Analysis of Trace Organic Contaminants in Water by Direct Injection Using Agilent 6490 LC/MS/MS with Pos/Neg Switching

Application Note

Environmental

Authors

Tarun Anumol, Shimin Wu,
Sylvain Merel, and Shane Snyder
University of Arizona

Sheher Bano Mohsin
Agilent Technologies, Inc.

Abstract

Trace organic contaminants (TOrCs) are ubiquitous in water and a potential health issue to the public and environment. With increasing concern about these TOrCs, a sensitive, robust, and expedient detection method is necessary for their monitoring. A fast and sensitive method for the monitoring of 21 TOrCs in water by direct injection has been developed using an Agilent 6490 Triple Quadrupole LC/MS system with positive and negative electrospray ionization. Minimal sample preparation is required with this instrument to measure 21 TOrCs at reporting limits of 1–200 ng/L. This method has been proven to be faster and less labor-intensive than conventional off-line solid phase extraction methods.

Agilent Technologies

Introduction

Organic contaminants are being found in water at trace levels and can be a concern for public health and the environment. These compounds come from residual consumer products and they are being detected more frequently and in greater concentrations than ever before. Further, developing a method to detect a suite of "indicator" chemicals that repesent the wider range of TOrCs is critical because they are not completely removed by conventional water treatment processes and the effects of many on humans are unknown. Traditional TOrC detection techniques include conventional offline solid phase extraction, which is extremely time consuming and labor intensive. In addition, this technique requires several additional steps that increase the possibility for errors to occur.

This application note describes a new technique for analyzing indicator TOrCs in wastewater. An Agilent 1260 Infinity High Performance Liquid Chromatography (HPLC) binary pump with a 100-µL autosampler coupled to an Agilent 6490 Triple Quadrupole LC/MS was used to develop a robust and sensitive method for analyzing these chemicals with ng/L limits of detection (LODs). Positive and negative electrospray ionization (ESI) is performed for quantification of 21 trace organic contaminants. It was expected that this method would give similar detection limits to conventional offline solid phase extraction for TOrCs in water.

Experimental

Twenty-one TOrC's including several wastewater indicator compounds such as artificial sweeteners, x-ray contrast media, and halogenated flame retardants were analyzed. Table 1 lists the compounds analyzed.

Nineteen isotopically labeled surrogate standards were used for increased accuracy in quantitation. These standards are listed in Table 2.

Table 1. Compounds Analyzed

Compound	Class
Acesulfame-K	Artificial sweetener
Atenolol	Anti-anginal
Benzophenone	UV-inhibitor
Benzotriazole	Corrosion-inhibitor
Caffeine	Stimulant
Carbamazepine	Anticonvulsant
DEET	Insect-repellant
Diphenhydramine	Antihistamine
Gemfibrozil	Anticholesterol
Iohexol	X-ray contrast media
Iopamidol	X-ray contrast media
Iopromide	X-ray contrast media
Meprobamate	Anti-anxiety
Naproxen	Pain-reliever
Sucralose	Artificial sweetener
Sulfamethoxazole	Antibiotic
TCEP	Flame-retardant
TCPP	Flame-retardant
Triclocarban	Anti-microbial
Triclosan	Anti-microbial
Trimethoprim	Antibiotic

Table 2. Surrogate Standards

Acesulfame-d_4	Diphenhydramine-d_5	Sucralose-d_6
Atenolol-d_7	Gemfibrozil-d_6	Sulfamethoxazole-$^{13}C_6$
Benzophenone-d_{10}	Iohexol-d_5	TCEP-d_{12}
Benzotriazole-d_4	Iopamidol-d_3	Triclocarban-$^{13}C_6$
Caffeine-$^{13}C_3$	Meprobamate-d_3	Triclosan-$^{13}C_{12}$
Carbamazepine-d_{10}	Naproxen-$^{13}C_1d_3$	Trimethoprim-d_3
DEET-d_6		

Instrumentation

The method was developed on an Agilent 1260 Infinity HPLC, coupled to an Agilent 6490 Triple Quadrupole LC/MS using both positive and negative ESI. Instrument conditions are listed in Table 3.

Sample preparation

A 2-mL sample was collected in an autosampler vial and stored at 4 °C to prevent degradation. A 900-µL amount of sample was weighed on an analytical balance for accuracy, and 100 µL of a 200 ppb surrogate standard mix (60:40 water: MeOH) was added and vortexed for 1 minute. The sample was filtered through 0.2-µm filters (Agilent Captiva PES filters; p/n 5190-5096). Methanol was added so that the amount in the final extract was < 5% of total sample volume. The samples were analyzed on an Agilent 6490 Triple Quadrupole LC/MS system coupled to an Agilent Infinity 1260 LC.

The multiple reaction monitoring (MRM) transitions for the 21 analytes and their surrogate standards are shown in Tables 4 and 5.

Table 3. Instrument Conditions

HPLC method		
Analytical column	Agilent Pursuit XRs C8, 100 × 2.0 mm (p/n A6011100X020)	
Column compartment temperature	30 °C	
Mobile phase	A: Water + 0.1% B: Acetonitrile	
Flow rate	0.4 mL/min	
Gradient	Time	B%
	0.00	2.0
	1.50	2.0
	8.00	60.0
	10.50	100.0
	10.80	100.0
	11.50	2.0
Post time	1.5 minutes	
MS conditions		
Acquisition parameters	ESI mode, postive and negative ionization, dynamic MRM	
Gas temperature	275 °C	
Gas flow	18 L/min	
Nebulizer	45 psi	
Sheath gas temperature	350 °C	
Sheath gas flow	11 L/min	
Capillary voltage	+3,000 V, –3,000 V	
Nozzle voltage	+1,500 V, –0 V	

Table 4. MRM Transitions for Target Analytes

Compound	Precursor ion	Product ion	Fragmentor voltage (V)	Collision energy (V)	Retention time	ESI Mode
Acesulfame	162	82.1	380	13	5.2	Negative
Atenolol	267.1	190.1	380	15	4.2	Positive
Atenolol	267.1	145	380	20	4.2	Positive
Benzophenone	183	105.1	380	10	10	Positive
Benzotriazole	118	90.1	380	16	6.1	Negative
Benzotriazole	118	50	380	28	6.1	Negative
Caffeine	195.1	138	380	16	5.4	Positive
Caffeine	195.1	110.1	380	24	5.4	Positive
Carbamezapine	237	194	380	15	8	Positive
Carbamezapine	237	179	380	35	8	Positive
DEET	192	119	380	15	8.8	Positive
DEET	192	91	380	30	8.8	Positive
Diphenylhydramine	256.2	167.1	380	4	6.5	Positive
Diphenylhydramine	256.2	165.1	380	44	6.5	Positive
Gemfibrozil	249.2	121	380	6	10.8	Negative
Iohexol	821.9	803.8	380	20	4.2	Positive
Iopamidol	777.9	558.9	380	22	2.3	Positive
Iopamidol	777.9	387	380	42	2.3	Positive
Iopromide	791.8	572.8	380	22	4.8	Positive
Iopromide	791.8	558.8	380	28	4.8	Positive
Meprobamate	219	158	380	5	7	Positive
Meprobamate	219	55	380	20	7	Positive
Sucralose	419	239	380	15	5.9	Positive
Sucralose	419	221	380	15	5.9	Positive
Sulfamethoxazole	254	156	380	10	7.1	Positive
Sulfamethoxazole	254	92	380	30	7.1	Positive
TCEP	285	223	380	10	9	Positive
TCPP	327	99	380	16	9.8	Positive
TCPP	327	81	380	70	9.8	Positive
Triclocarban	313	160	380	5	11	Negative
Triclocarban	313	126	380	25	11	Negative
Triclosan	289	37	380	5	11.1	Negative
Triclosan	287	35	380	5	11.1	Negative
Trimethoprim	291	261	380	25	5.1	Positive
Trimethoprim	291	230	380	25	5.1	Positive
Naproxen	229	170	380	4	9.1	Negative
Naproxen	229	169	380	24	9.1	Negative

Table 5. MRM Transitions for Surrogate Standards

Compound	Precursor ion	Product ion	Fragmentor voltage (V)	Collision energy (V)	Retention time	ESI Mode
Acesulfame-d_4	166.1	86.1	380	10	5.2	Negative
Atenolol-d_7	274	190.1	380	15	4.2	Positive
Benzophenone-d_{10}	193	110.1	380	10	10	Positive
Benzotriazole-d_4	122	94.1	380	16	6.1	Negative
Caffeine-$^{13}C_3$	198.1	140	380	16	5.4	Positive
Carbamezapine-d_{10}	247	204	380	15	8	Positive
DEET-d_6	198	119	380	15	8.8	Positive
Diphenhydramine-d_5	261.2	172.1	380	4	6.5	Positive
Gemfibrozil-d_6	255	121	380	6	10.8	Negative
Iohexol-d_5	826.9	810	380	20	4.2	Positive
Iopamidol-d_3	781	562	380	22	2.3	Positive
Meprobamate-d_3	222.1	161.1	380	5	7	Positive
Naproxen-$^{13}C_1d_3$	233	169	380	4	9.1	Negative
Sucralose-d_6	425	243	380	15	5.9	Positive
Sulfamethoxazole-$^{13}C_6$	260	162	380	10	7.1	Positive
TCEP-d_{12}	297	232	380	13	8.6	Positive
Triclocarban-$^{13}C_6$	318.9	160	380	5	11	Negative
Triclosan-$^{13}C_{12}$	299	35	380	5	11.1	Negative
Trimethoprim-d_3	294	264	380	25	5.1	Positive

Results and Discussion

The developed method was able to detect 21 TOrCs at ng/L levels in water samples, featuring analysis in both positive and negative ESI modes. The injection volume was 80 µL and the cycle time from analysis to post time was 13.25 minutes.

Figures 1 and 2 show examples of calibration curves for the method. In Figure 1, iopadimol was analyzed at 15 concentrations ranging from 100–50,000 ng/L with a linear fit and 1/x weighting. Figure 2 showed the calibration curve for triclocarban analyzed at 15 levels from 50–50,000 ng/L. This curve also provided a linear fit with duplicate injection for each calibration point. Both curves had $R^2 \geq 0.999$. All analytes had calibration curves with a linear regression and 1/X weighting with $R^2 > 0.99$.

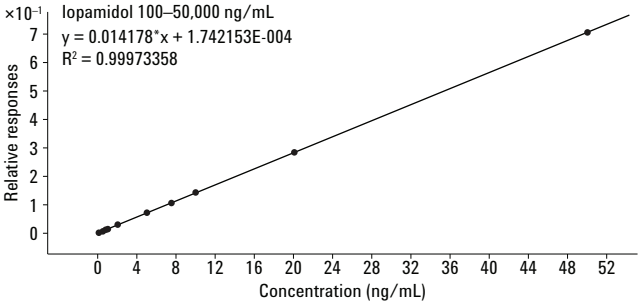

Figure 1. Calibration curve for iopamidol at 100–50,000 ng/L.

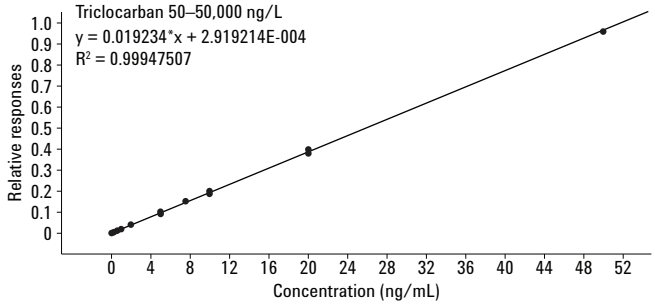

Figure 2. Calibration curve for triclocarban at 50–50,000 ng/L.

Figure 3 shows a chromatogram of the components analyzed in this study. As the peak shapes indicate, the method achieved good separation of all components tested. The inset chromatogram shows the 10 compounds detected in the sample at trace levels, illustrating the high sensitivity of the method.

The limits of detection (LOD) and limits of quantitation (LOQ) were determined for each target compound. The LOD is defined as the lowest concentration that provides a signal-to-noise (S/N) > 3 for the most abundant transitions. The limit of quantitation was defined as the lowest concentration providing S/N > 10 for all transitions. All values are shown in Table 6.

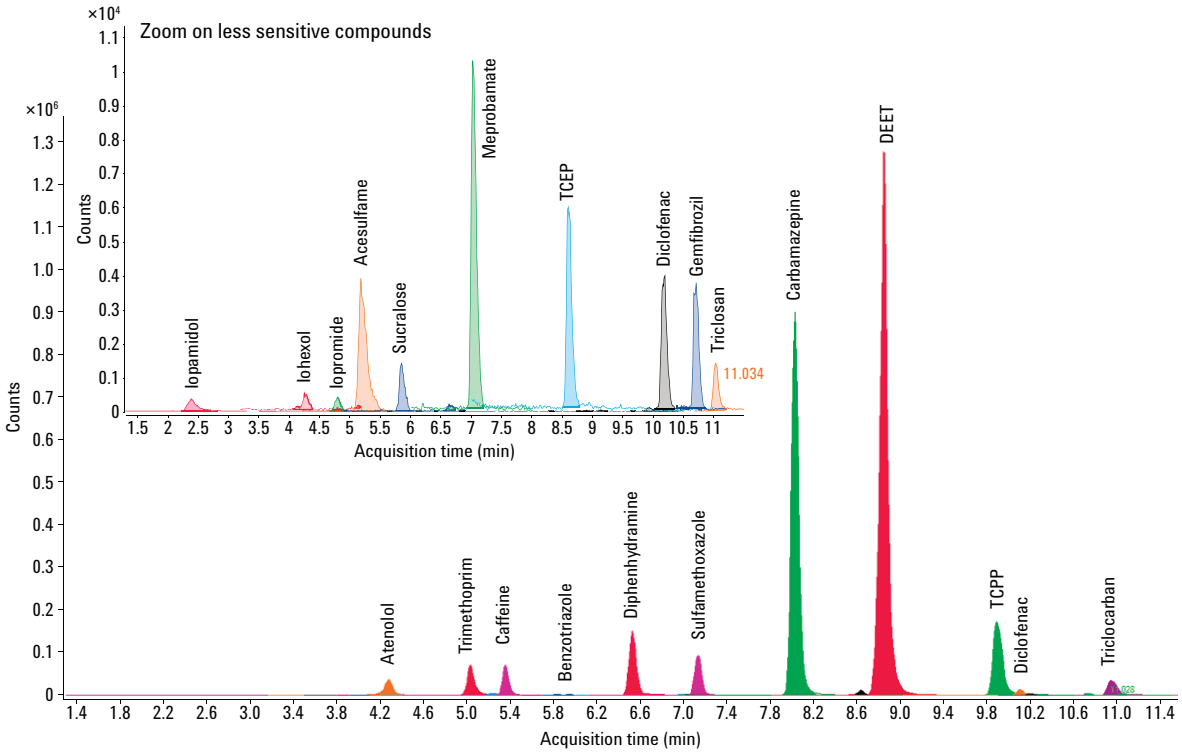

Figure 3. Chromatograms of analyzed components. Inset shows less sensitive compounds.

Table 6. Method Reporting Limits

Analyte	LOD (ng/L)	LOQ (ng/L)	Analyte	LOD (ng/L)	LOQ (ng/L)
Acesulfame-K	10	20	Meprobamate	200	500
Atenolol	10	20	Sucralose	75	100
Benzophenone	10	20	Sulfamethoxazole	2	10
Benzotriazole	75	100	TCEP	50	75
Caffeine	5	10	TCPP	5	10
Carbamazepine	5	10	Triclocarban	75	100
DEET	1	5	Triclosan	100	200
Diphenhydramine	5	10	Trimethoprim	5	10
Gemfibrozil	10	20	Iopamidol	50	100
Iohexol	100	200	Naproxen	750	1000
Iopromide	100	200			

LOD: S/N > 3 for most abundant transition
LOQ: S/N > 10 for all transitions

Wastewater analysis

Samples from two wastewater treatment facilities were analyzed using this method. Low (1 µg/L) and high (5 µg/L) concentrations of TOCs were spiked in wastewater effluent from one facility to verify method performance. In addition, grab samples at different treatment points of a second wastewater treatment plant were collected and analyzed.

Results of the spiked matrix are shown in Table 7.

The table shows the relative standard deviation (RSD %) for the effluent with no spike, low spiked recovery (1 µg/L), and high spiked recovery (5 µg/L). Recoveries were within 70–130% for more than 90% of the compounds tested, both in the low and high spike samples. Relative standard deviation for four replicates (n = 4) was less than 10% for all compounds with the exception of naproxen at the low spike.

Table 7. Matrix Spike (n = 4)

Sample	WWTP effluent: no spike (ng/L)	STDEV	RSD (%)	1 µg/L spike recovery (%)	RSD (%)	5 µg/L spike recovery (%)	RSD (%)
Iopamidol	6,110	111	1.8	59.2	2.0	90.1	0.4
Atenolol	380	9	2.4	80.9	0.6	98.0	2.0
Iohexol	320	25	8.0	100.2	0.3	98.3	4.2
Iopromide	540	19	3.4	97.4	2.1	98.0	1.2
Trimethoprim	< 70	–	–	90.1	2.5	97.8	5.2
Caffeine	< 10	–	–	74.7	5.1	71.2	3.0
Acesulfame	2,840	33	1.2	87.0	2.1	100.9	1.4
Sucralose	41,900	1,677	4.0	74.9	6.7	67.5	1.9
Benzotriazole	< 120	–	–	123.8	2.2	109.0	2.2
Diphenylhydramine	130	2	1.9	79.5	1.5	97.5	2.4
Meprobamate	8370	797	9.5	132.7	9.1	128.2	2.0
Sulfamethoxazole	860	35	4.0	102.3	3.7	105.5	4.1
Carbamezapine	290	8	2.6	106.2	1.2	99.9	1.7
TCEP	290	7	2.3	108.8	2.2	104.5	0.9
DEET	90	4	4.9	106.7	0.3	100.7	3.2
Naproxen	< 770	–	–	131.3	13.7	106.0	6.8
TCPP	1,500	24	1.6	58.9	2.6	73.2	1.8
Benzophenone	12,580	630	5.0	NA	NA	NA	NA
Gemfibrozil	130	3	2.2	102.2	4.8	94.4	4.6
Triclocarban	6,980	1,116	16.0	NA	NA	NA	NA
Triclosan	< 100	–	–	NA	NA	NA	NA

Table 8 shows the concentrations of target compounds in the grab samples from the second wastewater treatment facility. The grab samples were taken from the influent stream, after the primary treatment, after activated sludge treatment, and after chlorination. The data show that many of the TOCs have initial concentrations higher than 50,000 ng/L and some of these levels persisted at concentrations above 500 ng/L after water treatment. Detection of these compounds throughout the treatment stages in a plant show that the method is sensitive and robust enough to be used for wastewater indicators in real samples.

Table 8. Wastewater Grab Samples

Compound	Influent (ng/L)	After primary settling (ng/L)	After activated sludge treatment (ng/L)	After chlorination (ng/L)
Iopamidol	21,300	20,500	27,500	27,700
Iohexol	10,300	3,490	4,010	4,370
Atenolol	3,750	3,030	370	450
Iopromide	200	140	N.D	N.D
Trimethoprim	1,140	1,110	250	190
Acesulfame-K	49,400	45,200	800	940
Caffeine	103,000	82,500	N.D	N.D
Benzotriazole	1,990	1,070	1,250	1,440
Diphenylhydramine	2,820	3,250	550	430
Meprobamate	5,390	4,290	920	N.D
Sulfamethoxazole	2,980	2,520	1,420	710
Carbamezapine	870	940	330	330
TCEP	200	200	250	240
DEET	590	270	59	110
TCPP	930	1,030	1,550	1,390
Benzophenone	420	350	N.D	N.D
Gemfibrozil	4,070	3,840	62	100
Triclocarban	390	360	26	140
Triclosan	1,320	1,530	N.D	N.D

N.D. = Not detected

Conclusion

This application note provides a quick and sensitive method for monitoring trace levels of TOrCs in water. Twenty-one TOCs, which have been identified as wastewater indicator compounds such as artificial sweeteners, x-ray contrast media, and halogenated flame retardants were tested using an Agilent 1260 Infinity LC coupled with an Agilent 6490 Triple Quadrupole LC/MS. ESI+ and ESI- analysis with a fast-switching capillary reduced analysis time to less than 15 minutes. Calibration was linear, and quantification of all analytes were at ng/L levels with good recovery and Low %RSDs. Direct aqueous injection provides similar detection limits to those from conventional offline solid phase extraction. This method offers significant time, labor, and solvent savings while accurately detecting and quantifying TOCs in wastewater effluent.

References

1. M. Thurman, I. Ferrer, "EPA Method 538: Determination of selected Organic Contaminants in Drinking Water by Direct Aqueous Injection with the Agilent 6430 Triple Quadrupole LC/MS System", Agilent Application Note 5990-9670EN.

2. M. Thurman, I. Ferrer, "Direct Aqueous Analysis of Pharmaceuticals in Water at ppt Levels by LC/MS/MS with the Agilent 6490 Triple Quadrupole LC/MS System with Ion Funnel Technology", Agilent Application Note 5990-6431EN.

3. T. Anumol, S. Merel, S. Snyder, "High Sensitivity HPLC Analysis of Contaminants of Emerging Concern (CECs) in Water Using the Agilent 6460 Triple Quadrupole LC/MS System", Agilent Application Note 5991-1412EN.

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
August 22, 2013
5991-2732EN

Agilent Technologies