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Analysis of Pharmaceuticals in Water by Isotope Dilution Liquid Chromatography/Tandem Mass Spectrometry†

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A method has been developed for the trace analysis of 15 pharmaceuticals, four metabolites of pharmaceuticals, three potential endocrine disruptors, and one personal care product in various waters. 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. Unlike many previous LC−MS/MS methods, which suffer from matrix suppression, this method uses isotope dilution for each compound to correct for matrix suppression, as well as SPE losses and instrument variability. The method was tested in five matrices, and results indicate that the method is very robust. Matrix spike recoveries for all compounds were between 88 and 106% for wastewater influent, 85 and 108% for wastewater effluent, 72 and 105% for surface water impacted by wastewater, 96 and 113% for surface water, and 91 and 116% for drinking water. The method reporting limits for all compounds were between 0.25 and 1.0 ng/L, based on 500 mL of sample extracted and a final extract volume of 500 μL. Occurrence of the compounds in all five matrices is also reported.

Introduction

Due to a number of recent reports (*1*−*7*), sustained interest in the presence and fate of organic contaminants has been thoroughly established. As concern regarding the environmental impact of exogenous chemicals present in water continues to grow, so too have the number of analytical methods dedicated to their identification and quantification (*8*, *9*). This is especially true for the analysis of these compounds by liquid chromatography−mass spectrometry (LC−MS) (*10*−*12*). However, even though the number of methods using LC−MS has risen dramatically, discussion of matrix suppression and enhancement that can occur during the use of LC−MS for environmental analysis and the effect it can have on quantification accuracy and precision has been discussed in a relatively small number of publications.

Although the cause of matrix effects has been postulated to be natural organic matter (*13*), the mechanism still has not been fully resolved (*14*). Regardless, recent reports have shown drastic matrix effects when using LC−MS for the analysis of environmental and biological samples (*14*−*21*). There have been a number of methods proposed to compensate for matrix effects by using different calibration techniques, including standard addition (*13*, *17*, *22*), surrogate monitoring (*15*, *20*), and various forms of internal calibration (*14*−*16*, *19*, *23*). Still more have been developed to minimize matrix effects using different extraction, cleanup and elution techniques, including size-exclusion chromatography (*18*, *24*), solid-phase extraction (*22*), LC chromatographic procedures (*14*, *22*), ultra performance liquid chromatography (*25*), hollow fiber liquid-phase microextraction (*26*), flow-splitting and reduced eluent flow rates (*24*, *27*). However, most become problematic when applied to the simultaneous analysis of a broad range of compounds that encompass many different classes and structures in matrices having varying degrees of suppression and enhancement.

Isotope dilution has also been discussed as a means to compensate for matrix effects (*14*, *18*, *19*, *21*, *23*, *28*−*30*). Although is it generally considered the most highly recommended (*30*) and the most versatile approach (*19*) for dealing with matrix effects, it has only been used in the past for the analysis of individual compounds or small groups of similar compounds and has been characterized as being cost-intensive and having a lack of availability (*18*, *24*).

This paper presents a method that analyzes 15 pharmaceuticals, four metabolites of pharmaceuticals, three endocrine disruptors, and one personal care product in various waters using SPE coupled with liquid chromatography/tandem mass spectrometry (LC−MS/MS) and isotope dilution of every analyte (Table 1). Using the method, matrix effects were negligible and reporting limits for all compounds were at or below 1 ng/L.

Experimental Section

Materials. Sulfamethoxazole, atenolol, trimethoprim, meprobamate, dilantin, carbamazepine, diazepam, gemfibrozil, bisphenol A, diclofenac, naproxen, and triclosan were obtained from Sigma-Aldrich (St. Louis, MO). Linuron and atrazine were obtained from Ultra Scientific (North Kingstown, RI) and norfluoxetine, atorvastatin, o-hydroxy atorvastatin, p-hydroxy atorvastatin, risperidone, enalapril maleate, simvastatin, and simvastatin hydroxyl acid were obtained from Toronto Research Chemicals (Toronto, Canada). Fluoxetine was obtained from the United States Pharmacopeia (Rockville, MD). Sulfamethoxazole-d_4, meprobamate-d_3, atorvastatin-d_5, o-hydroxy atorvastatin-d_5, p-hydroxy atorvastatin-d_5, risperdione-d_4, trimethoprim-d_9, enalapril-d_5 maleate, gemfibrozil-d_6, simvastatin-d_6, simvastatin-d_6 hydroxy acid, and triclosan-d_3 were obtained from Toronto Research Chemicals. Fluoxetine-d_5, norfluoxetine-d_5, dilantin-d_{10}, atrazine-d_5, linuron-d_6, atenolol-d_7, and diclofenac-d_4 were obtained from C/D/N Isotopes (Pointe-Claire, Canada). Carbamazepine-d_{10}, diazepam-d_5, bisphenol A-d_{16}, and $^{13}C_1$-Naproxen-d_3 were obtained from Cambridge Isotope Laboratories (Andover, MA). The purity of all isotope standards used in this study was ≥98%. Trace analysis grade methanol was obtained from Burdick and Jackson (Muskegon, MI). Methyl-t-butyl ether (MTBE) was purchased from EM Science (Gibbstown, NJ) and ammonium acetate was obtained from J. T. Baker (Phillipsburg, NJ).

Sample Collection and Preservation. Samples were collected in 1 L silanized, amber glass bottles (Eagle-Picher, Miami, OK). Sample bottles were preserved with 1 g/L sodium azide to prevent microbial degradation and 50 mg/L ascorbic acid to quench any residual oxidant (e.g., chlorine, ozone, chloramine). Sample bottles were kept on ice and brought back to the laboratory within 4 h of collection where they

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TABLE 1. Target Compounds

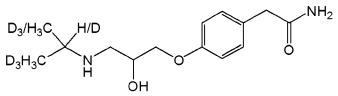
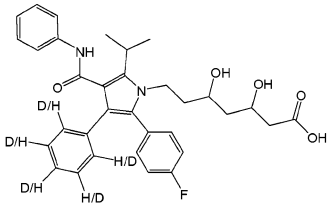
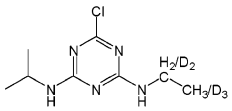
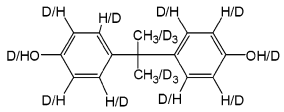
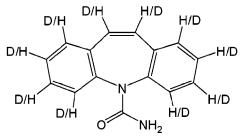
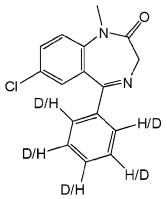
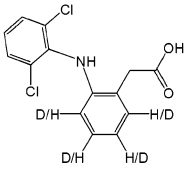
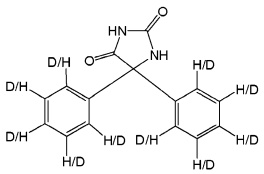
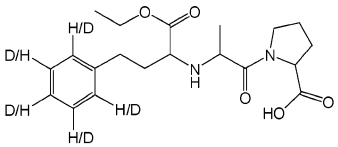
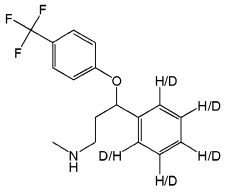
Unlabeled (Labeled)	Class	Structure (Unlabeled/Labeled)
Atenolol (Atenolol-d_7)	Beta-blocker	
Atorvastatin (Atorvastatin-d_5)	Antilipidemic	
Atrazine (Atrazine-d_5)	Herbicide	
Bisphenol A (Bisphenol A-d_{16})	Plasticizer	
Carbamazepine (Carbamazepine-d_{10})	Anticonvulsant	
Diazepam (Diazepam-d_5)	Benzodiazepine tranquilizer	
Diclofenac (Diclofenac-d_4)	Non-steroidal anti-inflammatory agent	
Dilantin (Dilantin-d_{10})	Antiepileptic	
Enalapril (Enalapril-d_5)	Angiotensin-converting enzyme (ACE) Inhibitor	
Fluoxetine (Fluoxetine-d_5)	Selective serotonin uptake inhibitor	

TABLE 1. (Continued)

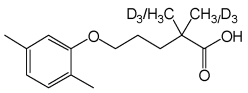
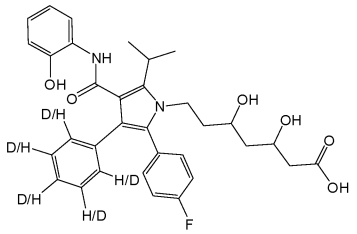
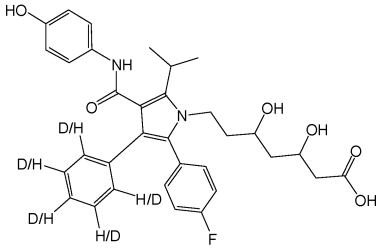
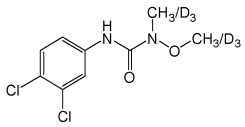
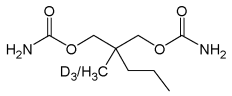
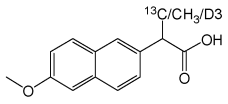
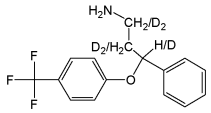
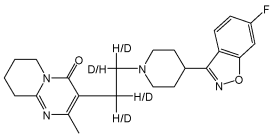
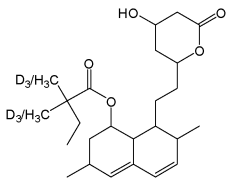
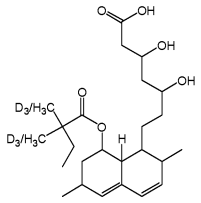
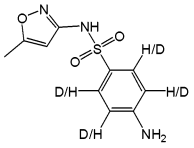
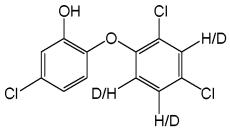
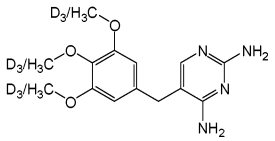
Unlabeled (Labeled)	Class	Structure (Unlabeled/Labeled)
Gemfibrozil (Gemfibrozil-d_6)	Antilipidemic	
o-Hydroxy atorvastatin (o-Hydroxy atorvastatin-d_5)	Atorvastatin metabolite	
p-Hydroxy atorvastatin (p-Hydroxy atorvastatin-d_5)	Atorvastatin metabolite	
Linuron (Linuron-d_6)	Herbicide	
Meprobamate (Meprobamate-d_3)	Anti-anxiety agent	
Naproxen ($^{13}C_1$-Naproxen-d_3)	Non-steroidal anti-inflammatory agent	
Norfluoxetine (Norfluoxetine-d_5)	Fluoxetine metabolite	
Risperidone (Risperidone-d_4)	Antipsychotic	
Simvastatin (Simvastatin-d_6)	Antilipidemic	
Simvastatin hydroxy acid (Simvastatin-d_6 hydroxy acid)	Simvastatin metabolite	

TABLE 1. (Continued)

Unlabeled (Labeled)	Class	Structure (Unlabeled/Labeled)
Sulfamethoxazole (Sulfamethoxazole-d_4)	Anti-infective	
Triclosan (Triclosan-d_3)	Anti-microbial	
Trimethoprim (Trimethoprim-d_9)	Anti-infective	

were stored at 4 °C. Samples were extracted within 3 days of collection.

Sample Locations. Five different aqueous matrices were tested to determine method performance over a wide range of water qualities. Wastewater influent (WWI) and effluent (WWE) were collected from the City of Las Vegas Water Pollution Control Facility. This 91 mgd plant serves approximately 650 000 people in the Cities of Las Vegas and North Las Vegas. The plant uses tertiary wastewater treatment, including chlorination as a final disinfectant. The Las Vegas Wash (LVW) is an urban waterway that is, in general, composed of >90% treated wastewater and a small percentage of shallow groundwater and urban runoff. The Boulder Basin (BB) of Lake Mead, Nevada, is composed primarily of water from the Colorado River; however, it is downstream from the confluence of the LVW and Lake Mead; therefore, water quality influence from the LVW is expected. It is also the primary source of drinking water for the Las Vegas metropolitan area. Finished drinking water (FDW) samples were taken at the River Mountains Water Treatment Facility, which uses both ozonation and chlorination for disinfection and the BB as its source of raw water.

Solid-Phase Extraction. Analytes were extracted in batches of six samples using 5 mL, 200 mg hydrophilic−lipophilic balance (HLB) glass cartridges from Waters Corporation (Millford, MA). All extractions were performed on an AutoTrace automated SPE system (Caliper Corporation, Hopkington, MA). The SPE cartridges were sequentially preconditioned with 5 mL of MTBE, 5 mL of methanol, and 5 mL of reagent water. Samples were spiked with a solution of isotopically labeled standards that contained a stable isotope of each analyte. 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 30 min. Next, the cartridges were eluted with 5 mL of methanol followed by 5 mL of 10/90 (v/v) methanol/MTBE into 15 mL calibrated centrifuge tubes. The resulting extract was concentrated with a gentle stream of nitrogen to an approximate volume of 400 μL. The extract was brought to a final volume of 500 μL using methanol.

Initial sample volumes and isotope spike amounts were determined based on the amount of wastewater present in the sample. Generally, 500 mL of sample with an addition of 50 μL of a 100 μg/L solution of isotopes (10 ng/L) were extracted. This resulted in a concentration factor of 1000 and a final extract concentration for the isotopes of 10 μg/L. Smaller volumes of wastewater samples were extracted, due to their complex nature and the expected levels of most of the pharmaceuticals. This, along with post-extraction dilutions, resulted in higher reporting limits for wastewater samples. Larger amounts of isotopes also were added to wastewater samples prior to SPE to allow post-extraction dilutions without the need to add more isotopes.

Liquid Chromatography. An Agilent (Palo Alto, CA) G1312A binary pump and an HTC- PAL autosampler (CTC Analytics, Zwingen, Switzerland) equipped with a 10 μL sample loop were used for all analyses. Analytes were separated using a 150 × 4.6 mm Luna C18 (*2*) column with a 5 μm particle size (Phenomenex, Torrance, CA). A binary gradient consisting of 5 mM ammonium acetate in water (A) and 100% methanol (B) at a flow rate of 800 μL/min was used. For ESI positive analyses, the gradient was as follows: 10% B held for 0.50 min, stepped to 50% B at 0.51 min and increased linearly to 100% B at 8 min, then held at 100% B for 2 min. For ESI negative analyses, the gradient was as follows: 10% B held for 0.50 min, stepped to 60% B at 0.51 min and increased linearly to 100% B at 8 min, then held at 100% B for 3 min. A 5 min equilibration step at 10% B was used at the beginning of each run to bring the total run time per sample to 15 and 16 min, for ESI positive and ESI negative, respectively. Analyte retention times are shown in Table 2 and representative chromatograms are shown in Figure 1.

Mass Spectrometry. Mass spectrometry was performed using an API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA). Techniques used for the optimization of the mass spectrometer parameters have been published previously (*20*). Briefly, optimization occurred in three separate steps: determination of the best ionization source and polarity and optimization of the compound- and source-dependent parameters. Results of this are shown in Tables 2 and 3. It must be noted that, due to the broad nature of our analyte list, several parameter 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, parameters were optimized to benefit the majority of analytes.

For ESI positive analysis, the 10 min run time was divided into two periods, in which a select group of compounds

TABLE 2. Compound-Dependent Parameters

compound	retention time (min)	precursor ion (*m/z*)	product ion (*m/z*)	declustering potential (V)	collision energy (eV)	collision cell exit potential (V)
			ESI Positive			
period 1						
Atenolol	3.7	267	145	61	37	10
Atenolol-d_7	3.7	274	145	61	37	10
Sulfamethoxazole	4.1	254	156	51	23	14
Sulfamethoxazole-d_4	4.1	258	160	56	25	12
Trimethoprim	4.7	291	261	81	37	8
Trimethoprim-d_9	4.7	300	234	71	35	16
period 2						
Meprobamate	5.8	219	158	36	13	16
Meprobamate-d_3	5.8	222	161	46	13	10
Enalapril	5.9−6.8	377	234	61	29	16
Enalapril-d_5	5.9−6.8	382	239	56	29	16
p-Hydroxy atorvastatin	6.6	575	440	91	31	12
p-Hydroxy atorvastatin-d_5	6.6	580	445	81	33	14
Dilantin	6.7	253	182	66	29	18
Dilantin-d_{10}	6.7	263	192	61	29	14
Carbamazepine	6.9	237	165	61	53	16
Carbamazepine-d_{10}	6.9	247	204	61	31	20
Risperidone	7.3	411	110	76	69	6
Risperidone-d_4	7.3	415	195	76	49	12
Fluoxetine	7.5	310	44	51	37	8
Fluoxetine-d_5	7.5	315	44	46	43	6
Norfluoxetine	7.5	296	134	31	11	10
Norfluoxetine-d_5	7.5	301	139	31	11	8
o-Hydroxy atorvastatin	7.6	575	440	91	31	12
o-Hydroxy atorvastatin-d_5	7.6	580	445	81	33	14
Atrazine	7.7	216	174	71	27	14
Atrazine-d_5	7.7	221	179	56	27	12
Atorvastatin	7.9	559	440	66	31	12
Atorvastatin-d_5	7.9	564	445	81	33	14
Linuron	8.4	249	160	61	27	10
Linuron-d_6	8.4	255	160	51	27	14
Diazepam	8.5	285	154	61	41	10
Diazepam-d_5	8.5	290	198	81	43	12
			ESI negative			
Naproxen	5.6	229	169	−30	−44	−17
$^{13}C_1$-Naproxen-d_3	5.6	233	169	−25	−38	−9
Bisphenol A	6.8	227	212	−80	−28	−21
Bisphenol A-d_{16}	6.8	241	142	−65	−36	−7
Diclofenac	6.9	294	250	−40	−16	−17
Diclofenac-d_4	6.9	298	217	−30	−30	−15
Cemfibrozil	9.1	249	121	−45	−28	−17
Gemfibrozil-d_6	9.1	255	121	−40	−20	−7
Simvastatin hydroxy acid	9.2	435	319	−60	−24	−7
Simvastatin-d_6 hydroxy acid	9.2	441	319	−60	−24	−7
Triclosan	9.7	287	35	−45	−38	−15
Triclosan-d_3	9.7	290	35	−30	−34	−5
Simvastatin	10.2	399	115	−90	−30	−7
Simvastatin-d_6	10.2	405	121	−70	−36	−7

was monitored. This was done to ensure that enough dwell time was spent on each transition to avoid any data loss. These periods are provided in Table 2. In ESI negative, all analytes were monitored for the entire run length.

MDL Study and Calibration. A method detection limit (MDL) study was performed by extracting 12 deionized water samples fortified with unlabeled analytes at either 0.1, 0.5, or 1.0 ng/L, depending on the expected MDL, and isotopically labeled standards at 10 ng/L. The MDL was calculated by multiplying the standard deviation of the replicate measurements by the appropriate student's T value for n − 1 degrees of freedom. The results of the MDL study are shown in Table 4. Reporting limits (Table 4) were chosen to be larger than the MDL and usually greater than three times the MDL. The instrument was calibrated with each analyte at 0.1, 0.5, 1.0, 5.0, 10, 50, and 100 μg/L. An isotopically labeled version of each analyte, corresponding to the isotopes added to each sample prior to extraction, was added to each calibration point at a concentration of 10 μg/L to generate a relative response ratio. Recoveries of the isotopes were compared with the relative response ratio and a concentration for the unlabeled analyte was calculated. Linear or quadratic regression with $1/x^2$ weighting was used for all analytes. Correlation coefficients were required to be at least 0.990 and typically exceeded 0.995.

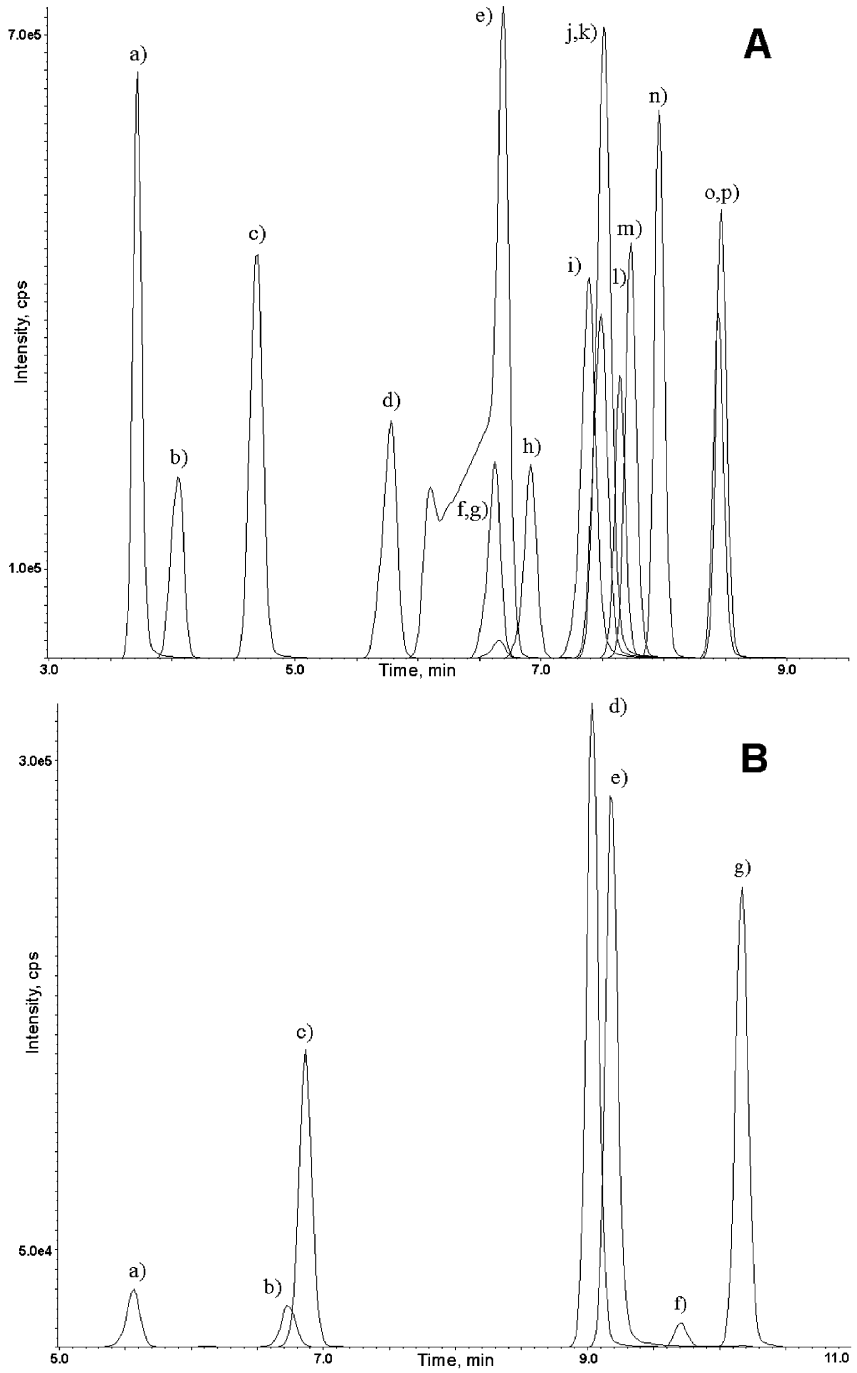


FIGURE 1. Overlaid target ion traces (isotopically labeled target ion traces not shown for clarity) of a 50 μg/L standard in (A) ESI positive mode (key: (a) atenolol, (b) sulfamethoxazole, (c) trimethoprim, (d) meprobamate, (e) enalapril, (f) p-hydroxy atorvastatin, (g) dilantin, (h) carbamazepine, (i) risperidone, (j) fluoxetine, (k) norfluoxetine, (l) o-hydroxy atorvastatin, (m) atrazine, (n) atorvastatin, (o) linuron, (p) diazepam); (B) ESI negative mode (key: (a) naproxen, (b) bisphenol A, (c) diclofenac, (d) gemfibrozil, (e) simvastatin hydroxy acid, (f) triclosan, (g) simvastatin).

Blank Analysis. Due to the ultra trace levels reported using this method, the possibility existed that the isotopically labeled standards used contained traces of unlabeled target analytes that could interfere with the MDL study or quantitation at or near the reporting limit. To determine whether this was the case, deionized water blanks were fortified with isotopically labeled versions of each target compound and extracted. Target compounds were not found at significant levels in these fortified blanks, indicating the isotopically labeled standards used were appropriate for this study. Chromatograms relating to this blank experiment are included in the Supporting Information (Figures S1 and S2). In addition, the possibility existed of low-level contamination from various other sources. Therefore, during the course of the study, several deionized water blanks were extracted and analyzed. All blanks tested were below the reporting limits shown in Table 4.

Results and Discussion

Chromatography. A mobile phase gradient of 5 mM ammonium acetate in water (A) and methanol (B) provided acceptable chromatography and sensitivity for all but one of the compounds tested (Figure 1). Enalapril exhibited multiple peaks with poor resolution (Supporting Information Figure

TABLE 3. Source-Dependent Parameters

	ESI positive	ESI negative
collision gas (psig)	8	8
curtain gas (psig)	19	19
ion source gas 1—nebulizer gas (psig)	60	60
ion source gas 2—turbo gas (psig)	50	50
ionspray voltage (V)	5500	−4500
temperature (°C)	550	550
probe X-axis position (mm)	5	5
probe Y-axis position (mm)	5	5
entrance potential (V)	10	−10

TABLE 4. Method Detection Limits and Reporting Limits for Target Compounds

compound	MDL spike (ng/L)	MDL (ng/L) $n = 12$	RL (ng/L)
Atenolol	0.1	0.047	0.25
Atorvastatin	0.1	0.061	0.25
Atrazine	0.1	0.022	0.25
Bisphenol A	1.0	0.49	1.0
Carbamazepine	0.5	0.13	0.50
Diazepam	0.1	0.042	0.25
Diclofenac	0.1	0.053	0.25
Dilantin	1.0	0.55	1.0
Enalapril	0.1	0.026	0.25
Fluoxetine	0.1	0.11	0.50
Gemfibrozil	0.1	0.045	0.25
Linuron	0.5	0.13	0.50
Neprobamate	0.1	0.069	0.25
Naproxen	0.5	0.17	0.50
Norfluoxetine	0.1	0.11	0.50
o-Hydroxy atorvastatin	0.5	0.10	0.50
p-Hydroxy atorvastatin	0.5	0.18	0.50
Risperidone	0.1	0.095	0.25
Simvastatin	0.1	0.050	0.25
Simvastatin hydroxy acid	0.1	0.058	0.25
Sulfamethoxazole	0.1	0.043	0.25
Triclosan	0.5	0.34	1.0
Trimethoprim	0.1	0.058	0.25

S3). As discussed in Trabelsi et al. (*31*), enalapril exists in solution as a mixture of cis and trans conformers around the amide bond that has very slow conformational change kinetics, typically resulting in poor chromatography without specific method parameters to improve it. Due to the number of compounds analyzed with this method, a sacrifice of chromatography was necessary for enalapril. Although other tested mobile phases (e.g., formic acid) provided better chromatography for enalapril, they resulted in poor chromatography and/or sensitivity for several other compounds (data not shown). Since the poor resolution did not impact quantification, enalapril remained in the method.

Precursor and Product Ions. All but one of the precursor ions in ESI positive and ESI negative were the result of a protonation ($[M + H]^+$) or deprotonation ($[M- H]^-$), respectively, of the intact, uncharged molecule. The only exception was simvastatin, which showed a loss of water in the source, to form the $[M- H_2O- H]^-$ precursor.

Since each analyte had an isotopically labeled counterpart, it presented an opportunity to assess previously published product ion structures and additionally propose novel product ion structures for analytes that have none in the literature. This information is provided in the Supporting Information (Text S1 and Table S1).

Recovery of Analytes by SPE. Recoveries for all of the target analytes are shown in Table 5. Matrix spikes were chosen at levels relative to the expected concentrations of analytes in the matrix. Six replicates were analyzed for each matrix, with the exception of BB ($n = 5$) due to an SPE pump failure during one of the sample extractions. Compound recoveries across all matrices were, with few exceptions, between 90 and 110%. Specifically, WWI recoveries ranged from 88 to 106%, WWE from 85 to 108%, LVW from 72 to 105%, BB from 96 to 113%, and FDW from 91 to 116% for all analytes. In comparison with previously published matrix spikes on samples from the same sites (*20*), these recoveries indicate that the negative effects of matrix suppression and SPE loss on quantification have been minimized by the use of isotope dilution. The degree to which isotope dilution standards compensated for matrix suppression and recovery loss was measured by comparing the area counts of isotope dilution standards in samples and matrix spikes presented in Tables 5 and 6 with those in the calibration curve. These results are presented in Supporting Information Table S2. Percent relative standard deviations (% RSD) were also excellent with all at or below 10% and most under 5%.

Occurrence. Six replicates were also analyzed for each matrix to determine the concentration of each analyte without fortification (Table 6). Sample % RSDs were generally greater than those for matrix spikes. This is likely due to the analyte higher concentrations of the matrix spikes and the tendency for background interference to have more of an effect on peaks with low signal-to-noise ratio that represent concentrations near the detection limit. In spite of this, the majority of the % RSDs were below 10% and all were below 25%.

Most were detected in WWI. Seven analytes were detected above 1000 ng/L with one above 20 000 ng/L. WWE samples showed a large drop in concentration for most analytes, probably due to the tertiary treatment and chlorination employed by the treatment plant. Only four compounds (atenolol, meprobamate, dilantin, and carbamazepine) remained above 100 ng/L in the final effluent. Downstream from this wastewater plant, many analytes exhibit a rise in concentration in the LVW. This is due to the effluent from a 110 mgd wastewater treatment plant that is located downstream from the WWE and upstream from the LVW sampling site. This treatment plant does not use chlorination as a final disinfectant, which could explain the higher concentrations of sulfamethoxazole, trimethoprim, carbamazepine, naproxen, diclofenac, gemfibrozil, and triclosan in the LVW. These compounds have previously been shown to be susceptible to chlorine oxidation (*32*−*34*).

Results in the BB show the effect of dilution and attenuation by the waters of Lake Mead. All concentrations were below 25 ng/L and only two, sulfamethoxazole and meprobamate, were above 10 ng/L. Finished drinking water concentrations reflect prior work on the effect of ozone and chlorination on some target analytes (*35*). Although the sampling effort was limited, sulfamethoxazole, trimethoprim, carbamazepine, naproxen, and gemfibrozil appear to have been degraded during drinking water treatment, whereas meprobamate, atrazine, and dilantin were detectable in the finished water.

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TABLE 5. Matrix Spike (Spike Amount in Parentheses) Recoveries of Pharmaceuticals in Various Waters (ng/L)

	wastewater influent (5000 ng/L)			wastewater effluent (500 ng/L)			Las Vegas Wash (100 ng/L)			Boulder Basin of Lake Mead, NV (raw drinking water) (50 ng/L)			finished drinking water (50 ng/L)		
compound	mean n=6	% RSD	% recovery	mean n=6	% RSD	% recovery	mean n=6	% RSD	% recovery	mean n=5	% RSD	% recovery	mean n=6	% RSD	% recovery
ESI positive															
Atenolol	7680	2.4	93	1360	1.8	96	939	2.0	80	56	1.6	99	50	2.4	100
Sulfamethoxazole	7020	2.3	99	524	2.6	104	775	1.0	102	77	0.7	110	54	1.4	108
Trimethoprim	5960	1.6	96	484	1.8	97	181	2.3	102	51	1.4	100	50	1.3	100
Meprobamate	6060	2.9	92	1740	1.2	95	680	1.7	90	69	4.3	102	55	3.2	98
Enalapril	4790	1.2	95	487	1.9	97	98	1.5	98	50	1.7	100	50	1.5	100
p-Hydroxy atorvastatin	5170	2.3	98	500	3.3	100	107	1.8	98	51	1.6	102	51	1.5	102
Dilantin	4970	2.9	91	712	5.8	85	242	3.4	72	57	6.4	102	50	6.4	97
Carbamazepine	4720	3.0	90	673	2.6	97	306	2.6	105	57	1.5	102	50	1.5	100
Risperidone	4720	3.2	95	490	2.1	98	98	2.7	98	50	2.2	100	49	2.0	98
Fluoxetine	4970	1.8	99	511	3.3	97	102	2.7	100	51	1.1	101	51	1.5	102
Norfluoxetine	4850	1.9	97	483	3.1	96	94	4.2	93	49	3.7	99	50	2.0	100
o-Hydroxy atorvastatin	5260	2.4	101	509	3.1	102	102	3.9	95	51	1.6	102	51	2.0	103
Atrazine	4390	2.5	88	456	2.2	91	93	4.0	92	50	3.1	96	46	1.8	91
Atorvastatin	5160	1.9	99	508	2.5	102	106	3.0	99	51	1.4	102	51	1.2	102
Linuron	4720	3.0	95	497	2.8	99	100	1.5	99	50	1.1	99	49	1.5	98
Diazepam	4820	2.2	96	469	2.1	93	98	1.6	95	51	1.1	101	52	1.4	104
ESI negative															
Naproxen	27600	7.1	102	502	3.1	100	174	4.0	100	51	3.0	100	51	1.2	102
Bisphenol A	5600	4.9	106	538	10	108	100	8.9	100	56	4.1	113	58	6.0	116
Diclofenac	5340	3.7	104	514	5.4	103	123	5.2	105	53	1.7	107	52	3.8	105
Gemfibrozil	9250	2.5	90	507	3.4	100	268	2.0	99	50	0.9	98	49	1.1	97
Simvastatin hydroxy acid	5010	2.0	100	510	3.1	102	99	3.0	98	52	2.6	103	53	2.1	106
Triclosan	6290	8.9	100	511	4.9	102	109	3.9	97	48	3.1	97	48	2.5	97
Simvastatin	4780	2.5	96	493	3.2	99	96	1.3	96	51	0.9	102	51	2.4	101

TABLE 6. Occurrence of Pharmaceuticals in Various Waters (ng/L)[a]

	wastewater influent		wastewater effluent		Las Vegas Wash		Boulder Basin of Lake Mead, NV (raw drinking water)		finished drinking water	
compound	n=6	% RSD	n=6	% RSD	n=6	% RSD	n=6	% RSD	n=6	% RSD
ESI positive										
Atenolol	3060	3.3	879	1.1	859	1.6	6.0	1.1	<0.25	N/A
Sulfamethoxazole	2060	2.7	5.0	11	672	1.6	22	1.9	<0.25	N/A
Trimethoprim	1140	3.2	<0.50	N/A	80	2.3	1.2	3.4	<0.25	N/A
Meprobamate	1440	4.2	1270	2.2	594	1.7	18	5.4	5.9	2.0
Enalapril	35	4.0	0.85	7.2	0.35	12	<0.25	N/A	<0.25	N/A
p-Hydroxy atorvastatin	280	4.0	<1.0	N/A	9.2	6.8	<0.50	N/A	<0.50	N/A
Dilantin	402	14	287	6.9	170	3.0	6.2	7.0	1.3	12
Carbamazepine	232	4.2	187	2.2	203	2.6	5.8	2.6	<0.50	N/A
Risperidone	<2.5	N/A	<0.50	N/A	<0.25	N/A	<0.25	N/A	<0.25	N/A
Fluoxetine	17	6.2	25	1.6	2.6	6.5	<0.50	N/A	<0.50	N/A
Norfluoxetine	9.9	12	3.9	3.4	1.3	25	<0.50	N/A	<0.50	N/A
o-Hydroxy atorvastatin	196	4.5	<1.0	N/A	6.9	5.0	<0.50	N/A	<0.50	N/A
Atrazine	<2.5	N/A	0.81	16	0.95	1.7	1.6	3.5	0.67	6.6
Atorvastatin	201	2.8	<0.50	N/A	7.3	4.6	<0.25	N/A	<0.25	N/A
Linuron	<5.0	N/A	0.73	25	0.89	5.6	<0.50	N/A	<0.50	N/A
Diazepam	<2.5	N/A	3.7	4.6	2.6	2.5	<0.25	N/A	<0.25	N/A
ESI negative										
Naproxen	22500	15	<1.0	N/A	75	4.5	0.54	6.3	<0.50	N/A
Bisphenol A	320	4.7	<2.0	N/A	<1.0	N/A	<1.0	N/A	<1.0	N/A
Diclofenac	116	3.4	<0.50	N/A	18	6.9	<0.25	N/A	<0.25	N/A
Gemfibrozil	4770	3.1	9.0	21	170	1.6	0.84	3.4	<0.25	N/A
Simvastatin hydroxy acid	10	10	<0.50	N/A	0.74	14	<0.25	N/A	<0.25	N/A
Triclosan	1280	4.2	<2.0	N/A	12	7.8	<1.0	N/A	<1.0	N/A
Simvastatin	<2.5	N/A	<0.50	N/A	<0.25	N/A	<1.0	N/A	<1.0	N/A

[a] N/A, not applicable.

Supporting Information Available

Discussion relating to and table of product ion structures for all analytes, table showing the degree of matrix suppression and recovery loss in samples, and chromatograms showing the chromatography of enalapril and the absence of target compounds in a deionized water blank spiked with isotopically labeled standards. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Daughton, C. G.; Ternes, T. A. Pharmaceuticals and personal care products in the environment: agents of subtle change? *Environ. Health Perspect.* **1999**, *107*, 907−938.

(2) Halling-Sorensen, B.; Nielsen, S. N.; Lanzky, P. F.; Ingerslev, F.; Lutzhoft, H. C. H.; Jorgensen, S. E. Occurrence, fate and effects of pharmaceutical substances in the environment—a review. *Chemosphere* **1998**, *36*, 357−393.

(3) Jobling, S.; Noylan, M.; Tyler, C. R.; Brighty, G.; Sumpter, J. P. Widespread sexual disruption in wild fish. *Environ. Sci. Technol.* **1998**, *32*, 2498−2506.

(4) Jones, O. A. H.; Voulvoulis, N.; Lester, J. N. Human pharmaceuticals in the aquatic environment: a review. *Environ. Technol.* **2001**, *22*, 1383−1394.

(5) Kolpin, D. W.; Furlong, E. T.; Meyer, M. T.; Thurman, E. M.; Zaugg, S. D.; Barber, L. B.; Buxton, H. T. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999−2000: a national reconnaissance. *Environ. Sci. Technol.* **2002**, *36*, 1202−1211.

(6) Snyder, S. A.; Kelly, K. L.; Grange, A. H.; Sovocool, G. W.; Snyder, E. M.; Giesy, J. Pharmaceuticals and personal care products in the waters of Lake Mead, Nevada. In *Pharmaceuticals and Personal Care Products in the Environment: Scientific and Regulatory Issues*; Daughton, C. G., Jones-Lepp, T. L., Eds.; ACS Symposium Series 791; American Chemical Society: Washington, DC, 2001; pp 116−140.

(7) Snyder, S. A.; Villenueve, D.; Snyder, E. M.; Giesy, J. Identification and quantification of estrogen receptor agonists in wastewater effluents. *Environ. Sci. Technol.* **2001**, *35*, 3620−3625.

(8) Koester, C. J.; Moulik, A. Trends in environmental analysis. *Anal. Chem.* **2005**, *77*, 3737−3754.

(9) Richardson, S. D.; Ternes, T. A. Water analysis: emerging contaminants and current issues. *Anal. Chem.* **2005**, *77*, 3807−3838.

(10) Petrovic, M.; Hernando, M. D.; Diaz-Cruz, M. S.; Barcelo, D. Liquid chromatography-tandem mass spectrometry for the analysis of pharmaceutical residues in environmental samples: a review. *J. Chromatogr. A* **2005**, *1067*, 1−14.

(11) Zwiener, C.; Frimmel, F. H. LC-MS analysis in the aquatic environment and in water treatment—a critical review, Part I: Instrumentation and general aspects of analysis and detection. *Anal. Bioanal. Chem.* **2004**, *378*, 851−861.

(12) Zwiener, C.; Frimmel, F. H. LC-MS analysis in the aquatic environment and in water treatment - a critical review, Part II: Applications for emerging contaminants and related pollutants, microorganisms and humic acids. *Anal. Bioanal. Chem.* **2004**, *378*, 862−874.

(13) Lindsey, M. E.; Meyer, M.; Thurman, E. M. Analysis of trace levels of sulfonamide and tetracycline antimicrobials in groundwater and surface water using solid-phase extraction and liquid chromatography/mass spectrometry. *Anal. Chem.* **2001**, *73*, 4640−4646.

(14) Benjits, T.; Dams, R.; Lambert, W.; De Leenheer, A. Countering matrix effects in environmental liquid chromatography-electrospray ionization tandem mass spectrometry water analysis for endocrine disrupting chemicals. *J. Chromatogr. A* **2004**, *1029*, 153−159.

(15) Ferguson, P. L.; Iden, C. R.; Brownawell, B. J. Analysis of alkylphenol ethoxylate metabolites in the aquatic environment using liquid chromatography-electrospray mass spectrometry. *Anal. Chem.* **2000**, *72*, 4322−4330.

(16) Lindberg, R.; Jarnheimer, P.-A.; Olsen, B.; Johansson, M.; Tysklind, M. Determination of antibiotic resistance substances in hospital sewage water using solid phase extraction and liquid chromatography/mass spectrometry and group analogue internal standards. *Chemosphere* **2004**, *57*, 1479−1488.

(17) Quintana, J. B.; Reemtsma, T. Sensitive determination of acidic drugs and triclosan in surface and wastewater by ion-pair reverse-phase liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 765−774.

(18) Schluesener, M. P.; Bester, K. Determination of steroid hormones, hormone conjugates and macrolide antibiotics in influents and effluents of sewage treatment plants utilising high-performance liquid chromatography/tandem mass spectrometry with electrospray and atmospheric pressure chemical ionisation. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 3269−3278.

(19) Stueber, M.; Reemtsma, T. Evaluation of three calibration methods to compensate matrix effects in environmental analysis of LC-ESI-MS. *Anal. Bioanal. Chem.* **2004**, *378*, 910−916.

(20) Vanderford, B. J.; Pearson, R. A.; Rexing, D. J.; Snyder, S. A. Analysis of endocrine disruptors, pharmaceuticals, and personal care products in water using liquid chromatography/tandem mass spectrometry. *Anal. Chem.* **2003**, *75*, 6265−6274.

(21) Stoob, K.; Singer, H. P.; Goetz, C. W.; Ruff, M.; Mueller, S. R. Fully automated online solid phase extraction coupled directly to liquid chromatography-tandem mass spectrometry quantification of sulfonamide antibiotics, neutral and acidic pesticides at low concentrations in surface waters. *J. Chromatogr. A* **2005**, *1097*, 138−147.

(22) Reemtsma, T. Liquid-chromatography-mass spectrometry and strategies for trace-level analysis of polar organic pollutants. *J. Chromatogr. A* **2003**, *1000*, 477−501.

(23) Delatour, T. Performance of quantitative analyses by liquid chromatography-electrospray ionisation tandem mass spectrometry: from external calibration to isotopomer-based exact matching. *Anal. Bioanal. Chem.* **2004**, *380*, 515−523.

(24) Kloepfer, A.; Quintana, J. B.; Reemtsma, T. Operational options to reduce matrix effects in liquid chromatography-electrospray ionization-mass spectrometry analysis of aqueous environmental samples. *J. Chromatogr. A* **2005**, *1067*, 153−160.

(25) Mezcua, M.; Aguera, A.; Lliberia, J. L.; Cortes, M. A.; Bago, B.; Fernandez-Alba, A. R. Application of ultra performance liquid chromatography-tandem mass spectrometry to the analysis of priority pesticides in groundwater. *J. Chromatogr. A* **2006**, *1109*, 222−227.

(26) Quintana, J. B.; Rodil, R.; Reemtsma, T. Suitability of hollow fibre liquid-phase microextraction for the determination of acidic pharmaceuticals in wastewater by liquid chromatography-electrospray tandem mass spectrometry without matrix effects. *J. Chromatogr. A* **2004**, *1061*, 19−26.

(27) Gangl, E. T.; Annan, M.; Spooner, N.; Vouros, P. Reduction of signal suppression effects in ESI-MS using a nanosplitting device. *Anal. Chem.* **2001**, *73*, 5635−5644.

(28) Reddy, S.; Iden, C. R.; Brownawell, B. J. Analysis of steroid conjugates in sewage influent and effluent by liquid chromatography-tandem mass spectrometry. *Anal. Chem.* **2005**, *77*, 7032−7038.

(29) Ren, Y.; Zhang, Y.; Jiao, J.; Cai, Z.; Zhang, Y. Sensitive isotope dilution liquid chromatography/electrospray ionization tandem mass spectrometry method for the determination of acrylamide in chocolate. *Food Addit. Contam.* **2006**, *23*, 228−236.

(30) Matuszewski, B. K.; Constanzer, M. L.; Chavez-Eng, C. M. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. *Anal. Chem.* **2003**, *75*, 3019−3030.

(31) Trabelsi, H.; Bouabdallah, S.; Sabbah, S.; Raouafi, F.; Bouzouita, K. Study of the *cis-trans* isomerization of enalapril by reverse-phase liquid chromatography. *J. Chromatogr. A* **2000**, *871*, 189−199.

(32) Joss, A.; Keller, E.; Alder, A. C.; Gobel, A.; McArdell, C. S.; Ternes, T. A.; Siegrist, H. Removal of pharmaceuticals and fragrances in biological wastewater treatment. *Water Res.* **2005**, *39*, 3139−3152.

(33) Petrovic, M.; Gonzalez, S.; Barcelo, D. Analysis and removal of emerging contaminants in wastewater and drinking water. *Trends Anal. Chem.* **2003**, *22*, 685−696.

(34) Snyder, S. A.; Leising, J.; Westerhoff, P.; Yoon, Y.; Mash, H.; Vanderford, B. J. Biological and physical attenuation of endocrine disruptors and pharmaceuticals: implications for water reuse. *Ground Water Monit. Rem.* **2004**, *24*, 108−118.

(35) Westerhoff, P.; Yoon, Y.; Snyder, S. A.; Wert, E. Fate of endocrine-disruptor, pharmaceutical, and personal care product chemicals during simulated drinking water treatment processes. *Environ. Sci. Technol.* **2005**, *39*, 6649−6663.

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