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Analysis of veterinary antibiotic residues in swine wastewater and environmental water samples using optimized SPE-LC/MS/MS

Lei Tong [a], Ping Li [a], Yanxin Wang [a,*], Kuanzheng Zhu [b]

[a] MOE Key Laboratory of Biogeology and Environmental Geology, School of Environmental Studies, China University of Geosciences, Wuhan 430074, PR China
[b] Provincial Institute of Quality Supervision, Inspection and Quarantine of Products, Wuhan 430061, PR China

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ABSTRACT

Strategies for sample preparation, solid-phase extraction (SPE), clean-up, and detection conditions of an optimized solid-phase extraction-liquid chromatography/mass/mass spectrometry (SPE-LC/MS/MS) method for determining multi-residues of four classes of widely used antibiotics in pig farms, sulfonamides (SAs), fluoroquinolone (FQs), tetracycline (TCs) and chloramphenicol (CAP) were presented. The multi-residue analysis was used in MS analysis, selecting two precursor ions to produce ion transitions for each target compound. Samples of swine wastewater, lake water and groundwater collected from two pig farms in central China were used to test the applicability of the multi-residue analysis method. The average antibiotics concentrations in groundwater, lake water, final effluent and influent swine wastewater were, respectively, 1.6–8.6, 5.7–11.6, 7.9–1172.3 and 8.5–21692.7 ng L^{-1} in summer; respectively, 2.0–7.3, 6.7–11.7, 5.8–409.5 and 32.8–11276.6 ng L^{-1} in winter. The limits of quantification were 0.8–4.1, 1.4–5.5, 1.8–11.5 and 6.4–104.4 ng L^{-1}, respectively, in groundwater, lake water, final effluent and influent swine wastewater. Results of multi-residue analysis of antibiotics in the samples indicate that SAs, FQs and TCs were widely used veterinary medicines in the pig farms. As compared with previous studies, higher elimination rates (more than 80%) of the antibiotics (except DC) were observed in effluent in this study. More detailed work is indispensable to investigate the fate and transport of antibiotics in the environment and to find out cost-effective approaches of removing antibiotics from swine wastewater and contaminated sites.

1. Introduction

In the last three decades, the demand for veterinary antibiotics has notably increased. Large quantities of veterinary antibiotics have been administered for disease control and disinfection in pig farms and different types of antibiotics used as sub-therapeutic for promoting pig growth. Despite the positive effects of the antibiotics, their improper use has become a new environmental problem (Christian et al., 2003; Giger et al., 2003). Residues of antibiotics used for animal husbandry enter the environment either by manure spreading or via sludge storage (Martínez-Carballo et al., 2007). Recent studies have detected residues of antibiotics in different water samples, including municipal wastewater, riverine water, lake water, and even groundwater (Batt et al., 2006; Gros et al., 2006). Once excreted from animal bodies either in unchanged forms or as metabolites (Wiuff et al., 2002), antibiotic residues show different behaviors under different environmental conditions (Managaki et al., 2007).

To mitigate the environmental and health risk of antibiotics, their use as animal growth promoters has been banned in EU countries (Alban et al., 2008) and the US (Neil et al., 2001; FDA, 2003). In China, the biggest producer of pigs in the world, most pig farms do not have facilities for treatment and disposal of manure and wastewater. With high loads of N and P, livestock wastewater effluent has become a major cause for eutrophication of surface waters in China. In recent years, more and more attention has been paid to antibiotics in livestock wastewater. Although the Chinese government has set maximum residue limits of some veterinary antibiotics in animal foodstuff, no regulations have been issued to control the discharge of antibiotics-containing livestock wastewater. Antibiotics such as sulfonamides, tetracycline, macrolide, chloramphenicol and fluoroquinolone are complicated compounds, and difficult to be detected in environmental samples (Hernández et al., 2007), because the co-extracted components depend on the analyte-sample combination and thus hamper the quantification, which is commonly referred to as ion suppression effects that affect precision and accuracy of a method (Jemal et al., 2003; Lindberg et al., 2004). Since monitoring data of antibiotic residues in the environment around pig farms in China have seldom been reported, as compared with those in EU countries (Castiglioni et al., 2006; Gómez et al., 2006; Vieno et al., 2007) and in the US (Batt and Aga, 2005; Kim et al., 2005), methods of sensitive, rapid and selective determination for antibiotics in swine wastewater and environmental water samples are urgently needed.

* Corresponding author. Tel.: +86 27 62879198; fax: +86 27 87481030.
E-mail address: yx.wang1108@gmail.com (Y. Wang).

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Several analytical methodologies have been tested for the determination of antibiotics in surface waters and wastewaters, and the levels of specific antibiotics ranged from ng L^{-1} to μg L^{-1} (Gros et al., 2006; Hernández et al., 2007; Managaki et al., 2007). Due to factors such as low analyte concentrations, complex environmental matrixes and diverse physicochemical properties of the antibiotics, their accurate determination remains a great challenge for environmental studies. The reported methods include liquid–liquid extraction (Lucchetti et al., 2005) and SPE (Gómez et al., 2006) to pre-concentration and clean-up extracts, and subsequent separation by HPLC, and detection by UV (Kay et al., 2005; Schubert et al., 2007), fluorescence (Golet et al., 2001) and mass spectrometry (Batt and Aga, 2005; Batt et al., 2006; Castiglioni et al., 2006; Gómez et al., 2006; Hernández et al., 2007; Vieno et al., 2006). The most frequently used mass detection technique has been single-stage quadrupole MS for years. However, it is currently superseded by triple-stage quadrupole (QqQ-MS) and ion-trap MS (IT-MS^n). And QqQ-MS and IT-MS^n are preferably used in confirmation studies with excellent selected reaction monitoring based selectivity (Stolker and Brinkman, 2005). Among these, SPE followed by LC/MS/MS is the most frequently used methods (Batt and Aga, 2005; Kim et al., 2005; Castiglioni et al., 2006; Gómez et al., 2006; Hernández et al., 2007; Martínez-Carballo et al., 2007; Vieno et al., 2007).

The target compounds for this study include 13 antibiotics belonging to four groups of widely used veterinary medicines in China, sulfonamide (SAs), fluoroquinolone (FQs), tetracycline (TCs) and chloramphenicol (CAP), which have different environmental behaviors and contribute to approximately 50% of the total amount of antibiotics used for human and livestock purposes, respectively. Although some multi-residue methods have been published (Castiglioni et al., 2006; Gómez et al., 2006; Gros et al., 2006; Vieno et al., 2006, 2007), few work has been done on simultaneous extraction and analysis of several classes of compounds covering a wide range of polarities, solubilities, pK_as, K_{ow}s, and stabilities (Ertl, 1997; Bruno et al., 2001; SilviaDíaz-Cruz et al., 2003; Batt and Aga, 2005; Malintan and Mohd, 2006; Connor and Aga, 2007) under acidic and basic conditions. This paper reports the results of our work on multi-residue analysis using liquid chromatography/mass/mass spectrometry (LC/MS/MS) to determine the concentrations of target antibiotics, with a single pre-treatment, chromatographic separation and mass detection method. The applicability of the method was tested by determining antibiotics in swine wastewater and environmental water samples collected from two pig farms in central China.

2. Experimental

2.1. Chemicals and materials

All of the SAs, FQs, TCs and CAP standards were purchased from the Laboratory of Dr. Ehrenstorfer (Augsburg, Germany). All chemicals used were of analytical and HPLC grades and ultrapure water was used in the analysis. The water was purified using a Milli-Q system at 18.3 MΩ cm^{-1}. Individual stock standard solution was prepared in a 1:1 (v/v) mixture of methanol and water and stored at −20 °C. Standard mixtures, at different concentrations, were prepared daily by appropriate dilution of the stock solutions using the same solvents as for stock solutions. The stock solutions of the antibiotic were renewed monthly to eliminate the effect of their instability. All solvents for HPLC application were filtered before use with 0.45 μm membrane filter papers (Millipore). Prior to use, all the glassware were first washed with diluted nitric acid, rinsed with distilled water and then dried in an oven at 180 °C for 4 h.

2.2. Sampling and sample preparation optimization

Water samples were collected from two pig farms in Hubei province in central China in winter (from November to January) and summer (from June to August) of the year 2007. One of the pig farms was new (P1) with a capacity of 20 000 heads slaughtered every year. The other (P2) was established more than 10 years ago and has ten thousand heads slaughtered every year. The wastes on pig house floors, walls and pig bodies were washed and the wastewater was then drained. The wastewater effluent was estimated to be, respectively, 80–100 $m^3 d^{-1}$ (P1) and 50–80 $m^3 d^{-1}$ (P2). Primary wastewater treatment system has been built at P1, which sequentially consists of a screen for solid–liquid separation, an anaerobic fermentation tank for COD removal and methane generation and a rapid geomaterial-slag infiltration pool for further removal of COD as well as N and P. Like many other pig farms in China, there are only solid–liquid separation pre-treatment facilities at P2, which are only capable of removing suspended solids and sediment from the wastewater. For this study, the raw wastewater samples from the hoggeries were collected at the two pig farms, and the final effluents from the treatment system were collected according to the hydraulic retention time of the system at P1. There is an anonymous lake around P1 and there is a 50-m deep well around P2. Considering the occurrence, transport and fate of antibiotics in the environment, the lake water from around P1 and groundwater from around P2 were sampled as well. All the samples were collected as a grab sample using 4 L amber glass bottles without head space. Back home in the laboratory, the samples were immediately filtered first through 1-mm filter and then through 0.45 μm glass fibber filter (50 mm diameter), preserved by adding 1% formaldehyde (water solution), and then stored at 4 °C before SPE extraction to be done within 24 h of storage to avoid any degradation. Preliminary experiments were conducted to assess the efficiency of sample storage procedures by adding 5 mL 5% (w/v) Na_2EDTA into the sample. As a chelating agent, Na_2EDTA was used to avoid bonding of antibiotics with metallic ions. For each type of water, the water samples were spiked in duplicate to test the recovery with and without the addition of Na_2EDTA.

2.3. Solid-phase extraction (SPE)

Experiments were conducted to assess the influence of the sample pH on extraction efficiency. The wastewater adjusted at pH 2.0, 4.0 (adjusted with hydrochloric acid) and 7.0 were spiked, respectively, at 2 μg L^{-1} to determine the recoveries of the target compounds of antibiotics. Recovery refers to the ratio of the experimental concentrations to the theoretical concentration (2 μg L^{-1}). The acidity is critical because recoveries for TCs were found to be higher than expected at low-extraction pH (Kim et al., 2005; Connor and Aga, 2007; Hernández et al., 2007), while recoveries for SAs and SPE co-extraction of humic and fulvic acid from water were influenced by the lower pH of the samples (Gros et al., 2006; Hernández et al., 2007; Managaki et al., 2007).

The cartridges used for SPE were Oasis Hydrophilic–lipophilic balanced (HLB) (60 mg, 3 mL, Waters, Milford MA, USA) which have been widely used (Bruno et al., 2001; FDA, 2003; Gómez et al., 2006; Gros et al., 2006; Malintan and Mohd, 2006; Vieno et al., 2006, 2007; Connor and Aga, 2007; Managaki et al., 2007). Antibiotics were extracted from groundwater (500 mL), lake water (250 mL) and piggery wastewater (50 mL). Before the sample loading, the solid-phase adsorbent was pre-conditioned with 3 mL of Methyl tertiary-butyl ether (MTBE), 3 mL of methanol and 3 mL of water (or hydrochloric acid at pH 2.0 or 4.0 according to the sample acidity) at a flow rate of less than 3 mL min^{-1}. The samples were introduced to the cartridges by means of PTFE tubes at flow rates of 1, 2.5 and 8 mL min^{-1} for wastewater, lake water, and

Table 1
LC/MS/MS conditions for the analytes by MRM in positive ion (PI) and negative ion (NI) mode.

Compound	Retention time (min)	Precursor ion (*m*/*z*)	Products ions (*m*/*z*)	Collision energy (eV)
PI mode				
Sulfadiazine (SDZ)	5.46	251 $[M+H]^+$	156.0 $[M,H-C_5H_6N_2]$	16
Oxytetracycline (OTC)	5.90	461 $[M+H]^+$	425.8 $[M,H-NH_3-H_2O]$	20
Sulfathiazole (STZ)	6.82	256 $[M+H]^+$	156.0 $[M,H-C_4H_5NS]$	14
Sulfamerazine (SMR)	8.37	265 $[M+H]^+$	156.0 $[M,H-C_6H_8N_2]$	17
Sulfamethazine (SMZ)	11.81	279 $[M+H]^+$	156.0 $[M,H-C_7H_{10}N_2]$	17
Norfloxacin (NOR)	12.67	320 $[M+H]^+$	276.0 $[M,H-CO_2]$	17
Ofloxacin (OFL)	12.70	362 $[M+H]^+$	318.0 $[M,H-CO_2]$	17
Ciprofloxacin (CIP)	13.13	332 $[M+H]^+$	288.0 $[M,H-CO_2]$	38
Enrofloxacin (ENR)	14.21	360 $[M+H]^+$	316.0 $[M,H-CO_2]$	17
Tetracycline (TC)	17.17	445 $[M+H]^+$	410.0 $[M,H-NH_3-H_2O]$	19
Doxycycline (DC)	17.33	445 $[M+H]^+$	427.6 $[M,H-NH_3]$	14
Chlortetracycline (CTC)	24.83	479 $[M+H]^+$	444.0 $[M,H-NH_3-H_2O]$	21
NI mode				
Chloramphenicol (CAP)	8.46	321 $[M-H]^-$	152.0	21

groundwater, respectively. After sample loading, the solid phase was washed with 3 mL of 5% methanol–water. Cartridges were then dried for more than 30 min under Supelco vacuum manifold (Sigma–Aldrich), which allowed for the parallel extraction of up to 12 samples and the analytes were then eluted with 2×3 mL of 1:9 (v/v) mixtures of methanol and MTBE. The extracts were rotary evaporated to near dryness at 36 °C under reduced pressure, re-dissolved in 2 mL of methanol and transferred to amber vials, evaporated to near dryness at 40 °C, and then re-dissolved and vortex mixed in 500 µL of methanol–water (1:1). The extracts were stored at −4 °C prior to analysis.

2.4. Instrumental analysis using LC/MS/MS

2.4.1. Liquid chromatography

The extracts were separated on the Dionex Acclaim C18 reversed phase column (2.1 mm i.d. × 150 mm, 4.6 µm) using Dionex HPLC system (Dionex UltiMate 3000 system) with a quaternary pump, a vacuum degasser, an autosampler, and a thermostated column oven kept at 35 °C. Sample aliquots of 10 µL were injected to the C18 column which was gradient-eluted with different solvents. For analysis in the positive ion mode, eluent A was acetonitrile and eluent B was 0.1% formic acid (water solution) at a flow rate of 0.2 mL min^{-1}. The concentration of the mobile phase was initially 5% ACN and maintained for 5 min; then changed linearly between 5 and 20 min to be 50% ACN; then changed in 0.1 min to be 5% ACN; and was then maintained for 6 min. Analysis in the negative mode was done with 20% acetonitrile as eluent A and 80% MilliQ water as eluent B at a flow rate of 0.2 mL min^{-1}.

2.4.2. Tandem MS analyses

The flow from the LC column was transferred to a triple-quadrupole mass spectrometer (TSQ Quantum Ultra AM, Finnigan, USA) equipped with an ESI source. The analyses were performed in the negative ion polarity mode for chloramphenicol and in the positive ion mode for other compounds. Instrument control, data acquisition and evaluation were done with the Xcalibur 2.0 software (Thermo). Determination was performed in multi-residue analysis using the two most intense and specific fragment ions with a scan time of 0.5 s and scan width of 0.05. The electrospray voltage was 5.0 kV and the capillary temperature 350 °C. Argon was used as the collision gas, with shealth gas pressure and auxiliary gas pressure at 35 MPa and 10 MPa, respectively. The collision energy was optimized separately for each analyte (Table 1).

2.5. Quantification and method validation

Concentrations in the samples were calculated by external standard method based on the peak area of the monitored product ion. The stock standard solutions of 13 analytes between 40 mg L^{-1} and 60 mg L^{-1} were prepared. A working solution was prepared by diluting the stock solutions in methanol. The repeatability of the LC/MS/MS method was evaluated by injecting standard solutions of five six concentrations (10, 20, 30, 40, 50, and 100 µg L^{-1}), respectively, into the system. The standard solutions were stable for one month at −20 °C. And to avoid photodegradation, they were kept in amber vials.

To determine the influence of different sample matrixes on LC/MS/MS analysis, the spiked amount of each compound was 0.4 µg L^{-1} for lake water and final effluent of the wastewater, 0.2 and 2.0 µg L^{-1} for groundwater and influent of the wastewater, respectively. For each matrix, recoveries were determined by comparing the obtained concentrations with the initial spiking levels. Since these spiked samples contained target compounds, blanks (samples not spiked) were analyzed to determine the concentrations. Procedural and instrumental blanks were also analyzed to avoid laboratory contamination and analytical interferences. The results obtained are discussed in a specific section below.

The instrument quantitation limit (IQL) for each analyte was determined using pure standards that were analyzed with the LC/MS/MS method. The limit of detection (LOD) was determined from a low concentration reference standard by continuous dilution and calculated using a signal-to-noise ratio of 3 (the ratio between the peak intensity and the noise was used), while limit of quantitation (LOQ) values were calculated with the equation proposed by Vieno et al. (2006), in which the concentration factors and matrix effect of different environmental samples were considered.

Table 2
Characteristic of the samples.

Water quality parameter	Wastewater P1		Wastewater P2	Lake water	Groundwater
	Influent	Effluent			
pH	8.1	8.7	7.6	7.5	7.9
SCOD or DOC (mg L^{-1})	3350 (COD)	267.5 (COD)	2830 (COD)	21.3 (DOC)	1.2 (DOC)
Copper (µg L^{-1})	255.2	14.8	257.7	20.0	4.8
Zinc (µg L^{-1})	2373.0	21.3	2249.0	209.2	10.1

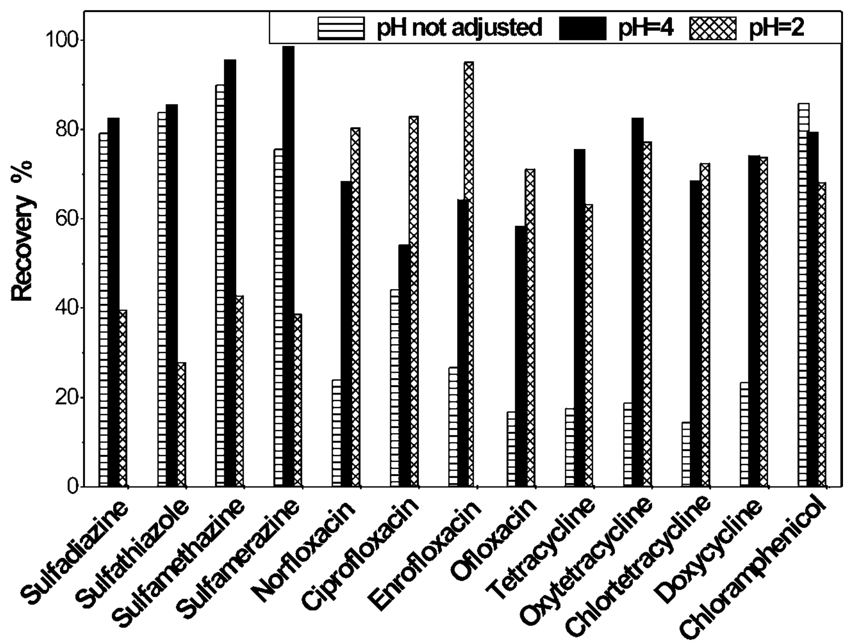

Fig. 1. Effects of pH on recovery rate of analyte (wastewater spiked at 2 μg L^{-1}). The recoveries of all analytes were within acceptable ranges between 54.21% and 98.65% under weakly acidic condition at pH 4.0, which meets the demand to extra all target analytes in one single step.

2.6. Analysis of environmental samples

To investigate the occurrence of the antibiotics in the wastewater treatment system and the natural environment by the optimized SPE-LC/MS/MS method, the four groups of antibiotics in groundwater (P2), lake water (P1), influent and effluent swine wastewater (P1) samples both in summer and in winter were, respectively, analyzed.

3. Results and discussion

3.1. Optimization of sample preparation procedure

Due to the high concentration of organic matters (Table 2) and pathogenic organisms in piggery wastewater, the bacterial activity has to be arrested in order to preserve the integrity of the sample. Some authors recommended the use of chemical preservatives, such as Hg^{2+}, formaldehyde and sodium azide, to halt bacterial activity (SilviaDíaz-Cruz et al., 2003). In this study, a small dose of formaldehyde was used for sample preservation. Heavy metals which were used as the necessities and growth promoters in pig breeding were liable to bonding with some antibiotics (Batt and Aga, 2005), making their extraction difficult. As can be seen from the results, the recoveries of TCs were enhanced nearly 20% and those of other target compounds were not more than 10% in samples with addition of Na_2EDTA, as compared with samples without addition of Na_2EDTA. The TCs contain four fused rings, which have two ketone groups and therefore are prone to chelate to metal ions and strongly bind to proteins and silanol groups (Andersen et al., 2005; Gu et al., 2007). As an excellent chelating agent, the Na_2EDTA was chosen in our study to diminish the interference of heavy metals.

3.2. Solid-phase extraction procedure

Effective sample pre-treatments which normally include extraction and purification steps are essential for detection of the targets at very low concentrations in complex matrixes. The results of recoveries of the 13 antibiotics at pH of 2.0, 4.0 and 7.0 were shown in Fig. 1. Except for CAP, the pH effect on the recoveries of most

Table 3
Linearity, IQLs (pg) and LOQs (ng L^{-1}) of the selected antibiotics.

Compound	Linearity (R^2)	IQL (pg on column)	LOQ (ng L^{-1})			
			Groundwater	Lake water	Wastewater	
					Influent	Effluent
SDZ	0.9934	37	4.1	5.5	6.4	4.5
OTC	0.9886	32	3.5	4.8	8.8	4.1
STZ	0.9996	23	2.8	3.1	7.4	3.8
SMR	0.9975	21	2.1	2.7	12.9	2.2
SMZ	0.9997	30	3.2	4.4	8.9	5.9
NOR	0.9862	19	1.8	2.7	6.4	4.3
OFL	0.9957	16	1.9	2.4	22.0	7.0
CIP	0.9932	17	1.8	1.5	10.3	2.1
ENR	0.9982	12	1.0	1.5	11.5	3.8
TC	0.9972	13	1.5	1.7	55.6	6.3
DC	0.9953	22	2.7	3.6	72.1	8.6
CTC	0.9875	13	1.0	2.2	104.4	11.5
CAP	0.9995	8	0.8	1.4	10.1	1.8

analytes was strong. The same classes of analytes followed similar patterns at different pH's. It can be seen that at pH 2.0, recoveries of FQs and TCs were more than 70% and 60%, respectively, whereas those of SAs lower than 40%. By contrast, under neutral condition, the recoveries of SAs were higher than 70%, while those of TCs and FQs less than 30% (except CIP). And the recoveries of all analytes were within acceptable ranges from 54.2% to 98.7% under weakly acidic condition at pH 4.0, which meets the demand to extract all target analytes in one single step.

The different recovery rates under different pH conditions could be attributed to the amphoteric properties that most of the analytes have. For instance, FQs have carboxylic acid ($pK_a \approx 5$) and one or more amine functional groups ($pK_a \approx 8$–9), and SAs show either characteristics of weak alkali due to anilinic nitrogen, or characteristics of weak acids due to N–H bond of the sulfonamidic group. Most of the analytes exhibited poor water solubility between pH 6 and 8, behaved as weak acids and formed salts in strongly acidic or alkaline solutions.

3.3. Instrumental analysis

The analysis of the antibiotics in the extracts obtained was done using HPLC, which is preferred to other instruments for thermoliable characteristic of these analytes. In order to prolong the column life, salts such as ammonium acetate (Malintan and Mohd, 2006) were not added in the mobile phase. Acetonitrile was chosen to separate the 13 antibiotics and formic acid added into the mobile phase to enhance peak resolution and sensitivity.

The electronic spray ionization (LC-ESI)-tandem MS offered high sensitivity and improved selectivity through multiple reaction monitoring (MRM) acquisition to detect antibiotics in real samples. The MRM transitions were determined for each compound with direct infusion of pure reference standards into the MS/MS compartment. Upon ionization, all the compounds except CAP produced positive precursor ions (Table 1).

3.4. Quantification and method validation

The optimized SPE-LC/MS/MS analysis method was validated by linearity, recoveries, precision and detection limits in each matrix. For the linearity validation, relative standard deviations (RSD) of five-point calibration tests less than 10% were considered to be reliable. The recoveries of the target compounds were different in different matrixes, ranging from 76.9% (OFL) to 115.1% (SMR), from 68.6% (OFL) to 92.6% (SMR), from 62.5% (OFL) to 93.1% (SMR) and from 58.4% (OFL) to 98.7% (SMR), respectively, for groundwater, lake water, effluent and influent wastewater samples. The wastewater showed the lowest recovery in these matrixes, probably due to large interference of co-extracted compounds. Among all the compounds, recoveries of OFL and SMR were the lowest and the highest, respectively.

The effective linearity, instrumental quantification limits (IQL) and detection limits of analytes for the entire method (LOQ) in different matrixes of groundwater, lake water, influent and effluent of the wastewater were determined (Table 3). The IQL was determined using pure standards that were analyzed with the LC/MS/MS method. The LOQ ranged from 0.8 to 4.1 $ng\,L^{-1}$ in groundwater

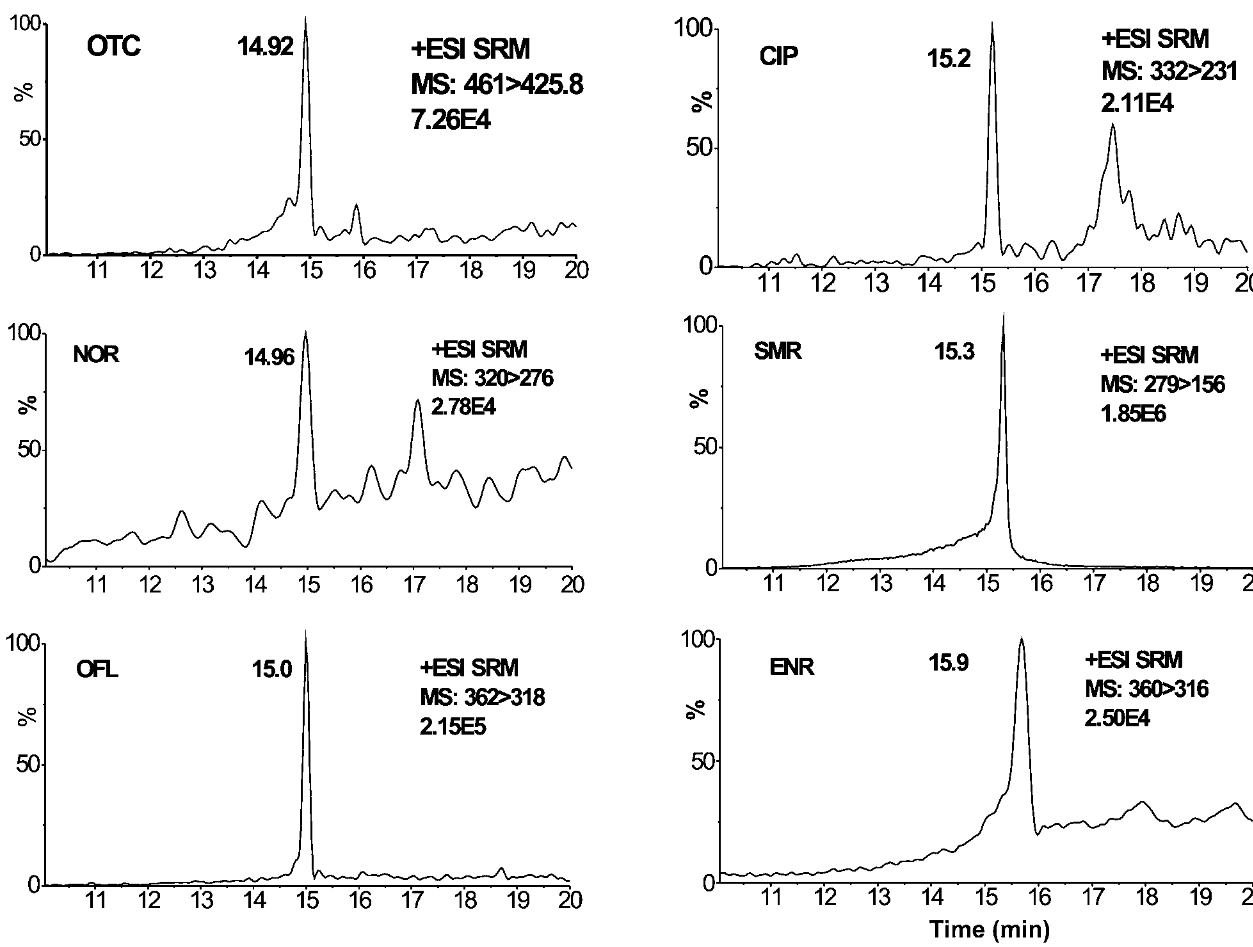

Fig. 2. MRM LC–MS/MS chromatogram resulting from analysis of monitored antibiotics in final effluent at P1.

and from 1.4 to 5.5 ng L^{-1} in lake water (Table 3). The influence of matrix effect was not obvious in groundwater as well as in lake water, but a little higher in wastewater. In final effluent of the wastewater, the LOQ ranged from 1.8 to 11.5 ng L^{-1}, while the LOQ ranged from 6.4 to 104.4 ng L^{-1} in influent wastewater. It seems that the co-eluting matrix components may cause suppression of the analyte signal during electrospray ionization (Vieno et al., 2006). The typical chromatogram of monitored antibiotics in effluent wastewater was shown in Fig. 2.

3.5. Environmental analysis application

In order to assess the occurrence of antibiotics in swine wastewater and the recipient environmental waters, the optimized SPE-LC/MS/MS method was applied to determine the antibiotic residues in environmental samples of groundwater, lake water and wastewater from P1 and P2 (Fig. 3). All of the compounds except SMZ were detected at considerable concentrations, and most of the concentrations of antibiotics, especially FQs and TCs, were higher in winter than those in summer. The estimated concentrations of individual antibiotic compounds from wastewater and environmental water ranged from 8.5 ng L^{-1} to 21692.7 ng L^{-1} and from 1.6 ng L^{-1} to 11.7 ng L^{-1}, respectively. The seasonal change of water usage in pig farms and difference in environmental conditions should be responsible for the observed concentration variations of the analytes. The effect of temperature on pollutant degradation in different seasons may be another important factor as well.

The detected antibiotics have also been reported in groundwater and lake water, as well as in swine wastewater in previous studies (Campagnolo et al., 2002; Meyer et al., 2000, 2003). For example, the concentration of CAP in our samples of lake water and groundwater was around 2.0 ng L^{-1} (Fig. 3), and not detected in any samples of the wastewater. This may be related to the banned use in livestock breeding since 2002 in China because of the awareness of the plastic anaemias in humans arising from the use of CAP (Stolker and Brinkman, 2005). But its earlier use and slow degradation resulted in its detection in our environmental water samples. A German group detected residues of CAP in one sewage treatment plant effluent and one small river in southern Germany at concentrations of 0.56 and 0.06 μg L^{-1}, respectively (Hirsch et al., 1999), although the use of CAP in livestock breeding has been banned since 1994 in the US and EU. SAs, FQs and TCs were also found in environmental water in our samples, although the concentrations of the individual compounds detected were commonly less than 12 ng L^{-1}. It is interesting to note that multiple classes of antimicrobial compounds (tetracycline, b-lactam, macrolide, sulfonamide) were also detected in and groundwater samples collected in places close to swine farms in the US (Campagnolo et al., 2002).

Four FQs antibiotics were detected in all samples, including lake water and groundwater (Fig. 3A), although the FQs do not have stronger persistence and their degradability was relatively fast in the aquatic environment (Xu et al., 2007). Among the FQs, concentration of CIP was higher than others in our environmental water samples. Seven antibiotics (NOR, OFL, CIP, ENR, TC, OTC, and CTC) were detected in samples from both P1 and P2 in summer and winter, as they were used extensively in pig farms. In an earlier work (Vieno et al., 2006), it has been found that the FQs and mainly CIP was detected and quantified in the STP influents, and that in the effluent samples they were present in concentrations of 5.7–36 ng L^{-1}, that are near or under the quantification limit.

On the other hand, SAs commonly used as veterinary medicine in the past were not found at significant concentrations except SMR. The result may be related to their limited use because the SAs were used as veterinary drugs for prophylactic and therapeutic purposes, and have potential carcinogenic and possible development of antibiotic resistance in animals. The SMR was frequently detected at μg L^{-1}

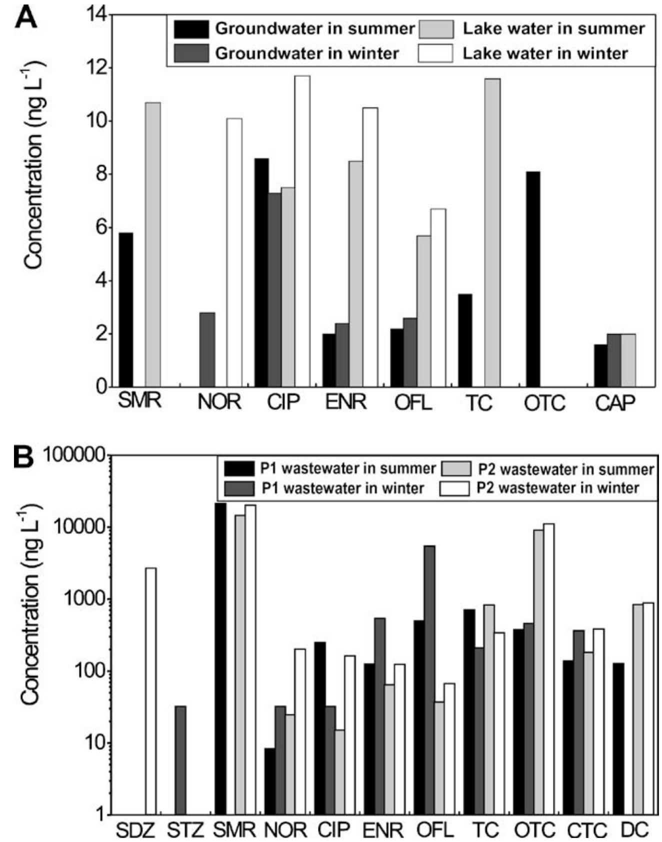

Fig. 3. Comparison of the different concentrations of antibiotics in groundwater, lake water and wastewater in summer and winter.

concentrations in wastewater in summer (Fig. 3B). Similar case of SAs contamination was reported in the US (Meyer et al., 2003).

A total of seven antibiotics, including four FQs, two TCs and one SAs, were detected in the final effluent in summer or winter at P1 (Fig. 4). The antibiotics were abundant in effluent, with concentrations from 5.8 to 1172.3 ng L^{-1}. The antibiotics showing the highest and lowest elimination rate of the treatment system were, respectively, CIP (96.9%) and DC (64.9%) in summer. Higher elimination rates of the FQs (more than 80%) were observed in effluent in this study as well as in other studies (Xu et al., 2007). Sorption to the sludge of the anaerobic process may be the main elimination process for FQs removal (Vieno et al., 2007). Given the elimination rates of FQs, their high residue contents (ranging from 5.8 to 409.5 ng L^{-1}; four FQs were detected) in the wastewater effluent was probably due to over loads from the pig farms. OTC and DC were also detected in the effluent. And the DC which had been reported to be highly persistent in the environment showed the lowest removal rate in this study. A similar previous study reported OTC concentrations in a river basin with livestock farms as high as 68 μg L^{-1} which increased in winter (Matsui et al., 2008). SMR, the major infectious medicine in the raw wastewater samples, was also the major detected infectious medicine in the effluent waters. And the SMR concentration was up to 21 692.7 ng L^{-1} in the effluent (Fig. 4), far exceeding the concentration levels commonly reported in the literature (Karthikeyan and Meyer, 2006; Malintan and Mohd, 2006). The STZ and CTC were commonly not detected in the effluent samples for this study, probably due to its presence in low concentrations near or under the LOQ or completely removal by the treatment system. It was found that they can be partly removed by sorption and photodegradation (Halling-Sørensen et al., 1998). Therefore, both low loads in the raw wastewater and dilution by rain water may explain the observed low levels of STZ and CTC.

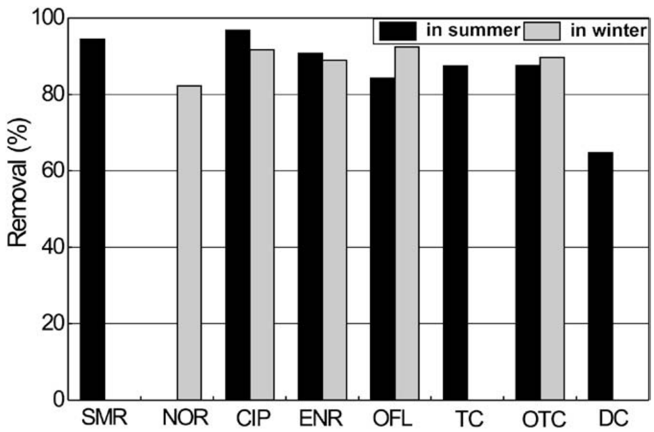

Fig. 4. Comparison of the removal efficiencies of selected antibiotics at P1 in summer and winter.

It should be noted that the data on the occurrence and fate of antibiotics from pig farms into the aquatic environment have been limited so far, and it is indispensable to investigate the fate and transport of the antibiotics in the environment and to find out cost-effective approaches to remove them from swine wastewaters and contaminated sites. Besides, although the analytes chosen for this study were typical indicators of antibiotic use livestock industry, characterization of other classes of antibiotics such as macrolids and β-lactams that have been widely used in pig breeding practice is also needed to determine the best indicators of antibiotic use and environmental contamination of swine feeding activities.

4. Conclusions

An optimized SPE-LC/MS/MS method to analyze multi-residues of selected TCs, SAs, FQs and CAP in groundwater, lake water and swine wastewater was presented in this paper. The concentrations of antibiotics residues in groundwater, lake water, final effluent and influent swine wastewater were, respectively, 1.6–8.6, 5.7–11.6, 7.9–1172.3 and 8.5–21 692.7 $ng\,L^{-1}$ in summer; and, respectively, 2.0–7.3, 6.7–11.7, 5.8–409.5 and 32.8–11 276.6 $ng\,L^{-1}$ in winter. The LOQ levels were 0.8–4.1, 1.4–5.5, 1.8–11.5 and 6.4–104.4 $ng\,L^{-1}$, respectively, in groundwater, lake water, final effluent wastewater and influent wastewater.

Overuse of the antibiotics is suspected and pollution control measures to protect the aquatic environment are urgently needed. Even though the antibiotics were detected at relatively low concentrations, there are high risks of their potential harms to non-target organisms and, finally, to human health. More intensive research is needed to better understand the fate and transport of these compounds in the aquatic environment and it is indispensable to develop cost-effective technologies to remove them from wastewaters and contaminated sites.

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