Online SPE Analysis of Pharmaceutical Compounds in Environmental Waters

The Agilent 1200 Infinity Series Online SPE Solution

Application Note

Environmental

Authors

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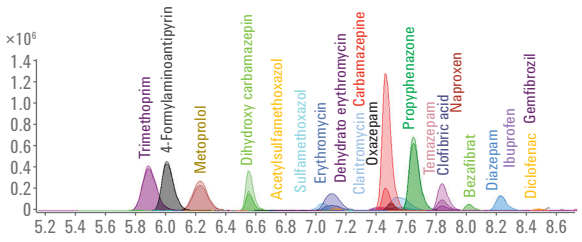
Abstract

Pharmaceuticals have been detected as contaminants in environmental water all over the world. There are many reasons for their occurrence in water, such as incomplete removal during water treatment or urban runoff.

In this Application Note, different environmental waters, including drinking water, surface water, and effluents from a wastewater treatment plant, were spiked with a suite of different pharmaceuticals and measured with the Agilent 1200 Infinity Series Online SPE Solution, coupled to an Agilent 6400 Series Triple Quadrupole LC/MS.

Performance data such as linearity, area and retention time precision, carryover, and accuracy, revealed a successful and robust analysis for a wide range of pharmaceutical compounds in environmental water samples.



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Introduction

Due to their increasing consumption worldwide, pharmaceuticals are ubiquitous in the aquatic environment, including wastewater, surface water, ground water, and drinking water. Some of these pharmaceuticals represent a potential risk and problem for humans and the environment, as even low concentrations can lead to unwanted biological effects. Of special concern is contaminated water used as a source for drinking water production[1]. One example of the indirect recycling of wastewater to drinking water, resulting in chronic exposure to pharmaceuticals, was reported in Berlin, Germany. In a study in 1996, clofibric acid was found in concentrations up to several ng/L in tap and ground water.[2]

To quantify and monitor low levels (ng/L) of pharmaceuticals in different environmental water samples, powerful, highly sensitive instrumentation and methods are necessary.

In this Application Note, an automated online solid-phase extraction (SPE) LC/MS/MS method was developed for the determination of 20 pharmaceutical compounds in different environmental water samples. While some of the samples were already contaminated with some of the targeted pharmaceuticals, all samples were additionally spiked with relevant concentrations of analytes and measured with respect to apparent recovery, linearity, carryover, area, and retention time (RT) precision.

Generally, online SPE is an environmentally friendly alternative to conventional offline SPE because solvents and hazardous waste are reduced. In addition, online SPE methods are reasonably priced compared to offline SPE[3]. The possibility of working with just a few mL of sample, and the simultaneous enrichment and desalting of samples, leads to an accurate quantification of analytes, and demonstrates the strength of the online SPE system. Additionally, online SPE methods save time and labor because little or no sample preparation is needed.

System configuration

The Agilent 1290 Infinity Flexible Cube is the heart of the 1200 Infinity Series Online SPE Solution. In this configuration, it hosts one 2-position/10-port valve with two trapping columns, as well as a piston pump and solvent selection valve that can draw up to three solvents. The two cartridges with Agilent PLRP-S material alternate in use, each cartridge delivering more than 300 enrichment and elution cycles[4].

PLRP-S (a cross-linked styrene divinylbenzene polymer) is a highly homogeneous material that is free from silanol groups and heavy metal ions and is perfectly suited for the enrichment of medium and nonpolar pharmaceuticals.

Experimental

Instrumentation

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

- Agilent 1290 Infinity Flexible Cube (G4227A) with an Online SPE Starter Kit (G4742A), which includes one 2-position/10-port valve, 600 bar, capillaries, cartridge holder, and cartridges
- Agilent 1260 Infinity Binary Pump (G1312B) and LAN card (G1369C)
- Agilent 1260 Infinity Standard Autosampler (G1329B) with 900-µL head (G1313-60007) and Agilent 1290 Infinity Thermostat (G1330B)
- Agilent 1260 Infinity Thermostatted Column Compartment (G1316A)
- Agilent 6400 Series Triple Quadrupole LC/MS (G6460A) with Agilent Jet Stream Technology

Software

- Agilent MassHunter Data Acquisition for triple quadruple 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

Chromatographic conditions

LC conditions	
Column	Agilent ZORBAX SB-Aq, 2.1 × 100, 3.5 µm (p/n 861753-914)
SPE cartridge	PLRP-S, 2.1 × 12.5 mm, 15-20 µm (p/n 5982-1271)
Column temperature	35 °C
Flow rate	0.4 mL/min
Mobile phase	A) 0.1 % acetic acid + 0.1 mM ammonium acetate B) 0.1 % acetic acid in ACN
Gradient	0 to 2 minutes 3 % B, 2 to 8 minutes 2 to 90 % B, 8 to 15 minutes 100 % B, 15 to 16 minutes 100 % B
Post time	14 minutes
Injection volume	900 µL
Sample temperature	5 °C

Agilent Flexible Cube timetable		
Time (min)	Function	Parameter
0	Pump volume	Pump 2.5 mL, 1.5 mL/min water, 0.1 % acetic acid
2	Left valve position change	Increase valve position
2.1	Pump volume	Pump 5 mL, 1.5 mL/min ACN
6	Pump volume	Pump 8 mL, 1.5 mL/min water, 0.1 % acetic acid

MS conditions, Agilent Jet Stream ESI, A	
Agilent Jet Stream ESI	
Gas temperature	300 °C
Gas flow	10 L/min
Nebulizer	40 psi
Sheath gas temperature	400 °C
Sheath gas flow	12 L/min
Capillary	Pos 3,000 V, neg 4,000 V
Nozzle voltage	Pos 0 V, neg 1,800 V
Delta EMV	Pos 400 V, neg 600 V

Samples were taken from tap water, rivers, and streams in the Cologne area. A water-control laboratory provided a mixed standard solution. The samples were stored at 5 °C and centrifuged for 5 minutes at 5,000 rpm prior to injection.

Besides contamination already included in the samples, the three water samples were spiked with different concentrations of pharmaceuticals. Samples with high wastewater content were diluted with drinking water (1:5).

The three samples were:

- Spiked drinking water (residual water disinfectant was quenched with 100 mg/L sodium thiosulfate)
- Original (2a) and spiked (2b) surface water
- Original (3a) and spiked (3b) wastewater

An external calibration was prepared in drinking water (Waldbronn, Germany) ranging from 0 to 1.0 µg/L. Calibration points were 0, 0.001, 0.005, 0.10, 0.025, 0.05, 0.1, 0.5, and 1.0 µg/L.

Table 1 lists the 20 targeted pharmaceuticals with their RT, ion polarity, precursor and product ions, fragmentor voltage, collision energy (CE), and cell accelerator voltage (CAV).

Chemicals

All solvents 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). Acetic acid and ammonium acetate were purchased from Sigma-Aldrich, Corp. (St Louis, MO, USA).

Table 1. Compound list and dMRM method.

Compound name	RT (min)	Precursor ion $(M+H)^+$	Product ion 1	CE (V)	CAV (V)	Product ion 2	CE (V)	CAV (V)	Fragmentor (V)	Polarity
4-Formylaminoantipyrine	6	232.1	214.1	8	2	56	28	4	90	Positive
Acetylsulfamethoxazol	7.12	296.1	198	12	2	134	20	4	90	Positive
Bezafibrate	8.04	362.1	316.2	8	3	139	24	5	50	Positive
Carbamazepine	7.47	237.1	194.2	12	4	179.1	36	2	90	Positive
Carbamazepine-10,11-dihydro-10,11-dihydroxy	6.55	271.1	253.4	4	3	180.1	32	3	70	Positive
Claritromycin	7.61	748.5	590.3	12	3	158.1	28	5	130	Positive
Clofibric acid	7.69	215	128.9	4	4	126.9	4	4	50	Negative
Dehydrato-erythromycin	7.48	716.5	558.5	8	3	158.1	28	4	95	Positive
Diazepam	8.23	285.1	193.1	32	5	154	28	4	50	Positive
Diclofenac	8.49	298	214	28	4	249.9	8	3	90	Positive
Erythromycin	7.14	734.5	576.3	12	3	158.1	24	5	130	Positive
Gemfibrozil	8.74	249.2	127	4	4	121	8	4	65	Negative
Ibuprofen	8.5	205.1	161	0	2				70	Negative
Metoprolol	6.23	268.2	74	20	4	56	28	4	110	Positive
Naproxen	8	229.1	170	8	2	168.9	40	2	50	Negative
Oxazepam	7.49	287.1	269.1	12	3	241.1	16	3	130	Positive
Propyphenazone	7.66	231.1	189.2	16	3	56.1	24	2	130	Positive
Sulfamethoxazole	7.02	254.1	156.1	0	2	92	24	5	90	Positive
Temazepam	7.83	301.1	283.1	8	3	255	20	3	70	Positive
Trimethoprim	5.89	291.2	261.1	20	3	230.1	16	5	130	Positive

Results and Discussion

Before measuring the samples, an optimization of the online SPE method was necessary. It was observed that the addition of 0.1 % acetic acid in water, which is used for the loading procedure, had a positive effect on the retention of ibuprofen and naproxen onto the SPE cartridge.[5]

Linearity, repeatability, and limits of detection

For most compounds the linearity ranges were between < 0.001 µg/L to 1 µg/L with excellent linearity (R^2) of 0.99 or greater. Figure 1 shows the calibration curve for erythromycin ranging from 0.001 to 1 µg/L in drinking water. Here, two replicates for each concentration and eight quality control standards at 0.1 µg/L are shown.

The repeatability of the method and online SPE cartridges is shown on eight quality control (QC) standards (shown as blue triangle in the calibration curve) measured within the work list. The QCs were spiked drinking water samples with a concentration of 0.1 µg/L. As shown in Figure 1, all QCs had excellent precision and accuracy over time and runs (> 120 injections, variability < 10 %). Recovery for all compounds was determined to be in the range of 90 to 105 % (n = 8). For the majority of compounds, limits of detection (LODs) with a signal-to-noise (S/N) ratio above 3:1 were determined to be ≤ 0.001 µg/L, except for oxazepam and naproxen, which showed LODs around ≤ 0.005 µg/L.

Recovery and precision

Several studies highlight the presence of pharmaceutical residues in environmental water[1,5]. As there is not yet any guideline for the evaluation of pharmaceutical residues in water samples, we decided to evaluate the performance of the method based on the criteria specified in SANCO/12571/2013[6]. This meant that acceptable mean recoveries were within the range of 70 to 120 %, with an associated precision of ≤ 20 % relative standard deviation (RSD).

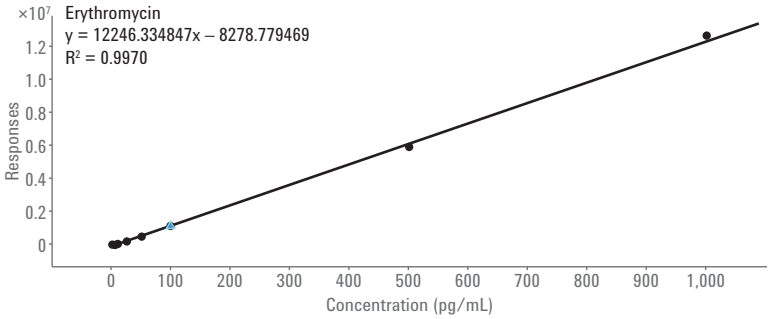

Figure 1. Calibration curve for erythromycin in drinking water (0.001 to 1 µg/L). Quality control standards are shown as a blue triangle at a concentration of 0.1 µg/L.

To evaluate precision data for area and RT, four replicates of each sample were analyzed. In Table 2, recoveries above 120 % are highlighted in red. Area RSDs above 20 % are also highlighted in red.

Table 2 shows the results for spiked drinking water (Sample 1). The expected concentration, the deviation of the calculated concentration (in the range 70 to 120 %) to the expected concentration, and the RSD for area and RT are shown.

Table 2. Results for spiked drinking water (Sample 1).

Compound name	Expected concentration (µg/L)	Calculated concentration (70–120 %) n = 4	Area RSD (%)	RT RSD (%)
Bezafibrate	0.085	108.15	2.52	0.08
Carbamazepine	0.085	179.47	5.11	0.09
Clofibric acid	0.085	104.41	3.05	0.11
Diazepam	0.085	115.65	1.65	0.06
Diclofenac	0.085	100.18	1.16	0.09
Ibuprofen	0.085	88.15	9.32	0.08
Metoprolol	0.085	121.30	0.87	0.16
Naproxen	0.085	112.21	2.02	0.08
Propyphenazone	0.085	147.56	1.38	0.05
Claritromycin	0.085	81.32	1.56	0.12
Dehydrato erythromycin	0.085	92.94	4.79	0.23
Erythromycin	0.085	87.50	9.47	0.11
Sulfamethoxazole	0.085	102.08	23.17	0.11
Trimethoprim	0.085	136.12	0.83	0.18
Oxazepam	0.085	103.65	3.30	0.09
Temazepam	0.085	116.41	1.29	0.06
Gemfibrozil	0.085	96.76	3.10	0.07
4-Formylaminoantipyrine	0.085	149.57	1.76	0.18
Carbamazepine-10,11-dihydro-10,11-dihydroxy	0.085	120.06	6.83	0.15
N4-Acetylsulfamethoxazol	0.085	96.18	18.35	0.11

The analysis of drinking water, spiked with 0.085 µg/L of each compound, showed good recovery for most compounds. Only carbamazepine, propyphenazone, trimethoprim, and 4-formylalminoantipyrine had recoveries > 120 %. This deviation can be explained by the different drinking water source of calibration standards and samples, and different matrix components such as salt content.

The area RSD over four runs was < 20 % for all compounds, except sulfamethoxazole with an RSD of 23 %. For the majority of compounds, an even better RSD of ≤ 5 % was obtained. RT RSDs for the two SPE cartridges used alternately were excellent, with an average value of 0.13 %. A higher RSD was found only for dehydrato-erythromycin, at < 0.25 %. Table 3 shows the results for original and spiked surface waters. In Table 3, recoveries below 70 % are highlighted in green, and recoveries above 120 % are highlighted in red.

For the surface water samples, background contamination was observed for several of the targeted compounds. Therefore, samples were measured before and after spiking with all pharmaceuticals. The reference values were based on an average concentration, which was estimated by the water-control laboratory that provided the samples. As spiked drinking water was used for the external calibration and was then compared to spiked environmental water samples, slight deviations from the spiked values could be expected.

Table 3. Results for original and spiked surface waters (Samples 2a and 2b).

	Original surface water (2a)				Spiked surface water (2b)			
Compound name	Expected concentration (µg/L)	Calculated concentration (70–120 %) n = 4	Area RSD (%)	RT RSD (%)	Expected concentration (µg/L)	Calculated concentration (70–120 %) n = 4	Area RSD (%)	RT RSD (%)
Bezafibrate	0.0320	83.67	4.71	0.06	0.157	106.83	3.45	0.06
Carbamazepine	0.0540	120.72	1.96	0.07	0.179	121.06	1.88	0.10
Clofibric acid					0.125	78.14	8.63	0.10
Diazepam					0.125	100.54	3.91	0.07
Diclofenac	0.1090	91.08	3.27	0.05	0.234	101.47	2.58	0.08
Ibuprofen					0.125	50.04	6.17	0.08
Metoprolol	0.1630	91.87	0.40	0.24	0.288	91.56	1.10	0.23
Naproxen					0.125	89.44	10.54	0.11
Propyphenazone					0.125	111.30	1.94	0.08
Claritromycin					0.125	133.72	3.68	0.15
Dehydrato erythromycin					0.125	115.98	5.48	0.13
Erythromycin					0.125	110.38	1.76	0.16
Sulfamethoxazole	0.0300	102.75	5.51	0.17	0.155	84.73	5.97	0.14
Trimethoprim					0.125	101.91	0.65	0.24
Oxazepam					0.125	155.88	5.66	0.10
Temazepam					0.125	114.42	2.36	0.08
Gemfibrozil					0.125	80.26	2.62	0.11
4-Formylaminoantipyrine	0.2510	100.63	0.85	0.21	0.376	92.09	1.29	0.27
Carbamazepine-10,11-dihydro-10,11-dihydroxy	0.1370	104.65	2.51	0.21	0.262	103.47	2.23	0.18
N4-Acetylsulfamethoxazol					0.125	122.70	6.07	0.15

The results for surface water show excellent recoveries for most compounds (Table 3). In the non-spiked surface water (2a), seven pharmaceuticals were found, and three compounds were determined in a relevant concentration of > 0.1 µg/L.

For the spiked surface water (2b), recoveries above 120 % were observed just for clarithromycin and oxazepam. Ibuprofen was found to be out of the expected concentration range, with 50 % recovery, which can be explained by matrix suppression caused by the more complex sample matrix of the surface water.

Area and RT RSDs were in the acceptable range for both surface samples (2a and 2b). The majority show area RSDs < 5 % and RT RSDs of < 0.2 %.

Table 4 shows that for the most complex sample with a high matrix content, good recoveries were observed for most compounds. Relevant concentration of analytes with > 0.1 µg/L and even > 1 µg/L were found in wastewater. However, as expected, some analytes were affected by ion suppression. In Table 4, recoveries below 70 % are highlighted in green.

For area and RT RSD, compounds in wastewater showed excellent precision with area RSD of typically ≤ 5 %. RT RSDs were determined with an average value of 0.1 %.

To reduce matrix effects, further improvements or changes in the chromatographic separation could be made. Additionally, the use of isotopically labeled internal standards or calibration with water with a similar characteristic is expected to further improve recoveries. This is shown here, when QC standards were prepared in the same drinking water as the calibration samples. The recovery, accuracy, and precision were excellent for all compounds.

Carryover

Carryover was not observed for the majority of compounds, except for bisoprolol, when a signal was observed in the blank samples after the analysis of the high-concentration samples. Carryover for bisoprolol was in the range of 0.02 to 0.28 % compared to the high-concentration sample.

Table 4. Results for original and spiked wastewater with high matrix content (Samples 3a and 3b).

	Original waste water (3a)				Spiked waste water (3b)			
Compound name	Expected concentration (µg/L)	Calculated concentration (70–120 %) n = 4	Area RSD (%)	RT RSD (%)	Expected concentration (µg/L)	Calculated concentration (70–120 %) n = 4	Area RSD (%)	RT RSD (%)
Bezafibrate	0.0420	76.79	15.41	0.07	0.9420	90.18	2.78	0.07
Carbamazepine	0.7030	120.00	1.65	0.07	1.6030	102.51	1.52	0.06
Clofibric acid					0.9000	90.33	1.05	0.08
Diazepam					0.9000	89.66	1.09	0.07
Diclofenac	1.3210	90.51	2.64	0.05	2.2210	87.71	1.99	0.11
Ibuprofen	0.0010	100.00			0.9000	73.54	4.00	0.11
Metoprolol	0.8440	75.52	0.65	0.09	1.7440	69.21	0.80	0.17
Naproxen	0.0570	101.23	6.37	0.19	0.9570	87.04	1.88	0.09
Propyphenazone					0.9000	99.64	1.81	0.04
Claritromycin	0.1530	64.41	0.58	0.18	1.0530	98.40	1.91	0.08
Dehydrato erythromycin	0.0840	60.30	9.45	0.33	0.9840	91.36	2.34	0.12
Erythromycin	0.0500	57.10	5.66	0.13	0.9500	76.70	1.56	0.10
Sulfamethoxazole	0.2040	86.36	7.40	0.09	1.1040	63.45	5.10	0.09
Trimethoprim	0.0460	63.62	3.12	0.12	0.9460	60.38	1.62	0.21
Oxazepam	0.0950	87.32	6.74	0.10	0.9950	102.33	1.62	0.06
Temazepam					0.9000	88.44	1.55	0.05
Gemfibrozil					0.9000	91.13	1.45	0.10
4-Formylaminoantipyrine	0.5450	76.02	1.46	0.10	1.4450	70.07	1.27	0.18
Carbamazepine-10,11-dihydro-10,11-dihydroxy	1.1900	45.96	3.24	0.12	2.0900	40.92	2.37	0.11
N4-Acetylsulfamethoxazol					0.9000	76.77	4.26	0.04

Conclusions

A suite of 20 pharmaceuticals was measured in different environmental water samples using HPLC/MS/MS with online SPE. These samples were analyzed before and after spiking with different concentrations of pharmaceutical standards.

Good linearity and LODs < 0.001 µg/L were determined for the majority of the compounds. Precision and recovery data were predominately in the range of 70 to 120 % for different environmental water samples. In the presence of complex matrixes, some compounds showed interferences, resulting typically in lower recoveries. The use of isotopically labeled internal standards or standard addition is suggested.

A low sample volume of 900 µL, a short preparation time, and a fast analysis time of 16 minutes including the preconcentration step, elution, and detection, were the most significant advantages of the Agilent 1200 Infinity Series Online SPE Solution compared to offline solid-phase extraction. The reusable PLRP-S cartridges had a long lifetime and robustness (typically 200 to 300 enrichment cycles) even with samples of heavy matrix.

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