

Analysis of Ecologically Relevant Pharmaceuticals in Wastewater and Surface Water Using Selective Solid-Phase Extraction and UPLC–MS/MS

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A rapid and sensitive method has been developed for the analysis of 48 human prescription active pharmaceutical ingredients (APIs) and 6 metabolites of interest, utilizing selective solid-phase extraction (SPE) and ultraperformance liquid chromatography in combination with triple quadrupole mass spectrometry (UPLC–MS/MS). The single-cartridge extraction step was developed using a mixed mode reversed-phase/cation-exchange cartridge (Oasis MCX) and validated in both wastewater effluent and surface water. Recoveries for the majority of compounds ranged from 80% to 125%, with relative standard deviations generally below 15%. Analytes were quantified using a multiple injection analysis with four chromatographic runs, with a combined run time of 48 min and SPE–UPLC–MS/MS method detection limits ranging from 1.0 to 51 ng/L. The analysis of seven wastewater effluents and one surface water sample revealed at least one detection for 38 of the 54 compounds, with effluent concentrations ranging from 7 to 2950 ng/L and surface water concentrations ranging from 10 to 140 ng/L. This initial data demonstrates that a significant number of the selected target analytes are present in wastewater treatment plant discharges.

The presence of many active pharmaceutical ingredients (APIs) in the environment has become of increasing concern due to their potential to cause undesirable ecological effects. Contamination of the aquatic environments by both veterinary and human pharmaceutical compounds through discharge from wastewater treatment plants and agricultural runoff has been reported in recent studies.^{1–3} Pharmaceuticals used in human medicine can enter the environment either by excretion or by disposal of surplus drugs into sewage systems. Presently, wastewater treatment plants (WWTPs) are not specifically designed to remove many classes of trace level contaminants such as pharmaceuticals,^{4–6} and many

of these compounds are consequently released into surface waters.^{3,7–10} There are a multitude of human APIs in use today with widely varying chemical and physical properties, uses, modes of action, and potency, and these compounds are expected to have different environmental fates and effects.

In order to investigate the occurrence and fate of these trace level contaminants, accurate and sensitive methods are needed. The separation technique that has been primarily implemented in the analysis of APIs in different water matrixes is high-performance liquid chromatography (HPLC),^{11–21} although gas chromatography (GC) has also been used.^{10,22} Due to its high selectivity and sensitivity, tandem mass spectrometry (MS/MS) is most frequently employed as the detection system.^{11,12,14,16–21} Typical environmental concentrations of pharmaceuticals are in submicrograms per liter, making preconcentration prior to detection imperative. For water samples, solid-phase extraction (SPE) is the method of choice for sample preparation, and both off-line and on-line SPE–LC–MS/MS have been used in the

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environmental analysis of antibiotics, antiepileptic drugs, antidepressants, and steroid hormones.^{10–13,15–17,20,22,23}

Because of the amount of time and resources needed to extract and analyze complex environmental samples for trace levels of contaminants, the most recent methods have focused on multi-residue analysis.^{12,16,18,20,22–24} However, a challenge is presented in the simultaneous extraction and analysis of several classes of compounds with a wide range of polarities, solubilities, pK_a 's, K_{OW} 's, and stabilities under acidic and basic conditions, and often compromises are made that may affect identification or quantification, such as reduced recoveries, elevated detection limits, or the monitoring of only one product ion. Furthermore, typical HPLC run times for the analysis of a larger number of analytes can make it difficult to assess greater quantities of compounds in a timely and cost-effective manner. The recent advent of ultraperformance liquid chromatography (UPLC), which uses small particle size columns and operates at a much higher pressure than HPLC, has been shown to be a promising solution for batch analysis.^{18,24,25} When directly compared to HPLC, UPLC has resulted in better resolution, sensitivity, and a significant reduction in sample analysis time and mobile phase solvent consumption.²⁵

With hundreds of active pharmaceutical ingredients currently in use today, complete monitoring of all APIs and their metabolites is impractical, and therefore the selection of the target list of analytes becomes crucial. The selection of analytes for many existing methods is sometimes based on which compounds can readily be included in a single method, such as those with similar charge, ionization mode, or available isotope standards, or is at times based on which analytes have previously been detected. Our group has recently described²⁶ the prioritization of a list of the most prescribed APIs in the United States based on the potential of their wastewater residues to cause biological effects. The top-rated APIs from that process and selected metabolites were targeted for method development, with the list of analytes shown in Table 1. This is the first environmental pharmaceutical analysis method targeting a list of analytes derived to reflect ecological importance, several of which to our knowledge have not previously been included in an environmental monitoring method. Our goal was to develop a rapid, sensitive analytical method for this specifically targeted list of analytes that not only includes a large number of analytes with a broad range of chemical properties but also ensures that we would be reporting relative and accurate information in the future. The following work describes the development and limited application of that method, which utilizes a single extraction, isotope dilution, and multiple injection UPLC–MS/MS analysis to assess trace levels of over 50 selected APIs and metabolites of interest in wastewater effluent and surface water. This method will be used in future studies to assess the exposure of ecosystems and aquatic organisms to APIs present in WWTP discharges.

EXPERIMENTAL SECTION

Chemicals and Reagents. API reference standards were purchased in the highest purity available (97% or greater) from

Sigma-Aldrich (St. Louis, MO), Toronto Research Chemicals (North York, Ontario, Canada), United States Pharmacopeia (Rockville, MD), and LGC Promochem (Teddington, Middlesex, United Kingdom). A 10 $\mu\text{g}/\text{mL}$ mixture of 32 stable isotopically labeled compounds was used as a procedural internal standard, and the compounds were purchased from Sigma-Aldrich, Toronto Research Chemicals, and C/D/N Isotopes, Inc. (Pointe-Claire, Quebec, Canada), as listed in Table 2. Glassware used in the preparation and storage of standards was silanized using Sylon CT (Sigma-Aldrich) to avoid any loss of analytes due to sorption to glass. The acetonitrile (ACN) and methanol (MeOH) used for the chromatographic mobile phase were HPLC grade high-purity solvents purchased from Fisher Scientific (Fair Lawn, NJ). Reagent grade ACN and MeOH used in sample preparation, formic acid, hydrochloric acid (HCl), disodium ethylenediaminetetraacetate (Na_2EDTA), ascorbic acid, and ammonium hydroxide were also purchased from Fisher Scientific.

Selection of Target Analytes. Our group has previously described²⁶ the use of publicly available human drug sales data and wastewater production rates to conservatively estimate the maximum likely concentrations of the most widely dispensed prescription active ingredients in U.S. wastewater. Pharmacological data on drug potency was used to prioritize these APIs with regard to the potential of their predicted concentrations to cause biological effects, and the target analytes were selected from the top of that prioritized list of APIs. Several of the top drugs were not selected for method development because they are known to be highly labile (insulin, isosorbide mononitrate, levothyroxine, liothyronine, and nitroglycerine) and unlikely to survive even rudimentary wastewater treatment. Several drugs with lower priorities were added based on frequent detection in other studies (cimetidine, ranitidine, and gemfibrozil) or potential to cause reproductive disruption (progesterone and testosterone). Six metabolites were chosen for monitoring along with their parents, because the metabolites are predicted to contribute a significant portion of total activity in patient urine and feces (desmethylsertraline, desmethyldiltiazem, 10-hydroxy-amitriptyline, norfluoxetine, and norverapamil), or because they account for a significant portion of the excreted mass of administered drug (2-hydroxy-ibuprofen). Predicted wastewater effluent concentrations, identities of metabolites, and relevant pharmacological properties of most of the APIs have been tabulated elsewhere.²⁶ Additional information on minimum daily dose, therapeutic free plasma concentrations, and metabolite proportions for cimetidine, ranitidine, paroxetine, fluoxetine, oxycodone, gemfibrozil, progesterone, and testosterone were identified from prescribing information (for Tagamet, Zantac, Paxil, Prozac, Oxycontin, Lipid, Prometrium, and Striant, respectively). Therapeutic free plasma concentrations of cimetidine²⁷ and gemfibrozil²⁸ were identified from the literature. Corresponding “safe” free plasma concentrations of testosterone and progesterone were chosen to be $1/10$ of the reference free plasma concentration in females or prepubescent males, respectively (historical ranges downloaded December 7, 2007 from <http://cclnprod.cc.nih.gov/dlm/testguide.nsf/Index?OpenForm>).

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Table 1. Summary of the Target Analytes and Their Assigned Procedural Internal Standards, Respective Retention Times, MS/MS Parameters, and Precursor and Product Ions

peak no.	compd	CAS no.	procedural internal standard	RT (min) ^a	segment	precursor ion	CV ^b	product ion 1	CE 1 ^c	product ion 2	CE 2 ^c
Basic APIs 1: Antidepressants, Antibiotics											
1	cimetidine	51481-61-9	trimethoprim- <i>d</i> ₉	1.5	I	253	22	159	13	95	30
2	ranitidine	66357-59-3	trimethoprim- <i>d</i> ₉	1.6	I	315	28	176	17	130	25
3	trimethoprim	738-70-5	trimethoprim- <i>d</i> ₉	2.5	II	291	46	230	25	123	30
4	sulfamethoxazole	723-46-6	sulfamethoxazole- <i>d</i> ₄	3.0	II	254	28	156	17	92	29
5	10-hydroxy-amitriptyline	64520-05-4	trimethoprim- <i>d</i> ₉	3.6	II	294	28	231	21	216	30
6	promethazine	58-33-3	promethazine- <i>d</i> ₄	6.2	III	285	22	86	17	198	21
7	paroxetine	110429-35-1	paroxetine- <i>d</i> ₄	7.0	III	330	40	70	30	192	21
8	alprazolam	28981-97-7	alprazolam- <i>d</i> ₅	7.4	III	309	43	281	26	205	40
9	amitriptyline	549-18-8	amitriptyline- <i>d</i> ₅	8.8	IV	278	34	91	21	233	17
10	benztropine	86-13-5	amitriptyline- <i>d</i> ₅	9.5	IV	308	50	167	30	98	30
11	norfluoxetine	83891-03-6	fluoxetine- <i>d</i> ₅	10.2	IV	296	16	136	9		
12	fluoxetine	59333-67-4	fluoxetine- <i>d</i> ₅	11.1	IV	310	20	44	9	148	9
13	desmethylsertraline	79902-63-9	sertraline- <i>d</i> ₃	11.8	IV	292	16	275	9	159	25
14	sertraline	79559-97-0	sertraline- <i>d</i> ₃	11.9	IV	306	16	158	25	275	13
Basic APIs 2: Cardiovascular Drugs, Pain Medications											
15	albuterol	18559-94-9	albuterol- <i>d</i> ₉	1.4	I	240	22	148	17	166	13
16	atenolol	29122-68-7	atenolol- <i>d</i> ₇	1.4	I	267	35	145	25	74	23
17	clonidine	4205-91-8	clonidine- <i>d</i> ₄	2.5	II	230	46	213	25	187	29
18	oxycodone	124-90-3	hydrocodone- <i>d</i> ₃	2.8	II	316	35	241	30	256	26
19	amphetamine	51-63-8	amphetamine- <i>d</i> ₃	2.9	II	136	20	91	15	119	9
20	hydrocodone	143-71-5	hydrocodone- <i>d</i> ₃	3.1	II	300	42	199	32	171	40
21	triamterene	396-01-0	triamterene- <i>d</i> ₅	3.6	III	254	50	237	26	104	38
22	metoprolol	56392-17-7	atenolol- <i>d</i> ₇	4.8	III	268	34	116	18	72	21
23	enalipril	76095-16-4	enalipril- <i>d</i> ₅	6.6	IV	377	34	234	21	160	29
24	propranolol	318-98-9	atenolol- <i>d</i> ₇	6.6	IV	260	34	116	17	72	21
25	desmethyl-diltiazem	130606-60-9	diltiazem- <i>d</i> ₃	7.6	IV	401	34	178	25	145	30
26	diltiazem	33286-22-5	diltiazem- <i>d</i> ₃	7.6	IV	415	40	178	17		
27	norverapamil	67814-42-4	verapamil- <i>d</i> ₆	8.3	V	441	50	165	29	150	30
28	verapamil	137862-53-4	verapamil- <i>d</i> ₆	8.4	V	455	50	303	29	165	30
29	propoxyphene	1639-60-7	verapamil- <i>d</i> ₆	8.6	V	340	16	58	16	266	9
30	amlodipine	111470-99-6	amlodipine- <i>d</i> ₄	8.7	V	409	16	238	9	294	9
Neutral APIs											
31	acetaminophen	103-90-2	acetaminophen- <i>d</i> ₄	1.1	I	152	28	111	17	65	29
32	prednisone	53-03-2	prednisolone- <i>d</i> ₄	3.0	II	359	22	267	13	131	13
33	prednisolone	50-24-8	prednisolone- <i>d</i> ₄	3.1	II	361	22	145	21	307	17
34	hydrocortisone	50-23-7	hydrocortisone- <i>d</i> ₂	3.1	II	363	34	121	24	97	24
35	carbamazepine	298-46-4	carbamazepine- <i>d</i> ₁₀	3.3	II	237	34	194	21	192	29
36	betamethasone	378-44-9	norethindrone- <i>d</i> ₆	3.5	II	393	16	355	13	373	9
37	methylprednisolone	83-43-2	prednisolone- <i>d</i> ₄	3.5	II	375	16	339	11	161	17
38	norethindrone	68-22-4	norethindrone- <i>d</i> ₆	4.1	III	299	34	109	25	213	25
39	testosterone	58-55-9	testosterone- <i>d</i> ₃	4.2	III	289	35	97	15	109	15
40	valsartan	396-01-0	valsartan- <i>d</i> ₃	4.6	III	436	22	235	22	291	22
41	flucanone	356-12-7	fluticasone- <i>d</i> ₃	4.8	III	495	22	337	16	319	22
42	atorvastatin	134523-00-5	atorvastatin- <i>d</i> ₅	5.4	IV	559	40	440	17	466	17
43	fluticasone	80474-14-2	fluticasone- <i>d</i> ₃	5.5	IV	501	16	293	18	131	18
44	progesterone	57-83-0	testosterone- <i>d</i> ₃	5.6	IV	315	35	97	20	109	25
45	simvastatin	79902-63-9	simvastatin- <i>d</i> ₆	7.0	V	419	25	199	15	243	17
Acidic APIs											
46	theophylline	58-55-9	hydrochlorothiazide- ¹³ C- <i>d</i> ₂	1.1	I	179	40	164.0	21	122	21
47	hydrochlorothiazide	58-93-5	hydrochlorothiazide- ¹³ C- <i>d</i> ₃	1.2	I	296	34	205	22	269	22
48	2-hydroxy-ibuprofen	51146-55-5	ibuprofen- <i>d</i> ₃	2.6	II	221	20	177	9		
49	furosemide	54-31-9	glipizide- <i>d</i> ₁₁	2.8	II	329	28	285	17	205	17
50	warfarin	81-81-2	glipizide- <i>d</i> ₁₁	2.8	II	307	34	161	17	250	25
51	glipizide	29094-61-9	glipizide- <i>d</i> ₁₁	3.0	II	44	40	319	21	170	30
52	ibuprofen	15687-27-1	ibuprofen- <i>d</i> ₃	3.4	II	205	16	161	9		
53	gemfibrozil	25812-30-0	gemfibrozil- <i>d</i> ₆	4.0	III	249	22	121	9	127	9
54	glyburide	10238-21-8	glipizide- <i>d</i> ₁₁	4.4	III	492	37	170	30	367	17

^a RT = retention time (min). ^b CV = cone voltage (V). ^c CE = collision energy (eV).

Sample Preparation. Samples were concentrated using 150 mg Oasis MCX mixed mode cartridges, which contain both a hydrophobic–lipophilic balanced copolymer for a reversed-phase interaction and a strong cation-exchange capacity for the selective retention of basic analytes (Waters, Milford, MA). Two milliliters of a solution containing 5.0 g/L Na₂EDTA and 25 mg/L ascorbic acid and 25 μ L of the 10 μ g/mL solution of the procedural internal

standards (corresponding to a final concentration of 500 ng/L) were added to 500 mL volume samples, which were extracted at a neutral (unadjusted) pH. The cartridges were conditioned with 6 mL of ACN, followed by 6 mL of distilled water. Samples were passed through the cartridges at a rate of 3–5 mL/min using a Supelco vacuum manifold (Sigma-Aldrich), which allowed for the parallel extraction of up to 24 samples. The cartridges were then

Table 2. Summary of the Employed Procedural Internal Standards and Their Respective Retention Times and MS/MS Parameters

procedural internal standard	supplier	LC-MS/MS method	RT (min) ^a	segment	precursor ion	CV ^b	product ion	CE ^c
trimethoprim- <i>d</i> ₉	Toronto Research	basics-1	2.5	II	300	46	234	25
sulfamethoxazole- <i>d</i> ₄	Toronto Research	basics-1	3.0	II	258	22	96	21
promethazine- <i>d</i> ₄	C/D/N Isotopes	basics-1	6.2	III	289	22	86	17
paroxetine- <i>d</i> ₄	Toronto Research	basics-1	7.0	III	334	46	74	29
alprazolam- <i>d</i> ₅	Sigma-Aldrich	basics-1	7.4	III	314	43	286	26
amitriptyline- <i>d</i> ₅	C/D/N Isotopes	basics-1	8.8	IV	281	40	91	21
fluoxetine- <i>d</i> ₅	C/D/N Isotopes	basics-1	10.9	IV	315	20	44	9
sertraline- <i>d</i> ₃	Toronto Research	basics-1	11.9	IV	309	16	275	12
albuterol- <i>d</i> ₉	Sigma-Aldrich	basics-2	1.4	I	249	22	148	17
atenolol- <i>d</i> ₇	C/D/N Isotopes	basics-2	1.4	I	274	35	145	25
clonidine- <i>d</i> ₄	C/D/N Isotopes	basics-2	2.5	II	234	46	217	25
amphetamine- <i>d</i> ₃	Sigma-Aldrich	basics-2	2.9	II	139	16	92	21
hydrocodone- <i>d</i> ₃	Sigma-Aldrich	basics-2	3.1	II	303	46	199	30
triamterene- <i>d</i> ₅	Toronto Research	basics-2	3.6	III	259	50	242	29
enalipril- <i>d</i> ₅	C/D/N Isotopes	basics-2	6.6	IV	382	35	239	21
diltiazem- <i>d</i> ₃	Toronto Research	basics-2	7.6	IV	418	40	178	25
verapamil- <i>d</i> ₈	Toronto Research	basics-2	8.4	V	461	50	165	29
amlodipine- <i>d</i> ₄	Toronto Research	basics-2	8.7	V	413	16	238	9
acetaminophen- <i>d</i> ₄	Toronto Research	neutrals	1.1	I	156	28	114	17
prednisolone- <i>d</i> ₄	C/D/N Isotopes	neutrals	3.1	II	367	34	150	21
hydrocortisone- <i>d</i> ₂	C/D/N Isotopes	neutrals	3.1	II	365	34	123	24
carbamazepine- <i>d</i> ₁₀	C/D/N Isotopes	neutrals	3.3	II	247	34	204	21
norethindrone- <i>d</i> ₆	Toronto Research	neutrals	4.1	III	305	34	113	25
testosterone- <i>d</i> ₃	Toronto Research	neutrals	4.2	III	292	46	97	29
valsartan- <i>d</i> ₃	Toronto Research	neutrals	4.6	III	439	22	294	22
atorvastatin- <i>d</i> ₅	Toronto Research	neutrals	5.4	IV	564	46	445	25
fluticasone- <i>d</i> ₃	Toronto Research	neutrals	5.5	IV	504	16	293	18
simvastatin- <i>d</i> ₆	Toronto Research	neutrals	7.0	V	425	25	199	15
hydrochlorothiazide- ¹³ C- <i>d</i> ₃	Toronto Research	acidics	1.2	I	299	34	206	25
glipizide- <i>d</i> ₁₁	Toronto Research	acidics	3.0	II	455	40	319	21
ibuprofen- <i>d</i> ₃	Toronto Research	acidics	3.4	II	208	16	164	9
gemfibrozil- <i>d</i> ₆	Toronto Research	acidics	4.0	III	256	22	121	9

^a RT = retention time (min). ^b CV = cone voltage (V). ^c CE = collision energy (eV).

slowly washed with 6 mL of 2% formic acid and allowed to vacuum-dry. Acidic and neutral analytes were first eluted with two 4 mL volumes of ACN into a silanized conical glass tube. Basic analytes were then collected in a separate silanized glass tube by two slow elutions with ACN containing 5% ammonium hydroxide. The volume of each eluate was reduced to dryness under a gentle stream of nitrogen at 40 °C. The first elution was reconstituted in 500 µL of 20% ACN in water, whereas the second elution was reconstituted in 20% MeOH in water. Samples were then transferred to a polypropylene vial for immediate UPLC-MS/MS analysis, as shown in Figure 1.

Ultraperformance Liquid Chromatography/Tandem Mass Spectrometry. Analysis was conducted using an Aquity UPLC coupled to a Quattro Micro triple quadrupole mass spectrometer equipped with an electrospray ionization source (ESI) (Waters, Milford, MA). The column used was a BEH C₁₈ column (100 mm × 1.0 mm i.d. with 1.7 µm particle size) equipped with a 0.2 µm in-line filter, also purchased from Waters. The flow rate for all applications was 100 µL/min, the column oven temperature was 40 °C, and the full loop injection volume was 10 µL. The analysis was divided into four injections, including one for basic antidepressants and antibiotics, one for basic cardiovascular drugs and pain medications, one for neutral APIs, and one for acidic APIs. Separations were performed using binary gradient mobile phases, consisting of water with 0.3% formic acid (A1) or 20 mM ammonium hydroxide (A2) and a mixture of (2:1) ACN/MeOH (B).

For the basic antidepressants and antibiotics, the initial mobile phase proportion was 90% (A1)/10% (B), which was held for 0.5 min. B was then linearly increased to 30% in 0.5 min and further increased to 35% over 2 min, which was then held for 7 min. B was then additionally increased to 90% over 1 min, which was held for 0.5 min. Initial mobile phase conditions were restored over 0.5 min, and the column was allowed to equilibrate for 3 min, for a total run time of 15 min. For the separation of the basic cardiovascular drugs, the initial mobile phase condition was also 90% (A1)/10% (B), which was held for 0.5 min. B was then linearly increased to 50% over 7 min, and further increased to 90% over 2 min, which was then held for 0.5 min. Initial mobile phase conditions were restored over 0.5 min, and the column was allowed to equilibrate for 3.5 min, for a total run time of 14 min. The initial mobile phase condition for the separation of the neutral APIs was 80% (A1)/20% (B), which was held for 0.5 min. B was then linearly increased to 40% over 0.5 min, and further increased to 85% over 5 min, which was then held for 0.5 min. Initial mobile phase conditions were restored over 0.5 min, and the column was allowed to equilibrate for 3.0 min, for a total run time of 10 min. Acidic APIs were separated with the initial mobile phase condition of 90% (A2)/10% (B), which was held for 0.5 min. B was then linearly increased to 40% over 1 min, which was held for 2.5 min. B was then increased to 90% over 0.5 min and held for 0.5 min. Initial mobile phase conditions were restored over 0.5 min, and the column was allowed to equilibrate for 3.5 min, for a total run time of 9 min.

Individual tune files were created for each standard in continuous flow mode to determine the optimum capillary voltages, collision energies, and fragment ions, which are listed in Table 1. Multiple reaction monitoring (MRM) was used to collect data for the two most intense and/or specific product ions for each precursor ion, with the exception of ibuprofen, 2-hydroxy-ibuprofen, norfluoxetine, and diltiazem, for which only one product ion was present. The desolvation temperature was 450 °C, source temperature was 140 °C, and the capillary voltage was 3.0 kV for positive ion analysis and 2.5 kV for negative ion analysis. Positive ESI was used for the analysis of the basic antidepressants and antibiotics, basic cardiovascular drugs and pain medications, and neutral APIs, whereas the ESI source was operated in negative ion mode for the acidic APIs. Nitrogen was used as a desolvation gas at a flow rate of 450 L/h and cone gas at 50 L/h, and argon gas was used to induce dissociation for the acquisition of MS/MS data. Each chromatographic run was divided into time segments (Table 1), with a dwell time of 20 ms for each transition. For the basic antidepressants and antibiotics, time segment I was from 0.5 to 2.2, segment II from 2.0 to 4.5, segment III from 4.5 to 8.5, and segment IV from 8.2 to 13 min. For the basic cardiovascular drugs, segment I was from 0.5 to 2.0, segment II from 1.8 to 3.7, segment III from 3.25 to 5.5, segment IV from 5.4 to 8.0, and segment V from 7.8 to 9.5 min. For the neutral APIs, segment I was from 0.5 to 2.0 min, segment II from 2.0 to 3.8 min, segment III from 3.8 to 5.2, segment IV from 5.0 to 6.5, and segment V from 6.5 to 7.5 min. For the acidic APIs, time segment I was from 0.5 to 2.0, segment II from 1.8 to 3.8, segment III from 3.5 to 5.0, and segment IV from 3.5 to 5.0 min.

Detection and Quantification. Detection was based on retention time and product ion ratios collected from the MRM transitions. For a positive identification, both product ions had to be present with a signal-to-noise ratio of at least 3 and a product ion ratio within $\pm 30\%$ of the expected ratio. Quantification of target analytes was based on internal calibration curves constructed from a plot of the peak area ratio of the analyte signal to the signal of the assigned stable isotope standard versus concentration. Calibration standards were prepared at eight different concentrations to cover the respective dynamic range for all analytes (1, 5, 10, 25, 50, 100, 1000, and 3000 ng/mL) and nonweighted, linear calibration curves typically displayed correlation coefficients greater than 0.99. The instrument limit of detection (ILOD) and instrument limit of quantification (ILOQ) were determined using 10 replicate injections of a distilled water blank. The ILODs and ILOQs were calculated as the average concentration measured for the blank plus 3 times and 10 times its standard deviation, respectively.

Method Validation. The performance of the extraction method was assessed by calculating the percent recovery and relative standard deviation (RSD) for five replicate distilled water samples fortified at 100 and 3000 ng/L. The method detection limit (MDL) for each analyte was determined as the minimum concentration of an analyte that can be identified and detected with a 99% confidence that the analyte concentration is greater than zero. Five replicate distilled water samples were fortified near the expected MDL and taken through the entire analytical method. Since the method includes a wide variety of compounds with different analyte responses, four concentration levels were needed

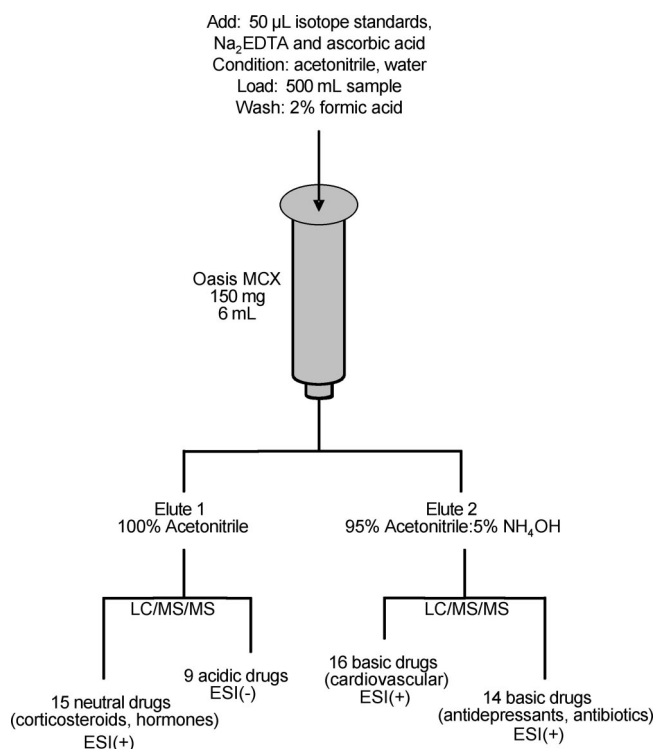


Figure 1. Schematic representation of the sample analysis procedure.

to cover the analyte range (5, 10, 25, and 50 ng/L). The MDL was then calculated by multiplying the standard deviation for the measured concentrations of the five replicate samples by the appropriate Student's *t* value for four degrees of freedom. The dynamic range (DR) of the method was identified as the lowest calibration point for each analyte (taking into account the 1000-fold concentration factor of the SPE) to the highest extraction concentration evaluated (3000 ng/L). Since isotopically labeled compounds were used as procedural internal standards, there is a possibility that the labeled standards may contain trace quantities of the unlabeled analytes that could interfere with quantification. Distilled water blanks at a volume of 500 mL were fortified with 500 ng/L of the 32 procedural internal standards and extracted along with each extraction batch. Blanks did not reveal any significant levels of the target compounds, indicating that contamination of the isotope standards was not a problem when spiked at this level.

To determine the influence of different sample matrixes on method performance, surface water from the North Shore Channel (Chicago, IL), and wastewater effluent from seven different WWTPs throughout New Mexico were tested using the optimized SPE conditions. Prior to fortification and SPE, water samples were passed through a Whatman 0.7 µm filter (Fisher Scientific, Waltham, MA). For the surface water, five 500 mL aliquots were spiked to obtain final concentrations of 500 ng/L. For the wastewater effluent, 500 mL of each of the seven different effluents was spiked to obtain final concentrations of 1000 ng/L. The procedural internal standards were added to each sample at a concentration of 500 ng/L, and the samples were extracted according to the conditions above. After extraction, the unevaporated extracts were stored at -4 °C for up to 3 months. Sample-based LODs and LOQ were determined

Table 3. Summary of the SPE Percent Recoveries and Relative Standard Deviations (RSD) in Distilled Water, Surface Water, and Wastewater Effluent^a

compd	distilledwater				surfacewater		effluent	
	recovery 100 ng/L	RSD	recovery 3000 ng/L	RSD	recovery 500 ng/L	RSD	recovery 1000 ng/L	RSD
cimetidine	80	1	83	2	109	12	87	9
ranitidine	61	11	68	10	48	24	60	16
trimethoprim	103	4	92	2	94	5	103	2
sulfamethoxazole	102	8	91	4	97	4	106	5
10-hydroxy-amitriptyline	85	10	81	4	128	3	121	9
promethazine	102	1	92	1	90	3	98	3
paroxetine	110	5	98	1	114	3	102	3
alprazolam	116	4	96	2	101	10	157	14
amitriptyline	97	10	99	4	120	4	98	4
benztropine	96	10	115	8	82	6	107	4
norfluoxetine	75	6	60	6	62	5	66	11
fluoxetine	98	11	96	1	93	7	101	4
desmethylsertraline	74	15	66	11	57	13	69	12
sertraline	113	4	81	8	89	3	95	6
albuterol	114	10	114	4	104	1	93	23
atenolol	101	3	114	11	102	9	98	7
clonidine	106	5	94	7	106	7	115	12
oxycodone	113	10	94	3	97	6	96	16
amphetamine	109	5	100	8	80	6	89	11
hydrocodone	103	10	91	1	98	7	98	4
triamterene	108	1	89	3	94	12	104	9
metoprolol	105	7	108	12	119	25	104	20
enalipril	94	3	98	1	85	19	100	12
propranolol	87	14	84	3	99	6	97	8
desmethyldiltiazem	78	10	70	4	94	7	81	9
diltiazem	100	3	92	2	99	4	100	4
norverapamil	84	9	80	4	77	10	99	18
verapamil	86	3	90	3	90	4	101	10
propoxyphene	117	10	93	7	94	9	102	7
amlodipine	110	5	102	5	100	3	102	2
acetaminophen	108	3	102	9	99	4	107	4
prednisone	93	10	123	7	123	11	115	6
prednisolone	103	5	100	3	98	6	94	6
hydrocortisone	97	10	91	5	104	4	97	12
carbamazepine	116	5	98	1	102	2	124	5
betamethasone	76	10	132	9	81	10	83	11
methylprednisolone	107	16	81	5	137	6	134	7
norethindrone	106	7	98	6	114	5	107	1
testosterone	111	3	95	2	100	3	102	3
valsartan	95	5	98	3	107	11	96	5
fluocinonide	65	7	118	4	118	19	134	9
atorvastatin	111	10	104	3	109	8	113	13
fluticasone	109	12	111	13	111	13	121	1
progesterone	104	15	95	4	93	8	84	8
simvastatin	90	13	108	8	119	8	124	3
theophylline	73	18	68	19	40	11	43	13
hydrochlorothiazide	105	10	93	7	107	5	105	19
2-hydroxy-ibuprofen	101	5	135	11	111	9	116	17
furosemide	101	5	136	15	102	13	142	27
warfarin	112	5	127	16	110	5	94	15
glipizide	102	2	98	11	101	12	103	11
ibuprofen	110	9	90	5	83	12	95	8
gemfibrozil	112	7	87	6	102	9	100	7
glyburide	127	11	144	19	82	5	90	23

^a Distilled water $n = 5$, surface water $n = 5$, effluent $n = 7$.

in both the surface water and wastewater effluent and were determined as the concentrations corresponding to a signal-to-noise ratio of 3 and 10, respectively. Since several of the analytes were already present in the extracted samples, LODs and LOQs were determined by extrapolation, assuming a linear correlation.

Sample Analysis. To test the applicability of the optimized SPE-LC-MS/MS method, samples were collected from the effluent of seven WWTPs in New Mexico and the surface water of the East Fork River in Cincinnati, OH. Samples were

collected in amber glass bottles and shipped overnight on ice. Upon arrival, 25 μL of the procedural internal standard mixture was added to each 500 mL sample volume and samples were stored at 4 °C until extraction. Samples were extracted within 2 days of storage. Matrix spike control samples at a concentration of 1.0 $\mu\text{g/L}$ and blanks prepared from laboratory distilled water were extracted along with the wastewater effluent and surface waters. The percent recovery of the matrix spike control samples was used to adjust the reported analyte concentrations.

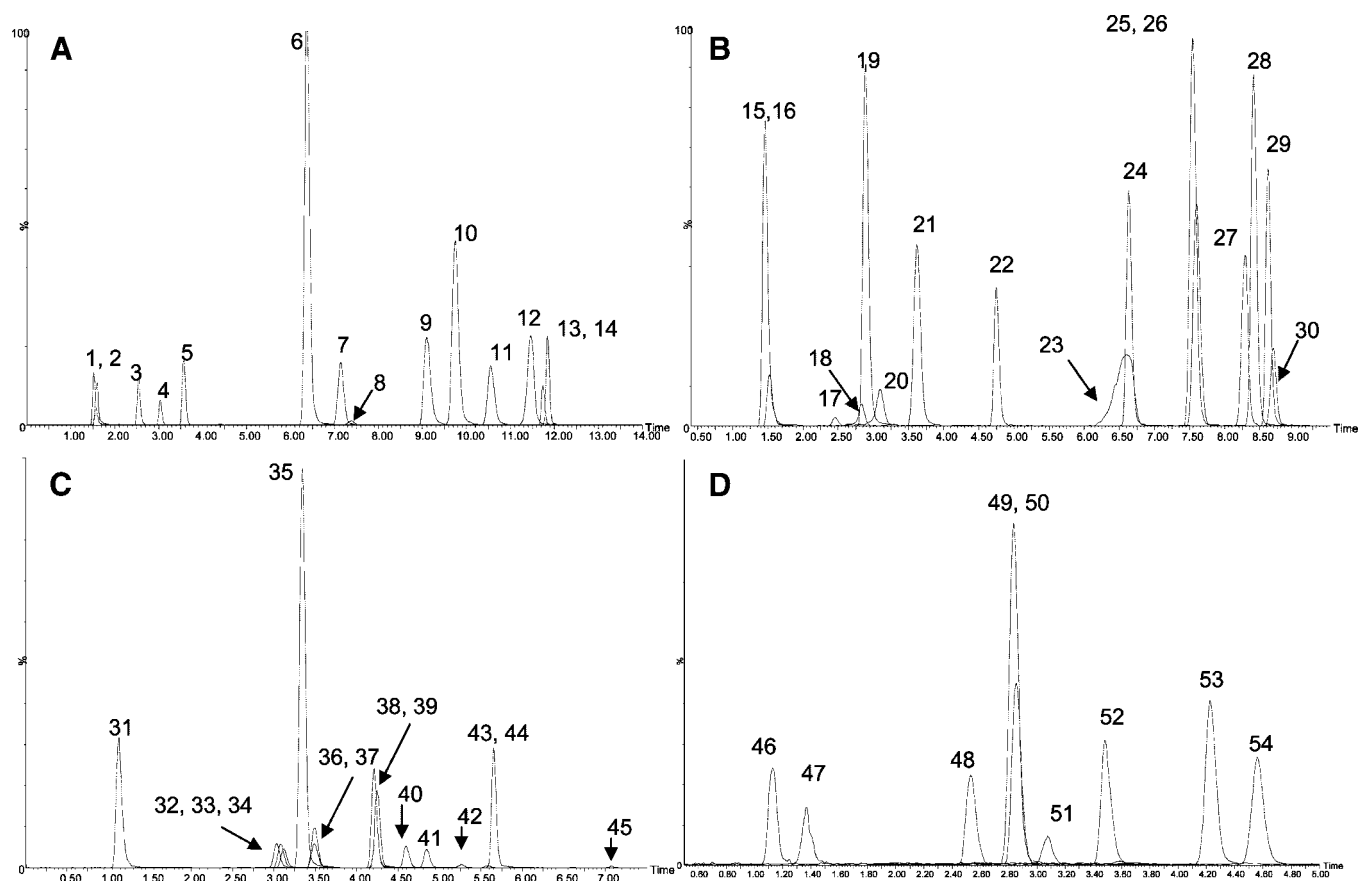


Figure 2. Typical combined MRM chromatograms of the product ion of greatest intensity for the 54 analytes using the multiple injection procedure: (A) basic APIs 1—antidepressants and antibiotics, (B) basic APIs 2—cardiovascular drugs and pain medications, (C) neutral APIs, and (D) acidic APIs. Individual peak assignments are listed in Table 1.

RESULTS AND DISCUSSION

Sample Preparation Procedure. Several different extraction cartridges were tested during preliminary experiments, including Oasis HLB (*N*-vinylpyrrolidone and divinylbenzene reversed-phase copolymer), Oasis MCX (HLB with a strong cation exchange), and the Oasis WCX (HLB with a weak cation exchange). The Oasis HLB and Oasis MCX provided better overall recoveries than the Oasis WCX. It should be noted that some APIs were not recovered from effluent using the Oasis MCX cartridges, including ciprofloxacin, enalaprilat, metformin, and lisinopril (data not shown); however, greater recoveries were observed for the highly polar compounds when compared to the Oasis HLB. Although the Oasis MCX extraction involves two separated elutions, this procedure provided significantly cleaner extracts in a matrix when compared to the Oasis HLB cartridge. Not only did this result in lower signal-to-noise ratios, but also resulted in considerably lower column backpressure. When analyzing effluent samples extracted with Oasis HLB cartridges, column backpressures would frequently exceed the 15 000 psi limit. However, a sequence of several effluent samples extracted with Oasis MCX cartridges could be run without a significant increase from the initial backpressure of about 8000 psi, resulting in an increase in UPLC column lifetime.

Na_2EDTA was added as a chelating agent and ascorbic acid was added to samples prior to extraction to remove any residual chlorine that may be present in disinfected effluent samples. Sample pH during extractions has also been shown to influence

analyte recovery, and therefore a sample pH of 3.0, 4.0, 5.0, and an unadjusted sample pH (neutral) were also investigated. In both distilled water and effluent, no significant difference in recoveries were found between acidic and neutral pH samples, and therefore samples were extracted at a neutral pH. Acetonitrile was used as the extraction solvent since atorvastatin may form esters by reaction with alcohols.²¹ The majority of the recoveries for five replicate distilled water samples fortified at 100 ng/L and 3000 ng/L ranged from 80% to 125% (Table 3); however, a few compounds did show recoveries outside of this range. The ideal internal or procedural internal standard for a compound is its isotopically labeled analog; however, isotopically labeled compounds were not available for each analyte. Differences in retention times or differing degree of ionization suppression or enhancement in the extract may result in reduced or enhanced recoveries for the compounds that did not have an exact match isotope procedural standard available. It should be noted that the percent recoveries reported were determined by adding the procedural internal standards before extraction, which accounts for any losses during the extraction procedure. However, during the extraction methods development, the internal standards were added after the extraction to assess the overall extraction efficiency (data not shown). With the exception of acetaminophen, which exhibited an average absolute recovery of 50%, the percent recoveries determined by adding the internal standards after extraction were not markedly different than those reported.

Table 4. Comparison of Instrument Limits of Detection and Quantification, Method Detection Limit, and the Limits of Detection and Limits of Quantification Achieved in Surface Water and Effluent

compd	distilled water				surface water		effluent		target
	ILOD ng/mL	ILOQ ng/mL	MDL ng/L	DR ng/L	LOD ng/L	LOQ ng/L	LOD ng/L	LOQ ng/L	LOQ ng/L
cimetidine	0.1	0.2	2.1	1–3000	1.8	5.9	0.9	2.9	32000
ranitidine	0.1	0.2	2.2	10–3000	5.0	17	1.2	4.1	38250
trimethoprim	0.2	0.7	3.0	1–3000	1.0	3.4	0.4	1.2	1500
sulfamethoxazole	0.2	0.4	5.5	1–3000	3.1	10	2.0	6.5	16000
10-hydroxy-amitriptyline	0.3	0.3	16	1–3000	2.8	9.4	0.5	1.6	797
promethazine	0.6	0.6	2.3	1–3000	0.2	0.8	0.4	1.4	26
paroxetine	0.6	0.6	6.5	1–3000	0.8	2.6	2.5	8.2	25
alprazolam	0.9	0.9	2.7	1–3000	4.1	14	2.4	8.0	230
amitriptyline	0.8	0.8	2.9	1–3000	3.1	10	2.2	7.3	263
benztropine	0.5	0.5	1.3	1–3000	0.6	1.9	0.9	3.0	15
norfluoxetine	1.3	1.3	9.0	1–3000	2.3	7.6	1.2	4.1	100
fluoxetine	1.1	1.1	4.0	1–3000	1.9	6.2	1.4	4.8	50
desmethylsertraline	1.3	1.3	6.9	5–3000	11	37	7.6	25	150
sertraline	0.7	0.7	3.6	5–3000	1.5	5.0	5.1	17	15
albuterol	0.2	0.4	1.3	1–3000	0.8	2.7	0.3	1.1	460
atenolol	0.2	0.5	2.0	1–3000	1.7	5.8	1.7	5.7	25000
clonidine	3.8	11	4.3	10–3000	5.3	18	2.4	8.1	22
oxycodone	0.5	1.2	8.3	5–3000	4.1	14	0.4	1.4	550
amphetamine	0.2	0.5	2.0	1–3000	0.3	1.1	0.2	0.6	1428
hydrocodone	1.4	2.9	2.3	1–3000	1.4	4.7	0.6	1.9	605
triamterene	0.2	0.5	4.5	1–3000	2.4	8.0	0.2	0.7	880
metoprolol	0.2	0.5	1.6	1–3000	1.1	3.6	0.3	1.0	6250
enalipril	0.1	0.3	1.1	1–3000	9.4	31	0.8	2.6	1250
propranolol	0.5	1.3	1.3	1–3000	0.6	2.1	0.4	1.4	40
desmethyldiltiazem	0.2	0.5	1.0	1–3000	0.1	0.5	0.2	0.7	14500
diltiazem	0.3	0.8	2.7	1–3000	0.1	0.4	0.1	0.3	4350
norverapamil	0.8	2.5	7.8	1–3000	0.8	2.5	0.2	2.6	2364
verapamil	0.4	1.1	3.0	1–3000	0.5	1.6	0.1	1.1	520
propoxyphene	0.3	0.7	9.2	1–3000	0.7	2.2	0.2	0.8	5520
amlodipine	0.5	1.3	6.1	1–3000	1.7	5.8	1.0	3.4	1250
acetaminophen	1.0	2.9	1.4	1–3000	6.6	22	9.4	31	600000
prednisone	1.9	4.7	5.7	5–3000	2.2	24	14	47	1300
prednisolone	3.3	7.6	5.4	5–3000	9.9	33	5.0	17	500
hydrocortisone	2.6	6.2	4.5	10–3000	16	54	11	39	87
carbamazepine	0.3	0.7	2.0	5–3000	0.4	1.3	0.3	0.8	30000
betamethasone	7.0	19	9.1	5–3000	25	84	9.0	30	75
methylprednisolone	2.5	6.7	14	1–3000	14	47	6.6	2.2	576
norethindrone	0.3	0.9	2.3	1–3000	2.3	7.6	1.4	4.6	9
testosterone	0.9	0.9	1.0	1–3000	1.7	5.8	2.1	6.9	0.4
valsartan	5.2	10	6.6	10–3000	1.6	5.3	1.5	4.9	3000
fluocinonide	4.3	12	12	5–3000	1.8	6.0	0.3	0.9	50
atorvastatin	3.3	9.0	3.7	10–3000	0.7	2.4	1.4	4.6	6.48
fluticasone	4.1	11	16	1–3000	13	42	1.2	3.9	6.25
progesterone	0.4	1.2	8.8	5–3000	0.8	2.6	1.4	4.7	0.12
simvastatin	1.1	2.4	4.5	5–3000	4.0	13	0.3	0.9	9
theophylline	1.1	3.0	28	50–3000	18	61	1.8	6.1	150000
hydrochlorothiazide	2.0	5.4	15	50–3000	11	35	2.0	5.4	4200
2-hydroxy-ibuprofen	9	23	45	25–3000	26	85	11	19	420000
furosemide	7.6	20	28	25–3000	12	39	3.1	1.9	1000
warfarin	2.2	5.4	38	25–3000	6.0	18	0.6	2.0	750
glipizide	25	50	11	50–3000	11	36	0.6	7.8	158
ibuprofen	3.7	10	33	10–3000	15	51	2.3	7.8	42000
gemfibrozil	2.0	5.0	17	25–3000	12	40	3.5	1.7	75000
glyburide	4.8	4.5	51	25–3000	12	41	0.4	1.4	85

UPLC–MS/MS. Two ions were monitored for the majority of the 54 APIs and one for each of the 32 isotope procedural internal standards, resulting in 136 total MRM transitions that needed to be monitored. Although UPLC can be used to achieve a significant reduction in analysis times, we are limited by the dwell time achievable by the Quattro Micro and therefore decided the best combination of speed and sensitivity could be achieved by separating the target analytes using multiple injections. Analytes were grouped first based on polarity, which determined both which fraction they elute during the SPE procedure and the optimum ESI mode for analysis, and then by similarities in

chemical structure and class. Since sensitivity typically decreases with an increasing number of transitions, each injection was further subdivided into time segments to allow the acquisition of a minimum of 15 data points per peak. Compounds were successfully separated to allow 3–5 time segments per chromatographic run, average peak widths (measured at half-height) of 0.07–0.3 min, and a total combined run time of 48 min. Combined chromatograms of the MRM transition for the product ion of highest intensity for each analyte are shown in Figure 2. Instrument detection limits ranged from 0.1 to 50 ng/mL and are listed in Table 4.

Table 5. Summary of the Concentrations of APIs Detected in Seven Wastewater Effluents and One Surface Water Sample^a

compd	WWTP1	WWTP2	WWTP3	WWTP4	WWTP5	WWTP6	WWTP7	SW
cimetidine	nd	14	410	250	12	12	210	nd
ranitidine	nd	nd	220	530	nd	550	450	nd
trimethoprim	40	39	39	96	69	140	120	det
sulfamethoxazole	98	1300	310	2200	1400	920	1500	140
10-hydroxy-amitriptyline	nd	nd	nd	13	42	64	det	nd
promethazine	det	det	det	det	16	det	det	nd
paroxetine	det	det	det	det	13	det	det	nd
alprazolam	10	15	12	14	14	18	det	nd
amitriptyline	25	44	57	77	79	79	86	nd
benztropine	nd	nd	nd	nd	det	nd	nd	nd
norfluoxetine	det	det	det	det	det	det	det	nd
fluoxetine	40	44	71	40	42	73	62	nd
sertraline	57	57	85	74	63	75	87	nd
albuterol	nd	38	55	48	60	38	41	nd
atenolol	960	390	120	440	890	730	530	35
oxycodone	120	140	53	89	150	76	93	det
amphetamine	det	det	det	det	det	det	det	nd
hydrocodone	28	190	120	100	73	67	120	10
triamterene	130	190	440	240	184	250	370	12
metoprolol	430	150	650	320	300	390	230	12
propranolol	46	32	63	77	50	50	64	23
desmethyldiltiazem	65	110	93	91	76	81	70	65
diltiazem	28	140	180	200	124	160	170	13
norverapamil	46	51	71	62	51	42	46	nd
verapamil	14	54	112	190	70	40	110	nd
propoxyphene	det	det	65	7	nd	det	nd	nd
acetaminophen	nd	nd	nd	nd	260	nd	nd	nd
carbamazepine	430	800	675	620	70	220	450	20
valsartan	81	120	60	150	250	1500	160	det
atorvastatin	nd	14	42	det	nd	30	nd	nd
hydrochlorothiazide	1300	2000	2950	2670	1460	1640	2420	75
2-hydroxy-ibuprofen	nd	nd	nd	nd	nd	200	67	nd
furosemide	180	190	930	760	570	710	340	nd
warfarin	nd	nd	50	nd	nd	nd	det	nd
glipizide	nd	nd	nd	nd	nd	30	nd	nd
ibuprofen	nd	nd	nd	nd	88	72	nd	nd
gemfibrozil	77	78	380	320	150	1220	47	nd
glyburide	nd	93	nd	nd	120	87	nd	nd

^a All concentrations are in ng/L. WWTP = wastewater treatment plant, SW = surface water.

Method Validation. Method performance was also evaluated in wastewater effluents and surface water, with the average percent recoveries reported in Table 3. Since wastewater treatment technology and therefore wastewater effluent can vary significantly between sampling locations, the method was evaluated in seven different effluents in order to fully investigate the influence of the matrix. The majority of the average recoveries ranged from 80% to 125%; however, a few compounds demonstrated recoveries below 70%, including ranitidine, norfluoxetine, desmethylsertraline, and theophylline. Although seven different effluents, with a single sample from each location potentially containing a different amount of matrix material, were spiked and extracted, the majority of the RSDs for the average of the percent recoveries for the seven effluents were below 15%. It has been widely demonstrated that pharmaceutical compounds analyzed in natural waters are susceptible to matrix effects, either ionization suppression or enhancement, when analyzed by LC-MS or LC-MS/MS using ESI.^{13,19,24} Isotopically labeled compounds were used as procedural internal standards to account for losses during the sample preparation and differences in ionization between standards and extracted samples. Again, isotope standards were not available for each analyte, and this could explain the reduced or enhanced recoveries or larger variations for those compounds without an

exact isotope match standard. For those compounds for which an isotopically labeled version was not available, the most suitable internal standard was determined by directly comparing the peak areas of a distilled water extract and a wastewater extract, with both being fortified after extraction (data not shown). When the spike effluent and distilled water extracts were compared, it was found that the vast majority of the analytes displayed effluent signal suppressions ranging from 20% to 40%. The most significant matrix effects were observed among the corticosteroids and hormones, which displayed signal suppressions from 50% to 60%. Valsartan and atorvastatin demonstrated signal enhancements ranging from 30% to 40% in effluent as compared to distilled water extracts, with signal enhancements for simvastatin exceeding 100%. Similarities in the extent of ionization suppression or enhancement in the wastewater along with similarities in chemical structure or class were used to assign the remaining procedural internal standards for compounds that did not have an exact match isotope standard available.

The MDLs and DRs of the SPE-LC-MS/MS were also determined. Since wastewater effluent and surface water typically contain levels of the target analytes at concentrations far exceeding the expected MDLs and these matrixes can vary greatly between sampling locations, the MDL was determined in distilled water.

MDLs ranged from 1.0 to 51 ng/L and are reported along with the DR in Table 4. Since ILODs and ILOQs are based mostly on instrument background levels and the MDL takes only the precision of the method and not accuracy into account, these determinations may not necessarily be indicative of the analyte levels that can be measured in environmental samples. Sample-based LODs and LOQs were also determined in both wastewater effluent and surface water based on measured signal-to-noise ratios, with the LODs ranging from 0.1 to 26 ng/L and the LOQs ranging from 0.8 to 85 ng/L (Table 4). When the respective ILOQs, MDLs, and sample-based LOQs are compared for each compound, the majority of the compounds exhibited similar quantification levels in the low nanogram per liter range for the different matrixes (Table 4).

Targets for limits of quantification were chosen for each analyte (Table 4) based on pharmacological potency, so as to encourage quantification of concentrations with similar potential for eliciting biological effects.²⁶ These LOQ targets correspond to the lesser of $1/10$ of the human free (not bound to plasma proteins or blood cells) plasma concentration after minimum therapeutic dosing (for nonhormones), or $1/10$ of the free plasma concentration present in relatively hormonally quiescent individuals (females for testosterone, prepubescent males for progesterone), or $1/10$ of the minimum inhibitory concentration for the most sensitive known microbe, or the concentration that would provide $1/1000$ of a minimum therapeutic adult dose in 2 L of sample. For all analytes except progesterone and testosterone, LOQs achieved in wastewater effluent met the LOQ targets (Table 4). The LOQ targets for these two analytes were both below 1 ng/L. For all analytes, actual LOQs were sufficient to detect concentrations at or above the previously predicted²⁶ wastewater effluent concentration ceilings. It should be noted that method development efforts also initially included estradiol, ethinyl estradiol, and equilin; however, ILOQs were far above the target LOQ (data not shown), and these compounds will be analyzed with a separate method.

Sample Analysis. The applicability of the method was evaluated by analyzing a range of wastewater effluents from seven different WWTPs in New Mexico and a surface water sample from the East Fork River in Cincinnati, Ohio, with the results shown in Table 5. It should be noted that the samples were filtered prior to extraction, so the reported concentrations are for the water-soluble fraction only. Thirty-eight of the 54 analytes were detected in at least one of the eight samples, with 36 of the analytes present in quantifiable concentrations ranging from 7 to 2950 ng/L. Of these detected APIs and metabolites, 21 were quantified in all

seven of the sampled effluents. This initial data set demonstrates that a significant portion of the selected target analytes is present in wastewater effluents and therefore is being discharged into surface waters. In order to arrive at more generalizable conclusions about ecological exposure to API residues originating from wastewater, we will soon conduct a monitoring study on a larger and more representative sample of wastewater effluents using the method described in this paper.

CONCLUSION

The optimized SPE method proved to be efficient and reproducible for the simultaneous extraction of the 54 target APIs of potential ecological concern in both effluent and surface water. The single extraction combined with UPLC–MS/MS provides a rapid, sensitive method for the trace level quantification of a wide variety of APIs. This method proved useful in monitoring several effluent and surface water samples, and this initial data set demonstrated that several of the target APIs are present in wastewater discharges. The method will be used in future studies to provide important information on the exposure of aquatic ecosystems to these compounds.

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NOTE ADDED AFTER ASAP PUBLICATION

This paper was posted on May 23, 2008. Ratios of buffers A to B that had been reversed in text were corrected, and a change in one Table 1 entry was corrected. The paper was reposted on June 7, 2008.

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