



# Quantitation of Trace Level Emerging Contaminants in Water Using Online SPE with LC/MS/MS

## Application Note

Environmental

### Authors

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### Abstract

This application note demonstrates the performance of an Agilent Infinity Series Online SPE Solution in combination with an Agilent 6460 Triple Quadrupole LC/MS System for the analysis of chemicals on US EPA's Contaminant Candidate List 3 at trace levels in water. This method shows excellent performance for detecting 12 challenging contaminants, with a limit of quantitation (LOQ) < 0.1 for all target compounds. Analysis of samples from real sources including ground water, lake, and flood control reservoir showed good results for tebucanzole.



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## Introduction

Recently, it has been reported that trace levels of pharmaceuticals and other unexpected chemicals can be found in drinking and surface water across the United States. Because these contaminants of emerging concern (CEC's) are often at very low levels, the number of samples presented often precludes the use of extensive offline sample preparation.

Online SPE has several well-known advantages over offline SPE, however, the setup for online SPE can be complicated. Multiple pumps for sample loading and analysis are required, as well as multiple valves for controlling the flow from each pump. This adds to the cost of online SPE and represents a technical barrier and potential technology transfer problems.

This application note describes a novel, integrated valving and liquid-metering system for online SPE, coupled to liquid chromatography triple quadrupole mass spectrometry (LC/MS/MS). The online SPE system presented here has a built-in single piston pump, with solvent selection capability, and user-selectable valves integrated in a single FlexCube module. A very low level detection is possible, without prior sample preparation, using the Flexible Cube solution for online SPE that significantly reduces the complexity, cost, and footprint of the HPLC system.

## Experimental

### Instrumentation

Agilent 1200 Infinity Online SPE Solution	Model number
Agilent 1260 Infinity Binary Pump with modular degasser	G1312B
Agilent 1260 Infinity Standard autosampler	G1329B
Agilent 1290 Infinity Flexible Cube with 2-position/10-port valves	G4227A
Agilent 6460 Triple Quadrupole LC/MS System with Agilent Jet Stream Technology	G6460A
Agilent 1290 Infinity Thermostatted Column Compartment	G1316C

### Chromatographic conditions – LC method

Parameter	Setting
Analytical column	Agilent ZORBAX Eclipse Plus C18, 3.0 × 50 mm, 1.8 μm
Mobile phases	A = Water + 0.1% formic acid B = Acetonitrile +0.1% formic acid
Analysis flow rate	0.4 mL/min
Auto SPE Injection volume	900 μL
Draw and eject speed	1,000 μL/min
Direct injection volume	90 μL

### Settings for the Agilent 1290 Infinity Flexible Cube

- Valve: 2 of 2-position/10-port Quick-Change valve
- Solvent selection valve:  
Solvent A1: 3% MeOH/Water,  
Solvent B1: ACN/IPA/MeOH/  
Water

## Principle of Operation

The Agilent 1290 Infinity Flexible Cube LC module depicted in Figure 1 has a single piston pump with a three-solvent selection valve. It can have one or two quick-change valves.

Figure 2 illustrates the plumbing configuration that allows direct on-column injection or online SPE. The left valve controls sample introduction either directly to the analytical column or to one of the SPE cartridges for online enrichment. For online SPE, the piston pump (or flush pump) is used to pump the sample from the autosampler onto the trapping columns, as well as clean those columns, with up to three different solvents, after each run. The autosampler is connected to the piston pump, which loads the sample directly onto one of the trapping columns (SPE1) while the other trapping column (SPE2) is in front of the analytical column and connected to the binary pump. When the first trapping column is loaded with sample, the 2-position/10-port valve is switched to change the positions of the trapping columns. After changing trapping column positions, the binary pump then delivers gradient to backflush the sample from the trapping column (SPE1) onto the analytical column. On the other side, the trapping column used in the previous run (SPE2) is cleaned and re-equilibrated. Using the solvent selection valve in the 1290 Infinity Flexible Cube, the operator can clean and re-equilibrate the trapping columns with up to three solvents [1].

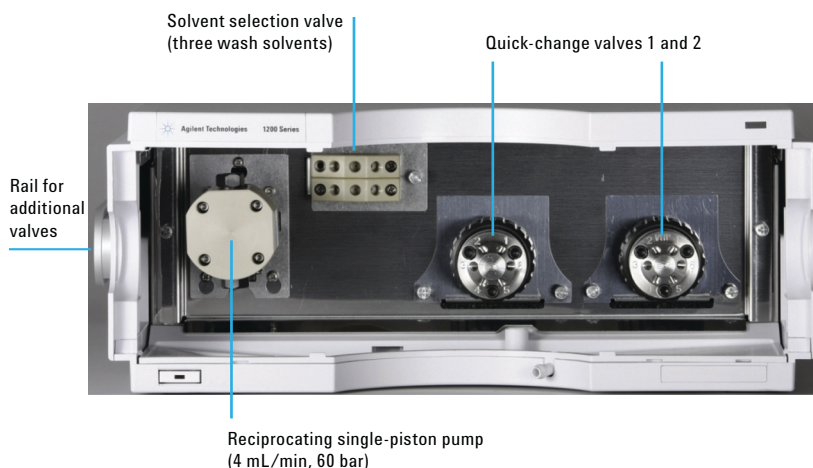


Figure 1. Agilent Flexible Cube with built-in single piston pump and valves.

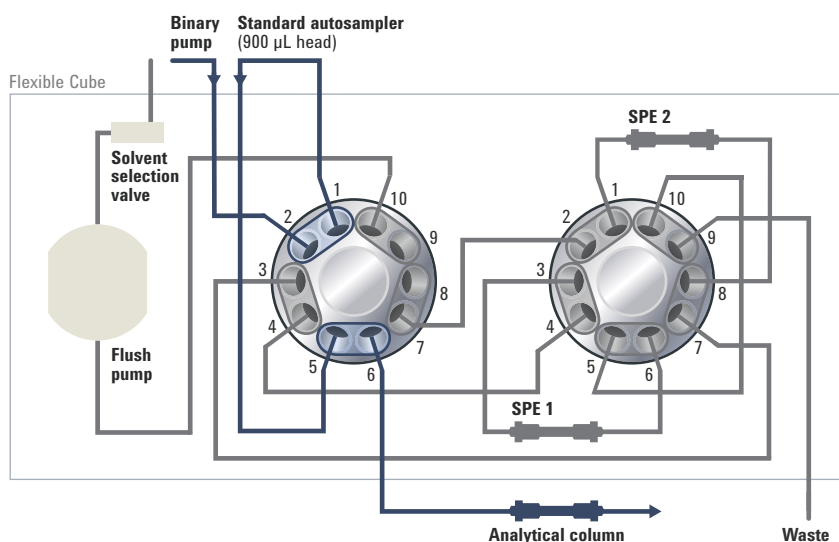


Figure 2. Plumbing for direct on-column injection or online SPE. Flow path in blue shows sample introduction directly to the analytical column..

Figures 3A and B show the flow path the sample takes through the 1290 Infinity Flexible Cube during different stages of analysis. Figure 3A depicts the beginning of the analysis, in which the binary pump moves sample through the left valve to the SPE2 trapping column, towards the analytical column (blue flow path). At the same time, the piston pump is delivering sample, backflushing from the autosampler through SPE1 then out to waste to clean SPE1 (red flow path). Figure 3B depicts a change in flow. At this point in the analysis, the binary pump is now pumping through SPE 1, which is now in front of the analytical column (blue path). Concurrently, the piston pump is delivering sample from the autosampler, back through SPE 2 for cleaning (red path).

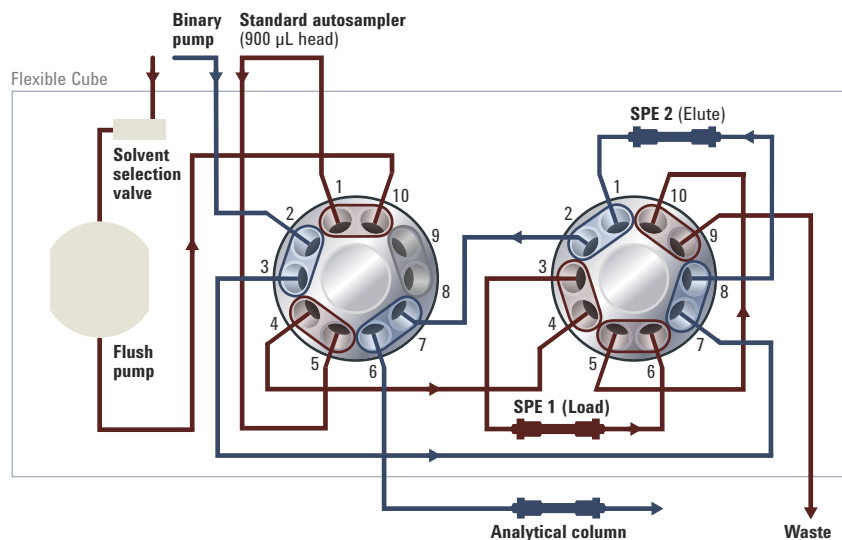


Figure 3A. Flowpath for injection on SPE trapping columns.

### Samples

Twelve compounds regulated by the Safe Drinking Water Act (SDWA) were evaluated in this study [2]:

- 4,4'-methylenedianiline
- 3-hydroxycarbofuran
- bensulide
- clethodim
- fenamiphos
- fenamiphos sulfone
- fenamiphos sulfoxide
- methomyl
- quinoline
- tebuconazole
- tebufenozide
- thiodicarb

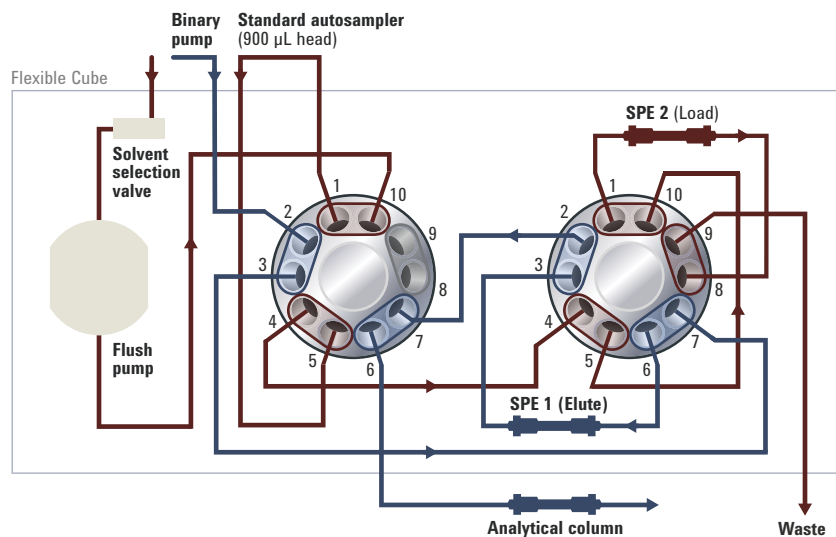


Figure 3B. Flowpath for injection on SPE trapping columns.

Table 1 lists the parameters for the dynamic MRM method used in this application, including the optimum fragmentor (V) and collision energy (eV) values for the individual compound transitions.

### Chromatographic conditions – MS method

Parameter	Setting
Gas temperature	300 °C
Gas flow	10 L/min
Nebulizer	45 psi
Sheath gas temperature	300 °C
Sheath gas flow	11 L
Capillary	4,000 volt
Nozzle	0 volt

Table 1. Dynamic MRM method conditions.

Compound name	Prec ion	Prod ion	Frag (V)	CE (V)	Cell acc (V)	Ret time (min)	Ret window	Polarity
4-4-diaminodiphenylmethane	199.1	106	120	28	3	6.74	1.81	Positive
Bensulide	398	356	100	1	2	11.06	1.11	Positive
Bensulide	398	314	100	6	2	11.06	1.11	Positive
Bensulide	398	158	100	26	2	11.06	1.11	Positive
Carbofuran-3-hydroxy	238.1	181	110	8	2	8.15	0.94	Positive
Carbofuran-3-hydroxy	238.1	163	110	11	2	8.15	0.94	Positive
Clethodim	360.1	206	120	12	4	11.67	1.15	Positive
Clethodim	360.1	166	130	27	4	11.67	1.15	Positive
Fenamiphos	304.1	234	150	15	3	10.28	1.03	Positive
Fenamiphos	304.1	217	150	23	3	10.28	1.03	Positive
Fenamiphos	304.1	202	150	31	3	10.28	1.03	Positive
Fenamiphos-sulfone	336.1	308	130	15	3	9.13	0.91	Positive
Fenamiphos-sulfone	336.1	266	130	18	3	9.13	0.91	Positive
Fenamiphos-sulfone	336.1	188	130	24	3	9.13	0.91	Positive
Fenamiphos-sulfoxide	320.1	292	150	9	3	8.64	0.99	Positive
Fenamiphos-sulfoxide	320.1	233	150	23	3	8.64	0.99	Positive
Fenamiphos-sulfoxide	320.1	171	150	18	3	8.64	0.99	Positive
Methomyl	163	107	170	17	2	8.15	1.14	Positive
Methomyl	163	95	170	17	2	8.15	1.14	Positive
Quinoline	130	103	120	30	3	6.97	1.81	Positive
Quinoline	130	77	120	30	3	6.97	1.81	Positive
Tebuconazole	308.1	125	130	42	3	10.49	1.05	Positive
Tebuconazole	308.1	70	130	23	3	10.49	1.05	Positive
Tebufenozide	353.2	297	85	1	3	10.85	1.09	Positive
Tebufenozide	353.2	133	85	15	3	10.85	1.09	Positive
Thiodicarb	355	163	80	2	3	9.17	1.28	Positive
Thiodicarb	355	108	80	14	3	9.17	1.28	Positive
Thiodicarb	355	88	80	17	3	9.17	1.28	Positive

## Results and Discussion

Twelve contaminants, considered to be the more challenging chemicals to analyze, were selected from the Contaminant Candidate List (CCL3) identified by the EPA. The CCL3 contaminants are currently not subject to any drinking water regulations but may require regulation under the Safe Drinking Water Act (SDWA) [2].

Figure 4 shows the superimposed chromatograms of a calibration standard with a concentration of 10 ppt (ng/L) each for all 12 compounds with quantifier and qualifier ions. Each run alternates between two different SPE cartridges according to the procedure outlined in the Principles of Operation. The chromatogram shows excellent reproducibility and good peak shape for all targets. For all analyses, the recoveries of the QC samples were in acceptable ranges for most of the 12 targets.

Figure 5 shows a chromatogram of the sample analysis at 0.1 ppt mix concentration. As the figure illustrates, there is no carryover between the compounds and the peaks have narrow width and good shape.

Figure 6 illustrates the superimposed chromatograms of 1 ppt and 0.1 ppt mix concentrations. As the figure demonstrates, the method provides excellent sensitivity for both concentrations.

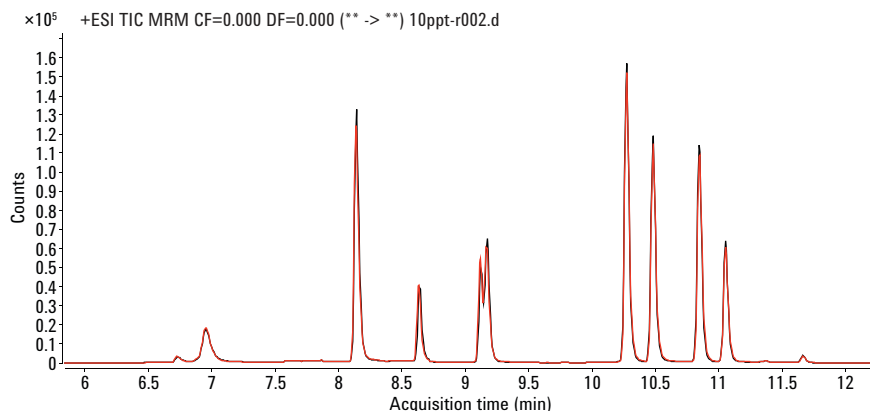


Figure 4. Superimposed chromatogram showing excellent reproducibility of retention time and area counts for two runs on the two alternating cartridges.

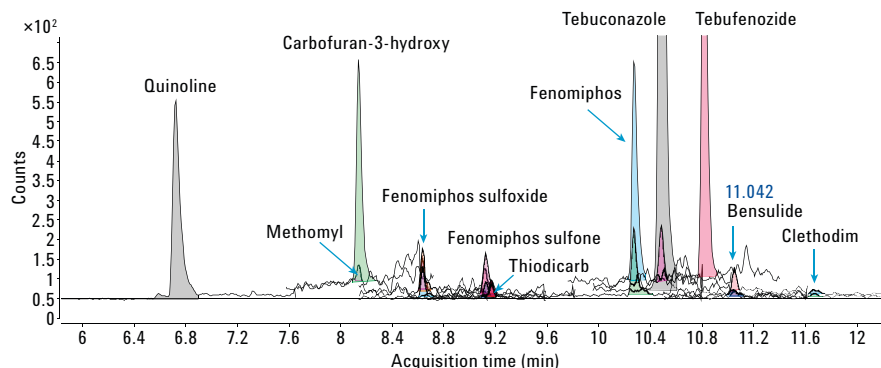


Figure 5. Chromatogram of sample at 0.1 ppt mix.

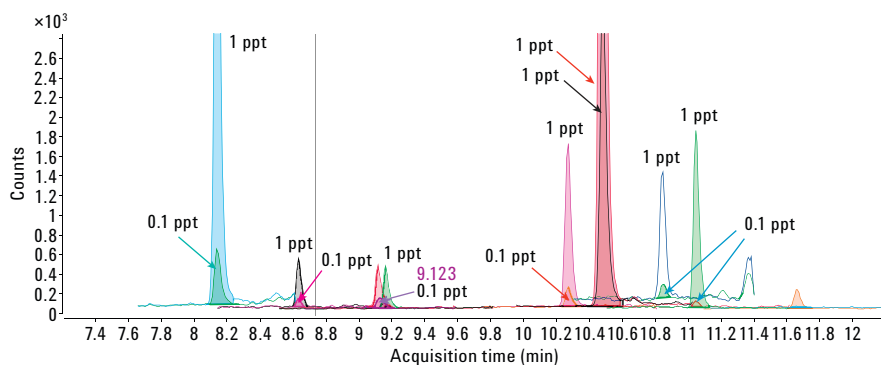


Figure 6. Superimposed chromatograms of 1 ppt and 0.1 ppt sample concentrations.

Figure 7 shows the chromatograms for the quantifier transition  $m/z$  308.1  $\rightarrow$  70.0, which corresponds to one of the target contaminants, tebuconazole, at concentrations of 0.1, 0.5, and 1.0 ppt. The trace shows the 0.1 ppt level with a signal-to-noise ratio of  $> 400:1$ . Since tebuconazole is a fungicide widely used for turf control in the US, it has been identified as a chemical typically tested in groundwater and other environmental analyses. Therefore, it was an important target compound in this application.

All calibration curves for the 12 compounds tested showed excellent linearity, with the majority of the calibration coefficients  $> 0.999$  for a seven-level calibration curve. No weighting was used, and the curves were forced through the origin. Quinoline had the lowest linearity coefficient of 0.96 with a 10-level calibration.

Figure 8 shows the calibration curve for tebuconazole based on individual solutions from 0.1 to 100 ppt through serial dilution. Each calibration standard was injected two times with a volume of 900  $\mu$ L and enriched on the SPE trapping column. The calibration curve shows excellent linearity with an  $R^2 > 0.999$ .

As part of this analysis, water samples were taken from a lake-fed municipal water supply (tap), residential well (ground), a suburban collection basin (WestLake), and a flood control reservoir (Spring Creek). Samples from the lake and flood control reservoir contained visible swimming organisms. The samples were filtered with 0.45- $\mu$ m disk filters prior to analysis. Results are based on the average of duplicate analyses, for the one quantifier transition (308.1  $\rightarrow$  70.0) and one qualifier transition (308.1  $\rightarrow$  125.0). These transitions correspond to tebuconazole, which is used as a fungicide for turf control. Notably, both WestLake and Spring Creek are in the drainage basin of nearby public and private golf courses, respectively. Figure 9 shows the chromatogram results of this analysis.

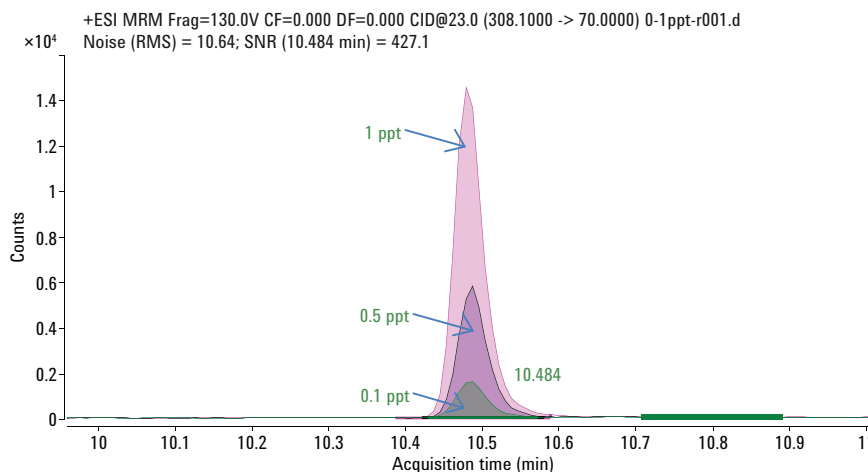


Figure 7. Chromatograms for the quantifier transition  $m/z$  308.1  $\rightarrow$  70.0 of tebuconazole at concentrations of 0.1, 0.5, and 1.0 ppt.

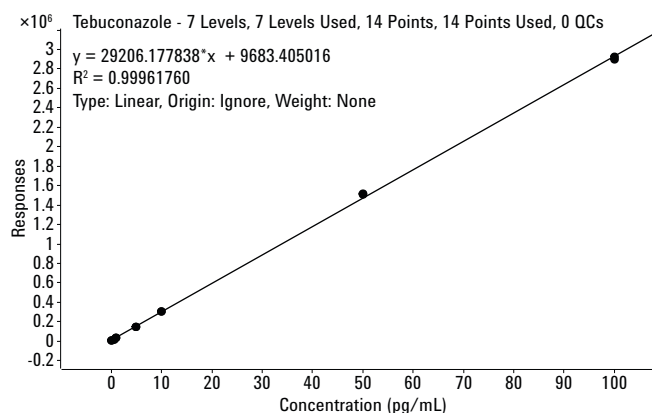


Figure 8. Calibration curve for tebuconazole based on individual solutions from 0.1 to 100 ppt through serial dilution.

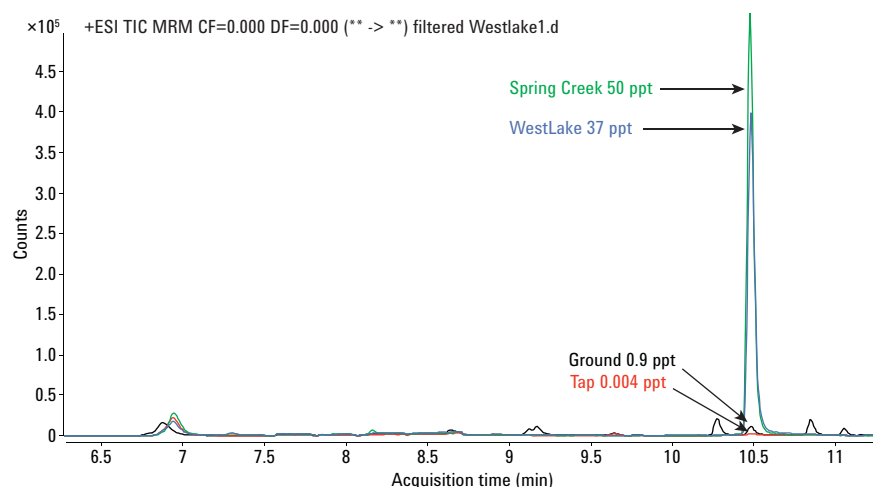


Figure 9. Water samples from multiple sources.

Table 2 lists performance data for all compounds present in the study based on the calculated signal-to-noise (S/N) at 0.1 ppt and assuming an LOQ at approximately 10:1 S/N. The S/N calculation is based on peak area and noise is  $\text{RMS} \times 3$ . The data show good precision of the method, with low S/N. LOQ for all components was nominally 0.1 ppt or lower, and as low as 0.002 ppt for two targets.

## Conclusion

This application note demonstrates an integrated, cost effective scheme for on-line SPE with LC/MS/MS using an Agilent 1200 Infinity Series Online SPE Solution coupled to an Agilent 6460 Triple Quadrupole LC/MS. This method does not seem to have the technical barriers or potential technology transfer issues typically associated with traditional SPE systems. The direct coupling of the SPE cartridges to the LC/MS/MS system was done through the novel integrated valve system of the Agilent 1290 Infinity Flexible Cube LC Module. The use of Dynamic MRM analyzes complex mixtures efficiently. This method shows excellent performance with the 12 challenging contaminants on the CCL3 Contaminant list. Results for real samples, including ground water, lake, and flood control reservoir produced measurable tebuconazole results as well.

## References

1. B. Schuhn, E. Naegle, T. Glauner, "Detection of Trace-Level Herbicides in Drinking, Surface, and Ground Water Using the Agilent Infinity series Online SPE Solution", Agilent Technologies Application Note 2013, Publication Number 5991-2405EN.
2. J. Shoemaker, D. Tettenhorst, "Evaluation of On-line Solid Phase Extraction -LC/MS/MS for the Analysis of Chemicals on U.S. EPA's Contaminant Candidate List 3".

Table 2. Performance data for all compounds present in the study.

Compound name	Level (ppt)	Signal-to-noise (S/N)	Nominal LOQ at 10:1 S/N
4-4-diaminodiphenylmethane	0.1	25	0.04
Bensulide	0.1	10.3	0.1
Carbofuran-3-hydroxy	0.1	34.4	0.03
Clethodim	0.1	7	0.15
Fenamiphos	0.1	301.2	0.005
Fenamiphos-sulfone	0.1	7.9	0.15
Fenamiphos-sulfoxide	0.1	8.2	0.15
Methomyl	0.1	8.3	0.15
Quinoline*	0.1	500	0.002
Tebuconazole	0.1	427	0.0025
Tebufenozide	0.1	9.4	0.15
Thiodicarb	0.1	9	0.15

\*Uncertainty due to unresolved carryover

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