0.9 minutes, stop time 21.00 minutes Post time 2 minutes
Agilent 6495B Triple Quadrupole Mass Spectrometer	
Ion source	Agilent Jet Stream ESI
Polarity	Positive and negative switching
Gas temperature	120 °C
Drying gas (nitrogen)	17 L/min
Nebulizer gas (nitrogen)	30 psi
Sheath gas (nitrogen)	300 °C
Sheath flow	12 L/min
Capillary voltage	3,500/–3,500V
Nozzle voltage	300/–500 V
iFunnel High/low pressure RF	150/60V
Scan type	Dynamic MRM (dMRM)
Q1/Q2 resolution	Unit (0.7 amu)
Delta EMV	200 V
Cell acceleration voltage	3–7 V
Total number of MRMs	542 (positive: 530, negative: 12)

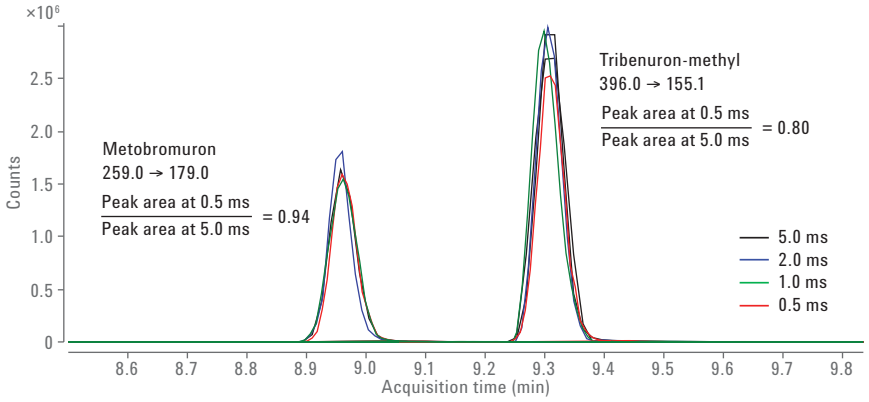


Figure 1. Overlaid MRM chromatograms of metobromuron and tribenuron-methyl (100 ppb) at dwell times of 5.0, 2.0, 1.0, and 0.5 ms using optimized inter-MRM delay times.

Method development and performance

An MRM method was first developed with new transitions optimized for mycotoxin and dye compounds of interest. Next, the new compound transition and observed RT data were used to update a dMRM method targeting >250 pesticides.

The combination of UHPLC with an optimized 6495B Triple Quadrupole Mass Spectrometer allowed the quantitation of the majority of analytes at a lower limit of quantitation (LLOQ) of 10 % of their allowed maximum residue levels (MRLs). The precision and accuracy of measurements were evaluated at 13 standard concentrations ranging from an LLOQ as low as 2 ppt, to the upper limit of quantitation (ULOQ) of 100 ppb, and were calculated from five replicate injections at each level. Excellent assay precision (RSD% < 20 % at the LLOQ and <15 % at the rest of the levels) as well as average accuracy (80–125 % at the LLOQ and 85–115 % at the rest of the levels) were obtained. Correlation coefficients (R^2) for calibration curves were higher than 0.99.

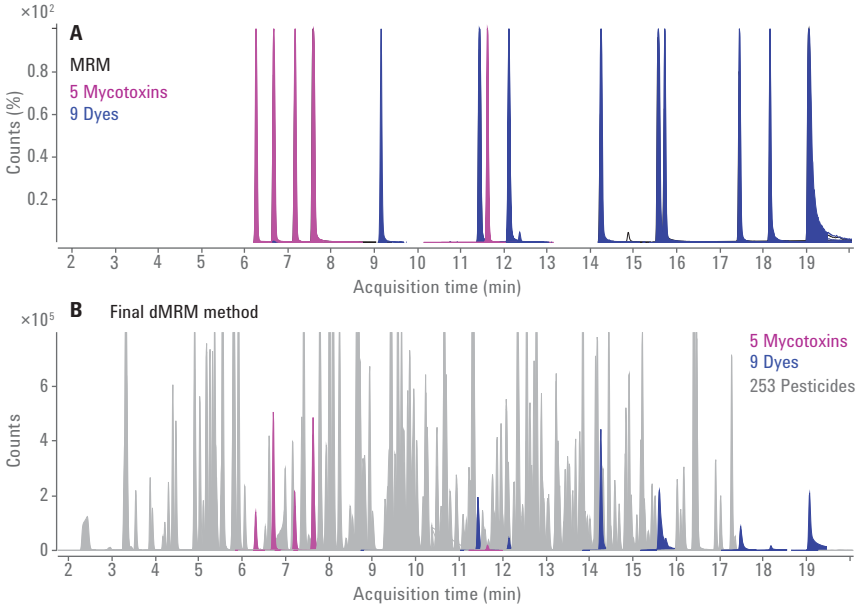
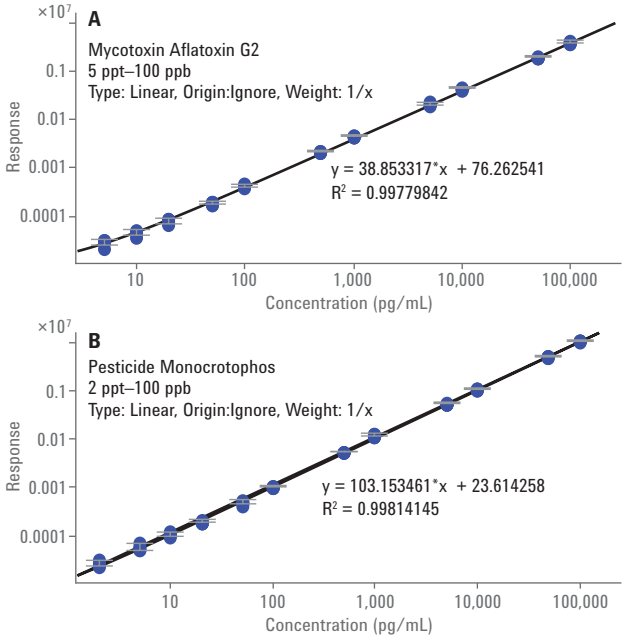


Figure 2. Overlaid MRM chromatograms of mycotoxins and dyes (A). Overlaid MRM chromatograms from a 10 ppb mixture of standards acquired with the final dMRM method targeting 267 total compounds (B).



Mycotoxin	LLOQ (ppt)	IDL (ppt)
Aflatoxin B1	10	3.7
Aflatoxin B2	20	2.2
Aflatoxin G1	5	2.3
Aflatoxin G2	5	2.6
Ochratoxin A	500	274.5

Dye	LLOQ (ppt)	IDL (ppt)
Dimethyl yellow	50	7.9
Orange II	1,000	733.5
Rhodamine B	500	294.1
Sudan I	50	24.0
Sudan II	50	8.8
Sudan III	500	158.8
Sudan orange G	500	190.5
Sudan red 7B	50	11.6
Sudan red G	100	24.1

Figure 3. Examples of the dynamic range and linearity achieved with the final dMRM method. The tables provide LLOQs and instrument-detection-limits (IDLs) for the mycotoxins and dyes.

Minimizing matrix effects by dilution of extracts

The ability to dilute complex sample extracts to minimize matrix effects enables more accurate quantification, while also reducing contamination of the LC/MS system, thereby increasing robustness. Table 1 shows the beneficial effect of dilution, where compound recoveries in black pepper improve as the sample is further diluted.

Spices are very challenging food matrices, as their complex nature results in significant ion suppression effects. The matrix effects were further enhanced by our inability to leverage conventional dispersive SPE cleanup to remove interfering matrix compounds, due to observed losses of dye compounds. Therefore, the dilution of extracts was critical to reduce suppression, and the comparison shown in Figure 4 demonstrates that the degree of dilution required varies considerably across types of spices.

Black pepper was identified as the most challenging spice matrix we analyzed. Figure 5 shows that a 1:100 dilution of the extract in acetonitrile was required to achieve acceptable recoveries for >90 % of the compounds. The innate sensitivity of the 6495B LC/MS system enabled analysis of the desired 1:100 black pepper dilution level while still maintaining the ability to detect the majority of the compounds with an area RSD < 20 %.

Table 1. Recoveries for selected compounds calculated for different dilution ratios based on a solvent calibration. Cells shaded in green comply with requirements of SANCO/12571/ 2013.

Analyte	No dilution	1:5 Dilution	1:10 Dilution	1:20 Dilution	1:50 Dilution	1:100 Dilution
Acetamiprid	47.1 ± 3.7	88.5 ± 2.3	94.6 ± 3	99.2 ± 6.8	104.5 ± 6.4	116.7 ± 5.9
Aflatoxin B1	31.1 ± 0.9	80 ± 1.3	90 ± 3.8	92.1 ± 7.7	110.2 ± 8.8	112 ± 3.4
Buprofezin	4.1 ± 0.3	16.6 ± 1.1	32.1 ± 1.2	47.3 ± 5.8	84.6 ± 9	109.8 ± 9.9
Chlorantraniliprole	8.2 ± 0.7	31.1 ± 1.1	50.9 ± 5.2	65.5 ± 10.9	94.2 ± 10.2	101.9 ± 20.4
Lenacil	16.9 ± 1.8	57.7 ± 5.9	75.7 ± 5.4	82.4 ± 3.6	97.5 ± 9.2	106.9 ± 11.5
Methomyl	24.9 ± 1.9	51.3 ± 3.5	65.3 ± 6.5	77.1 ± 7.5	107.3 ± 10.7	113.3 ± 14.6
Profenofos	2.6 ± 0.3	12.5 ± 0.6	20.4 ± 2.1	34.2 ± 1.7	59 ± 8.3	88.5 ± 15.5
Propamocarb	105.9 ± 8	102.4 ± 3.4	101 ± 3	100.2 ± 2.8	114.5 ± 3.4	111.5 ± 6.8
Proquinazid	11.4 ± 0.6	42.8 ± 0.9	57.9 ± 1	68.3 ± 3.9	96 ± 11.6	111.3 ± 6.8
Sudan red 7B	19.8 ± 2.5	57.4 ± 4.2	61 ± 6.9	70.5 ± 2.4	84 ± 5	81.5 ± 7.2

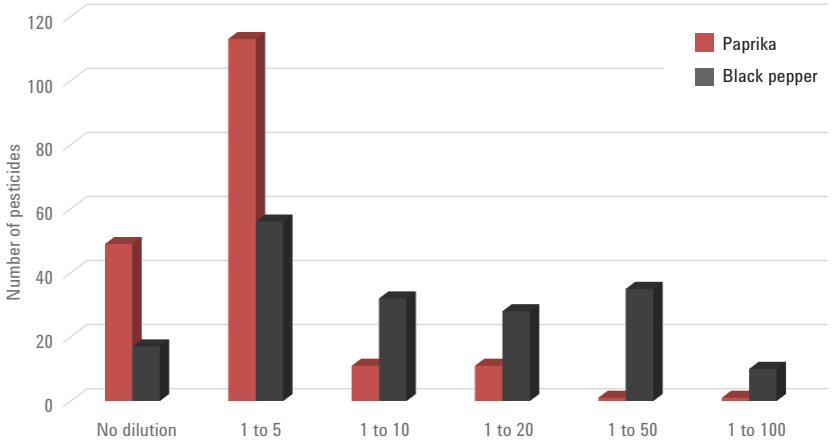


Figure 4. Acceptable recoveries (70–120 %) for 194 compounds were compared for paprika and black pepper extract dilutions.

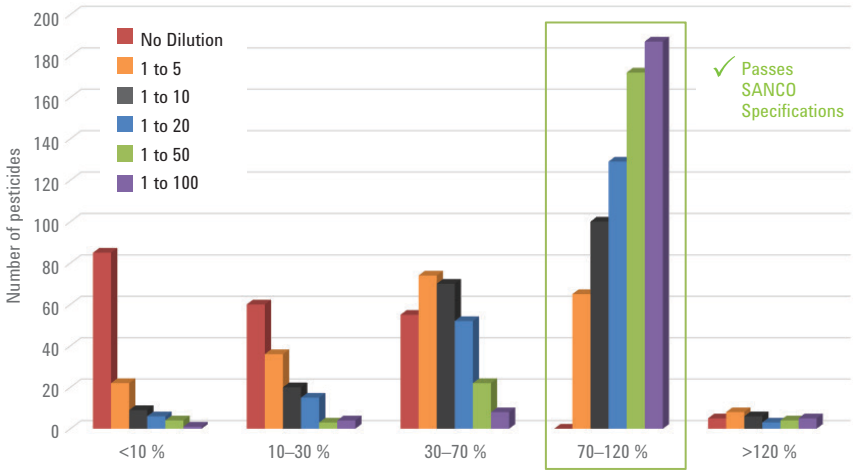


Figure 5. Histogram of recoveries for pesticides, mycotoxins, and dyes spiked into black pepper at 2 ppb (10 µg/kg), and diluted with acetonitrile. Two-hundred five compounds showed strong ion suppression, and significantly better recoveries were observed after dilution.

Confirmation with triggered MRM (tMRM)

While evaluating matrix effects with the dMRM method, we found >20 pesticides and mycotoxins present in the paprika blank extract (without spike-in of standards). For further confirmation of these compounds in this control sample, we applied a tMRM method where up to six transitions were acquired based on the presence of a single primary transition. The full compound spectrum was compared against a spectral library created from the pure standards. These same data were also used for quantitation. In the examples shown in Figure 6, Ochratoxin A and Aflatoxin B1 were calculated at 28 µg/kg and 8.1 µg/kg in the 1:20 diluted paprika extract.

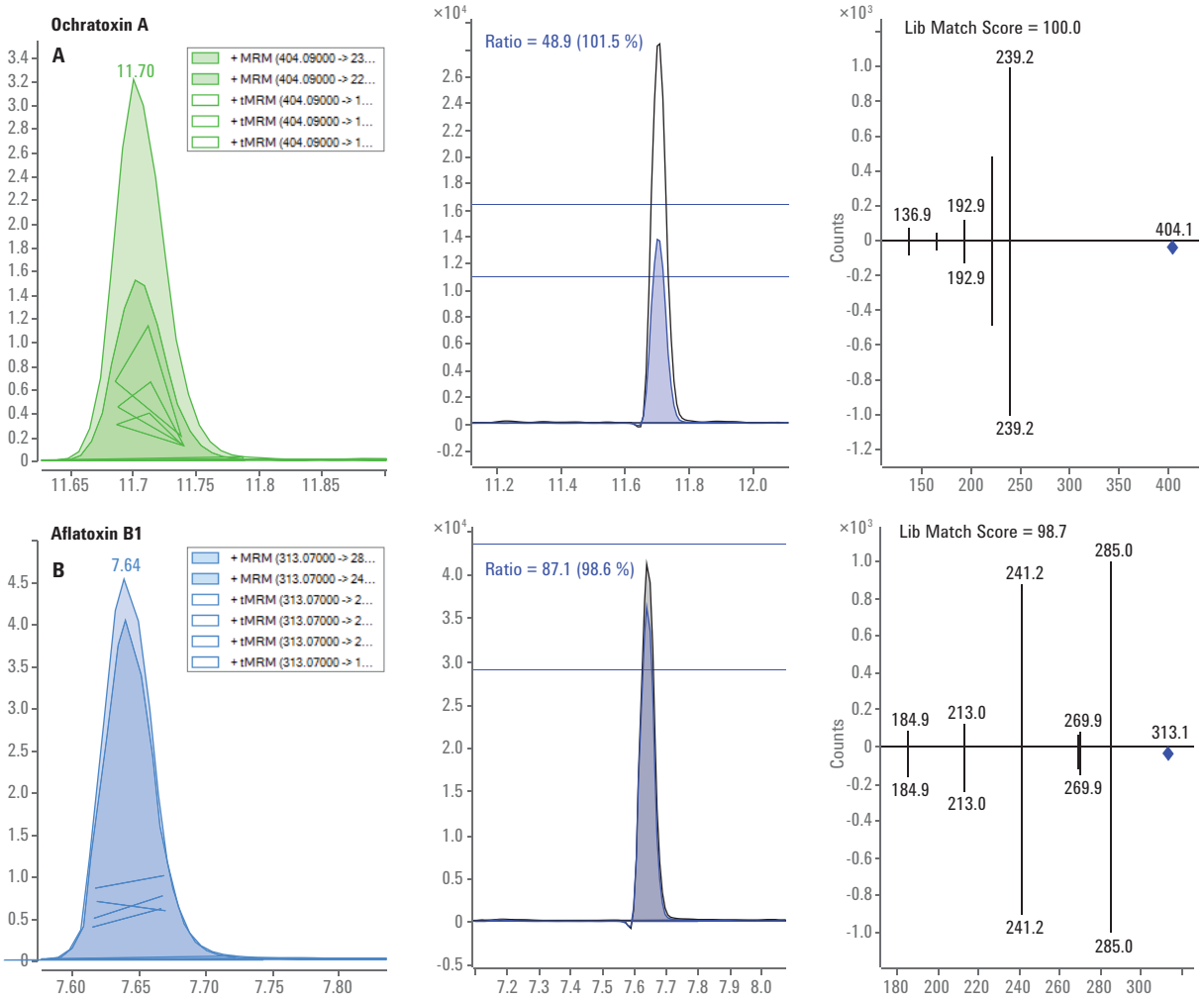


Figure 6. Triggered MRM data for Ochratoxin A (A) and Aflatoxin B1 (B) in paprika extract, displaying compound chromatograms, qualifier details, and reference library matching (left to right).

Conclusions

- Optimized inter-MRM delay times allowed the use of MRM dwell times as short as 0.5 ms with minimum analyte signal losses.
- Mycotoxin and dye compounds were incorporated into a UHPLC/MS/MS pesticide method for the determination of more than 260 compounds.
- The enhanced sensitivity achieved using the Agilent 6495B LC/MS system enabled precise and accurate quantitation of compounds with very high degrees of spice extract dilution, provided reduced matrix effects, and improved method robustness.
- The value of tMRM acquisition for confirmation of mycotoxin compounds in spices was demonstrated.

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