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Analysis of Endocrine Disruptors, Pharmaceuticals, and Personal Care Products in Water Using Liquid Chromatography/Tandem Mass Spectrometry

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A method has been developed for the trace analysis of 27 compounds from a diverse group of pharmaceuticals, steroids, pesticides, and personal care products. The method employs solid-phase extraction (SPE) and liquid chromatography/tandem mass spectrometry (LC/MS/MS), using electrospray ionization (ESI) in both positive and negative modes and atmospheric pressure chemical ionization in positive mode. Unlike many previous methods, a single SPE procedure using 1 L of water coupled to a simple LC method is used for all ionization modes. Instrument detection limits for most compounds were below 1.0 pg on column with reporting limits of 1.0 ng/L in water. Recoveries for most compounds in deionized water were greater than 80%. Sulfuric acid was found to be the preferred sample preservative, and structures of all MS/MS product ions are proposed. Matrix effects from waters with a high content of treated municipal effluent were observed in both ESI modes and are discussed in the paper.

In recent years, reports have shown that certain contaminants at trace concentrations in surface waters can have dramatic effects on the endocrine systems of aquatic organisms.[1−3] These compounds are collectively known as endocrine-disrupting compounds (EDCs). Pharmaceuticals, steroids, and personal care products have also been detected in the aquatic environment and may act as EDCs.[4−6] As environmental contaminants continue to be discovered, the need for comprehensive methods for their sensitive and selective identification has also increased. Traditional gas chromatography is of limited value without time-consuming and labor-intensive derivatization because many environmental contaminants are polar, have low volatility, and are thermally labile. This has led to the increased use of liquid chromatography/mass spectrometry (LC/MS) due to its ability to effectively analyze these types of molecules.

LC/MS methods for the analysis of pharmaceuticals, steroids, and personal care products in water have been developed for a number of different mass spectrometers using diverse extraction procedures and elution protocols. A complete review of methods for the analysis of these compounds in water using LC/MS is beyond the scope of this paper, and thorough reviews have previously been published.[7−9] The majority of concentration techniques involve solid-phase extraction (SPE) in which many solid phases, eluent schemes, and final solvents with and without ion-pairing reagents, buffers, and modifiers were used.[10−20] Additionally, both single quadrupole[11,15,16] and triple quadrupole[10,12−14,17−23]

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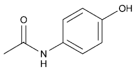
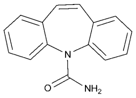
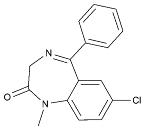
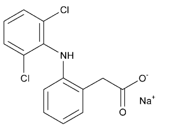
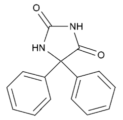
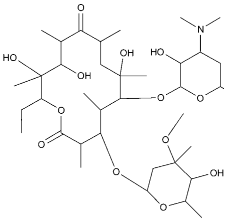
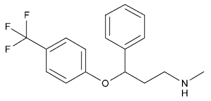
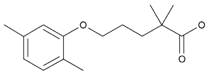
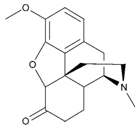
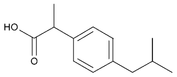
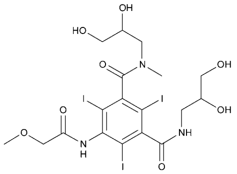
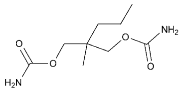
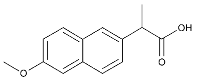
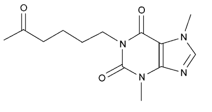
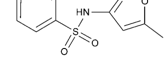
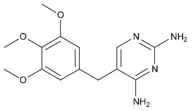
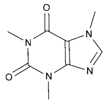
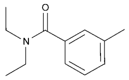
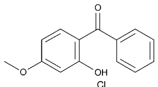
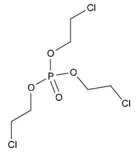
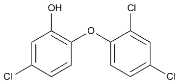
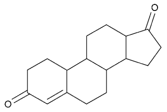
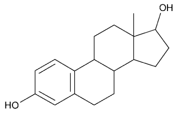
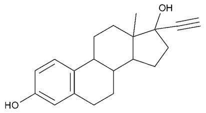
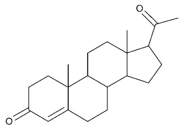
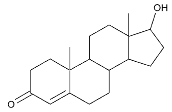
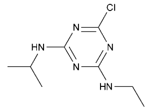
Table 1. Target Compounds

Class	Compound	Use	Structure
Pharmaceutical	Acetaminophen	Analgesic	
	Carbamazepine	Anti-seizure	
	Diazepam	Muscle relaxant	
	Diclofenac	Anti-arthritic	
	Dilantin	Anti-convulsant	
	Erythromycin-H$_2$O	Antibiotic	
	Fluoxetine	Anti-depressant	
	Gemfibrozil	Anti-cholesterol	
	Hydrocodone	Analgesic	
	Ibuprofen	Analgesic	
	Iopromide	X-ray contrast agent	
	Meprobamate	Anti-axiety	
	Naproxen	Analgesic	
	Pentoxifylline	Blood viscosity reducing agent	
	Sulfamethoxazole	Antibiotic	
Pharmaceutical	Trimethoprim	Antibiotic	
Personal Care Product	Caffeine	Stimulant	
	DEET	Insect repellant	
	Oxybenzone	Sunscreen	
	TCEP	Flame retardant	
	Triclosan	Antibiotic	
Steroid	Androstenedione	Androgen	
	Estradiol	Estrogen	
	Ethynylestradiol	Synthetic estrogen	
	Progesterone	Estrogen	
	Testosterone	Androgen	
Pesticide	Atrazine	Pesticide	

mass spectrometers were used, making it difficult to assess the relative selectivity of the methods due to their varying capabilities. Furthermore, most of these methods encompass only groups of compounds containing similar polarities,[10,14] similar structures,[11,18,19,21] or similar activities.[12,13,15,20,22,23] Several papers have proposed methods for the determination of broad groups of compounds.[17,24,25] However, these were either limited in scope,[16] used multiple extraction methods and liquid chromatography eluent systems,[17,24] or were developed for the pharmaceutical industry and were not applicable to environmental matrixes.[25] Although these methods, in general, describe the analysis of many classes of pharmaceuticals, steroids, and personal care products, no simple method has been developed that attempts to simultaneously extract and analyze representatives from each of these groups of compounds.

This paper presents a method for the sensitive detection of 27 pharmaceuticals, steroids, pesticides, and personal care products (Table 1). This method uses a single SPE method followed by a simple LC gradient coupled to a tandem mass spectrometer using both electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). The compounds studied were chosen based on their occurrence in the environment and their dissimilar structural and physicochemical properties.[4,5,26,27] Although not all classes of compounds are represented, this method analyzes a broad range of compounds that may provide a starting point for the development of new methods as novel classes of contaminants are identified. Instrument detection limits (IDLs) were generally below 1.0 pg on column and all compounds had a reporting limit of 1.0 ng/L in water. This low level provided sufficient sensitivity to detect many of the compounds in surface waters influenced by municipal effluent. The proper preservation of samples and the effect of matrix interferences on both ESI modes are also discussed.

EXPERIMENTAL SECTION

Materials. All standards were obtained from Sigma-Aldrich (St. Louis, MO) except atrazine and DEET from Accustandard (New Haven, CT); fluoxetine and iopromide from the United States Pharmacopeia (Rockville, MD); hydrocodone and hydrocodone-d_6 from Cerilliant (Round Rock, TX); and [$^{13}C_3$]-caffeine, [$^{13}C_1$]-erythromycin, [$^{13}C_3$]-atrazine, [$^{13}C_2$]-estradiol, and diazepam-d_5 from Cambridge Isotope Laboratories (Andover, MA). Trace analysis grade methanol was obtained from Burdick and Jackson (Muskegon, MI). Formic acid and methyl *tert*-butyl ether (MTBE) were purchased from EM Science (Gibbstown, NJ).

Sample Collection and Preservation. Samples were collected in 1-L silanized, amber glass bottles (Eagle-Picher, Miami, OK) as suggested by Ahrer et al.[16] Sample bottles were kept on ice and brought back to the laboratory within 4 h of collection. Immediately, they were preserved by adjusting to pH 2 with concentrated sulfuric acid and stored at 4 °C until extraction. Samples were extracted within 14 days of collection.

Solid-Phase Extraction. Analytes were extracted in batches of six samples using 500-mg hydrophilic−lipophilic balance (HLB) cartridges from Waters Corp. (Millford, MA). All extractions were performed on an AutoTrace automated SPE system (Zymark Corp., Hopkington, MA). The SPE cartridges were sequentially preconditioned with 5 mL of MTBE, 5 mL of methanol, and 5 mL of reagent water. One thousand-milliliter samples were spiked with 20 μL of a 2.5 mg/L solution of surrogate standards ([$^{13}C_3$]-caffeine, [$^{13}C_3$]-atrazine, [$^{13}C_2$]-estradiol). The sample was then loaded onto the cartridges at 15 mL/min after which the cartridges were rinsed with 5 mL of reagent water and then dried with a stream of nitrogen for 60 min. Next, the cartridges were eluted with 5 mL of 10/90 (v/v) methanol/MTBE followed by 5 mL of methanol into 15-mL calibrated centrifuge tubes. The resulting extract was concentrated with a gentle stream of nitrogen to a volume of ~750 μL. Then 20 μL of a 2.5 mg/L solution of internal standards (diazepam-d_5 and testosterone-d_3) were added, and the extract was brought to a final volume of 1 mL using methanol.

Liquid Chromatography. An Agilent (Palo Alto, CA) G1312A binary pump and an HTC-PAL autosampler (CTC Analytics, Zwingen, Switzerland) were used for all analyses. All analytes were separated using a 250 × 4.6 mm Synergi Max-RP C12 column with a 4-μm particle size (Phenomenex, Torrance, CA). A binary gradient consisting of 0.1% formic acid (v/v) in water (A) and 100% methanol (B) at a flow rate of 700 μL/min was used. The gradient was as follows: 5% B held for 3.5 min, increased linearly to 80% by 10 min and held for 3 min, and stepped to 100% and held for 8 min. A 9-min equilibration step at 5% B was used at the beginning of each run to bring the total run time per sample to 30 min. An injection volume of 10 μL was used for all analyses.

Mass Spectrometry. Mass spectrometry was performed using an API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA). Optimization of the mass spectrometer occurred in three separate steps: determination of the best ionization source and polarity, optimization of compound-dependent parameters, and optimization of source-dependent parameters.

To determine the best ionization source and mode for each analyte, all compounds were tested using ESI positive/negative and APCI positive/negative. For the ESI source, each analyte was infused directly into the mass spectrometer at a concentration of 100 ng/mL and a flow rate of 10 μL/min. For the APCI source, each analyte was prepared at a concentration of 1 μg/mL in methanol and infused at 10 μL/min into a faster flow rate of 700 μL/min methanol via a mixing tee due to the higher flow rate required by the APCI source. During the infusions, the first quadrupole of the mass spectrometer (Q1) was scanned while the declustering potential was raised and lowered. Typically, Q1 was scanned from m/z 50 to [M + 100]. This allowed the most intense precursor ion to be chosen for each source/mode. From these, the optimal source and polarity were chosen.

Once the best ionization source/polarity was found, the optimal compound-dependent parameters for each analyte were determined using that source/polarity (Table 2). Each compound was infused as discussed previously while the mass spectrometer was scanned to refine the declustering potential determined during the source/mode tests. While the collision energy for the second quadrupole (Q2) was ramped, the third quadrupole (Q3) was scanned to determine the four most intense product ions. Each

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Table 2. Compound-Dependent Parameters

compound	retention time (min)	declustering potential (V)	collision energy (eV)	collision cell exit potential (V)
ESI Positive				
period 1				
hydrocodone	11.2	91	41	20
trimethoprim	11.5	81	37	8
acetaminophen	12.7	56	23	10
period 2				
caffeine	13.7	41	29	14
[$^{13}C_3$]-caffeine surrogate	13.7	41	29	14
erythromycin − H_2O	13.8	66	21	16
sulfamethoxazole	13.9	51	23	14
fluoxetine	14.0	51	37	8
pentoxifylline	14.6	56	25	18
meprobamate	15.1	36	13	16
period 3				
dilantin	15.8	66	29	18
TCEP	15.8	61	19	6
carbamazepine	16.1	61	29	20
DEET	17.1	61	25	8
atrazine	17.4	71	27	14
[$^{13}C_3$]-atrazine surrogate	17.4	71	27	14
diazepam	18.3	61	41	10
diazepam-d_5 ISTD	18.3	61	41	10
oxybenzone	19.3	46	29	16
progesterone	19.6	86	37	10
ESI Negative				
iopromide	12.1	−58	−16	−17
naproxen	18.1	−85	−28	−17
ibuprofen	19.5	−35	−10	−17
diclofenac	19.6	−40	−28	−21
triclosan	20.2	−45	−30	−17
gemfibrozil	20.2	−45	−38	−15
APCI Positive				
ethynylestradiol	18.2	61	25	26
androstenedione	18.3	81	31	10
estradiol	18.4	41	25	4
[$^{13}C_2$]-estradiol	18.4	41	25	4
testosterone	18.8	71	43	20
testosterone-d_3 ISTD	18.8	71	43	20
progesterone	19.6	71	29	8

of these four precursor/product ion transitions was then tested to determine the optimal cell exit potential for the product ions leaving Q2. After each precursor/product ion pair was optimized, the pair with the most intense signal was chosen to represent the corresponding compound.

Once the compound-dependent parameters were determined, each source type and mode was tested individually to optimize the appropriate source-dependent parameters (Table 3). This was accomplished by repeatedly injecting 10 μL of a 10 μg/L solution of target compounds in methanol into the mass spectrometer without using any chromatographic separation. Before each injection, one of the parameters shown in Table 3 was slightly adjusted and the resulting change in intensity of the summed signals of all precursor/product ion analytes monitored was recorded. In this manner, each of the parameters in Table 3 was optimized to give the best possible signal for the compounds of interest. It was noted that, due to the broad nature of our analyte list, several adjustments were detrimental to some compounds while being beneficial to others. However, since the detection limits of all of the analytes were found to be acceptable, the adjustments were made to optimize the most compounds of interest for the sake of the simplicity of the method. In addition to the parameters listed in Table 3, the protrusion of the ESI needle was also optimized.

Table 3. Source-Dependent Parameters

	ESI positive	ESI negative	APCI positive
collision gas (psig)	7	7	6
curtain gas (psig)	10	10	14
ion source gas 1 nebulizer gas (psig)	50	50	55
ion source gas 2 turbo gas (psig)	50	50	n/a[a]
ion spray voltage (V)	4200	−4200	n/a
nebulizer current (μA)	n/a	n/a	3
temperature (°C)	450	450	500
probe *X*-axis position (mm)	5	5	5
probe *Y*-axis position (mm)	5	5	5
entrance potential (V)	10	−10	10

[a] n/a, not applicable.

For ESI positive analysis, the 21-min run time was divided into three periods in which only a select group of the compounds was monitored. These periods are provided in Table 2. This was done to ensure that enough dwell time was spent on each transition to avoid any data loss. This resulted in dwell times of 60 ms for each analyte in each period, well above the manufacturer's minimum recommendation of 25 ms. In ESI negative and APCI positive, all analytes were monitored for the entire run length and dwell times were all above 100 ms.

IDL Studies and Calibration. An IDL study was performed by consecutively injecting 2.5 pg on column 10 times. The IDL was then calculated by multiplying the standard deviation of the replicate measurements by the appropriate Student's *T* value for nine degrees of freedom (Table 4). Due to the varying IDLs from this study, a conservative lowest calibration point of 0.25 μg/L was chosen for all compounds. The remaining calibration points were at 1.0, 2.5, 5.0, 10, 25, 50, and 100 μg/L. Although this only covers 2 orders of magnitude, concentrations in the environment were expected to fall within this range. Therefore, it was decided to concentrate points in this narrow dynamic range rather than extending the curve to higher concentrations. All concentrations that were above the highest point in the calibration curve were diluted and reanalyzed. The method reporting limit was calculated by multiplying the reciprocal of the concentration factor of the SPE process by the concentration of the second calibration point. Since the SPE process concentrated the sample from 1000 to 1 mL for a concentration factor of 1000 and the second lowest calibration point was 1.0 μg/L, the method reporting limit was determined to be 1.0 ng/L. All analytes were calibrated externally using linear or quadratic regression with $1/x^2$ weighting. Correlation coefficients were required to be at least 0.990 and typically exceeded 0.995.

RESULTS AND DISCUSSION

Sample Preservation. It was discovered that the recoveries of androstenedione, testosterone, progesterone, trimethoprim, acetaminophen, and fluoxetine spiked into unpreserved surface waters would quickly decline over time. This degradation was presumed to be due to microbial degradation of the affected compounds. This phenomenon has also been reported in the literature by Baronti et al.,[21] who found that when estriol, estrone,

Table 4. IDLs, Percent Recoveries, and Percent RSD for Target Compounds

compound	IDL (pg) $n = 10$	recovery (%) $n = 17$	RSD (%)
	ESI Positive		
hydrocodone	0.23	112	7
trimethoprim	0.30	91	9
acetaminophen	0.30	41	14
caffeine	0.28	93	10
erythromycin − H_2O	0.17	71	10
sulfamethoxazole	0.35	72	16
fluoxetine	0.31	80	7
pentoxifylline	0.28	84	15
meprobamate	0.68	96	11
dilantin	0.94	86	11
TCEP	3.2	75	18
carbamazepine	0.43	91	12
DEET	0.24	81	19
atrazine	0.15	89	8
diazepam	0.28	80	19
oxybenzone	0.35	68	19
progesterone	0.86	81	17
	ESI Negative		
iopromide	0.72	91	9
naproxen	0.57	91	9
ibuprofen	0.32	96	15
diclofenac	0.12	83	11
triclosan	0.68	79	17
gemfibrozil	0.096	94	10
	APCI Positive		
ethynylestradiol	0.87	92	11
androstenedione	0.64	96	12
estradiol	0.62	92	14
testosterone	0.41	83	9
progesterone	0.38	90	12

Table 5. Results of Compounds Affected by Formaldehyde Preservation Study

compound	mean recovery (%)	
	with no preservative	with formaldehyde preservative
acetaminophen	48	12
meprobamate	95	154
dilantin	90	16
TCEP	98	46
iopromide	100	39

and estradiol were stored for 7 days in unpreserved river water, severe losses occurred. To preserve the samples and prevent degradation, this group added formaldehyde (1%, v/v) to freshly collected samples. To test the effects of formaldehyde on the nonsteroidal compounds in our target list, a preservation test was performed. Formaldehyde (1%, v/v) was added to 1 L of deionized water in a silanized amber glass bottle that had been fortified with 100 ng/L of our target compounds. The bottle was then thoroughly mixed and stored for 14 days at 4 °C, after which the sample was analyzed. Although the steroids were unchanged by the addition of formaldehyde, acetaminophen, meprobamate, dilantin, TCEP, and iopromide showed significant changes in recoveries when compared to a deionized water control. The results of the affected compounds are shown in Table 5.

Table 6. Sulfuric Acid Preservation Study

compound	mean surface water recovery (%)	
	unpreserved	sulfuric acid preserved
	Degradation Observed	
acetaminophen	10	26
trimethoprim	70	101
fluoxetine	49	76
androstenedione	56	97
testosterone	50	97
progesterone	22	93
	Unaffected Compounds	
caffeine	71	72
atrazine	70	73
ibuprofen	65	64
diazepam	60	62
ethynylestradiol	98	100

Since sulfuric acid is a commonly used preservative agent, it was tested to determine whether it would prevent degradation of the aforementioned compounds and not collaterally affect the recoveries of other compounds as was observed with formaldehyde preservation. Eight surface water samples from the Boulder Basin of Lake Mead, NV, were collected in 1-L silanized amber glass bottles. Four sets of samples, each containing one sample and one duplicate, were prepared. Two sets were fortified at 100 ng/L with the target compounds and left unpreserved and the other two sets were fortified at the same concentration and preserved to pH 2 with concentrated sulfuric acid. One set of preserved/unpreserved samples was held for 7 days at 4 °C, and the other duplicate set was held for 14 days. The samples were then extracted and analyzed as described previously. The sulfuric acid preservation both prevented the degradation of the affected compounds and did not adversely affect the recoveries of the other compounds being studied. Table 6 shows the results of the compounds that degraded in surface water and several other unaffected compounds for reference. All compounds not shown in Table 6 had recoveries that did not change in preserved versus unpreserved samples.

Surrogates and Internal Standards. In an attempt to represent the analytes most effectively, carbon-13- and deuterium-labeled surrogate and internal standards were chosen. Initially, the following compounds were chosen to be used as surrogate and internal standards: ESI positive, [$^{13}C_3$]-caffeine, [$^{13}C_1$]-erythromycin, [$^{13}C_3$]-atrazine, and diazepam-d_5; ESI negative, [$^{13}C_6$]-2,4-DP and [$^{13}C_6$]-2,4,5-T; APCI positive, [$^{13}C_2$]-estradiol and testosterone-d_3. It was determined, however, that some were not effective. [$^{13}C_1$]-Erythromycin − H_2O was found to rise and fall with increasing and decreasing amounts of unlabeled erythromycin − H_2O. This was attributed to [$^{13}C_1$]-erythromycin being labeled with only one carbon-13. Carbon-13 is a naturally occurring isotope of carbon and its abundance in the environment is $\sim$1.1%. Since erythromycin has 37 carbon atoms, the intensity of the [$^{13}C_1$]-erythromycin − H_2O peak will increase by 40.7% of the concentration of the unlabeled erythromycin peak. As the amount of erythromycin − H_2O in the samples increased, the amount of naturally occurring carbon-13-labeled atoms increased from negligible levels to amounts that were adversely affecting the usefulness of [$^{13}C_1$]-erythromycin as an internal or surrogate standard. [$^{13}C_6$]-2,4-DP and [$^{13}C_6$]-2,4,5-T were chosen to represent ESI negative compounds because of the intense signal seen when they were infused directly into the mass spectrometer. However, when they were injected using the final LC/MS conditions, only very weak signals were detected. We believe that this was due to

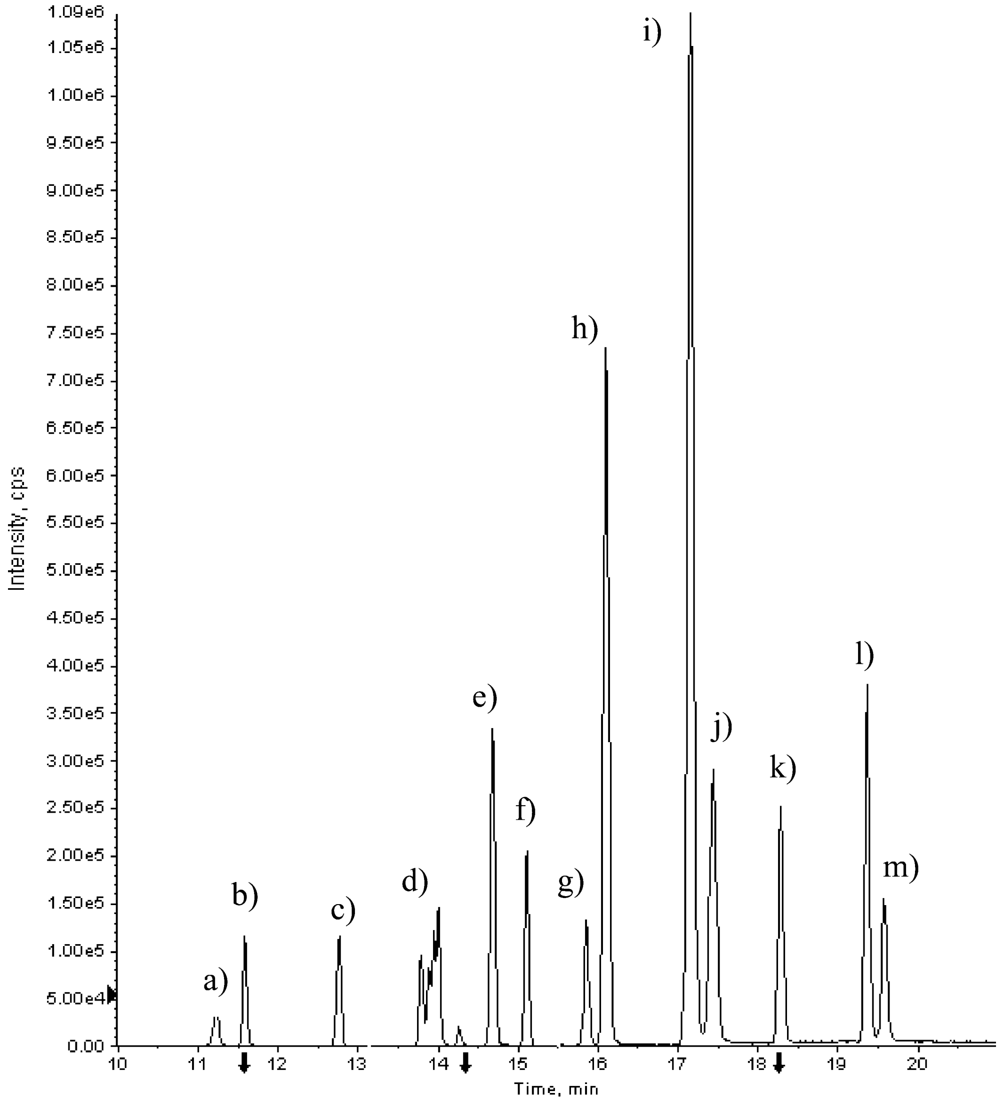

Figure 1. TIC of ESI positive. Key: (a) hydrocodone, (b) trimethoprim, (c) acetaminophen, (d) caffeine, erythromycin − H_2O, sulfamethoxazole, fluoxetine, (e) pentoxifylline, (f) meprobamate, (g) dilantin, TCEP, (h) carbamazepine, (i) DEET, (j) atrazine, (k) diazepam, (l) oxybenzone, and (m) progesterone.

the concentration of formic acid in the eluent. Since the compounds were being analyzed in negative mode via deprotonation, the abundance of protons in the eluent and the resulting acidic environment made it less favorable for the carboxylic acids [$^{13}C_6$]-2,4-DP and [$^{13}C_6$]-2,4,5-T to deprotonate. The remaining five were found to work well and were used to monitor the SPE and instrumental analysis for any egregious errors. Calibration curves and sample concentrations, however, were not corrected with surrogate or internal standards.

Recovery of Analytes by SPE. Analyte recoveries were determined by adding an appropriate amount of a stock solution of the target compounds to 1 L of deionized water such that the final concentration in the water was 50 ng/L. Over the course of three months, 17 batches of samples were extracted. Each batch included a deionized water blank and a spiked deionized water control sample. Table 4 shows the mean recovery and relative standard deviation (RSD) for all compounds. The number of determinations (n) used for all compounds in the recovery and RSD calculations was 17 except for hydrocodone and erythromycin − H_2O ($n = 9$) and TCEP and DEET ($n = 15$).

Chromatography. The LC method for ESI positive was developed first and was found to result in an adequate distribution of the target analytes within a run time of 21 min (Figure 1). When this method was applied to both ESI negative (Figure 2) and APCI positive (Figure 3), it was determined that it also provided sufficient separation of the compounds associated with these two modes. Although in ESI negative and APCI positive most of the analytes eluted within the final 4 min of the gradient, we believe that this is acceptable because the method was developed to be both simple and comprehensive.

It was observed that iopromide and erythromycin − H_2O each eluted as two separate peaks (Figure 4). Hirsch et al.[12] suggested that the two signals for iopromide were the result of the presence of two pairs of diastereomers that were the result of two chiral carbon atoms. Since erythromycin has 18 chiral centers, a reasonable explanation would be that it also showed more than one chromatographic peak for the same precursor/product ion transition due to stereoisomers with different physicochemical properties. As a result, the integration of these compounds was not as obvious as that of the others. Since the two peaks of

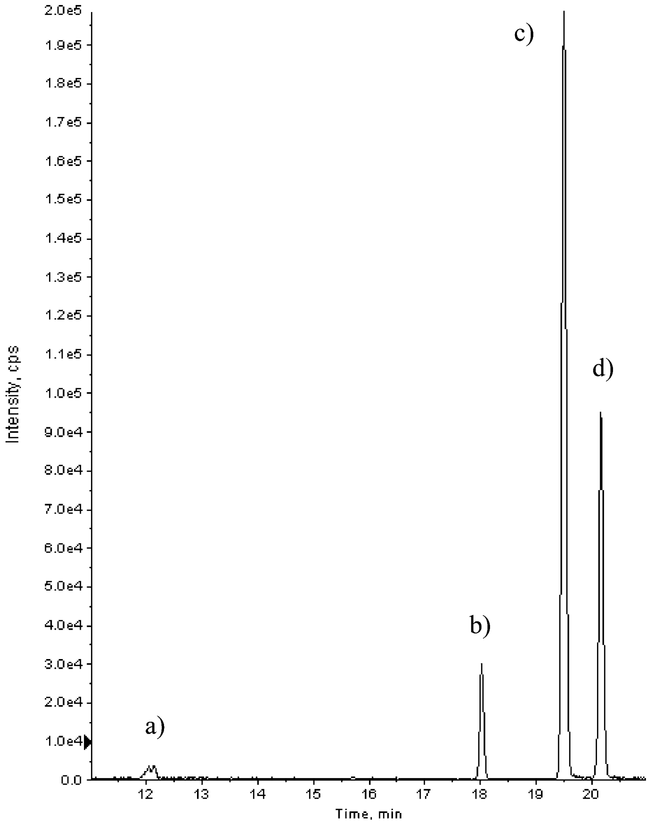

Figure 2. TIC of ESI negative. Key: (a) iopromide, (b) naproxen, (c) ibuprofen, diclofenac, and (d) triclosan, gemfibrozil.

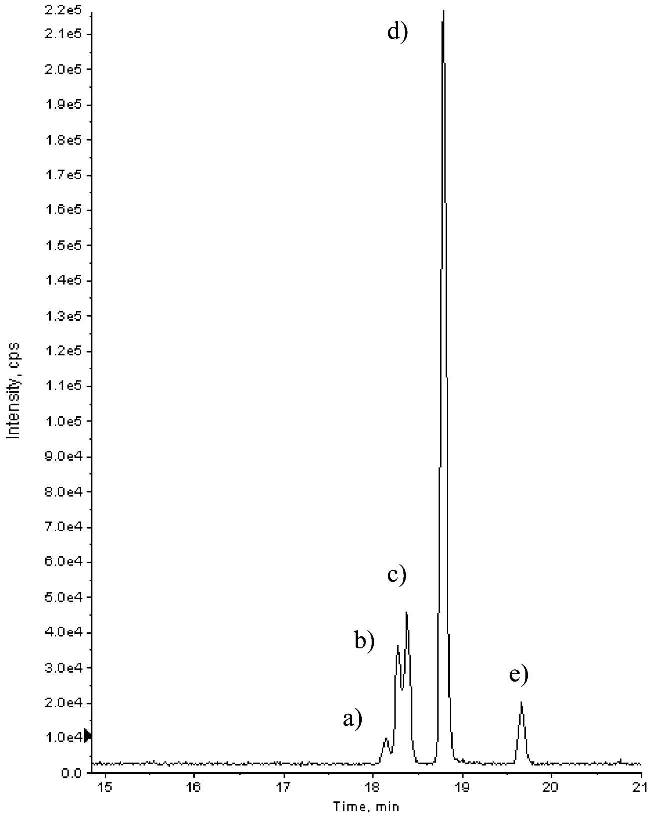

Figure 3. TIC of APCI positive. Key: (a) ethynylestradiol, (b) androstenedione, (c) estradiol, (d) testosterone, and (e) progesterone.

iopromide were not resolved, they were integrated as one compound. Erythromycin − H_2O, however, was fully resolved into two distinct peaks and therefore was integrated using only the earliest and largest of the two peaks.

Mass Spectrometry Observations. All precursor ions and their adducts, product ions, and proposed product ion structures can be found in Table 7. As a general rule, product ions had a structure that was able to support the stabilization of the charge through resonance.

MS/MS-ESI Positive. All but one of the precursor ions in ESI positive were the result of a protonation ($[M + H]^+$) of the intact, uncharged molecule. The only exception was erythromycin, which showed the loss of water to form erythromycin − H_2O. This finding is in agreement Hirsch et al.,[28] who showed that erythromycin has already lost a water molecule when present in the aquatic environment.

Other researchers have previously studied the product ions of several target compounds using ESI positive. Transitions in agreement with those presented in this paper were found for acetaminophen,[25] atrazine,[20,22,23] caffeine,[10,25] carbamazepine,[14] diazepam,[10] erythromycin − H_2O,[13,17] fluoxetine,[29,30] hydrocodone,[31] sulfamethoxazole,[11,17] and trimethoprim.[17] Although the same transition for erythromycin − H_2O was reported by Hirsch et al.,[13] we found that the transition was not the result of the loss of the desoamine group, but rather the loss of the cladinose ring. Through carbon-13 labeling of one of the methyl groups on the tertiary amine of the desoamine ring, it was found that the product ion of the labeled compound was at m/z 559. Since the product ion retained the M + 1 from the carbon-13-labeled methyl group, this group was not lost in Q2 and therefore the loss was from the removal of the cladinose ring.

To the knowledge of the authors, the product ions and proposed structures of pentoxifylline, meprobamate, DEET, dilantin, oxybenzone, and progesterone resulting from ESI positive LC/MS/MS presented in this paper have not been published previously.

MS/MS-ESI Negative. All precursor ions in ESI negative were the result of a simple deprotonation $[M - H]^-$, except for naproxen, which readily decayed in the source via the loss of carbon dioxide and a methyl group. Although the $[M - H]^-$ ion of naproxen was visible during the initial infusion, the $[M - H - CO_2 - CH_3]^-$ ion was selected because of its greater intensity. Iopromide also showed a much more intense potential precursor ion than the typical $[M - H]^-$. Since the peak was at m/z 127, it was assumed to be due to an iodide ion, probably the result of the decay of iopromide and resulting loss of iodide in the source. Since iodide cannot fragment to give product ions and the matrixes of interest would cause too much interference to allow the monitoring of the same ion through Q1 and Q3, the less intense $[M - H]^-$ ion was used.

Of the six compounds analyzed by ESI negative, four were carboxylic acids and each of them showed, to varying degrees,

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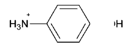
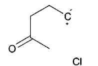
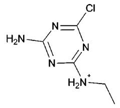
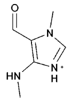
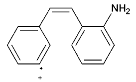
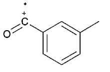
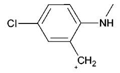
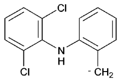
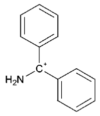
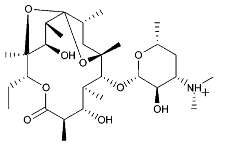
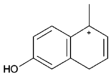
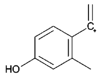
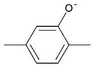
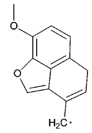
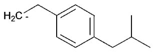
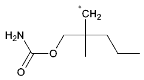
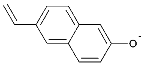
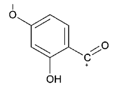
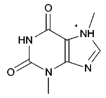
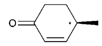
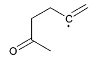
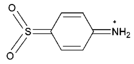
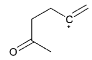
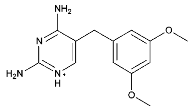
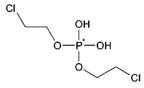
Table 7. Precursor Ion with Adduct, Product Ion, and Proposed Product Ion Structure for Compounds of Interest

Compound	Precursor Ion [Adduct]	Product Ion	Proposed Product Ion Structure
Acetaminophen	151.9 $[M + H]^+$	110.0	
Androstenedione	287.0 $[M + H]^+$	97.0	
Atrazine	216.1 $[M + H]^+$	173.8	
Caffeine	195.0 $[M + H]^+$	137.8	
Carbamazepine	237.0 $[M + H]^+$	193.9	
DEET	192.2 $[M + H]^+$	118.9	
Diazepam	285.0 $[M + H]^+$	153.9	
Diclofenac	294.0 $[M - H]^-$	249.8	
Dilantin	253.0 $[M + H]^+$	181.9	
Erythromycin – H_2O	716.4 $[M + H - H_2O]^+$	558.3	
Estradiol	255.1 $[M + H - H_2O]^+$	158.9	
Ethynylestradiol	279.1 $[M + H - H_2O]^+$	133.0	
Fluoxetine	310.0 $[M + H]^+$	44.2	
Gemfibrozil	249.2 $[M - H]^-$	121.1	
Hydrocodone	300.2 $[M + H]^+$	198.9	
Ibuprofen	205.0 $[M - H]^-$	161.0	
Iopromide	790.0 $[M - H]^-$	126.8	I^-
Meprobamate	219.2 $[M + H]^+$	157.9	
Naproxen	170.0 $[M - H - CO_2 - CH_3]^-$	168.9	
Oxybenzone	229.0 $[M + H]^+$	150.9	
Pentoxifylline	279.1 $[M + H]^+$	180.9	
Progesterone – ESI	315.1 $[M + H]^+$	109.0	
Progesterone – APCI	315.1 $[M + H]^+$	97.0	
Sulfamethoxazole	254.0 $[M + H]^+$	155.9	
Testosterone	289.1 $[M + H]^+$	97.1	
Triclosan	286.9 $[M - H]^-$	35.1	Cl^-
Trimethoprim	291.1 $[M + H]^+$	260.9	
TCEP	284.9 $[M + H]^+$	222.8	

the characteristic tendency to lose carbon dioxide (*m*/*z* 44) during collisions in Q2. This was used for the MS/MS transition of diclofenac and ibuprofen. The other two carboxylic acids each showed a loss of carbon dioxide in product ion scans; however, this loss did not result in the most intense product ion. As mentioned previously, naproxen easily lost both a carbon dioxide and a methyl group in the source. From this precursor ion, the optimal product ion was the loss of another proton $[M - H - CO_2 - CH_3 - H]^-$, most likely due to the formation of a double bond to stabilize the precursor. The final carboxylic acid, gemfibrozil, also showed a loss of carbon dioxide in the product ion scan. However, the signal for a loss of $C_7H_{12}O_2$, corresponding to a break of the ether bond, was much more intense and was used as the product ion. For iopromide and triclosan the product ions were both ionized halogen atoms: iodide and chloride, respectively.

MS/MS-APCI Positive. Of the five steroids that were analyzed by APCI positive, the three 4-ene-3-one-containing steroids (androstenedione, progesterone, testosterone) were seen as proton adducts $[M + H]^+$ during initial infusion tests. The other two steroids, estradiol and ethynylestradiol, underwent a loss of water in the source and were seen as the $[M + H - H_2O]^+$ ion in Q1. This seems to be a function of the source design itself as researchers with a source similar to the one used in this study reported this phenomenon[18] while others with a different source design did not.[19]

A pattern of common product ions emerged for the 4-ene-3-one-containing steroids. All three showed the loss of a large portion of the molecule, leaving a fragment with a *m*/*z* of ~97. To help determine the structure of the product ion, deuterium-labeled testosterone was used. This standard was labeled at the 16 (two deuteriums) and 17 (one deuterium) positions of the molecule. It was found that this labeling increased the mass of the precursor ion by 3 Da but did not change the mass of the product ion. Due to the positioning of the deuterium label, this indicates that the labeled D ring of testosterone was lost during the fragmentation process. This finding, combined with the similarity between the mass of the shared ene–one moiety and the mass of the unknown fragment, led to the structure shown in Table 7.

Product ion scans for estradiol and ethynylestradiol showed a heavy amount of fragmentation with few ions substantially larger than the others. After optimization, the fragments with the greatest abundance for estradiol and ethynylestradiol were found to be m/z 159 and 133, respectively. This is in agreement with Lagana et al.,[18] who hypothesized that the m/z 159 fragment corresponded to a dihydronaphthalenic structure and the m/z 133 fragment was consistent with a vinylbenzene structure as shown in Table 7.

Matrix Effects. During the course of the development of this method, it was noted that recoveries of the target compounds were, in most cases, severely reduced in the presence of natural waters. To test the extent of the effect, duplicate 1-L samples of surface water from the Boulder Basin of Lake Mead, NV, were extracted. The extracts were then spiked at a concentration of 50 μg/L and brought to a final volume of 1 mL. Simultaneously, trace methanol was added to a clean, empty vial, spiked with 50 μg/L of the target compound list and also brought to a final volume of 1 mL. The results of the test are shown in Table 8. All compounds ran in ESI positive and ESI negative, except hydrocodone, show a large degree of matrix suppression. Hydrocodone, conversely, shows signal enhancement. The data from erythromycin $-$ H_2O are not shown due to quality control failures. In contrast to ESI, all compounds ran in APCI positive showed no matrix effects. These results agree with other researchers and show the susceptibility of ESI to matrix effects. APCI, however, did not show this propensity.

Table 8. Matrix Suppression of Target Compounds

compound	mean methanol concn (ng/L)	mean surface water concn (ng/L)	change (%)
	ESI Positive		
hydrocodone	31	42	−36
trimethoprim	41	36	11
acetaminophen	48	26	46
caffeine	41	26	37
erythromycin − H_2O	54	37	32
sulfamethoxazole	56	22	61
fluoxetine	31	25	21
pentoxifylline	48	24	50
meprobamate	55	33	40
dilantin	46	20	57
TCEP	54	19	65
carbamazepine	54	29	46
DEET	51	37	27
atrazine	51	35	30
diazepam	51	24	53
oxybenzone	49	25	48
progesterone	49	30	38
	ESI Negative		
iopromide	48	20	58
naproxen	49	7.5	85
ibuprofen	49	23	53
diclofenac	49	27	46
triclosan	50	27	46
gemfibrozil	50	29	42
	APCI Positive		
ethynylestradiol	50	49	2
androstenedione	49	48	2
estradiol	50	48	4
testosterone	49	49	0
progesterone	50	50	0

Occurrence in Surface Water. Several surface waters with varying degrees of wastewater influence were sampled and analyzed using this method. The Las Vegas Wash is a waterway composed of ~90% treated municipal effluent that transports water from the city of Las Vegas, NV, to Lake Mead, NV. Samples were taken in the Las Vegas Wash (LVW), at the confluence of the LVW and Lake Mead (CWL), and in the Boulder Basin of Lake Mead (BB), ~10 km downstream from the CWL. A summary of the results of these samples is shown in Table 9. Matrix spikes were also extracted in two of the three water types, and the mean recoveries are shown in Table 9.

Most of the targeted compounds were detected in the LVW. As the flow from the wash is diluted by its convergence with Lake Mead, concentrations gradually decline until only 12 are detected

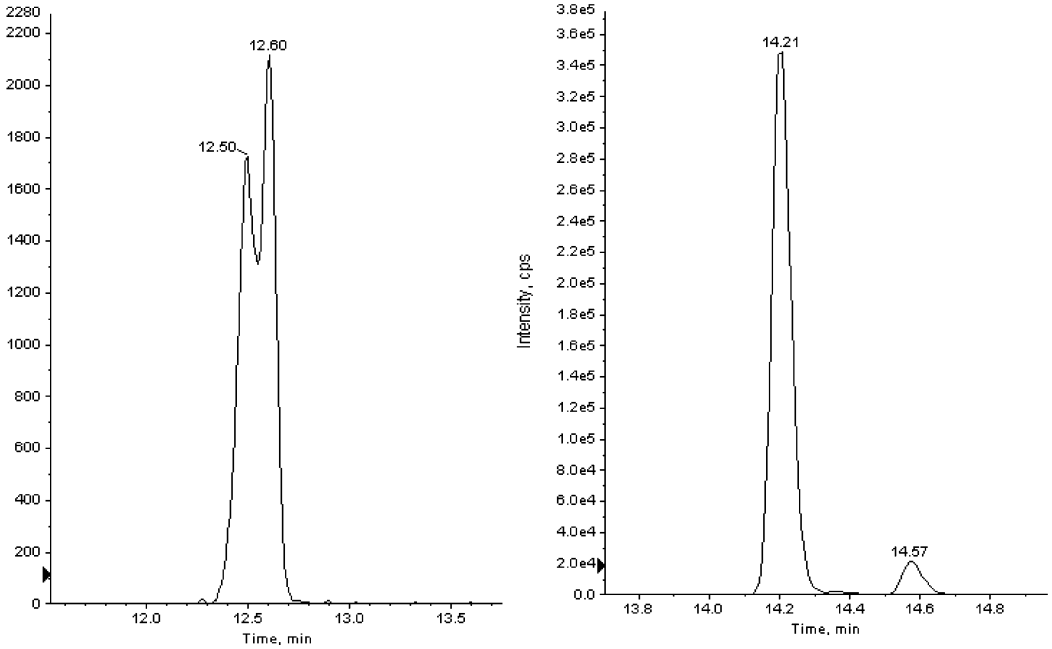


Figure 4. Chromatograms showing dual peaks for both iopromide (left) and erythromycin $-$ H_2O (right).

Table 9. Occurrence in and Matrix Spike Recoveries of Various Surface Waters (ng/L)

compound	Las Vegas Wash		confluence of Las Vegas Wash and Lake Mead		Boulder Basin		extracted blank		Las Vegas Wash matrix spike recovery		Boulder Basin matrix spike recovery	
	$n = 3$	% RSD	$n = 3$	% RSD	$n = 3$	% RSD	$n = 5$	% RSD	$n = 3$	% RSD	$n = 3$	% RSD
ESI Positive												
hydrocodone	19	8	7.3	24	<1.0	0	<1.0	0	40	4	73	2
trimethoprim	25	5	4.1	1	1.1	19	<1.0	0	28	3	66	7
acetaminophen	3.4	5	2.0	16	<1.0	0	<1.0	0	10	11	29	7
caffeine	27	6	1.5	39	3.0	14	<1.0	0	23	4	44	3
erythromycin $-H_2O$	98	11	3.0	29	<1.0	0	<1.0	0	27	5	55	5
sulfamethoxazole	20	15	33	23	5.4	11	<1.0	0	13	7	35	4
fluoxetine	5.5	27	<1.0	0	<1.0	0	<1.0	0	22	4	50	4
pentoxifylline	2.2	5	<1.0	0	<1.0	0	<1.0	0	22	0	48	2
meprobamate	220	3	190	1	14	0	<1.0	0	29	4	63	2
dilantin	52	2	48	0	2.6	0	<1.0	0	18	2	37	3
TCEP	81	5	68	1	2.0	20	<1.0	0	16	4	37	4
carbamazepine	92	5	76	3	3.9	5	<1.0	0	54	2	60	1
DEET	40	6	35	2	3.9	7	<1.0	0	39	4	67	2
atrazine	<1.0	0	1.0	0	1.2	13	<1.0	0	40	4	60	2
diazepam	1.3	4	<1.0	0	<1.0	0	<1.0	0	31	5	45	3
oxybenzone	5.2	2	4.8	4	3.1	3	<1.0	0	15	5	41	1
progesterone	3.9	89	14	76	12	92	200	135	19	8	55	3
ESI Negative												
iopromide	12	20	8.8	14	<1.0	0	<1.0	0	20	11	41	15
naproxen	4.2	14	5.5	9	<1.0	0	<1.0	0	8	8	23	10
ibuprofen	4.5	13	5.5	18	<1.0	0	<1.0	0	11	13	45	5
diclofenac	1.6	13	1.9	9	<1.0	0	<1.0	0	17	8	52	3
triclosan	5.0	9	5.4	9	<1.0	0	<1.0	0	18	11	45	3
gemfibrozil	56	11	48	3	1.8	10	<1.0	0	38	12	65	4
APCI Positive												
ethynylestradiol	<1.0	0	<1.0	0	<1.0	0	<1.0	0	128	4	128	3
estradiol	<1.0	0	<1.0	0	<1.0	0	<1.0	0	111	1	117	5
androstenedione	1.1	12	<1.0	0	<1.0	0	<1.0	0	89	1	102	4
testosterone	<1.0	0	<1.0	0	<1.0	0	<1.0	0	93	2	99	3
progesterone	14	90	44	61	14	148	217	136	84	3	98	4

in the BB. Some compounds show higher concentrations in the CWL than LVW; however, this can be explained by matrix suppression. As the concentration of wastewater in the sample increases, the amount of matrix suppression also increases as can be seen by the matrix spike recoveries in Table 9. Hence, although the concentration in the LVW is seemingly lower than the CWL, the degree of suppression from the LVW is larger. Considering this, the analyte concentrations in the LVW are probably greater than those in the CWL. The authors suggest using matrix spike recoveries, surrogate recoveries, or the method of standard additions to obtain more accurate quantitation using this method with waters containing high percentages of treated wastewater.

None of the extracted blanks showed contamination with the target compounds, except for progesterone (Table 9). Historically, this has not been seen using this method; however, the blanks associated with the data presented in this paper showed high and variable concentrations of progesterone. Since instrument blanks did not show the contamination, it is suspected that it was introduced during sample preparation. For this reason, the progesterone results presented in Table 9 should be viewed accordingly.

CONCLUSIONS

The analytical method described above provides a simple and sensitive method for the detection of a wide range of pharmaceuticals, personal care products, steroids, and various endocrine-disrupting compounds in several types of natural waters. Using automated SPE and a triple quadrupole mass spectrometer, this method gives the sensitivity and selectivity necessary for the detection of these compounds at environmentally relevant concentrations in the low-nanogram per liter range. Sulfuric acid was found to be the most effective and least destructive preservative agent. Most target compounds were detected in various surface waters, and the quantities of each reflect the percentage of treated wastewater in the sample. Matrix suppression in both ESI modes, due to the presence of treated municipal effluent in the samples, proved to have substantial effects on the recoveries of target analytes. To obtain more accurate surface water concentrations, correction using matrix spike recoveries, surrogate standards, or the method of standard additions is suggested. Research is ongoing to find ways of minimizing the impact of suppression on analyses that use ESI.

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