



Enhancing Throughput of Online SPE Analysis of Pesticides in Wastewater

The Agilent 1200 Infinity Series Online SPE Solution

Technical Overview

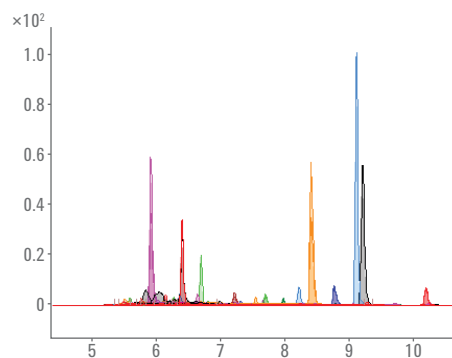
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Abstract

With the combination of two online solid phase extraction (SPE) Multi-Cartridge Kits, Agilent offers the possibility of higher sample throughput. Up to 12 cartridges can be installed in the system to enable the automated alternative use of cartridges. A high sample throughput with automated cartridge change is necessary, when, for example, many dirty samples such as wastewater need to be analyzed.

In this work, 14 pesticides were spiked into wastewater and measured 10 times for each cartridge. In total, more than 120 runs showed the performance and long lifetime of the online SPE cartridges. The Multi-Cartridge Kit increases the productivity of the system, but still offers the flexibility of direct injections due to the valve solutions.



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Introduction

For the determination of pollutants in wastewater, a cleaning and preconcentration step is often necessary to reduce matrix effects and to enrich analytes.

A very convenient, fast, and cost-effective solution is online solid phase extraction (SPE). Online SPE uses an approach that is similar to offline SPE but reduces time and labor because of the automated online enrichment process. For this purpose, Agilent has developed different online SPE kits with the Agilent 1200 Infinity Series Online SPE Solution, based on the Agilent 1290 Infinity Flexible Cube^{1,2,3}.

In this work, a combination of different online SPE kits is shown, which enables multi-online SPE, with up to 12 cartridges, and direct injection without re-plumbing the system. The Agilent Multi-Cartridge Kit (G4743A)⁴ was first introduced for easy online SPE method development. Now, with the setup of two Multi-Cartridge Kits and 12 cartridges, a higher sample throughput is possible.

To show the functionality and possibilities of the system, 12 polymeric phase (Agilent Bond Elut Online SPE, PLRP-S) online SPE cartridges were installed in the system to analyze a suit of pesticides in wastewater. Ten runs for each cartridge were performed. Area and retention time (RT) precision as well as carryover was determined.

Experimental

Instrumentation

All experiments were carried out on an Agilent 1200 Infinity Series Online SPE System comprising:

- Agilent 1290 Infinity Flexible Cube (G4227A) with an Online SPE Starter Kit (G4742A), which includes one 2-position/10-port valve, 600 bar, and an Online SPE Direct Injection Kit (G4744A), also including one 2-position/10-port valve, 600 bar
- Two Agilent 1290 Infinity External Valve Drives (G1170A) with two Online SPE Multi-Cartridge Kits (G4743A), which include two 6-position/14-port valves, 600 bar
- Agilent 1260 Infinity Binary Pump (G1312B) and LAN card (G1369C)
- Agilent 1260 Infinity Standard Autosampler (G1329B) with 900- μ L head (G1313-60007), multi-draw kit (G1313-68711), and an Agilent 1290 Infinity Thermostat (G1330B)

- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 6400 Series Triple Quadrupole LC/MS (G6490A) with Agilent Jet Stream Technology

Software

- Agilent MassHunter Data Acquisition for triple quadrupole mass spectrometer, version 06.00
- Agilent MassHunter Optimizer Software, version 06.00
- Agilent MassHunter Source and iFunnel Optimizer Software, version 06.00
- Agilent MassHunter Qualitative Software, version 06.00
- Agilent MassHunter Quantitative Software, version 06.00

Method

The method was first optimized regarding source conditions and multiple reaction monitoring (MRM) transitions by direct injection (Figure 1). Afterwards, the online SPE method was created (Table 1).

Table 1. HPLC method for online SPE.

Parameter	Online SPE
Mobile phase	A) water + 0.1 % formic acid B) acetonitrile + 0.1 % formic acid
Gradient	0 to 5 minutes 5 % B, 5 to 12 minutes 5 to 100 % B, 12 to 15 minutes 100 % B, 15.1 to 20 minutes 5 % B
Flow rate	0.5 mL/min
Injection volume	1,800 μ L
Sample temperature	5 °C
LC column	Agilent ZORBAX Eclipse Plus C18, 2.1 \times 50 mm, 3.5 μ m (p/n 959743-902)
Column temperature	40 °C
Enrichment cartridge	Agilent Bond Elut Online SPE, PLRP-S, 2.1 \times 12.5 mm, 15-20 μ m (p/n 5982-1271)

Agilent 1290 Infinity Flexible Cube configuration

Left 2-position/10-port valve:
position 1 → 10 (online SPE),
1 → 2 (direct injection)

Right 2-position/10-port valve:
position 1 → 10 (connection to right
6-position/14-port valve),
1 → 2 (connection to left
6-position/14-port valve)

System configuration and operation

Two Multi-Cartridge Kits were combined with the Online SPE Starter Kit and the Direct Injection Kit. Two Agilent 1290 Infinity External Valve Drives with 6-position/14-port valves were installed next to the 1290 Infinity Flexible Cube, and hosted up to 12 cartridges. The Flexible Cube itself hosted two 2-position/10-port valves to support direct injections and online SPE (Figures 1 to 3). With the built-in solvent selection valve in the 1290 Infinity Flexible Cube, up to three solvents can be used to condition and regenerate the cartridges.

To select the left or the right 6-position/14-port valve in the method, the right 2-position/10 port valve needs to be switched between position 1 or 2.

This setup enables rapid and convenient online SPE for a high sample throughput without changing the cartridges manually. Agilent offers one specialized online SPE material (PLRP-S), and different precolumns with a wide range of materials, which can be also used as SPE cartridges for sample enrichment.

Table 2. Timetable for an online SPE method with the Agilent 1290 Infinity Flexible Cube.

Time (min)	Function	Parameter
0	Pump for volume	Pump 5 mL, flow 1 mL/min, channel A1 (water)
5	Right valve change position	Increase valve position
5.1	Pump for volume	Pump 6 mL, flow 1 mL/min, channel A2 (ACN)
11.5	Pump for volume	Pump 7 mL, flow 1 mL/min, channel A1 (water)
20	Right valve change position	Change position 1 → 10 or 1 → 2 (as indicated)

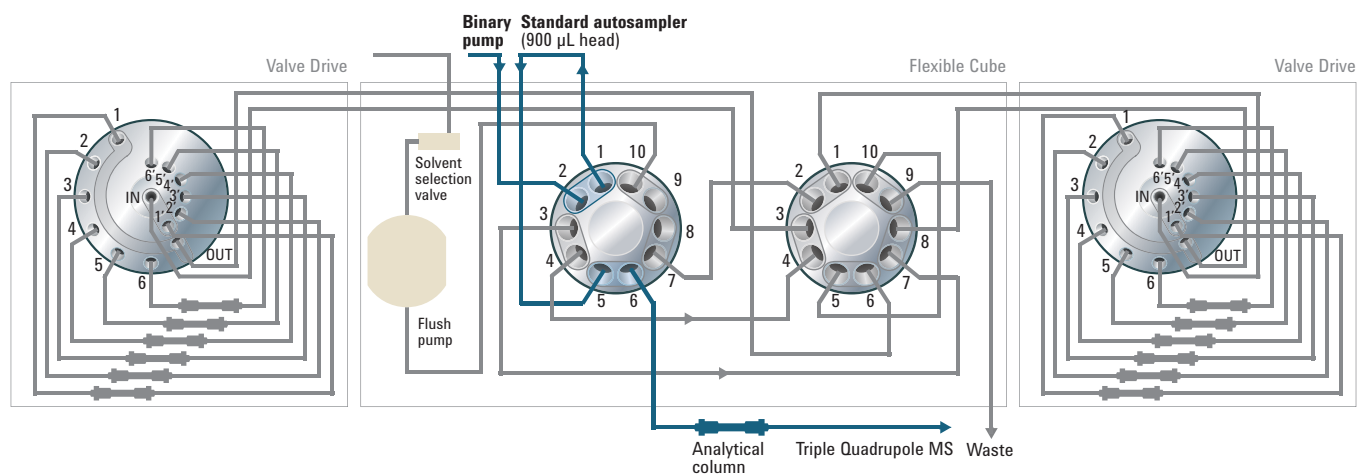


Figure 1. The Agilent 1290 Infinity Flexible Cube offers the possibility of direct injections and online SPE. Here, the flow path of the direct injection run is shown. In this position, the right 2-position/10 port valve and the additional 6-position/14-port valves on the external valve drives are disabled.

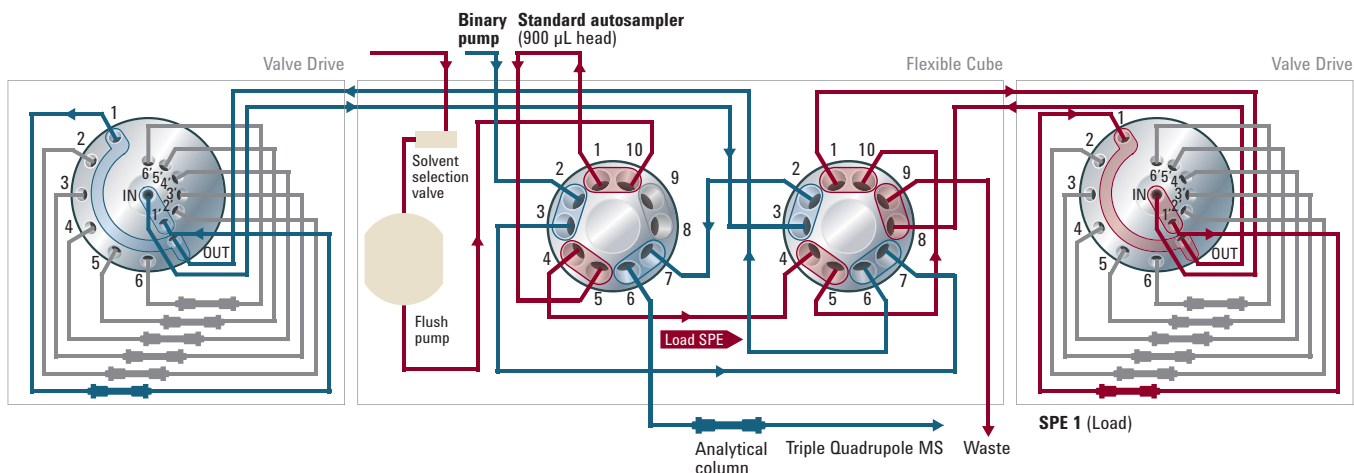


Figure 2. For online SPE, the flow path of the Agilent 1290 Infinity Flexible Cube goes through the left and right 2-position/10-port valve and afterwards to one of the 6-position/14-port valves. One of the 12 cartridges is loaded with sample.

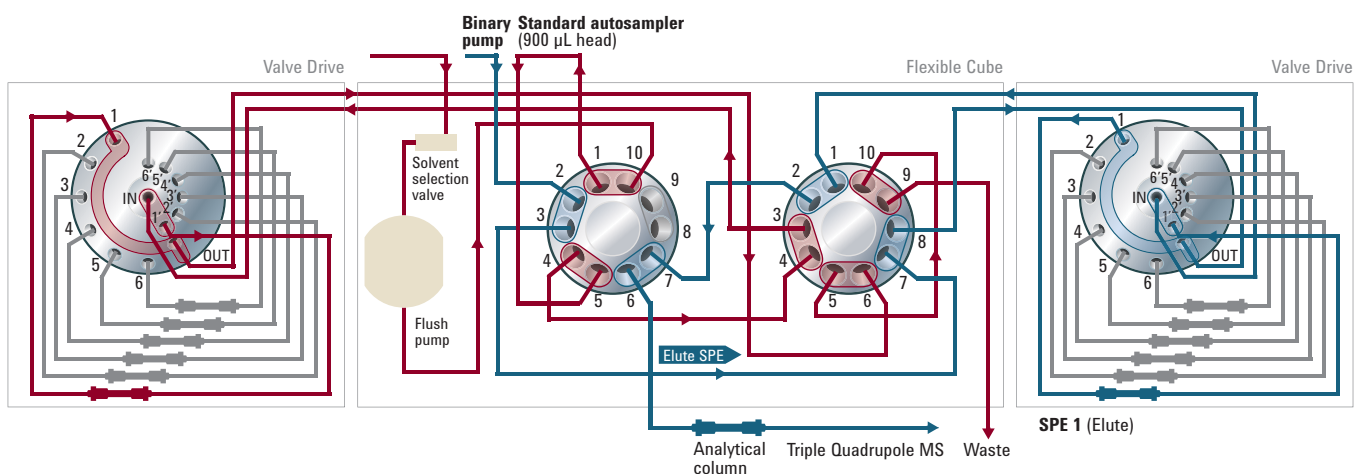


Figure 3. To elute the loaded cartridge, the right 2-position/10-port valve is switched.

Tip

To reduce carryover issues when using different samples, Agilent recommends installing one cleaning capillary in each 6-position/14-port valve (p/n 5067-4737) instead of one cartridge.

Samples

Wastewater (Bern, Switzerland) was first filtered (cellulose-nitrate, 0.45 µm) and then spiked at 10 ng/L with an equimolar mixture of 14 pesticides (the compounds are listed in Application Note 5991-4177EN⁵). Ten consecutive runs were performed for each cartridge as well as Milli-Q blanks and wastewater blanks.

Chemicals

All solvents used were LC/MS grade. Acetonitrile was purchased from Merck, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22-µm membrane point-of-use cartridge (Millipak). Formic acid (p/n G2453-85060) and a mixture of 14 basic pesticides (p/n 5190-0469) were purchased from Agilent Technologies, Inc.

Results and Discussion

A sample-sequence table was set up to measure 120 wastewater samples plus several blanks with an overall run time of more than 48 hours. The combination of two Multi-Cartridge Kits gave excellent and reliable results. Area and retention time RSD, carryover, and the comparison of 120 runs on 12 PLRP-S cartridges showed excellent repeatability and robustness of the system, even with wastewater.

Figure 4 displays 120 analyses of three pesticides, measuring 10 runs on each cartridge. Area/response is illustrated versus number of runs to show the repeatability over time, cartridge, and sample run.

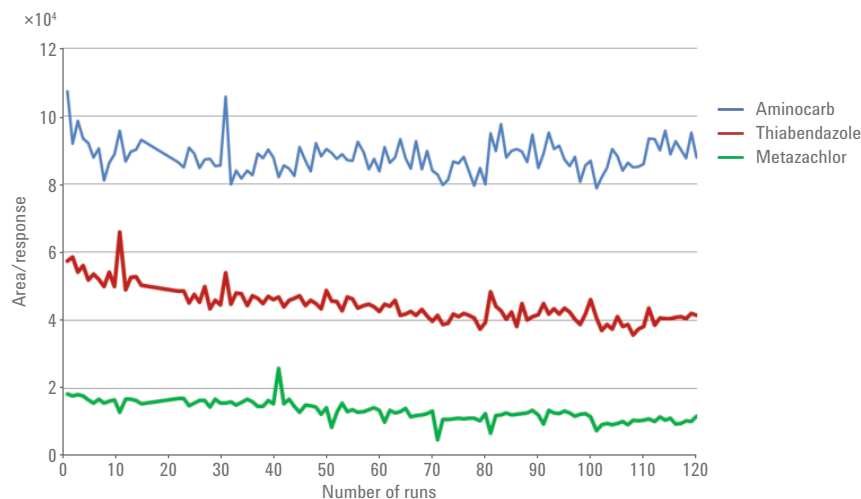


Figure 4. Aminocarb, thiabendazole, and metazachlor are shown with their area versus number of runs.

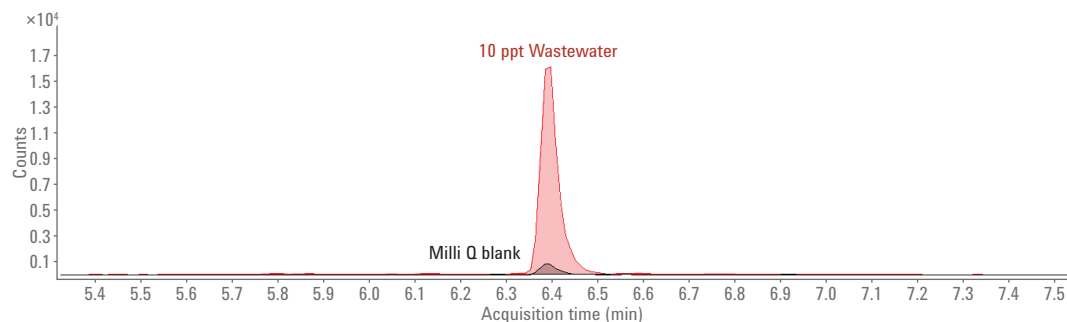


Figure 5. Thiabendazole at 10 ppt in wastewater followed by a Milli Q blank injection.

Aminocarb (RT 5.8 minutes), thiabendazole (RT 6.4 minutes), and metazachlor (RT 8.8 minutes) demonstrated good repeatability over time and runs, as with all other compounds, even in a highly difficult matrix such as wastewater. Area RSD was determined to be 5.4 % for aminocarb, 11.3 % for thiabendazole, and 12.8 % for metazachlor. Retention time RSD for all compounds was < 0.7 %.

In Figure 4, some deviations and spikes were visible for the response over time and runs for all analytes. These spikes occurred on different cartridges, which indicated either high matrix contamination or a short decrease in performance of the cartridge. Overall, a

good performance was achieved for all compounds over time and runs.

Carryover was determined for thiabendazole by injection of 10 wastewater runs followed by a Milli-Q blank injection and wastewater blank. Carryover for thiabendazole was < 5 % compared to the 10 ng/L wastewater spike (Figure 5). No other analyte showed carryover.

To minimize and reduce carryover, an additional capillary can be installed in the 6-position/14-port valve. This capillary enables a cleaning procedure for the autosampler and external loop. Here, one capillary would be installed instead of a cartridge in one 6-position/14-port valve.

Wastewater blanks showed no carryover even though we analyzed pesticides with up to 14 ng/L, for example carbofuran, diazinon, atrazine, imazalil, and dimethoate.

Conclusions

An online SPE system with two Agilent Multi-Cartridge Kits enables easy method setup and high sample throughput without changing cartridges. The combination of four online SPE kits makes it possible to switch between direct injection and online SPE with up to 12 cartridges. Two 6-position/14-port valves increase the capacity of the system without changing cartridges, and two 2-position/10-port valves enable direct injection and online SPE.

To show the functionality of the system, wastewater was spiked with pesticides and analyzed 10 times for each cartridge. Good area and retention time RSDs were found for 120 runs and 12 cartridges, even for difficult matrixes such as wastewater. Carryover was observed just for one compound and without using a cleaning procedure with an additional capillary.

The Multi-Cartridge Kit permits the use of up to 12 cartridges in the system for automated, productive, convenient, and rapid solid phase extraction.

References

1. Naegele, E. Quantification of trace-level herbicides in drinking water by online enrichment with the Agilent 1200 Infinity Series Online-SPE Solution and Triple Quadrupole MS Detection. *Agilent Technologies Application Note*, publication number 5991-1738EN, **2013**.
2. Naegele, E. Comparison of Direct Injection and Online SPE for Quantification by LC/MS of Trace-Level Herbicides in Water. *Agilent Technologies Application Note*, publication number 5991-2140EN, **2013**.
3. Schuhn, B. High Volume Injection up to 5 mL with the Agilent 1200 Infinity Series Online SPE Solution for Highest Sensitivity. *Agilent Technologies Technical Note*, publication number 5991-3173EN, **2013**.
4. Schuhn, B. Online SPE Multi-Cartridge Kit for Fast and Easy Method Development. *Agilent Technologies Technical Note*, publication number 5991-4005EN, **2014**.
5. Yang, D; Naegele, E. Detection of Basic and Acidic Pesticides and Herbicides at Trace Levels by Online SPE LC/MS in Drinking Water. *Agilent Technologies Application Note*, publication number 5991-4177EN, **2014**.

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