Multi-Residue Pesticide Screening and Quantitation in Difficult Food Matrixes Using the Agilent 6495 Triple Quadrupole Mass Spectrometer

Application Note

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Abstract

This Application Note describes a UHPLC/MS/MS-based multi-residue method for the determination of more than 250 pesticides and pesticide metabolites in food samples. The method benefits from the increased chromatographic resolution of the Agilent 1290 Infinity UHPLC System, the versatile ionization capabilities of the Agilent Jet Stream ionization source, and the innate sensitivity of the Agilent 6495 Triple Quadrupole LC/MS System. The method has been applied to the analysis of pesticide residues in complex matrixes such as black tea. Matrix effects in the ionization were controlled by extensive dilution of the sample extracts prior to injection.

Our results demonstrate that the increased sensitivity of the 6495 Triple Quadrupole LC/MS System enables the accurate quantitation of targeted pesticides below the maximum residue limits (MRLs) specified by the European Commission, most of them even in the 1:100 diluted extracts, with improved precision and excellent robustness.

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Introduction

The screening and quantitation of pesticide residues in food products is one of the most important and demanding applications in food safety. There are more than 1,000 pesticides and pesticide metabolites that can be present in food and, thus, are regulated and controlled. The European Commission regulation (EC) 396/2005 and its annexes set maximum residue limits (MRLs) for more than 170,000 matrix-pesticide combinations for food[1]. Similar regulations are in place in other regions. Most pesticides are analyzed with multi-residue methods covering hundreds of compounds, which are applied to various food commodities for both screening and quantitation. Therefore, fast and reliable analytical methods are required to allow identification and quantitation of hundreds of pesticides at low concentrations in a broad range of food matrixes with confidence. Criteria for the identification of pesticide residues and requirements for method validation and quality control procedures for quantitation are specified in guidance documents such as SANCO/12571/2013[2].

Matrix effects in electrospray ionization, which change considerably between different food samples, present a significant challenge to the accurate quantitation of pesticides. There are different strategies to compensate for matrix effects such as matrix matched calibrations, standard addition, or the use of internal standards. However, matrix matched calibrations do not fully compensate for variations in matrix effects within a commodity or a commodity group. Standard addition requires multiple injections for each sample, which reduces productivity. The use of isotopically labelled internal standards is probably the most attractive approach, however, it is not applicable for all target compounds in a multi-residue pesticide method. Sample dilution is another approach to minimize matrix effects[3] but requires the use of highly sensitive analytical instruments due to the need to detect contaminants below the MRLs stipulated by the EC. Furthermore, the extensive dilution of sample extracts requires very high precision, as even small deviations result in considerable inaccuracies when multiplied with high dilution factors.

This Application Note shows the development of an UHPLC/MS/MS method for the screening and quantitation of hundreds of pesticides in food samples. The method was developed using the Pesticide tMRM LC/MS Application Kit (p/n G1733BA). Transitions for all compounds in the comprehensive pesticide standard mix (p/n 5190-0551) and a few additional pesticides of interest were included in the method. An Agilent 1290 Infinity UHPLC System was coupled to the highly sensitive Agilent 6495 Triple Quadrupole LC/MS System operated with dynamic MRM mode with fast polarity switching. Several modifications to the previous high-end triple quadrupole mass spectrometer design resulted in higher analytical performance.

- New mass filter one (MS1) ion optics for increased precursor ion transmission
- An improved curved and tapered collision cell providing enhanced MS/MS spectral fidelity
- A new ion detector operating at dynode accelerating voltages of up to 20 kV
- A new autotune optimized for speed and sensitivity

In addition, the 6495 Triple Quadrupole LC/MS System uses the proven Agilent Jet Stream Ionization source and the dual stage ion funnel. Enhanced sensitivity gives enhanced peak area response and improved peak area precision, which ultimately leads to lower detection limits compared to previous designs. The enhanced sensitivity was used for the extensive dilution of complex food sample extracts to minimize matrix effects in the electrospray ionization. The improved precision of the analytical method is demonstrated for diluted black tea samples.

Experimental

Reagents and chemicals

All reagents and solvents were HPLC or LC/MS grade. Acetonitrile and methanol were purchased from Honeywell (Morristown, NJ, USA). Ultrapure water was produced using a Milli-Q Integral system equipped with a LC-Pak Polisher and a 0.22-µm point-of-use membrane filter cartridge (EMD Millipore, Billerica, MA, USA). Formic acid was from Fluka (Sigma-Aldrich Corp., St. Louis, MO, USA) and ammonium formate solution (5 M) was from Agilent (p/n G1946-85021). Pesticides were included in the Agilent comprehensive pesticide mixture (p/n 5190-0551). A limited number of additional pesticides were purchased from Fluka (Sigma-Aldrich Corp., St. Louis, MO, USA). Immediately before use, the eight submixes of the comprehensive pesticide mixture and the mixed stock solution of the additional pesticides were combined and further diluted with acetonitrile to a final pesticide working solution containing more than 250 pesticides at a concentration of 10 µg/mL. This solution was used for spiking the QuEChERS extracts and for the preparation of the calibration samples. Eight calibration samples with concentrations ranging from 0.02 to 100 ng/mL were prepared in pure acetonitrile.

Sample preparation

Tea, orange, and tomato samples were obtained from a local grocery store. Samples were extracted according to the citrate buffered QuEChERS protocol using Agilent BondElut QuEChERS kits (p/n 5982-5650). Ten grams of homogenized fruit and vegetable or 2 g of tea were weighed into 50-mL polypropylene tubes and extracted with 10 mL acetonitrile for 1 minute while shaking vigorously by hand. The tea samples were wetted with 8 mL ultrapure water for 2 hours prior to extraction. Raw extracts were cleaned up by dispersive SPE using primary

secondary amine (PSA, p/n 5982-5256). In black tea samples, graphitized carbon black (GCB) contained in the Agilent BondElut QuEChERS EN dispersive SPE tubes (p/n 5982-5356H) was also used for cleanup. Final extracts of blank samples were spiked in five relevant concentrations with the comprehensive pesticide working solution and then 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 measured with five technical replicates.

Equipment

Separation was carried out using an Agilent 1290 Infinity UHPLC system consisting of an Agilent 1290 Infinity Binary Pump (G4220A), an Agilent 1290 Infinity High Performance Autosampler (G4226A), a sample cooler (G1330B), and an Agilent 1290 Infinity Thermostatted Column compartment (G1316C). The UHPLC system was coupled to an Agilent G6495 Triple Quadrupole LC/MS System equipped with an Agilent Jet Stream electrospray ionization source. Agilent MassHunter Workstation Software was used for data acquisition and analysis (v. B.07.00).

Method

The 1290 Infinity UHPLC System conditions are summarized in Table 1, and a summary of the 6495 Triple Quadrupole parameters are shown in Table 2. Identification of polarity, precursor and product ions, as well as optimization of collision energies, was taken from the Agilent Pesticide tMRM LC/MS Application Kit, and was further optimized using Agilent MassHunter Optimizer Software. Analysis was carried out with positive and negative electrospray ionization in dynamic multiple reaction monitoring (dMRM) in a single analytical run. A 2-µL amount of the final extract was injected into the UHPLC/MS/MS.

Data were evaluated using the Agilent MassHunter Quantitative Analysis Software. Calibration was done using neat standard solutions and linear, 1/x weighted calibration curves. The lower limits of quantitation (LLOQs) correlate with the instrument detection limits (IDLs) in black tea matrix. IDL is calculated based on the relative standard deviation of a series of replicates of a low level sample that is not higher in concentration as 2 to 5 times the detection limit. The IDL is defined as the minimum amount of analyte required to produce a signal that is statistically distinguishable from background noise with a confidence level of 99 %. This approach has much more relevance for routine operation as it avoids ambiguity related to the variation in the chemical noise and subjectivity in the way signal-to-noise (S/N) is determined[4]. In addition, it is directly correlated to the precision of the analytical method, which is important when doing an extensive dilution of sample extracts.

Table 1. Instrument parameters.

Agilent 1290 Infinity 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
Speed	Draw 100 µL/min, Eject 200 µL/min
Autosampler temp	6 °C
Needle wash	10 s with acetonitrile/water (50/50; v/v)
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 B % 0 5 0.5 5 3.5 50 17.0 100 20.0 100 20.1 5
Stop time	20.1 minutes
Post time	3 minutes
Agilent 6495 Triple Quadrupole LC/MS System	
Ion mode	Positive and negative ESI with Agilent Jet Stream
Scan type	Dynamic MRM
Drying gas temperature	120 °C
Drying gas flow	17 L/min
Sheath gas temperature	300 °C
Sheath gas flow	12 L/min
Nebulizer pressure	30 psi
Capillary voltage	3,500 (pos/neg)
Nozzle voltage	300 V (pos); 500 V (neg)
Cycle time	500 msec
Total number of MRMs	532 (positive: 509/negative: 23)
Maximum number of concurrent MRMs	68
Minimum dwell time	5.10 ms
Maximum dwell time	249.09 ms
MS1 and MS2 resolution	Unit

Results and Discussion

Development of the UHPLC/MS/MS method

The pesticide screening method developed for the Agilent Pesticide tMRM LC/MS Application Kit was transferred to the 6495 Triple Quadrupole LC/MS System. The method was extended to include several relevant acidic herbicides, and fast polarity switching was employed. Compound-dependent parameters such as collision energy and cell acceleration voltage were fine optimized but only minor deviation from optimized values for previous models was observed. The prefilter and the detector were adjusted according to mass during the instrument's autotune. Sheath gas temperature was optimized using the MassHunter Source Optimizer Software to produce the highest abundance for the majority of target compounds, and to not compromise labile and ammonium adduct-forming compounds.

Figure 1 shows the chromatogram of a tea extract spiked with more than 250 pesticides at a concentration of 10 µg/kg, and diluted 1:20 with acetonitrile prior to injection.

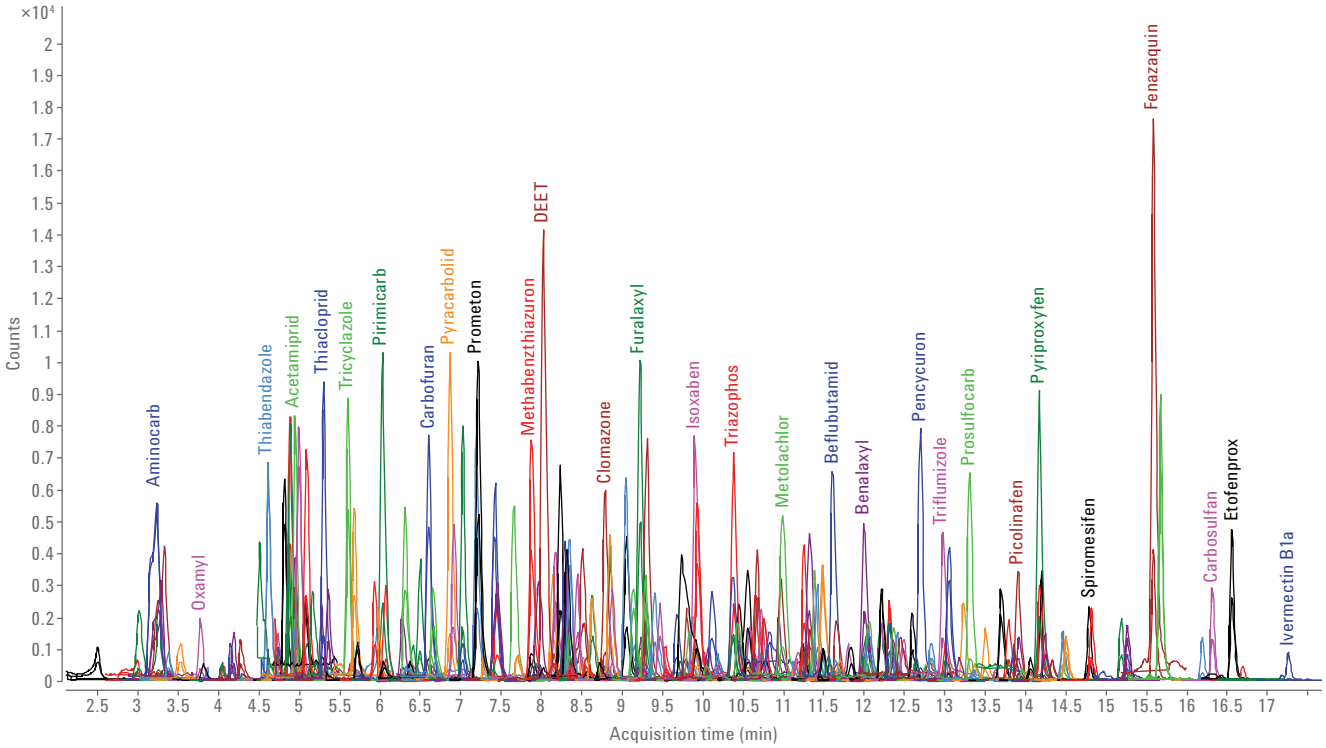

Figure 1. Chromatograms of more than 250 pesticides spiked into black tea at the MRL and diluted 1:20 with acetonitrile (corresponding to a concentration of 0.1 ng/mL). For the sake of clarity, only part of the chromatographic peaks are labelled.

Evaluation of increased instrument performance

The MS1 ion optics has demonstrated a noticeable increase in the precursor ion transmission, and an increase up to a factor of 3 has been observed, depending on the ion mass. In addition, the detector design results in signal gains especially for large fragment ions and negative ions across a broad mass range. When comparing the area response of pesticides acquired with the 6495 Triple Quadrupole System to results from the earlier model, a compound dependent area gain of up to a factor of 5 was observed.

Enhanced ion transmission not only resulted in increased peak areas but also in improved peak area precision. These enhancements ultimately lowered detection limits compared to previous designs. The empirical observation that supports this hypothesis is shown in Figure 2, which compares the obtained area RSDs on a 6495 Triple Quadrupole System versus a 6490 Triple Quadrupole for 50 pesticides spiked into black tea at the MRL and diluted in different ratios with acetonitrile. The selection of these 50 pesticides was based on relevance. Several of those compounds were found in official control samples above the MRL and thus, the import of tea into the European Union was blocked. The area of the blue polygon is considerably smaller than the area of the red polygon, which indicates that the improved ion statistics of the 6495 Triple Quadrupole System instrument translates into considerably lower RSD values for most pesticides at the same dilution levels. The relative standard deviation (RSD) of a series of replicates at a low concentration level is a universal measure of the ion efficiency, and can be used for the estimation of the quantitation limits. A low RSD value has much more relevance than S/N measurements, which can change based on the selected noise region and the software algorithm used for calculation. A particular RSD can be defined as the minimum amount that can be reliably detected, as long as the noise level does not significantly contribute to RSD values. For pesticide residues, a maximum RSD of 20 % has been specified as the minimum performance requirement in SANCO/12571/2013.

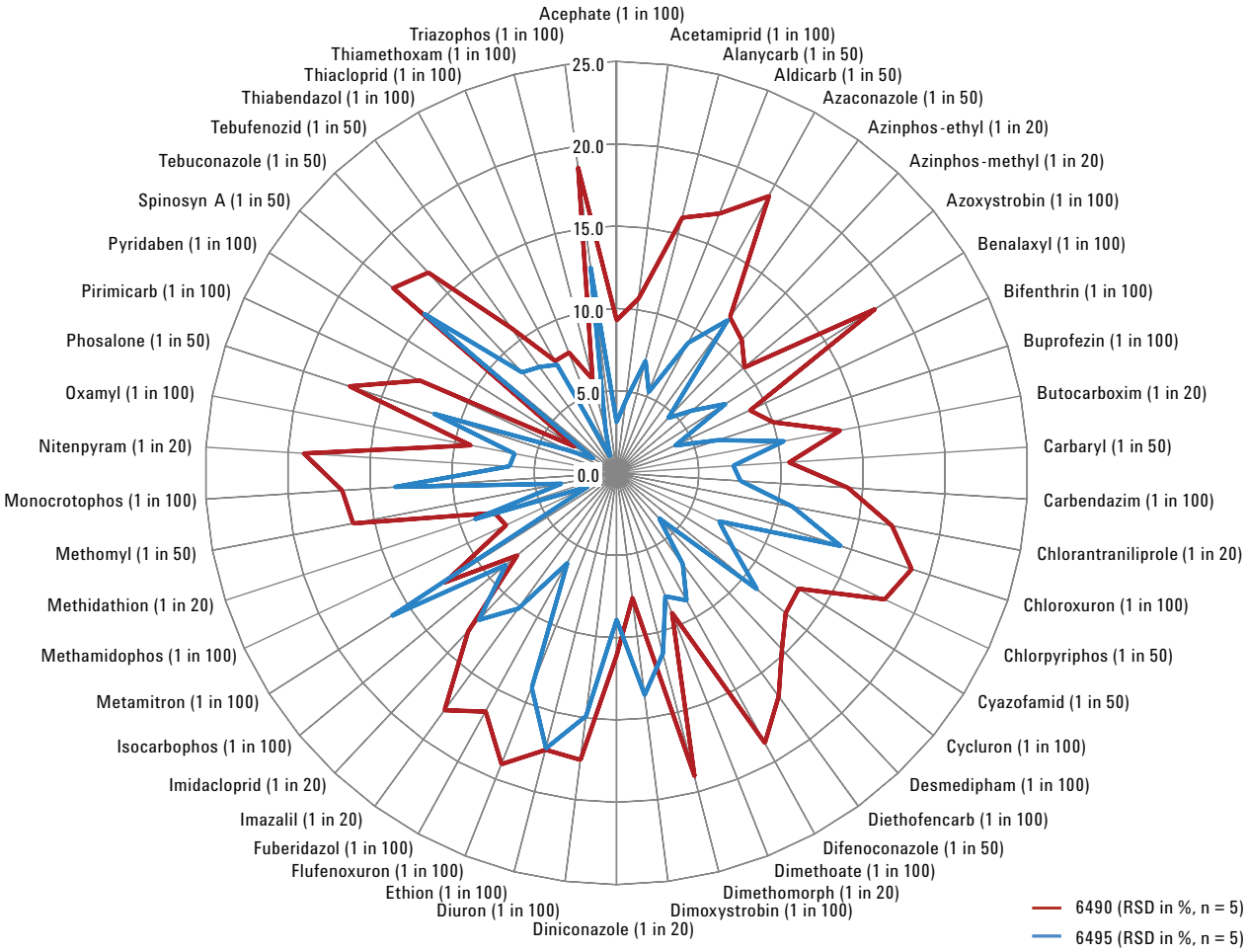

Figure 2. Comparison of area RSDs for pesticides spiked into black tea at the MRL and diluted in different ratios with acetonitrile for the Agilent 6495 LC/MS (blue) and the Agilent 6490 Triple Quadrupole (red).

With the updated design of the 6495 Triple Quadrupole LC/MS System, more pesticides can be detected at low concentrations in QuEChERS extracts of different food commodities according to the quality criteria specified in the SANCO guidelines. In tomato and orange extracts, all pesticides were easily detected at the lowest spiked concentrations of 1 ng/g. The tea matrix at this concentration has a slightly smaller detection rate due to the 5-fold lower sample amount and the more complex matrix. Figure 3 shows the detection rate of the pesticides for different dilution levels in the black tea matrix.

Under the applied experimental conditions, approximately 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. In addition, approximately 20 % were detected with acceptable precision in the 1:50 dilution, and another ~10 % in the 1:20 dilution. Excellent precision was observed for replicate injections of these samples within a 72-hour worklist.

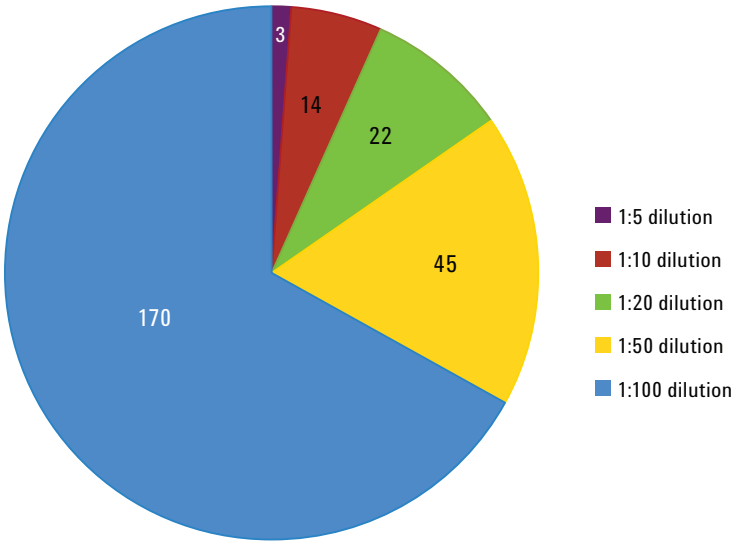

Figure 3. Pesticides spiked in black tea extract to 2 ng/mL and diluted at different levels. 170 pesticides can be detected at the 1:100 dilution level with an area RSD < 20 %. Additional compounds are detected at higher concentrations, that is, at lower dilution levels.

Minimizing matrix effects by dilution of sample extracts

The ability to extensively dilute sample extracts to remove matrix effects is an attractive capability to many routine testing labs. It enables quantitation of complex samples against a solvent calibration. A possible cause for matrix effects in electrospray ionization is the limited number of excess charges, and the limited space on the surface of the charged droplet. The dilution of the matrix frees up space at the surface, resulting in more efficient ionization of the target compounds. In addition, the amount of matrix injected to the LC/MS system is limited, which results in increased robustness of the analytical method, minimization of instrument contamination, and increased instrument uptime.

Figure 4 shows the chromatograms of alanycarb and oxamyl spiked in black tea extract corresponding to 10 µg/kg and diluted with acetonitrile prior to injection in different dilution ratios.

Upon 1:5 dilution, the signal for alanycarb increased. For oxamyl and the further dilution levels of alanycarb, the peak areas decreased less than the extent to which the target compounds were diluted. Dilution typically causes the final concentrations of the pesticides to increase until the point at which complete recovery is achieved. Table 3 shows the recoveries in black tea extract for 10 pesticides and different dilution ratios.

While a weak matrix effect was observed for diuron and flufenoxuron, the signal suppression for monocrotophos and alanycarb in the nondiluted tea was substantial. However, when diluting the final extract 1:10, more than half of the compounds showed adequate recoveries of over 70 %. Very few compounds required a larger dilution of 1:50, or even 1:100 to minimize the matrix effects to achieve acceptable recoveries based on a solvent calibration. In the 1:100 dilution, all detectable pesticides showed full recovery and basically no signal suppression. This is in agreement with published results, which showed that the Agilent Jet Stream Ionization required less dilution to eliminate matrix effects compared to equivalent techniques[3].

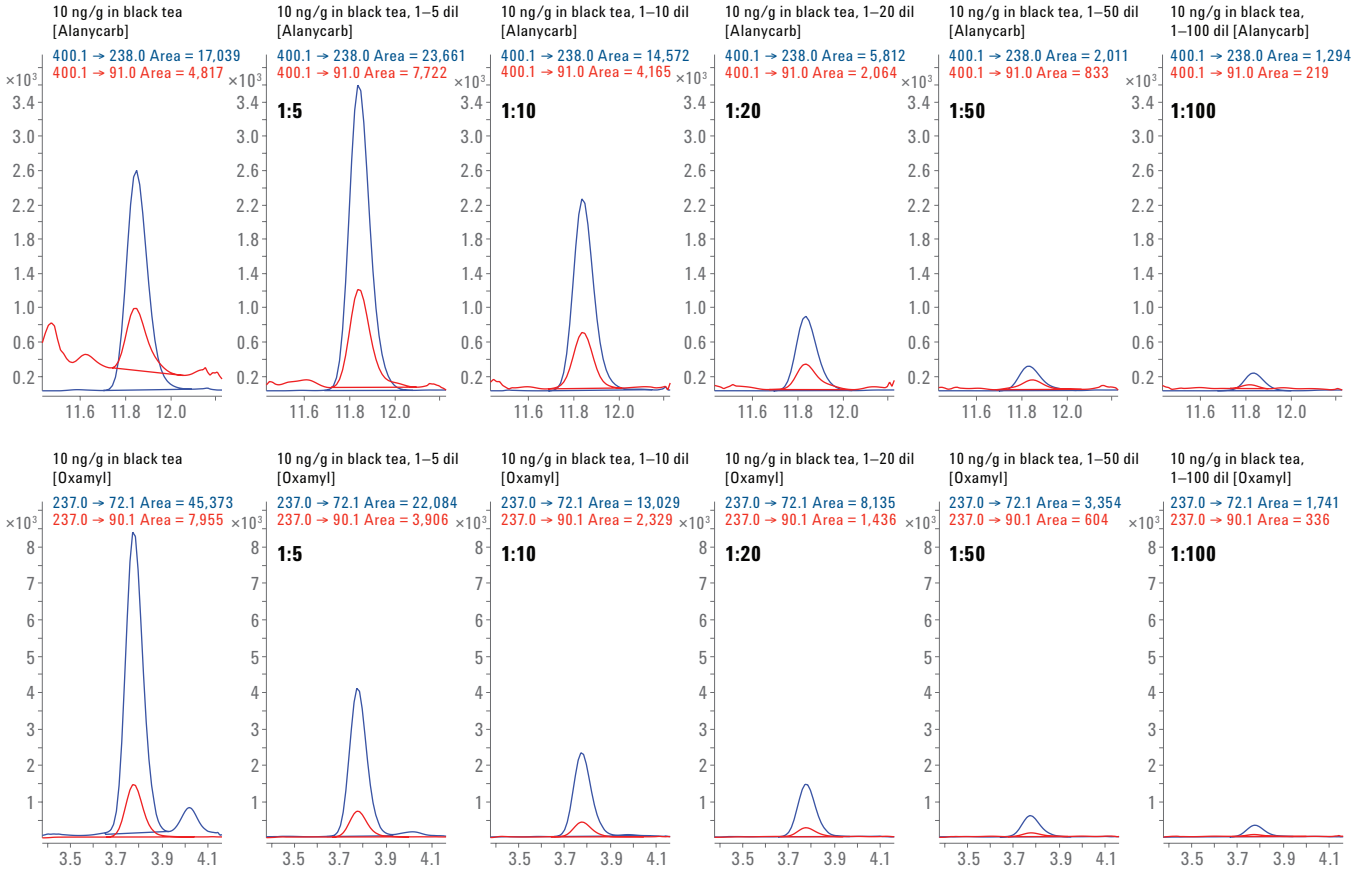

Figure 4. Comparison of peak areas for alanycarb and oxamyl spiked in black tea and diluted with acetonitrile 1:5, 1:10, 1:20, 1:50, and 1:100 prior to injection.

Table 3. Recoveries for selected pesticides calculated for different dilution ratios. Cells shaded in green comply with requirements of 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

Conclusions

An UHPLC/MS/MS based multi-residue method for the determination of more than 250 pesticides and pesticide metabolites has been developed. It takes full advantage of the low delay volumes of the Agilent 1290 Infinity LC System and its ability to handle high back pressures in UHPLC separations for increased chromatographic resolution. The method benefits from the highly sensitive Agilent 6495 Triple Quadrupole LC/MS System and from the versatile ionization capabilities of the Agilent Jet Stream ionization source. Dynamic MRM acquisition and fast polarity switching were used to maximize dwell times for each individual compound. Source parameters were optimized to achieve good sensitivity across the suite of target compounds.

The method was applied to the analysis of pesticides in complex matrixes such as black tea. Enhanced sensitivity allowed for more flexibility in the degree of sample dilution. With any dilution, a lower matrix amount is introduced into the LC/MS system leading not only to fewer matrix effects, but also to improved method robustness and increased instrument uptime. Extensive dilution of sample extracts was applied to minimize matrix effects, and to allow quantitation of all pesticides within the acceptable recovery range of 70 to 120 % based on a solvent calibration. The increased sensitivity of the 6495 Triple Quadrupole LC/MS System allowed the quantitation of the majority of all targeted pesticides below the maximum residue limits specified by the European Commission, even in the 1:100 diluted extracts with improved precision and excellent robustness.

References

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