Intern. J. Environ. Anal. Chem.
Vol. 92, No. 12, 15 October 2012, 1389–1402

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Simultaneous determination of fluoroquinolone and tetracycline antibacterials in sewage sludge using ultrasonic-assisted extraction and HPLC-MS/MS

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(*Received 10 April 2010; final version received 21 October 2010*)

A reliable method was proposed for the simultaneous determination of five fluoroquinolones (FQs) and two tetracyclines (TCs) in sewage sludge using ultrasonic-assisted extraction (USE) followed by SPE cleanup and high-performance liquid chromatography-mass spectrometry (HPLC-MS)/MS analysis with electrospray ionisation (ESI) in a positive mode. The USE conditions (e.g. extraction solvent, pH, and extraction cycles) and high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) parameters were optimised. Quantification was performed by internal standard calibration in multiple reaction monitoring mode. Recoveries of the antibacterials ranged from 41 to 123%, with relative standard deviations within 17%. The sample-based limits of quantification were 10–63 ng g^{-1} dry weight (dw) for FQs (ciprofloxacin, enrofloxacin, lomefloxacin, norfloxacin, and ofloxacin) and 250–500 ng g^{-1} dw for TCs (tetracycline and oxytetracycline). The method was applied to determine the antibacterials in sewage sludge and sediment samples were collected from the Pearl River Delta, China. Ciprofloxacin, norfloxacin, and ofloxacin were frequently detected, ranging from 1052 to 17740 ng g^{-1} dw in dewatered sludge samples, 585–3545 ng g^{-1} dw in untreated solids, and 98–258 ng g^{-1} dw in an urban stream sediment sample, respectively. Lomefloxacin and enrofloxacin were also occasionally detected.

Keywords: antibacterials; sewage sludge; ultrasonic-assisted extraction; liquid chromatography – tandem mass spectrometry

1. Introduction

Fluoroquinolones (FQs) are among the most important classes of antibacterial agents that are widely used to treat or prevent bacterial infections for humans and animals [1]. In China, FQs ranked the third most frequently hospital-prescribed antibacterials (about 13–14% of the total antibacterial consumption) next to cephalosporins and penicillins in recent years. Although tetracycline antibacterials (TCs) are less frequently used in human medicines today, they are still widely applied as growth promoters and therapeutic drugs for animals [2]. Their intensive use has led to ubiquitous presence of these antibacterials in the environment [3], which has become an issue owing to potential

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ISSN 0306–7319 print/ISSN 1029–0397 online

http://dx.doi.org/10.1080/03067319.2010.535124
http://www.tandfonline.com

ecological risks, e.g. promoting antibiotic resistance in organisms [4]. For instance, tetracycline-resistant genes have been detected in aquatic environment and marine sediment [5–7].

The occurrence and fate of FQs and TCs in aquatic environments have been extensively documented [8–12], benefiting from several methods available for their determination in aqueous matrices [13–20]. In contrast, data about these antibacterials in sewage sludge are still limited [21–25]. This is probably attributable to the great difficulties in analysing these compounds in highly complex sludge, which need comprehensive extraction, purification, sensitive, and specific detection for reliable identification and quantification.

Previous research has revealed a high tendency of FQs and TCs for sorption to soils, clay minerals, and humic-mineral complexes through cation exchange reactions and other surface complexation mechanism [26–30]. Sewage sludge is suggested to be a potentially important sink for these antibacterials. More than 70% of FQs mass flow in sewage has been observed to end up and persist in sludge during treatment in sewage treatment plants (STPs) [22,24]. TCs were also detected in sewage sludge [24], manure, and manure-fertilized soils [31–34]. Therefore, reliable and feasible methods for determination of these antibacterials in complex solid environmental matrices are indispensible for better understanding of their fate, transport, and risks in the environment.

Only a few works so far have been performed on analytical methods for determination of FQs and TCs in solid environmental matrices [21,23,31–34]. Mechanical shaking [34], ultrasonic- assisted extraction (USE) [23,33], and accelerated solvent extraction (ASE) [21,32] were commonly applied to extracted FQs and TCs from sewage sludge and soil. Since solid matrices, especially sewage sludge, are of great complexity, a further cleanup procedure, which is generally carried out by SPE, is always needed to minimise co-extracted matrix interferences and thus to increase precision and reproducibility of analysis [21,23,31–34]. The antibacterials were finally determined by (HPLC) coupled with fluorescence and ultraviolet detection [21,33], enzyme-linked immunosorbent assay [34], high-performance liquid chromatography-mass spectrometry (HPLC-MS) [25,34], and HPLC-MS/MS analysis [23,31,32]. However, relatively lower sensitivity (e.g. quantification limits ranging from sub- to low $\mu g\,g^{-1}$) and poorer reproducibility were usually observed [35], which might be attributable to the highly complex and variable matrix effects of sludge and soil. Although FQs and TCs show comparable tendency for sorption to solids [30], present research usually determined the two classes of antibacterials separately [21,31–34]. Recently, Lillenberg *et al.* [25] developed a method for simultaneous determination of ciprofloxacin, norfloxacin, ofloxacin, tetracycline, doxycycline, sulfadimethoxine, and sulfamethoxazole in sewage sludge using a self-designed pressurised liquid extraction system and LC-MS, with quantification limits of 80–160, 0.8–1.8, and 0.1 $ng\,g^{-1}$ wet weight for the TCs, FQs, and sulfonamides.

This work aimed to develop an efficient and reliable method for simultaneous determination of the commonly used FQs and TCs in complex sewage sludge. Ultrasonic-assisted extraction was selected based on the obtained comparable extraction efficiency to ASE for macrolide and sulfonamide antibacterials from sludge under optimal extraction condition [36]. In addition, relative to ASE, USE has advantages of being simple to handle, cost-effective, solvent saving, and easily accessible. Solid-phase extraction (SPE) was performed for purification and pre-concentration of the analytes. HPLC-MS/MS was used to determine the antibacterials because of the selectivity, high sensitivity, and

specificity [17]. Internal standard quantification was adopted to partly compensate for matrix effects. The method was applied to a preliminary investigation of the frequently consumed FQs and TCs in sewage sludge and sediment sampled from the Pearl River Delta, China. To the best of our knowledge, analysis of lomefloxacin in sewage sludge has not been reported previously.

2. Experimental

2.1 *Chemicals and materials*

Ciprofloxacin, enrofloxacin, lomefloxacin, norfloxacin, ofloxacin, oxytetracycline dihydrate, and tetracycline were all of high purity and were purchased from Sigma-Aldrich (St Louis, MO, USA). Ciprofloxacin-d_8 was bought from C/N/D Isotopes (Pointe-Claire, Quebec, Canada). HPLC grade acetonitrile, methanol, acetone, formic acid, and ammonium acetate were obtained from Merck (Darmstadt, Germany). Analytical grade ethylenediamine tetraacetate (EDTA) was bought from Bodi Chemical (Tianjin, China) and washed with methanol prior to use. High purity water (HPW) was produced by a Spring Ultrapure Water System (Ruishijie Scientific Instruments, Shanghai, China).

Individual stock standard solutions were prepared in methanol at 100 mg L^{-1} for FQs (including ciprofloxacin-d_8) and 400 mg L^{-1} for TCs, respectively. A standard mixture solution containing all the analytes was prepared in methanol/HPW (1 : 1) at 10 mg L^{-1}. All the standard solutions were stored at −20°C in darkness and renewed every month. Calibration solutions (1 to 1000 ng mL^{-1}) were prepared by serial dilution from the standard mixture solution every time prior to use. Stability of the standard solutions over the experimental period was checked through instrumental signal responses.

2.2 *Sample collection and preparation*

Sewage sludge samples were collected from two STPs located in Guangzhou, a metropolis in the Pearl River Delta, South China. GZSTPA has a designed capability of 30 000 $m^3\,d^{-1}$ and handles a mixture of domestic and industrial wastewater (~6 : 4), serving a population of 370 000. The industrial wastewater is mainly from manufacturers of chemical, food, electronic, automobile and is primarily treated before entering GZSTPA. This STP uses a conventional activated sludge treatment process consisting of grit removal, primary sedimentation, and oxic activated sludge treatment. GZSTPB, with a capacity of 550 000 $m^3\,d^{-1}$, treats predominantly domestic wastewater and serves a population of about 2.5 million. The bioreactor comprisea successively anaerobic, anoxic, and oxic tanks. Dewatered sludges were sampled in May and November 2008. Untreated solid samples from grit chambers were also collected in November.

Sediment samples were collected from an urban stream in Guangzhou and the Pearl River Estuary. The urban stream runs through a densely populated domestic area and receives sporadic discharge of domestic wastewater.

Samples were wrapped with pre-baked (450°C) aluminium foils, sealed in polyethylene bags, and kept on ice during transport to the laboratory, where they were stored at −20°C. The samples were afterwards lyophilised, ground and homogenised in an agate mortar, and stored in dark at −20°C until analysis.

2.3 *Sample extraction and cleanup*

The lyophilised and homogenised sewage sample was accurately weighed (0.2 g) into a 10 mL glass centrifugal tube and spiked with 100 ng ciprofloxacin-d_8. Eight millilitres of extraction solvent (50% acetonitrile solution in water with 1 mM EDTA at pH 2.0 adjusted with hydrochloric acid) was added. The slurry was successively vortexed for 1 min, ultrasonicated (YJ-5200D Ultrasonic Cleaner, Ningbo, China, 40 kHz, 300 W) for 10 min, and centrifuged (Avanti^{TM30} centrifuge, Beckman, California, USA) at 4000 rpm for 5 min. The clear supernatant was transferred to a 50 mL pear-shaped glass flask. The extraction procedure was repeated twice with 5 mL of the extraction solvent. The supernatants were combined and evaporated to reduce the acetonitrile content. The concentrated extract was transferred to an amber glass bottle and diluted with HPW to make the acetonitrile content to <2%. The diluted extract was then adjusted to pH 4.2 with hydrochloric acid (HCl) for further SPE cleanup.

The cleanup was performed with a 200 mg Oasis HLB cartridge (Waters, Milford, MA, USA). The cartridge was preconditioned successively by 3×2 mL of methanol and 3×2 mL of HPW (pH 4.2). The diluted extract was loaded on the cartridge at a flow rate of about 3 mL min^{-1}. The cartridge was then washed with 5 mL of 5% methanol solution and kept under vacuum evaporation for 5 min. The analytes were finally eluted with 3×1.5 mL of methanol. The eluent was concentrated to about 0.1 mL by a gentle flow of high purity nitrogen and reconstitute into 1 mL of HPW with 0.2% formic acid prior to HPLC-MS/MS analysis.

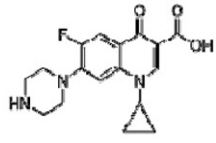
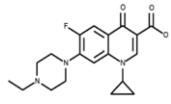
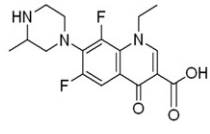
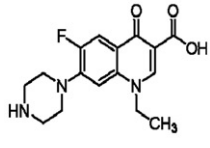
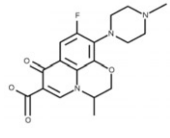
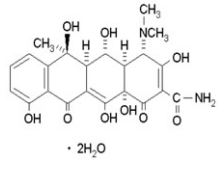
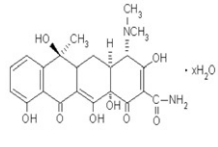
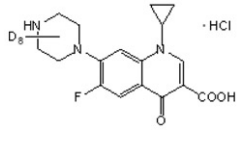
2.4 *HPLC-MS/MS analysis*

The antibacterials were determined by an Agilent 1200 LC system coupled to an Agilent 6410 triple quadrupole MS with electrospray ionisation in positive mode (Agilent, Palo Alto, CA, USA). Separation was achieved by injecting 5 μL of the sample on an Agilent Zorbax Eclipse XRD C18 column (150 mm × 3.0 mm i.d., particle size 3.5 μm) at 25°C and 0.25 mL min^{-1}. A 4.0 mm × 3.0 mm i.d. guard column (Phenomenex, Torrance, CA USA) containing the same sorbent was connected. The mobile phase consisted of HPW containing 0.2% formic acid and 5 mM ammonium acetate (mobile phase A, pH 2.6) and methanol (mobile phase B). A linear gradient was programmed from 10% B to 30% B in 10 min, to 80% B in 20 min, to 100% B in 30 min, isocratic for 2 min, and back to the initial condition in 2 min. A 10 min post-time was set for re-equilibration of the column. For MS detection, the capillary voltage was set at 4500 V. The MS temperature was 100°C. Nitrogen was used as dry gas with a flow rate of 10 L min^{-1} and temperature at 350°C. Nitrogen was also used as the collision gas. The protonated molecular ion ($[M+H]^+$) was selected as the precursor ion for each compound. Detection was carried out in multiple reaction monitoring (MRM) mode using the two most intense and specific ion transitions as listed in Table 1. A dwell time was set for each ion transition to maximise the sensitivity. Quantification was performed by internal standard method. Instrument control and data acquisition were managed with MassHunter Workstation.

2.5 *Method validation and quality control*

Since a sludge sample free of all the investigated antibacterials could not be obtained, extraction conditions (extraction solvent, pH, and extraction cycles) were optimised by

Table 1. Chemical structure and optimised HPLC-MS/MS parameters of the analytes.

Compound	Chemical structure	Retention time (min)	MRM transition[a]	Fragment (V)	Collision energy (eV)	Dwell time (ms)
Ciprofloxacin		19.38	**332.0 > 314.1** 332.0 > 288.1	135	23 20	50 50
Enrofloxacin		19.49	**360.2 > 342.1** 360.2 > 244.9	120	20 25	50 50
Lomefloxacin		19.92	**352.1 > 237.1** 352.1 > 222.9	140	25 25	50 50
Norfloxacin		18.90	**320.1 > 302.1** 320.1 > 276.0	140	20 25	50 50
Ofloxacin		18.19	**362.2 > 261.2** 361.2 > 318.1	135	25 20	50 50
Oxytetracycline dehydrate		18.83	**461.4 > 426.3** 461.4 > 154.1	140	20 26	150 50
Tetracycline		18.20	**445.2 > 410.1** 445.2 > 154.1	140	20 25	150 50
Ciprofloxacin-d8		19.29	**340.2 > 235.0** 340.2 > 296.0	140	35 25	50 50

[a]Quantification ion transition in boldface type.

spiking the analytes at 2500 ng g^{-1} dry weight (dw) in dewatered sludge samples. To verify the extraction efficiency, recovery tests were further performed by spiking the analytes at 500 and 100 ng g^{-1} dw in untreated solid samples . The spiked samples were stirred thoroughly and were kept at 4°C overnight to allow potential partition equilibrium. Both the spiked and the correspondingly unspiked sludge samples were added with ciprofloxacin-d_8 at 500 ng g^{-1} dw, extracted, and analysed in the same manner. The recovery was then calculated by Equation (1)

$$\text{Recovery}(\%) = \frac{C_{ss} - C_{us}}{C_s} \times 100 \quad (1)$$

where C_{ss} and C_{us} are measured concentrations in the spiked and correspondingly unspiked sludge samples, respectively. C_s is the spiking concentration.

Quantitative extraction of sewage sludge was assessed following the procedure described in the literature [21] with minor modifications. After three extraction cycles had been performed for a sewage sludge sample an additional two extraction cycles were further conducted for the same sample. The extracts were separately collected and analysed. Thermal stability of the antibacterials was evaluated by extracting spiked clean sand quartz with USE in the same protocol as the sludge samples.

Matrix effect was evaluated according to the strategy applied by Matuszewski *et al*. [37]. A known amount of standards was added into sample extracts. Matrix effect was then calculated by comparing the peak areas of the standards in sample matrix with those in HPW at the same concentration.

The instrumental quantification limit (IQL) was defined as the lowest concentration in pure water with a signal-to-noise ratio (S/N) of 10 for each compound. The sample-based limit of quantification (LOQ) of the method was estimated based on the IQL (ng mL^{-1}), recovery (%), final volume (V, 1 mL in this work), and sample weight (W, 0.2 g in this work) according to the strategy proposed by Kasprzyk-Hordern *et al*. [38] as the following:

$$\text{LOQ} = \frac{\text{IQL} \times V \times 100}{\text{Recovery} \times W} \quad (2)$$

A procedural blank was set for each batch of six samples to control laboratory contamination. An instrumental blank and a calibration solution at 100 ng mL^{-1} were run at the beginning, after every 10 samples, and at the end of each running sequence to check the instrumental performance and potential cross contamination during HPLC-MS/MS detection. The overall performance of the method was verified with replicate analyses of environmental samples.

3. Results and discussion

3.1 *USE optimisation and SPE cleanup*

Various extraction solvents comprising aqueous mixture with organic modifiers (acetonitrile, methanol, and acetone) were tested to achieve the optimal extraction efficiency. Pure organic solvents were not considered based on the reported poor efficiency in extracting the antibacterials from sludge and soil owing to minor roles of hydrophobic interactions on antibacterials sorption to solids [21,35]. FQs showed satisfactory recoveries (62–123%) except for enrofloxacin when using mixtures of water with acetonitrile and methanol at pH 2.0 (Table 2). However, addition of methanol in

Table 2. Recoveries (%) for the investigated antibacterials by various extraction solvents at pH 2[a].

	Spiking level (ng g^{-1} dry weight)						
	2500					500	100
	acetonitrile/ water (1 : 1)	acetonitrile/ methanol/ water (2 : 1 : 1)	acetonitrile/ methanol/ water (1 : 1 : 1)	methanol/ water (1 : 1)	acetone/ methanol/ water (2 : 1 : 1)	acetonitrile/ water (1 : 1)	acetonitrile/ water (1 : 1)
Ciprofloxacin	**103.1**(12.7)	75.8 (13.9)	79.1 (2.5)	62.2 (3.0)	61.0 (7.9)	**89.9 (5.8)**	**101.3 (1.9)**
Enrofloxacin	**43.9** (5.5)	34.2 (7.4)	29.2 (1.5)	9.7 (0.7)	33.0 (2.7)	**42.5 (5.0)**	**41.4 (4.7)**
Lomefloxacin	–[b]	–[b]	–[b]	–[b]	–[b]	**91.1 (8.2)**	**99.0 (11.9)**
Norfloxacin	**114.4** (7.2)	78.4 (16.6)	67.8 (3.7)	99.5(7.5)	59.6 (3.8)	**70.1(16.7)**	**64.4 (10.7)**
Ofloxacin	**123.3** (4.1)	92.9 (17.7)	79.3 (3.7)	101.7(9.2)	61.6 (4.1)	**65 (13.6)**	**77.4 (16.0)**
Oxytetracycline	**102.0**(14.4)	26.8 (15.9)	35.4 (13.6)	90.2(14.8)	25.0 (8.3)	–[b]	–[b]
Tetracycline	**82.5** (9.3)	13.6 (13.2)	19.0 (10.3)	41.3(11.6)	13.1 (4.4)	–[b]	–[b]

[a]The presented result is mean (RSDs, $n = 3$). The boldface data are recoveries of the extraction method eventually chosen. [b]Not analysed.

extraction solvent generally resulted in decreased extraction efficiency, especially for enrofloxacin and tetracycline. Addition of acetone in extraction solvent led to poorer extraction efficiency for all the analytes. As a whole, using the mixture of water and acetonitrile at a ratio of 1 : 1 as the extraction solvent gave acceptable recoveries (44–123%) with relative standard deviations (RSDs) of 4–14% for all the antibacterials.

Because of the zwitterionic character of FQs and TCs, pH values may affect the extraction efficiency [21,28]. Extraction efficiency was compared under acidic (pH = 2), neutral (pH = 7), and basic conditions (pH = 12). Better recoveries were obtained at pH 2 than at pH 7. Poor results were generally obtained at pH 12 owing to strong interference of co-extracted matrix (data not shown). Ionic interaction and surface complexation play significant roles in sorption of FQs and TCs to solids [26–30]. Golet *et al*. [21] suggested that the electrostatic repulsion between the FQs and sludge surface might result in better extraction efficiency because the anionic sites of the antibacterials and sludge are protonated in acidic condition. Furthermore, the sorption coefficients of TCs to clay minerals increase with pH value and reach maximum values at pH 8 [39], suggesting that higher aqueous solubility at low pH may also enhance the extraction efficiency of the antibacterials. In contrast, no significant pH dependence was observed in extraction efficiency of macrolides from sludge [40,41] and the highest extraction efficiency was achieved at pH 8.8 for extraction of sulfonamides from soil [42]. This may be attributed to the difference in physicochemical properties and sorption mechanism of these antibacterials [25–30,43].

Both FQs and TCs are known to form strong chelate complexes with metal ions [27,29] that may quite abundant in sewage sludge or sediment. Therefore, the addition of a chelating agent into the extraction solvent is indispensable in order to improve the extraction efficiency [35]. EDTA was used as the metal chelator, which has been proved effective in increasing recoveries for both FQs and TCs [21,31,34].

Phosphoric acid was preferred to adjust the pH value by Golet *et al*. [21] because it is friendlier to the steel components of the ASE extraction cells than HCl or other strong acids. Nevertheless, phosphoric acid may have harmful effect on the ESI source of the MS owing to its poor volatility. In addition, no steel facilities were used during ultrasonication in this work. Finally, 50% acetonitrile solution with 1 mM EDTA at pH 2.0 adjusted with HCl was selected as the extraction solvent in this work. Recoveries ranged from 41 to 101% in fortified sludge samples at 100 and 500 ng g^{-1} dw, with RSDs within 17% (Table 2).

The USE extracts of sewage sludge were typically dark green in colour and turbid with co-extracted various organic materials and inorganic salts that may not only interfere the analysis but also be harmful to the HPLC-MS/MS. Therefore, a cleanup step was required prior to HPLC-MS/MS analysis. The efficiency of SPE enrichment and purification for antibacterials in wastewater with a hydrophilic-lipophilic (HLB) cartridge is described in detail elsewhere [11]. Before SPE, the USE extracts were diluted with HPW to reduce the acetonitrile content to <2%, so that the cartridge breakthrough caused by organic solvent would not occur [21,33,40]. Satisfactory resolution and peak shapes were obtained after the purification (Figure 1).

3.2 *HPLC-MS/MS analysis*

For optimising LC separation and MS sensitivity of the analytes, the following MS compatible solutions were tested as mobile phases: HPW with 0.1% formic acid and 5 mM

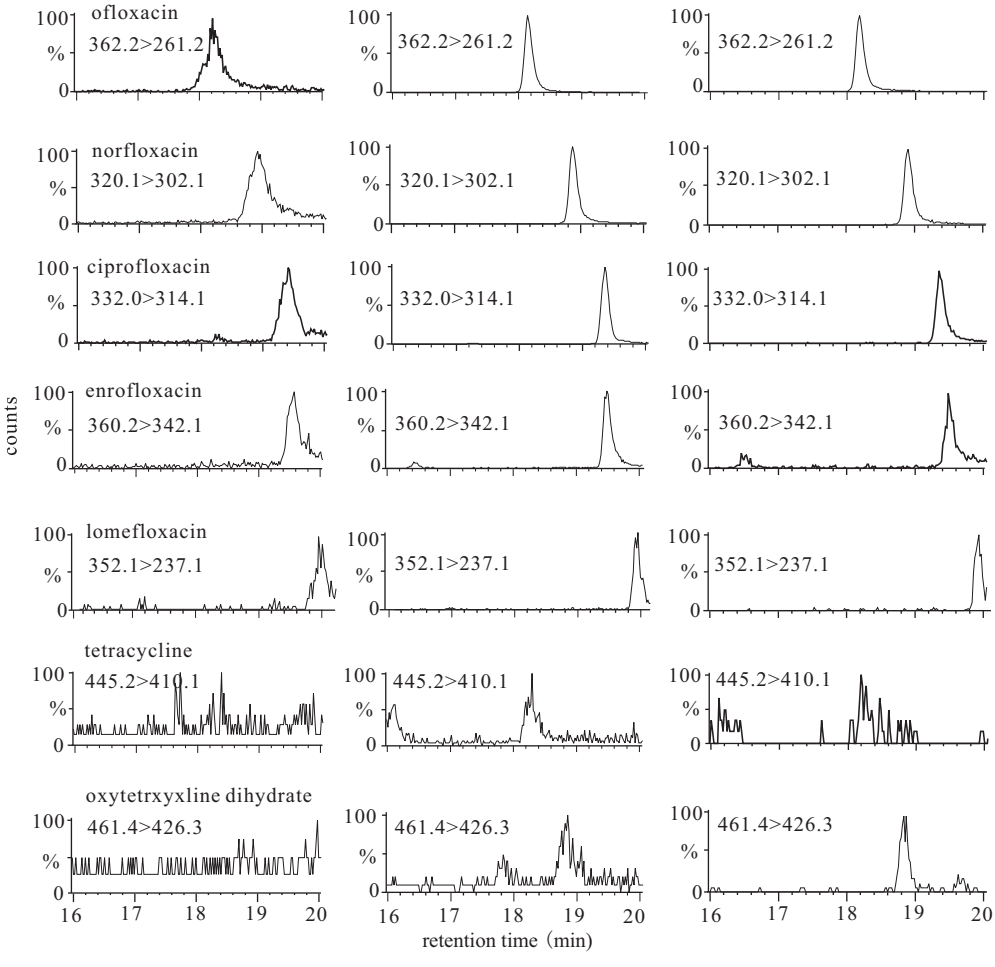


Figure 1. Multiple reaction monitoring chromatograms of the investigated antibacterials in the estuarine sediment (left), urban stream sediment (middle), and sewage sludge (right) samples from the Pearl River Delta, China.

ammonium acetate – methanol, HPW with 0.2% formic acid and 5 mM ammonium acetate – methanol, and HPW with 0.1% formic acid and 5 mM ammonium acetate – acetonitrile. Using methanol and acetonitrile as the organic mobile phase gave comparable resolution and reproducibility. Tiled peaks with substantial tailing were generated when the content of formic acid was 0.1% in the aqueous mobile phase. Increasing the content of formic acid to 0.2% significantly improved the peak shapes and sensitivity. Furthermore, addition of 2 mM oxalic acid into the aqueous mobile was also helpful to improve the peak shapes and resolution, especially for TCs. Nevertheless, crystal formation was observed on the surface of the capillary inlet port owing to the poor volatility of oxalic acid, which subsequently may affect the spray efficiency of the ESI source. Therefore, HPW with 0.2% formic acid and 5 mM ammonium acetate (mobile A)–methanol (mobile B) was finally selected as the mobile phase for determination of the investigated antibacterials.

Table 3. Linearity, analysis precision, and limit of quantification (LOQ, $ng\,g^{-1}$ dry weight) of the analytes in sewage sludge.

Antibiotics	Linear range (pg on column)	r^2 [a]	RSD (%)		LOQ
			Intra-day [b]	Inter-day [c]	
Ciprofloxacin	10 ~ 5000	0.9980	0.2–1.4	14.1	10
Enrofloxacin	5 ~ 5000	0.9987	1.5–8.9	21.5	12
Lomefloxacin	50 ~ 5000	0.9945	1.4–6.4	17.9	50
Norfloxacin	50 ~ 5000	0.9983	0.7–1.4	16.3	63
Ofloxacin	10 ~ 5000	0.9973	3.5–5.1	10.8	10
Oxytetracycline	500 ~ 5000	0.9994	1.2–7.9	14.0	500
Tetracycline	250 ~ 5000	0.9983	0.8–11.7	16.7	250

[a]Correlation coefficient. [b]Calculated through replicate analyses of standard solution ($100\,ng\,mL^{-1}$) at the beginning, in the middle, and at the end of each running sequence ($n \geq 3$).[c]Calculated through replicate analyses of standard solution ($100\,ng\,mL^{-1}$) over the experimental period ($n = 18$).

Various gradient elution programs and column temperature were also tested to optimise the chromatographic conditions. Optimal separation was achieved at 25°C with a gradient elution described in Section 2.3. Optimisation of the MS/MS parameters was performed by flow injection of standard solution for each compound. Identification of the precursor and product ions was performed in full scan mode and product scan mode, respectively. In order to maximise the sensitivity and to improve the peak shape, the fragmentor voltage for each compound, the collision energy, and the dwell time for each ion transition were also optimised. The optimum fragmentor voltage, quantitative and qualitative ion transitions, collision energy, and dwell time for each analyte are shown in Table 1.

3.3 *Quantification and method validation*

In identification of the analytes in environmental samples, the RSDs of retention times and ratios of the two specific MRM ion transitions with those of the standards were within 2% and 20%, respectively. For quantification, a nine-point calibration curve in the range of 1 to $1000\,ng\,mL^{-1}$ was constructed based on the quantification ion transition for each analyte using ciprofloxacin-d_8 as the internal standard. Good linearity was achieved ($r^2 > 0.990$, Table 3). Satisfactory recoveries were obtained for most analytes except for enrofloxacin (Table 2). The results suggest the suitability of ciprofloxacin-d_8 as the internal standard for quantification of the analytes except for enrofloxacin. However, an appropriate internal standard for each analyte is needed to improve the data quality in future work. The intra-day precision indicated by RSDs of analyses of at least three standard solutions at $100\,ng\,mL^{-1}$ (i.e. at the beginning, in the middle, and at the end of a running sequence) was <12%. The analysis precision over the experimental period (about one month) was monitored by replicate injections of standard solution at $100\,ng\,mL^{-1}$ and the RSDs were typically <18% except for enrofloxacin (21.5%, Table 3). The absolute deviations for duplicate analyses of sediment and sewage sludge samples were basically

Table 4. Concentrations (ng g^{-1} dry weight) of the investigated fluoroquilonones in sewage sludge and sediment samples collected from the Pearl River Delta, China in 2008[a].

Sample	Ciprofloxacin	Enrofloxacin	Lomefloxacin	Norfloxacin	Ofloxacin
GZSTPA dewatered sludge May	1052 ± 196	<LOQ[f]	<LOQ[f]	2002 ± 532	3724 ± 578
GZSTPA dewatered sludge November	1300 ± 88	<LOQ[f]	<LOQ[f]	3202 ± 933	4710 ± 138
GZSTPA untreated solid November[b]	585	<LOQ[f]	<LOQ[f]	967	1361
GZSTPB dewatered sludge May	10921 ± 2176	470 ± 102	1153 ± 111	13684 ± 2636	11744 ± 2586
GZSTPB dewatered sludge November	7202 ± 940	269 ± 53	2387 ± 680	11095 ± 768	17740 ± 3194
GZSTPB untreated solid November[b]	1456	<LOQ[f]	476	2765	3545
Sediment of an urban stream at Guangzhou	119 ± 15	27 ± 1	<LOQ[f]	258 ± 39	98 ± 18
Sediment from the Pearl River Estuary	<LOQ[f]	<LOQ[f]	not detected	<LOQ[f]	<LOQ[f]
Swedish STP sludge[c]	500–11 700	–[g]	–[g]	100–11 100	100–2000
Swiss STP sludge[d]	1400–3500	–[g]	–[g]	1540–3370	–[g]
Estonia STP sludge[e]	32.8–35.5	–[g]	–[g]	20.8–25	4–10.9

[a]Tetracyclines were not quantitatively detected in any sample and therefore were not listed in this table. The presented concentrations are mean ± absolute deviation of duplicate analyses. [b]Only one analysis. [c]Refs [23, 24]. [d]Ref. [21, 22]. [e]Ref [25], based on wet weight. [f]Detected but below the limit of quantification. [g]Not reported.

within 20%, with a few exceptions, e.g., the absolute deviations for duplicate analyses of norfloxacin were up to 27–29% in two dewatered sludge samples (Table 4). The greater uncertainty may be ascribed to complex matrix effect that was probably not completely the same for the analytes and the internal standard. In addition, sewage sludge is known to be among the most heterogeneous environmental matrices [35]. The high heterogeneity may also result in greater analysis uncertainty to some extent. No quantifiable amount of the analytes was detected in all procedural and instrumental blanks. The sample-based LOQs were estimated to be 10–63 ng g^{-1} dw for FQs and 250–500 ng g^{-1} dw for TCs, respectively in sewage sludge (Table 3).

Defining the total amounts measured in the five extraction cycles as 100%, the yield of the former three extraction cycles accounted for 83–100% ($n = 3$) for the FQs. Although small amounts of the FQs except lomefloxacin could still be detected in the fourth (7–10%) and fifth (2–7%) cycle, three cycles were finally chosen for extraction of sewage sludge, because too many extraction cycles might also lead to other problems, e.g. tailed HPLC-MS/MS peaks due to interference of complex co-extracts. The same phenomena have been described previously [40]. The TCs were not quantitatively detected in sludge samples and therefore were not included in the quantitative extraction test. No obvious decomposition of the antibacterials was observed during USE treatment. A certain degree of matrix interference was observed even after SPE cleanup with the calculated matrix effect ranging from 129 to 156% for the FQs in the sewage sludge samples.

3.4 *Method application*

The method was applied to investigate the occurrence of fluoroquinolone and tetracycline antibacterials in several sewage sludge and sediment samples collected from the Pearl River Delta, South China. Ciprofloxacin, norfloxacin, and ofloxacin were ubiquitously detected, ranging from 1052 to 17740 ng g^{-1} dw in the dewatered sludge, 585–3545 ng g^{-1} dw in the untreated solid, and 98 to 258 ng g^{-1} dw in the sediment from an urban stream, respectively. Enrofloxacin and lomefloxacin were only detected in sewage sludge from GZSTPB. Concentrations of FQs in sludge from GZSTPB appeared significantly higher than those in sludge from GZSTPA, which may be ascribed to the differences in both wastewater volumes and sources of the two STPs. GZSTPB has a great treatment capacity of 550 000 m^3 g^{-1} and treats predominantly domestic wastewater. In contrast, GZSTPA has a much lower treatment capacity and also receives a certain amount of industrial wastewater. Higher FQs concentrations in GZSTPB are therefore reasonable because these antibacterials are commonly human-used medicine and are consequently mainly discharged through domestic wastewater. The FQs concentrations did not show statistically seasonal difference. Tetracyclines were not quantitatively detected in either sludge or sediment samples (Figure 1, Table 4), probably because TCs are not frequently used for human medicine today. The detected FQ concentrations in the sewage sludge in this work were similar to or higher than those reported for sewage sludge in Switzerland and Sweden (Table 4). The high levels of FQs in the sewage sludge and sediment suggest their high sorption tendency onto and persistence in solid environmental matrices. The sorbed antibacterials may become an important secondary source of antibacterial contamination in environment, e.g. entering soils through application as fertilizer or landfill of sewage sludge. The fate and environmental risks of these sorbed antibacterials need further research.

4. Conclusions

An efficient method was developed and validated for simultaneous determination of frequently used human and veterinary fluoroquinolone and tetracycline antibacterials in sewage sludge using ultrasonic-assisted extraction coupled with SPE cleanup and HPLC-MS/MS detection. Parameters of extraction (extraction solvent, extraction cycles, and pH condition) and HPLC-MS/MS were optimised. The method accuracy and reproducibility were verified. The method can also be used to investigate the occurrence and fate of these antibacterials in sediment and soil. A preliminary study was performed using the method about the occurrence of the FQs and TCs in sewage sludge and sediment of the Pearl River Delta, South China. The result revealed the wide presence and high levels of the commonly used fluoroquinolones in sewage sludge and urban river sediment. This work may serve as a basis for in-depth research on the occurrence, fate, and transport of FQs and TCs during sewage treatment and in natural environment, and, subsequently, for assessment of their release and environmental risks.

Acknowledgements

This work was financially supported by the National Basic Research Program of China (No. 2009CB421604), GIG Innovation Program (No. GIGCX-07-01) and NSFC program (No. U0633005). We thank Mr Chen Huashan at the SKLOG for his assistance in

HPLC-MS/MS. The personnel of the studied STPs are thanked for their help in sampling. This is contribution No. IS-1254 from GIGCAS. The anonymous reviewers are also appreciated for their comments and recommendations that have greatly improved this paper.

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