

# Sensitive Detection of Trace Organic Contaminants in Water

Using Agilent 1290 Infinity FlexCube Online SPE and Agilent Ultivo Triple Quadrupole LC/MS

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## Abstract

This Application Note demonstrates the use of the Agilent 1200 Infinity Series Online-SPE solution coupled to an Agilent Ultivo triple quadrupole LC/MS for the detection of trace organic contaminants in water at low levels (ng/L). The method takes advantage of large volume injection of water samples to avoid tedious offline sample preparation and enrichment. The Ultivo houses many technological innovations that allowed us to reduce the instrument footprint, yet maintain sensitivity. In this study, 51 compounds were detected under simultaneous positive and negative electrospray ionization modes using fast polarity switching. System performance was demonstrated by linearity, limits of detection (LOD), and relative standard deviation (RSD).

## Introduction

The wide use of pesticides, pharmaceuticals, personal care products, and industrial chemicals in our daily lives results in contamination of our water sources. Systematic environmental pollution has raised health concerns worldwide. Governmental agencies have set regulations to monitor chemical contamination in surface and drinking waters. The control of chemical contaminants is directed by regulations such as the European Union Drinking Water Directive 98/83/EC<sup>1</sup>, US EPA guideline documents<sup>2,3</sup>, and so forth. The World Health Organization published Guidelines for Drinking-Water Safety, in which limits were set for >30 pesticides frequently found in water systems<sup>4</sup>. Globally, the requirements for determination of these organic contaminants, especially in drinking waters, can vary between sub- to low part-per-trillion (ng/L). To ensure this low level of quantification, it usually becomes necessary to use a sample enrichment step prior to LC/MS analyses. Typical offline enrichments use a large volume of samples with solid phase or liquid phase extractions. This is not only time-consuming, but also generates a large amount of chemical waste. The Agilent FlexCube online SPE solution provides an automated online sample enrichment, using a valve system that controls alternating sample loading onto an enrichment cartridge<sup>5,6</sup>. The module fits seamlessly into current LC/MS configurations, and is operated through a single integrated software platform.

Ultivo LC/TQ is designed to address many challenges faced by current routine users. Innovative technologies within Ultivo allowed us to reduce its overall footprint, without sacrificing sensitivity and performance compared to much larger MS systems. Advances in instrument design, such as the Cyclone Ion Guide, Vortex Collision Cell, Pre/Post-Virtual Filters, and the Hyperbolic Quadrupoles not only maximize quantitative performance, but also enhance instrument reliability and robustness. This all results in greater uptime and throughput. Ultivo reduces the need for user intervention during system maintenance, making system operation and maintenance manageable for nonexpert users. Agilent MassHunter Software simplifies data acquisition, method set up, data analysis, and reporting. Additionally, Quant-My-Way streamlines data processing and reporting to meet each user's workflow requirements, resulting in the fastest possible sample-to-reporting time.

In this study, a representative group of 51 contaminants were analyzed. The analytes studied included pesticides, pesticide metabolites, pharmaceutical products, perfluorinated compounds, plasticizers, and so forth. Approximately 25 % of compounds were analyzed in negative ionization mode, where fast polarity switching provided by Ultivo was critical to detect these organic contaminants at low levels.

## Experimental

### Instrumentation

Agilent Infinity Series Online-SPE Solution

- Agilent 1260 Infinity II Binary Pump (G7112B)
- Agilent 1260 Infinity II MultiSampler (G7167A) with 900  $\mu$ L head/loop and cooler
- Agilent 1290 Infinity Flexible Cube (G4227A) with online SPE starter set (G4742A) providing one 2 position/10 port Quick-Change valve heads and respective capillaries.
- Agilent 1260 Infinity II MCT (G7116A)

### MS Detection

Agilent Ultivo Triple Quadrupole LC/MS with Agilent Jet Stream ESI Source

### Analytical Column

- Agilent Poroshell 120 EC C18, 3.0  $\times$  50 mm, 2.7  $\mu$ m (p/n 699975-302)
- 2  $\times$  Guard Column Hardware Kit (p/n 820999-901)
- Trapping columns (part of G4742A), Agilent PLRP-S Cartridges, 2.1  $\times$  12.5 mm, 15–20  $\mu$ m (p/n 5982-1271)

Table 1 lists the Agilent 1260 Infinity II Binary Pump method and the FlexCube Online SPE method. The FlexCube online SPE only used an injection volume of 900  $\mu$ L of sample, compared to traditional EPA methods, which employ offline SPE and require several hundred milliliters of sample. The entire cycle time for enrichment and analysis was 21 minutes, compared to lengthy offline SPE methods that may require several hours. Table 2 lists the Agilent Jet Stream ESI Source parameters, and Table 3 lists the compounds in this study.

**Table 1.** Agilent Binary Pump and Agilent FlexCube conditions.

Parameter	Value														
Analytical column	Agilent Poroshell 120 EC C18, 3.0 × 50 mm, 2.7 μm														
Loading cartridge	Agilent PLRP 2.1 × 12.5 mm, 15-20 μm														
Mobile phase	Binary Pump: A) H <sub>2</sub> O+ 0.01 % acetic acid acid + 1 mM ammonium acetate + 0.1 mM ammonium fluoride B) 0.01 % acetic acid in acetonitrile FlexCube: A1) H <sub>2</sub> O + 0.1 % formic acid B2) ACN: IPA=1:1														
Column temperature	40 °C														
Injection volume	900 μL														
Binary pump gradient program	<table border="1"> <thead> <tr> <th>Time (min)</th> <th>%B</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>2</td> </tr> <tr> <td>4</td> <td>2</td> </tr> <tr> <td>6</td> <td>40</td> </tr> <tr> <td>16.5</td> <td>98</td> </tr> <tr> <td>18</td> <td>98</td> </tr> <tr> <td>18.1</td> <td>2</td> </tr> </tbody> </table>	Time (min)	%B	0	2	4	2	6	40	16.5	98	18	98	18.1	2
Time (min)	%B														
0	2														
4	2														
6	40														
16.5	98														
18	98														
18.1	2														
Stop time	19 minutes														
Post time	2 minutes														
FlexCube timetable	<table border="1"> <thead> <tr> <th>Time (min)</th> <th>Event</th> </tr> </thead> <tbody> <tr> <td>0.00</td> <td>Pump volume: Pump 240 seconds at 1.5 mL/min from Channel A1</td> </tr> <tr> <td>0.00</td> <td>Right valve change position: position 1 (FlexCube)</td> </tr> <tr> <td>4.05</td> <td>Left valve change position: increase valve position</td> </tr> <tr> <td>4.10</td> <td>Pump volume: Pump 240 seconds at 1 mL/min from channel B2</td> </tr> <tr> <td>8.15</td> <td>Pump volume: Pump 240 seconds at 1.5 mL/min from channel A1</td> </tr> </tbody> </table>	Time (min)	Event	0.00	Pump volume: Pump 240 seconds at 1.5 mL/min from Channel A1	0.00	Right valve change position: position 1 (FlexCube)	4.05	Left valve change position: increase valve position	4.10	Pump volume: Pump 240 seconds at 1 mL/min from channel B2	8.15	Pump volume: Pump 240 seconds at 1.5 mL/min from channel A1		
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Flow rate	0.40 mL/min														

**Table 2.** Agilent Jet Stream ESI Source parameters.

Parameter	Value
Drying gas temperature	250 °C
Drying gas flow	11 L/min
Sheath gas temperature	375 °C
Sheath gas flow	12 L/min
Nebulizer pressure	45 psi
Nozzle voltage (V)	500 (+); 500 (-)
Capillary voltage (V)	4,000 (+); 3,500 (-)

**Table 3.** Compounds detected in the study.

Compound	Compound	Compound
3-Hydroxy carbofuran	Bemfibrozil	PFHxA
Alachlor	Hydrocortisone	PFHxS
Atrazine	Ibuprofen	PFNA
Benzotriazole	Indoxacarb	PFOA
Caffeine	Isazofos	PFPeA
Carbamazepine	Meprobamate	PFUDA
Chinomethionat	Metalaxyl	Primidone
Clofibric acid	Methomyl	Propranolol
DEET	Mevinphos ( <i>cis</i> )	Propoxur
Diclofenac	Naproxen	Propylparaben
Diltiazem	Norgestrel	Simazine
Dimethoate	Oxadixyl	Sulfamethoxazole
Disulfoton-sulfoxide	Pendimethalin	TCEP
Ethion	PFBS	Testosterone
Ethoprophos	PFDA	Triclocarban
Fenamiphos	PFDoA	Trimethoprim
Fensulfothion	PFHpA	Vamidiothion sulfoxide

The Agilent 1290 Infinity FlexCube in this configuration consists of a 2-position/10 port valve. The two trapping columns are used in an alternating fashion for sample loading by programming the valve position in the method setup. The FlexCube also contains a reciprocating piston pump and solvent selection valves for flushing the sample onto the trapping columns, cleaning the cartridges, and equilibrating the cartridges (Figure 1). If the valve position for loading the sample is selected, the piston pump inside the FlexCube is connected to the autosampler to flush the sample onto one trapping column (SPE 1). The other trapping column (SPE 2) is connected to the analytical pump, and is eluted onto the analytical column. After loading the trapping column with sample, the valve is switched, and the positions of the trapping columns are exchanged. Now, the LC pump delivers the gradient to elute the enriched analytes from the trapping column (SPE 1) onto the analytical column. Simultaneously, the trapping column (SPE 2) which had been eluted in the previous run is further cleaned and reconditioned. This cleaning procedure is done by the piston pump of the FlexCube with the cleaning solvents by the solvent selection valve.

There were 51 contaminants, representing multiple classes of analytes, detected using the dynamic multiple reaction monitoring (dMRM) method.

### Chemicals and Standards

LC grade acetonitrile was purchased from Honeywell, USA (015-4). Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22- $\mu$ m membrane point-of-use cartridge (Millipak). Isopropanol (IPA) was from Honeywell (323-4). Ammonium acetate was purchased from Fluka as solid, then a 5 M solution was prepared (14267-25G). Acetic acid was purchased from Sigma-Aldrich (338826-25ML). The 5 M ammonium formate solution was from Agilent (G1946-85021).

The perfluoroalkyl substance standards were purchased from Wellington Laboratories (Canada). All other standards were purchased from Ultra Scientific (USA), Sigma-Aldrich (USA), or provided by collaborators.

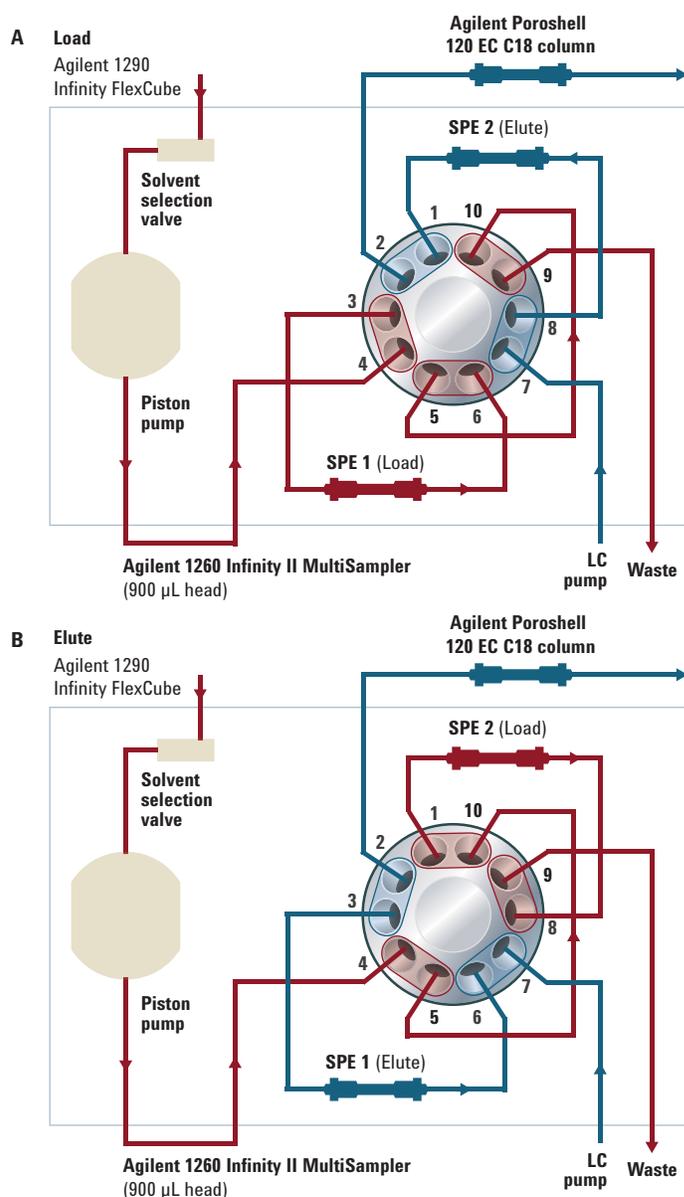


Figure 1. FlexCube online SPE valve diagram.

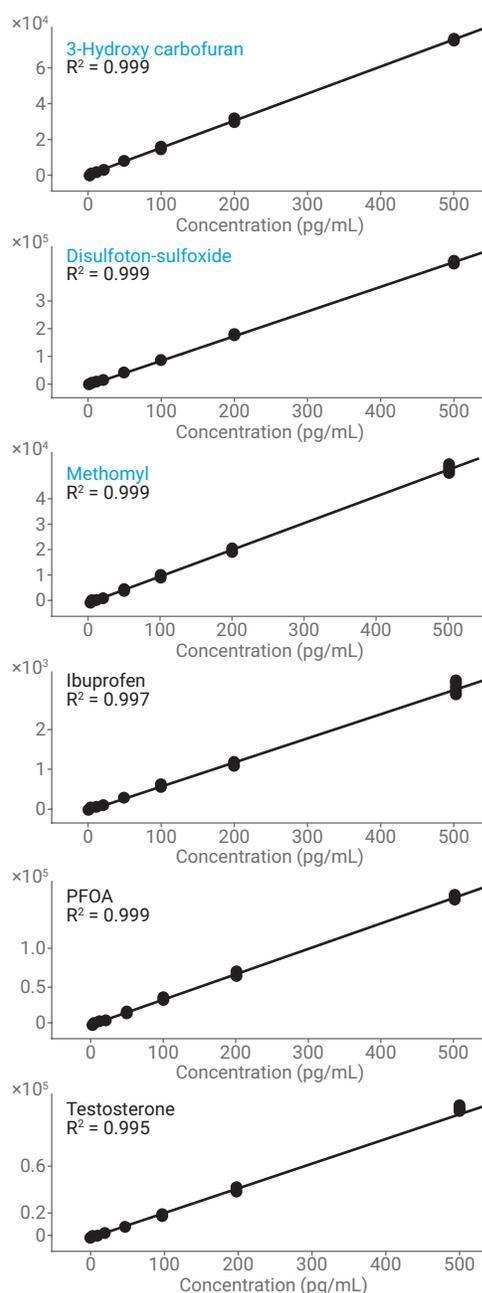
## Results and Discussion

### Method Linearity

A 0.5 ppm stock solution containing all analytes except the fluorinated compounds was prepared in acetonitrile. Fourteen microliters of the above stock solution, along with 3.5  $\mu\text{L}$  of fluorinated compounds (2 ppm stock) were added into 13.98 mL of Millipore water to make a spiking concentration of 500 ng/L. From this solution, eight calibration levels were created (200, 100, 50, 20, 10, 5, 2.5, and 1.25 ng/L). Six replicate injections were acquired per concentration level.

The  $R^2$  values for most calibration curves were better than 0.99 with linear fitting and 1/x weighting. Figure 2 depicts typical calibration curves obtained in this study for six compounds. 3-Hydroxy carbofuran, methomyl, and disulfoton-sulfoxides are compounds monitored in EPA Method 540. These three compounds could accurately be quantified at the lowest spiking level of 1.25 ng/L. Ibuprofen (pharmaceutical), perfluoroalkyl substance (PFOA), and testosterone (hormone), compound classes frequently found in water systems, could also be quantified at the lowest calibration levels. This demonstrates that the Ultivo LC/TQ and 1290 Infinity FlexCube online SPE system are the desired combination for trace organic analysis in water.

Some hydrophobic analytes, for example, indoxacarb and pendimethalin, required quadratic fitting, but still had  $R^2 = 0.994$ . This quadratic fitting is probably due to the loss of very-low-level analytes in water to container surfaces to which the analytes had direct contact.



**Figure 2.** Selected calibration curves. Compounds included in EPA Method 540 are highlighted in blue.

### Method Sensitivity

Figure 3 shows the signal response of the 51 compounds at 10 ng/L in water. It is evident that many of the compounds can be detected far below this concentration. The signal response for many compounds was excellent; some analytes could be detected at levels far below the lowest calibration point of 1.25 ng/L.

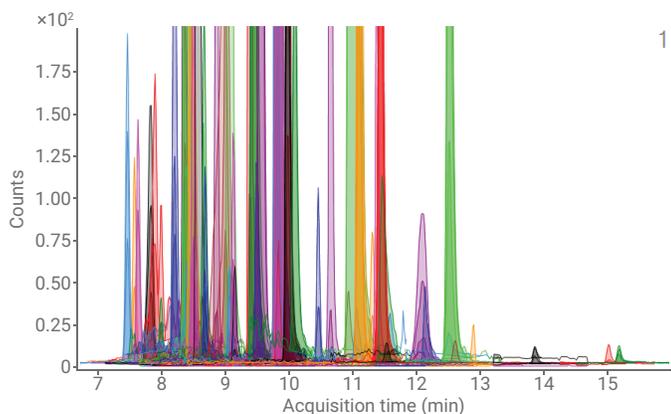


Figure 3. Signal response at 10 ppt with 900  $\mu$ L injection.

The limit of quantification (LOQ) was calculated from the lowest calibration point with a signal-to-noise ratio (S/N) above 10, and quantification accuracy within 80–120 % for at least four of six replicates. Figure 4 illustrates the detection sensitivity for the compounds in the study. It was observed that 86 % of the analytes could accurately be quantified under 10 ng/L.

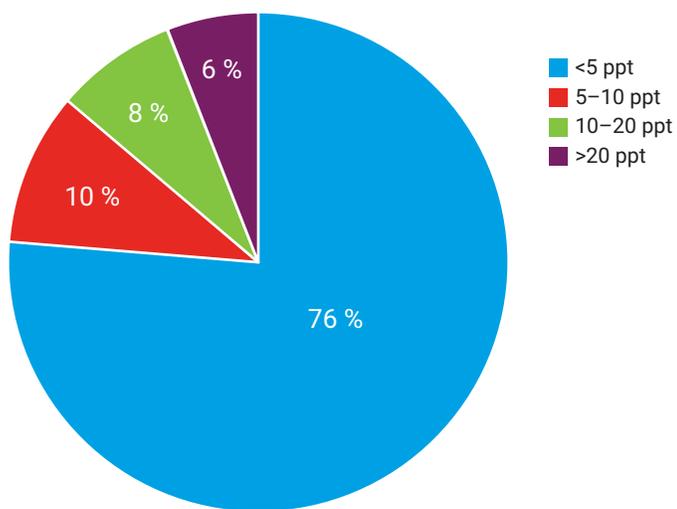


Figure 4. Distribution of the LOQ for 51 contaminants (accuracy 80–120 %).

### Method Precision

Six replicate injections were acquired at each concentration level from 1.25 ng/L to 500 ng/L. %RSDs were calculated at the lowest concentration for accurate quantitation (accuracy 80–120 % for at least four of six replicates). Except for one compound, all %RSDs were less than 20 %, as illustrated in Figure 5. The good %RSD was also an indication of consistent performance of the two alternating enrichment cartridges. Figure 6 demonstrates the stability of area and retention time of multiple injections. Blank sample occasionally contains analytes of interest. In this study, only DEET had significant presence in blank sample, but its concentration was much lower than 10 ng/L.

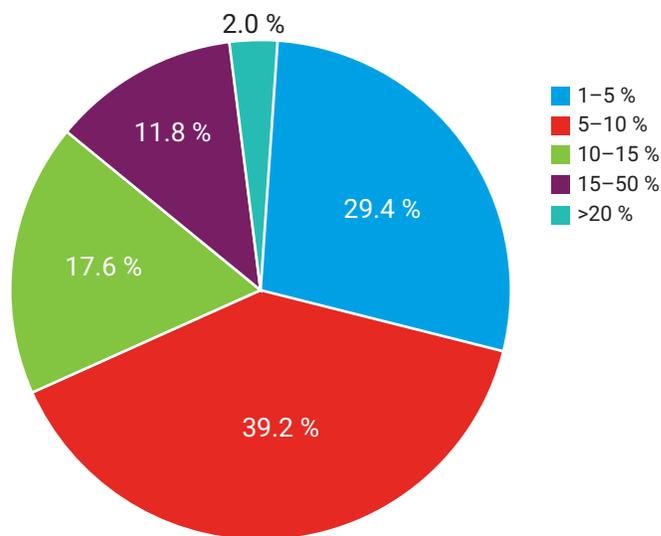
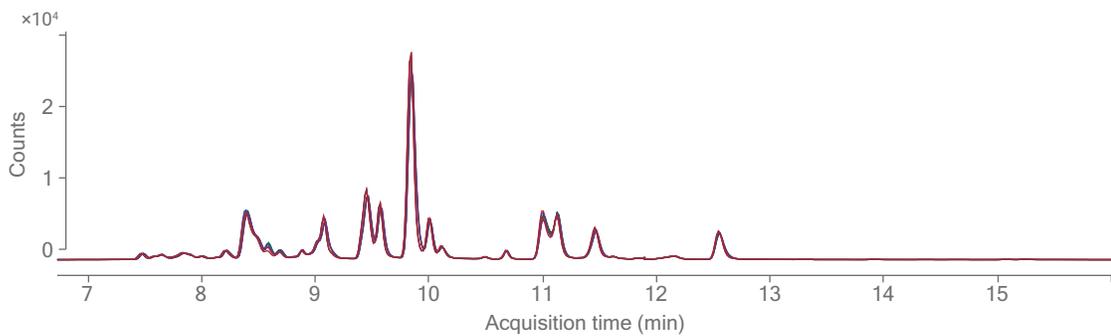


Figure 5. Measurement precision (%RSD, n = 6).



**Figure 6.** Chromatographic overlay of six injections at 20 ng/L with alternating enrichment cartridges.

## Conclusion

Our results demonstrate the sensitive detection of trace organic contaminants in water using the Agilent 1290 Infinity FlexCube online SPE and the Agilent Ultivo triple quadrupole LC/MS. Most of the analytes could accurately be quantified at low ng/L levels with a 900  $\mu$ L injection. Excellent linearity and precision were obtained, except for one compound. This method provides comparable detection levels to current offline SPE methods, and provides savings in analysis time, labor, and solvent usage.

## References

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