Routine Multiresidue Pesticide Analysis using the Agilent 6470 Triple Quadrupole Mass Spectrometer

Application Note

Food safety

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Abstract

This application note describes a UHPLC/MS/MS-based method for the screening and quantification of more than 250 pesticides and pesticide metabolites in food samples. The method leverages the:

- Increased chromatographic resolution of the Agilent 1290 Infinity UHPLC System
- Versatile ionization capabilities of the Agilent Jet Stream ionization source
- Innate sensitivity of the Agilent 6470 Triple Quadrupole LC/MS System.

The method was applied to the analysis of pesticide residues in complex food matrices. Sample dilution prior to injection was used as a means of maximizing method robustness and minimizing matrix effects.

Our results demonstrate that the increased sensitivity of the 6470 Triple Quadrupole LC/MS System enables the accurate and precise quantification of targeted pesticides below the maximum residue limits (MRLs) specified by the European Commission, even in 1:10 and up to 1:20 dilutions of black tea extracts.

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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 multiresidue methods. Major challenges in achieving accurate and precise quantitation include diversity of compound classes, matrix effects, low concentrations and poor ionization efficiencies of some pesticides.

This application note describes the development of an UHPLC/MS/MS method for the screening and quantitation of hundreds of pesticides in complex food matrices using the Agilent Pesticide tMRM LC/MS Application Kit (p/n G1733BA). Transitions for all compounds in the comprehensive pesticide standard mixture (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 an Agilent 6470 Triple Quadruple LC/MS system operated in dynamic MRM (DMRM) and fast polarity switching mode. Several hardware modifications to previous designs resulted in increased quantitative performance. Improvements were achieved by optimized 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 of the instrument.

Enhanced sensitivity achieved by the design translates into enhanced peak area response and improved area precision, leading ultimately to lower detection limits compared to previous designs. We achieved rugged and high performance quantitation at low levels in tomato, orange, and black tea. Moreover, we evaluated the use of dilution as a means of minimizing matrix effects, and demonstrated that the increased sensitivity achieved by this design allowed a high degree of sample dilution while still allowing to achieve the maximum residue level (MRL) stipulated by the European Union.

Experimental

Reagents and chemicals

The Agilent comprehensive pesticide mixture (p/n 5190-0551) was used, and several additional pesticides were purchased from Fluka (Sigma-Aldrich Corp., St. Louis, MO, USA). The eight submixes of the comprehensive pesticide mixture and 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 (10 ppm). This solution was used for spiking the QuEChERS extracts and for the preparation of the calibration samples. For instrument detection limit (IDL) and low limit of quantitation (LLOQ) determination in solvent, 13 calibration levels with concentrations ranging from 1 ppt to 100 ppb were prepared in pure acetonitrile. For recovery calculations in matrices, a solvent calibration set ranging from 10 ppt to 100 ppb was prepared in acetonitrile.

All reagents and solvents were HPLC or LC/MS grade. Acetonitrile and methanol were purchased from Honeywell (Morristown, NJ, USA). Ultrapure water was produced with 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 and ammonium formate were from Fluka (Sigma-Aldrich Corp., St. Louis, MO, USA).

Sample preparation

Organic tomato, orange, and black tea samples were obtained from a local grocery store. Two grams of tea samples were wetted with 8 mL of water and incubated for 2 hours at room temperature. Well-blended homogenized fruit samples were prepared with a ceramic homogenizer (p/n 5982-9312), and 10 g were weighed. The fruit and tea samples were extracted with 10 mL acetonitrile for 1 minute with vigorous shaking. One pouch of Agilent EN extraction salts (p/n 5982-6650) was added to each mixture, and shaken for 1 minute followed by centrifugation at 3,000xg for 5 minutes. Six mL of tea supernatant were added into Agilent QuEChERS Dispersive SPE for high pigment EN (p/n 5982-5356), 6 mL of tomato supernatant were added into Agilent QuEChERS Dispersive SPE for general fruits and vegetables EN (p/n 5982-5056), and 6 mL of orange supernatant were added into Agilent QuEChERS Dispersive SPE for fruits and vegetables with fats and waxes EN (p/n 5982-5156), followed by shaking for 1 minute and centrifuged at 3,000xg for 5 minutes. Supernatant was

collected and passed through a 0.45 µm syringe filter. Final extracts were spiked at 10 ng/g in relation to starting matrix quantity with the comprehensive pesticide working solution and diluted 1:2, 1:5, 1:10, and 1:20 with acetonitrile. Matrix matched standards and dilutions were prepared immediately before injection and measured with five technical replicates.

Equipment

Separation was carried out using an Agilent 1290 Infinity UHPLC system consisting of:

- Agilent 1290 Infinity Binary Pump (G4220A)
- Agilent 1290 Infinity High Performance Autosampler (G4226A)
- Agilent sample cooler (G1330B)
- Agilent 1290 Infinity Thermostatted Column compartment (G1316C)

The UHPLC was coupled to an Agilent 6470 Triple Quadrupole Mass Spectrometer equipped with an Agilent Jet Stream electrospray ionization source. Agilent MassHunter Acquisition (ver. B.08.00) and Agilent MassHunter Quantitative Analysis (Ver B.07.00) software was used for data acquisition and analysis.

Methods

The LC/MS conditions and parameters are provided below. MRM parameters, such as polarity, precursor, and product ions as well as optimal collision energies were imported from the Agilent Pesticide tMRM LC/MS Application Kit, and source conditions were optimized for selected poor-responding analytes using Agilent Source Optimizer Software. Data acquisition was carried out in fast polarity switching DMRM mode. A 2-µL amount of the final extract was injected into the LC/MS system.

Data was evaluated using the Agilent MassHunter Quantitative Analysis Software. Calibration was done using neat standard solutions. For calibration curves, linear fit with weight = $1/x$ or $1/x^2$ was used.

Chromatography

Agilent 1290 Infinity UHPLC System

Column	Agilent EclipsePlus C18, RRHD, 2.1 × 150 mm, 1.8 µm (p/n 959759-902)
Column temperature	40 °C
Injection volume	2 µL
Autosampler temperature	4 °C
Needle wash	8 seconds in wash port (75:25 methanol/H_2O)
Mobile phase	A) 5 mM ammonium formate + 0.1% formic acid B) 5 mM ammonium formate + 0.1% formic acid in methanol
Flow rate	0.400 mL/min
Gradient program	Time B (%) 0.00 5 0.50 5 3.50 40 17.00 98 20.00 98 20.10 5
Post time	3 minutes

Mass spectrometry

Agilent 6470 Triple Quadrupole Mass Spectrometer

Ion source	Agilent Jet Stream
Polarity	Positive and negative switching
Gas temperature	140 °C
Drying gas (nitrogen)	5 L/min
Nebulizer gas	30 psi
Sheath gas	375 °C
Sheath gas flow	12 L/min
Capillary voltage	4,000 V/–3,000 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

Development and performance of the UHPLC/MS/MS method

The pesticide screening method developed for the Agilent Pesticide tMRM LC/MS Application Kit was transferred to the 6470 Triple Quadrupole LC/MS system. The method was extended to include several relevant acidic herbicides. DMRM and fast polarity switching mode were employed. Sheath gas temperature, drying gas temperature, capillary voltage, and nozzle voltage were optimized using the MassHunter Source Optimizer Software to produce the highest abundance for a selected subset of labile and poor-responding analytes. Figure 1 shows the overlapped MRM chromatograms of a black tea extract spiked with more than 250 pesticides to a concentration of 10 µg/kg, and diluted 1:10 with acetonitrile prior to injection.

The improved ion optics and detector allowed the quantitation of the majority of pesticides at an LLOQ of 10% of their MRL. The precision and accuracy of measurements were evaluated at 10 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 four orders of linear dynamic range. These results are well within the criteria set by bioanalytical method validation guidelines.

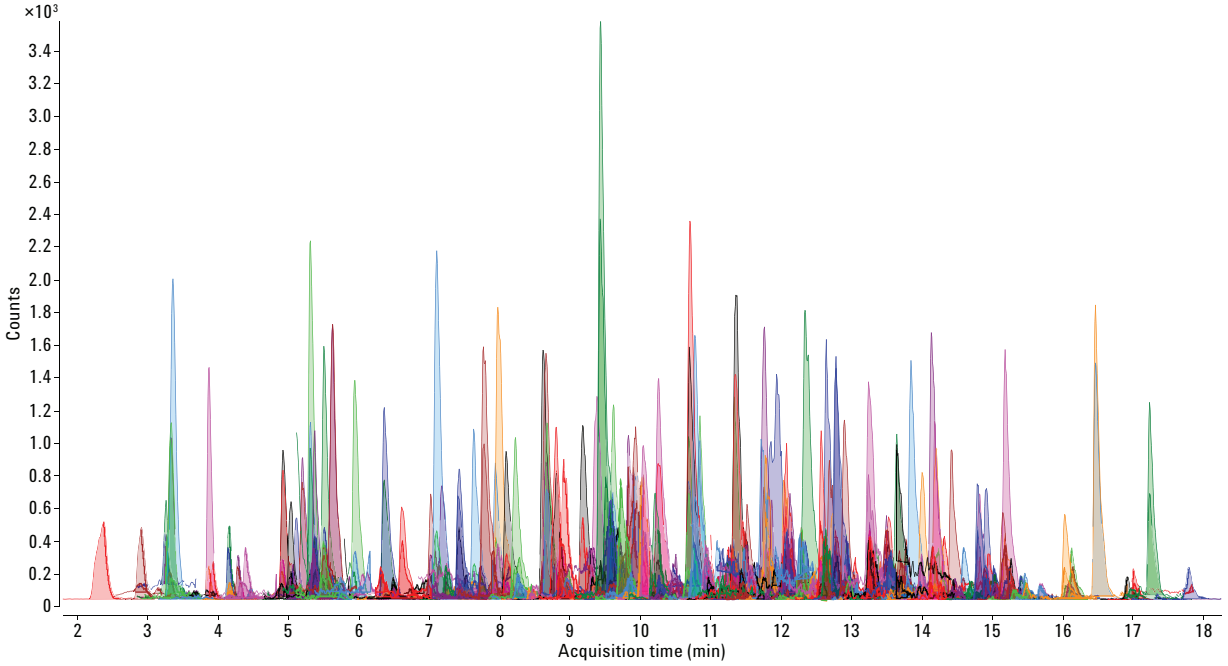

Figure 1. Overlapped MRM chromatograms of more than 250 pesticides spiked into black tea at the MRL (10 µg/kg) and diluted 1:10 with acetonitrile (corresponding to a concentration of 0.2 ng/mL).

Evaluation of increased instrument performance

The improved hardware design of the 6470 Triple Quadrupole LC/MS system showed sensitivity gains of up to factor 4 compared to the previous 6460 Triple Quadrupole model. The observed sensitivity gain was compound-dependent.

Enhanced ion transmission not only resulted in increased area reponse but also in improved area precision. Area relative standard deviation (RSD) can be used as an indirect measure of the relative number of ions under a chromatographic peak and therefore can be used to estimate the instrument detection limit (IDL) [1]. Performance based on signal response precision gives a much clearer indication of sensitivity compared to signal to noise ratios in quantitative applications. 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.

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 2 shows the histogram of LLOQs in the solvent standard and food extracts. LLOQs in the black tea extract were found to be higher due to the 5-fold lower sample amount and the complex matrix. Even in this challenging matrix, the majority of pesticides achieved an LLOQ of ≤10% of the default MRL.

Table 1. IDL for 50 Relevant Pesticides in Black Tea Based on a Dilution Series Prepared in Acetonitrile

	IDL (ppt)				IDL (ppt)		
Pesticide	**Agilent 6470**	**Agilent 6460**	**Fold improvement**	**Pesticide**	**Agilent 6470**	**Agilent 6460**	**Fold improvement**
Acephate	7.1	10.4	1.5x	Fenarimol	264.5	4023.8	15.2x
Acetamiprid	1.8	32.9	18.0x	Fipronil	74.5	339.7	4.6x
Aldicarb	645.0	43.3	0.1x	Flufenoxuron	58.0	521.6	9.0x
Azinphos-methyl	165.1	278.9	1.7x	Flusilazole	56.1	204.5	3.6x
Bifenthrin	267.5	67.8	0.3x	Hexaflumuron	1003.8	20771	20.7x
Bosclid	220.2	665.8	3.0x	Imazalil	30.1	203.2	6.7x
Buprofezin	6.2	36.0	5.8x	Imidacloprid	57.6	191.1	3.3x
Butocarboxim	645.0	107.3	0.2x	Isocarbophos	14.6	129.5	8.9x
Carbendazim	3.7	13.5	3.6x	Metamitron	172.6	558.4	3.2x
Chloroxuron	7.4	98.4	13.3x	Methamidophos	1.7	43.1	25.9x
Chlorpyriphos	68.9	401.1	5.8x	Methidathion	71.9	340.1	4.7x
Cycluron	8.4	71.1	8.4x	Methomyl	4.1	21.5	5.2x
Cyprodinil	182.1	61.9	0.3x	Monocrotophos	10.8	52.3	4.8x
Desmedipham	11.2	32.4	2.9x	Myclobutanil	10.3	356.2	34.5x
Diazinon	32.1	31.4	1.0x	Omethoat	2.1	6.1	2.9x
Diethofencarb	11.1	59.0	5.3x	Oxamyl	1.0	5.8	5.7x
Difenoconazole	315.1	620.7	2.0x	Phosalone	36.9	2044.3	55.3x
Dimethoate	2.8	32.8	11.8x	Pirimicarb	1.3	2.8	2.2x
Dimethomorph	59.0	310.7	5.3x	Pyridaben	0.8	0.6	0.8x
Dimoxystrobin	6.3	23.6	3.8x	Tebuconazole	45.8	41.9	0.9x
Diniconazole	30.9	237.4	7.7x	Tebufenozid	175.4	52.3	0.3x
Dioxacarb	103.7	139.1	1.3x	Teflubenzuron	291.6	2012.1	6.9x
Diuron	22.6	37.4	1.7x	Thiacloprid	1.5	6.0	4.1x
Epoxyconazol	21.4	144.8	6.8x	Thiamethoxam	8.7	38.8	4.5x
Ethion	11.0	166.3	15.2x	Triazophos	10.1	65.9	6.6x

Five injection replicates were used for calculation.

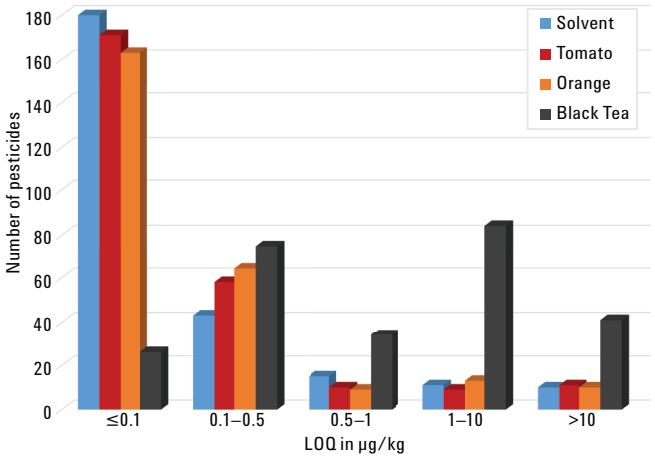

Figure 2. LLOQs for the >250 pesticides in solvent and spiked tomato, orange, and black tea extracts. Results were classified in five relevant concentration ranges and are shown as a histogram.

Minimizing matrix effects by dilution of extracts

Evaluation of matrix effects (suppression and enhancement) was done by comparing the response of the targeted compound in a solvent calibration series against the spiked black tea sample extract. Primarily, signal suppression was observed, with a significant number (n = 104) of target compounds exhibiting a suppressed signal, where the analyte response in undiluted black tea was less than 70% of the analyte response in neat solvent.

The ability to dilute complex sample extracts to minimize matrix effects enables more accurate quantification against a solvent calibration, and is an attractive capability to many routine testing labs. 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 minimization of instrument contamination, translating into increased instrument uptime and robustness of the analytical method. Table 2 shows the beneficial effect of dilution, where pesticide recoveries in black tea improve as the sample is further diluted. For compounds such as chloroxuron and myclobutanil, only weak matrix effects were observed, whereas compounds such as aldicarb and methomyl experienced significant signal suppression, and required a dilution of 1:20 to achieve acceptable recoveries. There were a few of compounds, such as monocrotophos, that would require a higher degree of sample dilution to achieve acceptable recovery, previously shown to be achieved with the Agilent 6495 Triple Quadrupole LC/MS System [2].

Table 2. Recoveries for Selected Relevant Pesticides in Black Tea, Calculated for Different Dilution Ratios Based on a Solvent Calibration

Analyte	No Dilution	Dilution 1:2	Dilution 1:5	Dilution 1:10	Dilution 1:20
Acephate	57 ± 1.6	68.9 ± 1.9	78.5 ± 3.9	83.6 ± 3.4	90 ± 1.9
Aldicarb	24.1 ± 5.8	37.5 ± 4.6	58.3 ± 8.9	68.5 ± 4	84 ± 6.1
Carbofuran	45.3 ± 0.5	60.6 ± 2.9	74.5 ± 4	81 ± 1.6	96.9 ± 7.2
Chloroxuron	75.4 ± 2.3	78.5 ± 9.5	81.8 ± 5.8	83.9 ± 9.4	97.8 ± 7
Dimethoate	25.1 ± 2.2	37.9 ± 2.7	57.8 ± 3.8	70.4 ± 6.5	84.9 ± 6.4
Epoxyconazol	66.1 ± 3.6	75.5 ± 6.4	73.4 ± 12.6	84.6 ± 11	89.3 ± 11.9
Ethion	54 ± 3.1	73.3 ± 4.3	81 ± 5.8	83 ± 5.3	88.2 ± 9
Methamidophos	42.6 ± 0.8	54.9 ± 1	67.9 ± 1	76.7 ± 1	88.2 ± 1.2
Methidathion	67.3 ± 4.1	79.3 ± 7.8	83.3 ± 9.5	88.8 ± 5	108.6 ± 3.3
Methomyl	10.4 ± 3.2	20.9 ± 2.7	42.1 ± 1.2	60.7 ± 2.6	76.4 ± 11.2
Monocrotophos	5.5 ± 5.6	9.9 ± 9.4	18.7 ± 6.9	31.4 ± 14.5	48.4 ± 8.7
Myclobutanil	84.6 ± 4.8	84.6 ± 3.8	86.8 ± 9	90.3 ± 13.4	99.7 ± 9.7
Oxamyl	14.1 ± 1.6	23.4 ± 2.2	44.1 ± 1.8	60.2 ± 1.7	76.8 ± 4
Pirimicarb	50.7 ± 1.2	62.9 ± 2	75.2 ± 1.7	81.9 ± 1.5	88.9 ± 2.4
Pyridaben	50.8 ± 1	60.4 ± 1.7	71.3 ± 1	79.4 ± 2	89.8 ± 2.9
Thiacloprid	24.1 ± 0.5	37.3 ± 0.7	56.8 ± 1.9	69.3 ± 2.7	82.4 ± 1.2

Cells shaded in green comply with requirements of SANCO/12571/2013.

Figure 3 demonstrates that most pesticides spiked in black tea achieve acceptable recoveries at the 1:20 dilution. In the 1:20 dilution, 93% of the detectable pesticides showed acceptable recoveries, basically showing minimal signal suppression.

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. Figure 4 shows the detection rates of pesticides for different dilution levels in the black tea matrix. Under the applied experimental conditions, approximately 67% of the spiked pesticides were easily detected in the 1:20 dilution with RSD values below 20%, corresponding to a concentration of 0.1 ng/mL. In addition approximately 7% were detected with acceptable precision in the 1:10 dilution, and another ~9% in the 1:5 dilution.

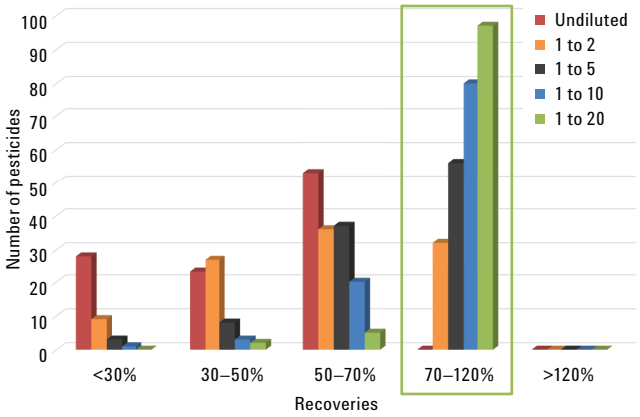

Figure 3. Histogram of recoveries for pesticides spiked into black tea at the MRL of 10 µg/kg and diluted with acetonitrile. 104 pesticides showed strong ion suppression and significantly better recoveries were observed after dilution. The green box denotes acceptable recoveries according to SANCO specifications.

Conclusion

An UHPLC/MS/MS based multiresidue method for the determination of more than 250 pesticides and pesticide metabolites has been developed. The obtained results demonstrate the increased chromatographic resolution of the Agilent 1290 Infinity LC System, the high sensitivity of the Agilent 6470 Triple Quadrupole LC/MS System, and the proven ionization enhancement capabilities of the Agilent Jet Stream Ionization Source.

Acquisition in DMRM, and fast polarity switching mode allowed maximized dwell times for each compound. Source parameters were optimized to improve detection of poor-responding analytes.

The method was applied to the analysis of pesticides in complex matrices including black tea, and the enhanced sensitivity enabled the appropriate dilution of the sample, which is required to minimize ionization suppression effects. With any dilution, a lower matrix amount is introduced into the LC/MS system leading not only to fewer matrix effects, but also improved method robustness, increased instrument uptime, and lab productivity. Dilution of sample extracts was applied to minimize matrix effects, while still allowing quantification of the majority of pesticides with acceptable recovery ranges of 70 to 120% based on a solvent calibration. The increased sensitivity of the 6470 Triple Quadrupole LC/MS System allowed the quantification of the majority of all targeted pesticides below the maximum residue limits specified by the European Commission, even in 1:20 diluted extracts with improved precision and method robustness.

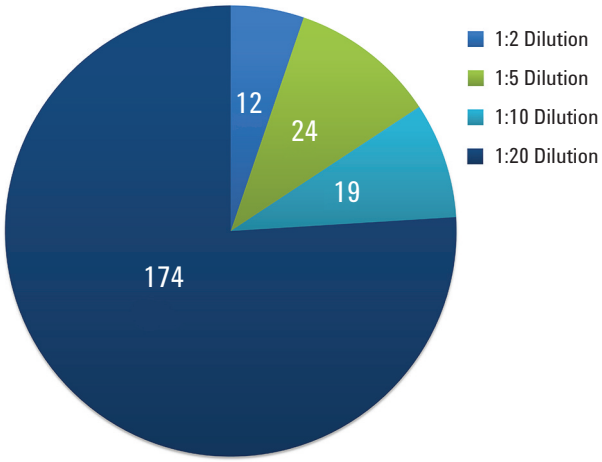

Figure 4. Detection rates of pesticides spiked into black tea extracts at the MRL of 10 µg/kg and diluted with acetonitrile. 174 pesticides were detected at the 1:20 dilution level with an area RSD <20%. Additional compounds are detected at lower dilution levels (higher concentrations).

References

1. N. P. Parra, L. Taylor, Why Instrument Detection Limit (IDL) is a Better Metric for Determining the Sensitivity of Triple Quadrupole LC/MS Systems. *Agilent Technologies Technical Overview*, publication number 5991-4089EN (**2014**).
2. D. D. Yang, *et al*. Multi-Residue Pesticide Screening and Quantitation in Difficult Food Matrixes Using the Agilent 6495 Triple Quadrupole Mass Spectrometer. *Agilent Technologies Application Note*, publication number 5991-4687EN (**2014**).

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