

# Analysis of Pharmaceuticals and Personal Care Products in Groundwater by Online SPE and LC/MS/MS

## Application note

Water analysis

### Authors

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### Abstract

An online SPE method for the identification and quantitation of trace levels of pharmaceuticals and personal care products in groundwater was developed. This SPE method used the Agilent 1290 Infinity Flexible Cube solution, followed by detection with triple quadrupole mass spectrometry. A small volume (900  $\mu$ L) of filtered groundwater was preconcentrated on a polymeric cartridge and directly analyzed. More than 100 samples were rapidly analyzed as part of a field study on groundwater sampling techniques. Seven compounds were analyzed including the food ingredients caffeine and sucralose, the antibiotic sulfamethoxazole, three antidepressants, and one metabolite. Recoveries ranged from 34 to 164 %, and the method detection limits varied from 1 to 10 ng/L.



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## Introduction

Automation of a manual solid phase extraction (SPE) method can provide many benefits, such as reduced operator exposure to solvents, improved recoveries, cost savings, and rapid analysis. Automating a manual SPE method with online SPE removes the analyst from extended contact with hazardous environmental samples such as wastewater, as well as contact with organic elution solvents. Furthermore, there is no evaporation of organic solvents to the atmosphere during sample preparation. Operator error from repeated motions of SPE is eliminated, as an automated robotic system performs sample handling. Finally, overnight runs are possible, maximizing sample preparation and analysis time. The most important benefit of online SPE is the ability to maximize the recovery of analytes, since all the sample is directly eluted onto the analytical column from the SPE cartridge.

The importance of SPE in water sample analysis has been known since the early 1980s, because of the need to improve the detection limit for trace organic compounds. This resulted in large volumes, up to 1 L, being analyzed. Typically, manual methods used a vacuum manifold and large volume preconcentration and elution, which results in sample losses. Both of these issues are removed with online SPE and use of the sensitive LC/MS/MS system, the Agilent 6460 triple quadrupole LC/MS. Sample volumes are reduced from liters to less than 1 mL, and the sample is placed directly into the SPE cartridge. The sample is then eluted with the mobile phase of the chromatographic system directly into the LC column, and detected by the mass spectrometer using MS/MS analysis.

This application note demonstrates an automated SPE method for groundwater samples that contain pesticides and pharmaceuticals. A hundred samples of alluvial groundwater were analyzed using online SPE coupled to LC/MS/MS. The samples came from groundwater associated with the South Platte River near Denver, Colorado. The method is rapid, automated, and sensitive so samples can be analyzed quickly and accurately using the Agilent online SPE Flexible-Cube system with the Agilent 6460 Triple Quadrupole LC/MS.

## Experimental

### Standards and reagent

Calibration standards were obtained from Cerilliant and AccuStandards at the highest available purity. Calibration standard solutions were prepared in the range of 1–1,000 ng/L. A labeled surrogate internal standard, carbamazepine d-10, was from Cambridge Isotopes. All solvents used were of highest purity available. Pesticide-grade water, methanol, and acetonitrile were obtained from Burdick & Jackson.

### Instrumentation

An Agilent 1290 Infinity Series online SPE solution system was used, comprising:

- Agilent 1290 Infinity binary pump with internal degasser (G4220A)
- Agilent 1260 Infinity standard autosampler with 900 µL loop (G1329B #20) and thermostat (G1330B)
- Agilent 1290 Infinity Flexible Cube (G4227A) with 2-position/10-port valve (G4232C)
- Agilent 1290 Infinity thermostatted column compartment (G1316C)

The MS detection was carried out with the 6460 Triple Quadrupole LC/MS with Agilent Jet Stream technology. Figure 1 and Figure 2 show the schematic of the different modules and the actual Infinity Flexible Cube instrument. Two cartridges were used in this system; while one column is loading, the second column is eluted, thus minimizing the total analysis time.

Table 1 shows the analytical operating conditions for both the 1290 Infinity Flexible Cube and the LC/MS system.

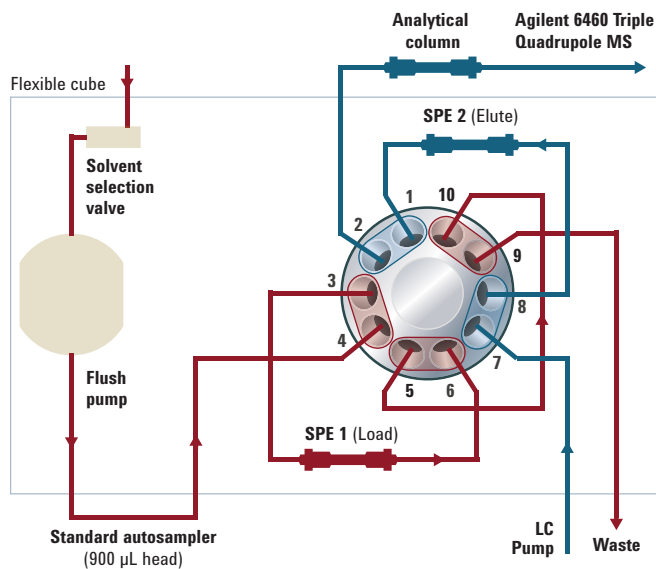


Figure 1. Schematic of the two-cartridge online process.

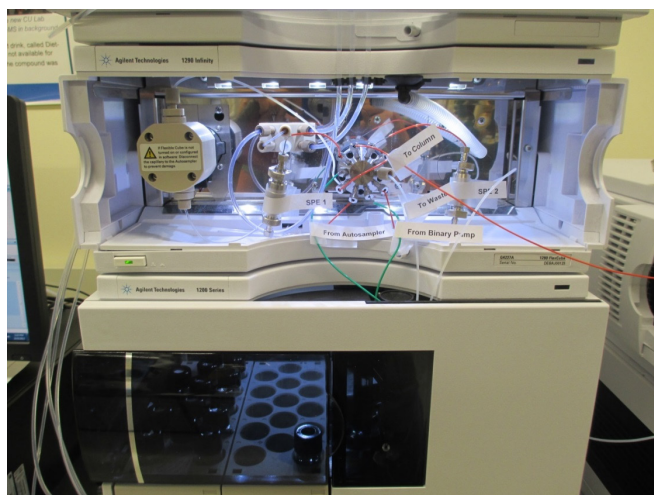


Figure 2. Photo of the Agilent 1290 Infinity online SPE solution, the Agilent Infinity Flexible Cube.

Table 1. Online SPE Conditions

#### Agilent 1290 Infinity Flexible Cube

Online SPE cartridges	Agilent PLRP-S, 4.6 × 12.5 mm (p/n 5982-1270)
Temperature	25 °C
Volume	900 µL
Injection draw speed	1,000 µL/min
Draw position	0.5 mm
Flow rate	1 mL/min
Solvents	A1) Water A2) MeOH B2) ACN
Cartridge conditioning	2 mL MeOH 4 mL Water
Cartridge wash	2 mL ACN

#### HPLC conditions

Analytical column	Agilent InfinityLab Poroshell 120 EC-C8, 150 × 2.1 mm, 2.7 µm (p/n 693775-902)	
Temperature	25 °C	
Mobile phase	A) Water (0.1% acetic acid) B) Acetonitrile	
Flow rate	0.4 mL/min	
Gradient for elution from SPE cartridge	Time (min)	%A %B
	0	90 10
	2	90 10
	12	40 60
	14	0 100
	14.3	0 100

#### MS conditions

Acquisition parameters	MRM positive mode
Sheath gas temperature	350 °C
Sheath gas flow rate	11 L/min
Drying gas temperature	250 °C
Drying gas flow rate	10 L/min
Nebulizer pressure	45 psig
Nozzle voltage	0 V positive
Vcap	4,000 V
EMV	200 V positive

## Sample preparation

Colleagues at the Colorado Department of Agriculture collected groundwater samples using one of three methods:

- Purge and sample with a peristaltic pump (PP)
- Purge and sample with a bladder pump (BP)
- Collection of a no-purge sample with a HydraSleeve (HS)

Filtration in the field was carried out only for the water samples collected with the peristaltic pump and the bladder pump, using 0.45-mm Sterlitech nylon membrane filters. The HydraSleeve sample was filtered in the laboratory, using a 0.45-mm laboratory filter. A 6-mL aliquot of each sample was used for online SPE analysis.

Using this online system minimizes the total analysis time to less than 20 minutes per sample; while one cartridge was loaded with sample, the second cartridge was eluted.

## Analysis parameters

Table 2 shows the MRM transitions for all the analytes studied in this work. Two transitions for each compound were used for the correct identification of the pharmaceuticals and personal care products.

## Results and Discussion

### Online SPE performance

Table 3 shows the recoveries obtained for the pharmaceuticals and pesticides studied in the groundwater samples. For most analytes, acceptable recoveries were obtained, except for gabapentin. Due to the polarity of gabapentin, as well as its low affinity for the polymeric phase, it is somewhat removed during the loading phase.

Table 2. MRM ESI Analysis Parameters for all Analytes Studied

Compound	Precursor ion	Product ion	Retention time (min)	Fragmentor	Collision energy	Polarity
Caffeine	195	138	3.90	110	15	Positive
Caffeine	195	110	3.90	110	25	Positive
Carbamazepine	237	194	8.96	120	15	Positive
Carbamazepine	237	179	8.96	120	35	Positive
Carbamazepine D-10	247	204	8.96	120	15	Positive
Carbamazepine metabolite	271	180	6.32	70	15	Positive
Carbamazepine metabolite	271	236	6.32	70	5	Positive
Gabapentin	172	154	6.53	90	10	Positive
Gabapentin	172	137	6.53	90	15	Positive
Lamotrigine	256	211	6.00	120	25	Positive
Lamotrigine	258	213	6.00	120	25	Positive
Sucralose	419	239	5.33	110	15	Positive
Sucralose	419	221	5.33	110	15	Positive
Sulfamethoxazole	254	156	7.18	80	10	Positive
Sulfamethoxazole	254	92	7.18	80	30	Positive

Table 3. Recoveries Obtained After Preconcentration of 900  $\mu$ L Onto the PLRP-S Cartridges

Compound	% Recovery	Limits of detection (LODs) (ng/L)
Caffeine	106	5
Carbamazepine	100	1
Carbamazepine metabolite	86	5
Gabapentin	34	10
Lamotrigine	114	5
Sucralose	164	5
Sulfamethoxazole	124	10

Another Agilent phase, such as C-18 or another polymer, may be a more suitable choice for this analyte. A larger loading of solid phase, PLRP-S, or a smaller volume of sample would also work. The remaining pharmaceuticals, which are more hydrophobic, were well recovered, varying from 86 to 164 %. The unusually high recovery of sucralose was likely to be caused by variation in sodium recovery, which affects the sodium adduct used for quantitation. It is necessary to use a labeled standard for this analyte in future work. No carryover was found for any of the analytes in the blanks after a rinse with 2 mL of acetonitrile.

### Sample analysis

Figure 3 shows a chromatogram of a groundwater sample from one of the alluvial wells along the South Platte River. All seven analytes were found in well 009. Sucralose was the predominant compound found, with a concentration of 3,360 ng/L, followed by lamotrigine, with 279 ng/L, sulfamethoxazole at 141 ng/L, carbamazepine metabolite at 103 ng/L, carbamazepine at 77 ng/L, gabapentin at 18 ng/L, and caffeine at 5 ng/L. The analysis of these compounds was rapid, which is an excellent feature of online SPE followed by LC/MS/MS analysis.

One purpose of this study was the analysis of 100 groundwater samples for comparison of the three sampling methods. The usefulness of the online method was that it made the analysis cost-effective since analysis time was halved by using online SPE versus offline SPE. In general, the three sampling methods gave similar results.

However, this study reveals that variation within alluvial groundwater wells may far exceed the reproducibility of the chemical analysis and sampling technology. For example, Figure 4 shows the variability in the concentration of carbamazepine and its metabolite in well 009 from May to August of 2015. The range of concentration for carbamazepine (red) was from a low of 78 ng/L, to a high of 131 ng/L. The metabolite of carbamazepine varied from 100 to 200 ng/L over the same time period. Thus, the variation in concentration of both carbamazepine and its metabolite varied by 100 % over the six sampling events.

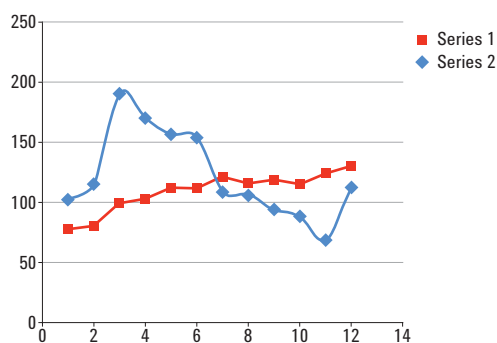


Figure 4. The variation in well 009 over a 2-month sampling period, from 12 August to 22 September 2015, for the concentration of carbamazepine (red) and its metabolite (blue), an antidepressant found in the well. Samples 1–2, 3–4, 5–6, 7–8, 9–10, and 11–12 are sample pairs comparing HS to BP.

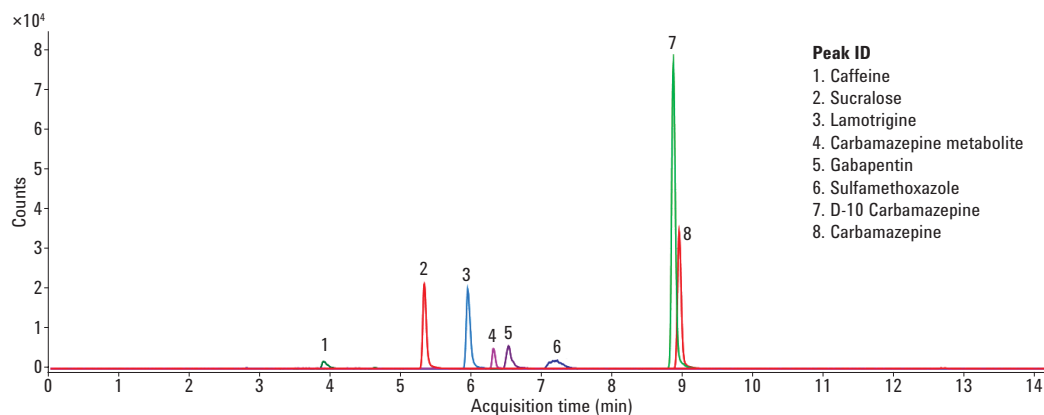


Figure 3. LC/MS/MS analysis of a groundwater sample from well 009 in the alluvium of the South Platte River, Colorado.

The chemical analysis of duplicate water samples using two different pumps (HS and BP) varied by approximately 10 % during these same six sampling events. Samples 1–2, 3–4, 5–6, 7–8, 9–10, and 11–12 are sample pairs comparing the HS sample to the BP sample, in well 009. Thus, the variation in groundwater sampling is an important variable to consider. While the chemical analysis with online SPE was only a small part of the total variation in groundwater analysis, sample pairs varied approximately 10 %, and the concentration changed by 100 % over the 2-month sample period.

It is important to note that the South Platte River performs a major role in the transport of pharmaceuticals and personal care products to alluvial groundwater wells in the South Platte River valley. Several metropolitan sewage processing facilities, including Metro Wastewater Reclamation, which contributes up to 100,000,000 gallons per day of secondary sewage, affect the South Platte River. There can also be substantial variation in the discharge of the river due to natural rainfall, which causes the concentration of pharmaceuticals and personal care products to vary widely in the river. Furthermore, the wastewater outfall can vary in both concentration and volume. Therefore, there can be drastic differences in the concentration of pharmaceuticals and personal care products in the alluvial groundwater wells, as shown in Figure 4.

The efficient and accurate use of online SPE makes the work of following these variations in concentration relatively easy and cost-effective for future evaluation. The online SPE method is valuable for the monitoring of these pharmaceuticals and personal care products down the South Platte River valley. This valley contains wells similar to those used for drinking water in Northeastern Colorado and Western Nebraska.

## Conclusions

This application note shows that automated online SPE is a valuable tool for groundwater analysis for trace levels of pharmaceuticals and personal care products. We analyzed 100 water samples for seven analytes with detection limits in the low ng/L level using only 900 µL of filtered groundwater. The combination of the Agilent 1290 Infinity Flexible Cube and the Agilent 1260 Infinity autosampler allowed the analyst to do online SPE with chromatography while the second cartridge was being loaded. Thus, no time was lost in sample preparation. Proper cleaning and elution techniques allowed the same SPE cartridge to be used in multiple applications before replacement. In this study, 20 injections were used on each cartridge, with no detection of pharmaceuticals in the blanks.

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