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Determination of pharmaceutical compounds in surface- and ground-water samples by solid-phase extraction and high-performance liquid chromatography–electrospray ionization mass spectrometry

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Abstract

Commonly used prescription and over-the-counter pharmaceuticals are possibly present in surface- and ground-water samples at ambient concentrations less than 1 μg/L. In this report, the performance characteristics of a combined solid-phase extraction isolation and high-performance liquid chromatography–electrospray ionization mass spectrometry (HPLC–ESI-MS) analytical procedure for routine determination of the presence and concentration of human-health pharmaceuticals are described. This method was developed and used in a recent national reconnaissance of pharmaceuticals in USA surface waters. The selection of pharmaceuticals evaluated for this method was based on usage estimates, resulting in a method that contains compounds from diverse chemical classes, which presents challenges and compromises when applied as a single routine analysis. The method performed well for the majority of the 22 pharmaceuticals evaluated, with recoveries greater than 60% for 12 pharmaceuticals. The recoveries of angiotensin-converting enzyme inhibitors, a histamine (H2) receptor antagonist, and antihypoglycemic compound classes were less than 50%, but were retained in the method to provide information describing the potential presence of these compounds in environmental samples and to indicate evidence of possible matrix enhancing effects. Long-term recoveries, evaluated from reagent-water fortifications processed over 2 years, were similar to initial method performance. Method detection limits averaged 0.022 μg/L, sufficient for expected ambient concentrations. Compound-dependent matrix effects on HPLC/ESI-MS analysis, including enhancement and suppression of ionization, were observed as a 20–30% increase in measured concentrations for three compounds and greater than 50% increase for two compounds. Changing internal standard and more frequent ESI source maintenance minimized matrix effects. Application of the method in the national survey demonstrates that several pharmaceuticals are routinely detected at 0.010–0.100 μg/L concentrations.

Keywords: Water analysis; Environmental analysis; Drugs

1. Introduction

The discovery, production, and medicinal use of pharmaceuticals, synthetically derived or extracted from natural materials, are one of our society's greatest medical assets [1]. Human and animal health has been improved substantially by the introduction of antibiotics, analgesics, and condition-specific formulations for heart disease, hypertension, and other episodic and chronic health problems. An unsurprising but unintended consequence of the near universal use of pharmaceuticals has been the inadvertent introduction of these compounds or their metabolites into surface water and ground water, initially identified in European studies [2–4]. The presence of pharmaceuticals in aquatic environments typically results from human excretion of metabolized and unmetabolized drug passing into sewage or septic systems and subsequent discharge of wastewater [5] and percolation of septic-system leachate into surface water or ground water. Animal waste in pasturage or confined animal-feeding operations is another potential source of pharmaceuticals. Direct discharge to ground from excretion by livestock can contaminate surface water, or collection of waste in lagoons can infiltrate to ground water.

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Recent data from Europe indicate that the normal operation of sewage-treatment plants results in the incomplete removal of pharmaceuticals, hence as much as 80% of the total load of pharmaceuticals entering sewage-treatment plants may be discharged into surface water [6,7]. The pharmaceutical concentrations measured in wastewater prior to and after treatment have been about several micrograms per liter. The concentrations measured in surface-water samples downstream from sewage-treatment plant discharges typically have been in the tens of nanograms per liter, although concentrations in the μg/L range are possible. Although these concentrations are much lower than typical maximum contaminant concentrations (in the tens of micrograms per liter) reported for other industrial contaminants, the effects of continuous exposure to mixtures of pharmaceuticals on aquatic biota is unknown [8].

High-performance liquid chromatography coupled to mass spectrometry (HPLC–MS) and particularly tandem mass spectrometry (HPLC–MS–MS) have been the primary techniques used to determine ultratrace concentrations of many pharmaceuticals in wastewater, surface water, and ground water [5,9,10]. The long-term method performance of HPLC–MS and HPLC–MS–MS for routine monitoring of environmental pharmaceutical concentrations previously has not been described.

In this report, the development of a combined solid-phase extraction (SPE) isolation and HPLC–electrospray ionization (ESI) MS analytical procedure for routine determination of pharmaceuticals, primarily those associated with human health, in surface- and ground-water samples, is described. This method development was part of a larger study to assess the presence and distribution of organic wastewater contaminants in surface water and ground water in the USA. Some of the goals of the study are as follows: (1) to develop a list of compounds that reflect the prescription and over-the-counter pharmaceuticals most likely found in USA surface water and ground water; (2) to develop a method that would be sufficiently sensitive and selective to measure a variety of classes of compounds in the low ng/L range; (3) to apply the method to samples from a range of surface- and ground-water sites throughout the USA; and (4) to describe the long-term performance of the method and assess its suitability to routine monitoring. Goals 1, 2, and 4 are the focus of this report. Goal 3 is the subject of a separate report [8].

2. Experimental

2.1. Compounds analyzed

The prescription pharmaceuticals included in the method (Table 1) were selected in part based on data estimating the number of prescriptions written per year [11], the typical dose, and typical number of doses per prescription. An estimate of the annual mass prescribed was calculated by using the following formula:

$$\left(\frac{\text{number of prescriptions}}{\text{year}}\right) \times (\text{unit dose}) \times \left(\frac{\text{doses}}{\text{prescription}}\right) = \text{annual mass prescribed}$$

There is uncertainty in these estimates because of variations in dosing for individuals, variations in the size of the

Table 1
Pharmaceutical compounds determined by this method in order of elution

Target compound	Generic name	Drug name	Classification
Metformin	Metformin	Glucophage	Antihyperglycemic
Amoxicillin	Amoxicillin	Trimox	Antibacterial
(–)-Cotinine	Nicotine	Nicotine	CNS stimulant (metabolite)
Albuterol	Albuterol	Albuterol aerosol	Antiasthmatic
Cimetidine	Cimetidine	Tagamet	Histamine H2 Inhibitor
Ranitidine	Ranitidine	Zantac	Histamine H2 Inhibitor
Acetaminophen	Acetaminophen	Acetaminophen	Analgesic
1,7-Dimethylxanthine, caffeine	1,7-Dimethylxanthine, caffeine	1,7-Dimethylxanthine, caffeine	CNS stimulant (metabolite) CNS stimulant
Lisinopril	Lisinopril	Zestril	Antihypertensive
Enalaprilat	Enalapril	Vasotec	Antihypertensive (metabolite)
Trimethoprim	Trimethoprim	Trimethoprim/sulfa	Antibacterial
Digoxigenin	Digoxin	Lanoxin	Cardiac glycoside (metabolite)
Paroxetine metabolite	Paroxetine	Paxil	Antidepressant (metabolite)
Sulfamethoxazole	Sulfamethoxazole	Trimethoprim/sulfa	Antibacterial
Diltiazem	Diltiazem	Cardizem CD	Antiangina, antihypertensive
Furosemide	Furosemide	Furosemide Oral	Diuretic
Fluoxetine	Fluoxetine	Prozac	Antidepressant
Dehydronifedipine	Nifedipine	Procardia XL	Antianginal (metabolite)
Warfarin	Warfarin	Coumadin Tabs	Anticoagulant
Ibuprofen	Ibuprofen	Ibuprofen	Analgesic, NSAID
Gemfibrozil	Gemfibrozil	Gemfibrozil	Antihyperlipidemic

CNS: central nervous system; NSAID: non-steroidal anti-inflamatory drug.

dose, and the limited availability of proprietary prescription data. In some cases the excreted metabolite is the primary form in wastewater; therefore, primary metabolites also were considered in developing the list of pharmaceuticals analyzed.

Standards were obtained from commercial sources or were provided by the manufacturer and typically were at purities of 95% or greater. An injection internal standard, ^{13}C-labeled caffeine (Cambridge Isotope Labs., Cambridge, MA, USA), was used to quantify all compounds. ^{13}C-labeled Phenacetin (Cambridge Isotope Labs.) was used as the method-performance surrogate. Stock solutions of all compounds, fortification solutions, and surrogate solutions were dissolved in HPLC-grade methanol (Burdick & Jackson, Muskegeon, MI, USA). Calibration and fortification stock solutions were diluted in 10 mM, pH 3.7 aqueous formic acid/ammonium formate buffer.

2.2. Sample collection

Surface-water samples were collected using standard US Geological Survey (USGS) procedures for trace organic analysis [12]. Samples were shipped to the laboratory by overnight express. Samples that could not be filtered in the field were filtered in the laboratory, typically within 48 h. All samples were filtered using baked (450 °C for 8 h) Whatman 0.7 μm GF/F (glass-fiber filters) (Whatman, Clifton, NJ, USA).

2.3. Sample preparation

Several SPE stationary phases were tested under a range of elution conditions for optimum recovery (data not shown). Upon selection, pharmaceuticals were isolated from 1-L water samples using Waters (Milford, MA, USA), Oasis HLB, 0.5 g, 6-mL SPE cartridges with a positive-displacement pump (Fluid Metering, Syosset, NY, USA). Prior to extraction, a performance surrogate containing 1.0 μg of [^{13}C]1-ethoxy-phenacetin in 100 μL of methanol was added to each filtered sample. The SPE cartridges were conditioned with 6 mL of methanol, followed by vacuum drying at 1.6 kPa on a vacuum manifold for 10–15 s, followed by 6 mL of HPLC-grade water. Samples were processed through the SPE cartridge at 15 mL/min. Following isolation, 1 mL of 5% methanol in reagent-grade water was passed through the cartridge using a vacuum manifold, and the water discarded. The cartridge then was sequentially eluted with two 3-mL aliquots of methanol followed by two 2-mL aliquots of methanol acidified to pH 3.7 with trifluoroacetic acid (Sequanal grade, Pierce Biotechnology, Rockford, IL, USA). The combined aliquots were evaporated to near dryness (≅100 μL) with a TurboVap (Zymark, Hopkinton, MA, USA) evaporative concentrator, using nitrogen at a pressure of 34.5 kPa, in a 40 °C bath. Samples were reconstituted with 800–900 μL of formate buffer and 100 μL of [^{13}C]caffeine-3-methyl internal standard, at a concentration of 0.010 μg/μL. The reconstituted sample was filtered through a polytetrafluoroethylene/Teflon (PTFE) 0.2-μm syringe filter (Acrodisc [^{13}C]R, Pall Corp., East Hills, NY, USA). Samples were extracted in sets of up to 10 environmental samples, with two laboratory quality-control (QC) samples: an HPLC-grade water sample containing only the performance surrogate and an HPLC-grade water sample containing the performance surrogate and fortified at 1.0 μg/L of all compounds measured in this study. These laboratory samples were used to assess possible laboratory contamination of the sample, formation of analytical artifacts, and the recovery of pharmaceuticals under controlled conditions.

2.4. Chromatography and mass spectrometry

HPLC–MS was applied using a Hewlett-Packard (now Agilent Technologies, Palo Alto, CA, USA) Series 1100 HPLC/MSD system. An ammonium formate/formic acid buffer (10 mM, pH 3.7) aqueous phase and acetonitrile were used to produce a multistep binary elution gradient (Table 2). The flow rate was 0.200 mL/min, and all flow was directed to the mass spectrometer. Separations were made using a Metasil Basic 3 μm, 150 mm × 2.0 mm, C_{18} analytical column coupled to either a Metasil Basic Safeguard (MetaChem Technologies), 3 μm, 2.0 mm guard column, or NewGuard RP-18, 7 μm, 15 mm × 3.2 mm guard column (Perkin-Elmer).

The HPLC system was interfaced with the mass spectrometer using ESI in the positive ionization mode. The ESI source conditions were as follows: source temperature 150 °C, nebulizer gas pressure of 100 kPa, drying gas (nitrogen) flow rate of 9 L/min, and drying gas temperature of 350 °C. The potential difference between the source and the capillary was held at 3500 V. Programmed capillary exit voltage changes were used to produce sufficient fragmentation of each compound (Table 3) so that characteristic fragments were produced. In the instrument used in this study, the capillary exit voltage is referred to as the fragmentor voltage. A series of flow injection analyses were used to optimize

Table 2
Elution gradient used for high-performance liquid chromatographic separation of pharmaceutical compounds, flow rate of 0.200 mL/min

Time (min)	Proportion of acetonitrile (%)
0	6
5	6
9	14
10	24
15	41
16	51
26	70
27	100
34	100
39	6
50	6

Table 3
Instrumental parameters for pharmaceutical compounds determined by using high-performance liquid chromatography–mass spectrometry under selected-ion monitoring conditions

Time (min)	Fragmentor voltage	Compound(s)	Ion assignments	Relative ion abundance (%)	Electrometer gain
2.00	80	Metformin	113.0, **130.1**[a]	15, 100	2
5.00	80	Amoxicillin	**114.0**, 208.0	63, 60, 100	2
		Cotinine	349.0[a] 80.1, 98.1, **177.1**	3, 6, 100	
6.30	70	Albuterol	**166.1**, 222.1, 240.1[a]	38, 62, 100	2
7.80	88	Cimetidine	**159.0**, 253.1[a]	100, 38	2
		Acetaminophen	**110.0**, 152.0[a]	100, 73	
		Ranitidine	**176.0**, 315.1[a]	100, 88	
		1,7-Dimethylxanthine	124.0[a], **181.1**	100, 83	
13.00	90	Lisinopril	84.1, 246.1, **406.1**[a]	16, 2, 100	3
16.00	110	Caffeine	138.1, **195.1**[a]	58, 100	2
		[^{13}C]caffeine-3-methyl	139.1, **198.1**[a]	58, 100	
17.70	100	Enalaprilat	230.1, 303.1,	29, 19, 100	2
		Trimethoprim	**349.1**[a] 206.1, **291.1**	15, 100	
20.00	70	Digoxigenin	355.1, 373.2, **391.2**[a]	24, 25, 100	2
21.70	100	Paroxetine metabolite	192.1, **332.1**[a]	11, 100	2
		Phenacetin [^{13}C]	139.1, **181.1**[a]	79, 100	
		Sulfamethoxazole	156.1, **254.1**[a]	43, 100	
22.85	110	Diltiazem	178.1, **415.1**[a]	73, 100	2
		Furosemide	81.1, **352.9**[b]	5, 100	
24.35	70	Fluoxetine	148.1, **310.1**[a]	43, 100	2
25.40	120	Dehydronifedipine	268.0, 284.0, **345.0**[a]	17, 39, 100	2
26.65	70	Warfarin	163.1, 251.1, **309.1**[a]	44, 17, 100	2
28.50	60	Ibuprofen	**161.1**, 207.2[a]	100, 101	2
30.00	50	Gemfibrozil	205.2, **233.2**, 273.0[b]	61, 100, 80	2

Quantitation ions in bold; confirmation ions in normal font. [^{13}C]caffeine-3-methyl—internal standard. Phenacetin [^{13}C]—surrogate.
[a] Mass + hydrogen.
[b] Mass + sodium.

the fragmentor voltage for each compound. For each compound, the optimal detection conditions for the protonated molecular ion and at least one confirming fragment ion were used when collecting data in the selected-ion monitoring (SIM) mode, thereby increasing the sensitivity of detection (Table 3).

A multipoint internal standard calibration, from 0.010 to 2.0 μg/L, was used for each sample set analyzed. Calibration was monitored through the use of continuing calibration verification (CCV) samples, and if the calibration was within ±20%, analysis of environmental and laboratory QC samples was continued. Instrument blanks to monitor potential carryover between injections were interspersed between every 10 injections prior to a CCV. The HPLC–MS analyses were quantified using commercial quantitation software (Target 4.0; Thru-Put Systems, Boca Raton, FL, USA).

3. Results and discussion

Information for the most frequently prescribed drugs for the year 1998 from a compilation by commercial market-tracking sources was obtained from literature [13–15]. The source of the prescription data was an audit that tracked 2.486×10^9 prescriptions dispensed by 35 000 community pharmacies from December 1997 through November 1998, and projected to provide national estimates. Selected pharmaceuticals were drawn from the top twenty most commonly prescribed drugs and the most commonly used nonprescription drugs [16]. Except for albuterol and digoxigenin, all compounds were selected on the basis of these usage criteria. Albuterol and digoxigenin were included in the selected compound list as a negative control, because it was hypothesized that these compounds would not be detected in surface-water samples because of the low unit dosage (Table 1).

An effective chromatographic separation (Fig. 1) was achieved by using the stepped gradient listed in Table 2. The response of the individual compounds varied substantially (Fig. 1). This variation was expected because of the diversity of chemical classes among the selected pharmaceutical compounds under electrospray ionization conditions. However, overall response for all compounds was sufficient for analysis at the expected ambient environmental concentrations.

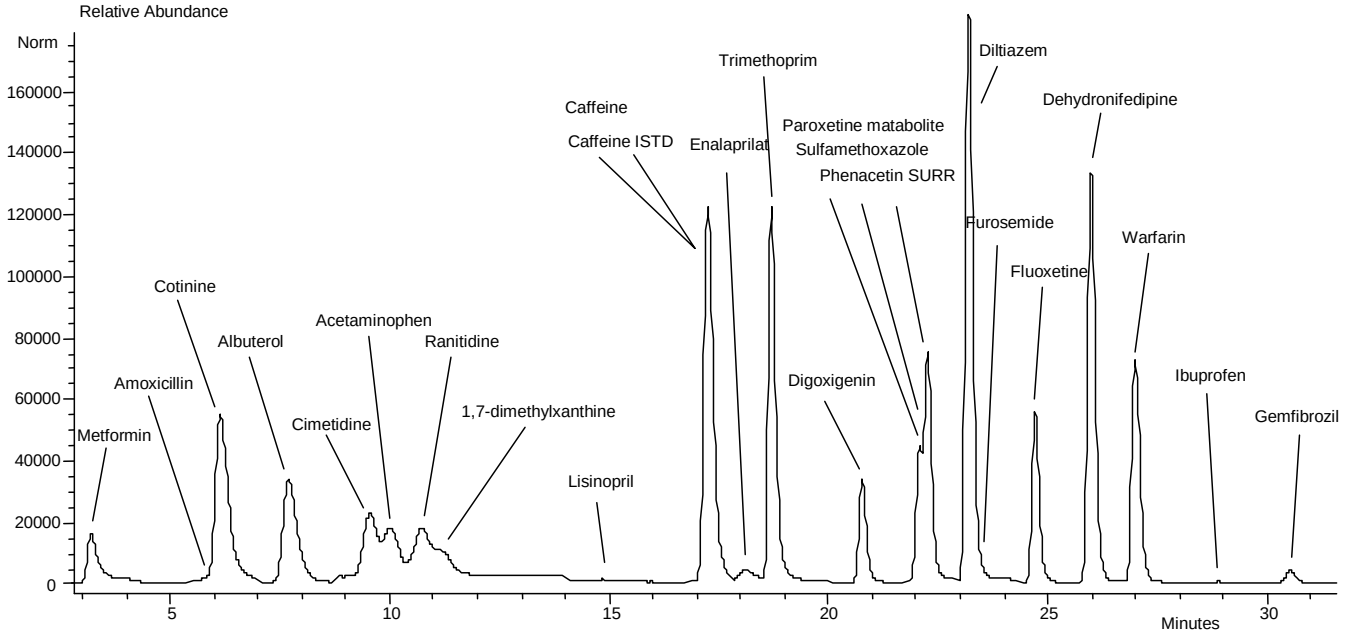

Fig. 1. Representative high-performance liquid chromatography/mass spectrometry chromatogram of a standard solution of the pharmaceuticals determined in this study. The amount of each compound injected was 0.050 μg, equivalent to a 1-μg/L concentration in a 1-L sample.

Flow injection analysis of each compound (Fig. 2) was used to determine the fragmentor voltage required to produce a definitive mass spectrum at optimal sensitivity. The characteristics of a definitive mass spectrum for qualitatively identifying compounds consisted of a protonated molecular ion and at least one confirming fragment ion in excess of 20% abundance of the molecular ion, and elution within 0.1 min of standard retention times. Fragmentor voltages were set for groups of compounds because closely eluting compounds could not always be separated. For some compounds this procedure resulted in a compromise fragmentor setting, which had the effect of either yielding fragment ions in less than 20% relative abundance or fragment ions in greater abundance than the molecular ion. The final

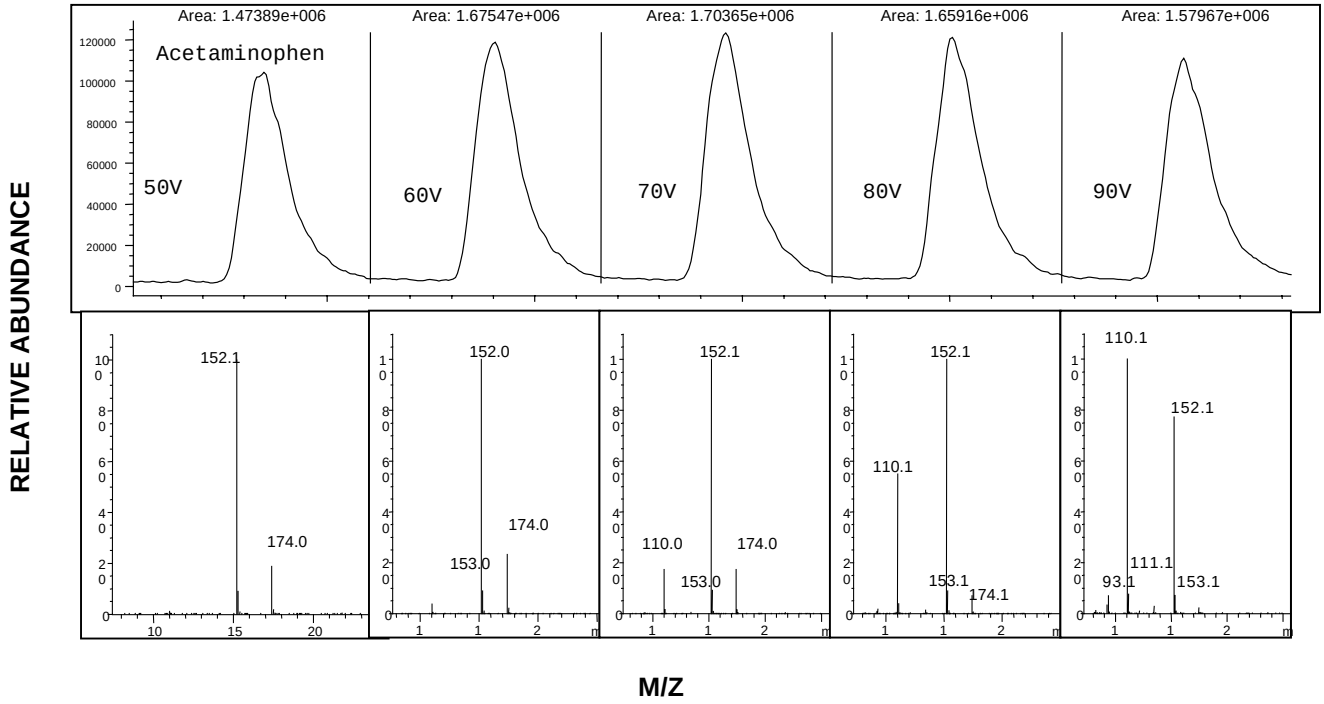

Fig. 2. The effect of fragmentor voltage on the fragmentation of acetaminophen, a representative pharmaceutical compound.

Table 4
Percent recoveries and relative standard deviation of pharmaceutical compounds from solid-phase extraction compared to long-term recoveries of pharmaceuticals determined from fortified reagent-water samples analyzed with environmental samples

Compound	Mean recovery SPE trials ($n = 8$, %)	Relative standard deviation of SPE trials	Recovery year 1999 set spikes ($n = 16$, %)	Relative standard deviation of year 1999 set spikes	Recovery year 2000 set spikes ($n = 28$, %)	Relative standard deviation of year 2000 set spikes
Cotinine	108	9.5	78	6.9	68	8.4
Albuterol	118	6.6	69	9.2	78	12.9
Cimetidine	52	1.0	34	6.0	21	8.4
Acetaminophen	78	8.2	62	7.8	85	19.3
Ranitidine	54	14.4	41	7.8	27	8.4
1,7-Dimethylxanthine	105	3.0	87	9.6	166	61.4
Lisinopril	29	1.8	32	9.6	13	12.9
Enalaprilat	12	2.8	11	3.6	25	16.4
Trimethoprim	124	15.1	81	4.5	71	7.8
Digoxigenin	122	13.5	85	4.4	94	20.0
Paroxetine metabolite	8	7.5	16	19.1	12	15.4
Diltiazem	99	5.1	78	6.7	70	10.3
Furosemide	65	11.8	67	7.4	69	11.4
Fluoxetine	78	4.0	74	10.1	47	18.2
Warfarin	127	13.1	86	5.2	85	9.8
Ibuprofen	65	15.9	79	6.8	72	12.9
Gemfibrozil	65	20.8	73	12.2	60	30.0
Mean	77	9.1	62	8.1	62	16.7

Solid-phase extraction trial and 1999 spikes fortified with 1.0 mg of each compound and 2000 spikes fortified with 1.0 or 0.5 μg/L. Five unrecovered compounds not listed.

fragmentor voltages, quantitation and confirmation ions, and their relative abundances used to confirm identification are listed in Table 3.

The variety of chemical classes represented in the analyte list provided varying results among the solid-phase media tested in preliminary trials and required compromises in making a final choice of extraction media. The best overall solid-phase extraction recoveries were achieved using Oasis HLB extraction cartridges with no pH adjustment of the sample. The average recoveries for the compounds tested are listed in Table 4. Twelve of the 22 compounds tested were recovered at greater than 60%. Highly polar compounds, such as the histamine (H2) receptor antagonists ranitidine and cimetidine, were recovered at less than 50%. The extremely polar compound metformin ($C_4H_{11}N_5$) and the unstable β-lactam antibiotic amoxicillin were not recovered at all. Low recovery for polar compounds is believed to be a result of poor retention on the polymeric sorbent as a result of not adjusting pH of the sample for extraction. Cimetidine and ranitidine and metformin did however have recoveries greater than 75% from octadecyl (C_{-18}) SPE cartridges. All three compounds have a more linear molecular shape, which may better suit retention on C_{18}. Amoxicillin, with an unstable β-lactam structure, is known to degrade in solution. Recoveries of less than 30% were observed for the amphoteric angiotensin-converting enzyme (ACE) inhibitors, lisinopril and enalaprilat, and for paroxetine metabolite. Enalaprilat and lisinopril likewise are believed to be poorly retained in the polymeric sorbent without pH adjustment. Enalaprilat did recover greater than 90% using a graphitized carbon SPE sorbent. Enalaprilat and lisinopril recoveries improved only slightly, 45–88%, but with poor reproducibility using C_{18} SPE sorbent. Subsequent studies on the effect of sample pH did not reveal any significant improvements to overall extraction efficiency (data not shown). The final choice for the HLB cartridges was based on overall recovery and consistency of recoveries over multiple months. In addition, some compounds were recovered using HLB cartridges that were not recovered using other SPE sorbents tested, such as acetaminophen, which is suspected to be ubiquitous in wastewater-effected surface water. Compounds that were recovered less than 60% were retained in the method to investigate possible sample matrix effects and to compare with related methodologies applied to different matrices, such as sediments and other solids, planned for subsequent projects. These compounds are qualified as estimated concentrations and any compounds that are recovered less than 30% are reported only as qualitatively being present and are only retained in the method if they are deemed sufficiently important in regards to possible environmental impact or human health.

Future strategies in SPE development would include use of tandem or mixed modes of extraction sorbents to enhance multiclass methodology. In addition, the development of newer mixed mode phases with ion-exchange capability provide a possible means to isolate specific classes of compounds and better eliminate interferences from dissolved organic matter (DOM).

The method detection limit (MDL) was determined according to US Environmental Protection Agency guidelines [17]. The MDL is defined as the minimum concentration of a substance that can be measured and reported with a 99%

Table 5
Method detection limits (MDL) for pharmaceutical compounds, in micrograms per liter (μg/L) determined in this method and listed in order of elution

Compound	MDL (μg/L)
Metformin	0.0034
Cotinine	0.023
Amoxicillin[a]	–
Albuterol	0.030
Cimetidine	0.0067
Acetaminophen	0.0086
Ranitidine	0.010
1,7-Dimethylxanthine	0.019
Lisinopril[a]	–
Caffeine	0.014
Enalaprilat	0.15
Trimethoprim	0.014
Digoxigenin	0.0077
Paroxetine metabolite[a]	–
Sulfamethoxazole	0.023
Phenacetin [^{13}C] surrogate	0.013
Diltiazem	0.012
Furosemide[a]	–
Fluoxetine	0.018
Dehydronifedipine	0.0095
Warfarin	0.0061
Ibuprofen	0.018
Gemfibrozil	0.015

[a] Not detected under experimental conditions.

confidence that the compound concentration is greater than zero, and is determined from at least seven replicate analyses of samples containing the compounds of interest. Eight 1-L water samples, collected upstream from known or suspected sources of contamination of the compounds of interest, were amended to a concentration of 0.10 μg/L for each compound. The samples were processed as a single set through the entire extraction and analytical procedure. The MDL for each compound was determined from the standard deviation of concentration for the replicate measurements, which is multiplied by the Student's *t*-value for $(n - 1)$ degrees of freedom. The resulting MDL for each of the compounds is listed in Table 5. A few compounds, such as metformin, are poorly recovered, but have relatively low MDLs (0.0034 μg/L for metformin) because the MDL is determined using the absolute standard deviation of concentration. This result indicates that the recovery and MDL need to be evaluated together to determine the performance of the compound in the method. The mean MDL for all compounds is 0.022 μg/L and the median 0.014 μg/L. The MDLs for compounds previously identified as chromatographing poorly, such as lisinopril, the metabolite of paroxetine, and compounds that were not recovered by SPE, were not determined, and are not listed in Table 5. The high MDL for enalaprilat, 0.15 μg/L, is likely caused by variability in recovery, resulting in a high standard deviation of concentration and the resulting high MDL. MDLs for the other compounds in Table 5 range between 0.006 and 0.030 μg/L, comparing favorably with published limits of detection (LODs) for other studies that use HPLC–MS–MS, which range between 0.010 and 0.050 μg/L [5,18].

Recoveries from an initial validation of the method were compared to the recoveries from quality-control samples processed with environmental samples. The quality-control sample was a reagent-water sample amended to a concentration of 1.0 μg/L of each compound. Sixteen of these reagent-spike samples were extracted in 1999. In 2000, 28 reagent-spike samples were amended at 0.50 μg/L, extracted, and analyzed. These set spikes are used to evaluate method performance over an extended time. Several potential sources of variation are built into these long-term recoveries, including multiple operators and instruments (Table 4). Recoveries for SPE trials, extracted and analyzed in triplicate, ranged from 8 to 127%, with a mean of 77%. The relative standard deviation (R.S.D.) ranged from 1 to 20.8%, with a mean of 9.1%. Recoveries for the year 1999 set spikes ranged from 11 to 87%, with a mean of 62%. The R.S.D. for year 1999 set spikes ranged from 3.6 to 19.1%, with a mean of 8.1%. Lower recoveries for the year 1999 reagent set spikes, compared to the initial SPE trials, likely are attributable to the addition of a syringe-filtering step. This step was applied consistently to all sample extracts and set spikes to prevent clogging of the HPLC column from precipitates that formed in environmental sample extracts during concentration. The decrease in R.S.D. observed between the SPE trials and the year 1999 set spikes is not substantial. These results indicate that the recoveries are sufficiently similar before and after addition of the required syringe-filtration step and that this method is suitable for short- and long-term application.

Set-spike recoveries for year 1999 also were compared to data for the year 2000 set spikes to indicate long-term performance (Table 4). The mean recovery for all set spikes in 2000 for all compounds was 62%. Mean set-spike recoveries for individual compounds ranged from 12 to 166%. The R.S.D.s of year 2000 set spike recoveries for individual compounds ranged from 7.8 to 61.4%, with a mean of all R.S.D.s of 16.7%. Mean recovery changes between 1999 and 2000 varied by compound. Mean recovery decreases of greater than 20% between 1999 and 2000 were observed for five compounds, and mean recovery increases of greater than 20% were observed for three compounds. Mean recovery changes between 1999 and 2000 were less than 20% for nine compounds. These data indicate that there is no systematic change in recoveries between years, given the multiple potential sources of variation in the data. However, there is evidence in this data set that the effects leading to signal enhancement, which are thought to occur in the HPLC–MS, are producing enhanced recoveries for some compounds. These enhanced recoveries are observed as greater than expected spike recoveries and continuing calibration verification (CCV) standard concentrations (data not shown) for some compounds. The compounds that demonstrate substantial signal enhancement are acetaminophen, 1,7-dimethylxanthine, digoxigenin, and gemfibrozil (in-

creased CCV concentration). Enhancement was seen only after running surface-water sample extracts, and no compounds were detected in instrument blanks and method blanks. Peak areas and recoveries fell within expected limits for CCV standards when calibration standards were analyzed just prior to a CCV standard and prior to sample extracts, whereas enhanced peak areas were measured in the same standards when analyzed after sample extracts. Signal enhancement was as low as 20–30% increase for acetaminophen, digoxigenin, and gemfibrozil, and as high as 50–100% for acetaminophen and 1,7-dimethylxanthine. Replacing the analytical HPLC guard column and analytical HPLC column improved the apparent enhancement slightly. Decreasing sample analyses to only one set of 10 sample extracts, cleaning the MS source, and recalibrating between each sample set resulted in the most important improvement, as evidenced in normal or only slightly elevated CCV recoveries.

Compound specific matrix interferences have been noted by measuring concentrations of spiked compounds in serial dilutions of surface water, containing high organic matter concentrations, with reagent grade water. Some compounds were seen to increase in measured concentration by up to 100% while others remained within ±20% of the average measured concentration using an isotopically labeled caffeine internal standard. The response of the caffeine internal standard in the MS detector was seen to decrease as dilution of surface water decreased. The variability of effect on the MS signals of compounds as affected by the aqueous matrix reflects the variability in measured concentrations. While some compounds are affected in the same manner that the internal standard is, other compounds are not and therefore produced significant differences in observed concentrations (Fig. 3). In order to eliminate or at least minimize such affects another internal standard was used that would not be affected by complex matrices. Isotopically labeled nicotinamide was substituted as an internal standard because it elutes much earlier in the chromatogram and is therefore much less affected by later eluting interferences. It was tested against labeled caffeine as an internal standard in serial dilutions of surface water with high concentrations of DOM measuring concentration of an isotopically labeled surrogate, ethyl nicotinate. The observed concentration remained within ±20% using the labeled nicotinamide internal standard while the observed concentration using labeled caffeine rose to 160% recovery from undiluted water (data not shown).

Recoveries of pharmaceuticals from surface-water samples prepared as a single set and spiked at 1.000 μg/L were compared to mean spike recoveries for 16 set spikes prepared using the same method for about 6 months (Fig. 4). Recoveries ranged from 0 to 123% with a mean of 57%, including zero recoveries, and 73% for all nonzero recoveries. Matrix enhancement as well as suppression of recoveries occurred although the enhancement is neither as marked nor as consistent as that observed in similar methodology applied to detection of herbicides [19]. Lisinopril and enalaprilat are amphoteric compounds that chromatograph poorly under present conditions. Lisinopril as well as paroxetine metabolite were not quantifiable greater than the baseline signal. Enalaprilat had a substantially enhanced signal and recovery from environmental matrices. Cimetidine, which is highly

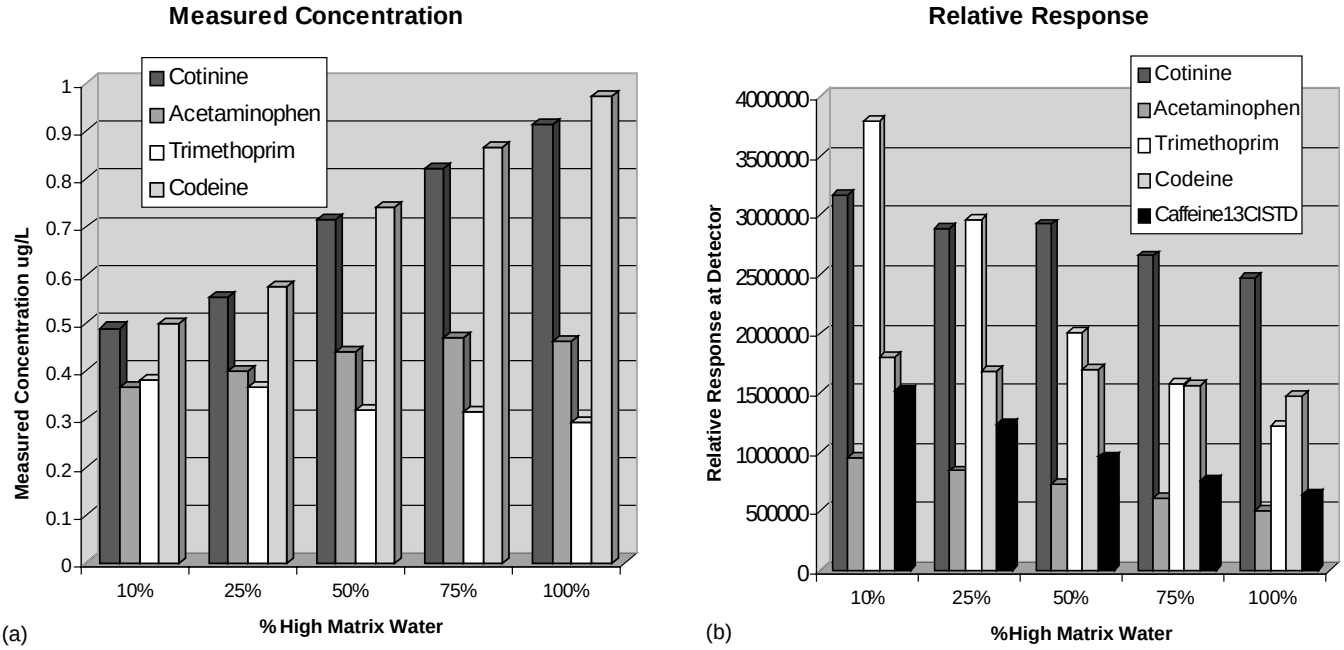

Fig. 3. (a) Measured concentration and (b) relative response of selected pharmaceuticals in serial dilutions of surface water high in dissolved organic matter spiked at 0.5 μg/L (codeine not included in initial study).

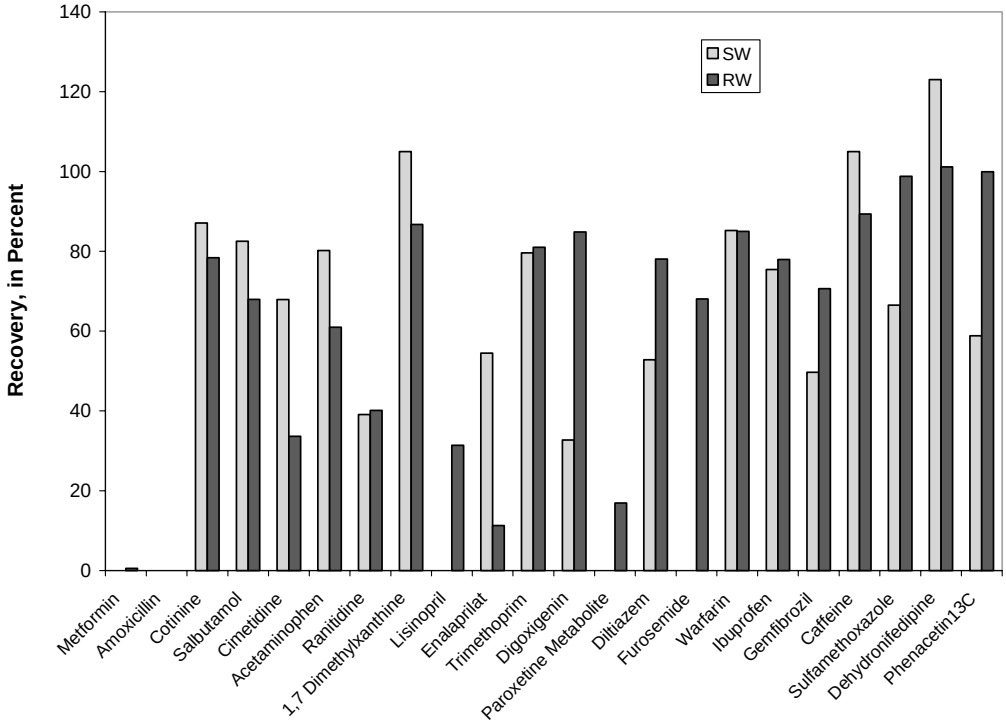

Fig. 4. Effect of natural environmental matrix components in surface-water (SW) samples on the high-performance liquid chromatography/mass spectrometric determination of pharmaceutical compounds as compared to organic-free reagent-water (RW) samples.

polar, also shows substantial enhancement, which appears to be the trend among the more polar basic compounds. Amphoteric and neutral and acidic compounds show an overall tendency toward matrix-related suppressed recoveries. Furosemide was suppressed the most as the signal could not be detected above the baseline.

Five pairs of duplicate field samples were analyzed to characterize method precision at ambient environmental concentrations and in the presence of interferences. Duplicate samples were analyzed within a sample set (intraset replicates) or in separate sample sets (interset replicates), and results for both were combined for data analysis. Boxplots of the relative percent difference (RPD) for six pharmaceuticals that were detected in four or more of the duplicate samples, and the performance surrogate are shown in Fig. 5. With the exception of caffeine, all compounds averaged 39% RPD or less. The performance surrogate ([^{13}C]1-ethoxy-phenacetin) that was added at a concentration of about 1.000 μg/L had a RPD of 9% and was accounted for in all 10 samples. The concentrations of the pharmaceuticals detected ranged from 0.009 to 1.02 μg/L with a median concentration of 0.082 μg/L. The low concentrations detected, together with a low number of samples for comparison, likely result in the somewhat higher RPDs observed in this report when compared to those reported for sulfonylurea, imidazolinone, and sulfonamide herbicides determined using similar methodology [19].

The method described herein has been applied to samples collected as part of a national reconnaissance of pharmaceuticals in USA streams in 1999 and 2000 [10]. The pharmaceuticals measured by this method were detected frequently, with individual compounds present in as many as 61.9% of samples tested. The most frequently detected compounds were nonprescription compounds, such as acetaminophen (23.8%), caffeine (61.9%), and cotinine (a nicotine metabolite, 38.1%). Median concentrations mea-

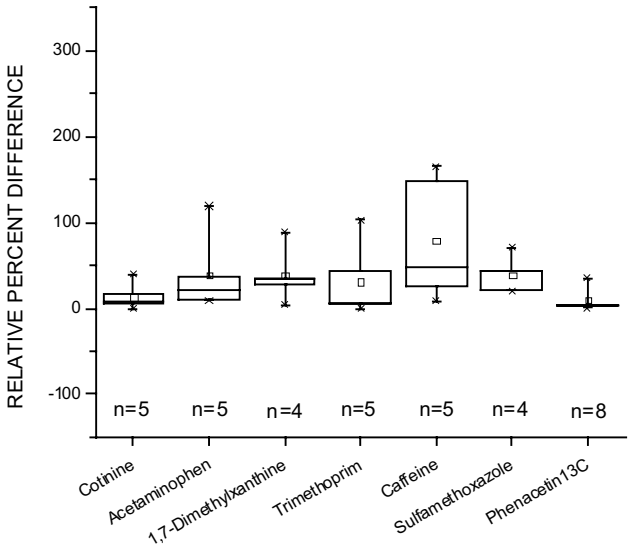

Fig. 5. Relative percent differences between individual pharmaceutical compounds determined in duplicate environmental water samples.

sured were 0.110 μg/L for acetaminophen, 0.081 μg/L for caffeine, and 0.024 μg/L for cotinine; maximum concentrations for these compounds were 10, 6, and 9 μg/L, respectively.

Prescription pharmaceuticals were less frequently detected, with the highest frequencies of detection for trimethoprim (27.4%) and sulfamethoxazole (19%), two antibiotics used in combination for treating urinary tract and ear infections. Median concentrations for these antibiotics were 0.013 μg/L for trimethoprim and 0.066 μg/L for sulfamethoxazole, with maximum concentrations of 3 and 5.2 μg/L, respectively. Other pharmaceuticals detected included diltiazem, 1,7-dimethylxanthine (a caffeine metabolite), dehydronifedipine, cimetidine, ibuprofen, metformin, and gemfibrozil. Median concentrations for these compounds were all less than 0.20 μg/L. The detection of metformin suggests recovery improvements related to environmental sample matrix because reagent-spike recoveries typically were very low.

4. Conclusions

The routine determination of multiple classes of pharmaceuticals in surface- and ground-water samples at ambient environmental concentrations using SPE and HPLC–MS has been shown to be practical and effective. The prescription and over-the-counter pharmaceuticals most likely found in USA surface- and ground-waters was evaluated from prescription data and resulted in a potential analyte list consisting of multiple chemical classes. The method performance and quality-control data presented indicate that the techniques applied to routine analysis of surface- and ground-water samples for pharmaceuticals is robust and sensitive for the majority of compounds tested, at concentrations less than 0.10 μg/L, with method detection limits averaging 0.022 μg/L. Highly polar and amphoteric compounds were the most difficult to isolate and detect. Precision under controlled conditions, as reflected in mean spike recovery R.S.D. of 16.7% is good, but the limited amount of data obtained from replicate environmental samples indicate that a larger data set is required to characterize precision at ambient environmental concentrations. Extensive QA/QC data collection and interpretation, particularly of replicate samples, are necessary to validate the performance of this method and to assess matrix effects on recoveries of the selected compounds. SPE recovery data in this report are useful in describing the directions for further exploration in developing methods to extract more specific classes of compounds and enhance extraction recoveries, as well as to indicate what chemical characteristics are amenable to existing methodology.

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