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Rapid analysis of trace organic compounds in water by automated online solid-phase extraction coupled to liquid chromatography–tandem mass spectrometry

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ABSTRACT

A fully automated online solid-phase extraction (SPE) with directly coupled liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for analysis of 34 trace organic compounds in diverse water matrices has been developed. The current method offers several advantages over traditional offline SPE methods including low sample volume (1.7 mL), decreased solvent use, higher throughput, and increased reproducibility. The method uses simultaneous positive and negative ESI for analysis of all compounds in one injection, which reduces cycle time (extraction+analysis) to <15 min. Method optimization included testing different online SPE cartridges, mobile phase compositions, and flow rates. The method detection limits (MDLs) ranged from 0.1 to 13.1 ng/L with 80% of the compounds having an MDL <5 ng/L. Matrix spike recoveries in three different water qualities were evaluated and ranged from 61.2% to145.1% with 95% of the recoveries ranging between 70–130%. As part of the method validation studies, linearity (0.9911–0.9998), intra-day variability (1.0–10.4%), inter-day variability (1.0–11.9%), and matrix effects were also assessed. The use of 26 isotopically-labeled standards increased the reliability of the method while retention time locking and use of two transitions for most compounds increased the specificity. The applicability of the method was tested on samples across treatment points from two wastewater plants, a septic tank, surface water and groundwater.

1. Introduction

The extensive utilization of organic compounds in modern society combined with increasing demands for water resources have resulted in widespread contamination of water resources. Many of these compounds have anthropogenic sources and are being produced in greater quantities than ever before around the globe [1,2]. These compounds including pharmaceuticals, personal-care products, pesticides, hormones and industrial contaminants, and are collectively termed as trace organic compounds (TOrCs). Several studies have shown that conventional water treatment processes generally are not effective in removal of TOrCs thus leading to their release into the environment with potential for contamination of drinking water sources [3,4]. While the adverse effects associated with exposure to individual compounds from drinking water is not expected to pose significant risk to public health [5], the long term exposure to mixtures of TOrCs and potential for synergistic effects is largely unknown [6–8]. Due to the vast number of TOrCs detected in water, regulatory actions to determine acceptable levels of all detected TOrCs is not feasible despite studies documenting adverse effects to the environment and wildlife [9–11]. Thus, it is prudent to monitor indicator TOrCs in water while sufficient toxicological data can be collected and studies on any potential mixture effects performed.

Currently, methods utilizing liquid chromatography–tandem mass spectrometry (LC–MS/MS) are considered the gold standard for sensitive analysis of multiple TOrCs in water [12–14]. However, to achieve requisite detection limits of low ng/L for many TOrCs, time consuming sample preparation steps are often required before LC–MS/MS analysis. The sample preparation step generally involves extraction and concentration of the target analytes with commensurate elimination of many interferences in the original matrix. Traditional off-line solid-phase extraction (SPE) and liquid–liquid extraction (LLE) are the most commonly used concentration and clean-up steps for analysis of TOrCs in environmental matrices [12,15,16]. These two techniques require a relatively large sample volume, are extremely time consuming, laborious, and require considerable amounts of organic solvents to perform. Further, these methods can potentially decrease the reproducibility and accuracy of analysis due to multiple sample manipulations that are required [17]. Considering the high degree of temporal [18] and geographical [19] variability, high-throughput time-sensitive analytical screening

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methods would greatly advance the resolution of environmental monitoring. Also, considering the variability of methods currently used for TOrC analyses [20], the reproducibility and accuracy of the data must be enhanced by introducing automation into the entire analytical procedure.

The online SPE process offers several advantages over the offline techniques including reduction in sample preparation and thus increase in productivity [21]. Further, online SPE minimizes operator contact which reduces the possibility of contamination while also eliminating a solvent evaporation step that can result in loss of target analytes [22]. The introduction of the entire mass of analyte to the detector allows injection of much lower sample volumes while increasing the sensitivity of analysis [23]. Automation of the entire process (extraction+analysis) also improves the reproducibility of the method. In addition, online SPE cartridges can be reused as they are amenable to backwashing and have been shown to perform well even after treating several hundred water samples [24].

Over the last few years, several manufacturers have developed fully automated online SPE systems coupled to LC–MS/MS which has allowed for integration of sample clean-up, extraction and analysis. Consequently the use of online SPE methods in bioanalytical [25,26] and environmental [21,27] applications has increased. However, most of these methods have focused on analysis of specific classes of TOrC such as pharmaceuticals [28–30], hormones [24,31] or pesticides [32] only. Other methods still require multiple injections for analysis of all analytes [33], large sample volumes [34], require derivitization [35], or are not completely automated which may reduce the reproducibility and/or increase time of analysis.

The focus of this study was to develop a single method for rapid analysis of 34 indicator compounds comprising several classes of TOrCs in different water matrices with a fully automated online SPE coupled to LC–MS/MS. The objectives were to (i) use low sample volume (<2 mL), (ii) employ minimal sample preparation and automate the extraction step to reduce variability, (iii) perform simultaneous analysis of TOrCs both in electrospray (ESI) positive and negative mode with one injection, (iv) rapid cycle (extraction+analysis) time (<20 min) for high throughput analysis of samples, (v) achieve desired sensitivity of low ng/L detection limits for all analytes, and (vi) provide 'cleaner' and 'greener' methods for analysis of TOrCs in aqueous matrices compared to conventionally used offline pre-concentration techniques.

2. Experimental

2.1. Chemicals and reagents

All analytical standards used during the study were at least $>97\%$ purity and every effort was made to use standards of the highest purity commercially available. All native standards were purchased from Sigma-Aldrich (St. Louis, MO) except DEET and triclosan from Alfa Aesar (Ward Hill, MA) and meprobamate from Cerilliant (Round Rock, TX). Labeled surrogate standards were purchased from Cambridge Isotope Laboratories (Andover, MA) except $^{13}C_2$-PFHxA, $^{13}C_4$-PFOA and $^{13}C_4$-PFOS from Wellington Laboratories (Ontario, Canada); primidone-d_5, meprobamate-d_3, triclocarban-$^{13}C_6$ and $^{13}C_6$-diclofenac from Toronto Research Chemicals (Ontario, Canada); and gemfibrozil-d_6, benzophenone-d_{10}, benzotriazole-d_4, and diphenhydramine-d_5 from C/D/N Isotopes (Quebec, Canada). All solvents used were of purity suitable for LC–MS analysis. Methanol, isopropyl alcohol (IPA), and acetic acid were obtained from Fisher Scientific (Pittsburgh, PA), while acetonitrile (ACN) and HPLC grade water were purchased from Burdick and Jackson (Muskegon, MI). Detailed information about all target analytes and isotopes used in this study are shown in Table 1.

2.2. Selection of target list

The selection of TOrCs analyzed in this study was based on data presented in previous literature that rely on occurrence data, detection frequency, toxicological relevance, availability of robust analytical methods and ability to pose as 'indicators' of water quality [36–38]. Additionally, emerging classes of TOrCs like perfluorinated compounds and glucocorticoid steroids were also represented in our target list to show that the method can be used for their detection. The aim was to select a diverse list of compounds in terms of physical, chemical properties while representing traditionally monitored and frequently detected TOrCs along with newer classes of interest.

2.3. Sample collection and preparation

During this study, grab samples were collected from two wastewater treatment plants, a surface water, groundwater, and septic tank from various locations across the USA. All samples were collected in 20 mL amber glass vials dosed with 1 g/L of sodium azide as a preservative and stored in ice before transport to the laboratory. The samples were stored in a refrigerator at 4 °C on arrival at the lab. Five-ml of sample was then aliquoted into a 6-ml glass vial before being spiked with a mixture of all isotopically-labeled surrogates to achieve a final concentration of 100–200 ng/L in the sample depending on the type of analyte and sample matrix. All samples were doped with the surrogate standards within 72 h of collection to account for losses due to storage and degradation of analytes. The samples were then filtered using a 0.2 μm Captiva polyethylene styrene (PES) filter from Agilent Technologies (Santa Clara, CA) prior to analysis. All samples were analyzed within two weeks of collection.

2.4. Online SPE configuration

Automated pre-concentration of all samples was performed using an Agilent flexible cube (FlexCube) module coupled to a large volume autosampler (900 μL) with a programmable multi-draw capacity. In this method, 1.7 mL of sample was injected into the sample loop using two 850 μL autosampler injections. A schematic of the different valve positions on the online SPE system is shown in Supplementary materials (Fig. S1). The FlexCube online SPE unit consists of a built in single piston pump with four solvent lines and a 10-port switching valve that houses two online SPE cartridges for simultaneous loading of one cartridge while the other is being eluted or backwashed. Initially, the switching valve on the FlexCube was set to the LOAD position (position 1) during which the sample was loaded onto the SPE cartridge with 95/5 (v/v) water/ACN mixture containing 0.1% acetic acid (line A) from the FlexCube pump. During this time, the aqueous waste and matrix not retained on the SPE cartridge was allowed to go to waste. After loading was complete, the valve switched to the ELUTE position which resulted in the binary pump back-flushing SPE cartridge 1 to allow a gradient elution of the target analytes onto the analytical column. At the same time, SPE cartridge 2 was being cleaned with a strong solvent mix of 1:1:1 (v/v/v) isopropyl alcohol, methanol and ACN (line B) by the FlexCube pump to remove any retained target analytes or matrix from causing blank contamination and interferences in subsequent runs. A few minutes before the end of the run, the solvent channel on the FlexCube pump was switched back to its original line (line A) to allow SPE cartridge 2 to equilibrate and prepare it for the next sample.

Table 1
Class and use of TOrCs selected in this study with isotopes used for quantification.

Compound	Use	Category	Isotope[a]
Atenolol	β-Blocker	Pharmaceutical	Atenolol-d_7
Carbamazepine	Anti-seizure	Pharmaceutical	Carbamazepine-d_{10}
Clofibric acid	Lipid regulator metabolite	Pharmaceutical	Bisphenol A-$^{13}C_{12}$
Diclofenac	Anti-arthritic	Pharmaceutical	Diclofenac-$^{13}C_6$
Diphenhydramine	Antiarrhythmic	Pharmaceutical	Diphenylhydramine-d_5
Diltiazem	Anti-histamine	Pharmaceutical	Diltiazem-d_3
Fluoxetine	Anti-depressant	Pharmaceutical	Fluoxetine-d_5
Gemfibrozil	Anti-cholesterol	Pharmaceutical	Gemfibrozil-d_6
Hydrochlorothiazide	Antihypertensive	Pharmaceutical	Benzotriazole-d_4
Ibuprofen	Analgesic	Pharmaceutical	Ibuprofen-d_3
Meprobamate	Anti-anxiety	Pharmaceutical	Meprobamate-d_7
Naproxen	Analgesic	Pharmaceutical	Naproxen-$^{13}C_1d_3$
Primidone	Anticonvulsant	Pharmaceutical	Primidone-d_5
Propranolol	β-Blocker	Pharmaceutical	Atenolol-d_7
Sulfamethoxazole	Antibiotic	Pharmaceutical	Sulfamethoxazole-d_6
Trimethoprim	Antibiotic	Pharmaceutical	Trimethoprim-d_3
Benzophenone	UV blocker	Personal care product	Benzophenone-d_{10}
Caffeine	Stimulant	Personal care product	Caffeine-$^{13}C_3$
N,N-Diethyl-m-toluamide (DEET)	Insect repellant	Personal care product	DEET-d_6
Propylparaben	Preservative in cosmetics	Personal care product	Propylparaben-d_4
Tris (2-chloroethyl) phosphate (TCEP)	Flame retardant	Personal care product	TCEP-d_{12}
Tris (2-chloropropyl) phosphate (TCPP)	Flame retardant	Personal care product	TCEP-d_{12}
Triclocarban	Antibiotic	Personal care product	Triclocarban-$^{13}C_6$
Triclosan	Anti-microbial	Personal care product	Triclosan-$^{13}C_{12}$
Benzotriazole	Corrosion inhibitor	Industrial compound	Benzotriazole-d_4
Bisphenol A	Plasticizer	Industrial compound	Bisphenol A-$^{13}C_{12}$
Perfluoro hexanoic acid (PFHxA)	Fluorosurfactant	Industrial compound	PFHxA-$^{13}C_2$
Perfluoro octanoic acid (PFOA)	Fluorosurfactant	Industrial compound	PFOA-$^{13}C_4$
Perfluoro octane sulfonate (PFOS)	Fluorosurfactant	Industrial compound	PFOS-$^{13}C_4$
Hydrocortisone	Anti-inflammatory glucocorticoid	Hormone	Carbamazepine-d_{10}
Norgestrel	Hormonal contraceptive	Hormone	Primidone-d_5
Testosterone	Androgen	Hormone	Carbamazepine-d_{10}
Atrazine	Herbicide	Pesticide	Atrazine-d_3
Simazine	Herbicide	Pesticide	Atrazine-d_3

[a] Used for quantification.

2.5. Liquid chromatography

An Agilent 1290 UHPLC binary pump was used to perform liquid chromatography for all analyses. Polypropylene solvent lines with metal frits were used to minimize potential system contamination of perfluorinated materials. An Agilent Poroshell 120 EC C-18 (2.1 mm × 50 mm, 2.7 μm) column was used for chromatographic separation of all analytes. The column was maintained at 30 °C throughout the run. A dual eluent mobile phase comprising of water with 0.1% acetic acid (A) and ACN with 0.1% acetic acid (B) at 350 μL/min was used for separation. For the first 4 min, the gradient was held at 5% B while the sample was loaded onto the online SPE cartridge and the binary pump was conditioning cartridge 2. At 4 min, the switching valve turned to the ELUTE position (position 2) and solvent B was linearly increased to 100% at 11 min. This gradient was held till 12 min before returning to the initial condition at 12.5 min. A post-time of 2 min was added to allow the column to re-equilibrate before the next analysis. This resulted in a total cycle time (extraction + analysis) of 14.5 min per sample.

2.6. Mass spectrometry

Mass spectrometry was performed on an Agilent 6460 triple quadrupole mass spectrometer. The optimization of the mass spectrometer was divided into two: (i) compound-specific optimization and (ii) source-dependent optimization. Details of the optimization process have been published previously [14]. The optimized compound parameters and retention times (RT) are shown in Supplementary material (Table S1) while source-dependent parameters for both ESI positive and negative modes (run simultaneously) are shown in Table S2.

The mass spectrometer was run in dynamic multiple reaction monitoring (DMRM) mode with a delta RT of 0.7 min for each compound. Fast polarity switching with the dielectric capillary allowed for simultaneous analysis in ESI positive and negative in the same run. Two transitions: a quantifier (most-abundant product) and qualifier were used for most of the compounds to increase specificity of the method. Data acquisition and analysis was performed using Agilent MassHunter software (version Rev B.06.00). Isotope dilution was used for quantification of all analytes [39]. In cases where the identical isotopic standard was not available, a closely-related isotope was used as a replacement (Table 1). RT locking and product ion ratio monitoring reduced the possibility of false positives in the method.

3. Results and discussion

3.1. Solid phase extraction cartridge selection

The selection of the online SPE cartridge is critical to the success of the entire analysis. Presently, the availability of different online SPE cartridges is significantly lower than conventional offline cartridges. Three online SPE cartridges from Agilent and an experimental cartridge packed in the lab were tested for their suitability to extract the target analytes from aqueous samples. PLRP-s (styrene-polydivinylbenzene, 2.1 mm × 12.5 mm, 15–20 μm), ZORBAX SB-AQ (reversed-phase alkyl group bonded to high-purity porous silica microsphere, 2.1 mm × 12.5 mm, 5 μm), ZORBAX Phenyl-hexyl (dimethylphenylhexylsilane, 2.1 mm × 12.5 mm, 5 μm) from Agilent and Carbon-X (experimental phase with activated charcoal, 2.1 mm × 12.5 mm 5 μm) were evaluated. To

assess which of these cartridges was most appropriate for extraction of target analytes, the absolute recoveries were calculated by comparing the peak areas obtained in online SPE mode and direct injection mode with the same amount of analyte in ultrapure water in five replicate injections. Fig. 1 shows the range of absolute recoveries obtained for the 32 target analytes tested with each cartridge (propranolol and hydrochlorothiazide were not tested). An absolute recovery between 70–130% was considered as acceptable and used as the criteria for cartridge selection. The PLRP-s cartridge had 26 analytes in this range and good reproducibility (RSD < 10%) for all but three analytes (bisphenol A, propylparaben and TCEP) so was the most suitable choice. The SB-AQ cartridge performed similarly but had slightly less reproducibility while extraction efficiency of some compounds with –NH group was lower than the PLRP-s. The Carbon-X cartridge had poor extraction recoveries (< 50%) for several of the analytes and it was later discovered that many of the target analytes were very strongly bound onto the cartridge and not entirely eluted. This was confirmed by passing 100% ACN through the Carbon-X cartridge at the end of the five replicates and monitoring the spectrum. Further, several analytes (like meprobamate, atrazine and carbamazepine) had very high extraction recoveries (> 150%) and large %RSDs probably due to carryover from one injection to the next for the Carbon-X. It should be noted that low absolute recoveries for some compounds are automatically corrected as both the calibration standards and samples are processed in exactly the same manner and go through the entire analytical process (extraction + analysis) in an automated online SPE setup. However, it is always desirable to get the maximum recoveries for best sensitivity but with a highly diverse analyte list trade-offs are inevitable. With this in mind, the PLRP-s cartridge was selected for all further analyses. The complete dataset of absolute recoveries for all four online SPE cartridges is provided in the supplementary material (Table S3).

3.2. Washing mobile phase composition and volume

The presence of organic substances in the matrix can cause severe matrix suppression especially in complex water matrices such as wastewater [40]. Hence a wash step is often needed after the sample has been loaded onto the online SPE cartridge. The wash mobile phase composition in the FlexCube pump was tested at three different ACN concentrations (0%, 5% and 10%) in water with 0.1% acetic acid. An aqueous mobile phase of 5% ACN with 0.1% acetic acid was finally used as it provided the best recoveries with high reproducibility. The 10% ACN mobile phase resulted in significant loss of the early-eluting polar compounds while the 0% ACN phase had lower recoveries for the apolar compounds. Similarly, the wash volume is another important parameter for optimization as too small a volume may not eliminate the interferences in the matrix while too large a volume can cause the target analytes to be washed off the cartridge. Six different wash volumes (2, 2.5, 3, 3.5, 4, and 5 mL) were tested with the loading flowrate maintained at 1 mL/min. Fig. 2 provides the range of absolute recoveries of all target analytes with the different wash volumes. It was found that a 4 mL wash volume provided best recoveries for most of the target analytes. The 2 mL load volume had poor recoveries for all compounds tested possibly due to the fact that there was not enough washing time to elute the target analytes off the cartridge. The 5 mL load volume had very low recoveries for the polar compounds that were likely washed out of the cartridge into waste before the switching valve sent the sample onto the analytical column. The supplementary material (Table S4) contains information of specific recoveries of each analyte with all six loading volumes.

3.3. Loading flowrate

In online SPE analysis, the autosampler injects the sample into the large-volume loop which is then carried onto the cartridge by the loading mobile phase solvent from the FlexCube pump. A high flowrate may result in less adsorption of target analytes onto the cartridge while a slow flowrate increases time of analysis and also provides competing matrix elements time to attach onto the cartridge. In this study, three different loading flowrates were tested by comparing the peak areas of each analyte with direct chromatographic injection. This analysis was run in multiple-reaction monitoring (MRM) mode where RTs were not locked due to the fact that different flowrates with same loading volume

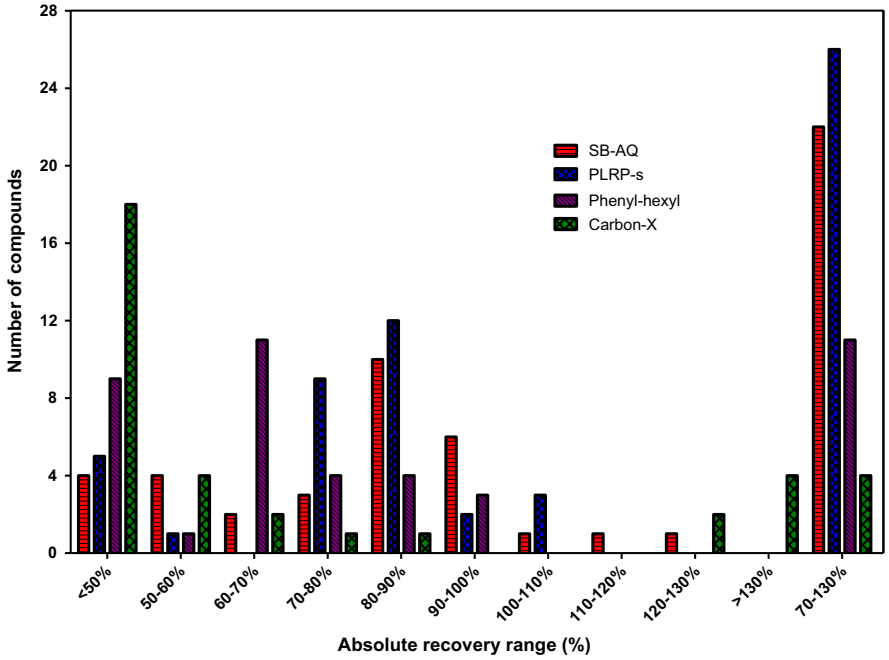

Fig. 1. Range of absolute recoveries for target analytes with four different online SPE cartridges.

caused the target analyte RT to shift. The absolute recoveries of all target analytes for the three different loading flowrates in order of increasing RT is illustrated in Fig. 3. It was found that the 1 mL/min flowrate provided best recoveries for the target analytes compared to the other flowrates. After all replicates for the condition were run, a set of blanks were injected to look for carryover. No carryover was found in the 1 mL/min samples indicating that the strong solvent mix was successful in washing any remaining analytes of the cartridge. The 1.5 mL/min flowrate had higher recoveries for the late-eluting analytes but some recoveries were extremely high with low reproducibility possibly due to carryover from previous injections. However, since 1 mL/min provided better results the potential carryover for the 1.5 mL/min flowrate was not investigated further.

3.4. Limit of detection (LOD) and method detection limit (MDL)

The instrument limit of detection (LOD) was based on an analyte signal to noise ratio (SNR) greater than three. A set of standards at 0.1, 0.2, 0.5, 1.0, 2.5, 5.0, 10 and 20 ng/L were analyzed to determine the LOD. The method detection limit (MDL) was calculated based on previous literature [41] by injecting eight

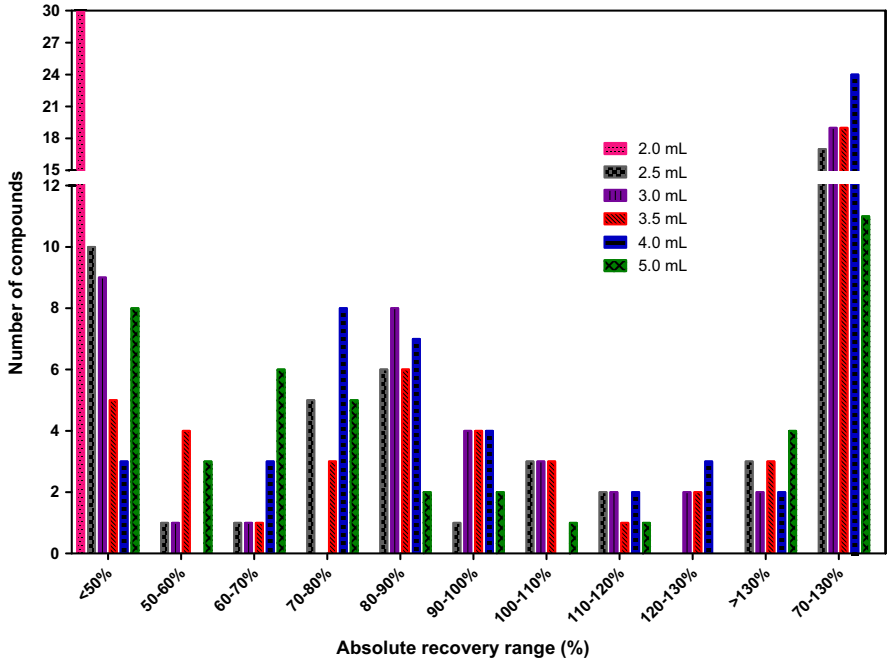

Fig. 2. Range of absolute recoveries for target analytes with different loading volumes.

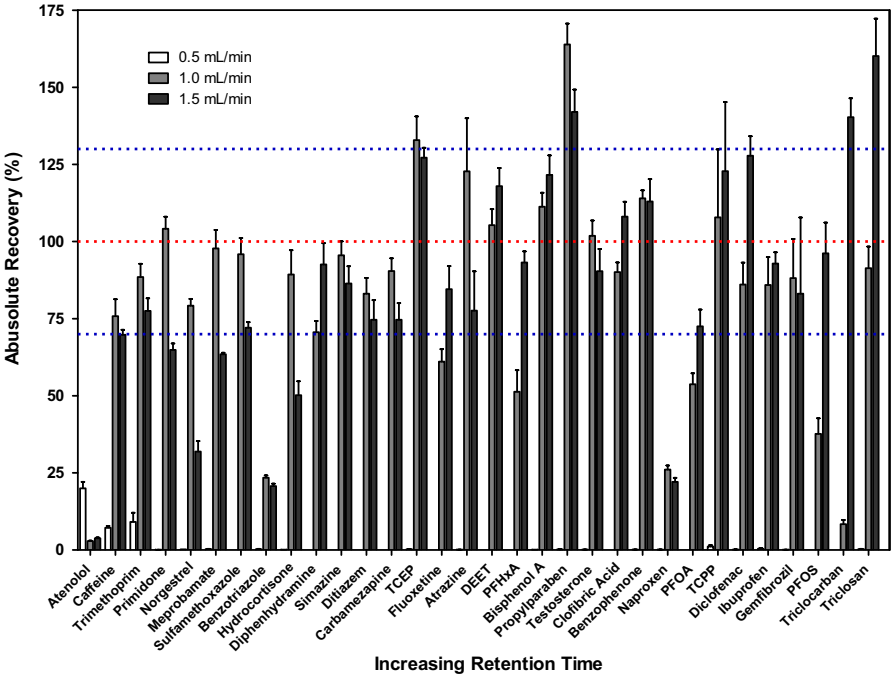

Fig. 3. Absolute recoveries of target analytes with three loading flowrates.

samples fortified with isotopically-labeled surrogate standards and target analytes at 2–5 times the LOD. The standard deviation of the eight replicates was multiplied by the student's *t*-test value for $n-1$ degrees of freedom at 99% confidence levels to determine the MDL of each target analyte. The calculated MDL for most analytes was <5 ng/L with only four compounds (benzophenone, benzotriazole, bisphenol A, and norgestrel) being >10 ng/L. The MDLs in this study (Table 2) were comparable and in most cases lower than previously published literature while using lower sample volumes of 1.7 mL [33,42]. A further advantage of using low sample volume was that the mass of isotopically-labeled standard added to each sample was much lower when compared to other published methods. The cost of isotopically-labeled IS standards can be prohibitively expensive often resulting in having to select a few IS to represent many compounds. With addition of lower masses of IS per sample, analysis costs are significantly lowered.

The method reporting limits (MRL) were determined individually for each sample as each water quality has different matrix effects on each analyte. When analyzing several different samples, it is not practically feasible to determine the MRL for each water quality. Hence, this method has been used to provide a realistic MRL for each sample while avoiding time-consuming MRL studies for each specific water quality. The details of this process have been described earlier [14]. Briefly, to calculate the MRL, the lowest calibration standard (usually at or close to MDL) was divided by isotope standard recovery (calculated by comparing peak area in ultrapure water) in that matrix to give the true MRL for each analyte in a sample. For analytes where matrix enhancement (IS recovery $>100\%$) was encountered, the isotope recovery was assumed to be 100% so not to get artificially low MRLs. For samples, where dilution was performed, the MRLs were suitably adjusted taking this into account. This method provides a realistic MRL for each sample without having to perform MRL studies on each water matrix.

Table 2
LODs, MDLs and practical MRLs in ultrapure water 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[a]	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
Hydracortisone	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[a]	0.1

[a] Assumed as the lowest calibration standard (SNR $\gg 3$ at this concentration).

3.5. Matrix spike recoveries

Three different water matrices (ultrapure water, surface water and wasterwater effluent) were tested to evaluate the suitability of the method for analyte spike recoveries. Five replicate samples of ultrapure water and surface water (SW) were spiked with all target analytes at two levels (30 ng/L and 100 ng/L) while a wastewater effluent ($5\times$ diluted) was spiked at 100 ng/L. A summary of the recoveries for each analyte is shown in Table 3. In ultrapure water, recoveries were acceptable (70–130%) for all target analytes except norgestrel (61.2%) and fluoxetine (132.6%) in the low spike of 30 ng/L. The %RSD for both spiking levels in ultrapure water was $<10\%$ for more than 90% of the target analytes with most compounds having an RSD $<5\%$. In SW, recoveries varied from 69.2–137.1% for the 30 ng/L spike and 68.4–136.3% in the 100 ng/L spike with only two compounds outside the acceptable range in both spikes. The reproducibility for all compounds in SW was good as RSDs were $<12\%$ in both spike levels. All target analytes recoveries were within the acceptable range for the wastewater effluent (WWE) spike with the exception of PFOS (145.1%) while RSDs were less than 10% for all compounds but hydrocortisone (13.1%). The data shown is for the isotope corrected recoveries while absolute recoveries were lower as seen in earlier sections.

3.6. Matrix effect assessment

Matrix effects can pose severe challenges for low level quantification of analytes using ESI-LC–MS/MS [43]. Generally caused by interferences of co-eluting constituents in the matrix, they can result in loss of sensitivity and reproducibility. In this study, the matrix effect was calculated using 26 isotopically labeled standards in the analytical method. The isotopically labeled standards were spiked at 100 ng/L into three different matrices (surface water, wastewater effluent, 1:5 diluted wastewater effluent) and the matrix effect was calculated by comparing the peak area obtained in the matrix with peak area obtained in an ultrapure water sample according to Eq. (1).

$$\text{Matrix effect } (\%) = \frac{(PA_S - PA_M) \times 100}{PA_S} \quad (1)$$

where PA_s is the peak area in the standard (ultrapure water) and PA_M is the peak area obtained in the matrix. A positive value of matrix effect indicates signal suppression while a negative value indicates signal enhancement. Five replicate samples were analyzed in each matrix and of the standard in ultrapure water. The matrix effects for each analyte in the three different water qualities are represented in Fig. 4. The results indicate that while all the analytes were affected by suppression or enhancement in the three water qualities, the magnitude of effect was vastly different. For example, diltiazem-d_3 had $<10\%$ matrix effect in the three different water qualities whereas meprobamate-d_3 experienced much stronger suppression in all three water qualities (73.7%, 92.1% and 47.9% in SW, WWE and 1:5 WWE, respectively). Generally, the effects were greater in more complex matrices with the average matrix effect in the WWE (53%), being higher than SW (38%) and 1:5 diluted WWE (19%). The results from this study are in agreement with other studies and show the propensity of ESI methods to matrix effects. Hence, the authors strongly recommend the use of isotopically labeled surrogate standards for quantification in aqueous samples. For analytes where an isotopically labeled standard is not available, assigning an

Table 3
Compound matrix recoveries in three different water matrices.

Compound	Ultrapure water				Surface water				Wastewater effluent (1:5 dilution)	
	30 ng/L ($n=5$)		100 ng/L ($n=5$)		30 ng/L ($n=5$)		100 ng/L ($n=5$)		100 ng/L ($n=5$)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Atenolol	102.2	1.2	110.8	4.5	115.8	2.4	90.9	5.8	86	3.4
Atrazine	88.9	3.3	98.8	1.2	103	0.2	97.4	2.6	94.7	1.9
Benzophenone	98.9	4.8	128.9	2.7	93.8	1.8	111.6	8.6	120.4	7
Benzotriazole	108.6	1.9	114.7	9.7	81.5	8.8	98	7.1	75.2	7.4
Bisphenol A	127.9	3.1	101.9	6.7	101.8	3.6	88.6	10.3	76.2	5.4
Caffeine	88.8	3	96.1	2.9	118.5	0.9	100.4	4.9	99.7	3.5
Carbamazepine	96.6	0.9	101.3	0.9	105.8	0.3	99.3	3.3	95.3	0.9
Clofibric acid	85.5	4.2	93.9	1.5	117.5	1.2	118	4.6	115.3	1.9
DEET	95.1	0.8	100.1	1.3	109.9	2.3	128.2	3.1	111.2	1.1
Diclofenac	81	1.8	78.1	3	106.2	2.2	99.1	1.9	111.1	2.8
Diphenhydramine	88.1	0.3	93.2	0.8	123.9	0.2	94.4	0.8	95.9	6.1
Ditiazem	81.1	0.7	87.4	2	118.7	0.3	102.2	1.4	104.9	1.8
Fluoxetine	132.6	2.4	128.3	4.2	92.4	1.5	68.4	4.5	112.7	3.5
Gemfibrozil	83.8	3.1	92.6	2.1	96.7	0.8	92.9	3.2	73.7	2.2
Hydrochlorothiazide	90.1	4.1	93.2	3.2	90.4	4.2	96.3	3.3	86.2	4.7
Hydrocortisone	98.1	9.7	86.5	10.3	137.1	11.5	95.7	3.8	97.6	13.1
Ibuprofen	90.7	5.8	91	3.8	103	1.6	91.8	1.6	89.8	3.5
Meprobamate	86.7	1.5	98.2	1.1	105.1	1.6	96.9	2.9	105.2	1.6
Naproxen	86.3	5.5	94.8	2.5	104.7	1.5	98.2	3.8	99.9	3.1
Norgestrel	61.2	4.9	91.7	1.6	119.5	2.5	101.4	5.4	75.1	6.8
PFHxA	91.6	5.3	93	3	69.2	8.3	91.3	3.4	88.3	2.4
PFOA	100.4	5.4	107.3	4.2	88.1	1	124.6	0.8	85.5	2.6
PFOS	121.2	7.3	128.6	4	125.6	0.3	136.3	0.8	145.1	1.8
Primidone	85.8	9.1	92.3	4.7	79.2	3.8	98.6	5.2	91.6	5.8
Propranolol	84.0	6.1	89.2	3.9	80.3	4.4	86.8	4.1	83.1	5.6
Propylparaben	86.2	2.2	91.2	3.3	116.1	1.8	108.3	9.6	100	5.3
Simazine	96.6	7	104.3	2.7	127.7	1.6	117.6	2.7	103.3	5.7
Sulfamethoxazole	80.9	4.2	90.5	2.1	119.7	2	101.5	4.1	102.1	5.9
TCEP	102.2	3.3	73.7	12	NA	NA	103.1	4.1	90.1	7.2
TCPP	119.7	4.9	109.4	3.1	113.7	1.6	111.8	3.9	88.3	5
Testosterone	103	10.3	116	5.4	123.4	2.3	117.5	6.1	91.8	9.9
Triclocarban	121.6	11.3	78.8	5.3	104.7	1.9	80.4	3.5	102.8	1.4
Triclosan	91.5	10.4	102.6	4.4	114.9	2.3	105.9	3.8	91.3	3.1
Trimethoprim	75.1	1.1	83.5	1	90	3.6	75.7	11.2	74.8	5.9

NA: not analyzed.

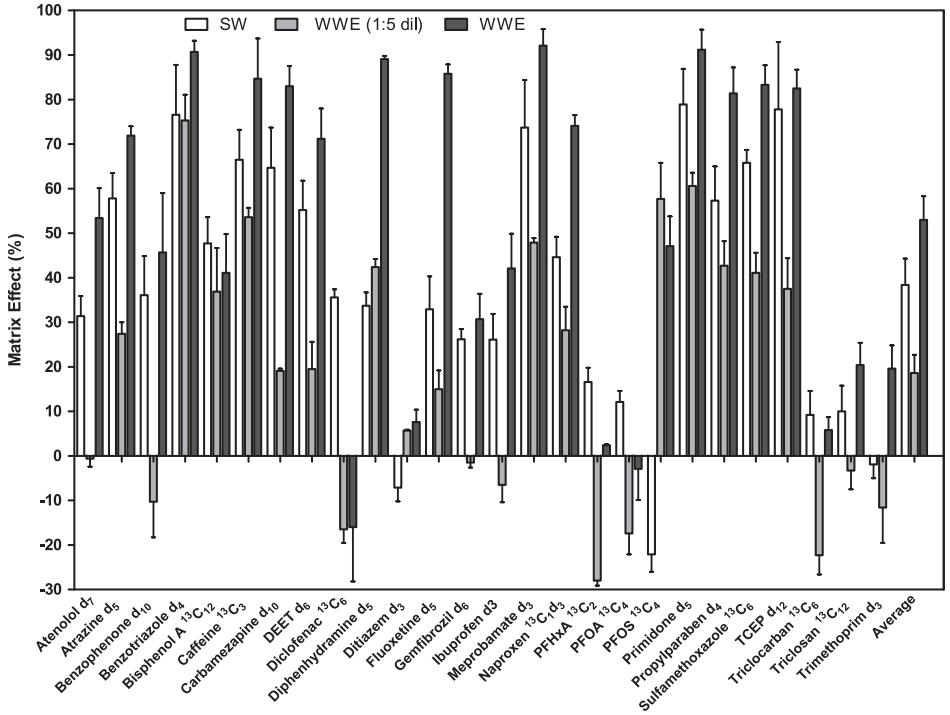

Fig. 4. Matrix effect (%) of isotopically labeled standards in different water qualities.

appropriate one is critical as matrix effects are different for every analyte in each matrix and depend on chemical structure, retention time and nature of ionization [16,44].

3.7. Blanks, linearity and precision

The analysis of compounds at trace levels in any matrix is always susceptible to contamination. The use of automated extraction and analysis techniques reduces the possibility of contamination due to sample handling and human error. In this study, blanks with and without isotopically labeled standards were analyzed to check for the presence of target analytes. Except for DEET, none of the target analytes were detected in blanks. Only a small trace of DEET was detected similar to that reported previously [14]. Subsequently, the MDL and MRL for DEET were adjusted to minimize the possibility of false positives. Special precautions were taken to avoid contamination of prefluorinanted compounds including replacing all Teflon lines in the instrument with PEEK or stainless steel, use of metal solvent liner frits in the mobile phase bottles and use of a Poroshell EC 120 C-18 (2.1×30 mm^2, 2.7 μm) trapping column after the mixer of the pump to avoid potential contamination from solvents. Finally, a series of blanks ($n=5$) were analyzed for all the target analytes and all values were below the MDL. For analysis of real water samples, true blanks, method blanks and matrix spikes were run frequently to monitor contamination, instrument carry-over and robustness of the method.

The linearity of the calibration curves for each analyte was verified by preparing seven standards from the MDL to 100 ng/L (six points if the MDL > 10 ng/L) fortified with isotopically-labeled standards. The correlation coefficient (R^2) for all analytes was > 0.99 with 24 compounds (71%) having an $R^2 > 0.995$. The precision of the analytical method was verified using the intra-day and inter-day reproducibility (Table S7). Intra-day reproducibility calculated as % RSD was evaluated by analyzing four replicate injections of a 50 ng/L standard injected over the same day approximately three hours apart. The intra-day variability ranged from 1 to 10.4% with all the analytes except fluoxetine within 10%. The inter-day variability (% RSD) was determined by analyzing a 50 ng/L standard, prepared fresh everyday over four consecutive days and was < 10% for all compounds except atenolol with a range of 1–11.9%.

3.8. Analysis of water samples

The optimized method was used to determine the concentration of the 34 TOrCs in different environmental waters. Two conventional wastewater treatment plants (WWTP) were sampled at different treatment points to study treatment efficacy. WWTP 1 serves a largely urban population and has a capacity of 70 million gallons per day (MGD). It employs flocculation/sedimentation as primary treatment followed by an activated sludge process (sludge retention time ~4 days) as a secondary treatment and disinfection with free chlorine. WWTP 2 has a capacity of 2 MGD and serves a much smaller population (~17,000 people) with > 70% of the residents over 65 years in age. The first step in treatment of raw sewage is bar screens for grit removal followed by a biological oxidation ditch treatment (primary effluent), the mixed liquor is then sent to secondary clarifiers (secondary effluent) after which the water is disinfected with chlorine as hypochlorite before discharge (final effluent).

All pharmaceuticals analyzed were detected in the influent of both WWTPs with the anti-inflammatory drug Naproxen (avg. conc.:61,000 ng/L) and pain reliever Ibuprofen (35,000 ng/L) detected at highest concentrations. The metabolite clofibric acid however was not detected in both plants. Generally, most pharmaceuticals were present at > 1000 ng/L in the influent possibly indicating significant loading from humans as both plants served a domestic population. Similarly, all personal-care products analyzed were detected in the influent of the two WWTPs. Caffeine was the highest detected TOrC in the influent with an average concentration of 74,500 ng/L while none of the hormones tested were detected. No pesticides or perfluoroalkyl substances tested in this study was detected in the two plants. This could be due to the highly domestic nature of the wastewater indicating a lack of industrial and agricultural contribution to the waste.

The removal of TOrCs in wastewater depends on a number of factors including type of treatment process used, characteristics of the plant (like sludge retention time, pH and temperature) and physicochemical properties of the compound like biodegradability, sorption capacity, and water solubility [45,46]. The pharmaceuticals atenolol, trimethoprim, carbamazepine and sulfamethoxazole have low sorption coefficients and had poor removal after primary treatment in WWTP 1[47]. However these compounds with the exception of carbamazepine are well removed by biological processes as shown in the data (secondary effluent in WWTP 1 and primary effluent in WWTP 2) and corroborated by previous studies [48,49]. Similarly, caffeine has been shown to be easily biodegraded in activated sludge processes and this was evinced here as well with concentrations > 50,000 ng/L reduced to < 5 ng/L after biological treatment in both plants [50]. Moderate to high removals were seen for personal-care products like benzophenone and DEET in the primary and secondary treatments which is in agreement with previously published literature [51,52]. Finally, disinfection by chlorine can also oxidize some TOrCs leading to additional removal. Antimicrobial agents triclosan and triclocarban are extremely well removed by chlorine while flame retardants (TCEP and TCPP) have been shown to be resistant to most oxidants used in water treatment [4] and this was seen in the results as well. While several factors at each individual plant dictate the removals of these TOrCs, it can be seen that conventional treatment processes are not effective in complete attenuation of these compounds.

Analysis of a surface water revealed the presence of seven pharmaceuticals (carbamazepine, diltiazem, meprobamate, naproxen, primidone, sulfamethoxazole, and trimethoprim) ranging from 1.4 to 18 ng/L (Table 4). Five personal-care products (Benzophenone, caffeine, DEET, triclocarban and TCEP) were detected, with the UV-screen benzophenone (47 ng/L) being the highest detected TOrC in the SW. Both pesticides (atrazine and simazine) analyzed were present at < 5 ng/L. PFOA was detected at 27 ng/L in the SW while the other two PFCs analyzed were absent.

The groundwater (GW) sample collected from Tucson, AZ had the pharmaceutical-metabolite clofibric acid (22 ng/L) and insect-repellant DEET (5.4 ng/L) present at low concentrations while all other TOrCs analyzed were < MRLs. A grab sample collected from a septic tank showed the presence of all the personal-care products tested at concentrations > 100 ng/L. Caffeine (19,000 ng/L) was the TOrC detected at highest concentration with benzophenone (1600 ng/L) and triclocarban (1400 ng/L) also present at > 1000 ng/L in this sample. Only two pharmaceuticals (diphenhydramine and primidone) were present in this sample while all the hormones, pesticides and industrial compounds analyzed were not detected. The occurrence for all waters tested is presented in Table 4.

The presence of several trace-organic contaminants in surface and ground waters indicates that release of these compounds into the environment can enter water supplies and hence it would be prudent to monitor these chemicals while relevant toxicological studies are performed to evaluate health risk from water exposures.

4. Conclusions

A method utilizing fully automated online SPE coupled to LC–MS/MS with simultaneous positive and negative ESI for

Table 4
Concentration of analytes (ng/L) in different water matrices.

Compounds	WWTP 1				WWTP 2				SW	Septic tank	GW
	Influent	1° Effluent	2° Effluent	Final effluent	Influent	1° Effluent	2° Effluent	Final effluent			
Pharmaceuticals											
Atenolol	4000	3400	510	620	14,000	600	1000	590	< 3.5	< 19	< 2.5
Carbamazepine	1300	1400	350	340	300	320	300	300	< 0.7	< 1.9	< 0.2
Clofibric acid	< 40	< 37	< 5.1	< 4.8	< 18	< 16	< 12	< 2.8	< 1.2	< 21	< 1.1
Diclofenac	1400	1200	1300	680	9000	9300	7800	1800	< 4.9	< 76	< 6.3
Diltiazem	280	260	130	59	320	110	80	60	< 0.2	< 5.0	< 4.8
Diphenhydramine	4900	3800	820	670	25,000	1500	1100	340	< 1.4	710	< 1.1
Fluoxetine	60	< 24	< 17	< 11	92	74	19	< 16	< 4.4	< 73	< 3.5
Gemfibrozil	5200	5000	120	49	6800	200	190	21	< 0.7	< 2.8	< 0.7
Hydrochlorothiazide	3800	3100	540	360	7400	5100	5300	5100	< 0.6	< 23	< 0.6
Ibuprofen	33,000	32,000	27	45	35,000	64	63	53	< 2.4	< 21	< 1.9
Meprobamate	980	970	790	820	460	160	180	180	< 1.4	< 36	< 0.9
Naproxen	46,000	44,000	< 39	< 41	76,000	80	64	59	1.4	< 57	< 5.4
Primidone	350	360	300	53	2900	840	840	730	18	310	< 3.6
Propranolol	19	< 14	< 6.9	< 1.6	460	110	120	11	< 1.3	< 31	< 1.3
Sulfamethoxazole	3200	3200	1500	820	2600	2700	1500	470	4.5	< 35	< 0.6
Trimethoprim	1800	1900	320	250	2000	150	110	19	4.3	< 2.5	< 0.1
Personal-care products											
Benzophenone	2200	620	420	200	2500	300	190	94	47	1600	< 14
Caffeine	64,000	44,000	< 4	< 3	85,000	40	< 4	< 3	4.4	19,000	< 0.8
DEET	800	340	80	120	1100	21	29	48	4.9	760	5.4
Propylparaben	530	320	< 30	< 25	540	230	< 27	< 22	< 3.0	490	< 2.0
Triclocarban	540	520	100	< 14	6800	200	190	21	19	1400	< 1.1
Triclosan	2000	1800	81	28	3000	750	26	< 13	< 2.8	770	< 3.0
TCEP	5000	4900	4200	4000	810	370	210	190	19	370	< 3.9
TCPP	6600	5100	4900	4100	850	640	450	440	< 25	190	< 16
Industrial compounds											
Benzotriazole	1800	2300	1600	850	5600	2700	1700	1500	< 54	< 100	< 11
Bisphenol A	190	< 120	< 89	< 67	< 160	< 150	< 73	< 48	< 24	< 190	< 39
PFHxA	< 38	< 18	< 4.6	< 3.9	< 36	< 19	< 4.8	< 4.0	< 4.3	< 21	< 3.6
PFOS	< 60	< 52	< 13	< 10	< 66	< 57	< 19	< 11	< 3.4	< 51	< 6.1
PFOA	< 22	< 15	9	< 5	< 32	< 37	< 13	< 6	27	< 44	< 3.1
Hormones											
Hydrocortisone	< 102	< 64	< 51	< 21	< 85	< 75	< 57	< 23	< 28	< 31	< 10
Norgestrel	< 86	< 79	< 23	< 21	NA	NA	NA	NA	< 17	< 46	< 12
Testosterone	< 150	< 140	< 32	< 29	NA	NA	NA	NA	< 12	< 41	< 11
Pesticides											
Atrazine	< 8.3	< 6.4	< 1.5	< 1.4	< 5.2	< 4.1	< 4.6	< 1.0	2.1	< 1.5	< 0.3
Simazine	< 9.6	< 10	< 1.9	< 2.1	< 5.9	< 5.4	< 6.1	< 1.2	4.1	< 1.1	< 0.4

NA: not analyzed.

analysis of 34 diverse trace organic contaminants including pharmaceuticals, personal-care products, hormones, pesticides, and industrial compounds in water has been developed. The method presented provides rapid screening and low-level quantification of analytes without sacrificing sensitivity, with MDLs ranging from 0.1 to 13.1 ng/L. Further, a method to determine the MRL of each sample using isotope recovery data without having to perform time-consuming MRLs in each matrix tested is described. A low sample volume (1.7 mL) coupled with a cycle time of < 15 min allows for high-throughput analysis. Critically, the use of fast polarity switching allowed for analysis of all 34 TOrCs in both ESI positive and negative mode with just one injection resulting in large time savings that is unique compared to other published OSPE methods. This fully automated online extraction coupled to LC–MS/MS method provides significant time, labor and solvent savings compared to previously published methods while also increasing the reproducibility of analysis. Results indicate that optimization of the type of online SPE cartridge, washing mobile phase composition, washing volume and flowrate are critical to obtain best sensitivity for analysis. The method has been validated with analysis of TOrCs across several different environmental water matrices.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.08.011.

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