Eliminating Matrix Effects During Multi-residue Pesticide Analysis by Extensive Dilution Using the Agilent 6495 Triple Quadrupole MS With Enhanced Sensitivity

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Introduction

Most pesticides are analyzed with multi-residue methods covering hundreds of compounds in various food commodities for both screening and quantitation. Matrix effects in electrospray ionization present a significant challenge to accurately quantify the analytes. Sample dilution is one approach to minimize matrix effects but requires the use of highly sensitive instruments due to the need to detect contaminants below the maximum residue levels (MRL) stipulated by the regulations. Furthermore, the extensive dilution of sample extracts requires high precision, as even small deviations would result in considerable inaccuracies.

We have developed an UHPLC/MS/MS method for the screening and quantitation of hundreds of pesticides in black tea. An Agilent 1290 Infinity UHPLC system was coupled to the new, highly sensitive Agilent 6495 triple quadrupole mass spectrometer. Several modifications to the triple quadrupole mass spectrometer have resulted in higher analytical performance. Improvements were achieved by a new MS1 ion optics, an improved curved and tapered collision cell, a new ion detector operating at dynode accelerating voltages of up to 20 kV, and a new autotune optimized for speed and sensitivity. Enhanced performance gives enhanced peak area response and improved peak area precision, leading ultimately to lower detection limits. The high sensitivity allowed the extensive dilution of sample extracts to minimize matrix effects with excellent precision.

Experimental

Sample preparation

Tea samples were obtained from a local grocery store and extracted according to the citrate buffered QuEChERS protocol using Agilent BondElut QuEChERS kits. Two grams of tea were wetted with 8 mL water and extracted with 10 mL acetonitrile. Raw extracts were cleaned up by dispersive SPE using primary secondary amine and graphitized carbon black. Final extracts of blank samples were spiked in five relevant concentrations with the comprehensive pesticide working solution and diluted 1:5, 1:10, 1:20, 1:50 and 1:100 with acetonitrile. Matrix matched standards and dilutions were prepared immediately before injection and were analyzed with 5 technical replicates.

Equipment and Method Setup

An Agilent 1290 Infinity UHPLC system was coupled to an Agilent 6495 triple quadrupole LC/MS system equipped with an Agilent Jet Stream electrospray ionization source. Two MRM transitions per compound as well as the 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 summarized in Table 1.

Figure 1. The new 6495 Triple Quadrupole LC/MS system with new MS1 ion optics, improved collision cell and new ion detector for enhanced sensitivity

Table 1. UHPLC and 6495 LC/MS Method Parameters

Column	Agilent ZORBAX RRHD Eclipse Plus C18 2.1 x 150 mm, 1.8 µm (p/n 959759-902) @ 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 program	Time 0 0.5 3.5 17.0 20.0 20.1 Stop Time Post Time	B% 5 5 50 100 100 5 20.1 min 3 min
Ion mode	Positive and negative ESI with Agilent Jet Stream	
Total number of MRMs	532 (positive: 509 / negative: 23)	
Minimum dwell time	5.10 ms	
Maximum dwell time	249.09 ms	
MS1 and MS2 resolution	Unit	

Results and Discussion

Method Performance

More than 250 pesticides were targeted using fast polarity switching and dynamic MRM acquisition. Under the method conditions, the new MS1 ion optics in combination with the new collision cell and detector design resulted in a compound dependent area gain of up to 5x compared to 6490.

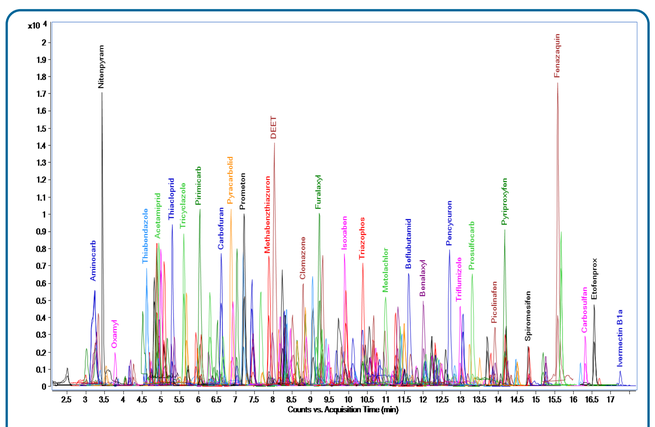

Figure 2. Chromatograms of more than 250 pesticides spiked into black tea at the MRL and diluted 1:20 with acetonitrile (corresponding to a concentration of 0.1 ng/mL).

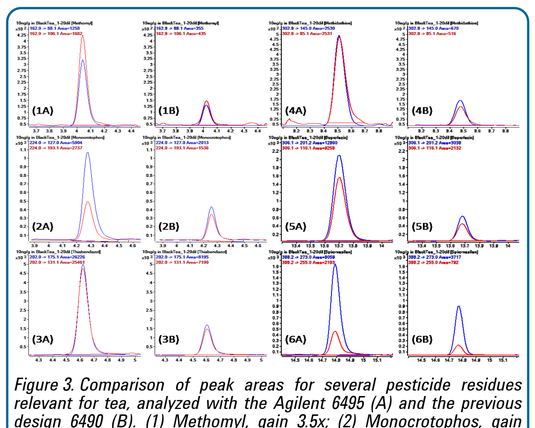

Figure 3. Comparison of peak areas for several pesticide residues relevant for tea, analyzed with the Agilent 6495 (A) and the previous design 6490 (B). (1) Methomyl, gain 3.5x; (2) Monocrotophos, gain 2.9x; (3) Thiabendazole, gain 3.2x; (4) Methidathion, gain 3.7x; (5) Buprofezine, gain 4.2x; (6) Spiromesifen, gain 2.1x.

Results and Discussions (Continued)

Minimizing Matrix Effects by Dilution of Extracts

The positive effect of sample dilution is shown in Figure 4. Upon 1:5 dilution, the signal for alanycarb even increased compared to the non-diluted sample. For oxamyl, the peak areas decreased less than the extent to which the target compound was diluted. Dilution typically causes the pesticides recovery against solvent calibration to increase until complete recovery is achieved. This is obvious from the recovery values in Table 2.

Weak matrix effect was observed e.g. for diuron. Monocrotophos and alanycarb suffered from strong matrix effects in the non-diluted tea extract. When diluting the final extract 1:10 more than half of the compounds showed adequate recoveries of more than 70% and only very few compounds required an even larger dilution to achieve acceptable recoveries based on a solvent calibration.

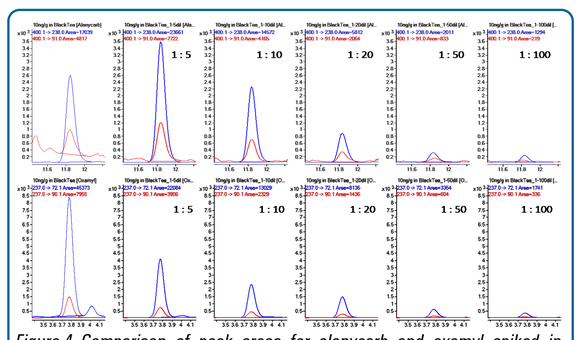

Figure 4. Comparison of peak areas for alanycarb and oxamyl spiked in black tea and diluted with acetonitrile 1:5, 1:10, 1:20, 1:50, and 1:100 prior to injection

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

Analytes	No dilution (n = 5)	Dilution 1:5 (n = 5)	Dilution 1:10 (n = 5)	Dilution 1:20 (n = 5)	Dilution 1:50 (n = 5)	Dilution 1:100 (n = 5)
Acetamiprid	29.4 ± 0.8	57.3 ± 1.4	67.5 ± 3.7	79.9 ± 2.9	91.8 ± 5.2	109.5 ± 3.4
Alanycarb	10.4 ± 1.3	73.9 ± 2.2	81.5 ± 14.3	85.7 ± 11.1	87.6 ± 4.7	121.7 ± 10.8
Aldicarb	36.9 ± 1.0	69.9 ± 1.4	78.0 ± 3.5	91.0 ± 4.2	95.2 ± 8.8	104.9 ± 14.1
Carbaryl	56.9 ± 1.8	80.1 ± 3.8	80.8 ± 4.1	96.1 ± 7.2	102.6 ± 6.6	116.4 ± 9.6
Dimethoate	33.9 ± 1.7	68.6 ± 2.4	84.1 ± 5.4	89.0 ± 7.9	88.2 ± 8.8	84.7 ± 7.5
Diuron	79.7 ± 4.0	90.4 ± 7.0	91.7 ± 4.9	94.9 ± 7.2	89.2 ± 7.3	100.9 ± 13.5
Flufenoxuron	95.4 ± 1.1	88.8 ± 1.6	89.4 ± 3.8	93.3 ± 5.8	100.0 ± 6.1	119.2 ± 13.9
Monocrotophos	4.6 ± 0.3	13.9 ± 0.3	21.8 ± 0.8	33.8 ± 1.1	58.5 ± 2.0	95.1 ± 5.7
Oxamyl	20.8 ± 0.7	52.6 ± 1.9	65.0 ± 2.0	79.7 ± 3.0	91.2 ± 4.6	110.6 ± 5.2
Thiamethoxam	40.0 ± 1.4	45.9 ± 0.9	46.6 ± 3.8	52.2 ± 1.7	70.9 ± 2.9	97.3 ± 2.0

Improved Precision and Detection Rates in Complex Matrices

Enhanced ion transmission resulted in increased peak area response and improved peak area precision. Figure 5 compares the relative standard deviation (RSD) of area for 50 pesticides spiked into black tea at the MRL and diluted in different ratios with acetonitrile. The area of the blue polygon is considerably smaller than the area of the red polygon indicating that the 6495 triple quadrupole had significantly lower RSD values compared to the previous design. The area RSD at a low concentration is a universal measure of ion efficiency and can be used to estimate the limits of detection. An LOD estimation based on the precision has much more relevance than the estimation based on signal to noise which can change depending on the noise region and the selected algorithm for noise calculation. RSD values can be correlated to the minimum amount that can be reliably detected as long as the noise is not significantly contributing to RSD. For pesticide residues, a maximum RSD of 20% has been specified as minimum performance requirement in SANCO/12571/2013.

Figure 6 shows the pesticides detection at different dilution levels in the black tea matrix. Under the experimental conditions, 67% of all the spiked pesticides were easily detected with an RSD below 20% in the 1:100 dilution corresponding to a concentration of 0.02 ng/mL. An additional 18% were detected with acceptable precision in the 1:50 dilution and another 9% in the 1:20 dilution. Excellent precision was observed for replicate injections of these samples within a 72 hour work list demonstrating the robustness of the system.

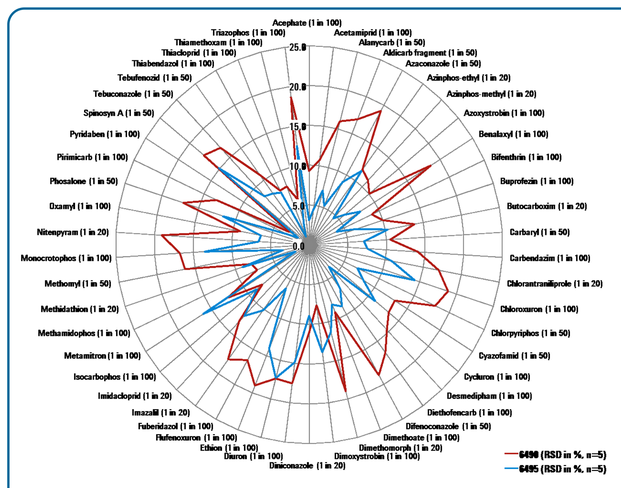

Figure 5. Comparison of area RSDs for pesticides spiked into black tea at the MRL and diluted in different ratios with acetonitrile for the Agilent 6495 (blue) and the 6490 triple quadrupole (red).

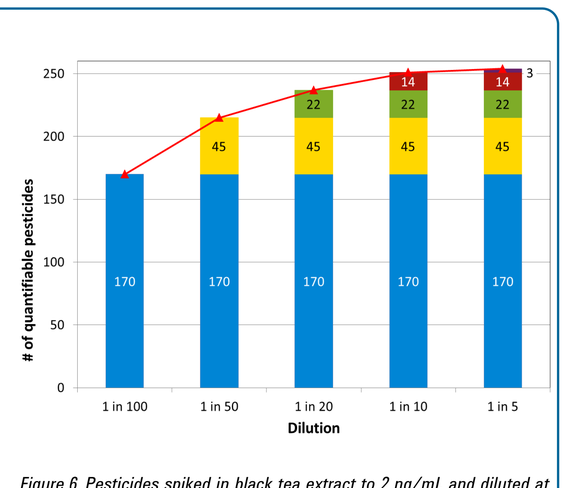

Figure 6. Pesticides spiked in black tea extract to 2 ng/mL and diluted at different levels. 170 pesticides can be detected at the 1:100 dilution level with an area RSD <20%. Additional compounds are detected at higher concentrations, i.e. lower dilution levels.

Conclusions

A UHPLC/MS/MS based multi-residue method for the determination of more than 250 pesticides and pesticide metabolites has been developed using a 1290 Infinity UHPLC and the highly sensitive 6495 triple quadrupole LC/MS system. The enhanced instrument sensitivity allows for the extensive dilution of black tea extracts resulting in:

- minimized matrix effects
- the possibility of using solvent-based calibration
- increased robustness and instrument uptime

These improvements will ultimately increase the throughput and the efficiency for a testing laboratory.