High Sensitivity HPLC Analysis of Contaminants of Emerging Concern (CECs) in Water Using the Agilent 6460 Triple Quadrupole LC/MS System

Application Note

Environmental

Authors

Tarun Anumol, Sylvain Merel, and Shane Snyder
Department of Chemical and Environmental Engineering
BIO5 Institute
University of Arizona
Tucson, AZ
USA

Abstract

A fast and sensitive method for the monitoring of CECs across the wastewater treatment process has been developed for a suite of 36 compounds. Most of the compounds have limits of detection and quantitation well below one part per billion (ppb), and method reporting limits (MRLs) range from 0.1 to 15 parts per trillion (ppt). Total cycle time is less than 20 minutes.

Agilent Technologies

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. The United States Environmental Protection Agency (EPA) is working to improve its understanding of a number of CECs, particularly pharmaceuticals and personal care products (PPCPs) and perfluorinated compounds, among others. Since CECs are found with increasing frequency in the world's streams, lakes, and ground water, this improved understanding is necessary to measure the potential threat to the environment and human health.

Some CECs are known endocrine disruptors, while others affect glucocorticoid activity. 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, UCMR, 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].

This comprehensive program requires methods that are sensitive, accurate, reproducible, and fast. This application note describes a method for the rapid and sensitive detection of 36 CEC analytes in less than 20 minutes per sample. This UHPLC/MS/MS method was developed on an Agilent 1290 Infinity LC System coupled to an Agilent 6460 Triple Quadrupole LC/MS system, using both positive and negative electrospray ionization. It provides limits of detection (LODs) and limits of quantitation (LOQs) for most of the 36 compounds that are at or even well below one ppb, and method reporting limits (MRLs) that range from 0.1 to 15 ppt. The method has been used to monitor CEC levels across the wastewater treatment process, from crude wastewater to dechlorinated final effluent. Several of the CECs had initial concentrations in wastewater higher than 1,000 ng/L (ppt), and many of these persisted at concentrations above 500 ng/L after water treatment.

Experimental

Reagents and Standards

Calibration standards were obtained from Sigma-Aldrich, except for perfluorohexadecanoic acid (PFHxDA) from Matrix Scientific, meprobamate from Cerilliant (Round Rock, TX), 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 HPLC pesticide grade methanol (Burdick & Jackson). Subsequent calibration and fortification solutions were prepared by mixing of all the standards in methanol at 10 µg/mL, followed by successive dilutions to obtain the required concentrations. Labeled internal standards were purchased from Cambridge Isotope Laboratories, except for: $^{13}C_4$-PFOA, $^{13}C_4$-PFOS, $^{13}C_2$-PFHxA, $^{13}C_4$-PFBA (Wellington Laboratories); primidone-d_5 and $^{13}C_6$-diclofenac (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. Methyl tertiary-butyl ether (MTBE), formic acid, and ammonium hydroxide were obtained from Fisher Scientific, while acetonitrile was obtained from Burdick and Jackson.

Table 1. Labeled Internal Standards

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

Instruments

This method was developed on an Agilent 1290 Infinity HPLC System coupled to an Agilent 6460 Triple Quadrupole LC/MS system, using both positive and negative ESI. The instrument conditions are listed in Tables 2 and 3.

Table 2. HPLC and ESI+ MS Instrument Conditions

HPLC conditions	
Analytical column	Agilent ZORBAX Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm particle size (p/n 959757-902)
Guard column	Agilent 1290 Infinity In-line filter with 0.3 µm SS frit (p/n 5067-4638)
Column temperature	30 °C
Injection volume	3 µL
Mobile phase	A = Water + 0.1% (v/v) formic acid B = Acetonitrile + 0.1% (v/v) formic acid
Run time	8 minutes + 1.45 minutes post time
Flow rate	0.4 mL/min
Gradient	Time (min) Mobile phase 0 5% B 1.5 5% B 3 20% B 4 45% B 6.1 65% B 7 100% B 7.45 5% B Post time: 1.45 minutes

MS conditions	
Acquisition parameters	ESI mode, positive ionization; Dynamic MRM
Sheath gas temperature	375 °C
Sheath gas flow rate	11 L/min
Drying gas temperature	275 °C
Drying gas	11 L/min
Nebulizer pressure	45 psig
Nozzle voltage	0 V positive; 500 V negative
Vcap	4,000 V positive; 3,500 V negative
Δ EMV	400 V

Table 3. HPLC and ESI- MS Instrument Conditions

HPLC conditions	
Analytical column	Agilent ZORBAX Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm (p/n 959757-902)
Guard column	Agilent 1290 Infinity In-line filter with 0.3 µm SS frit (p/n 5067-4638)
Column temperature	30 °C
Injection volume	3 µL
Mobile phase	A = Water + 5 mM ammonium acetate B = (90% Acetonitrile + 10% water) + 5 mM ammonium acetate
Run time	6.4 minutes + 1.5 minutes post time
Flow rate	0.4 mL/min
Gradient	Time (min) Mobile phase 0 20% B 4.5 96% B 5 100% B 6.3 100% B 6.4 20% B Post time: 1.5 minutes

MS conditions	
Acquisition parameters	ESI mode, negative ionization; Dynamic MRM
Sheath gas temperature	350 °C
Sheath gas flow rate	11 L/min
Drying gas temperature	225 °C
Drying gas	10 L/min
Nebulizer pressure	45 psig
Nozzle voltage	0 V positive; 1,500 V negative
Vcap	4,000 V positive; 3,600 V negative
Δ EMV	400 V

Sample Preparation

Water samples were taken from two wastewater treatment plants, across several stages of treatment. A 1-L amount of each sample was spiked with 50–150 ng/L of internal standard and then extracted using 200 mg Hydrophilic Lipophilic (HLB) SPE cartridges. Sample preparation was performed on an automated SPE extraction system by elution with 5 mL of methanol and 5 mL of 10/90 (v/v) MTBE.

The sample was then evaporated using nitrogen to 100 µL and reconstituted to a final volume of 1 mL in methanol.

Analysis Parameters

The multiple reaction monitoring (MRM) transitions for the 36 analytes and their internal standards are shown in Tables 4 and 5.

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

Compound	Precursor ion	Product ion	Fragmentor voltage (V)	Collision energy (V)
Caffeine	195.1	138[a]	104	16
		110.1[b]	104	24
Caffeine-$^{13}C_3$	198.1	140	104	16
Trimethoprim	291	261	75	25
		230	75	25
Trimethoprim-d_3	294	264	75	25
Sucralose+Na	419	239	110	15
		221	110	15
Sucralose-d_6	425	243	110	15
Triamcinolone	395.2	375.1	95	4
		225.1	95	12
Primidone	219.3	162.1	70	9
		91.1	70	25
Primidone-d_5	224	167	70	9
Sulfamethoxazole	254	156	80	10
		92	80	30
Sulfamethoxazole-d_6	260	162	80	10
Meprobamate	219	158	70	5
		55	70	20
Meprobamate-d_7	226	165	70	5
Diphenylhydramine	256.2	167.1	60	4
		165.1	60	44
Diltiazem	415.2	178	130	24
		150	130	48
Hydrocortisone	363.2	327	130	13
		120.9	130	24
Prednisone	359.2	171	95	36
		147.1	95	24
Simazine	202.1	132	72	16
		68.1	72	36
Fluoxetine	310	148	90	5
Fluoxetine-d_5	315	153	90	5
Carbamazepine	237	194	120	15
		179	120	35
Carbamazepine-d_{10}	247	204	120	15
Dexamethasone	393.2	373.2	87	4
		355.2	87	5
TCEP	285	222.8	95	10
Atrazine	218	176	140	15
		174	140	15
Atrazine-d_3	221	179	140	15
DEET	192	119	110	15
		91	110	30
DEET-d_6	198	119	110	15
Testosterone	289	109	115	25
		97	115	25
Norethistrone	299.2	109.1	104	28
		91.1	104	56
TCPP	327	99	72	16
		81	72	70
Norgestrel	313.2	91	130	60
		77.1	130	75
Benzophenone	183	105.1	85	10

[a]Quantifier ion
[b]Qualifier ion

Results and Discussion

Method Performance

An LC MS/MS method has been developed for the sensitive and robust detection of 36 Contaminants of Emerging Concern (Table 6), using multiple transitions and labeled internal standards for most of the compounds (Tables 4 and 5). It provided highly accurate calibration curves over a concentration range of 1 to as much as 2,400 ng/L, with R^2 values > 0.99 for linear fit (Figure 1).

Table 5. Multiple Reaction Monitoring (MRM) ESI-Analysis Parameters

Compound	Precursor ion	Product ion	Fragmentor voltage	Collision energy
PFOA	412.9	368.9[a]	86	5
		169[b]	86	5
PFOA-$^{13}C_4$	416.9	371.9	86	5
PFDA	512.9	469	102	5
Gemfibrozil	249.2	121	75	6
Gemfibrozil-d_6	255	121	75	6
PFOS	498.9	99	210	50
		80	210	50
PFOS-$^{13}C_4$	502.9	99	210	50
Triclocarban	313	160	110	5
		126	110	25
Triclocarban-$^{13}C_6$	318.9	159.9	110	5
Triclosan	289	37	75	5
	287	35	75	5
PFHxDA	813	769	100	10
PFBS	298.8	98.9	133	29
		80	133	45
PFBA	213	169	60	0
PFBA-$^{13}C_4$	217	172	50	5
Ibuprofen	205	161	50	0
Ibuprofen-d_3	208	164	50	0
Bisphenol A	227	212	115	11
		133	115	19
Bisphenol A-$^{13}C_{12}$	239	224	115	11
Naproxen	229	170	55	4
		169	55	24
Naproxen-$^{13}C_1d_3$	233	169	55	24
Diclofenac	294	250	75	4
		214	75	16
Diclofenac-$^{13}C_6$	316	272.1	75	5

[a]Quantifier ion
[b]Qualifier ion

Table 6. Target Analytes

Compound	Use
Atrazine	Pesticide
Benzophenone	UV Blocker
Bisphenol A	Plasticizer
Caffeine	Stimulant
Carbamazepine	Anti-seizure drug
DEET	Insect repellant
Dexamethasone	Anti-inflammatory
Diclofenac	Anti-arthritic
Diltiazem	Anti-arrhythmic
Diphenylhydramine	Antihistamine
Fluoxetine	Antidepressant
Gemfibrozil	Anti-cholesterol
Hydrocortisone (Cortisol)	Anti-inflammatory
Ibuprofen	Analgesic
Meprobamate	Anti-anxiety
Naproxen	Analgesic
Norethisterone	Contraceptive pill Ingredient
Norgestrel	Hormonal contraceptive
PFBA	Perfluorinated compound
PFBS	Perfluorinated compound
PFDA	Perfluorinated compound
PFHxDA	Perfluorinated compound
PFOA	Perfluorinated compound
PFOS	Perfluorinated compound
Prednisone	Anti-inflammatory
Primidone	Anticonvulsant
Simazine	Herbicide
Sucralose	Artificial sweetener
Sulfamethoxazole	Antibiotic
TCEP	Flame retardant
TCPP	Flame retardant
Testosterone	Androgen
Triamcinolone	Synthetic corticosteroid
Triclocarban	Antibiotic
Triclosan	Antimicrobial
Trimethoprim	Antibiotic

The LOD, (LOQ), and (MRL) were determined for each target analyte. The LOD was defined as the lowest concentration that provides a signal/noise > 3 (peak height) for three successive injections, and the LOQ was defined as the lowest concentration providing S/N >10 for both the quantifier and the qualifier transitions. Both values are listed in Table 7 for all 36 target analytes.

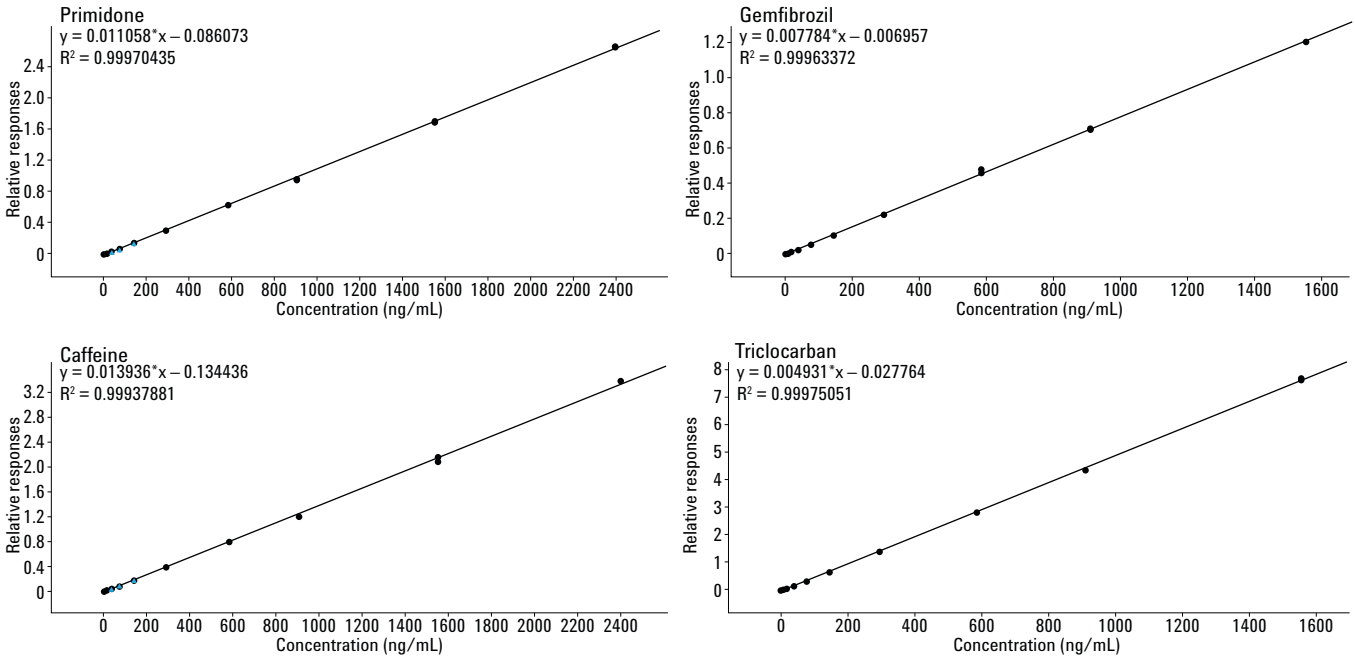

Figure 1. Calibration curves (in duplicate) for primidone (2.5–2,400 ng/L), caffeine (1–2,400), gemfibrozil (1–1,550 ng/L), and triclocarban (1–1,550 ng/L), all with R^2 values > 0.999.

The method reporting limit (MRL) for each analyte was determined using eight aliquots of HPLC water spiked at concentrations two to three times higher than the LOQ. The samples were extracted using the SPE procedure and injected into the UHPLC. The MRL was then calculated using the standard deviation and t-test at 99% confidence, and the value was rounded off to a single decimal place. The MRLs for all 36 analytes are shown in Table 7.

All but one of the LOD values were at or below 1 µg/L ppb. Several were as low as 0.02 µg/L, or 20 ppt. Most LOQ values were at or below 5 ppb, with a few at or below 100 ppt. All MRL values were at or below 15 ng/L ppt, and several were as low as 0.1 to 0.5 ppt.

Table 7. LODs, LOQs and MRLs for 36 Contaminants of Emerging Concern

Compound	LOD (µg/L)	LOQ (µg/L)	MRL (ng/L)
ESI positive analysis			
Caffeine	0.5	1	1
Trimethoprim	0.05	0.1	0.1
Sucralose	1	5	7.5
Triamcinolone	1	5	5
Primidone	1	2.5	2.5
Sulfamethoxazole	0.02	1	1
Meprobamate	0.1	1	1
Diphenylhydramine	0.02	0.1	1
Diltiazem	0.02	0.1	0.5
Hydrocortisone	0.5	1	1.5
Prednisone	10	20	15
Simazine	0.1	0.5	0.5
Fluoxetine	0.02	0.05	0.5
Carbamazepine	0.05	0.1	0.25
Dexamethasone	0.05	0.5	1
TCEP	0.5	1	2.5
Atrazine	0.5	0.5	0.5
DEET	0.05	0.1	1
Testosterone	0.5	1	0.5
Norethistrone	0.1	0.5	0.5
TCPP	0.05	1	2.5
Norgestrel	0.5	2.5	1
Benzophenone	0.02	0.5	1
ESI negative analysis			
PFOA	0.02	0.5	1
PFDA	0.02	0.5	0.5
Gemfibrozil	0.05	0.5	1
PFOS	0.02	0.05	0.1
Triclocarban	0.1	0.5	1
Triclosan	0.5	2.5	5
PFHxDA	0.02	0.1	0.5
PFBS	0.02	0.05	0.5
PFBA	0.02	0.02	NA
Ibuprofen	5	10	15
Bisphenol A	1	5	15
Naproxen	0.1	1	1.5
Diclofenac	0.1	0.5	1.5

NA = Not analyzed

Wastewater Analysis

Samples from two wastewater treatment plants in Arizona and California were analyzed using this method, at different treatment points in each plant. Samples were extracted using automated SPE to a volume of 1 mL in methanol. Samples were diluted and re-run if the initial concentration of the compound was above the range of the calibration curve. All concentration values were then corrected using the appropriate internal standard. The concentrations determined for the 36 target analytes across several stages of wastewater treatment are shown in Table 8. It shows that several of the CEC's had initial concentrations in wastewater higher than 1,000 ng/L, and some of these persisted at concentrations above 500 ng/L after water treatment.

Table 8. Measurement of CECs (ng/L) in Wastewater Across Several Stages of Treatment

	Wastewater treatment plant 1				Wastewater treatment plant 2		
Compound	After Bar screens	After BNROD	After Sand filter	After Chlorination	After Clarifier	Secondary effluent After activated sludge	Dechlorinated final effluent
ESI positive analysis							
Caffeine	>4,000	22	6	9	>4,000	343	492
Trimethoprim	1,368	30	12	<MRL	1,108	930	847
Sucralose	8,979	8,171	7,573	7,954	>6,000	>6,000	>6000
Primidone	1,118	617	579	582	197	179	188
Triamcinolone	14	<MRL	<MRL	<MRL	4	<MRL	<MRL
Sulfamethoxazole	6,078	3,909	3,013	39	2,287	843	1134
Meprobamate	597	421	570	190	1,600	654	482
Diphenylhydramine	<MRL	15	27	<MRL	<MRL	<MRL	<MRL
Diltiazem	NA	NA	NA	NA	NA	NA	NA
Hydracortisone	NA	NA	NA	NA	<MRL	<MRL	<MRL
Prednisone	59	<MRL	<MRL	<MRL	20	20	18
Simazine	1	3	4	3	<MRL	<MRL	<MRL
Carbamezapine	578	467	457	262	262	227	229
Fluoxetine	79	38	23	34	155	32	57
Dexamethasone	94	<MRL	<MRL	<MRL	19	<MRL	<MRL
TCEP	547	332	329	262	775	685	647
Atrazine	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
DEET	2248	193	174	158	3,569	539	630
Testosterone	15	4	3	3	14	1	4
Norethistrone	103	1	3	6	<MRL	<MRL	<MRL
TCPP	5,665	3,874	3,408	2,907	1654	2,903	3035
Norgestrel	19	6	7	3	18	10	93
Benzophenone	4,541	314	318	219	>4,000	2,318	1708
ESI negative analysis							
PFBA	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
Naproxen	>6,000	114	28	6	>6,000	552	297
PFBS	24	9	8	5	17	10	9
Diclofenac	834	531	417	14	96	<MRL	<MRL
Ibuprofen	>8,000	50	<MRL	<MRL	>6,000	1,810	1589
PFOA	0	46	49	60	9	7	9
Bisphenol A	352	20	57	36	635	142	323
Gemfibrozil	>6,000	399	234	193	2,747	2,382	2188
PFDA	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
PFOS	464	3	2	4	1,083	200	187
Triclocarban	738	201	68	106	334	31	42
Triclosan	4,642	103	69	15	2,252	441	162
PFHxDA	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL

Conclusions

A rapid and sensitive method for monitoring trace levels of CECs across the wastewater treatment process has been developed using an Agilent 1290 Infinity LC System coupled to an Agilent 6460 Triple Quadrupole GC/MS system. Analysis of 36 analytes at low ppt levels is possible in less than 20 minutes per sample. The MRM approach enables validation of many of the results, using a second transition, and the use of 20 labeled internal standards provides further precision and accuracy in quantification.

References

1. Contaminants of Emerging Concern, http://water.epa.gov/scitech/cec/, accessed October 3, 2011.

2. Unregulated Contaminant Monitoring Rule 3 (UCMR3), http://water.epa.gov/lawsregs/rulesregs/sdwa/ucmr/ucmr3/index.cfm, accessed October 3, 2011.

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
November 6, 2012
5991-1412EN

Agilent Technologies