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Simultaneous extraction and analysis of 11 tetracycline and sulfonamide antibiotics in influent and effluent domestic wastewater by solid-phase extraction and liquid chromatography-electrospray ionization tandem mass spectrometry

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Abstract

Wastewater treatment plants (WWTPs) in which antibiotic compounds are not totally eliminated are considered to be point sources of antibiotic contamination in surface and ground waters. Therefore, there is a need for sensitive and reliable analytical methods for measuring these compounds in WWTP water matrices. This paper describes a simultaneous method for the determination of six tetracyclines (TCs) (oxytetracycline (OTC), tetracycline (TC), demeclocycline (DMC), chlortetracycline (CTC), doxycycline (DXC), meclocycline (MCC)) and five sulfonamides (SAs) (sulfathiazole (STZ), sulfamethazine (SMT), sulfachloropyridazine (SCP), sulfamethoxazole (SMX) and sulfadimethoxine (SDM)) using solid-phase extraction followed by liquid chromatography-ion trap tandem mass spectrometry. The average recovery of 11 antibiotics for simultaneous extraction was 83.3 ± 12.6 and 89.8 ± 11.5% for six TCs, and 95.2 ± 11.4 and 97.7 ± 10.6% for five SAs in the influent and effluent water, respectively. Matrix effects were found to be significant when measuring TCs but not SAs. The accuracy and day-to-day variation of the method fell within an acceptable range of 15% absolute. Method detection limits in wastewater matrices were between 0.03 and 0.07 μg/L. For the investigated 11 antibiotic compounds TC, DMC, CTC, DXC, SMT, SMX and SDM were found in the influents with a concentration range of 0.05–1.09 μg/L. CTC, DXC and SMX were also detected in the effluents with a concentration range of 0.06–0.21 μg/L. These results were compared with those in WWTP effluents of Canada, Germany and Switzerland.

Keywords: Antibiotics; Tetracyclines; Sulfonamides; Wastewater; WWTPs

1. Introduction

Tetracyclines (TCs) and sulfonamides (SAs) are widely used antibiotics in today's human and veterinary medicine practice. TCs (e.g. oxytetracycline (OTC), tetracycline (TC), demeclocycline (DMC), chlortetracycline (CTC), doxycycline (DXC), meclocycline (MCC)) are broad-spectrum bacteriostatic agents active against Gram-positive and Gram-negative bacteria that act by inhibiting protein synthesis. Their basic structures consist of a hydronaphthacene backbone containing four fused rings (Fig. 1). The various analogues differ primarily by substitutions of the fifth, sixth or seventh position on the backbone (Fig. 1). SAs (e.g. sulfathiazole (STZ), sulfamethazine (SMT), sulfachloropyridazine (SCP), sulfamethoxazole (SMX) and sulfadimethoxine (SDM)) are N-substituted derivatives of the substance sulfanilamide and compete with *p*-aminobenzoic acid in enzymatic synthesis of dihydrofolic acid (Fig. 1). This leads to a decreased availability of the reduced folates that are essential in the synthesis of nucleic acids. TCs in human medicine are continuing to be useful in treating a broad range of infections, including malaria and SAs are routinely used to treat human infection such as bronchitis, urinary tract and ear infections [1]. These compounds have been widely used both for prevention and treatment of disease and as feed additives

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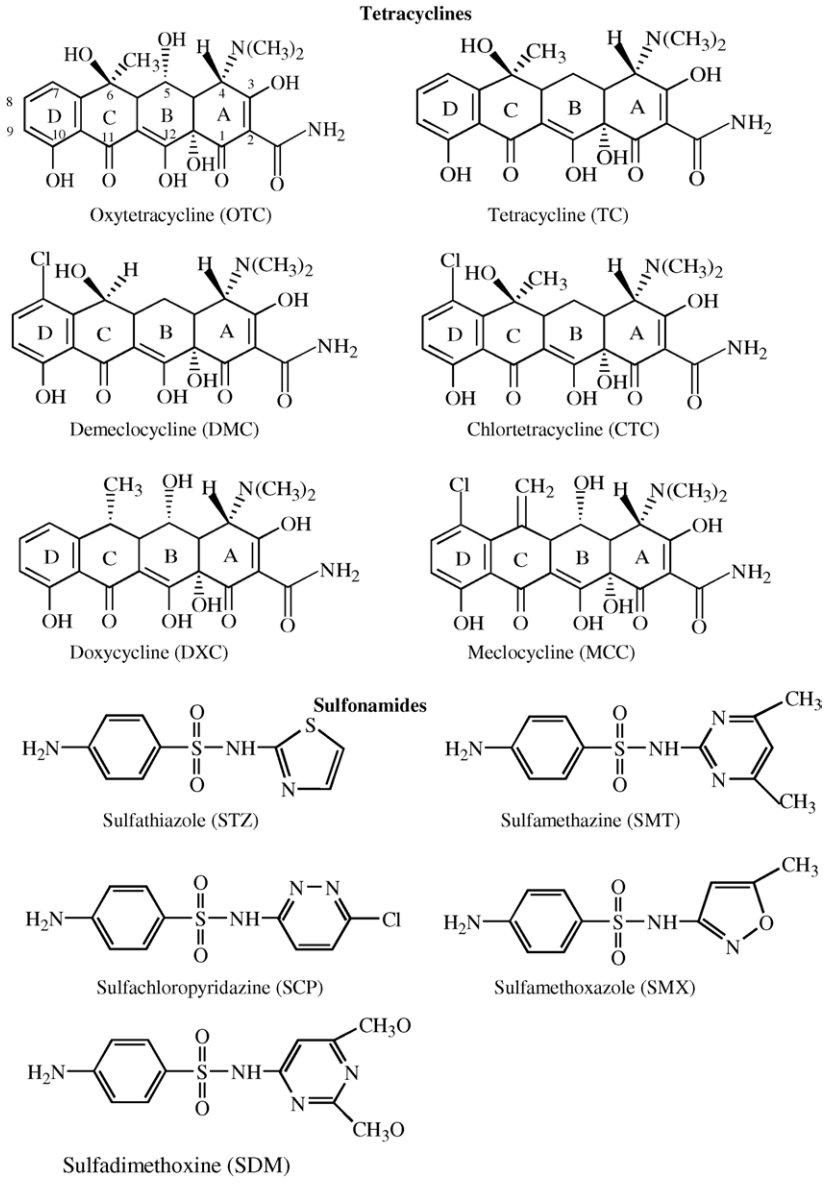

Fig. 1. Chemical structures of tetracyclines and sulfonamides.

to promote growth in animal feeding operations (AFOs) and concentrated animal feeding operations (CAFOs) [2].

A high percentage of antibiotics consumed by humans are ultimately excreted unchanged via urine and feces into domestic sewage, and are discharged to wastewater treatment plants (WWTPs). In WWTPs, these compounds are only partially eliminated and there is the potential for residues of antibiotics to be released in WWTP effluent into the aquatic environment. SMX has been found in WWTP effluents of Germany with a maximum concentration of 2.0 μg/L [3]. Miao et al. [4] measured maximum concentrations of 0.98 and 0.87 μg/L for TC and SMX in WWTP effluents of Canada. Researchers have shown that several classes of antibiotics (e.g. TCs and SAs) are present in hog waste lagoons at concentrations as high as 0.7 mg/L [5]. The U.S. Geological Survey reported that 95 organic wastewater contaminants containing antibiotics were found in 80% of the 139 streams sampled during 1999 and 2000 [6]. Antibiotic concentrations as high as 1.9 μg/L were found with the frequency (22%) of detection of at least one antibiotic in the 84–104 streams sampled and only 10 of 24 antibiotic compounds measured were not detected in any of the streams. Other studies by our research group have reported a substantial increase of TCs, SAs, macrolides and ionophore antibiotics along the flow path of the Cache La Poudre River in northern Colorado that is influenced by WWTP effluents and agricultural landscapes [7–9].

WWTPs are considered to be point sources of antibiotic contamination in surface and ground waters. Concerns have been raised regarding public health issues over the occurrence of antibiotics in the aquatic environment, and the potential exists for proliferation of resistant bacteria in

WWTP effluents [4,10,11]. To predict the concentrations of these antibiotic compounds in the aquatic environment and to design strategies to minimize exposure to these compounds, there is a need for sensitive and reliable analytical methods to measure concentrations of TCs and SAs in the influent and effluent wastewater of WWTPs.

Numerous methods for analytical determination of one or more of TCs and/or SAs in environmental matrices (e.g. natural and waste water, soil, manure) have been reported in the literature [3,4,6,7,12–17]. Liquid chromatography–mass spectrometry (LC–MS) [6,12,13] or LC–MS–MS [3,4,7,14–17] has been used in the analysis of antibiotics because of its high sensitivity and ability to provide compound confirmation. Researchers have varied methodological approaches for a variety of matrices and site-specific equipment. Most analytical methods for TCs and SAs in evironmental matrices are for single or triple quadrupole mass spectromers [3,4,6,12,13,16,17], although some research has been conducted with ion trap tandem mass spectrometers [7,14,15]. In addition, most of these analytical methods are for each class of antibiotics (e.g. TCs, SAs).

Some researchers have developed simultaneous analytical methods coupled with simultaneous extraction as a sample preparation for two or more antibiotic classes in the environmental matrices. For example, Hamscher et al. [15] reported simultaneous LC–MS–MS method for OTC, TC, CTC and tylosin in soils and manures. For TCs and SAs in WWTPs, Renew and Huang [12] reported a simultaneous LC–MS method for SAs (sulfamerazine (SMR), SMT, SMX), trimethoprim and fluoroquinolones in secondary and final effluent, followed by a LC–MS–MS method for SAs (STZ, SMR, SMT, SMX) and macrolides in primary, secondary and final effluent by Gobel et al. [17]. A challenge is presented in the simultaneous extraction and analysis of multiple classes of compounds due to the wide range of polarities, solubilities, pK_{as} and others under the acidic and basic conditions. Analytical methods for quantifying these compounds in WWTP influent wastewater are complex and not well defined. No study has been conducted on simultaneous analytical methods for multiple TCs and SAs in WWTP influent wastewaters.

To investigate the occurrence of six TCs (OTC, TC, DMC, CTC, DXC, MCC) and five SAs (STZ, SMT, SCP, SMX, SDM) in the influent and effluent water of a WWTP, a simultaneous analytical method for 11 TC and SA compounds was developed using simultaneous solid-phase extraction (SPE) followed by ion trap tandem LC–MS–MS with positive ion electrospray ionization, ESI (+) and selected reaction monitoring (SRM). This paper details a sensitive and reliable analytical method for the determination of six TCs and five SAs in the WWTP influents and effluents. Several product ions for MS–MS detection have been identified and the different product ions in an ion trap and a triple quadrupole LC–MS–MS are compared. This paper describes the procedures for optimizing mass peak detection and integration of analyte for exact quantitation in SRM using two different manual and autofilter modes in the Qual Browser window of Xcalibur software employed in ion trap tandem mass spectrometry. The paper also describes the impact of matrix effects when measuring these compounds at environmentally relevant concentrations and statistical analysis for determination of the method detection limit (MDL), accuracy and precision of the method is shown. Finally, antibiotic compounds found in the WWTP effluents of this study are compared with those in Canada, Germany and Switzerland.

2. Experimental

2.1. Materials and reagents

All antibiotics (purity, 95–99%), citric acid (purity, 99%) and Na_2EDTA (purity, 99%) were obtained from Sigma–Aldrich (St. Louis, MO). Simatone, the internal standard (1000 mg/L in methanol) was purchased from Absolute Standards Inc. (Hamden, CT). Stock solutions of the standards were prepared by dissolving each compound in methanol at a concentration of 100 mg/L and stored at −20 °C in the dark. Fresh stock solution was prepared monthly. Working solutions (10.0, 5.0, 1.0 and 0.1 mg/L) were prepared fresh weekly by diluting the stock solution with deionized water and stored at 4 °C in the dark. Internal standard working solutions (0.3 mg/L) were prepared by diluting the standard solution with deionized water, stored at 4 °C, and replaced with a fresh solution each week.

2.2. Description of wastewater treatment plant

Drake Water Reclamation Facility (DWRF) is the WWTP that is studied in this paper. The plant serves a population of approximately 125,000 in Fort Collins, Colorado. It treats 45,000–50,000 m^3/d of domestic (95%) and industrial sewage (5%) by pretreatment, primary clarification, intermediate clarification, secondary clarification and chlorine disinfection.

2.3. Sample collection and preparation

Twenty four-hour composite samples of raw influent and final effluent were collected from the DWRF twice a month over a period of 8 months from 1 March 2004 to 31 October 2004. Sampling was carried out by a flow proportioned automatic sampler, whereby the 24-h composite samples of the final effluent were collected time-related to the influent. The samples of triplicates collected twice a month during 8 months were a minimum of 48 influents and effluents each. The influent water samples were centrifuged at 3000 rpm for 40 min at 4 °C in a centrifuge (IEC Centra CL 3R, MA, USA) with a cooling system. All influent and effluent water samples were filtered through 0.4-μm glass fiber filters (Millipore, MA) and stored at 4 °C in refrigerators until they were extracted, typically within 2 days to minimize microbial degradation.

2.4. Solid-phase extraction

Water samples were prepared for extraction by adding 1.0 mL of 5% Na_2EDTA to a flask containing 120 mL of water and 30 mL of 0.1 M citric acid. For controls and calibration curves, appropriate amounts of the working solution containing each of 11 analytes were added to the water samples including deionized water. To test the behavior of TCs and SAs spiked into the DWRF influent and effluent water matrix, several DWRF samples were analyzed using this method (TCs and SAs). Influent and effluent samples of DWRF water containing the minimum concentrations of CTC and SMX were used as the matrix.

Each 120 mL sample was extracted through a 60 mg/3 mL Oasis HLB cartridge (Waters, Millford, MA). Cartridges were preconditioned with 3 mL of MeOH, 3 mL of 0.5N HCl and 3 mL of deionized water. Water samples then were passed through the cartridges at a flow rate of approximately 5 mL/min on a vacuum manifold (PrepSep 12 port, Fisher scientific, PA). Extraction using the cartridges was performed with the sample pH adjusted with 40% H_2SO_4 to <3.0 immediately prior to extraction because extraction at the sample pH adjusted below the pK_a (3.3–9.5 for six TCs and 2.5–7.5 for five SAs) increases retention on the SPE cartridges [7,13,18]. SAs were also extracted by the HLB cartridges at pH < 3.0 to provide a simultaneous SPE method for TCs and SAs.

After isolation, cartridges were rinsed with 3 mL of deionized water. The analytes were eluted with 5 mL of MeOH into a test tube containing 12 ng of the internal standard. The extracts were concentrated under a flow of N_2 gas to about 50 μL using a nitrogen evaporation system (N-Evap, Organermation Associates Inc., MA). To this, 70 μL of mobile phase A was added. The resulting solutions were transferred to 0.5 mL amber autosampler vials to prevent photodegradation of TCs and SAs. For determination of recovery during the SPE procedure, appropriate amounts of TCs and SAs were spiked in 120 mL of deionized water, influent or effluent matrix before extraction and in 5 mL extracts after extraction. The concentrations were measured with the LC–MS–MS method developed in this study. SPE and measurement were performed on the same day since the solubility of the extracted TCs and SAs during freezing and thawing was variable.

2.5. Liquid chromatography and mass spectrometry

The LC system was a HP 1100 LC (Agilent, Palo Alto, CA) equipped with a cooled autosampler (4 °C). TCs and SAs were separated using a 2.1 mm × 50 mm Xterra MS C_{18} column with a 2.5 μm pore size (Waters, Millford, MA) in combination with a guard column of the same type (2.1 mm × 4 mm) from Phenomenex Inc. (Torrance, CA). Column temperature was 15 °C. 0.1% formic acid in water (mobile phase A) and acetonitrile (mobile phase B) was used to produce a multistep binary elution gradient with a flow rate of 0.35 mL/min. Simultaneous separations of TCs and SAs were achieved with the following mobile phase gradient program: at 0 min A/B = 100:0, 25 min A/B = 89:11, 29 min A/B = 85.5:14.5, 49 min A/B = 78:22 and 50 min A/B = 100:0. The investigated TCs and SAs eluted within 50 min. A 10-min post time allowed re-equilibration of the column. The injection volume was 40 μL.

All mass spectrometric measurements were performed on a Finnigan LCQ Duo ion trap mass spectrometer (ThermoQuest, CA) equipped with a heated capillary interface and an electrospray ionization (ESI) source. The instrument was operated in the positive ion mode and coupled to the outlet of the LC column via PEEK tubing. ThermoQuest Xcalibur software was employed to control the mass spectrometric conditions and quantify TCs and SAs. Full scan MS–MS mode was used to acquire full scan MS–MS spectra, to select precursor ions, and then to record product ions from standard solution of TCs and SAs on the mass spectrometer with ESI (+) source. Infusion into the ion trap tandem mass spectrometer was performed as follows: the flow of standard compounds (3 mg/L) coming from an integrated syringe pump at a flow rate of 5 μL/min was mixed with mobile phases A/B at a 80:20 ratio through a T-piece for tuning the mass spectrometer and optimizing the ESI source. The ESI source and MS–MS parameters were automatically optimized and saved in a tune file. Spray needle voltage was set at 4.5 kV for all applications, automatic gain control (AGC) was on, maximum isolation time was 300 ms and three microscans per scan were acquired. Voltages on capillary and tube lens were 29 and 10 V, respectively. These were set by automatic optimization using the LCQ autotune program on the mass spectrometer instrument. Nitrogen was used as a sheath and auxiliary gas. Helium was used as the collision gas in the ion trap. The optimized tune conditions were as follows: sheath gas flow rate was set at 40 units (a scale of arbitrary units in the 0–100 range defined for the LCQ system), the auxiliary gas was turned off, and capillary temperature was 188 °C. MS–MS parameters for TCs and SAs including their precursor and product ions, collision energy and isolation width (m/z) are summarized in Table 1.

2.6. Quantitation

The product ion producing the highest intensity was used for SRM and quantitation to increase analytical sensitivity and selectivity in the LC–MS–MS mode. For the internal standard, the protonated molecular ion $[M + H]^+$ was chosen for SIM. For SRM, the product ion of the highest intensity for the investigated TCs and SAs is reported in boldface in Table 1. Quantitation was based on a detector response defined as the ratio of the base peak ion (the specific product ion of interest) to the base peak ion of the internal standard. Calibration curves constructed for TCs and SAs spiked into water samples before extraction ranged from 0.05 to 5 μg/L in deionized water, WWTP influent and effluent. Because the DWRF influent and effluent water when used as a matrix already contained CTC and SMX, calibration curves for these

Table 1
MS–MS parameters for analysis of tetracyclines and sulfonamides

	Simatone[a]	OTC	TC	DMC	CTC	DXC	MCC	STZ	SMT	SCP	SMX	SDM
Nominal molecular mass (Da)	197	460	444	464	478	444	476	255	278	284	253	310
Isolation width (*m/z*)		1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Normalized collision energy (%)		23	24	25	26	27	27	32	35	36	36	38
Precursor ions, $[M+H]^+$, (*m/z*)	198	461	445	465	479	445	477	256	279	285	254	311
Product ions (*m/z*)												
(Relative abundance, %)												
$[M+H-NH_3]^+$		444 (10.6)	428 (12.6)	**448**[d] (100)	**462**[e] (100)	**428**[f] (100)	**460**[g] (100)					
$[M+H-H_2O]^+$		**443**[b] (100)	**427**[c] (100)	430 (11.5)	461 (30.2)							
$[M+H-NH_3-H_2O]^+$		426 (20.2)	410 (16.9)		444 (22.5)							
$[M-RNH_2]^+$								**156**[h] (100)	156 (4.4)	**156**[i] (100)	156 (40.3)	**156**[j] (100)
$[M-RNH_2-SO]^+$								108 (6.0)		108 (14.1)	108 (7.4)	108 (9.2)
$[M-RNH_2-SO_2]^+$								92 (2.4)	92 (4.7)	92 (4.3)	92 (3.8)	92 (3.6)
$[RNH_2+2H]^+$									124 (6.9)			
$[RNH_2+SO_2]^+$								163 (1.4)	186 (13.9)			218 (32.4)
$[RNH_2+SO_2+H_2O]^+$								189 (3.0)	**204**[i] (100)			
$[M+H-H_2SO_2]^+$											**188**[k] (100)	245 (39.3)

[a] Protonated molecular ion, $[M+H]^+$, *m/z* of simatone, internal standard in SIM is 198.
[b–l] Product ions (*m/z*) of the highest intensity for SRM and quantitation are reported in boldface. R in product ions represents N-substituted derivatives of sulfonamides (see Fig. 1).

antibiotics in these matrices were constructed by subtracting the initial concentration from the spiked concentration. Concentrations of TCs and SAs in the DWRF influent and effluent water samples were determined reproducibly by using the standard calibration curves for the influent and effluent samples used as the matrix.

2.7. Method detection limit, accuracy and precision

The method detection limit (MDL) was determined by analyzing seven influent and effluent extracts each spiked at 0.2 μg/L of TCs and SAs. MDL determination in this study was based on the US EPA method using the variability of multiple analyses for these extracts [19]. To assess the accuracy and day-to-day variation of the method, aliquots of six influent and effluent samples spiked with 0.1, 1.0 or 2.0 μg/L of TCs and SAs were extracted to obtain independent replicates of the two wastewater matrices. These six replicates were all run ($n = 6$) on three different days.

3. Results and discussion

3.1. Liquid chromatography and mass spectrometry

The biggest consideration when analyzing multiple analytes simultaneously is to have adequate chromatographic separation for each analyte such that more actual data points (DPs) depending on the extent of mass peak separation quality can be used to determine the peak area (AA) of analyte for quantitation. To improve mass peak separation quality of analytes, TCs and SAs were separated at different volumetric flow rates with different mobile phase gradient programs during development of the analytical method. For example, 11 antibiotic compounds of TCs and SAs were separated at 0.32 mL/min with a mobile phase gradient program: at 0 min A/B = 98:2, 49 min = 70:30 and 50 min A/B = 98:2 (Fig. 2 (A)), indicating that SMT, simatone, SCP and DMC were not separated adequately. This result indicates that a higher aqueous percentage in the mobile phase is needed to increase separation efficiency for the more polar SMT, SCP and DMC compounds. To increase separation quality of these four compounds, TCs and SAs were separated at a higher aqueous percentage in the mobile phase and a flow rate (0.35 mL/min) using a mobile phase gradient: at 0 min A/B = 100:0, 25 min A/B = 89:11, 29 min A/B = 85.5:14.5, 49 min A/B = 78:22 and 50 min A/B = 100:0 (Fig. 2 (B)). As shown in the TICs for TCs and SAs (Fig. 2 (B)), SMT, simatone, SCP and DMC exhibited higher quality separation at the higher aqueous percentage in the mobile phase compared to those at the lower value (Fig. 2 (A)). These results indicate the more polar TCs (OTC, TC, DMC) and SAs (STZ, SMT, SCP), which possess rapid elution times necessitate the use of a higher aqueous percentage in the mobile phase to obtain good chromatographic peak resolution.

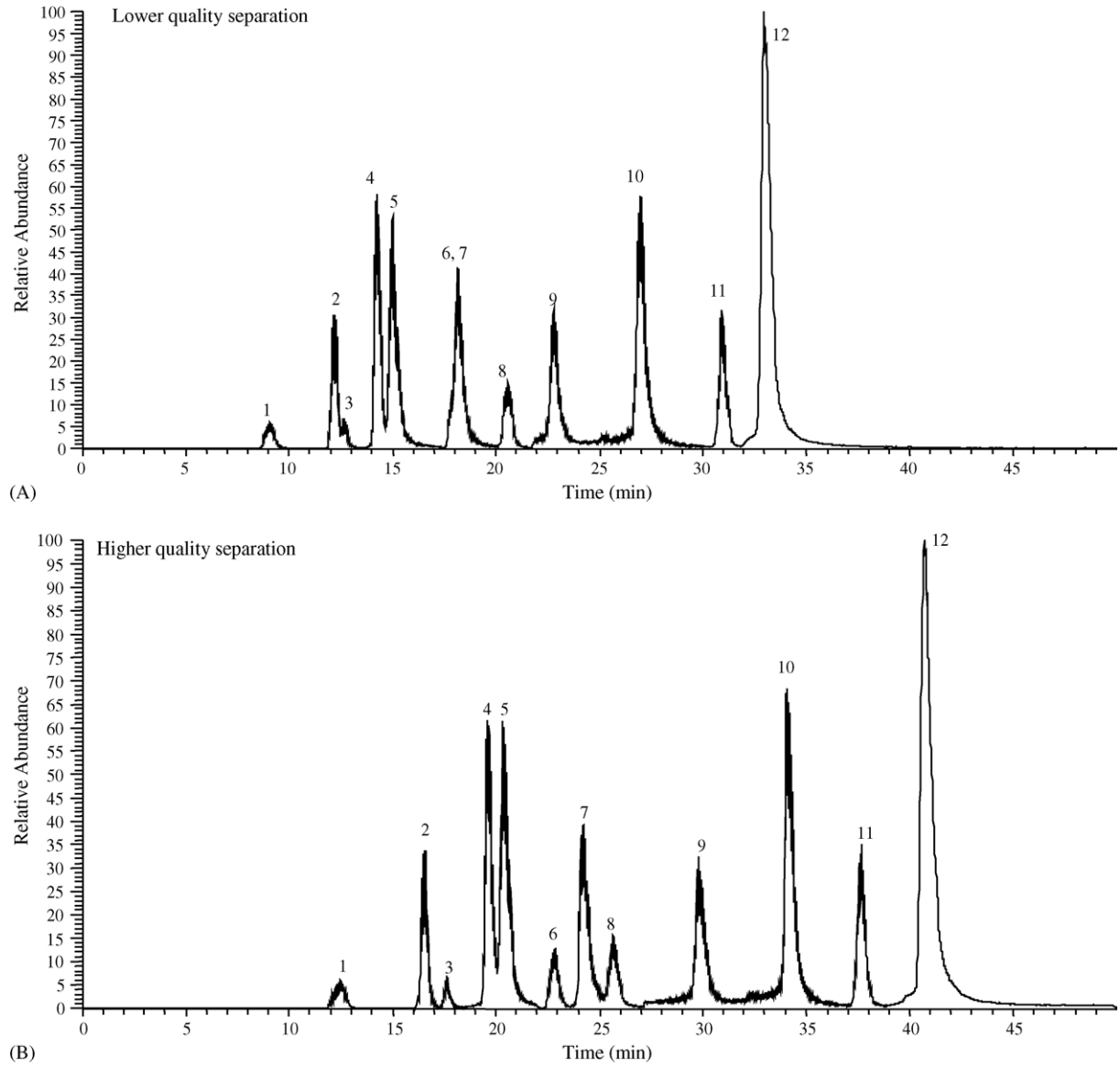

Fig. 2. Total-ion chromatograms (TICs) for tetracyclines and sulfonamides of 4 μg/L by varying flow rate and mobile phase gradient. Simatone was spiked at 0.1 μg/L. (1) STZ, (2) SMT, (3) Simatine, (4) OTC, (5) TC, (6) SCP, (7) DMC, (8) SMX, (9) CTC, (10) DXC, (11) SDM and (12) MCC.

A problem encountered in chromatography with TCs is that CTC and DXC are eluted as long and broad mass peaks at short retention times. The extent of this undesired effect depends on the type of LC column used and the chromatographic conditions selected, especially the temperature at which the LC column operates. Another challenge with quantifying TCs is the formation of epimers (e.g., e-OTC, e-TC, e-CTC, e-DMC and e-DXC) as a function of sample pH [20]. These metabolites are thermally labile. Another study by the authors [7] fully reported the effects of LC column temperature (15, 25 and 35 °C) on the mass peak quality for TCs. Briefly, the decreased temperature (15 °C) resulted in better mass peak symmetry for CTC and DXC as compared to the increased temperatures (25 and 35 °C). CTC and DXC at these higher temperatures exhibited anomalous peak distortions due to chemical conversion processes (e.g. tautomerization and epimerization of CTC and DXC), potentially catalyzed by residual silanol groups [7,21–23]. For the other investigated TCs, no effect on mass peaks was observed using the three LC column temperatures [7]. Thus, the LC column temperature in this study was also maintained at 15 °C to minimize undesired isomer production and peak distortion for CTC and DXC (Fig. 2).

3.2. Fragmentation of TCs and SAs in the ion trap tandem mass spectrometer

Mass spectra and product ions for six TCs and five SAs were clearly observed in the full scan mode of the ion trap tandem mass spectrometer. Another study by the authors [7]

fully reported full scan MS–MS spectra for TCs and SAs with an ESI (+) source. Precursor ions and product ions with relative abundance (%) for TCs and SAs including collision energy (%) and isolation width (*m*/*z*) are listed in Table 1. Each of the TCs and SAs exhibited characteristic fragmentation with the ESI (+) source and the precursor ion observed for all analytes was $[M + H]^+$.

All of six TCs exhibited product ions corresponding to $[M + H–NH_3]^+$ due to the loss of NH_3 (17 Da) during fragmentation (Table 1). Hamscher et al. [15] reported the product ion for OTC, TC and CTC in an ion trap MS–MS, and Hirsch et al. [24] reported the product ion for CTC and DXC in a triple quadrupole MS–MS. OTC, TC and CTC exhibited neutral losses of 17 and 35 Da corresponding to the loss of NH_3, $[M + H–NH_3]$, with the subsequent loss of H_2O (18 Da), $[M + H–NH_3–H_2O]^+$. Both of these losses agree with the findings of other research groups [13–15,23]. The product ion, $[M + H–NH_3–H_2O]^+$ for OTC, TC and CTC in this study is also consistent with the findings of Hamscher et al. [15] and Hirsch et al. [24]. DXC and MCC exhibited only the loss of 17 Da corresponding to $[M + H–NH_3]$. Fragmentation of OTC, TC, DMC and CTC with ion trap MS–MS in this study also produced $[M + H–H_2O]^+$ due to the loss of 18 Da without the loss of 17 Da. The results of DMC and DXC in this study are in contrast to the findings of Zhu et al. [14] and Hirsch et al. [24], who reported $[M + H–NH_3–H_2O]^+$ for DMC or DXC. These results indicate that specific ions for each analyte may vary according to the ion trap MS–MS or triple quadrupole MS–MS mode.

After fragmentation, each of the five SAs exhibited the 156 ion and the 92 ion corresponding to $[M–RNH_2]^+$ and $[M–RNH_2–SO_2]^+$. All of the SAs except SMT exhibited the 108 ion corresponding to $[M–RNH_2–SO]^+$. Fragmentation of SMT produced the 124 ion, the 186 ion and the 204 ion corresponding to $[RNH_2 + 2H]^+$, $[RNH_2 + SO_2]^+$ and $[RNH_2 + SO_2 + H_2O]^+$ due to the loss of 155 and 93 Da, and the subsequent gain of 18 Da for $[RNH_2 + SO_2]^+$. The 204 ion in the ion trap MS–MS (this study) is in contrast to the findings of Hirsch et al. [24] who reported only the 186 ion and the 124 ion in a triple quadrupole MS–MS. Fragmentation of SMX and SDM produced the loss of 66 Da corresponding to the loss of H_2SO_2, $[M + H–H_2SO_2]^+$. SMX exhibited both the 108 ion and 92 ion in the ion trap (this study) and triple quadrupole MS–MS [24,25]. The 156 ion and 188 ion of SMX in this study are in contrast to the findings of Hirsch et al. [24] who reported only the 108 and 92 ion in a triple quadrupole MS–MS. The 156 ion and 188 ion of SMX agrees with those in a triple quadrupole MS–MS reported by Verzegnassi et al. [25]. In addition, the 190 ion of SMX in a triple quadrupole MS–MS [24] was not observed in the ion trap MS–MS (this study). STZ, SMT and SDM exhibited the product ion corresponding to $[RNH_2 + SO_2]^+$ due to the loss of 93 Da. All of the product ions of SDM observed in ion trap MS–MS (this study) agree with those in a triple quadrupole MS–MS reported by Verzegnassi et al. [25].

3.3. Optimization of mass peak detection and integration of analyte for quantitation using a LCQ Duo ion trap tandem mass spectrometry with SRM

For LC–MS–MS with SRM the starting point for preparing the mass chromatographic plot of an analyte, adjusting the data display and then following with data measurements (e.g. peak height (AH), peak area (AA), signal-to-noise ratio (SN)) is to use the 'manual mode' and/or 'autofilter mode' of the Qual Brower window in the Xcalibur software employed in the ion trap tandem mass spectrometry. The manual mode requires analysts to set both the scan filter operation (mass spectrometer mode of acquisition specified to scan product ions exhibited by a precursor ion) for precursor ion (*m*/*z*) and the mass range (*m*/*z*) of specific product ions of the analyte in the 'chromatogram ranges' window of Qual Browser. This means that analysts must specify both a specfic *m*/*z* ion as well as the scan filter in either mode when optimizing mass peak detection and the integration of the analyte for exact quantitation with SRM since the system is potentially more selective when using both of these parameters together when selecting the mass chromatographic trace for the analyte. An erratic performance of each mode and/or both modes prevents analysts from optimizing mass peak detection and the integration process of the analyte for exact quantitation. Therefore, this study evaluated deviations of the data display and the integration process through each mode and/or both modes using the Xcalibur raw data files.

Fig. 3 shows the results of the data display and integration process performed in different options for setting the manual or autofilter mode to determine the CTC concentration in the DWRF influent. Fig. 3(A) represents mass peak and data sets of CTC obtained from an option of the manual mode that sets only the specific product ion at *m*/*z* 462.0 in the 'chromatogram ranges' window. In other words, only the product ion (*m*/*z*) of CTC was specified for quantitation of CTC, but not scan filter for the precursor ion at *m*/*z* 479.1, indicating that this option attempts to show the presence of the specified product ion in any scan filter available since an analyst specified only the specific product ion at *m*/*z* 462.0 and not a scan filter for CTC. This implies that any data from different scan events of interest would be displayed and calculated in this option (e.g. Fig. 3(A)), especially when acquiring data with multiple scan events for multiple compounds of interest. This could result in too large of a signal and become a problem.

For the other option of the manual mode, setting only the scan filter for the precursor ion of CTC but not the specific *m*/*z* ion, the resulting chromatographic plot would include any other background ion that also exhibited the precursor ion at *m*/*z* 479.1 of CTC that could have been isolated in the first stage of MS–MS. This would also be a problem, and could also make the observed signal too large.

Fig. 3(B) represents mass peak and data sets of CTC obtained from another option of the manual mode that set both the specific product ion at *m*/*z* 462.0 and the scan filter

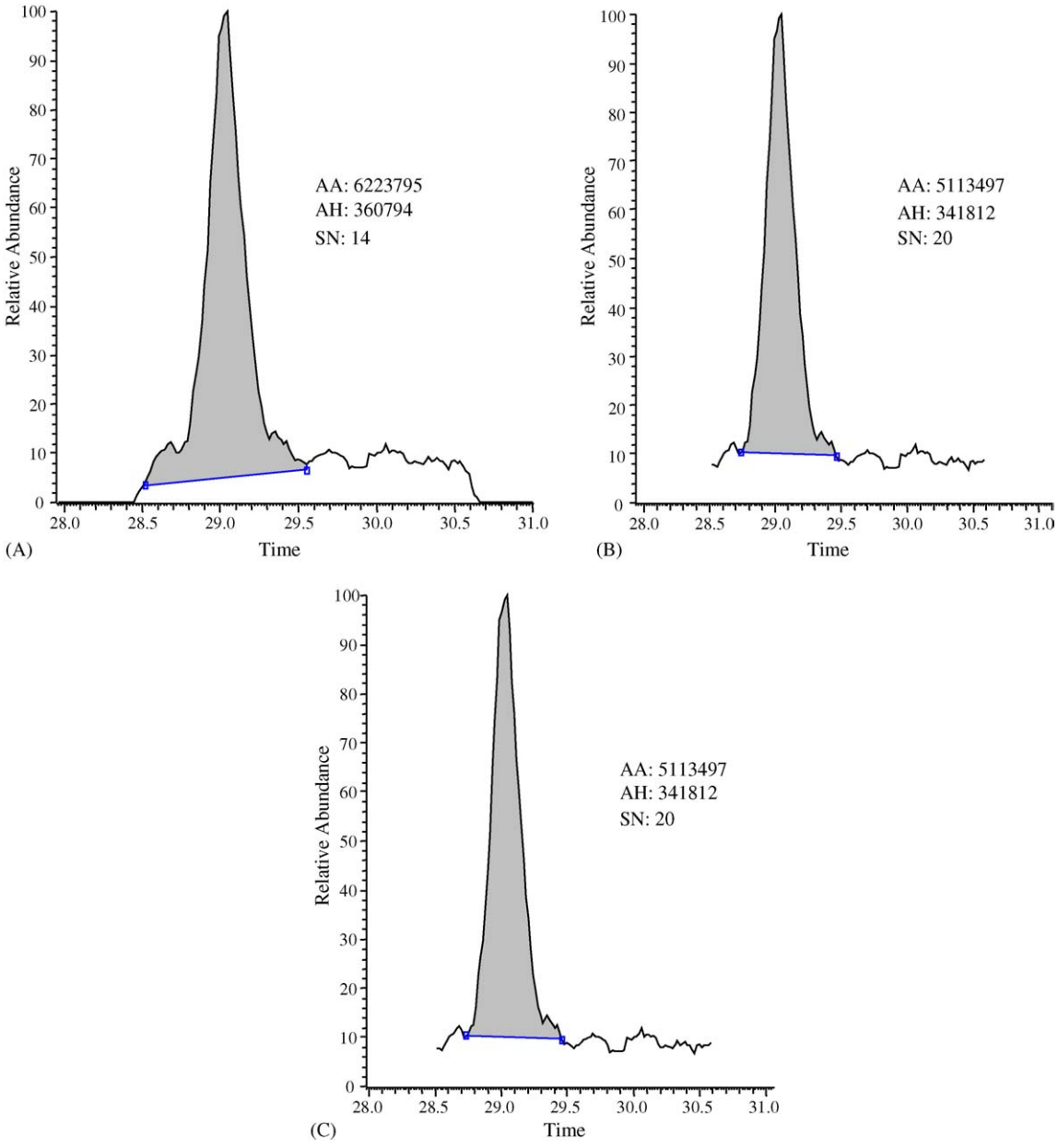

Fig. 3. Deviations of data displays for CTC in WWTP influent from both of 'manual mode' and 'autofilter mode' used in optimizing peak detection and integration process of an analyte for quantation using a LCQ Due ion trap mass spectrometry. (A) Manual mode (the setting of only the specific product ion (*m*/*z*)). (B) Manual mode (the setting of the specific product ion (*m*/*z*) and scan filter for precursor ion (*m*/*z*)). (C) Autofilter mode (the setting of the specific product ion (*m*/*z*) and scan filter for precursor ion (*m*/*z*)). AA: peak area; AH: peak height; SN: signal-to-noise ratio.

for precursor ions at *m*/*z* 479.1. In this option, the scan filter for the precursor ion and the specific product ion of CTC were specified, indicating that any chance of data from the other scan event (which used a different precursor ion and thus has a different scan filter) affecting the results was eliminated. The possibility of having any matrix signal that may also have the precursor ion at *m*/*z* 479.1 of CTC from affecting the results was also reduced. The major difference between the plots is that the CTC plot (Fig. 3(B)) only shows the data points for the specific scan event filtered by an analyst, but the CTC plot (Fig. 3(A)) shows the presence of the specified product ion (*m*/*z*) in any scan filter available. Thus, peak area and peak height of CTC in Fig. 3(A) are greater but SN is lower than those in Fig. 3(B). A value (6,223,795) of peak area displayed in the mode option of Fig. 3(A) was overestimated by 22.0% relative to the 5,113,497 displayed in the mode option of Fig. 3(B). These results indicate that if either the scan filter for the precursor ion or the specific *m*/*z* ion is not set appropriately, the data displayed and processed will not be representative of the data for only that single analyte (e.g. CTC). Thus, the mass chromatographic plot (Fig. 3(B)) from the mode option setting the specific precursor-product

ion pair must be used in quantifying the analyte (e.g. CTC) with SRM accurately.

Fig. 3(C) represents mass peak and data sets of CTC obtained from autofilter mode that set both the specific product ion at *m*/*z* 462.0 and the scan filter for precursor ions at *m*/*z* 479.1, indicating that the results are identical to those of the manual mode (Fig. 3(B)), since the scan filter and the specific product ion for CTC were specified. In other words, regardless of which mode an analyst starts with, manual or autofilter mode, an analyst will get to the same data display and processed results when both the scan filter and the specific *m*/*z* ion are correctly set in the 'chromatogram ranges' window of Qual Brower. These results indicate that to develop an assay coupled to LCQ Duo ion trap tandem mass spectrometer reproducibly and accurately, the specific precursor-product ion pair in 'manual mode' or 'autofilter mode' must be used to quantitate analytes with SRM.

3.4. Recovery and matrix effects

HLB cartridges were selected for simultaneous extraction of TCs and SAs in the water matrices because they do not contain silanols to which metal ions may bind. TCs can potentially sorb to residual metals in the sample matrix, SPE cartridges and glassware, resulting in irreversible binding lower recovery. Na_2EDTA was utilized in this study to chelate metals that are sufficiently soluble in water and prevent interference with the extraction of TCs. Because citric acid chelates metals and lipids, 30 mL of 0.1 M citric acid was also added to 120 mL of WWTP influent and effluent water samples. Using SPE with only SAs spiked at appropriate amounts in the influent and effluent wastewater matrix (this study) as well as a natural water matrix (previous studies), the addition of HCl, H_2SO_4, Na_2EDTA has been shown by the authors to not affect the extraction efficiency of these compounds implying no pH dependence [7]. Therefore, we extracted SAs in this study with HLB cartridges using the identical conditions discussed above for TCs.

The recoveries of TCs and SAs from the HLB cartridges were measured by extracting analytes from 120 mL of deionized water spiked at 0.05–200.0 μg/L before and after extraction. WWTP influent and effluent water was also spiked at 0.1–5.0 μg/L of analytes before and after extraction. Raw influent and effluent wastewater samples, which showed typical characteristics (e.g. NH_4–N, TOC, BOD, SS) for the DWRF influent and effluent constituents studied in this work, were selected as the reference matrices (Table 2). Recoveries of TCs and SAs for simultaneous SPE were determined using the ratio of the concentration of analyte for extract spiked before extraction to the concentration of analyte for extract spiked after extraction. Because CTC and SMX were detected in the influent and effluent using the developed method, recovery of TCs and SAs in these matrices over a period of 8 months was determined using a concentration calculated by subtracting the level concentration from the spiked concentration.

Table 2
Concentrations of constituents in the raw influent and effluent used as the reference matrices

Constituents	Raw influent	Effluent
NH_4–N (mg/L)	17.3	0.7
TOC (mg/L)	87.9	9.1
BOD (mg/L)	205.6	6.7
SS (mg/L)	208.3	7.8

Recoveries of TCs and SAs are the average of triplicates of 0.05, 0.1, 0.5, 1.0, 2.0, 5.0, 10.0, 25.0, 50.0, 100.0, 150.0 and 200.0 μg/L of TCs and SAs spiked in deionized water before and after extraction, and 0.1, 0.5, 1.0, 1.5, 3.0 and 5.0 μg/L of TCs and SAs spiked in the influent and effluent wastewater before and after extraction. For TCs and SAs (Table 3), the average recovery from all the sample matrices was generally above 80%. No concentration dependence was observed. Another study by the authors reported recoveries of TCs and SAs in deionized water and surface water by individual extraction for TCs or SAs [7]. Recoveries (more than 95%) of TCs and SAs spiked at 0.05–50.0 μg/L in deionizd water by the individual extraction [7] are similar to those in the spiked concentration range (0.05–200.0 μg/L) using simultaneous extraction (Table 3) of this study.

Recoveries of SAs in the influent and effluent were similar to those in deionized water indicating that matrix effects were minimal. Recoveries of SAs in the influent were similar to those in the effluent, indicating that SAs did not exhibit matrix effects in the more complex influent water matrix. These results indicate that HLB cartridges gave reproducible recoveries for SAs and were effective for the isolation of the SAs.

The lower recovery of TCs from WWTP influent and effluent samples relative to deionized water indicates that matrix effects were important due to the presence of organic matter (OM) and/or natural organic matter (NOM) in the WWTP influent and effluent matrices. Because the WWTP influent is a complex mixture of various organic and inorganic substances, the recovery efficiency corresponds to the solubility of these substances (e.g. OM (amines, organic acids), NOM (humic and fulvic acid)) in the solvent used to elute TCs in the SPE cartridges [7,13]. These findings indicate that TCs associate with OM and/or NOM, which may bind to the SPE cartridges irreversibly, and cannot be easily eluted separately. Thus, it is desirable that these organic compounds be separated when analyzing more complex environmental samples (e.g. the WWTP influent). The average recovery of TCs in the effluent was greater (by more than 5%) than that of the influent over a period of 8 months due to a lower total organic carbon (TOC) concentration in this matrix. The TOC concentration in the DWRF effluent ranged from 7 to 10 mg/L compared with a concentration range of 80–100 mg/L in the raw DWRF influent.

Most matrix effects result in suppression or enhancement of the analyte signal [27,28]. Co-eluting, undetected matrix components such as OM and/or NOM in the aqueous matrix

Table 3
Recovery of TCs and SAs in 120 mL of water samples over a period of 8 months

Antibiotics	Recovery, $X \pm$ SD (%)[a]								
	Deionized water (ug/L)			WWTP influent (ug/L)			WWTP effluent (ug/L)		
	0.05–200.0	0.05	1.5	0.1–5.0	0.1	1.5	0.1–3.0	0.1	1.5
TCs									
OTC	94.9 ± 10.4	95.6 ± 9.8	95.2 ± 9.6	79.8 ± 11.7	77.9 ± 11.8	80.6 ± 10.4	87.7 ± 9.4	83.6 ± 10.9	87.1 ± 10.2
TC	94.6 ± 9.9	95.2 ± 10.1	94.9 ± 10.3	84.1 ± 12.6	83.7 ± 13.8	85.9 ± 13.1	91.6 ± 11.8	87.5 ± 12.7	89.9 ± 9.5
DMC	95.1 ± 7.7	94.8 ± 8.4	97.5 ± 8.9	84.3 ± 13.3	84.4 ± 13.1	87.5 ± 13.9	87.4 ± 12.6	83.8 ± 11.5	85.3 ± 12.8
CTC	99.5 ± 9.2	97.3 ± 9.9	101.6 ± 8.5	81.6 ± 10.9	80.5 ± 12.2	81.2 ± 11.6	86.2 ± 10.5	85.9 ± 9.8	85.4 ± 11.7
DXC	98.3 ± 7.2	96.7 ± 8.6	97.2 ± 6.7	86.7 ± 13.8	82.1 ± 12.4	83.4 ± 12.2	90.8 ± 12.4	84.2 ± 12.8	89.1 ± 9.3
MCC	97.7 ± 10.3	100.5 ± 9.4	99.6 ± 7.9	83.4 ± 13.1	85.6 ± 13.9	82.2 ± 12.7	94.9 ± 12.1	93.7 ± 13.4	94.5 ± 12.9
Average recovery	96.8 ± 9.1	96.9 ± 9.2	97.6 ± 8.6	83.3 ± 12.6	82.4 ± 12.9	83.5 ± 12.3	89.8 ± 11.5	86.5 ± 11.9	88.6 ± 11.1
SAs									
STZ	98.7 ± 7.9	96.4 ± 8.6	97.9 ± 8.2	97.7 ± 11.9	96.2 ± 11.1	99.8 ± 12.5	98.5 ± 9.1	96.7 ± 10.9	96.1 ± 11.8
SMT	99.6 ± 6.5	98.5 ± 7.9	99.2 ± 6.8	92.8 ± 12.1	93.4 ± 10.3	93.8 ± 10.8	97.7 ± 12.6	94.2 ± 9.6	92.8 ± 8.3
SCP	100.5 ± 9.6	97.1 ± 8.8	96.6 ± 7.3	91.1 ± 10.5	92.3 ± 12.4	93.5 ± 11.2	93.3 ± 11.5	91.4 ± 10.6	92.9 ± 8.2
SMX	98.8 ± 8.4	96.7 ± 7.5	97.5 ± 8.1	97.4 ± 10.6	94.9 ± 11.8	97.4 ± 9.1	98.9 ± 9.3	96.5 ± 11.6	103.5 ± 8.8
SDM	96.4 ± 7.2	98.9 ± 8.2	98.3 ± 8.9	96.5 ± 11.3	94.1 ± 9.9	96.7 ± 10.4	102.8 ± 8.4	96.9 ± 7.2	95.7 ± 11.5
Average recovery	98.7 ± 8.1	97.5 ± 8.4	98.1 ± 8.2	95.2 ± 11.4	94.1 ± 11.0	96.0 ± 10.6	97.7 ± 10.6	94.8 ± 10.2	95.9 ± 9.8

[a] Recovery and standard deviation (SD) is given ($n = 3$) at the spike concentration levels.

may reduce or enhance the ion intensity of the analytes and affect the reproducibility and accuracy of the assay. Previous studies have reported that matrix effects are ionization mode (ESI or APCI) dependent in a LC–MS or LC–MS–MS assay, indicating ionization suppression in ESI and enhancement in APCI [26,27].

For TCs, this study using LC-ESI–MS–MS with SRM also confirmed suppression of the analyte signal in WWTP influent and effluent water as compared to that in deionized water. The suppression in the wastewater is likely due to the higher TOC concentration and the corresponding matrix effects. These results confirm that the matrix components (e.g. organic matter) in WWTP water matrices potentially eluted at the same time as target compounds, resulting in ion suppression in the ESI mode. Given that ESI is a liquid phase ionization technique, anything also in the liquid can 'get in the way' of the target compounds being ionized since both are trying to become ionized at the same time in the ESI spray needle. Sometimes this is helpful, but most times it is harmful and results in lower ionization efficiency in ESI for the target compound (present at a much lower concentration than the matrix), resulting in ionization suppression in ESI.

3.5. Quantification

It has been reported that both a compound as an internal standard and target compounds should have structural similarities such that it reflects the properties of the target compounds during the entire analytical procedure. This indicates that it would be more desirable to have an internal standard (e.g. isotopically labeled compound, structurally similar compound) for each class of antibiotics. Unfortunately, this adds cost and complexity in obtaining the ideal compound. Thus, simatone was chosen as an internal standard for TCs and SAs in this study and previous studies [7] because it eluted within the same chromatographic time frame as the analytes, responded well in ESI (+) mode and did not exhibit noticeable matrix effects. As shown in the TICs (Fig. 2) of this study, simatone (spike concentration of 0.1 μg/L) also eluted earlier in the chromatogram and was therefore less affected by later eluting interferences. Lindsey et al. [13] and Kolpin et al. [6] reported that internal standard simatone did not exhibit matrix effects in analysis of TCs and SAs in surface water, ground water and U.S. streams. To evaluate matrix effects of the internal standard, we compared the peak area of the internal standard (0.1 μg/L) in extracts including internal standard, TCs and SAs for 120 mL water samples. The average peak area and standard deviation of the internal standard in deionized water, influent and effluent water was 13,153,881 ± 8.6, 12,895,816 ± 13.6 and 13,039,976 ± 11.1% in analyzed extracts over a period of 4 months, respectively. These values in the influent and effluent did not differ statistically from those in deionized water. Standard deviation (8.6, 13.6 and 11.1%) of peak areas for the internal standard in the three water matrices is within the recommended acceptable values difference of 15% [26]. This indicates that the internal standards do not exhibit matrix effects in the influent and effluent wastewater. Thus, concentrations for TCs and SAs were calculated reproducibly by using the standard calibration curves, which were constructed using a detector response defined as the ratio of the base peak ion (the specific product ion of highest intensity) to the base peak ion of the internal standard. TCs and SAs measured in the influent and effluent water were not corrected for the matrix effect of the internal standard.

Calibration curves were constructed for the TC and SA extracts spiked at the range from 0.05 to 5 μg/L in 120 mL of

deionized water, DWRF influent and effluent, respectively. The calibration curves were linear with correlation coefficients (R^2) > 0.99 for the MS–MS procedure. Concentrations for 11 antibiotic compounds in the influent and effluent water were determined using calibration curves from each water matrix to correct for matrix effects, resulting in reproducible quantitation for TCs and SAs. The results of the method development conducted for this study indicated that water matrix effects are significant when measuring TCs but not SAs.

3.6. Method detection limit, accuracy and precision

The MDL was determined by multiplying the sample standard deviation calculated from each group of the extracts spiked at the concentration of 0.2 μg/L for each of TCs and SAs by the Student's *t*-variate for a one-sided *t*-test at the 99% confidence level with $n - 1$ degrees of freedom [19]. The MDL for six TCs extracted from 120 mL of water sample was 0.04 and 0.03 μg/L for OTC in the DWRF influent and effluent, 0.05 and 0.03 μg/L for TC, 0.06 and 0.04 μg/L for DMC, 0.05 and 0.03 μg/L for CTC, 0.07 and 0.04 μg/L for DXC and 0.07 and 0.05 μg/L for MCC, respectively. The MDL for five SAs extracted from 120 mL of water sample was 0.04 and 0.03 μg/L for STZ and SMT in the DWRF influent and effluent, 0.05 and 0.04 μg/L for SCP, 0.06 and 0.04 μg/L for SMX and 0.06 and 0.05 μg/L for SDM, respectively. In addition, for sensitivity of the present method, we did not employ limits of detection (LOD) or limits of quantlfication (LOQ) based on a signal-to-noise (S/N) ratio of 3 or 10 with the consideration of the lowest and/or the second lowest concentration in the calibration curve, but the MDL determined by the statistical criteria of the US EPA [19]. Therefore, we considered the determined MDL as the minimum concentration of the analyte that can be quantified with acceptable accuracy and precision of the method.

The accuracy and the variability of the method were determined using six influent and effluent extracts spiked with 0.1, 1.0 and 2.0 μg/L of six TCs and five SAs over a period of 3 days. These influent and effluent water samples were used to assess the accuracy and precision of the simultaneous method. The results are summarized in Table 4. The accuracy range in the DWRF influent water was −9.5 to +13.2% for TCs and −7.6 to +9.4% for SAs. The accuracy range in the DWRF effluent water matrix was −8.4 to +10.7% for TCs and −5.8 to +7.0% for SAs. These accuracy ranges are well within the recommended acceptable values of −30 to +20% [29,30]. The precision as the relative standard deviations (RSDs) calculated from these experiments ranged from 7.6 to 15.5% for TCs and 5.1 to 12.8% for SAs in the DWRF influent water matrix. The precision (RSDs) in the DWRF effluent water matrix ranged from 6.2 to 13.9% for TCs and 4.3 to 10.6% for SAs. No concentration dependence for TCs and SAs in the influent and effluent water matrices were observed.

3.7. Occurrence, distribution and fate of 11 antibiotics of TCs and SAs in the DWRF

The simultaneous LC–MS–MS method was utilized to determine the occurrence and fate of the investigated six tetracycline and five sulfonamide compounds in the wastewater from the DWRF. Fig. 4 shows RTICs for 11 antibiotics for TCs and SAs in the DWRF influent and effluent wastewater samples that were reconstructed on the basis of each class of six tetracyclines and five sulfonamides. Antibiotics reported in the grey-colored mass chromatograms represent the commonly detected compounds in the influent and effluent water samples over a period of 8 months (Fig. 4).

The average concentrations of six TC and five SA compounds measured in the influents and effluents are shown in Fig. 5. For the investigated six TCs TC, DMC, CTC and DXC were found in the influent with a concentration range of 0.05–0.27 μg/L. CTC (0.06 μg/L) and DXC (0.07 μg/L) were found in the effluent water, indicating that these compounds were substantially eliminated during activated sludge treatment and/or chlorination with a removal efficiency of 78% for CTC and 67% for DXC. No OTC or MCC was detected in the influent and effluent. For the investigated five SAs SMT, SMX and SDM were found in the influent wastewater with a concentration range of 0.07–1.09 μg/L and no STZ or SCP was detected. Only SMX (0.21 μg/L) was measured in the effluent samples, indicating that the activated sludge treatment and/or chlorination appears to have removed 81% of the compound. For the investigated 11 antibiotic compounds, SMX (1.09 and 0.21 μg/L) was found at the highest concentrations in the influent and effluent samples. It has been reported that in the case of SMX, the fraction present as human metabolite, N^4-acetylsulfamethoxazole has to be taken into account to better assess the occurrence of SMX in WWTPs [17]. This study verified the presence of CTC, DXC and SMX in treated effluent discharged from the DWRF (Fig. 5).

Little is known about the environmental behavior of TCs and SAs in WWTPs. Hydraulic retention time (HRT), degradation and adsorption to sludge of the target compound in WWTPs are generally considered to impact the fate of the TCs and SAs in WWTPs. The HRTs are generally shorter than the degradation half-lives of many pharmaceuticals and personal care products (PPCPs) that enter WWTPs [31], resulting in discharge of some relatively soluble compounds in effluent before degradation can occur. In addition, the concentration reduction in WWTP effluent may be due to adsorption of the target compounds (e.g. TCs) to sludge (solid-phase) during activated sludge treatment, rather than degradation in the solution phase. These results indicate that further studies are necessary to evaluate the efficiency and mechanism of removal for TCs and SAs at full-scale treatment facilities.

Concentrations of antibiotic compounds found in the DWRF effluents of this study were compared to those found by other research groups [3,4,17]. For TCs, OTC was not detected in any WWTP effluents of this study, Canada [4] or

Table 4
The accuracy and day-to-day variation of LC–MS–MS method in the WWTP influent and effluent extracts from 120 mL spiked with 0.1, 1.0 and 2.0 μg/L of TCs and SAs before extraction

Samples	*n*	Spike concentration (μg/L)	OTC accuracy (%)	RSDs[a] (%)	TC accuracy (%)	RSDs (%)	DMC accuracy (%)	RSDs (%)	CTC accuracy (%)	RSDs (%)	DXC Accuracy (%)	RSDs (%)	MCC Accuracy (%)	RSDs (%)
Day 1														
Influent	6	0.1	−6.2	12.4	10.7	13.8	−6.7	14.3	−6.6	14.6	−4.9	14.0	12.2	11.6
	6	1.0	12.9	13.8	−9.0	10.6	8.1	10.7	12.9	13.3	−7.4	7.6	10.5	15.3
	6	2.0	−5.1	10.5	−6.8	15.1	12.4	8.8	−8.3	9.1	13.2	12.9	8.6	8.7
Effluent	6	0.1	−7.5	13.3	−4.4	13.9	−4.8	13.8	−7.2	12.9	−5.3	13.8	6.6	10.9
	6	1.0	9.1	11.8	8.9	12.5	−3.5	10.4	−3.6	10.6	−3.9	8.6	9.3	7.8
	6	2.0	−5.3	7.9	−2.7	7.8	5.9	6.6	4.3	6.8	−6.6	10.3	4.8	13.6
Day 2														
Influent	6	0.1	13.0	14.9	13.1	9.5	−2.4	15.5	12.1	14.9	11.8	15.4	10.4	14.3
	6	1.0	−7.6	12.7	11.4	13.3	−6.8	13.3	8.7	10.8	−8.0	13.8	−6.2	10.5
	6	2.0	−8.3	9.1	8.9	11.7	−9.5	8.4	4.4	8.2	−5.3	9.1	−3.0	9.8
Effluent	6	0.1	9.3	12.4	5.3	9.7	−5.3	12.3	8.7	11.7	2.4	11.2	−2.5	12.1
	6	1.0	−7.8	9.6	−4.6	12.4	9.4	10.5	−2.8	8.3	9.5	9.4	−1.2	10.5
	6	2.0	3.9	8.1	−6.5	6.2	5.2	8.2	−4.5	7.6	3.2	8.8	−4.6	8.3
Day 3														
Influent	6	0.1	−7.4	14.0	12.5	14.9	6.3	14.7	−3.2	10.7	12.3	13.3	9.7	14.9
	6	1.0	−9.2	8.3	−8.6	10.1	−5.7	9.0	11.8	12.8	10.2	11.8	10.3	9.3
	6	2.0	12.8	11.4	−7.0	13.8	−8.6	12.5	−7.5	7.5	−8.8	10.7	−2.8	12.0
Effluent	6	0.1	10.6	13.7	−4.5	12.0	6.2	10.6	10.7	12.5	−4.6	13.5	10.4	13.4
	6	1.0	4.5	8.2	−8.4	9.3	8.7	12.8	−8.1	9.1	−3.2	10.7	7.9	11.2
	6	2.0	6.4	9.8	3.9	6.9	−4.1	6.5	3.8	8.2	−7.8	9.4	−5.1	9.9

Samples	*n*	Spike concentration (μg/L)	STZ accuracy (%)	RSDs (%)	SMT accuracy (%)	RSDs (%)	SCP accuracy (%)	RSDs (%)	SMX accuracy (%)	RSDs (%)	SDM accuracy (%)	RSDs (%)
Day 1:												
Influent	6	0.1	8.5	12.0	−6.0	12.5	7.3	11.6	−5.0	11.5	−7.3	12.8
	6	1.0	−4.2	7.1	8.8	6.2	8.9	5.3	−6.4	10.9	7.8	10.3
	6	2.0	7.0	10.6	−7.6	11.0	6.5	6.2	7.5	5.5	4.6	7.2
Effluent	6	0.1	6.2	8.8	3.2	8.9	4.9	8.0	6.3	10.2	5.6	8.8
	6	1.0	1.9	9.2	−2.3	6.4	6.7	5.7	4.2	7.1	−4.7	9.7
	6	2.0	−0.8	4.9	1.5	4.5	−1.4	6.8	1.6	5.4	4.8	10.4
Day 2:												
Influent	6	0.1	−5.1	11.8	8.5	11.6	9.4	11.5	9.3	9.7	−1.6	12.4
	6	1.0	8.7	8.5	2.2	9.8	8.2	7.5	7.1	6.2	8.8	10.7
	6	2.0	2.5	6.7	6.1	8.7	5.8	6.9	5.6	11.1	1.4	8.6
Effluent	6	0.1	−5.8	9.4	−2.4	10.2	6.3	9.5	3.4	9.8	1.7	9.2
	6	1.0	−2.3	8.8	1.7	8.1	−1.6	7.1	1.6	6.7	4.1	5.6
	6	2.0	3.6	6.5	2.8	4.6	1.8	5.6	−0.7	4.5	5.2	4.3
Day 3:												
Influent	6	0.1	8.9	11.9	5.4	5.1	7.2	12.1	8.8	12.4	4.3	10.1
	6	1.0	1.6	10.3	8.3	7.4	3.3	7.9	6.2	6.6	9.2	8.5
	6	2.0	6.3	6.2	6.2	10.3	4.6	11.4	4.5	9.3	−5.5	5.9
Effluent	6	0.1	4.5	10.1	2.3	9.7	1.4	10.0	−2.8	10.6	4.0	10.3
	6	1.0	4.1	8.6	−4.6	7.6	−0.1	9.3	4.4	7.1	2.8	9.9
	6	2.0	2.7	4.7	7.0	6.3	4.7	5.9	1.2	6.2	5.9	6.1

[a] RSDs: relative standard deviations.

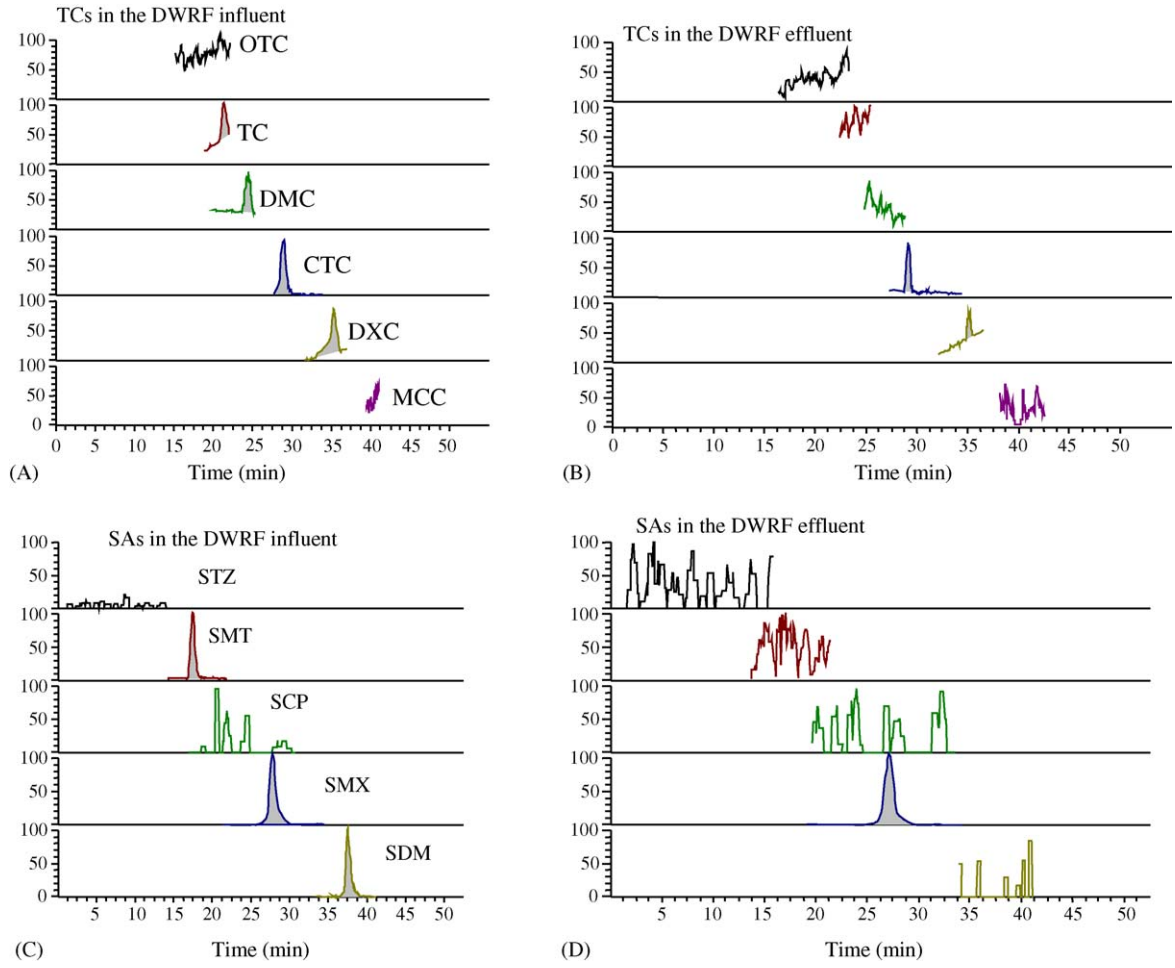

Fig. 4. Reconstructed total-ion chromatograms (RTICs) for 11 antibiotics for TCs and SAs in the DWRF influent and effluent. RTICs were based on each class of six tetracyclines and five sulfonamides. Antibiotics reported in grey-colored chromatograms represented the commonly detected compounds in the influent and effluent water samples over a period of 8 months.

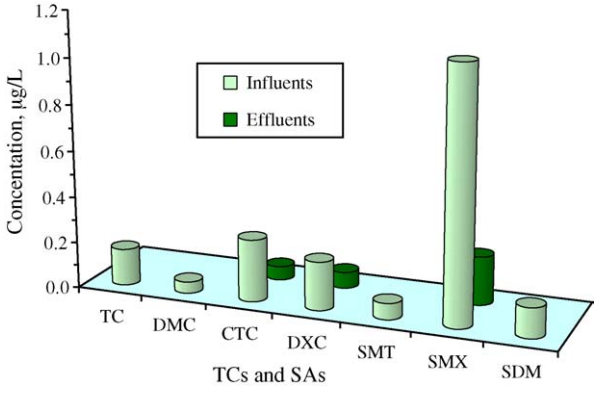

Fig. 5. Occurrence of TCs and SAs in the DWRF influents and effluents. Concentrations shown are the triplicate average of three samples over a period of 8 months. *N* (number of samples for the influent and effluent each) = triplicates × three samples × sixteen frequencies = 144).

Germany [3]. CTC was not found in effluents of Canada [4] or Germany [3] as compared with detection (0.06 μg/L) close to the MDL (0.05 μg/L) in this study. TC was not detected in effluents of this study and Germany [3], but Canada [4] at a median concentration of 0.15 μg/L. DXC was measured in effluents of this study and Canada [4] with 0.07 and 0.04 μg/L, respectively. For SAs, SMX has been frequently detected in WWTP effluents in this study, Canada [4], Germany [3] and Switzerland [17] with similar concentrations of 0.21, 0.24, 0.40 and 0.35 μg/L, respectively. SMT was not detected in effluents of this study or Germany [3], but has been reported in Canada [4] and Switzerland [17] at a median concentration of 0.36 and 0.02 μg/L, respectively. STZ, SCP and SDM were not detected in WWTP effluents examined (this study and Canada [4]). Deviations in the concentrations of TCs and SAs detected in the WWTP effluents reflect differences, which may been caused by prescription patterns of these antibiotic compounds for humans in the countries, sampling date on the basis of seasonal variations in the prescriptions of antibiotics for humans, the extent of removal of the antibiotics by WWTPs, and method detection limit (MDL). No other study has reported concentrations of TCs

and SAs in WWTP influents. The LC–MS–MS method in the present study will be applied to fully investigate the fate of these compounds through the various steps of wastewater treatment.

4. Conclusions

The occurrence of human pharmaceutical compounds in WWTP influent and effluent water is an important emerging environmental issue. Antibiotic compounds are not totally eliminated in WWTPs. This indicates that WWTP effluents are relevant point sources for residues of these compounds in the aquatic environment. This paper describes a sensitive and reproducible analytical method for simultaneously quantifying six tetracycline and five sulfonamide compounds in WWTP influent and effluent water matrices using SPE and LC–MS–MS with ESI (+) and SRM.

Average recovery of 11 antibiotic compounds for simultaneous extraction in the influents and effluents indicates that matrix effects were found to be significant when measuring TCs but negligible for analysis of SAs. The MDL in wastewater matrices determined using the EPA method was between 0.03 and 0.07 μg/L for TCs and SAs. The method developed in this study was applied to evaluate the occurrence of these compounds in a WWTP in northern Colorado. For the investigated 11 antibiotic compounds TC, DMC, CTC, DXC, SMT, SMX and SDM were found in the influents with a concentration range of 0.05–1.09 μg/L. CTC, DXC and SMX were also found in the effluents with a concentration range of 0.06–0.21 μg/L. SMX was present at the highest concentration at 1.09 and 0.21 μg/L in the influent and effluent wastewater, respectively. Adequate repeatability, reproducibility, linearity and MDL for each analyte moiety in this study indicate that this assay can be extended to more complex environmental matrices such as hospital wastewater, sewage sludge, lagoon wastewater and manure with SPE and/or liquid–liquid extraction procedures. Ongoing studies with this method will evaluate the occurrence and fate of these compounds in a relatively small watershed in northern Colorado that is influenced by WWTP effluents and agricultural landscapes (Larimer and Weld Counties, Colorado have a high density of AFOs and CAFOs) as well as to fully assess the fate of these compounds in WWTPs.

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