Sensitive LC/MS Quantitation of Trace Organic Contaminants in Water with Online SPE Enrichment

Application Note

Environmental

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Abstract

An online SPE method has been developed for the rapid, sensitive, reproducible, and robust analysis of trace organic compounds in water, eliminating large sample volumes and tedious extraction procedures. Method reporting limits (MRLs) ranged from 0.10 to 15 ppt; recoveries varied primarily from 70 to 130%; most relative standard deviations (RSDs) were below 10%. The results were comparable to those obtained using the conventional SPE method. The method also provided reliable results across the wastewater treatment process, including influent.

Introduction

Chemicals are being discovered in water that previously had not been detected, or are being detected at levels that may be significantly different than expected [1]. These are generally referred to as contaminants of emerging concern (CECs) because the risk to human health and the environment, associated with their presence, frequency of occurrence, or source may not be known. Diverse mixtures of trace organic compounds have been detected in water, some of which impact aquatic wildlife at ng/L parts per trillion (ppt) concentrations.

Some CECs are known endocrine disruptors, while others affect glucocorticoid activity, and the synergistic effects of long-term exposure to low doses of CECs is yet unknown. As a result, the EPA has instituted Unregulated Contaminant Monitoring Rules (UCMRs) to assess the potential threat of CECs in the water supply. The latest, UCMR3, requires monitoring for 30 contaminants from 2013 to 2015. States, laboratories, and public water systems will participate in assessment monitoring, a screening survey, and prescreen testing [2].

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One of the challenges to monitoring water sources for trace contaminants is the size of the sample and the sample preparation required for adequate detection. Conventional analytical approaches often require 1L of water, solid-phase extraction and extract concentration before instrumental analysis can be applied. These methods require transportation of large sample volumes, are labor intensive, and
consume high volumes of organic solvents.

This application note describes an online SPE Trace enrichment method that requires minimal sample preparation and significantly reduces the volume of sample required. This results in robust and sensitive detection of ng/L levels of trace organic contaminants. This UHPLC/MS/MS method uses a reusable polymeric solid phase extraction cartridge that is attached online to an Agilent 1290 Infinity LC System, which in turn is coupled to an Agilent 6460 LC/MS Triple Quadrupole system. It uses simultaneous positive and negative electrospray ionization (ESI) to provide significant time savings. Multiple reaction monitoring (MRM) enables MRLs for 24 organic contaminants as low as 0.10 ppt and no higher than 15 ppt. Most recoveries range from 70 to 130%, are comparable to conventional extraction, and have relative standard deviations lower than 10% for most compounds. The method is robust, as it has been used to monitor the levels of these contaminants across the wastewater treatment process, from
influent to chlorinated effluent

Experimental

Standards and Reagents

All but two of the calibration standards were obtained from Sigma Aldrich at the highest available purity. Meprobamate was procured from Cerilliant and triclosan from Alfa Aesar. Calibration standard solutions were prepared by first making 500 µg/mL stock solutions of each standard from the neat solid in methanol. This was followed by making a mix of all the target analytes at a concentration of 5 µg/mL. This stock solution was diluted with HPLC water to obtain the desired concentration for the calibration curve.

Labeled surrogate internal standards were purchased from Cambridge Isotope Laboratories, except for: $^{13}C_4$-PFOA and $^{13}C_4$-PFOS (Wellington Laboratories); primidone-d_5 (Toronto Research Chemicals); and gemfibrozil-d_6 (C/D/N Isotopes) (Table 1). All solvents used were of highest purity available, suitable for LC/MS analysis. Pesticide-grade methanol and acetonitrile were purchased from Burdick & Jackson, while HPLC water was purchased from Fisher.

Table 1. Labeled Surrogate Internal Standards

Compound	Compound
Atrazine-d_5	Naproxen-$^{13}C_1d_3$
Bisphenol A-$^{13}C_{12}$	PFOA-$^{13}C_4$
Caffeine-$^{13}C_3$	PFBA-$^{13}C_4$
Carbamazepine-d_{10}	PFOS-$^{13}C_4$
DEET-d_6	Primidone-d_5
Fluoxetine-d_5	Sulfamethoxazole-d_6
Gemfibrozil-d_6	Triclocarban-$^{13}C_6$
Ibuprofen-d^3	Triclosan-$^{13}C_{12}$
Meprobamate-d_7	Trimethoprim-d_3

Instruments

The system was built using Agilent 1200 Infinity LC System modules coupled to an Agilent 6460A Triple Quadrupole LC/MS. The online enrichment system used the Agilent 1200 Series Quaternary Pump, Agilent 1200 Autosampler with 900 µL metering device and multidraw capability, a programmable six position selection valve (valve 1), a 12 port/2 position cartridge selection valve (valve 2), and an Agilent 1290 Infinity LC as shown in Figure 1.

Figure 2 shows the loading and elution valve positions. Tables 2 and 3 show the system operating conditions.

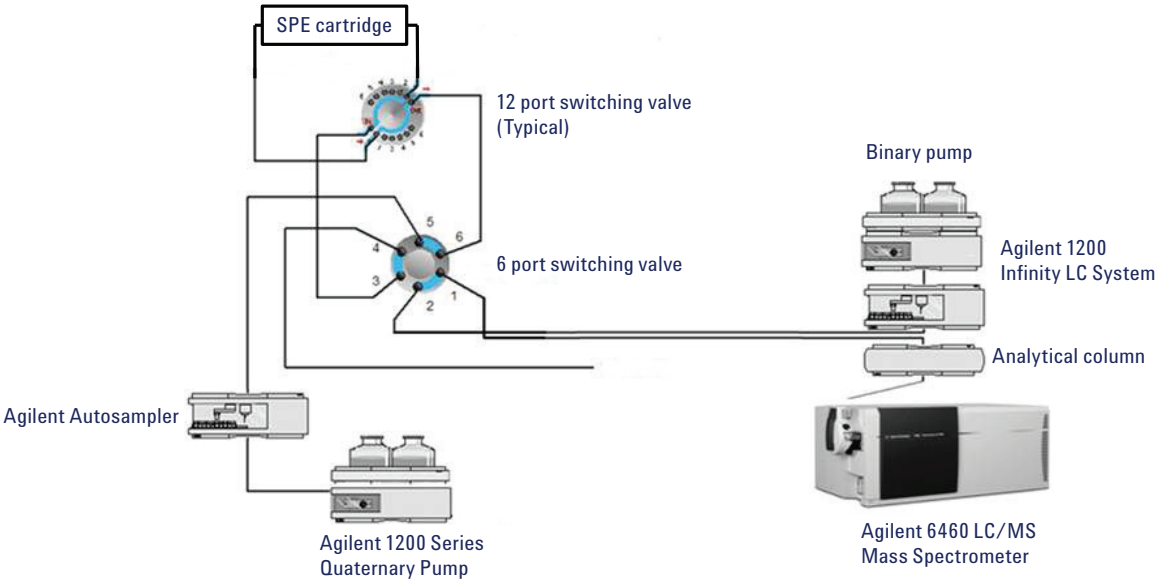


Figure 1. Online SPE LC/MS system configuration.

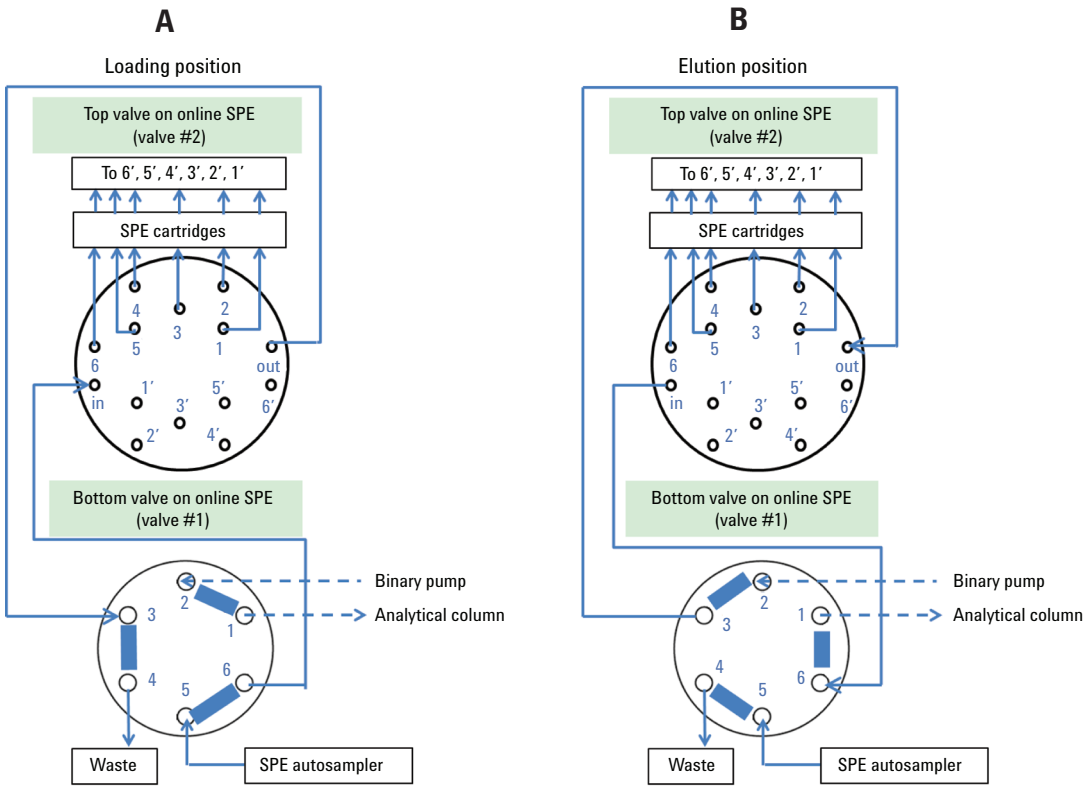


Figure 2. Loading (A) and Elution (B) valve positions (Positions 1 and 2) of the online SPE system.

Table 2. Online SPE Conditions

Injection	
SPE cartridge	PLRP-S, 15 µm
Temperature	30 °C
Injection volume	2 × 750 µL, 1.5 mL total
Injection draw speed	500 µL/min
Eject speed	200 µL/min
Draw position	0.5 mm

Quaternary pump				
Flow rate	0.7 mL/min			
Mobile phase	A = Water + 0.1% (v/v) acetic acid B = Acetonitrile/isopropanol/methanol (1:1:1) C = Acetonitrile + 0.1% (v/v) acetic acid			
Gradient for elution from SPE column	**Time (min)**	**A**	**B**	**C**
	0	95%	0%	5%
	5.5	0%	100%	0%
	11.1	95%	0%	5%
	Post time: 2.9 minutes			
Valve positions	**Time (min)**	**Position**		
	1	1 (Elution)		
	5.5	2 (Loading)		
	13.5	1 (Elution)		
Injector program	Command			
	DRAW default volume from sample			
	EJECT default volume into seat with maximum speed using default offset			
	DRAW default volume from sample with default speed using default offset			
	VALVE Switch valve to "Mainpass"			
	WAIT 3.5 minutes			
	REMOTE: Set remote line "Start"			

Table 3. HPLC and Simultaneous ESI- and ESI+ MS Instrument Conditions

HPLC conditions		
Analytical column	Agilent Poroshell 120 EC 50 mm x 2.1 mm dia., (p/n 699775-902)	
Column temperature	30 °C	
Mobile phase	A = Water + 0.1% (v/v) acetic acid B = Acetonitrile 0.1% (v/v) acetic acid	
Run time	14 minutes + 1.5 minutes post time	
Flow rate	0.7 mL/min	
Gradient for elution from SPE column	**Time (min)**	**Mobile phase**
	0	5% B
	2	5% B
	3.5	25% B
	9	100% B
	11.5	5% B
	Post time: 1.5 minutes	

MS conditions	
Acquisition parameters	ESI mode, simultaneous positive and negative ionization; Dynamic MRM
Sheath gas temperature	375 °C
Sheath gas flow rate	12 L/min
Drying gas temperature	250 °C
Drying gas	11 L/min
Nebulizer pressure	45 psig
Nozzle voltage	0 V positive; 1,500 V negative
Vcap	4,000 V positive; 3,500 V negative
Δ EMV	400 V

Sample Preparation

Wastewater samples were taken from a treatment plant, across several stages of the treatment process. Samples were fortified with a surrogate standard stock to obtain a final concentration of 100 ppt within two days of collection. Samples were subsequently filtered through 0.2-µm syringe filters from GE Whatman. Two sets of samples were prepared, a 1.5-mL sample and a sample diluted 5x with HPLC water (300 µL sample + 1,200 µL water) so as to obtain concentrations of all analytes within the linear range. Conventional SPE using 1 L water samples for comparison was performed as described previously [2].

Analysis Parameters

Table 4 shows the MRM transitions for the 24 analytes and their surrogate internal standards.

Table 4. Multiple Reaction Monitoring (MRM) ESI Analysis Parameters

Compound	Retention time	Precursor ion	Product ion	Fragmentor voltage	Collision energy	ESI mode
Atenolol	3.1	267.1	190.1	130	15	Positive
			145	130	20	Positive
Atrazine	6.4	218	176	140	15	Positive
		216	174	140	15	Positive
Atrazine-d_5	6.4	221	179	140	15	Positive
Bisphenol A	6.54	227	212	115	11	Negative
			133	115	19	Negative
Bisphenol A-$^{13}C_{12}$	6.54	239	224	115	11	Negative
Caffeine	4.3	195.1	138	104	16	Positive
			110.1	104	24	Positive
Caffeine-$^{13}C_3$	4.3	198.1	140	104		Positive
Carbamazepine	6.06	237	194	120	15	Positive
			179	120	35	Positive
Carbamazepine-d_{10}	6.54	247	204	120	15	Positive
DEET	6.44	192	119	110	15	Positive
			91	110	30	Positive
DEET-d_6	6.44	198	119110	15		Positive
Estrone	6.91	269.2	183.1	120	30	Negative
			145.1	120	37	Negative
Fluoxetine	6.28	310	148	90	5	Positive
Fluoxetine-d_5	6.28	315	153	90	5	Positive
Gemfibrozil	7.93	249.2	121	75	6	Negative
Gemfibrozil-d_6	7.93	255	121	75	6	Negative
Ibuprofen	7.52	205	161	50	0	Negative
Ibuprofen-d_3	7.52	208	164	50	0	Negative
Meprobamate	5.4	219	158	70	5	Positive
			55	70	20	Positive
Meprobamate-d_7	5.4	226.1	165.1	70	5	Positive
Naproxen	6.72	229	170	55	4	Negative
			169	55	24	Negative

Table 4. Multiple Reaction Monitoring (MRM) ESI Analysis Parameters (continued)

Compound	Retention time	Precursor ion	Product ion	Fragmentor voltage	Collision energy	ESI mode
Naproxen-$^{13}C_1d_3$	6.72	233	169	55	24	Negative
PFBS	6.11	298.8	98.9	133	29	Negative
			80	133	45	Negative
PFOA	6.72	412.9	368.9	86	5	Negative
			169	86	5	Negative
PFOA-$^{13}C_4$	6.72	416.9	371.9	86	5	Negative
PFOS	7.79	498.9	99	210	50	Negative
			80	210	50	Negative
PFOS-$^{13}C_4$	7.79	502.9	99	210	50	Negative
Primidone	4.85	219.3	162.1	70	9	Positive
			91.1	70	25	Positive
Primidone-d_5	4.82	224	167	70	9	Positive
Simazine	5.85	202.1	132	72	16	Positive
			68.1	72	36	Positive
Sulfamethoxazole	5.35	254	156	80	10	Positive
			92	80	30	Positive
Sulfamethoxazole-d_6	5.35	260	162	80	10	Positive
TCEP	7.1	285	222.8	95	10	Positive
TCPP	7.13	327	99	72	16	Positive
			81	72	70	Positive
Testosterone	6.5	289.2	97	100	25	Positive
Triclocarban	8.22	313	160	110	5	Negative
			126	110	25	Negative
Triclocarban-$^{13}C_6$	8.22	318.9	159.9	110	5	Negative
Triclosan	8.33	289	37	75	5	Negative
			35	75	5	Negative
Triclosan-$^{13}C_{12}$	8.33	299	35.1	75	8	Negative
Trimethoprim	4.48	291	261	75	25	Positive
			230	75	25	Positive
Trimethoprim-d_3	4.48	294	264	75	25	Positive

Results and Discussion

Online SPE Sequence of Operation

Aqueous samples (particulate free) are contained in 2-mL amber glass vials in the autosampler. Samples are injected (1.5 mL volume) onto the reuseable polymer based enrichment cartridges attached to the output 12 port/2 position column selection valve (Upper valve, valve #2, Figure 2A). A total of six different cartridges can be attached. Aqueous effluent is sent to waste (Figure 2A). Typically, after approximately 3 minutes, sample loading is complete. The bottom valve (#1) is then switched to the elute position (Figure 2B), and the gradient is started on the high pressure gradient pump.

Trapped analytes are then desorbed onto the Agilent Poroshell 120 EC analytical column before LC/MS/MS analysis. This also washes the cartridge in solvent B (acetonitrile/isopropanol/methanol (1:1:1, Figure 2B). During the analytical run, with valve #1 returned to the loading position (Figure 2A), it is also possible to further wash the enrichment cartridge if required, using Solvent B on the quaternary loading pump and then returning the solvent conditions to 100% solvent A (water + 0.1% acetic acid) in readiness for the next sample.

For wastewater samples, each reusable SPE cartridge can be used typically for at least 100 injections. The 12 port valve (#2, Figure 2) can then index to the next cartridge for the next series of injections.

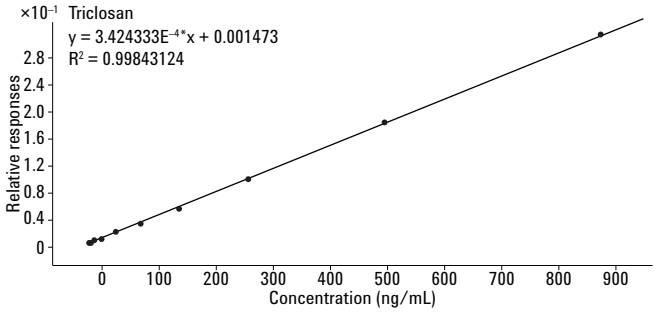
Method Performance

An online solid phase extraction (SPE) LC/MS/MS method has been developed for the rapid, sensitive and robust detection of 24 organic contaminants (Table 5) at ppt concentrations, using multiple transitions and labeled surrogate internal standards for many of the compounds. With a 19 minute cycle time, it provided highly accurate calibration curves over a concentration range of 3 to as much as 880 ng/L (ppt), with R^2 values >0.995 for linear fit (Figure 3).

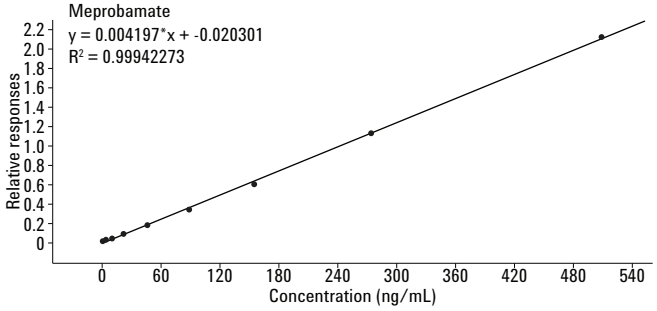
Table 5. Target Analytes

Compound	Class
Atenolol	Beta blocker drug
Atrazine	Herbicide
Bisphenol A	Plasticizer
Caffeine	Stimulant
Carbamazepine	Antiseizure drug
DEET	Insect repellant
Diltiazem	Anti-histamine drug
Estrone	Hormone
Fluoxetine	Antidepressant drug
Gemfibrozil	Anticholesterol drug
Ibuprofen	Analgesic
Meprobamate	Antianxiety drug
Naproxen	Analgesic
PFBS	Fluoro-surfactant
PFOA	Fluoro-surfactant
PFOS	Fluoro-surfactant
Primidone	Anticonvulsant
Simazine	Herbicide
Sulfamethoxazole	Antibiotic
TCPP	Flame retardant
Testosterone	Androgen
Triclocarban	Antibiotic
Triclosan	Antibiotic
Trimethoprim	Antibiotic

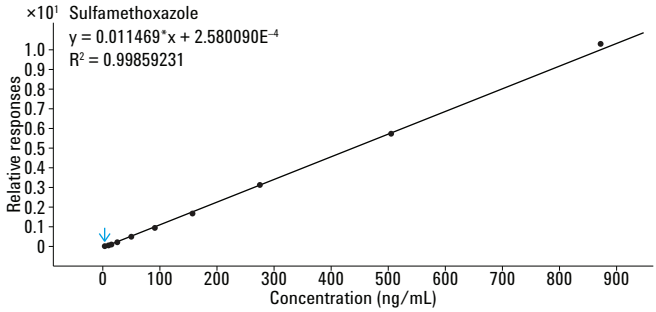
Triclosan



Meprobamate



Sulfamethoxazole



DEET 3-880 ppt

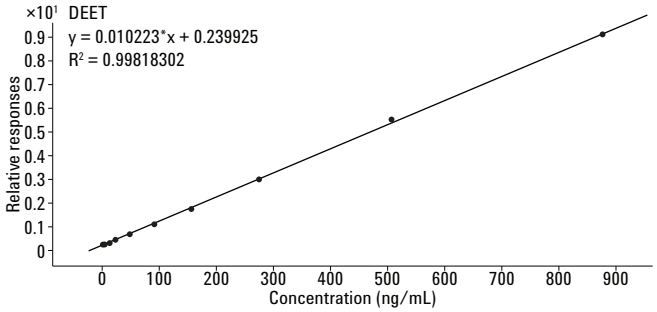


Figure 3. Typical calibration curves for four of the analytes: triclosan and sulfamethoxazole (5-880 ppt), meprobamate (3-510 ppt), and DEET (3-880 ppt).

The MRL for each analyte was determined using three aliquots of HPLC water spiked at various different concentrations, and was defined as the lowest concentration for which a signal-to-noise ratio (S/N) greater than 10 was obtained for three successive injections at the same concentration. Table 6 shows the MRLs for all 24 analytes.

An ultrapure water sample was spiked with the standards mix at 20 and 100 ppt to determine recoveries, which were within 70–130% for >90% of the compounds, both in the low and high spike sample. The relative standard deviations (RSDs) for five replicates were less than 10% for about 80% of the compounds (Table 7).

Table 6. MRLS (ppt) for the Target Analytes

Analyte	MRL	Analyte	MRL
Atenolol	15	**Meprobamate**	0.5
Atrazine	5	**Naproxen**	10
Bisphenol A	10	PFBS	10
Caffeine	0.5	**PFOA**	10
Carbamazepine	2.5	**PFOS**	10
DEET	0.1	**Primidone**	15
Estrone	20	Simazine	1.5
Fluoxetine	10	**Sulfamethoxazole**	2.5
Gemfibrozil	1.5	**TCPP**	0.5
Ibuprofen	10	**Triclocarban**	1
Trimethoprim	5	**Triclosan**	5

The bold font indicates those analytes for which a surrogate standard was used.

*Table 7. Recoveries at Two Analyte Concentrations in Ultrapure Water**

	20 ppt		100 ppt	
Compound	**Recovery**	**RSD (%)**	**Recovery**	**RSD (%)**
Atenolol	89	0.8	85	6.2
Caffeine	92	2.8	96	1.4
Trimethoprim	88	5.5	108	2.9
Primidone	70	11.9	86	2.3
Sulfamethoxazole	107	2.3	100	0.9
Meprobamate	115	1.5	103	0.7
Simazine	106	2.0	111	0.6
Ditiazem	115	1.2	74	1.6
Carbamezapine	89	1.5	99	2.5
PFBS	94	3.5	98	2.2
Fluoxetine	85	5.5	93	4.8
Atrazine	86	2.3	99	1.9
DEET	138	18.4	113	1.9
Bisphenol A	93	21.5	119	11.9
Testosterone	115	9.3	94	2.2
Naproxen	96	6.5	93	3.6
PFOA	86	23.7	91	14.1
Estrone	99	13.3	152	19.9
TCPP	NA	NA	80	15.8
Ibuprofen	100	7.4	98	3.7
PFOS	125	4.2	117	3.6
Gemfibrozil	99	6.7	111	2.3
Triclocarban	109	1.2	102	3.2
Triclosan	91	3.8	100	2.4

*5 replicates
NA = Not analyzed

Table 8. Comparison of Recoveries for the Online and Conventional SPE Methods

	Online SPE		Conventional SPE	
Compound	**Recovery (%)**	**RSD (%)**	**Recovery (%)**	**RSD (%)**
Atrazine	108	0.8	94	1.9
Bisphenol A	87	8.1	97	11.6
Caffeine	105	5	95	4.3
Carbamezapine	114	6.4	117	1.3
DEET	106	0.8	96	1.6
Diltiazem	NA	NA	NA	NA
Estrone	NA	NA	NA	NA
Fluoxetine	129	5.2	97	2.3
Gemfibrozil	122	4.8	93	2.7
Ibuprofen	105	3.2	92	5.7
Meprobamate	97	0.6	74	1.5
Naproxen	111	2.9	89	1.4
PFBS	105	2.5	111	8
PFOA	135	3.8	121	6.4
PFOS	127	6.2	94	9
Primidone	110	17.1	96	1.5
Simazine	81	0.9	73	2
Sulfamethoxazole	99	1.4	98	1.7
TCPP	87	1.6	119	2.9
Testosterone	NA	NA	42	2.4
Triclocarban	111	2	97	1.5
Triclosan	82	10.3	112	2.8
Trimethoprim	116	1.4	102	0.9

NA = Not analyzed

Comparison with Conventional SPE

Surface water samples from the Colorado River were spiked with 200 ppt of the target analyte standards mix and analyzed using both the online and conventional SPE methods. Recoveries for both extraction methods are comparable (Table 8). Recoveries for online SPE for all analytes tested except PFOA were within 80–130%. RSDs were less than 10% for all analytes using either method, except for primidone and triclosan.

Wastewater Analysis

Online SPE allows rapid analysis of a variety of trace organic contaminants in complex water matrixes. Wastewater samples were analyzed in triplicate using the online SPE method, across the treatment process from influent to chlorinated effluent (Table 9). Samples were diluted and rerun if the initial concentration of the compound was above the range of the calibration curve. All concentration values were then corrected using the appropriate surrogate internal standard. Table 9 shows that several of the organic compounds had initial concentrations in wastewater higher than 1,000 ng/L, with a few being higher than 8,000 ng/L, and some of these persisted at concentrations above 500 ng/L after water treatment. However, some of the compounds were not detectable above their MRLs.

Table 9. Measurement of Organic Contaminants (ppt) in Wastewater Across Several Stages of Treatment

Compound	Influent	Secondary effluent	After sand filtration	Chlorinated effluent
Atenolol	721	43	22	30
Caffeine	>8000	41	37	26
Trimethoprim	2030	61	47	8
Primidone	1676	514	443	670
Sulfamethoxazole	6552	3941	4223	62
Meprobamate	693	618	636	612
Simazine	<MRL	<MRL	<MRL	<MRL
Ditiazem	158	78	85	71
Carbamezapine	375	307	310	275
PFBS	36	67	97	84
Fluoxetine	71	22	23	27
Atrazine	<MRL	<MRL	<MRL	<MRL
DEET	1383	150	93	156
Bisphenol A	310	20	30	<MRL
Testosterone	45	<MRL	<MRL	<MRL
Naproxen	>8000	180	117	<MRL
PFOA	<MRL	<MRL	<MRL	<MRL
Estrone	<MRL	<MRL	<MRL	<MRL
TCPP	1791	1338	1213	1299
Ibuprofen	>8000	88	55	55
PFOS	148	<MRL	<MRL	<MRL
Gemfibrozil	>8000	119	85	66
Triclocarban	765	25	32	11
Triclosan	2227	73	124	97

Conclusions

The use of online SPE enables sensitive and automated analysis of trace organics in water while providing significant time and labor savings. In addition, the method is robust enough to provide reliable results across the wastewater treatment process, including influent. MLRs were very low, ranging from 0.10 to 15 ppt. Recoveries ranged primarily from 70 to 130%, most RSDs were below 10%, and the results were comparable to those obtained using the conventional SPE method. Automated online SPE/HPLC/MS/MS is, therefore, an ideal choice for sensitive, fast, reproducible, and robust analysis of trace organic contaminants in a variety of water sources.

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2. T. Anumol, S. Merel, S. Snyder. "High sensitivity HPLC analysis of contaminants of emerging concern (CECs) in water using Agilent 6460 triple quadrupole LC/MS system" Agilent Technologies Application Note 5991-1124EN.

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