Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1042 (2004) 113–121
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Simultaneous determination of fluoroquinolone, sulfonamide, and trimethoprim antibiotics in wastewater using tandem solid phase extraction and liquid chromatography–electrospray mass spectrometry

Jay E. Renew, Ching-Hua Huang*

School of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA

Received 22 April 2003; received in revised form 9 February 2004; accepted 17 May 2004

Abstract

A robust and sensitive method for the detection of fluoroquinolones, sulfonamides and trimethoprim has been developed. Wastewater samples were acidified and extracted through an anion-exchange cartridge in tandem with a hydrophilic–lipophilic balance (HLB) cartridge, a procedure that reduced interferences from wastewater organic matter. The extracted antibiotics were analyzed using liquid chromatography electrospray mass spectrometry and selected ion monitoring. Quantification of antibiotics was assessed both by internal standard and standard addition methods. Average recoveries for a range of wastewater matrices were 37 to 129% for a 1 μg/L spiking concentration. The method detection limits (MDLs) of antibiotics in deionized water, final and secondary effluent ranged from 2 to 7 ng/L, from 20 to 50 ng/L, and from 30 to 90 ng/L, respectively. Assessment of matrix interference shows that signal suppression and MDL increases with higher amounts of organic matter in the sample. Analyses of samples from two municipal wastewater treatment plants indicate that ciprofloxacin, ofloxacin, sulfamethoxazole and trimethoprim are present in the secondary effluents at median concentrations of 100–160, 205–305, 395–575, and 40–705 ng/L, respectively.

Keywords: Water analysis; Signal suppression; Fluoroquinolones; Sulfonamides; Trimethoprim

1. Introduction

Large quantities of antibiotics are administered to humans and animals to treat diseases and infections every year. Antibiotics are also widely used at sub-therapeutic levels to promote growth in livestock. Often a high percentage of the administered antibiotics is excreted from the dosed animals without metabolism or excreted in conjugated forms that can be readily converted back to the parent compounds [1]. Recently several studies have indicated the presence of antibiotic residues in water sources including municipal wastewater effluents and surface waters [2–10]. These findings merit concerns because antibiotic contaminants may perturb microbial ecology, increase the proliferation of antibiotic-resistant pathogens and pose threats to human health [1]. A better understanding of the occurrence and fate of antibiotics in natural and engineered water systems is imperative to assess the risks associated with these compounds.

Fluoroquinolones, sulfonamides and trimethoprim represent classes of synthetic antibiotics that are widely used in human and veterinary medicine. This study focuses on these groups of antibiotics in developing a sensitive method that can be utilized to investigate their fate in complicated water matrices. Prior studies show that these antibiotics are rather resistant to microbial degradation [11–13], providing an indication as to why these compounds might persist within municipal wastewater effluents. On the other hand, abiotic degradation including photolysis [14–17] and chemical oxidation [18–20] may be significant in their environmental fate. Among the antibiotics selected in this study (Fig. 1), ciprofloxacin, sulfamethoxazole, and trimethoprim have been among the top 200 drugs prescribed in the US from 1995 to 2002 [21]. Trimethoprim, a dihydrofolate reductase inhibitor which differs structurally from fluoroquinolones and sulfonamides, is commonly prescribed in combination

* Corresponding author. Tel.: +1 404 894 7694; fax: +1 404 894 8266.
E-mail address: ching-hua.huang@ce.gatech.edu (C.-H. Huang).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.05.056

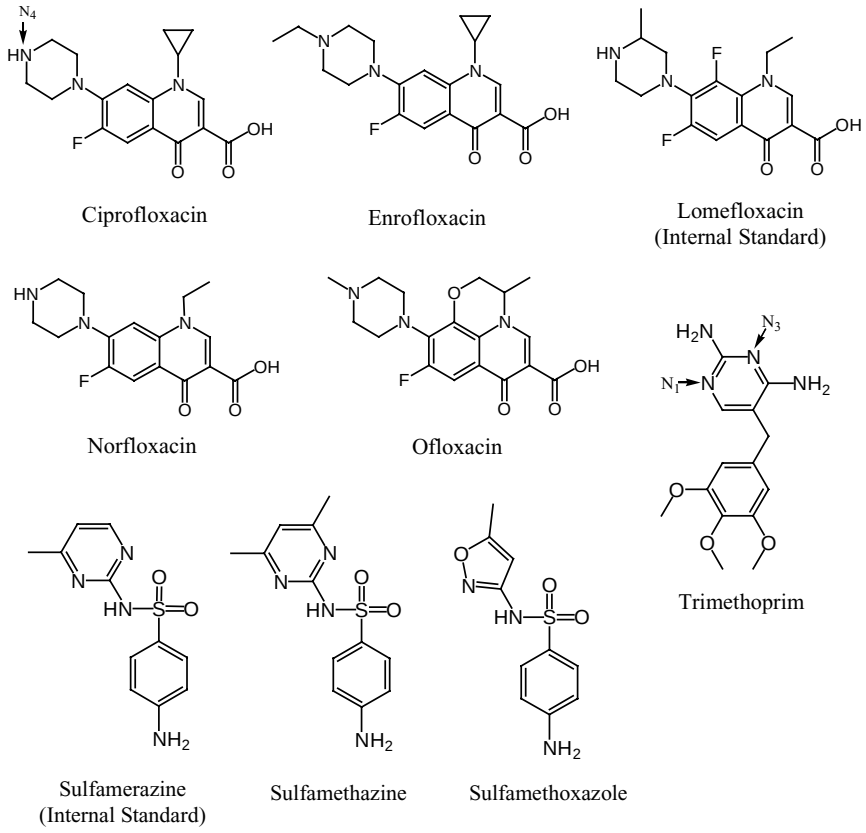

Fig. 1. Structures of antibiotics selected in this study.

with sulfamethoxazole (as co-trimoxazole, which contains SMX:TMP in a 5:1 ratio) or on its own. Levofloxacin, the (−)-*S*-enantiomer of the racemic ofloxacin, has also been among the top 200 drugs since 1998 [21]. Enrofloxacin and sulfamethazine are two popular veterinary drugs in their groups.

A number of analytical methods are currently available to detect the fluoroquinolone and sulfonamide classes of antibiotics separately in manure, surface water, wastewater and groundwater [3,6,8,22,23]. An analytical method to detect fluoroquinolones in wastewater was developed using cation-exchange cartridges [8]. Recoveries reported for this method were over 70% in both primary and tertiary wastewater effluent for 0.067–0.4 μg/L spiking concentrations. The method relies on fluorescence detection for quantification and identification and uses tandem LC–MS for additional confirmation. The instrumental quantification limit varies from 150 to 450 pg per 200 μL injection. This method is successful at detecting fluoroquinolones in complex wastewater matrices and at the low concentrations that are expected in aquatic environments. However, this analytical method is not suitable for extracting and detecting sulfonamide and trimethoprim antibiotics.

LC–MS techniques to detect sulfonamides are readily available [3,6,23]. Hirsch et al. [23] and Hartig et al. [6] developed methods based on tandem MS. The method developed by Hirsch et al. uses lyophilization and solid-phase extraction (SPE) to concentrate the sulfonamides. The average recoveries reported for this method were 15 and 75% for sulfamethazine and sulfamethoxazole, respectively in mountain spring water, and were 40 and 60%, respectively in surface water samples. No recoveries for wastewater effluent were reported. Hartig et al. reported average recoveries in secondary effluent of 68 ± 7 and 81 ± 12% for sulfamethazine and sulfamethoxazole, respectively for a 1 μg/L spiking concentration. This method provides excellent extraction efficiency for sulfonamides in complex matrices. The limit of detection for this method varies from 0.2 to 3.7 μg/L. Another LC–MS method was recently developed to detect sulfonamides and tetracyclines in surface water and groundwater [3]. This technique utilizes SPE and reported mean recoveries of 130 ± 17 and 91 ± 13% for sulfamethazine and sulfamethoxazole, respectively in distilled water. However, no recoveries are available for more complex matrices such as surface water or wastewater. Therefore, the performance of this method in analyzing wastewater samples cannot be evaluated.

In contrast to the earlier studies, the method developed in this study provides a technique to detect fluoroquinolones, sulfonamides and trimethoprim simultaneously at sub micrograms per liter concentrations in wastewater effluents. In addition, the use of single quadrupole LC–MS allows the method to be more widely adapted since single quadrupole LC–MS is currently more common than tandem LC–MS in

many water quality laboratories. SPE followed by LC–MS analysis are utilized by this method. Minimization of matrix effects and signal suppression is a major component of the method development. As will be shown later, application of this method allows preliminary determination of the occurrence of these antibiotics in municipal wastewater treatment plants.

2. Experimental

2.1. Chemicals

Ciprofloxacin, enrofloxacin, norfloxacin, ofloxacin, and sulfamethoxazole were purchased from ICN Biomedicals (Aurora, CA), whereas lomefloxacin, sulfamerazine, sulfamethazine, and trimethoprim were from Sigma–Aldrich (St. Louis, MO). The antibiotics were in >98% purity and used without further purification. Ammonium acetate, glacial acetic acid, phosphoric acid, sodium chloride, sodium thiosulfate ($Na_2S_2O_3$), and HPLC-grade methanol (MeOH) and acetonitrile (ACN) were obtained from Fisher (Pittsburgh, PA). Deionized reagent water (18.3 MΩ cm resistivity) was prepared by a Barnstead Nanopure water purification system (Dubuque, IA). Antibiotic stocks were prepared in 10:90 (v/v) MeOH/H_2O mixture at around 100 mg/L, stored at 4 °C, and used within 7 days. The Suwannee River organic matter (SROM) was provided by Dr. E.M. Perdue at the Georgia Institute of Technology.

2.2. Sample collection and preparation

Grab wastewater samples were collected in 1 L amber glass bottles with Teflon-lined caps from various municipal wastewater treatment plants in Georgia, California and Arizona. Minimum losses of antibiotics via adsorption to the container walls were observed in glass bottles when compared to fluorinated polypropylene sampling bottles. Effluent samples were collected after a range of processes including primary (screening and sedimentation), secondary (activated sludge and trickling filter), tertiary (biological nutrient removal and disinfection) and advanced treatment (microfiltration, reverse osmosis, granular activated carbon, and ozonation) processes. The wastewater samples were stored on ice in coolers during shipping. In collecting wastewater effluents that contained residual chlorine, 2 mg/L of $Na_2S_2O_3$ was added as a quenching agent to consume the residual chlorine. Experiments were conducted to confirm that the antibiotics did not react with $Na_2S_2O_3$. The wastewater samples were filtered through 0.5 μm glass fiber filters (Pall, Ann Arbor, MI) immediately after being brought back to the laboratory. The filtered samples were then added with 0.1 M NaCl, acidified to pH 2.5 with H_3PO_4, and spiked with the antibiotics for recovery samples. The samples were stored in dark at 4 °C and extracted within 3 days.

2.3. Sample extraction

Each 1 L sample was extracted through a 500 mg anion-exchange cartridge (Isolute, Mid Glamorgan, UK) stacked on top of a 500 mg hydrophilic–lipophilic balance (HLB) cartridge (Waters, Taunton, MA). Each cartridge was pre-conditioned with 6 mL MeOH followed by 6 mL 4.38 mM H_3PO_4. The samples were extracted using a Visiprep apparatus (Supelco, Bellefonte, PA) at a flow rate of approximately 6 mL/min. To evaluate the accuracy and precision of the method, blank (i.e. deionized water), duplicates, and recovery samples were analyzed with each batch of samples. Recoveries were evaluated by amending the samples with 1 μg/L of the antibiotics prior to extraction. To calculate recoveries, concentrations of antibiotics measured in the samples were subtracted from the concentrations measured in the recovery samples.

The antibiotics were eluted from the HLB cartridges with 10 mL of 95% MeOH/5% 4.38 mM H_3PO_4 solution to high-density polyethylene conical tubes. Eluting the anion-exchange cartridges with the same eluent yielded negligible amounts of antibiotics. The analytes were evaporated to dryness in a 30 °C water bath under a gentle stream of nitrogen gas and then reconstituted in 1 mL of 20% MeOH/80% 4.38 mM H_3PO_4 containing 1 mg/L of internal standards, sulfamerazine and lomefloxacin. When standard addition quantification was employed, an aliquot (300 μL) of the sample extract was added with a known amount of each antibiotic. All samples were transferred to amber HPLC vials and stored at 0 °C before LC–MS analysis.

2.4. Chemical analysis

The samples were analyzed using an Agilent 1100 Series HPLC with a diode-array UV detector and a mass spectrometer detector (MSD) (Palo Alto, CA). The antibiotics were separated using a 2.1 mm × 150 mm, 5 μm Zorbax SB-C18 column at 30 °C. A binary gradient at a flow rate of 0.25 mL/min was used: mobile phase A contained 1 mM ammonia acetate, 0.007% (v/v) glacial acetic acid and 10% ACN, and mobile phase B was 100% ACN. The gradient started with 0% B for the first 2 min. B was then increased to 8.5% by 8 min, 18% by 20 min, 50% by 25 min, and 100% by 30 min. After the gradient had completed, the column was flushed with 100% B for 10 min. A 15 min post-time was used between sample runs to allow the column to re-establish equilibrium.

Positive mode electrospray ionization and selected ion monitoring (SIM) were used. The MSD drying gas was set at a temperature of 350 °C and a flow rate of 10 L/min. The nebulizer pressure and the capillary voltage were 30 psig and 3500 V, respectively. To prevent the MS system from contamination and clogging, the eluent was allowed to enter the MSD only between 6 and 30 min after sample injection. Analytes were detected at fragmentation voltages of 85 and 120 V, respectively. Analytes were confirmed based on

Table 1
The pK_a, retention time, molecular ion and fragment ions of the antibiotics

Compound	pK_a values	Typical retention time (min)	$[M+H]^+$ ion (*m*/*z*)	Confirming ion 2 (*m*/*z*)	Confirming ion 3 (*m*/*z*)
Ciprofloxacin	6.09, 8.74[a]	17.2	332	314	288
Enrofloxacin	5.86, 8.24[b]	20.6	360	342	316
Lomefloxacin	5.82, 9.30[a]	17.9	352	334	
Norfloxacin	6.30, 8.38[a]	16.3	320	302	276
Ofloxacin	6.05, 8.22[a]	16.3	362	318	261
Sulfamerazine	2.17, 6.77[c]	9.5	265	156	
Sulfamethazine	2.28, 7.42[c]	12.6	279	156	
Sulfamethoxazole	1.83, 5.57[c]	18	254	156	
Trimethoprim	1.32[d], 7.12[e]	14.1	291	261	

[a] Reference [24].
[b] Reference [25].
[c] Reference [26].
[d] Reference [27].
[e] Reference [28].

the retention time, presence and relative abundance of the molecular and confirming ions as listed in Tables 1 and 2. At the lower voltage, the antibiotics yield less fragments and the molecular ion ($[M + H]^+$) is detected with the greatest sensitivity. The higher voltage causes more fragmentation of the antibiotics and yields higher abundance of the confirming ions, providing additional confirmation for the antibiotics. The relative abundances of the ions were required to be within 25% of the values shown in Table 2.

A Hewlett-Packard 8452A diode-array spectrophotometer was used to measure samples' UV absorbance at 254 nm.

3. Results and discussion

3.1. Solid-phase extraction (SPE)

One of the problems frequently encountered in extracting organic contaminants (e.g. antibiotics) from wastewater is matrix interference due to high amounts of organic matter in the samples. Organic matter reduces extraction efficiency and interferes with detection. The SPE method developed in this study in which an anion-exchange cartridge and a HLB cartridge are used in tandem can simultaneously extract fluoroquinolone, sulfonamide and trimethoprim antibiotics and reduce organic matter interference.

Solution pH is expected to significantly influence speciation of the antibiotics owing to the presence of acidic and basic functional groups in their structures (Fig. 1). Their acidity constants (Table 1) [24–28] indicate that protonation and deprotonation of these antibiotics occur readily in the environmental pH range. The pK_{a_1} and pK_{a_2} values correspond to (i) deprotonation of the carboxylic acid group and protonation of the piperazinyl amino group (N_4) of fluoroquinolones, respectively (ii) protonation of the aniline group and deprotonation of the sulfonylamido group of sulfonamides, respectively, and (iii) protonation of the two heterocylic nitrogen atoms (N_1 and N_3) of trimethoprim. Acidification of wastewater samples to near pH 2.5 prior to SPE yielded predominantly neutral sulfonamides and cationic fluoroquinolones and trimethoprim. As a result, the neutral and cationic antibiotics were not retained in the anion-exchange cartridges while some of the highly negatively-charged natural organic matter was. Elution of the anion-exchangers yielded negligible amounts of antibiotics, confirming that antibiotics were not retained in these cartridges. Visual inspection of the anion-exchangers after extraction showed that a significant amount of organic matter had accumulated

Table 2
The relative abundance of molecular and confirming ions (*m*/*z*) at the two fragmentation voltages

Compound	$[M+H]^+$ ion relative abundance (%)		Confirming ion 2 relative abundance (%)		Confirming ion 3 relative abundance (%)	
	85 V	120 V	85 V	120 V	85 V	120 V
Ciprofloxacin	100	100	4	71	9	73
Enrofloxacin	100	100	1	36	9	90
Lomefloxacin	100	100	2	25		
Norfloxacin	100	97	5	100	13	90
Ofloxacin	100	92	10	100	1	33
Sulfamerazine	100	52	15	100		
Sulfamethazine	100	100	5	71		
Sulfamethoxazole	100	27	41	100		
Trimethoprim	100	100	1	9		

in the polymers. This sample clean-up during SPE was necessary for most wastewater samples examined in this study. Wastewater samples that were extracted by the HLB cartridge alone experienced elevated baseline and severe matrix interference that prohibited accurate analysis by LC–MS.

The investigation also found that salt addition (0.1 M NaCl) improved antibiotic extraction efficiency, particularly for sulfonamides and trimethoprim. Although the amount of salt added was not sufficient to salt out the antibiotics, the presence of additional electrolytes appeared to facilitate sorption of the antibiotics to the HLB polymers.

3.2. Detection by LC–MS and signal suppression

In LC–MS analysis, the signal intensity of antibiotics was considerably suppressed in wastewater matrices. The signal suppression may be caused by several phenomena. Firstly, the antibiotics may sorb to organic matters in the samples, causing the concentrations of freely dissolved antibiotics to be lower and thus more difficult to detect. Prior studies have reported declining aqueous concentrations of fluoroquinolones in the presence of humic acids as a result of fluoroquinolones partitioning to the dissolved organic carbon [29,30]. Secondly, contaminants in the sample matrix may mask the analyte peaks by raising the chromatogram baseline. As a result, the area under the chromatographic curve is underestimated. Thirdly, contaminants may reduce ionization efficiency of the analytes by taking up some of the limited number of excess charged sites on the surfaces of electrosprayed droplets [31–33]. Humic substances in particular have been shown to cause signal suppression in the analysis of polar pesticides in surface and estuarine waters when analyzed by electrospray ionization [33].

Signal suppression is a complex effect that can vary with instrumental conditions (e.g. the geometry and voltage of the ion source, etc). Despite that, evaluation of signal suppression was conducted in order to assess its effect on antibiotic quantification in this study. The signal suppression observed with each antibiotic was calculated using Eq. (1), as the percentage decrease in signal intensity in a sample matrix versus in deionized water:

$$\text{signal suppression (\%)} = \left(1 - \frac{I_s - I_x}{I_{DI}}\right) 100 \quad (1)$$

where I_s was the antibiotic signal intensity in a sample extract where S amount of the antibiotic was spiked after extraction, I_x was the antibiotic signal intensity in the unspiked sample extract, and I_{DI} was the antibiotic signal intensity in deionized water matrix (20% MeOH/80% 4.38 mM H_3PO_4) spiked with S amount of the antibiotic. As shown by a few examples in Fig. 2A, signal suppression for every antibiotic increases approximately linearly as the wastewater's UV_{254} absorbance increases. Ultraviolet absorbance at 254 nm correlates positively with the organic carbon content of water [34]. These results show that a higher amount of organic matter causes greater signal suppression.

Similar experiments were conducted to assess the influence of Suwannee River organic matter (SROM) on the signal intensity of antibiotics. Solutions of varying concentrations of SROM (0.13–1.04 g/L, comparable to the amount of organic matter in the wastewater extracts) were spiked with the antibiotics. The signal suppression of the antibiotics was calculated by Eq. (1), where the I_x was near zero in this case. Similarly, higher concentration of SROM (i.e. higher UV_{254} absorbance) causes greater signal suppression for the antibiotics and the relationship is approximately linear (Fig. 2B).

Overall, the wastewater samples have considerably higher UV_{254} absorbance than the SROM solutions despite that both sample sets contain comparable levels of total organic carbon (TOC). The different light-absorbing properties indicate different structural characteristics among these organic matters. The overall greater signal suppression in the wastewater samples also shows that the wastewater organic matter exerts a stronger matrix effect than the SROM for the antibiotics. This observation suggests that method development using surrogate organic matter like SROM may underestimate the matrix effect from wastewater organics. Comparison of signal suppression among the antibiotics indicates that antibiotics within the same class generally exhibit a similar degree of signal suppression (i.e. fluoroquinolones versus sulfonamides versus trimethoprim) and that fluoroquinolones are more susceptible to signal suppression than sulfonamides and trimethoprim.

Another complication encountered in the LC–MS analysis was that the retention time for fluoroquinolones and trimethoprim might drift up to 2 min in some samples. However, the molecular and confirming ions provided enough evidence to identify the antibiotics in these cases. The eluent buffer concentration was adjusted to minimize this drift in retention time and improve the peak shape. Increasing the buffer concentration, however, reduces signal intensity. Therefore, it was necessary to optimize the buffer concentration to maintain signal strength without sacrificing chromatographic peak shape or retention time stability. For several highly complex wastewater matrices, the buffer concentration was reduced in order to detect the antibiotics.

3.3. Quantification, recoveries and detection limits

Compounds were quantified based on their $[M + H]^+$ ion peaks by internal standard and standard addition methods. In the internal standard method, the ratios of the analyte and internal standard signals were use to develop the calibration curve. To ensure quantification accuracy, the internal standards should have similar ion evaporation properties as the analytes [35] and thus will experience the same degree of signal suppression in different matrices as the analytes. For this reason, radio-labeled analytes are frequently the choice of internal standards. However, radio-labeled analogs of most of the antibiotics are not available. Lomefloxacin and sulfamerazine (Fig. 1) are selected as the internal standards

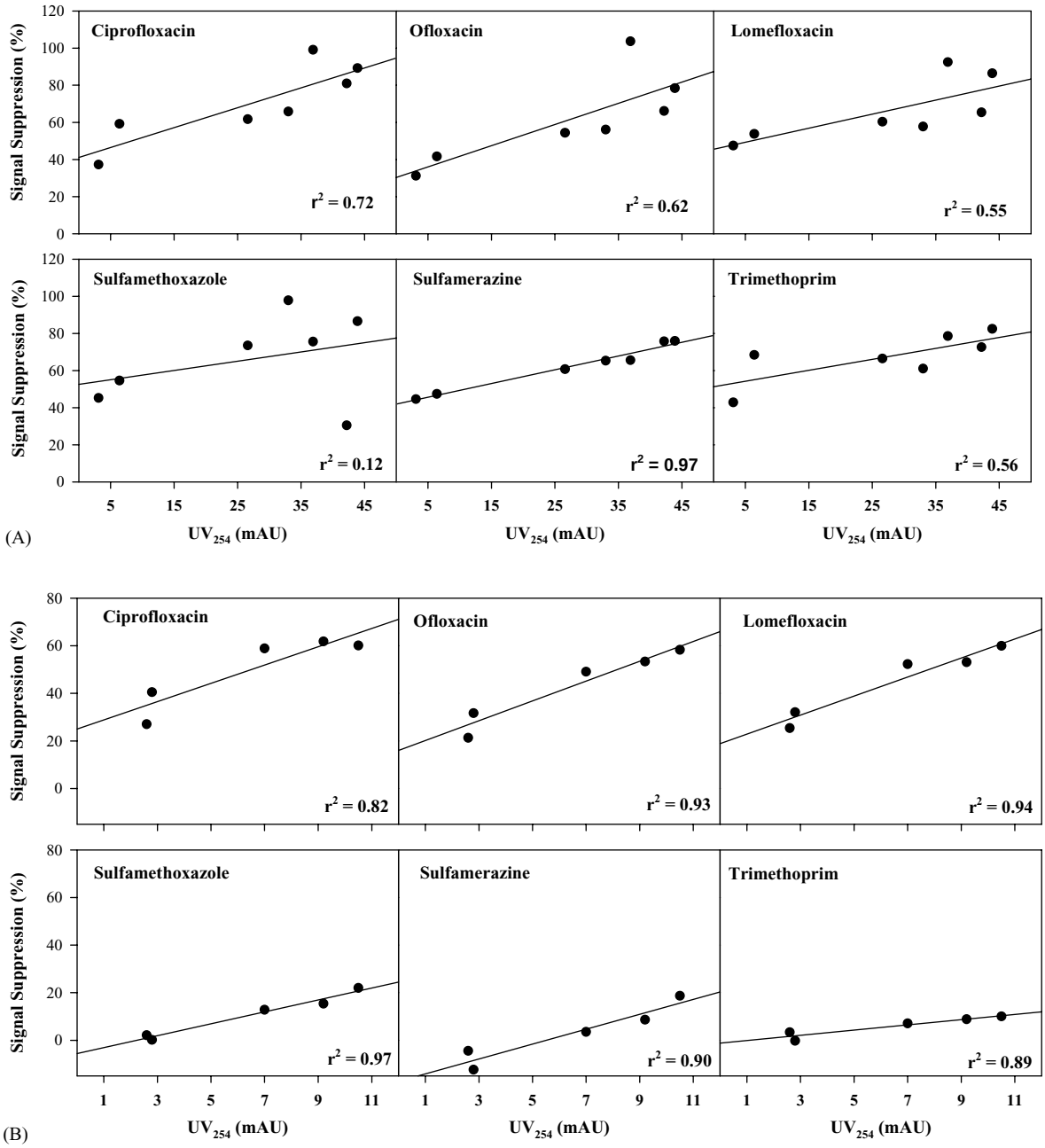

Fig. 2. Signal suppression for the antibiotics in (A) wastewater extracts (concentration factor = 1000), and (B) solutions containing the Suwannee River organic matter (SROM) at 0.13–1.04 g/L. The UV_{254} absorbance was measured after diluting 10 μL of the sample in 25 mL of deoionized water.

because they are not commonly used in human therapy in the US [21] and thus are not expected at significant concentrations in municipal wastewaters. Neither lomefloxacin nor sulfamerazine was at detectable levels in any of the wastewater samples examined in this study.

The similar signal suppression observed with lomefloxacin and the other fluoroquinolones (Fig. 2) indicates that lomefloxacin served as an appropriate internal standard. Lomefloxacin and the other fluoroquinolones have closely related structures and thus likely share similar ion evaporation properties. Additionally, the chromatographic retention times of the fluoroquinolones are close together (Table 1). Thus, the constituents co-eluting with these analytes are likely similar, yielding comparable matrix effects during ionization. The above two factors work together for lomefloxacin to be a good internal standard for the fluoroquinolone antibiotics.

Sulfamerazine closely resembles sulfamethazine and sulfamethoxazole. However, the chromatographic retention times of these three sulfonamides span a wider range than those of fluoroquinolones (Table 1) and may lead to different matrix effects that are caused by co-eluting constituents.

Table 3
Recoveries (%) of antibiotics in wastewater samples

Compound	DI water ($n = 8$)	Secondary effluent ($n = 13$)	Final effluent ($n = 13$)
Ciprofloxacin[a]	102 ± 15	90 ± 28	98 ± 25
Enrofloxacin[a]	84 ± 17	92 ± 24	97 ± 24
Norfloxacin[a]	99 ± 15	106 ± 29	95 ± 31
Ofloxacin[a]	101 ± 10	129 ± 25	114 ± 24
Sulfamethazine[a]	86 ± 19	37 ± 16	64 ± 24
Sulfamethoxazole[a]	63 ± 30	56 ± 31	65 ± 14
Trimethoprim[b]	92 ± 17[c]	109 ± 56[d]	98 ± 39[d]

[a] Quantification based on internal standard.
[b] Quantification based on standard addition.
[c] $n = 4$.
[d] $n = 7$.

Nevertheless, the results showed that sulfamerazine served as a satisfactory internal standard for the sulfonamides in most wastewater matrices examined.

In contrast, sulfamerazine was not an appropriate internal standard for trimethoprim. Using sulfamerazine as an internal standard generally overestimates the concentration of trimethoprim because the signal for sulfamerazine is often more suppressed than trimethoprim in a given matrix. This overestimation was evident by the fact that the extraction recoveries determined for trimethoprim were generally greater than 150% if using sulfamerazine as an internal standard. The standard addition method (Eq. (2)) was used instead to quantify the concentration of trimethoprim:

$$[X] = \frac{[S]I_x}{I_s - I_x} \quad (2)$$

where $[X]$ is the antibiotic concentration in the unknown sample $[S]$ the antibiotic concentration added to the unknown sample, I_x the antibiotic signal intensity in the unknown sample, and I_s is the antibiotic signal intensity in the sample spiked with $[S]$ amount of the antibiotic. The standard addition method helped reduce inaccuracies caused by matrix effects.

In calculating the method recoveries, the internal standard method was used for fluoroquinolones and sulfonamides, and the standard addition method for trimethoprim. The average recoveries for deionized water, secondary effluent, and final effluent are shown in Table 3. Final effluent was defined as wastewater that has received tertiary and/or advanced treatment. The recoveries were above 55% for all compounds except for sulfamethazine in secondary effluents. The average recoveries for the fluoroquinolone antibiotics were above 90% for all samples. Ciprofloxacin, enrofloxacin and norfloxacin have comparable recoveries (90–106%) while ofloxacin generally has higher recoveries (114–129%). The higher recoveries for ofloxacin may be due to the slightly lower degree of signal suppression for ofloxacin than lomefloxacin in the wastewater matrices. The average recoveries for trimethoprim ranged from 98 to 109%. The average recoveries for the sulfonamide antibiotics were lower, at 37–65%.

Table 4
Method detection limits (ng/L) for antibiotics

Compound	Deionized water	Final effluent	Secondary effluent
Ciprofloxacin	4	20	30
Enrofloxacin	3	40	40
Norfloxacin	7	30	30
Ofloxacin	3	20	30
Sulfamethazine	2	40	90
Sulfamethoxazole	4	50	60
Trimethoprim	2	40	50

The method detection limits (MDLs) for the antibiotics were calculated using the EPA method [36] in deionized water (i.e. 20% MeOH/80% 4.38 mM H_3PO_4), final effluent and secondary effluent matrices, respectively. The deionized water sample was spiked with the antibiotics at 20.4–22.7 μg/L. For MDLs in secondary and final effluents, the effluent extracts were first analyzed by LC–MS to determine the antibiotic concentrations. Next, additional antibiotics were spiked at 450–670 μg/L into the matrix by amending 10 μL of an antibiotic cocktail to 320 μL of the effluent extracts. The samples were then analyzed by LC–MS for the antibiotic concentrations. These samples were analyzed seven times consecutively at 85 V fragmentation voltage. The standard deviation of the measured antibiotic concentrations was calculated and multiplied by the *t*-value of 3.14 to yield the corresponding MDL. Clearly, the MDL increases as the matrix becomes more complex, from 2 to 7 ng/L in deionized water, from 20 to 50 ng/L in the final effluent, from 30 to 90 ng/L in the secondary effluent (Table 4). The increase in MDL is resulted from increasing signal suppression in the matrices.

3.4. Concentrations of antibiotics in wastewater effluent

The described method has been applied to many wastewater and surface water samples to assess the occurrence and fate of the antibiotics. The details of the occurrence results are being reported in a separate paper. The results from two municipal wastewater treatment plants (WWTPs) are included here to illustrate the applicability of this method for wastewater matrices (Table 5). Both treatment plants utilize primary and secondary (activated sludge) treatment. After the secondary treatment, WWTP I utilizes chlorination for disinfection whereas WWTP II utilizes UV disinfection. The concentrations of antibiotics were quantified based on the standard addition method when possible and were not corrected for recovery. The internal standard method was used for a couple of samples that were collected during the earlier stage of this study.

Among the seven antibiotics examined in this study, four of them (ciprofloxacin, ofloxacin, sulfamethoxazole, and trimethoprim) were frequently detected in the secondary effluents of both plants. Norfloxacin was detected at less than 60 ng/L in the secondary effluent of WWTP I and was

Table 5
Occurrence results from two WWTPs

Compound	*n*	Secondary			Chlorination		
		Maximun	Minimum	Median	Maximum	Minimum	Median
WWTP I							
Ciprofloxacin	4	100	<MDL	100	<MDL	<MDL	NA
Ofloxacin	4	350	<MDL	305	50	<MDL	45
Sulfamethoxazole	4	640	<MDL	575	70	<MDL	60
Trimethoprim	3	1210	30	40	<MDL	<MDL	NA
		Secondary			UV		
WWTP II							
Ciprofloxacin	6	370	80	160	<MDL	<MDL	NA
Ofloxacin	4	260	140	205	210	100	180
Sulfamethoxazole	6	1600	100	395	2140	330	660
Trimethoprim	4	1220	270	705	1760	<MDL	1070

MDL: method detection limit; median: median detectable concentration (samples below the MDL were not included in the median calculation); NA: not available; *n*: number of samples collected between February 2002 and August 2002; the numbers were not the same because different members of antibiotics were included in the analyses.

not found in WWTP II. This observation agrees with the less frequent usage of norfloxacin in the US according to the prescription data [21]. The two veterinary antibiotics, enrofloxacin and sulfamethazine, were non-detectable, consistent with the fact that the two plants received predominantly municipal input. In general, sulfamethoxazole and trimethoprim were detected at higher concentrations than ciprofloxacin and ofloxacin. The median concentrations of sulfamethoxazole, trimethoprim, ciprofloxacin and ofloxacin in the secondary effluent were 395–575, 40–705, 100–160 and 205–305 ng/L, respectively.

Although the occurrence of antibiotics in the secondary effluents at these two plants was comparable, the levels of antibiotics in their tertiary effluents differed considerably. In general, the concentrations of antibiotics were significantly lower in the chlorination effluent (WWTP I) than in the UV disinfection effluent (WWTP II), with the exception of ciprofloxacin, which was non-detectable at both plants. These preliminary data suggest that chlorine may eliminate the antibiotics more efficiently than UV treatment. These results are consistent with bench-scale experiments that illustrate high susceptibility of fluoroquinolones, sulfonamides and trimethoprim to reactions with chlorine [18–20], and low susceptibility of sulfonamides and trimethoprim to photolysis at typical dosages of UV disinfection [18]. Further studies, however, are necessary to confirm this hypothesis and understand the efficiency and mechanism of removal for the antibiotics at full-scale treatment facilities.

4. Conclusions

The study results show that the developed method is robust and sensitive for simultaneous detection and quantification of four fluoroquinolone, two sulfonamide and trimethoprim antibiotics in wastewater matrices at the nanograms-per-liter concentration range. Tandem SPE followed by LC–MS is used to accomplish this task. The internal standard method using lomefloxacin and sulfamerazine as standards works well for the quantification of fluoroquinolones and sulfonamides, respectively. However, standard addition method is necessary for the quantification of trimethoprim due to matrix interferences. An increase in signal suppression and consequently the MDL is seen with increasing UV_{254} absorbance of the sample matrix, indicating increasing amounts of organic matter yield greater interference with analysis. Although this method is demonstrated with seven antibiotics, it can be readily applied to other members of fluoroquinolones and sulfonamides. Applying this developed method to samples from wastewater treatment plants yields important information on the occurrence and fate of antibiotics after wastewater treatment processes.

Acknowledgements

This work was supported by grants from the American Water Works Association Research Foundation. The authors thank the project advisory committee and Dr. David L. Sedlak for comments on this work. Laboratory assistance from Jason Ritchie and James Day is also acknowledged.

References

[1] C.G. Daughton, T.A. Ternes, Environ. Health Perspect. 107 (1999) 907.
[2] D.W. Kolpin, E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber, H.T. Buxton, M.E. Lindsey, Environ. Sci. Technol. 36 (2002) 1202.
[3] M.E. Lindsey, M. Meyer, E.M. Thurman, Anal. Chem. 73 (2001) 4640.

[4] A. Hartmann, A.C. Alder, T. Koller, R.M. Widmer, Environ. Toxicol. Chem. 17 (1998) 377.
[5] R. Hirsch, T. Ternes, K. Haberer, K.-L. Kratz, Sci. Total Environ. 225 (1999) 109.
[6] C. Hartig, T. Storm, M. Jekel, J. Chromatogr. A 854 (1999) 163.
[7] M.T. Meyer, J.E. Bumgarner, J.L. Varns, J.V. Daughtridge, E.M. Thurman, K.A. Hostetler, Sci. Total Environ. 248 (2000) 181.
[8] E.M. Golet, A.C. Alder, A. Hartmann, T.A. Ternes, W. Giger, Anal. Chem. 73 (2001) 3632.
[9] E.M. Golet, A.C. Alder, W. Giger, Environ. Sci. Technol. 36 (2002) 3645.
[10] E.R. Campagnolo, K.R. Johnson, A. Karpati, C.S. Rubin, D.W. Kolpin, M.T. Meyer, J.E. Esteban, R.W. Currier, K. Smith, K.M. Thu, M. McGeehin, Sci. Total Environ. 299 (2002) 89.
[11] A. Al-Almad, F.D. Daschner, K. Kummerer, Arch. Environ. Contam. Toxicol. 37 (1999) 158.
[12] F. Ingerslev, B.H. Sorensen, Environ. Toxicol. Chem. 19 (2000) 2467.
[13] K. Kummerer, A. Al-Ahmad, V. Mersch-Sundermann, Chemosphere 40 (2000) 701.
[14] W. Zhou, D.E. Moore, Int. J. Pharm. 110 (1994) 55–63.
[15] J. Burhenne, M. Ludwig, M. Spiteller, Chemosphere 38 (1999) 1279–1286.
[16] E. Fasani, F.F.B. Negra, M. Mella, S. Monti, A. Albini, J. Org. Chem. 64 (1999) 5388.
[17] M. Mella, E. Fasani, A. Albini, Helv. Chim. Acta 84 (2001) 2508–2519.
[18] C. Adams, Y. Wang, K. Loftin, M. Meyer, J. Environ. Eng. 128 (2002) 253.
[19] M.M. Huber, S. Canonica, G.-Y. Park, U. von Gunten, Environ. Sci. Technol. 37 (2003) 1016.
[20] M.C. Dodd, C.H. Huang, Environ. Sci. Technol. (2004) in press.
[21] IMS Health, http://www.rxlist.com/, accessed October 2003.
[22] M. Haller, S.R. Muller, C.S. McArdell, A.C. Alder, M.J.F. Suter, J. Chromatogr. A 952 (2002) 111.
[23] R. Hirsch, T.A. Ternes, K. Haberer, A. Mehlich, F. Ballwanz, K.L. Kratz, J. Chromatogr. A 815 (1998) 213.
[24] D.L. Ross, C.M. Riley, Int. J. Pharm. 83 (1992) 267.
[25] D. Barrón, E. Jiménez-Lozano, J. Cano, J. Barbosa, J. Chromatogr. B 759 (2001) 73.
[26] C.-E. Lin, C.-C. Chang, W.-C. Lin, J. Chromatogr. A 768 (1997) 105.
[27] J. Cao, R.F. Cross, J. Chromatogr. A 695 (1995) 297.
[28] B. Roth, J.Z. Strelitz, J. Org. Chem. 34 (1969) 821.
[29] H.C.H. Lutzhoft, W.H.J. Vaes, A.P. Fredig, B. Halling-Sorensen, J.L.M. Hermens, Environ. Sci. Technol. 34 (2000) 4989.
[30] P. Schmitt-Kopplin, J. Burhenne, D. Freitag, M. Spiteller, A. Kettrup, J. Chromatogr. A 837 (1999) 253.
[31] C.G. Enke, Anal. Chem. 69 (1997) 4885.
[32] A.P. Bruins, J. Chromatogr. A 794 (1998) 345.
[33] R.J.C.A. Steen, A.C. Hogenboom, P.E.G. Leonards, R.A.L. Peerboom, W.L. Confino, U.A.T. Brinkman, J. Chromatogr. A 857 (1999) 157.
[34] L.S. Clesceri, A.E. Greenberg, A.D. Eaton, Standard Methods for the Examination of Water and Wastewater, APHA, AWWA, WEF, Baltimore, 1998.
[35] L. Tang, P. Kebarle, Anal. Chem. 65 (1993) 3654.
[36] Chapter 40, Code of Federal Regulations. Part 136, Appendix B. 49 Federal Register 43234, 1984.