Application Note

Environmental

Direct Injection and Online SPE LC/MS/MS for the Determination of Pharmaceuticals and Personal Care Products (PPCPs) in Water Samples

Using the Agilent InfinityLab Online SPE Solution

Authors

Gerd Vanhoenacker and
Koen Sandra
Research Institute for
Chromatography
President Kennedypark 26
B-8500 Kortrijk
Belgium

Thomas Glauner
Agilent Technologies, Inc.

Abstract

The Agilent InfinityLab Online SPE solution using the Agilent Online SPE Starter Set with the Agilent Online SPE Direct Inject Kit has been combined with the Agilent 6470A Triple Quadrupole LC/MS for the determination of traces of pharmaceuticals and personal care products (PPCPs) in environmental water and drinking water samples. With the Online SPE Direct Inject Kit, switching between direct injection and online SPE is completely automated.

A selection of water samples was analyzed with both direct injection and online SPE. Results of both techniques were compared. The data demonstrate that, for most compounds, the chromatographic performance was not significantly influenced by the SPE step, and a substantial gain in sensitivity was achieved.

Introduction

PPCPs are widespread in daily life, and traces can be found in most environmental water samples. Some of these compounds are detected as such, and others as degradation products or metabolites (metabolized by intake or in the environment, for example, by bacteria). According to a review by Petrie; *et al.*[1] on these emerging contaminants in the environment, the presence of over 200 different pharmaceuticals has been reported in river waters in various countries. The chemicals are present in the environment as complex mixtures, and this situation can potentially lead to synergistic effects[1]. Therefore, the influence of these contaminants on the environment is difficult to define and predict.

The analysis of PPCPs is an important step to gain insight into this matter[2]. As these compounds are present in low concentration in environmental samples, there is a demand for sensitive analytical methods. They must detect these chemicals at levels below ng/L (ppt) in various water matrices and, if possible, with minimal impact of these matrices on sensitivity and uptime of the analytical system. An additional challenge is that the compounds under investigation do not originate from one chemical family or class, and that their physicochemical properties are diverse. As a result, a single technique will not be able to cover all PPCPs present in the environment.

Guidelines and regulations on water quality are becoming more stringent because of the potential for contamination and the associated environmental risks[3]. The number of analyses required is increasing significantly, and the quest to automate sample preparation procedures is gaining momentum. Regarding increasing sensitivity and automating sample preparation, online SPE is an interesting alternative to offline and manual pretreatments. The online SPE step concentrates and cleans up large volumes of water samples that can then be injected with no or only minimal manual intervention.

This Application Note demonstrates the benefit of online SPE for a selection of different PPCPs. Analyses were carried out using the Agilent 1290 Infinity II LC system coupled to a 6470A Triple Quadrupole LC/MS. The system was equipped with an Agilent Online SPE Starter Set and the Agilent Online SPE Direct Inject Kit. The advantage of the latter is that switching between online SPE (with generally large injection volumes) and direct injection with reduced delay volume can be fully automated without any hardware modification. This creates a flexible system for use in a wide variety of applications.

Experimental

Instrumentation

A 1290 Infinity II LC system and a 6470A Triple Quadrupole LC/MS with an Agilent Jet Stream ESI source were used. The 1290 Infinity II LC system was configured as follows:

- Agilent 1290 Infinity II High Speed Pump (analytical) (G7120A)
- Agilent 1260 Infinity II Quaternary Pump (SPE) (G7111B)
- Agilent 1290 Infinity II Multisampler (G7167B)
  - 100-µL analytical head and 100-µL sample loop
- Agilent 1290 Infinity Valve Drive (G1170A)
  - Agilent InfinityLab Quick Change 2-Position/6-Port valve (G4231C)
  - 1.4-mL seat extension loop (G1313-87308)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent 1290 Infinity Flexible Cube (G4227A)
  - Second valve drive installed (G4227A option #058)
  - Agilent Online SPE Starter Set, 800 bar (G4742A)
  - Agilent Online SPE Direct Inject Kit, 1,300 bar (G4744B)

The following columns were installed:

- **SPE:** two Agilent Bond Elut Online SPE cartridges, PLRP-S, 2.1 × 12.5 mm, 15–20 µm (p/n 5982-1271)
- **Analytical:** Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm (p/n 695775-902)

The configuration shown in Figure 1 enables analyses with online SPE with injection volumes up to 1.5 mL, and analyses using direct injection with injection volumes up to 100 µL. Switching between the two modes is fully automated, and the delay volume during direct injection analysis is greatly reduced using this setup. The principle of operation for this setup is described in more detail in the Application Note 5991-8017EN[4].

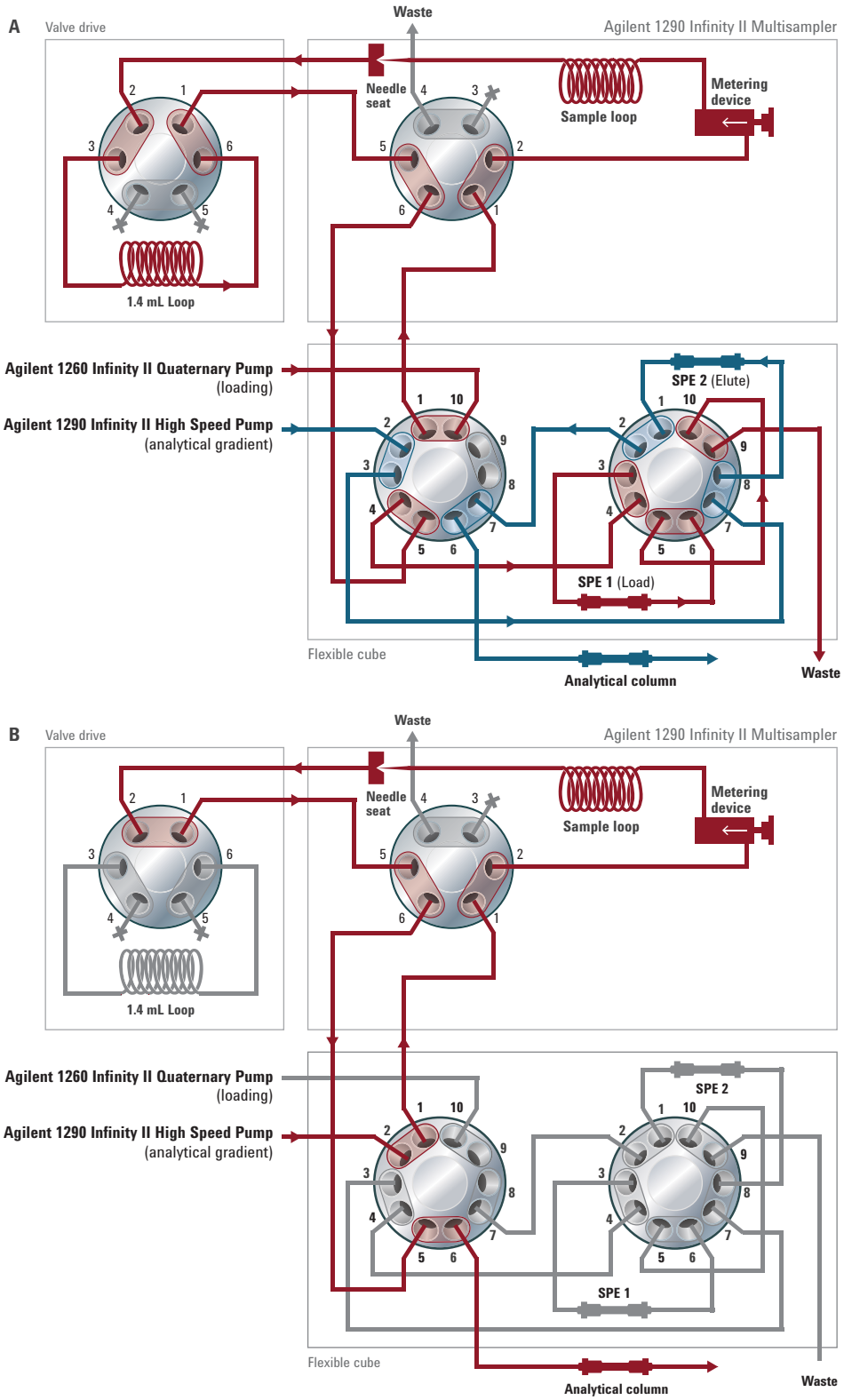

Figure 1. Schematic overview of the configuration during online SPE (A) and direct injection with reduced delay volume (B) operation.

Chemicals

The PPCPs (Table 1) were from Sigma-Aldrich (St. Louis, MO, USA) except for 10,11-dihydro-10,11-dihydroxy carbamazepine, which was from Toronto Research Chemicals (Toronto, ON, Canada). Formic acid and ammonium formate for LC/MS were from Sigma-Aldrich, and HPLC grade water, HPLC-S grade acetonitrile, and LC/MS grade methanol were from Biosolve (Valkenswaard, The Netherlands).

Standard solutions

Individual stock solutions of the standards were prepared in methanol and acetonitrile. These solutions were mixed and diluted to 5 ppm in methanol. This standard mix was further diluted in tap water with 0.1 % formic acid (referred to as solvent) to 10 ppb. All further dilutions were also prepared in the solvent.

Samples

Two lake samples and two canal samples were evaluated. Samples were stored in the dark at 2–8 °C.

- Surface water, Lake Zwevegem
- Surface water, Lake Steenhuffel
- Surface water, Canal Zwevegem
- Surface water, Canal Tessenderlo

To these samples, 0.1 % v/v formic acid was added, and the sample was vortexed and filtered through an Agilent Captiva Premium Syringe Filter (regenerated cellulose, 25 mm, 0.45 µm, p/n 5190-5111).

Spiking was carried out in prepared samples by dilution of the 100 ppb standard in the respective sample solution to 1 ppb. This spiking stock solution was further diluted to the required level using the prepared sample at hand as a diluent.

Table 1. PPCPs under investigation.

Compound	CAS	Use
Atenolol	29122-68-7	Beta blocker
Carbamazepine	298-46-4	Antiepileptic
10,11-Dihydro,10,11-dihydroxy carbamazepine	58955-93-4	Antiepileptic metabolite
Diclofenac	15307-79-6	NSAID
Propranolol	13071-11-9	Beta blocker
Sulfadimethoxine	122-11-2	Sulfonamide antibiotic
Sulfamerazine	127-79-7	Sulfonamide antibiotic
Sulfamethoxazole	723-46-6	Sulfonamide antibiotic
N4-Acetylsulfamethoxazole	21312-10-7	Sulfonamide antibiotic metabolite
Tramadol	36282-47-0	Analgesic
Valsartan	137862-53-4	Hypertension treatment

Table 2. LC method parameters.

	Direct	Online SPE
Column	Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm (p/n 695775-902)	
Mobile phase	A) 5 mM Ammonium formate + 0.05 % formic acid in water B) Acetonitrile	
Gradient	0 to 1 minute: 5 %B, 0.4 mL/min 1 to 8 minutes: 5 to 95 %B, 0.4 mL/min 8 to 10 minutes: 95 %B, 0.4 mL/min 4 minutes post time at 5 %B, 0.4 mL/min	0 to 0.25 minutes: 5 %B, 0.2 to 0.4 mL/min 0.25 to 4.5 minutes: 5 %B, 0.4 mL/min 4.5 to 11.5 minutes: 5 to 95 %B, 0.4 mL/min 11.5 to 15 minutes: 95 %B, 0.4 mL/min
Column temperature	35 °C	
Injection temperature	8 °C	
Injection	100 µL Needle wash methanol/acetonitrile 5 Seconds flush port	**Injector program** Draw 1,500 µL Needle wash methanol/acetonitrile 5 Seconds flush port
SPE column		Bond Elut Online SPE, PLRP-S 15-20 µm, 2.1 × 12.5 mm (p/n 5982-1271)
Mobile phase		A) 0.1 % Formic acid in water B) Methanol
SPE gradient		0 to 0.25 minutes: 0 %B, 0.2 to 1.2 mL/min 0.25 to 4 minutes: 0 %B, 1.2 mL/min 4 to 5 minutes: 0 to 100 %B, 1.2 mL/min 5 to 8 minutes: 100 %B, 1.2 mL/min 8 to 9minutes: 100 to 0 %B, 1.2 to 0.8 mL/min
Flexible Cube		3.5 minutes: Increase valve position
Column temperature		Ambient (Flexible Cube)

Table 3. MS method parameters.

Parameter	Value
Detection	MS/MS
Ionization	Agilent Jet Stream Technology, electrospray, positive ionization
Source settings	
Drying gas temperature	290 °C
Drying gas flow	8 L/min
Nebulizer pressure	45 psi
Sheath gas temperature	380 °C
Sheath gas flow	11 L/min
Capillary voltage	2,500 V (pos)/3,500 V (neg)
Nozzle voltage	0 V (pos)/500 V (neg)
Acquisition settings	
Dynamic MRM	
Cycle time	200 ms
Time filter	0.04 minutes
EMV	100 V (pos)/300 V (neg)

Table 4. Method transitions; the first transition for each compound was used as the quantifier.

Compound	Frag.	Polarity	RT direct (min)	RT SPE (min)	Delta RT	Precursor ion	MS1 res.	Product ion	MS2 res.	Collision energy	Cell accelerator voltage
Atenolol	150	Positive	2.93	5.96	0.8	267.2	Unit	190.1	Unit	20	4
						267.2	Unit	145.1	Unit	20	5
Sulfamerazine	150	Positive	3.82	6.68	0.8	265.1	Unit	92	Unit	40	3
						265.1	Unit	156	Unit	10	5
						265.1	Unit	108	Unit	20	6
Tramadol	118	Positive	4.44	7.18	0.8	264.2	Unit	58.2	Unit	16	5
						264.2	Unit	42.2	Unit	60	7
10,11-DiH-10,11-diOH-carbamazepine	74	Positive	4.48	7.21	0.8	271.1	Unit	180	Unit	36	4
						271.1	Unit	253.1	Unit	4	4
						271.1	Unit	236	Unit	10	4
Sulfamethoxazole	150	Positive	4.75	7.51	0.8	254.1	Unit	156	Unit	10	5
						254.1	Unit	108	Unit	20	5
						254.1	Unit	92	Unit	20	6
N4-Acetylsulfamethoxazole	105	Positive	4.89	7.61	0.8	296.1	Unit	65.1	Unit	46	4
						296.1	Unit	134	Unit	26	4
Propranolol	150	Positive	5.14	7.85	0.8	260.2	Unit	56	Unit	40	3
						260.2	Unit	183.1	Unit	20	3
						260.2	Unit	116.1	Unit	20	3
Sulfadimethoxine	150	Positive	5.23	7.96	0.8	311.1	Unit	156.1	Unit	20	4
						311.1	Unit	108	Unit	40	2
						311.1	Unit	92	Unit	40	3
Carbamazepine	146	Positive	5.6	8.3	0.8	237.1	Unit	194.1	Unit	16	4
						237.1	Unit	179.1	Unit	36	4
Valsartan	116	Positive	6.51	9.2	0.8	436.2	Unit	235	Unit	18	4
						436.2	Unit	291.1	Unit	14	4
						436.2	Unit	207	Unit	30	4
Diclofenac	80	Negative	7.13	9.82	0.8	294	Unit	250	Unit	5	6
						294	Unit	214	Unit	16	6

Results and Discussion

Due to the large volumes injected on the SPE column and the SPE process as such, care must be taken to maintain the chromatographic performance of the analysis. Therefore, the loading and elution conditions must be carefully selected and optimized. The impact of the loading solvent (for example, addition of acid, type of acid, and so forth) on the peak shape and recovery of analytes can be significant. This was the case here, where compounds featuring different chemistries were analyzed. The use of 0.1 % formic acid in water proved to be a good compromise for the compounds under investigation. The application of other solvents can increase recovery for some compounds, but will reduce recovery for others, and potentially lead to peak broadening. Figure 2 shows a comparison between direct injection (100 µL, 200 ng/L) and online SPE (1.5 mL, 20 ng/L) for standard solutions made in tap water. The results were generated in a single sequence with automated switching between the two modes. The chromatographic performance of the analytical column is maintained when the SPE cartridge is eluted in backflush mode, except for sulfamerazine and sulfamethoxazole, which suffer from slight peak broadening. The first eluting compound, atenolol, shows some lower recovery in online SPE due to its polar nature. However, the overall result is satisfactory, and the method should be suitable for real sample analyses. The retention time distribution is similar due to the comparable delay volumes in direct injection mode and online SPE. The approximately three-minute delay of the online SPE retention times is caused by the time needed for loading the 1.5 mL of sample onto the cartridge, and subsequent washing.

The performance of the methods was evaluated for injection precision, linearity, and sensitivity. Table 5 summarizes the results obtained with both techniques. The injection precision with online SPE is in general slightly inferior compared to direct injection. Considering that offline SPE (manual or automated) will not result in better precision[5-7], these values can be considered acceptable.

The sensitivity gain is also illustrated in Table 5 (limit of detection (LOD) and slope of the calibration curve), and is visualized for a standard solution in Figure 3, where the TIC is displayed for the analysis of a 20 ng/L solution with both modes. Figure 4 gives a comparison of the signal-to-noise ratio (S/N) for direct injection and online SPE for valsartan and the carbamazepine metabolite.

Table 5. Method performance data for standard solution in tap water.

		Injection precision (RSD%)	LOD (ng/L)	Slope calibration
Atenolol	Direct	0.51	0.20	161.8
	Online SPE	9.17	0.10	564.8
Sulfamerazine	Direct	0.51	0.10	88.3
	Online SPE	0.97	0.10	799.8
Tramadol	Direct	0.24	0.20	2147.1
	Online SPE	1.52	0.05	16725.3
10,11-Dihydro, 10,11-dihydroxy carbamazepine	Direct	0.88	0.10	192.4
	Online SPE	2.40	0.05	2044.5
Sulfamethoxazole	Direct	1.90	1.00	38.7
	Online SPE	3.04	0.20	189.0
N4-Acetylsulfamethoxazole	Direct	1.29	0.10	48.4
	Online SPE	1.63	0.05	505.2
Propranolol	Direct	0.61	0.10	201.6
	Online SPE	8.13	0.02	1273.7
Sulfadimethoxine	Direct	0.21	0.20	195.7
	Online SPE	2.76	0.02	2299.9
Carbamazepine	Direct	0.79	0.50	1326.4
	Online SPE	0.70	0.10	8398.8
Valsartan	Direct	1.44	1.00	13.1
	Online SPE	3.92	0.10	265.0
Diclofenac	Direct	0.92	5.00	15.2
	Online SPE	3.72	<1	174.4

Injection precision:
Direct: 100 µL of 200 ng/L
SPE: 1.5 mL of 20 ng/L

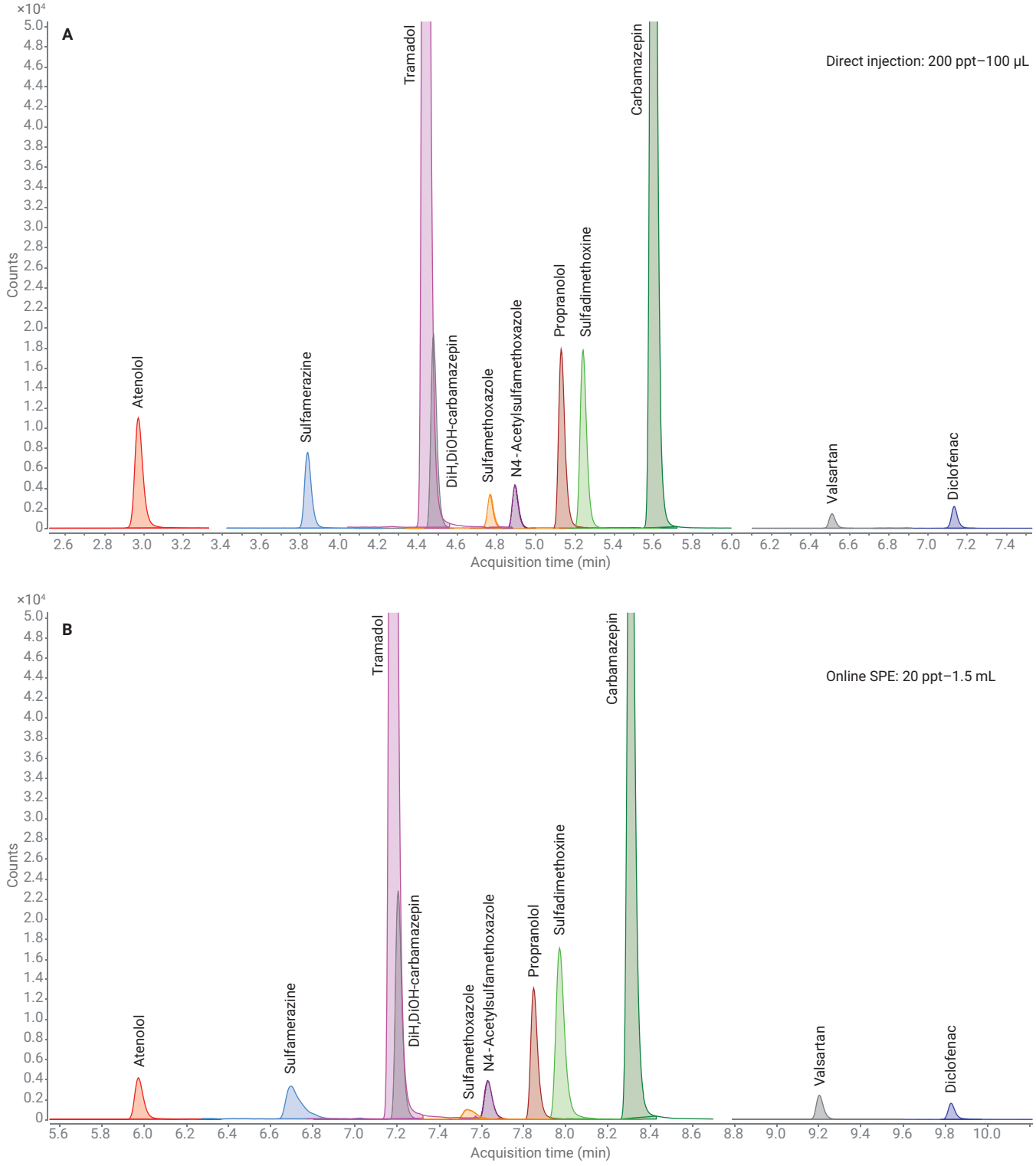

Figure 2. Comparison of performance for direct injection (A) and online SPE (B) for a spiked tap water sample. Signals for quantifier transitions are shown.

The environmental water samples were analyzed with both methods. Spiking experiments showed that recoveries are not always optimal under the applied conditions. This is a consequence of the selected loading conditions, which are a compromise to cover the diversity of the selected analytes. However, the absolute result in the samples is similar for direct and online SPE. Table 6 shows the final concentrations of the PPCPs in the samples, and Figures 5A and 5B show an example of the sensitivity improvement in a real sample. Valsartan and sulfadimethoxine are significantly easier to detect using the online SPE approach. With direct injection, these compounds appear at or below the LOD level.

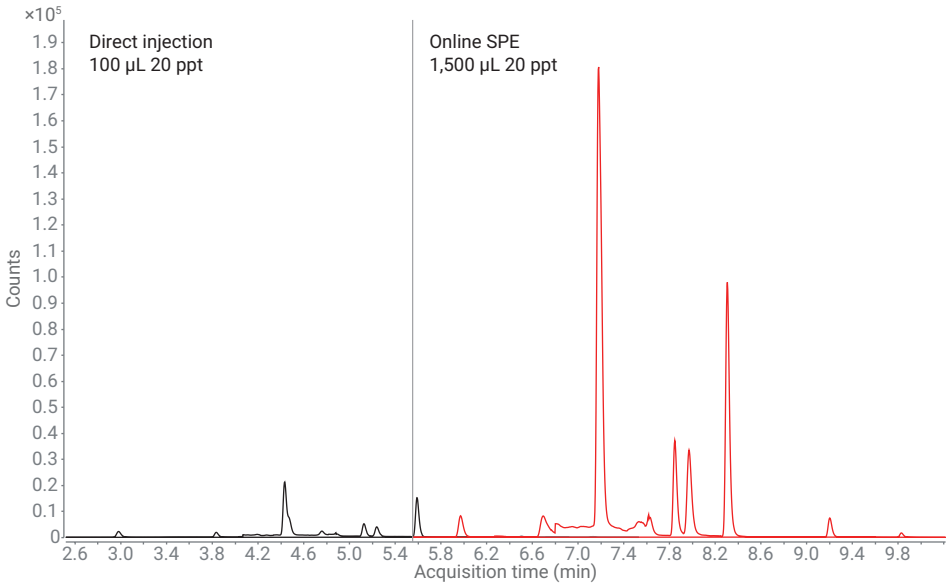

Figure 3. Comparison of sensitivity for direct injection and online SPE for a standard solution in tap water.

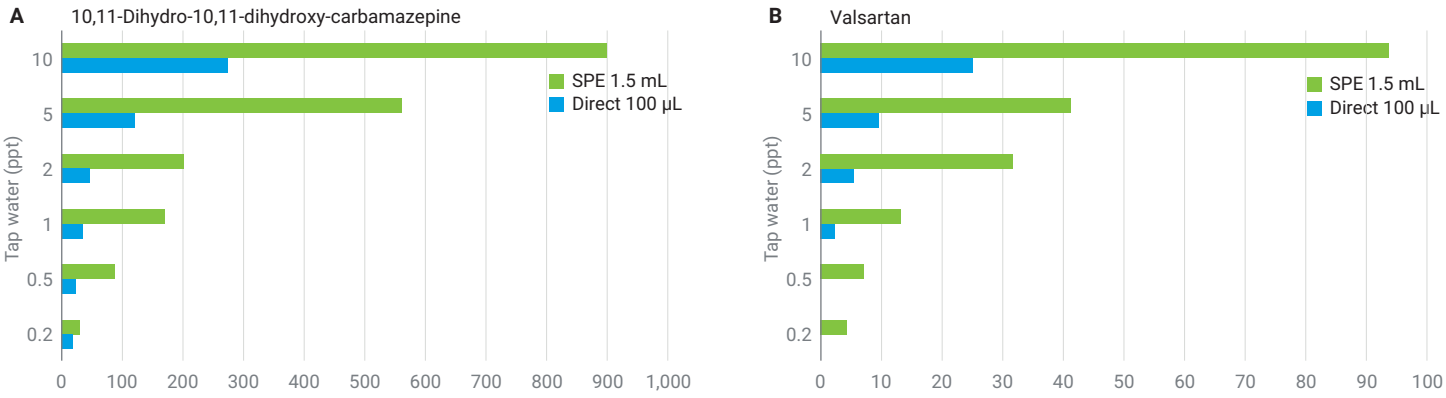

Figure 4. Comparison of S/N for direct injection and online SPE for a standard solution in tap water. Noise calculation: ASTM noise, noise window 0.1 to 0.2 minutes before peak.

Table 6. Result for the environmental samples obtained after direct injection and online SPE.

	Lake Steenhuffel		Lake Zwevegem		Canal Zwevegem		Canal Tessenderlo	
Concentration (ppt)	Direct	SPE	Direct	SPE	Direct	SPE	Direct	SPE
Atenolol	–	0.4	5.9	3.6	20.8	12.2	–	0.4
Sulfamerazine	–	–	–	–	–	–	–	–
Tramadol	3.7	4.5	281.6	274.0	245.4	232.8	79.7	85.8
DiH-DiOH-carbamazepine	87.8	79.2	385.3	202.6	293.2	146.1	69.6	48.4
Sulfamethoxazole	–	1.7	32.3	28.5	29.8	23.9	3.5	5.2
N4-Ac-sulfamethoxazole	–	–	2.9	1.4	11.8	5.3	–	–
Propranolol	–	0.3	2.7	2.8	6.5	7.0	0.9	1.0
Sulfadimethoxine	–	0.1	1.8	0.5	0.6	0.4	–	0.1
Carbamazepine	20.0	19.9	139.5	114.6	96.4	80.8	30.2	26.6
Valsartan	0.5	0.4	9.0	5.0	15.1	8.2	–	0.1
Diclofenac	–	–	237.5	269.2	112.4	95.9	–	3.2

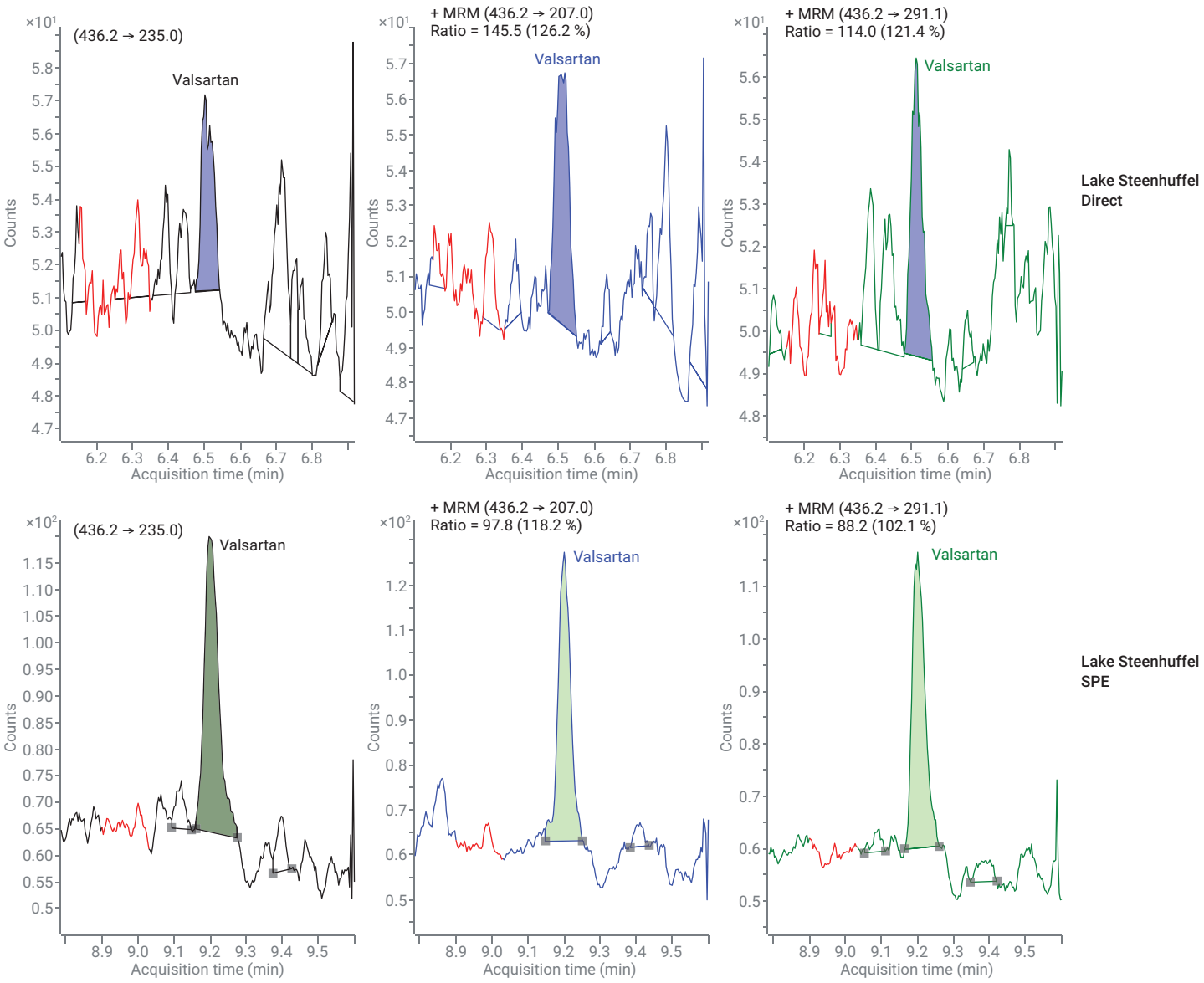

Figure 5A. Quantifier and qualifier transitions for valsartan in a lake sample after direct injection and online SPE.

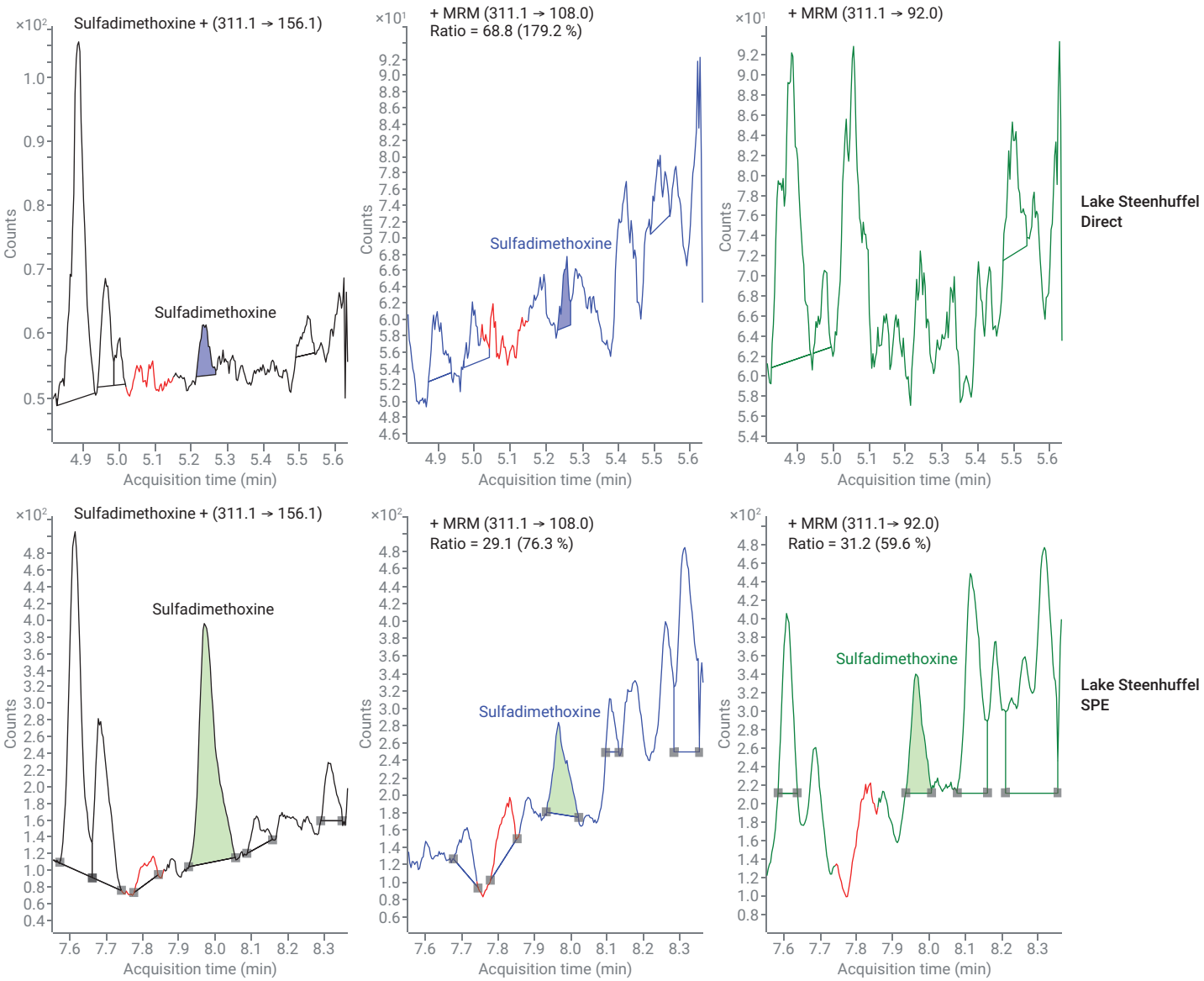

Figure 5B. Quantifier and qualifier transitions for sulfadimethoxine in a lake sample after direct injection and online SPE.

During the study, several control samples (standards diluted in tap water) were analyzed with direct injection. The excellent signal stability over a sequence of 15 environmental samples, which were analyzed with large volume injections (1.5 mL/sample), demonstrates the high cleanup efficiency of the online SPE approach. As an example, the TICs for three analyses carried out on two different days and before and after sample analysis are shown as an overlay in Figure 6. The first injection was carried out on day 1, and was followed by the analysis of 13 environmental samples with direct injection of 100 µL. The second direct injection of the control sample was performed on day 2 at the start of a sequence with online SPE. During this sequence, 26 solutions of standards in tap water and 13 environmental water samples were injected at 1.5 mL, and analyzed with online SPE. The system then automatically switched back to the direct injection mode, and the third direct injection of the control sample was executed.

Conclusion

A method was developed for the analysis of a selection of PPCPs in water samples. Analyses were carried out on a 1290 Infinity II LC coupled to a 6470A Triple Quadrupole LC/MS system. A comparison was made between direct injection (100 µL) and online SPE (1.5 mL). The switching between the two approaches was fully automated, with a significant reduction of delay volume for the direct injection analyses.

Switching to online SPE enabled the detection of trace amounts in environmental samples that could not be detected by the direct injection approach. Although recovery was not optimized for all compounds, a notable gain in sensitivity could be observed without significant loss in chromatographic performance, and no excessive increase in analysis and sample preparation time. The amounts of PPCPs detected in real water samples were very similar with both techniques.

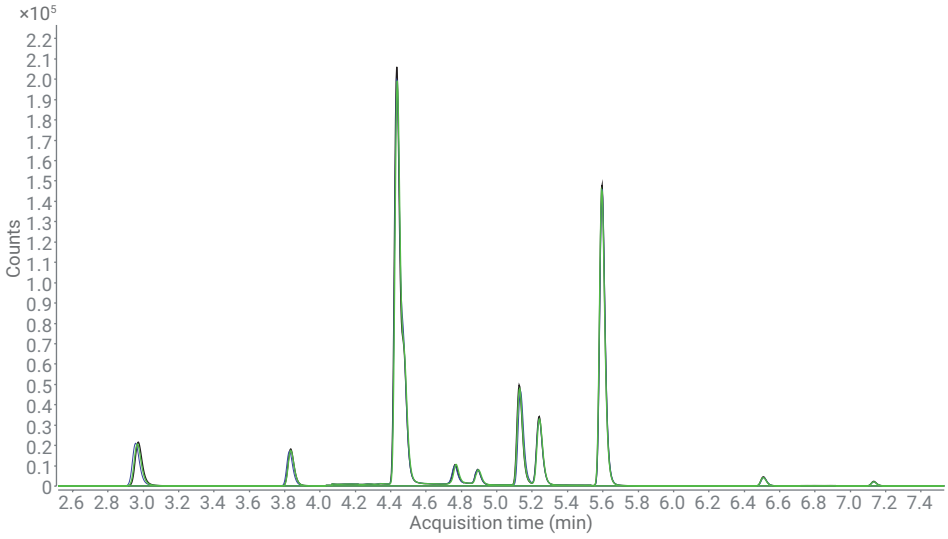

Figure 6. Overlay of direct injection analysis of a 200 ppt standard solution in tap water. The analyses were carried out on two different days and before and after injection of real samples with direct injection and online SPE.

References

1. Petrie, B.; Barden, R.; Kasprzyk-Hordern, B. A review on emerging contaminants in wastewaters and the environment: Current knowledge, understudied areas and recommendations for future monitoring. *Water Res.* **2015**, *72*, 3–27.
2. US EPA Method 1694 (**2007**) Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS. http://www.epa.gov/sites/production/files/2015-10/documents/method_1694_2007.pdf
3. Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy.
4. Timm, A.; *et al.* Analysis of Four β-Lactam Antibiotics in Water Using an Agilent 6470 TQ LC/MS with Direct Injection and Online SPE. *Agilent Technologies Application Note*, publication number 5991-8017EN, **2017**.
5. Yang, S.; Cha, J.; Carlson, K. Quantitative determination of trace concentrations of tetracycline and sulfonamide antibiotics in surface water using solid-phase extraction and liquid chromatography/ion trap tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2004**, *18(18)*, 2131–2145.
6. Castiglioni, S.; *et al.* A multiresidue analytical method using solid-phase extraction and high-pressure liquid chromatography tandem mass spectrometry to measure pharmaceuticals of different therapeutic classes in urban wastewaters. *J. Chromatogr. A* **2005**, *1092(2)*, 206–215.
7. Maldaner, L.; Jardim, I. C. S. F. Determination of some organic contaminants in water samples by solid-phase extraction and liquid chromatography–tandem mass spectrometry. *Talanta* **2012**, *100*, 38–44.

www.agilent.com/chem

Printed in the USA, November 1, 2018
5994-0382EN

Agilent
Trusted Answers