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Reducing Matrix Effects in Multi-Residue Pesticide Analysis by Sample Dilution Using a Newly Developed QQQ MS with Enhanced Sensitivity

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Introduction

An important application in food safety is the screening and quantitation of hundreds of pesticides from a wide variety of food commodities using multi-residue methods. Major challenges in achieving accurate quantitation include diversity of compound classes, matrix effects, low concentrations and poor ionization efficiencies of some pesticides.

Here we demonstrate the use of an UHPLC/MS/MS method for the screening and quantitation of hundreds of pesticides in complex food matrices. An Agilent 1290 Infinity UHPLC system was coupled to the new Agilent 6470 triple quadruple LC/MS system operated with dynamic MRM with fast polarity switching. Several modifications to the triple quad mass spectrometer have resulted in higher analytical performance. Improvements were achieved by new mass filter one (MS1) ion optics, an improved curved and tapered collision cell, a detector operating at dynode accelerating voltages of up to 20 kV, and a new autotune optimized for speed and sensitivity. In addition the use of a curved collision cell resulted in a smaller physical footprint

Enhanced sensitivity achieved by the new design gives enhanced peak area response and improved area precision, leading ultimately to lower detection limits compared to previous designs. Moreover we evaluated the use of dilution as a means of minimizing matrix effects and achieving rugged and high performance quantitation at trace levels in tomato, orange and black tea. Increased sensitivity achieved by the new design allowed a higher degree of sample dilution while still achieving the maximum residue level (MRL) stipulated by the European Union.

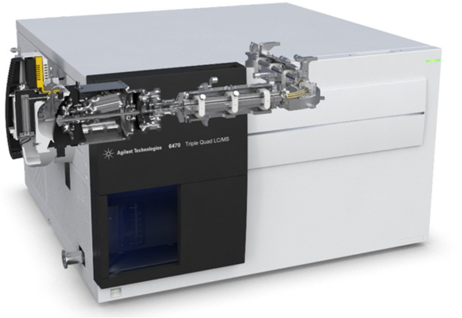


Figure 1 *The new 6470 Triple Quadrupole LC/MS system with new MS1 optics, improved collision cell, and new ion detector for enhanced sensitivity.*

Experimental

Sample Preparation:

Organic tomato, orange, and black tea samples were obtained from a local grocery store and extracted according to the citrate buffered QuEChERS protocol using Agilent BondElut QuEChERS kits. Final extracts were spiked at 10 ppb with the Agilent comprehensive pesticide mixture (p/n 5190-0551) working solution and were diluted 1:2, 1:5, 1:10, and 1:20 with acetonitrile.

Method Design

An Agilent 1290 Infinity UHPLC system was coupled to an Agilent 6470 triple quadrupole LC/MS system equipped with an Agilent Jet Stream electrospray ionization source. At least two MRM transitions per compound and the corresponding MS conditions were taken from the Agilent Pesticide Triggered MRM LC/MS Application Kit. Analysis was carried out with positive and negative electrospray ionization in dynamic multiple reaction monitoring (DMRM) in a single analytical run. Major UHPLC and MS parameters are as follows:

Agilent UHPLC 1290 System	
Column	Agilent Eclipse Plus C18, RRHD, 2.1x150mm 1.8 um (p/n 959759-902)
Column temperature	40 °C
Injection volume	2 µL
Mobile phase	A= 5mM ammonium formate + 0.1% formic acid B= 5mM ammonium formate + 0.1% formic acid in methanol
Flow rate	0.4 mL/min
Gradient	95% A hold for 0.5 min, to 40% B in 3.5 min, to 98% B in 17 min, hold for 3 min, down to 5% B in 0.1 min, post time 3 min

Agilent 6470 Triple Quadrupole Mass Spectrometer	
Ion source	Agilent Jet Stream ESI
Polarity	Positive and Negative Switching
Gas temperature	140 °C
Drying gas (nitrogen)	5 L/min
Nebulizer gas (nitrogen)	30 psi
Sheath gas (nitrogen)	375 °C
Sheath flow	12 L/min
Capillary voltage	4000/-3000 V
Nozzle voltage	0 V
Scan type	Dynamic MRM (DMRM)
Q1/Q2 resolution	Unit (0.7 amu)
Delta EMV	200 V
Cell acceleration voltage	3-7 V
Cycle time	500 ms
Total number of MRMs	525 (positive: 505, negative: 20)
Min/max dwell time	2.3/246.5 ms

Results and Discussion

Method Performance

More than 250 pesticides were targeted using fast polarity switching and dynamic MRM acquisition.

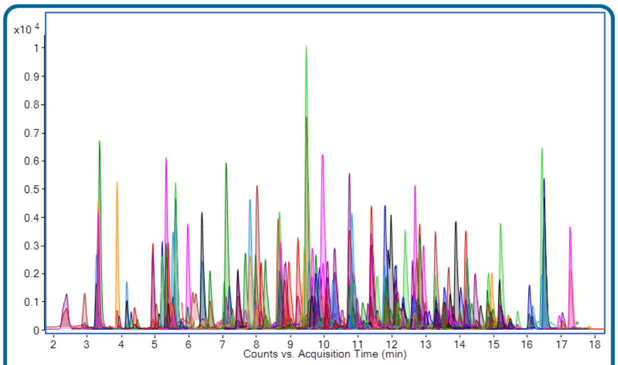


Figure 2. *Chromatograms of more than 250 pesticides spiked into black tea and diluted 1:20 with acetonitrile (corresponding to a concentration of 0.5 ppb).*

The improved ion optics and detector allowed the quantitation of the majority of pesticides at an LLOQ of 10% of their maximum residue level (MRL). The precision and accuracy of measurements were evaluated at twelve standard concentrations ranging from the LLOQ as low as 10 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 LLOQ and <15% at the rest of the levels) as well as average accuracy (80-125% at LLOQ and 85–115% at the rest of the levels) were obtained. Correlation coefficients (R^2) for calibration curves were higher than 0.99 over up to 4 orders of linear dynamic range. These results are well within the criteria set by bioanalytical method validation guidelines.

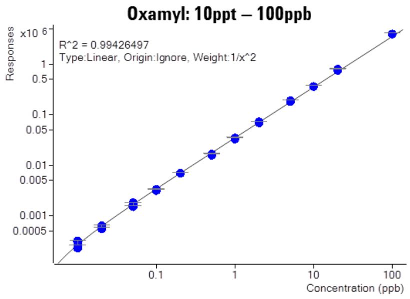


Figure 3. An example of the dynamic range and linearity achieved for the spiked tomato matrix. The concentration range was 0.1-100 ppb (0.1-100 µg/kg) and 12 levels were used to generate the curve.

Increased Sensitivity Improves Detection Limits

The new MS1 ion optics of the 6470 triple quadrupole LC/MS system in combination with the new detector design resulted in a compound dependent gain of up to 4X compared to the previous 6460 design.

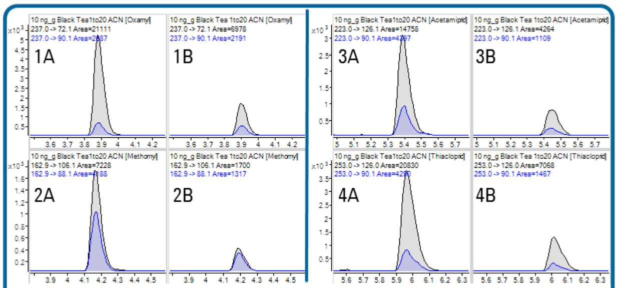


Figure 4. *Comparison of peak areas for several relevant pesticides spiked into black tea and diluted 1:20 (corresponding to 0.5 ppb), analyzed with the Agilent 6470 (**A**) and the previous design 6460 (**B**). (**1**) Oxamyl, gain 2.8x, (**2**) Methomyl, gain 3.9x, (**3**) Acetamiprid, gain 3.4x, (**4**) Thiacloprid, gain 2.7x*

Enhanced ion transmission resulted in increased peak area response and improved area precision. The area relative standard deviation (RSD) at a low concentration is a universal measure of ion efficiency and can be used to estimate the Instrument Detection Limit (IDL) as a superior metric compared to signal-to-noise calculations for sensitivity assessment. Table 1 shows the fold-improvement in IDL for individual relevant pesticides comparing the 6470 to the previous instrument design. A median fold-improvement of **3.6x** was observed across all >250 pesticides.

Pesticide	IDL (ppt)		Fold-Improvement	Pesticide	IDL (ppt)		Fold-Improvement
	6470	6460			6470	6460	
Acephate	0.71	1.04	**1.5x**	Fipronil	7.45	33.9	**4.6x**
Azinphos-methyl	16.5	27.89	**1.7x**	Flufenoxuron	5.80	52.1	**9.0x**
Buprofezin	0.62	3.60	**5.8x**	Hexaflumuron	100.3	2077.1	**20.7x**
Carbendazim	0.37	1.35	**3.6x**	Imazalil	3.01	20.32	**6.7x**
Chloroxuron	0.74	9.84	**13.3x**	Imidacloprid	5.76	19.11	**3.3x**
Cycluron	0.84	7.11	**8.4x**	Isocarbophos	1.46	12.95	**8.9x**
Desmedipham	1.12	3.24	**2.9x**	Metamitron	17.2	55.8	**3.2x**
Diethofencarb	1.11	5.90	**5.3x**	Methidathion	7.19	34.0	**4.7x**
Difenoconazole	31.5	62.07	**2.0x**	Methomyl	0.41	2.15	**5.2x**
Dimethoate	0.28	3.28	**11.8x**	Monocrotophos	1.08	5.23	**4.8x**
Dimethomorph	5.90	31.07	**5.3x**	Pirimicarb	0.13	0.28	**2.2x**
Dimoxystrobin	0.63	2.36	**3.8x**	Pyridaben	0.08	0.06	**0.8x**
Diniconazole	3.09	23.74	**7.7x**	Tebuconazole	4.58	4.19	**0.9x**
Dioxacarb	10.3	13.91	**1.3x**	Teflubenzuron	29.1	201.2	**6.9x**
Diuron	2.26	3.74	**1.7x**	Triazophos	1.01	6.59	**6.6x**

Table 1. *Instrument detection limits (IDL) for 30 pesticides relevant as residues in black tea based on a dilutions series prepared in acetonitrile. Five injection replicates were used for calculation.*

Results and Discussion

The increased sensitivity of the 6470 LC/MS system enabled the quantitation of most targeted pesticides in tomato, orange, and black tea extracts below the default MRL of 10 µg/kg specified by the European Commission.

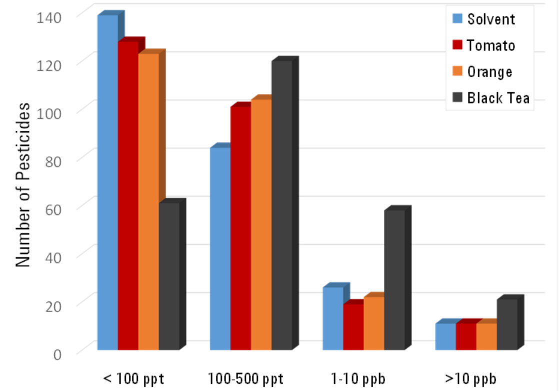


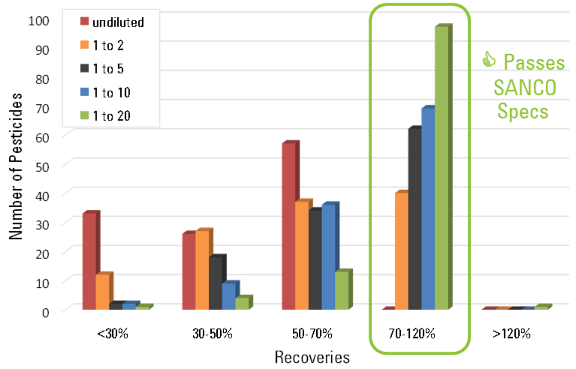
Figure 5. *Histogram of LLOQs for evaluated pesticides in solvent and spiked tomato, orange, and black tea extracts. Black tea represents the most challenging matrix.*

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. The beneficial effect of dilution is shown in Table 2, where pesticide recoveries in black tea improve as the sample is further diluted. Figure 5 demonstrates that most pesticides spiked in black tea achieve acceptable recoveries at the 1:20 dilution.

Analyte	No Dilution	Dilution 1:2	Dilution 1:5	Dilution 1:10	Dilution 1:20
Acephate	59.5 +/- 0.7	70.5 +/- 1.2	77 +/- 1.3	74.8 +/- 0.8	83.5 +/- 2.4
Aldicarb	26.3 +/- 1.3	42 +/- 1.9	64.1 +/- 2.7	71.3 +/- 6.1	83.9 +/- 6
Carbofuran	45 +/- 0.3	60.5 +/- 0.4	73.1 +/- 0.6	73.3 +/- 0.7	82.9 +/- 0.7
Diethofencarb	86.2 +/- 1.4	92.8 +/- 1	89.1 +/- 1.1	80.4 +/- 1.7	86.7 +/- 2.1
Dimethoate	27.2 +/- 0.4	40.9 +/- 0.5	57.2 +/- 0.9	63.3 +/- 1.2	75.4 +/- 1.9
Epoxyconazol	65.2 +/- 1	71.9 +/- 1.6	77 +/- 2.3	75.3 +/- 3.1	84.6 +/- 7.3
Ethion	46.7 +/- 0.8	66.7 +/- 0.9	82.7 +/- 1.8	78 +/- 1.4	85.1 +/- 5.3
Flufenoxuron	92.3 +/- 1	93.1 +/- 2.7	89.6 +/- 3	80.2 +/- 5.4	87.1 +/- 10.6
Methamidophos	44.9 +/- 0.8	56.5 +/- 0.2	66 +/- 0.4	68.4 +/- 0.4	78 +/- 1.1
Methidathion	66.2 +/- 1.8	76.7 +/- 0.9	82 +/- 3.3	83.9 +/- 2.2	82.7 +/- 10.9
Methomyl	11.7 +/- 0.2	25 +/- 0.3	45.4 +/- 0.9	56.8 +/- 1.2	70.2 +/- 1
Oxamyl	15.7 +/- 0.2	27.8 +/- 0.3	47.6 +/- 0.3	58.5 +/- 0.6	71.8 +/- 0.6
Pirimicarb	50.2 +/- 0.3	62.6 +/- 0.4	72.6 +/- 0.3	71.5 +/- 0.8	80.3 +/- 0.6
Pyridaben	51.1 +/- 0.5	62.7 +/- 0.3	71.2 +/- 0.7	70.5 +/- 0.8	79.1 +/- 0.8
Thiacloprid	22.6 +/- 0.1	35.2 +/- 0.5	52.1 +/- 0.4	58.7 +/- 0.8	71 +/- 1.2

Table 2. *Recoveries for selected pesticides calculated for different dilution ratios based on a solvent calibration. Cells shaded in green comply with requirements of SANCO/12571/2013.*



Passes SANCO Specs

Figure 6. *Histogram of recoveries for pesticides spiked into black tea at 10 ppb and diluted with acetonitrile. 116 pesticides showed strong ion suppression and significantly better recoveries were observed after dilution. The green box denotes acceptable recoveries according to SANCO specifications.*

The enhanced sensitivity of the 6470 LC/MS system enabled analysis of the desired black tea dilution level while still maintaining the ability to detect the majority of pesticides. Under the experimental conditions, ~70% of the spiked pesticides were easily detected in the 1:20 dilution.

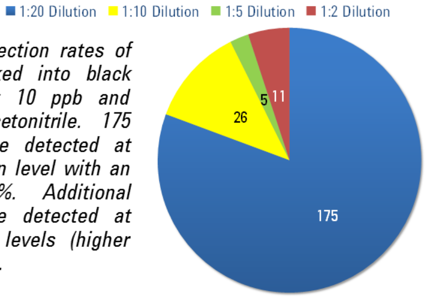


Figure 7. *Detection rates of pesticides spiked into black tea extract at 10 ppb and diluted with acetonitrile. 175 pesticides were detected at the 1:20 dilution level with an area RSD <20%. Additional compounds are detected at lower dilution levels (higher concentrations).*

Conclusions

- A UHPLC/MS/MS based multi-residue method for the determination of more than 250 pesticides and pesticide metabolites has been developed.
- The improved sensitivity achieved using the new 6470 LC/MS system enabled precise and accurate quantitation of pesticides with high degrees of sample dilution which allowed reduction in matrix effects and improved method robustness.