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Simultaneous Analysis of Multiple Classes of Antibiotics by Ion Trap LC/MS/MS for Assessing Surface Water and Groundwater Contamination

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Solid-phase extraction (SPE) and liquid chromatography in combination with ion trap mass spectrometry (LC/MS/MS) conditions were optimized for the simultaneous analysis of 13 antibiotics belonging to multiple classes and caffeine in 3 different water matrixes. The single-cartridge extraction step was developed using a reversed-phase cartridge, resulting in recoveries for the 14 compounds ranging from 71 to 119% with relative standard deviations of 16% or lower. The analytes were separated in one chromatographic run, and the SPE-LC/MS/MS detection limits ranged from 0.03 to 0.19 μg/L. The SPE procedure was validated in groundwater, surface water, and wastewater. The analysis of samples from each of the three water matrixes revealed clindamycin (1.1 μg/L) in surface water and multiple antibiotics in wastewater (0.10−1.3 μg/L). The use of identification points to unambiguously assign the identity of antibiotics in various water matrixes was applied to an ion trap data-dependent scanning method, which simultaneously collects full scan and full scan MS/MS data for the unequivocal identification of target analytes.

The occurrence of antibiotics in aquatic environments is of ecotoxicological concern because of potential ecosystem alteration. Prolonged exposure to low doses of antibiotics leads to the selective proliferation of resistant bacteria, which could transfer the resistance genes to other bacterial species.[1] Contamination of the aquatic environments by antibiotics and other pharmaceutical compounds has been reported in recent studies through discharge from domestic sewer systems and agricultural runoff.[2−4] A nationwide survey conducted by the United States Geological Survey (USGS) Toxic Substances Hydrology Program reported the presence of human and veterinary drugs in 80% of the streams sampled.[2] These streams consisted of areas susceptible to contamination from wastewater sources, such as downstream from intense urbanization or livestock production.

Antibiotics have a wide range of uses in both human and veterinary medicine. In the livestock industry, the use of antibiotics as growth promoters as well as therapeutic agents is very common. Animal manure containing excreted antibiotics is frequently applied to agricultural fields where antibiotics may potentially contaminate groundwater and eventually enter surface water, such as rivers and lakes. Antibiotics used in human treatment can also enter the environment, either by excretion or by disposal of surplus drugs into sewage systems. Effluent from wastewater treatment plants (WWTPs) is released into the local aquatic surroundings.[5] Presently, WWTPs are not designed to completely remove most pharmaceuticals, and these compounds are consequently released into surface waters,[6] making it important to monitor their presence in recycled wastewaters.

The target compounds in this study are 13 antibiotics and caffeine that have previously been detected in surface waters[2] and wastewaters.[3,4] The selected compounds are representative of several different classes used in both human and veterinary medicine, including tetracycline, fluoroquinolone, sulfonamide, lincosamide, and macrolide antibiotics, and are expected to have different environmental fates and effects. Caffeine concentrations will be monitored because it can be used as an anthropogenic marker of untreated domestic wastewater contamination, and its detection in surface waters can indicate contamination from untreated sewage overflows.[7]

The separation technique that has been primarily implemented in the detection of these target compounds in different water matrixes is liquid chromatography (LC). LC has been used to separate fluoroquinolones from surface waters[2,8,9] and wastewaters,[9,10] often with mass spectrometry (MS) as the detection system, but ultraviolet[8] and fluorescence[9] detection have also been used. LC/MS and LC/MS/MS have also been used to detect

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Table 1. Summary of the Target Compounds and Their Selected Physical Properties

compound	molecular formula	MW	CAS registry number	log K_{OW}	pK_a	class
caffeine	$C_8H_{10}N_4O_2$	194.2	58−08−2	−0.06[16]	nf[a]	stimulant
sulfamethazine	$C_{12}H_{14}N_4O_2S$	278.3	57−68−1	0.25[17]	2.07, 7.49[23]	sulfonamide
sulfadimethoxine	$C_{12}H_{14}N_4O_4S$	310.3	122−11−2	1.4[17]	2.13, 6.08[23]	sulfonamide
sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	253.3	723−46−6	nf	1.85, 5.60[23]	sulfonamide
trimethoprim	$C_{14}H_{18}N_4O_3$	290.3	738−70−5	0.91[18]	3.23, 6.76[23]	used in combination with sulfonamides
ciprofloxacin	$C_{19}H_{22}FN_3O_3$	331.3	85721−33−1	−1.74[19]	3.01, 6.14, 8.70, 10.58[23]	fluoroquinolone
enrofloxacin	$C_{17}H_{18}FN_3O_3$	359.4	93106−60−6	nf	3.85, 6.19, 7.59, 9.86[23]	fluoroquinolone
tetracycline	$C_{22}H_{24}N_2O_8$	444.4	60−54−8	0.09[20]	3.32, 7.78, 9.58[23]	tetracycline
oxytetracycline	$C_{22}H_{24}N_2O9$	460.5	79−57−2	−0.90,[19] 0.08[20]	3.22, 7.46, 8.94[23]	tetracycline
chlortetracycline	$C_{22}H_{23}ClN_2O_8$	478.8	57−62−5	0.41[20]	3.33, 7.55, 9.33[23]	tetracycline
clindamycin	$C_{18}H_{33}ClN_2O_5S$	424.9	21462−39−5	nf	7.77[24]	lincosamide
erythromycin	$C_{37}H_{67}NO_{13}$	733.9	114−07−8	3.0[21]	8.90[23]	macrolide
roxithromycin	$C_{41}H_{76}N_2O_{15}$	837.1	80214−83−1	nf	9.17[23]	macrolide
tylosin	$C_{46}H_{77}NO_{17}$	916.1	1401−69−0	2.5[22]	7.5[23]	macrolide

[a] nf, not found.

tetracyclines,[10−12] sulfonamides,[12,13] and macrolides[13,14] in environmental water matrixes, utilizing many different types of mass spectrometers, including the single quadrupole (q), triple quadrupole (QqQ), and ion trap (IT) mass analyzers. The QqQ typically offers the lowest detection limits of these three instruments, but its cost limits its accessibility. The q is the simplest and least expensive of the three, but its inability to perform tandem mass spectrometry does not allow it the selectivity of the QqQ and IT. A recent publication by Hernández et al.[15] compared different MS techniques for the confirmation of pesticides in water and discussed the importance of having an appropriate number of identification points (IPs) to confidently establish the identity of an analyte, as defined by European Commission Guidelines for the detection of organic residues in animals and animal products. While the IT does not provide the same sensitivity as the q or QqQ for trace analysis, the high selectivity of the IT can improve signal-to-noise ratios such that the detection limits of the IT are applicable to the quantification of environmentally relevant concentrations of contaminants. The unique ability of the IT to provide MS^n data increases the number of identification points and provides valuable information in the analysis of complex environmental samples at an affordable cost.

Typical concentrations of antibiotics found in the environment 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.[8−12,14] However, a challenge is presented in the simultaneous extraction and analysis of several classes of compounds due to the wide range of polarities, solubilities, pK_as, K_{OW}s, and stabilities under acidic and basic conditions. For this reason, most previously published SPE techniques and detection methods have been directed toward the analysis of only one or two classes of antibiotics. The work presented in this paper will focus on the development of an analytical method, including SPE and IT-LC/MS/MS, needed to simultaneously analyze 13 antibiotics belonging to 5 different classes, along with caffeine, in a single extraction step and one chromatographic run for analysis of groundwaters, surface waters, and wastewaters. The list of analytes is shown in Table 1 along with their selected physical properties. The applicability of the method in environmental analysis is demonstrated by the determination of antibiotic and caffeine concentrations in several environmental aquatic matrixes and will be used in future studies to assess the impact of WWTP discharges on the ecological health and quality of the surface waters and groundwaters.

EXPERIMENTAL SECTION

Chemicals and Reagents. Enrofloxacin (ENR), tetracycline (TC), oxytetracycline (OTC), tylosin (TYL), and sulfadimethoxine (SDM), were purchased from Fluka (St. Louis, MO). Roxithromycin (ROX), erythromycin (ERY), sulfamethazine (SMZ), sulfamethoxazole (SMX), and caffeine (CAF) were purchased from Sigma-Aldrich (St. Louis, MO); chlortetracycline (CTC) and trimethoprim (TRI) were from ICN Biomedicals, Inc. (Aurora, OH); clindamycin (CLD) was from MP Biomedicals (Aurora, OH); and ciprofloxacin (CIP) was supplied by Bayer (Stillwell, KS). Individual standard solutions at a concentration of 1 mg/mL were prepared in methanol, with the exception of ciprofloxacin and enrofloxacin, which were prepared in methanol with 5% 0.1 M sodium hydroxide. The standard solutions were stored at −40 °C for a maximum of three months. Working standard solutions were prepared daily by dilution with water. An isotopically labeled sulfonamide, $^{13}C_6$-sulfamethazine, was used as the internal standard and was purchased from Cambridge Isotope Laboratories (Andover, MA).

The acetonitrile (ACN) and methanol (MeOH) used for the chromatographic mobile phase were HPLC grade high-purity solvents purchased from Burdick and Jackson (Muskegon, MI). The ACN and MeOH used in the sample preparation were HPLC grade solvents purchased from Fisher Scientific (Fair Lawn, NJ).

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Table 2. Summary of the Retention Times in Surface Water, MS/MS Parameters, and Precursor and Product Ions Observed for Each of the Target Compounds

compound	retention time (min)	precursor ion	product ion 1	product ion 2	optimal collision energy (%)	isolation width
caffeine	8.8	195	138	149	44	1.0
sulfamethazine	9.6	279	203	156	34	1.0
sulfadimethoxine	19.5	311	156	245	42	1.0
sulfamethoxazole	15.9	254	188	156	34	1.0
trimethoprim	11.9	291	230	258	44	1.0
ciprofloxacin	15.0	332	288	268	44	1.0
enrofloxacin	16.1	360	316	342	42	1.0
tetracycline	14.3	445	410	427	34	1.0
oxytetracycline	13.4	461	426	443	44	1.0
chlortetracycline	18.7	479	444	462	40	1.0
clindamycin	20.8	425	377	126	40	1.0
erythromycin-h_2o	24.6	716	522	558	30	1.5
roxithromycin	26.0	837	679	522	32	2.0
tylosin	24.0	916	772	754	42	2.0

Formic acid and orthophosphoric acid (H_3PO_4) were purchased from Fisher Scientific. Disodium ethylenediamine tetraacetate (Na_2EDTA) was purchased EMD Chemicals (Darmstadt, Germany), disodium phosphate was purchased from Lancaster Synthesis, INC. (Pelham, NH), and citric acid was purchased from J. T. Baker (Phillispsburg, NJ). Water was prepared using a NANOpure DIamond Ultrapure water system with a 0.2-μm filter, purchased from Barnstead International (Dubuque, IA).

Solid-Phase Extraction. Preliminary experiments were conducted to assess the extraction efficiency of two SPE cartridges, namely, the 500-mg Oasis HLB cartridges and 1-g tC_{18} Sep-Pak cartridges (Waters, Milford, MA). Six different extraction conditions were tested, as summarized in Table S-1 (Supporting Information). Aqueous solutions of 500-mL volume containing the 13 antibiotics and caffeine were prepared in different media, such as 0.1 M EDTA−McIlvaine buffer (method I), NANOpure water (methods II and III), or NANOpure water containing 2 mL of 5% (w/v) Na_2EDTA (methods IV−VI). For methods II, IV, V, and VI, the pH was adjusted between 2.8 and 3.0 using 40% orthophosphoric acid. The cartridges were conditioned with 6 mL of either MeOH (methods I and II) or ACN (methods III−VI), followed by 6 mL of NANOpure water. The samples were passed through the cartridges at a rate of ~3 mL/min using a Supelco vacuum manifold (Sigma-Aldrich), which allowed for the parallel extraction of up to 12 samples. The analytes were eluted twice using 4 mL of either MeOH with 10 mM oxalic acid (method I), MeOH with 1% TFA (method II), ACN (methods III and VI), ACN with 1% ammonia (method IV), or ACN with 1% TFA (method V) into a tube containing 50 μL of the internal standard (a 10 μg/mL solution of $^{13}C_6$-sulfamethazine). The volume of each eluate was reduced to 0.2 mL under a stream of air at 40 °C. NANOpure water was added to each eluate to a final volume of 1 mL for LC/MS analysis.

Liquid Chromatography/Mass Spectrometry. The LCQ Advantage IT-MS system was equipped with an electrospray ionization source (ESI) and connected to a Surveyor LC system (ThermoFinnigan, San Jose, CA). The column used was a BetaBasic-18 C_{18} column (100 × 2.1 mm internal diameter with 3-μm particle size) equipped with a Uniphase guard cartridge (10 × 2.1 mm internal diameter with 3-μm particle size), both purchased from Thermo Hypersil-Keystone (Bellefonte, PA). The flow rate was 200 μL/min, the column oven temperature was 30 °C, and the full loop injection volume was 20 μL. The ESI-IT-MS was operated in positive ion mode. The capillary temperature was 235 °C, and the spray voltage was 5.25 kV for all applications. Nitrogen was used as a sheath gas at a flow rate of 20 arbitrary units, and helium gas was used to induce dissociation for the acquisition of MS/MS data. Individual tune files were created for each standard in continuous flow mode to determine the optimum capillary voltages, lens settings, collision energies, isolation widths, and fragment ions, which are listed in Table 2.

Table 3. Summary of the Mobile-Phase Compositions for the Chromatographic Separation[a]

minutes	% A	% B	% C
0	5	5	90
2	5	5	90
10	10	10	80
30	45	45	10
36	45	45	10
40	5	5	90
55	5	5	90

[a] Solvent A, acetonitrile; solvent B, methanol; solvent C, water with 0.3% formic acid.

The separation was performed using a ternary gradient mobile phase consisting of ACN (A), MeOH (B), and water with 0.3% formic acid (C), using the gradient solvent program listed in Table 3. Full scan and full scan MS/MS data were collected simultaneously using data-dependent scanning. The acquisition was divided into three time segments, each with two scan events, including an independent full scan and a dependent full MS/MS scan. The data-dependent criteria for all time segments included a minimum MS signal of 10^4 counts for the specified precursor masses, a repeat count of 5, a repeat duration of 2.0 min, and a collision energy of 44%. For segment I (0−11 min), the mass range was 185−300 m/z and the isolation width was 1.0, for segment II (11−23 min) the mass range was 200−500 m/z and the isolation width was also 1.0, and for segment III (23−30 min), mass range was 700−1000 m/z with an isolation width of 2.0. The full scan data were used for quantification, while the full scan MS/MS data were used for structural confirmation of target compounds.

Method Validation. SPE method VI provided the best recoveries for the target analytes and was therefore validated. The method limit of detection (LOD), limit of quantification (LOQ), and dynamic range (DR) were determined using calibration curves prepared from spiked NANOpure water samples. The 500-mL volumes of NANOpure water were spiked with the 14 target analytes at 4 levels to contain 0.1, 0.25, 1.0, and 2.5 μg, with duplicate samples extracted at the higher concentrations (0.25, 1.0, and 2.5 μg) and 4 replicates extracted for the lowest concentration (0.1 μg). The calibration curves were plotted, and the LOD was defined as the concentration corresponding to signal at the *y*-intercept plus 3 times its standard deviation. The LOQ was defined as the concentration corresponding to the signal at the *y*-intercept plus 10 times its standard deviation. The DR was identified to include the LOD and the highest concentration tested (2.5 μg/L).

Quantification of target analytes was based on internal calibration curves constructed from the SPE extraction of spiked NANOpure water samples. Duplicate samples were prepared at five different concentrations covering the dynamic range (0.1, 0.25, 0.5, 1.0, and 2.5 μg/L). The calibration curves were a plot of the peak area ratio of the analyte signal to the $^{13}C_6$-sulfamethazine signal versus concentration.

To determine the influence of different sample matrixes on SPE recoveries, groundwater from a local private drinking well (Boston, New York), surface water from the Niagara River (Tonawanda, NY), and wastewater effluent from the Amherst Sewage Treatment Plant (Amherst, New York) were tested using the optimized SPE conditions. Prior to spiking and SPE, water samples were passed through a Whatman 1.2-μm filter (VWR Scientific, Bridgeport, NJ). For each type of water, two 1-L aliquots were spiked in duplicate to obtain final concentrations of 0.25 and 2.5 μg/L. The 50-μL samples of $^{13}C_6$-sulfamethazine were added to each sample along with 10 mL of 5% (w/v) Na_2EDTA, and the samples were extracted according to SPE method VI.

Analysis of Environmental Samples. To test the applicability of the optimized SPE-LC/MS/MS method, groundwater samples were collected from five private drinking wells located in Weiser, ID, near a confined animal feeding operation. Wastewater was also collected from the effluent of four WWTPs, located in Holland, East Aurora, Fredonia, and Dunkirk, New York. Surface water samples were also collected from a second location along the Niagara River (Tonawanda, NY). To each 1-L sample, 50 μL of the $^{13}C_6$-sulfamethazine was added and all samples were stored at 4 °C until extraction. Samples were extracted within 2 days of storage.

RESULTS AND DISCUSSION

Solid-Phase Extraction Procedure. Based on the overall recoveries of the 14 target analytes, the best extraction conditions involved use of HLB cartridges with the sample pH adjusted below 3.0, the addition of Na_2EDTA into the sample, and use of ACN as the elution solvent (method VI). It has been reported that macrolide antibiotics exhibit a decreased recovery when extracted from water with little or no salt content;[13] therefore, Na_2EDTA was added as a chelating agent (methods IV−VI). This approach has been previously used in the extraction of macrolides from water.[14] It should be noted that, at pH below 7.0, erythromycin is immediately converted into its main degradate, erythromycin−H_2O (ERY−H_2O),[14] and thus, this degradate was quantified in the extracts rather than the parent molecule. Although total extraction time could be up to 5 h/1 L of sample, the vacuum manifold used allowed for the parallel extraction of up to 12 samples. Furthermore, vacuum manifolds that permit the simultaneous extraction of up to 24 samples are available for use in large-scale environmental monitoring programs.

Several different extraction conditions were tested in preliminary experiments (see Table S-1, Supporting Information), which began with an adaptation of the procedure described by Zhu et al.[11] for the extraction of tetracyclines from water. This included a comparison of tC_{18} to HLB cartridges. Although no macrolides were recovered from the HLB cartridges, they seemed to provide more reproducible recoveries for the majority of the compounds. Sulfamethazine, sulfamethoxazole, trimethoprim, and clindamycin were only added to the method after investigating methods I and II, and therefore, recoveries were not available at this point.

For the subsequent SPE optimization, ACN was used as the eluent to ensure that all compounds were eluted from the HLB cartridges, which improved the recoveries for erythromycin−H_2O and roxithromycin. In method III, the pH of the sample was not adjusted to examine the effect of pH on the extraction efficiency. The sample without pH adjustment did not affect the extraction of the majority of the compounds on the HLB cartridge; however, the recovery of the fluoroquinolones was reduced to below 35%. This indicates that the mechanism for interaction of the fluoroquinolones with the HLB cartridges is different from the remainder of the compounds, suggesting that it is based on electrostatic rather than hydrophobic interactions. Fluoroquinolones have exhibited acceptable recoveries in both basic and acidic pH, but it has also been reported that recoveries for tetracyclines are much improved in an acidic rather that a basic solution,[10] and therefore, the pH of the samples were adjusted to between 2.8 and 3.0. This pH range was chosen because it is below the lowest reported pK_a for ciprofloxacin (3.01) and enrofloxacin (3.85).[23] Different compositions of eluents, including ACN with 1% ammonia (method IV), ACN with 1% TFA (method V), and ACN (method VI) were also tested.

To further determine the accuracy and precision of the optimized SPE method (method VI), five replicates of NANOpure water were spiked at two different levels, one at a low, environmentally relevant concentration (0.25 μg/L) and another at a high concentration (2.5 μg/L) to determine whether the breakthrough level of the cartridge would be reached. The recoveries for the compounds at both concentrations ranged between 71 and 119%, with relative standard deviations of 16% or lower (Table 4).

LC/MS/MS. The 13 target antibiotics, caffeine, and the internal standard were separated using a C_{18} column and a ternary mobile phase. First, a binary mobile-phase gradient was used, with

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Table 4. Summary of the Solid-Phase Extraction Recoveries and Relative Standard Deviations for Method VI ($n = 5$), Limits of Detection, Limits of Quantification, and Dynamic Range of the SPE-LC/MS/MS Method

compound	recovery % 0.25 μg/L	recovery % 2.5 μg/L	LOD (μg/L)	LOQ (μg/L)	dynamic range (μg/L)
caffeine	108 ± 6	115 ± 3	0.071	0.24	0.071−2.5
sulfamethazine	95 ± 3	102 ± 6	0.053	0.18	0.053−2.5
sulfadimethoxine	93 ± 1	107 ± 5	0.050	0.17	0.050−2.5
sulfamethoxazole	97 ± 3	107 ± 3	0.083	0.27	0.083−2.5
trimethoprim	95 ± 9	71 ± 13	0.091	0.31	0.091−2.5
ciprofloxacin	112 ± 16	107 ± 10	0.030	0.10	0.030−2.5
enrofloxacin	117 ± 16	103 ± 5	0.034	0.12	0.034−2.5
tetracycline	113 ± 3	109 ± 3	0.043	0.15	0.043−2.5
oxytetracycline	108 ± 5	110 ± 2	0.060	0.20	0.060−2.5
chlortetracycline	119 ± 6	117 ± 4	0.059	0.20	0.059−2.5
clindamycin	87 ± 11	85 ± 4	0.027	0.10	0.027−2.5
erythromycin-H_2O	108 ± 8	94 ± 6	0.075	0.25	0.075−2.5
roxithromycin	98 ± 5	86 ± 11	0.19	0.65	0.19−2.5
tylosin	85 ± 15	72 ± 12	0.040	0.12	0.036−2.5

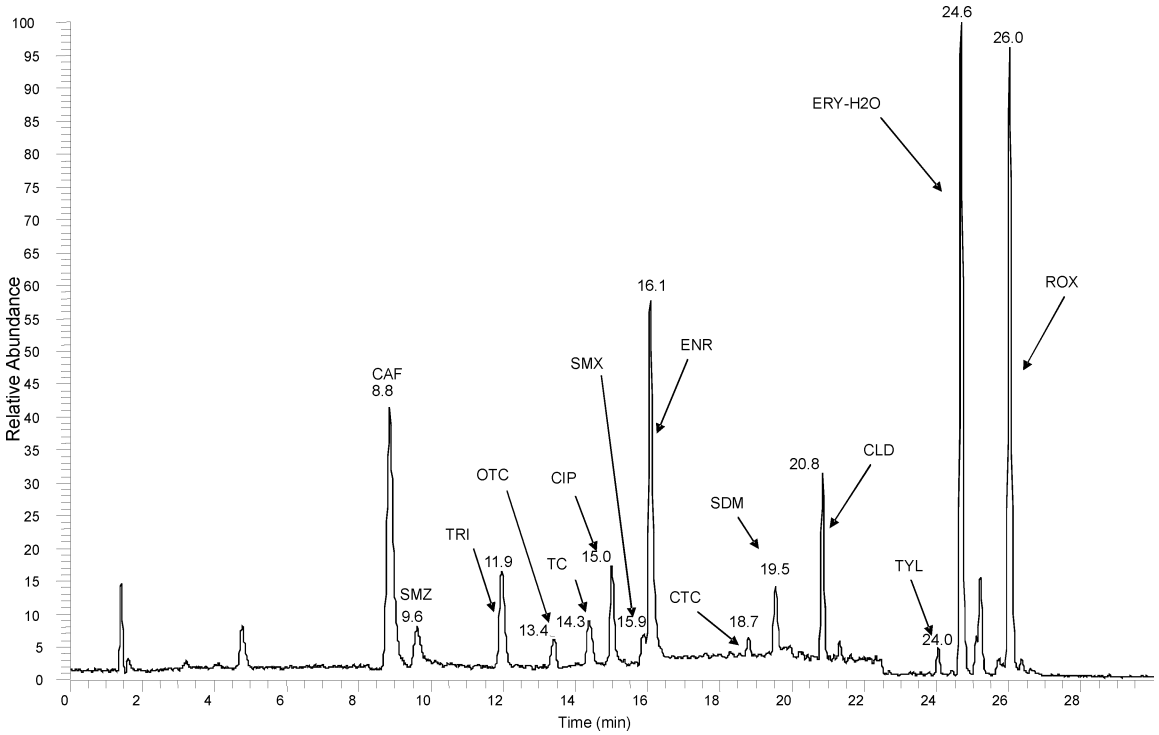

Figure 1. Chromatogram obtained for the separation of the target analytes in Niagara River water spiked at a concentration of 0.25 μg/L.

ACN as the organic portion of the mobile phase; however, not all compounds were separated using this mobile-phase composition. MeOH was then used to replace ACN, but the more hydrophobic compounds did not elute from the column after 30 min even after 90% MeOH was reached. A gradient of ACN, MeOH, and water with 0.3% formic acid, provided the best separation with all 15 compounds eluting within 27 min, as shown in the base peak-extracted ion chromatogram of a spiked and extracted Niagara River water sample (0.25 μ/L) (Figure 1). The fragment ions observed are in agreement with those previously reported.[13,26−30]

The MS data for the quantification of the 14 target compounds were collected in full scan mode given that selected ion monitoring (SIM) does not improve sensitivity in an IT-MS.[25] Since the analytes have a wide range of m/z (195−916), the data acquisition was divided into three time segments to allow the full scan data to be collected over reduced mass ranges. Scanning over a small mass range greatly increases sensitivity for the target analytes. Selected reaction monitoring (SRM) was not used for quantification due to the large number of analytes that each would require a separate scan event for each analyte, resulting in a reduced

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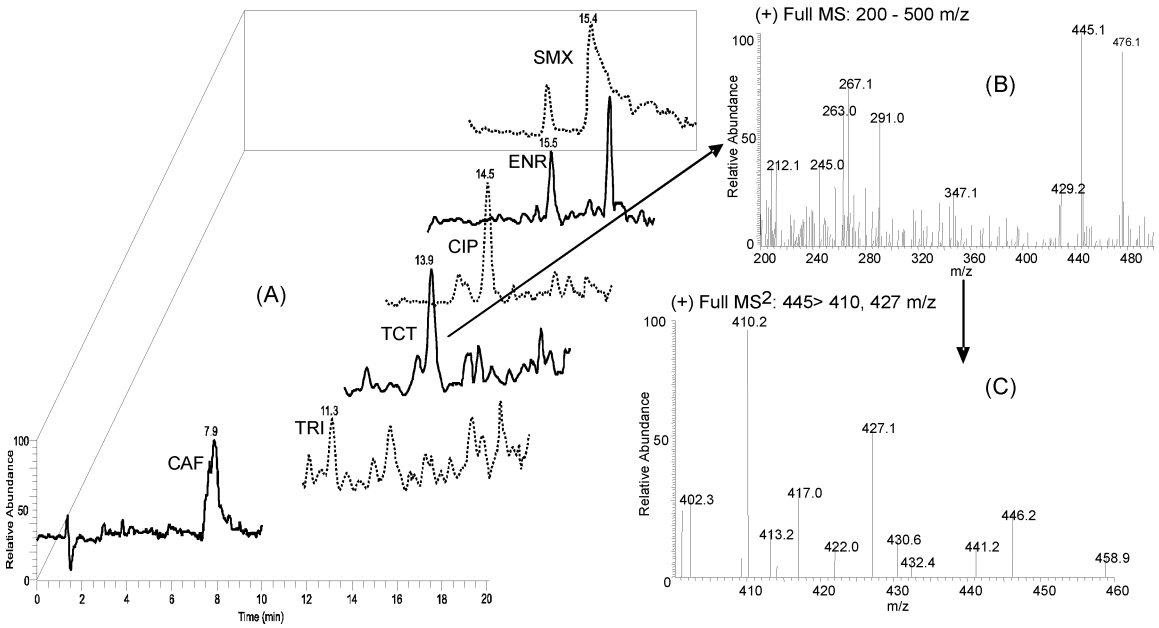

Figure 2. Base peak-extracted ion chromatograms (A) for the six target analytes detected in the WWTP effluent collected from Holland, NY. The full scan mass spectra (B) and full scan MS/MS spectra (C) collected for tetracycline at a concentration of 0.62 μg/L using the data-dependent scanning technique are also shown.

number of data points collected across each peak. Although the MS acquisition in an IT can be divided into different time segments to reduce the number of scan events in each segment, any shift in retention time due to matrix effects could lead to nondetection or misidentification of target analytes. To circumvent this problem, data-dependent scanning was used to collect full scan and full scan MS/MS data with only two scan events, providing the important identification information and a more accurate quantification.

In the LCQ IT, data-dependent scanning includes at least two scan events, the first of which is an independent scan, usually a full scan, and the second scan, which in this case is a full scan MS/MS, is dependent upon the data collected in the first scan. If a specified precursor ion is present in the independent scan above a user-defined background threshold (for this method, 10^4 counts), the IT then isolates and fragments the specified m/z. If no specified precursor mass is present or if no precursor mass is specified, then the IT will perform an MS/MS experiment on the mass peak of highest intensity. Figure 2 shows the base peak-extracted ion chromatograms (A) for six analytes that were detected in wastewater effluent from Holland, NY. The implementation of the data-dependent scanning is shown here, with the full scan mass spectra ranging from 200 to 500 m/z (B) containing the precursor mass for tetracycline and the subsequent full scan MS/MS spectra (C) showing the characteristic fragment ions at 410 and 427 m/z. The collision energy was set to 44% to allow fragmentation of all target compounds, and the isolation width was either 1.0 or 2.0. To ensure the full scan MS/MS data are collected for each desired m/z, dynamic exclusion was also enabled. This allows the IT to collect a specified number of dependent scans (repeat count) before a precursor ion is placed on an exclusion list. A repeat count of five was specified in order to average a sufficient amount of tandem data. Once an ion has been placed on the exclusion list, it will not trigger the collection of dependent data for a specified amount of time (in this case, a repeat duration of 2.0 min). Variations of data-dependent scanning techniques have been used in the identification of complex mixtures of peptides[31] and isotopic dependence data scans can be used in the identification of halogenated compounds.

The lower detection limits of the q and QqQ are achieved using SIM or multiple reaction monitoring (MRM), which do not collect full scan data. This limits the availability of full scan data, which can be used not only to identify target analytes but also to detect additional unknown compounds. Full scan MS/MS data are also more valuable for identification than SRM since the full scan MS/MS spectra contain all of the fragment ions for an isolated parent mass, providing multiple product ions for more complete identification. Since data-dependent scanning can collect full scan MS/MS data for many compounds in addition to the target analytes, this method proves to be especially useful in the identification of unknown contaminants. Furthermore, because the q mass analyzer does not have the tandem capabilities of the IT, the fragment ions in a q are collected using ion source collision-induced dissociation (CID). This technique applies a voltage to fragment ions in the ionization source, and therefore, the q does not have the MS/MS capabilities of the IT. This is due to the fact that there is no isolation of the precursor mass prior to fragmentation, so in source CID is not as specific or efficient as mass analyzer CID.

Method Validation. The limits of detection, limits of quantification, and dynamic range of the SPE-LC/MS/MS method were determined. The LODs ranged from 0.027 to 0.19 μg/L, while the LOQs ranged from 0.10 to 0.65 μg/L (Table 4). Compounds demonstrated good linearity between the LODs and the highest concentration tested, which was 2.5 μg/L (Table 4). Concentrations higher than 2.5 μg/L were not tested because antibiotics are not typically found in surface water or groundwater above this level.

The extraction procedure was tested in groundwater, river water, and wastewater to validate its applicability in matrixes from

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Table 5. Summary of the Solid-Phase Extraction Recoveries for Duplicate Samples Using the Optimized SPE Conditions Observed in Three Different Water Matrixes

	groundwater (%)		surface water (%)		wastewater (%)	
compound	0.25 μg/L	2.5 μg/L	0.25 μg/L	2.5 μg/L	0.25 μg/L	2.5 μg/L
caffeine	120 ± 1	104 ± 2	108 ± 2	110 ± 0	116 ± 1	125 ± 1
sulfamethazine	96 ± 2	102 ± 1	99 ± 1	107 ± 2	99 ± 1	114 ± 2
sulfadimethoxine	106 ± 3	102 ± 3	99 ± 2	102 ± 3	107 ± 1	96 ± 3
sulfamethoxazole	102 ± 1	115 ± 7	108 ± 2	103 ± 0	102 ± 2	111 ± 1
trimethoprim	88 ± 1	88 ± 6	88 ± 2	90 ± 13	94 ± 1	85 ± 7
ciprofloxacin	111 ± 7	92 ± 1	106 ± 5	99 ± 6	91 ± 11	93 ± 1
enrofloxacin	86 ± 8	77 ± 2	104 ± 1	98 ± 4	92 ± 23	100 ± 4
tetracycline	106 ± 1	102 ± 1	106 ± 1	103 ± 5	113 ± 2	99 ± 8
oxytetracycline	104 ± 4	90 ± 2	108 ± 1	99 ± 1	111 ± 2	96 ± 3
chlortetracycline	109 ± 2	114 ± 3	127 ± 1	124 ± 8	126 ± 0	123 ± 6
clindamycin	81 ± 5	83 ± 3	92 ± 2	92 ± 6	96 ± 3	90 ± 2
erythromycin-H_2O	75 ± 11	86 ± 6	112 ± 8	129 ± 5	85 ± 5	114 ± 1
roxithromycin	51 ± 10	76 ± 1	78 ± 3	94 ± 6	82 ± 2	99 ± 1
tylosin	65 ± 11	67 ± 2	74 ± 8	87 ± 9	83 ± 4	75 ± 5

three different aquatic systems (Table 5). The sulfonamides, trimethoprim, the tetracyclines, and clindamycin all exhibited recoveries in the three types of natural waters similar to those in the NANOpure water, with recoveries ranging from 81 to 127%. A blank from the Niagara River and the Amherst WWTP revealed caffeine at concentrations of 0.22 and 0.78 μg/L, respectively. These initial concentrations were subtracted from the calculated concentrations of the spiked samples, after which the recoveries were similar to those found in the spiked NANOpure water. The fluoroquinolones and the macrolides demonstrated a 20−40% decrease in recovery in the groundwater, indicating vulnerability of some compounds to matrix effects. It should be noted that concentrations of all analytes were calculated based on the $^{13}C_6$-sulfamethazine internal standard, which may not necessarily have the same mechanism of interaction with the HLB cartridge as all of the analytes. Sulfamethazine may also be affected by the matrix to suppress ionization to a different extent as compared to the fluoroquinolones and macrolides. Therefore, it would be more ideal to have an internal standard for each class of antibiotics to compensate for losses and matrix effects. Unfortunately, this equates to the added cost of obtaining labeled reference materials. The data-dependent scanning of the spiked groundwater, surface water, and wastewater used in the method validation revealed the same diagnostic product ions for all target analytes as spiked distilled water, indicating that the matrix did not influence the MS/MS fragmentation significantly.

Environmental Application. Groundwater samples from Weiser, ID, along with wastewater effluent from two sources, the Dunkirk and the Fredonia WWTPs, did not reveal any of the target analytes. Surface water from a second location on the Niagara River revealed clindamycin at a concentration of 1.1 μg/L. Clindamycin is a lincosamide antibiotic often used in the topical treatment of acne[32] and has been detected in surface waters.[33] Wastewater effluents from East Aurora and Holland, NY, exhibited caffeine and several antibiotic concentrations, ranging from 0.10 to 1.3 μg/L and are listed in Table 6. The differences in the occurrence of antibiotics in wastewater effluents reflect the different removal efficiencies of varying operation conditions in the treatment plants.

Table 6. Concentrations of Antibiotics Detected in the Effluent of Two Local WWTPs (in μg/L)

compound	East Aurora	Holland
caffeine	0.46	1.56
sulfamethazine		
sulfadimethoxine		
sulfamethoxazole	1.3	1.3
trimethoprim	0.12	0.16
ciprofloxacin		0.36
enrofloxacin		0.10
tetracycline		0.62
oxytetracycline		
chlortetracycline		+[a]
clindamycin		+
erythromycin-H_2O		+
roxithromycin		
tylosin		

[a] The false positive results are also indicated. +, false positive result.

The study by Hernández et al.[15] discussed the 2002/657/EC European Commission Decision, which created guidelines for the identification of organic contaminants in animals and animal products. At least three IPs are needed for a positive confirmation, and these guidelines were applied to the detection of pesticides in water samples. A low-resolution MS (LR-MS) precursor ion has the value of 1.0 IP, and each LR-MS product ion has a value of 1.5 IPs; therefore, at least two diagnostic product ions in LR-MS must be monitored to meet the minimum number of IPs. Furthermore, the use of SIM, SRM, or MRM with only one product ion being monitored in a LR-MS would not meet the 3.0 IP requirement, as is the case with many of the current pharmaceutical detection methods. The importance of the added confidence offered by the data-dependent full scan MS/MS capability of IT can be demonstrated in the analysis of the complex matrix from WWTP samples. The mass spectral information becomes particularly valuable in eliminating both false-negative and false-positive detection of analytes when quantification is based only on SIM or MRM.

For example, in the WWTP effluent from Holland, NY, the presence of six of the target analytes were confirmed by their

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characteristic full scan MS/MS spectra. The implementation of the data-dependent scanning is exhibited by the base peak-extracted ion chromatograms (A) for each of the six detected analytes in Figure 2, along with the full scan (B) and data-dependent full scan MS/MS (C) mass spectra for tetracycline. However, false positive results based only on the retention time of precursor masses were found for chlortetracycline, clindamycin, and erythromycin$-H_2O$. With a q MS, these peaks could have been misidentified when using SIM. The data-dependent scanning revealed a full scan MS/MS fragmentation pattern very different from that of the standard solutions, as illustrated for erythromycin$-H_2O$ in a wastewater effluent sample (Figure S-1, Supporting Information), which revealed a peak with m/z of 716 at a retention time of 24.6 min. Also consider the case of false negative results, as shown by the example of caffeine, which was present in a wastewater effluent (0.78 μg/L) used for method validation. Based only on the base peak ion chromatogram, the presence of caffeine in this effluent is ambiguous because of the shift in retention time due to matrix effects. However, the data-dependent scanning showed the presence of the characteristic fragments ions (Figure S-2, Supporting Information), confirming that the peak at 8.3 min is caffeine.

CONCLUSION

The optimized SPE method proved to be efficient and reproducible for the simultaneous extraction of the 14 target analytes in water from various environmental matrixes, using only one type of SPE packing material and a single elution step. IT-LC/MS/MS with data-dependent scanning is a powerful analytical tool that offers reliability to simultaneously separate and identify target compounds in groundwater, surface water, and wastewater in the submicrogram per liter range. Although the IT does not offer the lower detection limits achieved by other mass spectrometers, the ability of IT to simultaneously collect full scan and full scan MS/MS data that can be used to more accurately identify compounds in complex samples makes this instrumentation very valuable. This method proved useful in monitoring a wide range of antibiotics in various aquatic matrixes and could be used to provide important information on the susceptibility of different water systems to wastewater contamination. With the introduction of newer IT configurations, such as the linear IT recently described by Hager et al.,[34] improved sensitivity and LODs in IT-MS may be achieved.

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SUPPORTING INFORMATION AVAILABLE

Additional results (SPE preliminary experiment recoveries) and two additional figures (chromatograms and MS/MS spectra). This material is available free of charge via the Internet at http://pubs.acs.org.

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