Identification of a Panel of 20 Indicator Compounds in Wastewater Effluent Using Rapid, Direct Injection LC/MS/MS on the Agilent 6490 Triple Quadrupole LC/MS

Application Note

Environmental

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Abstract

A rapid LC/MS/MS method for the detection of trace organic compounds in wastewater using direct aqueous injection and only 80 µL of sample has been used to identify a set of 20 indicator compounds for their presence in wastewater effluent. Cycle time was less than 14 minutes, and the method reporting limits (MRLs) of most compounds were from 3 to 39 ng/L. Matrix effects were less than 30 % for most compounds, and intra- and inter-day variation for all but one of the 20 indicator compounds was <5 % as well.

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Introduction

Wastewater effluents worldwide contain trace organic compounds (TOrCs), including pharmaceuticals, pesticides, industrial compounds, and personal care products. With implementation of water reuse schemes involving treated wastewater, it may be possible for TOrCs to be discharged into drinking water sources, providing a pathway to contaminate them. Such contamination has been documented by several studies in the US [1]. This problem will only get worse as freshwater sources dwindle, and several thousand new chemicals are introduced into the environment each day.

While some TOrCs have known adverse health effects on wildlife and humans, the risk of long-term exposure to mixtures of these compounds at low concentrations in drinking water is unknown. However, given the large and increasing number of TOrCs, it is not practical to monitor each of them in water supplies. A list of indicator compounds that signal the presence of wastewater contamination in drinking supplies is needed. Potential indicator compounds can be evaluated on the basis of frequency of occurrence, risk assessment, impact of environmental conditions, and wastewater treatment efficacy.

This application note summarizes a published study to identify a practicable indicator compound list [1] using a literature review of frequency of detection and occurrence in US wastewater effluents, followed by a monitoring study of four wastewater treatment plants to validate the utility of the list.

Conventional analysis of TOrCs often involves laborious and time-consuming extraction and concentration steps that can increase analysis cycle time and degrade reproducibility. The method used in this study eliminates the extraction step by using direct large volume injection of sample into the high performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) system, thus optimizing reproducibility and decreasing analysis time. The volume of sample required is still quite small, eliminating the need for large volume sample collection, and reducing the amount (and cost) of isotopically labeled surrogate standard required. The simultaneous positive and negative ionization feature of the Agilent 6490 Triple Quadrupole LC/MS with fast polarity switching and Dynamic MRM enabled rapid optimization of acquisition parameters for all 20 compounds, and contributed to the short cycle time of 13 minutes.

This method enabled the identification of 20 TOrCs, all of which were detected at least once in a minimum of three of the four wastewater plants. Some of the compounds were present in all samples analyzed and at high enough concentrations to be detected in drinking water after dilution.

Experimental

Reagents and standards

All reagents and standards were obtained as described [1]. Isotopically labeled surrogate standards (ILSS) were used to determine matrix effects.

Instruments

The analysis method was performed using an Agilent 1260 Infinity HPLC pump fitted with a 100 μL syringe loop. The chromatography column (Agilent Pursuit XRs C-8, 100 × 2.0 mm, 3 μm) was coupled to an Agilent 6490 Triple Quadrupole LC/MS system. The system operating conditions were optimized using the Agilent Source Optimizer feature, and are shown in Table 1.

Sample collection and preparation

At four different times over the course of a year, grab samples were taken from four different wastewater treatment plant effluents. All samples were collected between 8 and 10 AM to avoid diurnal variations. Ascorbic acid and sodium azide were added as a quenching agent and microbial growth inhibitor, respectively. One mL of each sample was spiked with an ILSS mixture at 2 μg/L and passed through an Agilent 0.2 μm polyethylene sulfonate (PES) syringe filter into an autosampler vial for analysis.

Table 1.   HPLC and MS Instrument Conditions

HPLC conditions

Analytical column	Agilent Pursuit XRs C-8, 100 × 2.0 mm, 3 µm (p/n A6011100X020)
Injection volume	80 µL
Column temperature	30 °C
Mobile phase	A) 0.1 % (v/v) formic acid in water B) Acetonitrile
Run time	11.5 minutes + 1.5 minutes post time = 13 minutes cycle time
Flow rate	0.4 mL/min
Gradient	Time (min) %B 0 2 1.5 2 8 60 10.5 100 11.5 2
Post time	1.5 minutes

MS conditions

	ESI Positive	ESI Negative
Acquisition parameters	ESI mode, simultaneous positive and negative ionization with fast polarity switching; Dynamic MRM	
Delay time	0.5 minutes	
Sheath gas temperature	350 °C	350 °C
Sheath gas flow rate	11 L/min	11 L/min
Drying gas temperature	275 °C	275 °C
Drying gas flow rate	18 L/min	18 L/min
Nebulizer pressure	45 psig	45 psig
Nozzle voltage	1,500 V	0 V
Vcap	3,000 V	3,000 V
High pressure RF	150 V	90 V
Low pressure RF	60 V	60 V
Δ EMV	400 V	400 V

Analysis parameters

Table 2 shows the multiple reaction monitoring (MRM) transitions for the 20 compounds selected for the indicator compound list and their surrogate internal standards.

Table 2.   MRM ESI Analysis Parameters

Compound	Retention time (min)	Precursor ion (m/z)	Product ion (m/z)	Fragmentor voltage (V)	Collision energy (V)	ESI Mode
Acesulfame	5.2	162	82.1	380	13	Negative
Acesulfame-d_4	5.2	166.1	86.1	380	10	Negative
Atenolol	4.2	267.1	190.1 145	380 380	15 20	Positive Positive
Atenolol-d_7	4.2	274	190.1	380	15	Positive
Caffeine	5.4	195.1	138 110.1	380 380	24 16	Positive Positive
Caffeine-$^{13}C_3$	5.4	198.1	140	380	16	Positive
Carbamazepine	8.0	237	194 179	380 380	15 35	Positive Positive
Carbamazepine-d_{10}	8.0	247	204	380	15	Positive
DEET	8.8	192	119 91	380 380	15 30	Positive Positive
DEET-d_6	8.8	198	119	380	15	Positive
Diclofenac	10.3	294	250 214	380 380	4 16	Negative Negative
Diphenhydramine	6.5	256.2	167.1 165.1	380 380	4 44	Positive Positive
Diphenhydramine-d_5	6.5	261.2	172.1	380	4	Positive

Compound	Retention time (min)	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision energy (V)	ESI Mode
Gemfibrozil	10.8	249.2	121	6	Negative
Gemfibrozil-d$_6$	10.8	255	121	6	Negative
Hydrochlorothiazide	6.0	296	268.9	10	Negative
			204.7	15	Negative
Iohexol	4.2	821.9	803.8	20	Negative
Iopromide	4.8	791.8	572.8	22	Positive
			558.8	28	Positive
Iopamidol-d$_3$	2.3	781	562	22	Positive
Meprobamate	7.0	219	158	5	Positive
			55	20	Positive
Meprobamate-d$_3$	7.0	222.1	161.1	5	Positive
Primidone	6.3	219.3	162.1	9	Positive
			91.1	25	Positive
Sucralose	5.9	419	239	15	Positive
			221	15	Positive
Sucralose-d$_6$	5.9	425	243	15	Positive
Sulfamethoxazole	7.1	254	156	10	Positive
			92	30	Positive
Sulfamethoxazole-^{13}C$_6$	7.1	260	162	10	Positive
TCEP	9.0	285	223	10	Positive
TCEP-d$_{12}$	8.6	297	232	13	Positive
TCPP	9.8	327	99	16	Positive
			81	70	Positive
Triclocarban	11.0	313	160	5	Negative
			126	25	Negative
Triclocarban-^{13}C$_6$	11.0	318.9	160	5	Negative
Triclosan	11.1	289	37	5	Negative
		287	35	5	Negative
Triclosan-^{13}C$_{12}$	11.1	299	35	5	Negative
Trimethoprim	5.1	291	261	25	Positive
			230	25	Positive
Trimethoprim-d$_3$	5.1	294	264	25	Positive

Results and Discussion

Selection of the indicator compounds

Based on a literature search of US studies, a list of 48 potential indicator TOrCs was assembled and assessed using a priority scoring system (PSS). It was based on the detection frequency (DF) across the studies, mean concentration (Occurrence), and number of relevant studies (Research) in which the compound was detected in wastewater effluents. Each of the three criteria was given a maximum score of 3, so that the highest PSS that a compound could attain was 9. Selecting only those compounds with a score >6, 20 candidate indicator compounds were chosen, as shown in Table 3.

Table 3.   Twenty Indicator Compounds Selected from a Review of US Studies

Compound	Score			
	DF*	Occurrence†	Research‡	Total
Acesulfame	3	3	1	7
Atenolol	3	3	2	8
Caffeine	1	3	3	7
Carbamazepine	3	1	3	7
DEET	3	3	3	9
Diclofenac	2	2	3	7
Diphenhydramine	3	3	1	7
Gemfibrozil	2	2	3	7
Hydrochlorothiazide	3	3	1	7
Iohexol	3	3	1	7
Iopromide	3	2	2	7
Meprobamate	3	2	2	7
Primidone	3	2	2	7
Sucralose	3	3	2	8
Sulfamethoxazole	3	3	3	9
TCEP	3	2	2	7
TCPP	3	3	2	8
Triclocarban	3	2	2	7
Triclosan	3	1	3	7
Trimethoprim	3	1	3	7

* DF (detection frequency) across the US studies: 1 (51–70 %), 2 (71–85 %), 3 (>85 %)
† Occurrence scoring (based on mean concentration): 1 (51–200 ng /L), 2 (201–500 ng /L), 3 (>500 ng/L)
‡ Research scoring (based on no. of studies): 1 (2–4), 2 (5–9), 3 (>9)

Method performance

The method reporting limit (MRL) and instrument limit of quantification (LOQ) for each analyte were determined as described [1], and are shown in Table 4. The MRLs for the 20 compounds covered a range from 3 ng/L to 37 ng/L, with the exception of sucralose (Table 4). While the MRL for sucralose was relatively high (302 ng/L), the levels detected in wastewater effluent are several times higher. The LOQs for most of the 20 compounds were <20 ng/L, and they ranged from 2 to 100 ng/L (Table 4).

Table 4.   MRLs and LOQs for the 20 Indicator Compounds

Indicator compound	MRL (ng/L)	LOQ (ng/L)
Acesulfame	4	5
Atenolol	37	20
Caffeine	8	5
Carbamazepine	3	2
DEET	34*	10
Diclofenac	14	10
Diphenhydramine	7	5
Gemfibrozil	23	50
Hydrochlorothiazide	15	10
Iohexol	13	10
Iopromide	39	20
Meprobamate	8	10
Primidone	9	10
Sucralose	302	100
Sulfamethoxazole	5	5
TCEP	20	20
TCPP	22*	10
Triclocarban	14	10
Triclosan	15	10
Trimethoprim	11	5

*MRL adjusted to 2× blank level due to low level instrument contamination.

Spike recovery was determined for all 20 indicator compounds in effluent from two wastewater treatment plants (WWTPs), at 1,000 and 5,000 ng/L. With the exception of sucralose (67 % at 5,000 ng/L for WWTP 1) and iohexol (46 % at 1,000 ng/L for WWTP 1), the recoveries ranged from 83 to 138 % for the two WWTPs at both spiking levels. The WWTP 1 effluent contained concentrations more than 20 times higher than the lower spiking level for sucralose and iohexol, which may explain their low recoveries. Precision of spike recovery measurements was quite good, with most of the 20 compounds having relative standard deviations (RSDs) <5 % at each spiking level and each WWTP.

Matrix effects in wastewater effluent were determined using the ratio of area counts for a spiked isotopically labeled surrogate standard (ILSS) in effluent over the area counts of the same ILSS spiked into ultrapure water. Most of the 20 indicator compounds exhibited matrix effects <30 % in wastewater, although the results indicated higher suppression (up to 60 %) for the iodinated X-ray contrast media iohexol, iopamidol, and iopromide (Figure 1). Wastewater matrix enhanced signals for acesulfame and sucralose, while the other 18 compounds exhibited matrix suppression.

Using calibration curves starting just above the MRL for each compound and extending to 10 µg/L, 15 of the 20 indicator compounds had correlation coefficients (R^2) >0.995, with all of them having R^2 values >0.99 (Table 5). The precision of quantification was determined at 2 µg/L using standards for each compound, and the relative standard deviations (RSDs) were all <5 % for intra-day determinations (Table 5). Of the 20 compounds, 14 had inter-day RSDs <5 %, and all of them had inter-day RSDs <10 % except TCEP (14 %) and sucralose (12 %).
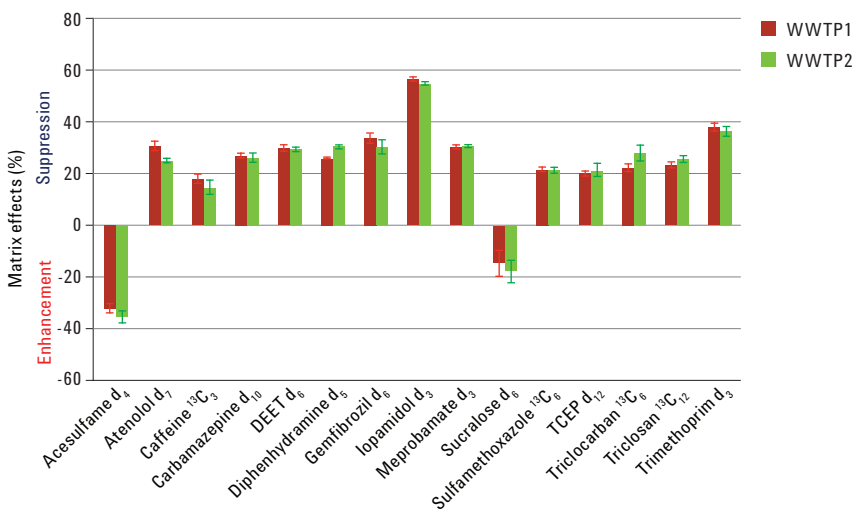

Figure 1.   Matrix effects in wastewater effluent on isotopically labeled surrogate standards (ILSS) (n = 5).

Table 5. *Precision and Linearity of Calibration for the 20 Indicator Compounds*

Compound	Inter-day variability (%)	Intra-day variability (%)	Linearity (R^2)
Acesulfame	1.4	0.35	0.9987
Atenolol	7.7	1.3	0.9964
Caffeine	0.69	3.8	0.9967
Carbamazepine	6.2	1.5	0.9989
DEET	0.47	4.6	0.9951
Diclofenac	2.0	1.0	0.9982
Diphenhydramine	2.7	2.1	0.9904
Gemfibrozil	3.3	3.8	0.9974
Hydrochlorothiazide	1.0	2.5	0.9983
Iohexol	0.48	1.9	0.9988
Iopromide	2.2	1.8	0.9975
Meprobamate	1.2	1.5	0.9975
Primidone	0.12	2.7	0.9963
Sucralose	12	0.55	0.9970
Sulfamethoxazole	0.30	2.2	0.9971
TCEP	14	0.84	0.9974
TCPP	5.6	2.4	0.9924
Triclocarban	9.9	2.3	0.9929
Triclosan	1.1	1.0	0.9927
Trimethoprim	0.97	2.8	0.9941

Wastewater effluent analysis

Effluent sampling and analysis was done across four WWTPs, with a total of four samples taken from each plant over the course of a year. All of the 20 TOrC indicator compounds were detected at least once in a minimum of three of the four plants. Compounds such as hydrochlorothiazide, acesulfame, sucralose, TCPP, and iohexol and were detected in all samples at high enough concentrations to theoretically be detected when diluted into drinking water supplies. These are good candidates for universal indicators of wastewater contamination.

Other indicator compounds were often present at concentrations above their MRLs, making them useful as indicators of WWTP treatment process efficacy, usage and consumption patterns, and seasonal variation. These include triclosan, sulfamethoxazole, caffeine, carbamazepine, and DEET. This 20-compound indicator set is divided into groups of chemicals that are in the same or similar class to provide redundancy, and allow for seasonal and geographic differences in the compound composition of wastewater effluent. A robust indicator list such as the one identified in this study is essential for studying the fate and attenuation of TOrCs and their influence on water reuse.

Conclusions

A rapid and robust method for evaluating a set of potential indicator compounds for wastewater effluent has been developed using direct injection LC/MS/MS. Only 80 µL of sample is used, eliminating the need for tedious extraction steps that can increase analysis time, decrease throughput, and reduce the accuracy and precision of the method. MRLs ranged from only 3 to 37 ng/L for all but one of the 20 indicator compounds (sucralose at 302 ng/L). Precision for most compounds was <5 % for intra- and inter-day variation. Matrix effects were well characterized and limited to less than 30 % change in signal for most compounds. This method enables the use of a set of indicator compounds to detect wastewater contamination of drinking water supplies, as well as for monitoring the efficiency and seasonal and geographical variations of wastewater treatment plant processes.

Reference

1. T. Anumol, *et al*. "Rapid direct injection LC-MS/MS method for analysis of prioritized indicator compounds in wastewater effluent" *Environ Sci: Water Res Technol DOI: 10.1039/c5ew00080g* (2015).

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