Automated Online SPE for LC/MS/MS Analysis of Trace Organic Contaminants in Water Using the Agilent 1290 Infinity Flexible Cube Module

Application Note

Environmental

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Abstract

The Agilent 1290 Infinity Flexible Cube module has enabled a fully automated online SPE extraction LC/MS/MS method for ng/L level detection and quantitation of 34 trace organic compounds (pharmaceuticals, personal-care products, pesticides, perfluorinated compounds and so forth) in a wide variety of water sources. It requires only 1.7 mL of sample and provides a cycle time less than 15 minutes, enabled in part by the simultaneous positive and negative ionization feature of the Agilent 6460 Triple Quadrupole LC/MS. The automated online sample preparation embodied in this method provides unparalleled throughput and reproducibility, as well as time, labor, and solvent savings.

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Introduction

Modern society has become highly dependent on the use of organic chemicals for everything from personal care products (makeup, toothpaste), pharmaceuticals (ibuprofen), hormones, pesticides (bug and weed killers), and a plethora of industrial materials. Unfortunately, these chemicals, collectively referred to as trace organic compounds (TOrCs), often end up in water resources. While they are not expected to pose significant adverse health effects, the synergistic effects of long-term exposure to mixtures of these compounds are unknown [1].

Regulatory actions to establish acceptable levels of TOrCs, which typically take significant time to be formulated, are not feasible at this time due to the very large number of compounds. However, while studies on the toxicity of these compounds and the effects of exposure to mixtures of them are ongoing, it makes sense to monitor their presence in water sources.

Liquid chromatography-tandem mass spectrometry (LC/MS/MS) methods are the most widely used for detecting TOrCs at very low levels in water. However, these approaches have been hampered by the need to extract and concentrate the target compounds, which can be very time-consuming, labor-intensive, and consume large amounts of organic solvents. Because they require several human intervention steps, they can also reduce the accuracy and reproducibility of the analysis. Automation of the sample preparation step is needed to decrease time to result, increase throughput, and enhance the accuracy and reproducibility of TOrC analyses.

This application note summarizes a published study of the feasibility of automating TOrC analysis in water, and its applicability to the monitoring of water resources. The key to enabling automation was the use of the Agilent 1290 Infinity Flexible Cube LC module that contained two online SPE cartridges. This configuration facilitated the simultaneous backwashing of one cartridge after elution while the second cartridge was being loaded with the next sample.

Most of the current methods for analyzing TOrCs have been developed for specific classes, such as pesticides or hormones. The goal of this study was to develop a single automated method for the rapid analysis of 34 indicator compounds that represented several classes of TOrCs in a variety of water matrices. Using a sample volume of less than 2 mL, this automated method attained low ng/L detection limits with a cycle time less than 15 minutes, without manual sample preparation or the use of large volumes of organic solvents.

Experimental

Reagents and standards

All reagents and standards were obtained as described [1]. Isotopically labeled surrogate standards were used to provide realistic Method Reporting Limits (MRLs) for each water matrix type, as previously described [2]. Table 1 shows the 34 TOrCs analyzed in this study and their classes.

Table 1. TOrCs Targeted by the Automated Online SPE Method

Compound	Class
Atenolol	Pharmaceutical
Carbamazepine	Pharmaceutical
Clofibric Acid	Pharmaceutical
Diclofenac	Pharmaceutical
Diphenhydramine	Pharmaceutical
Diltiazem	Pharmaceutical
Fluoxetine	Pharmaceutical
Gemfibrozil	Pharmaceutical
Hydrochlorothiazide	Pharmaceutical
Ibuprofen	Pharmaceutical
Meprobamate	Pharmaceutical
Naproxen	Pharmaceutical
Primidone	Pharmaceutical
Propranolol	Pharmaceutical
Sulfamethoxazole	Pharmaceutical
Trimethoprim	Pharmaceutical
Benzophenone	Personal Care Product
Caffeine	Personal Care Product
N,N-Diethyl-m-toluamide (DEET)	Personal Care Product
Propylparaben	Personal Care Product
Tris-(2-chloroethyl) phosphate (TCEP)	Personal Care Product
Tris-(2-chloropropyl) phosphate (TCPP)	Personal Care Product
Triclocarban	Personal Care Product
Triclosan	Personal Care Product
Benzotriazole	Industrial Compound
Bisphenol A	Industrial Compound
Perfluoro hexanoic acid (PFHxA)	Industrial Compound
Perfluoro octanoic acid (PFOA)	Industrial Compound
Perfluoro octane sulfonate (PFOS)	Industrial Compound
Hydrocortisone	Hormone
Norgestrel	Hormone
Testosterone	Hormone
Atrazine	Pesticide
Simazine	Pesticide

Instruments

An Agilent 1290 Infinity Series LC was coupled to an Agilent 6460 Triple Quadrupole LC/MS. The online enrichment system used the Agilent 1200 Binary LC Pump, an Agilent 1200 Autosampler with 900 µL metering device and multi-draw capability, and the Agilent 1290 Infinity Flexible Cube (G4227A) configured with a single piston pump, a 10-port switching valve, and two SPE cartridges to maximize sample throughput as shown in Figure 1. Tables 2 and 3 show the system operating conditions.

Table 2. Online SPE Conditions

Injection	
SPE cartridge	PLRP-S, 21.1 × 12.5 mm, 15 µm (p/n 5982-1271) (Several cartridges were tested; this one was optimal)
Temperature	30 °C
Injection volume	2 × 850 µL, 1.7 mL total
Injection draw speed	500 µL/min
Eject speed	500 µL/min
Draw position	0.0 mm
Quaternary Pump (Flexible Cube pump)	
Flow rate	1.0 mL/min
Mobile phase	A) Water (95 %) + acetonitrile (5 %) + 0.1% (v/v) acetic acid B) 1/1/1 (v/v/v):methanol/iso-propyl alcohol/ acetonitrile
Step gradient	Time (min) Mobile phase 0 100 %A 4 100 %A 4.01 100 %B 8.0 100 %B 8.01 100 %A
Valve positions	Time (min) Position 0 LOAD 4 ELUTE
Injector program	Command: Draw 850 µL at default speed Eject to seat at default speed Draw 850 µL at default speed Inject

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

HPLC conditions	
Analytical column	Agilent Poroshell 120 EC C18, 50 mm × 2.1 mm, 2.7 µm (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	12.5 minutes + 2 minutes post time = 14.5 minutes cycle time
Flow rate	0.350 mL/min
Elution gradient	Time (min) Mobile phase 0 5 %B 4 Start of linear gradient 11 100 %B 12.5 5 %B
Post time	2.0 minutes
MS conditions	
Acquisition parameters	ESI mode, simultaneous positive and negative ionization with fast polarity switching; Dynamic MRM
Solvent delay	0.7 minutes
Sheath gas temperature	375 °C
Sheath gas flow rate	12 L/min
Drying gas temperature	250 °C
Drying gas flow rate	11 L/min
Nebulizer pressure	45 psig
Nozzle voltage	4,000 V positive; 3,500 V negative
Vcap	4,000 V positive; 3,500 V negative
Δ EMV	400 V

Sample collection and preparation

Samples were taken from two water treatment plants, across several stages of the treatment process, as well as a septic tank, a surface water source, and a ground water source. Samples were dosed with 1 g/L sodium azide to preserve them, and spiked within 72 hours with a surrogate standard stock to obtain a final concentration of 100–200 ng/L. Samples were subsequently filtered through 0.2-µm Agilent syringe filters, and analyzed within two weeks of collection.

Analysis parameters

Table 4 shows the multiple reaction monitoring (MRM) transitions for the 34 analytes and their surrogate internal standards.

Table 4. MRM ESI Analysis Parameters

Compound	Retention time (min)	Precursor ion	Product ion	Fragmentor voltage (V)	Collision energy (eV)	Cell accelerator voltage (V)	ESI Mode
Atenolol	5.2	267.1	190.1 (145)	130	15 (20)	2	Positive
Atenolol-d_7	5.2	274	190.1	130	15	2	Positive
Atrazine	7.6	218	176 (174)	140	15 (15)	2	Positive
Atrazine-d_5	7.6	221	179	140	15	2	Positive
Benzophenone	8.0	183	105.1	85	15	2	Positive
Benzophenone-d_{10}	8.0	183	110	85	15	2	Positive
Benzotriazole	6.5	90.1 (50)	85	16 (28)	7	2	Negative
Benzotriazole-d_4	6.5	94	85	16	7	2	Negative
Bisphenol A	7.7	227	212 (13)	115	11 (19)	7	Negative
Bisphenol A-$^{13}C_3$	7.7	239	224	115	11	7	Negative
Caffeine	5.7	195.1	138 (110.1)	104	16 (24)	2	Positive
Caffeine-$^{13}C_3$	5.7	198.1	140	104	16	2	Positive
Carbamazepine	7.2	237	194 (179)	120	15 (35)	2	Positive
Carbamazepine-d_{10}	7.2	247	204	120	15	2	Positive
Clofibric Acid	7.8	213	127	80	10	7	Negative
DEET	7.6	192	119 (91)	110	15	2	Positive
DEET-d_6	7.6	198	119	110	15	2	Positive
Diclofenac	8.6	294	250 (214)	75	4 (16)	7	Negative
Diclofenac-$^{13}C_6$	8.6	316	272.1	75	5	7	Negative
Diltiazem	7.0	415.2	178 (150)	130	24 (48)	2	Positive
Diltiazem-d_3	7.0	418.2	178	130	24	2	Positive
Diphenylhydramine	7.1	256.2	167.1 (165.1)	60	4 (44)	2	Positive
Diphenylhydramine-d_5	7.1	261.2	172.1	60	4	2	Positive
Fluoxetine	7.50	310	148	90	5	2	Positive
Fluoxetine-d_5	7.50	315	153	90	5	2	Positive
Gemfibrozil	9.2	249.2	121	75	6	7	Negative
Gemfibrozil-d_6	9.2	255	121	75	6	7	Negative
Hydrochlorothiazide	5.9	296	268.9 (204.7)	130	10 (15)	7	Negative
Hydrocortisone	6.9	363.2	327 (120.9)	130	13 (24)	2	Positive
Ibuprofen	8.8	205	161	50	0	7	Negative
Ibuprofen-d_3	8.8	208	164	50	0	7	Negative
Meprobamate	6.5	219	158 (55)	70	5 (20)	2	Positive
Meprobamate-d_7	6.5	226.1	165	70	5	2	Positive
Naproxen	8.0	229	170 (169)	55	4 (24)	7	Negative
Naproxen-$^{13}C_1d_3$	8.0	233	169	55	24	7	Negative
Norgestrel	6.2	313.2	91 (77.1)	130	60 (75)	2	Positive
PFHxA	7.4	312.9	268.9	66	5	7	Negative

Compound	Retention time (min)	Precursor ion	Product ion	Fragmentor voltage (V)	Collision energy (eV)	Cell accelerator voltage (V)	ESI Mode
PFHxA-$^{13}C_2$	7.4	314.9	269.9	66	5	7	Negative
PFOA	8.0	412.9	368.9 (169)	86	5 (5)	7	Negative
PFOA-$^{13}C_4$	8.0	416.9	371.9	86	5	7	Negative
PFOS	9.2	498.9	99 (80)	210	50 (50)	7	Negative
PFOS-$^{13}C_4$	9.2	502.9	99	210	50	7	Negative
Primidone	6.1	219.3	162.1 (91.1)	70	9 (25)	2	Positive
Primidone-d_5	6.1	224	167	70	9	2	Positive
Propranolol	6.6	260	116 (56)	122	13 (29)	2	Positive
Propylparaben	7.7	179.1	137.1 (92)	80	7 (20)	7	Negative
Propylparaben-d_4	7.7	183.1	141.1	80	7	7	Negative
Simazine	7.0	202.1	132 (68.1)	72	16 (36)	2	Positive
Sulfamethoxazole	6.5	254	156 (92)	80	10 (30)	2	Positive
Sulfamethoxazole-d_6	6.5	260	162	80	10	2	Positive
TCEP	7.5	285	222.8	95	10	2	Positive
TCEP-d_{12}	7.5	297	232	95	10	2	Positive
TCPP	8.4	327	99 (81)	72	16 (70)	2	Positive
Testosterone	7.8	289	109 (97)	115	25 (25)	2	Positive
Triclocarban	9.4	313	160 (126)	110	5 (25)	7	Negative
Triclocarban-$^{13}C_6$	9.4	318.9	159.9	110	5	7	Negative
Triclosan	9.4	289 (287)	37 (35)	75	5 (5)	7	Negative
Triclosan-$^{13}C_{12}$	9.4	299	35	75	5	7	Negative
Trimethoprim	5.8	291	261 (230)	75	25 (25)	2	Positive
Trimethoprim-d_3	5.8	294	264	75	25	2	Positive

() = secondary transition

Results and Discussion

Method development

All aspects of the method were optimized, from SPE cartridge selection, wash conditions and loading flow rate, to chromatography conditions, data acquisition parameters for the target compounds, and matrix effects. The Agilent PLRP-S SPE cartridge was selected for use due to its high reproducibility and recovery for most compounds. The Agilent Poroshell 120 EC C18 column provided sufficient separation in only 12 minutes, and the simultaneous positive and negative ionization feature of the 6460 Triple Quadrupole LC/MS with fast polarity switching and Dynamic MRM enabled rapid optimization of acquisition parameters for all 34 compounds.

The Agilent 1290 Infinity Flexible Cube enables automated online SPE

The Flexible Cube module was coupled to a large volume Agilent autosampler, which is capable of automated multidraw capacity. The sample was injected with the Flexible Cube switching valve in the LOAD position (Figure 1), and mobile phase A (Table 2), and material not bound to the first SPE cartridge (SPE1) was sent to waste. Once loading was finished, the switching valve was automatically moved to the ELUTE position (Figure 1), and the retained sample was eluted from the SPE1 with a step gradient from the binary pump to the analytical column (Table 2).

Simultaneously, the second SPE cartridge (SPE2) was being cleaned with a strong solvent (mobile phase B, Table 2) with the valve in the LOAD position to remove any contaminants and prepare it for loading. As the first sample was eluted from SPE1 to the separation column with the valve in the ELUTE position, SPE2 was equilibrated with mobile phase A (The second sample could also be loaded onto it if desired.) (Figure 1). At the same time, SPE1 was cleaned with mobile phase B, making it ready for loading the third sample.

Method performance

The instrument limit of detection (LOD) and method detection limit (MDL) for each analyte were determined as described [1], and they are shown in Table 5. The MDLs for most of the 34 compounds were <5 ng/L. Only four compounds had MDLs >10 ng/L: norgestrel, bisphenol A, benzotriazole, and benzophenone. While using much lower sample volumes than previously published studies, this method also provided MDLs that were lower in most cases. The low sample volume also greatly reduced the amount of internal standard required, substantially lowering the cost of analysis.

The linearity of calibration was excellent, using seven standards, from the MDL concentration to 100 ng/L. For all target analytes, the calibration coefficient (R^2) was > 0.99, with 71 % (24) of the analytes having R^2 values > 0.995 (Table 6). The precision of the method was also excellent, as determined using the intra- and inter-day reproducibility. The range of intra-day reproducibility was 1 to 10.4 %, with only fluoxetine having a value above 10 % (Table 6). All compounds except atenolol gave inter-day reproducibility below 10 %, with a range of 1 to 11.9 %.

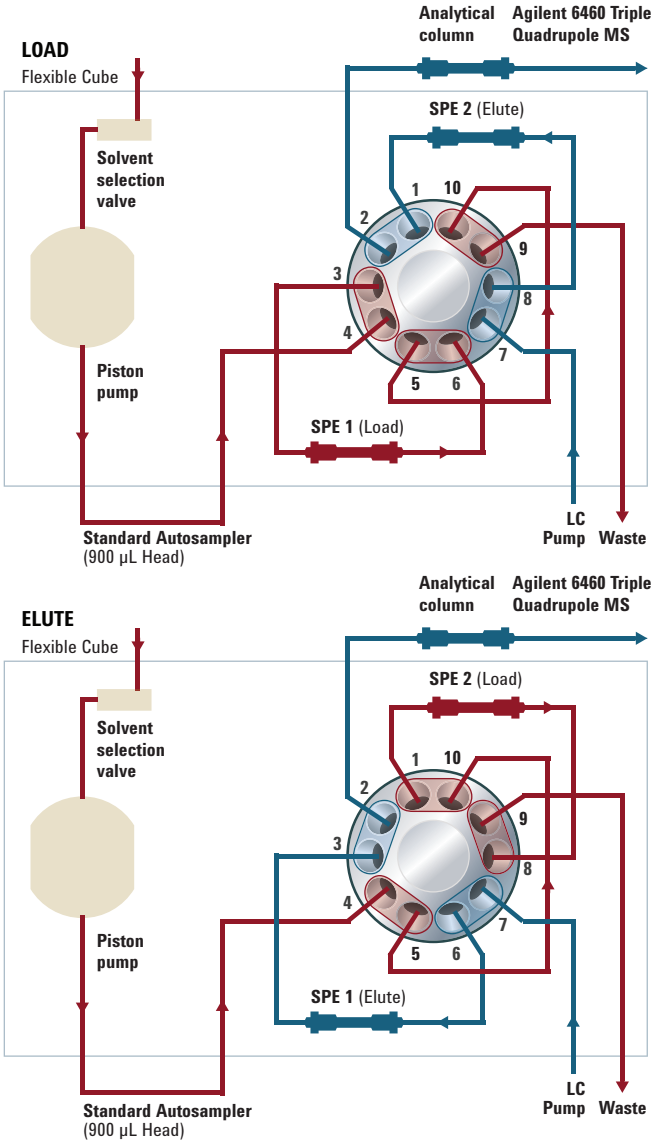

Figure 1. Valve diagram for the Agilent 1290 Infinity Flexible Cube, showing the LOAD and ELUTE positions.

Table 5. LODs and MDLs for all Target Analytes

Analyte	LOD (ng/L)	MDL (ng/L)
Atenolol	1	2.5
Atrazine	0.2	0.3
Benzophenone	5	11.3
Benzotriazole	10	10.8
Bisphenol A	10	13.1
Caffeine	0.2	0.5
Carbamazepine	0.1#	0.1
Clofibric Acid	0.2	0.7
DEET	0.1	0.3
Diclofenac	2	2.8
Diphenhydramine	0.5	0.9
Ditiazem	0.1	0.2
Fluoxetine	1	3
Gemfibrozil	0.2	0.5
Hydrocortisone	5	9.3
Hydrochlorothiazide	0.2	0.4
Ibuprofen	0.5	1.9
Meprobamate	0.2	0.4
Naproxen	1	2.5
Norgestrel	10	11.6
PFHxA	1	3.6
PFOA	0.5	3
PFOS	5	6.1
Primidone	0.5	2
Propranolol	1	1.2
Propylparaben	1	1.4
Simazine	0.2	0.4
Sulfamethoxazole	0.2	0.5
TCEP	1	2.1
TCPP	5	9
Testosterone	2.5	4.4
Triclocarban	0.5	1.1
Triclosan	1	2.6
Trimethoprim	0.1#	0.1

S/N >>3 at this concentration

Table 6. Linearity and Precision of Calibration for Target Analytes

Compound	Linearity R^2	Intra-day variability* RSD‡ (%)	Inter-day variability* RSD‡ (%)
Atenolol	0.9996	6.1	11.9
Atrazine	0.9998	3.9	3.7
Benzophenone	0.9911	9.0	7.7
Benzotriazole	0.9939	2.0	3.2
Bisphenol A	0.9924	2.8	7.2
Caffeine	0.9978	7.1	2.0
Carbamazepine	0.9996	2.1	1.4
Clofibric Acid	0.9992	4.8	2.9
DEET	0.9997	1.0	1.3
Diclofenac	0.9918	6.2	9.8
Diphenhydramine	0.9968	1.3	1.0
Ditiazem	0.9976	3.5	4.3
Fluoxetine	0.9946	10.4	5.6
Gemfibrozil	0.9987	2.0	2.5
Hydrochlorothiazide	0.9972	3.3	4.1
Hydrocortisone	0.9960	7.8	7.7
Ibuprofen	0.9949	4.5	6.1
Meprobamate	0.9997	1.1	2.2
Naproxen	0.9949	6.3	1.9
Norgestrel	0.9962	3.8	5.0
PFHxA	0.9972	3.2	5.8
PFOA	0.9983	4.8	2.7
PFOS	0.9932	6.1	2.3
Primidone	0.9930	9.9	3.7
Propranolol	0.9989	2.6	3.9
Propylparaben	0.9993	1.5	1.5
Simazine	0.9997	2.5	2.9
Sulfamethoxazole	0.9980	7.7	3.6
TCEP	0.9918	5.3	3.9
TCPP	0.9954	7.3	7.2
Testosterone	0.9979	8.8	4.4
Triclocarban	0.9983	2.9	2.3
Triclosan	0.9962	5.3	3.8
Trimethoprim	0.9967	6.8	7.2

* 4 replicates
‡ Relative standard deviation

Water sample analysis

The target panel of 34 TOrCs was analyzed in five different water sources using the optimized method: surface water, ground water, septic tank water, and two conventional wastewater treatment plants (WWTPs) at four stages of treatment.

This method showed its utility for detecting TOrCs in drinking water sources, as seven pharmaceuticals, one personal care product, and both pesticides in the target panel were detected in a surface water sample [1]. Some of the compounds were also detected in a groundwater sample at low levels.

The influent from both WWTPs contained all of the target pharmaceutical and personal care product compounds in the target panel, with ibuprofen and naproxen having the highest concentrations. Most of the pharmaceuticals were well removed by either WWTP 1 or WWTP 2 after biological or chorine oxidation, or after both, as were most personal care products.

Conclusions

A fully automated online SPE online extraction method using the Agilent 1290 Infinity Flexible Cube enables the analysis of 34 diverse TOrCs at ng/L levels, using LC/MS/MS on the Agilent 6460 Triple Quadrupole LC/MS with simultaneous positive and negative ESI. The MRLs were measured without having to implement time-consuming MRL determinations in each matrix, using isotope recovery data. A low sample volume requirement (1.7 mL) and a 15-minute cycle time enabled high-throughput analysis. The use of fast polarity switching also provided significant time savings by enabling the analysis of all 34 TOrCs in both ESI positive and negative mode with only one injection. This is the only published online SPE method that uses this feature. The benefits of this unique approach to automated online SPE analysis of TOrCs include increased reproducibility and substantial time, labor, and solvent savings compared to previously published methods. The utility of this approach has been demonstrated by the analysis of a wide variety of water sample types.

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