Front. Environ. Sci. Eng. 2014, 8(3): 357–371
DOI 10.1007/s11783-013-0580-6

RESEARCH ARTICLE

Simultaneous quantification of several classes of antibiotics in water, sediments, and fish muscles by liquid chromatography–tandem mass spectrometry

Yimei WEI[1,2], Yuan ZHANG[2], Jian XU (✉)[2], Changsheng GUO[2], Lei LI[2], Wenhong FAN[3]

1 College of Environment, Liaoning University, Shenyang 110036, China
2 State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing 100012, China
3 Department of Environmental Science and Engineering, School of Chemistry and Environment, Beihang University, Beijing 100191, China

Abstract Precise and sensitive methods for the simultaneous determination of different classes of antibiotics, including sulphonamides, fluoroquinolones, macrolides, tetracyclines, and trimethoprim in surface water, sediments, and fish muscles were developed. In water samples, drugs were extracted with solid-phase extraction (SPE) by passing 1000 mL of water through hydrophilic lipophilic balanced (HLB) SPE cartridges. Sediment samples were solvent-extracted, followed by tandem SPE (strong anion exchange (SAX) + HLB) clean-ups. Fish muscles were extracted by a mixture of acetonitrile and citric buffer (80:20, v/v) solution, and cleaned by SPE. Liquid chromatography–tandem mass spectrometry (LC-MS/MS) with multiple reaction monitoring (MRM) detection was employed to quantify all compounds. The recoveries for the antibiotics in the spiked water, sediment, and fish samples were 60.2%–95.8%, 48.1%–105.3%, and 59.8%–103.4%, respectively. The methods were applied to samples taken from Dianchi Lake, China. It showed that concentrations of the detected antibiotics ranged from limits of quantification (LOQ) to 713.6 $ng \cdot L^{-1}$ (ofloxacin) in surface water and from less than LOQ to 344.8 $\mu g \cdot kg^{-1}$ (sulphamethoxazole) in sediments. The number of detected antibiotics and the overall antibiotic concentrations were higher in the urban area than the rural area, indicating the probable role of livestock and human activities as important sources of antibiotic contamination. In fish muscles, the concentration of norfloxacin was the highest (up to 38.5 $\mu g \cdot kg^{-1}$), but tetracyclines and macrolides were relatively low. Results showed that the methods were rapid and sensitive, and capable of determining several classes of antibiotics from each of the water, sediment, and fish matrices in a single run.

Keywords antibiotics, liquid chromatography–tandem mass spectrometry (LC-MS/MS), water, sediment, fish muscle

1 Introduction

Antibiotics are commonly used in veterinary and human medicine for the prevention and treatment of microbial infections. In the natural environment antibiotics are not readily biodegradable [1], resulting in the frequent detection of these compounds in surface waters, groundwater, wastewater effluent, landfill leachate, and soils irrigated with reclaimed water [2,3]. Sulphonamides, fluoroquinolones, macrolides, tetracyclines, and trimethoprim are several groups of typical antibacterial agents widely employed in treating bacterial infections in livestock, poultry production, fish farming, and human health [4]. These compounds are introduced into the environment via biosolid application to agricultural farmlands, land irrigation with reused wastewater, and the release of wastewater effluent into receiving water bodies [5,6]. The residues of these compounds in the environmental matrix are of great concern to both scientific and regulatory communities, because the presence of these drugs can result in an increase of drug-resistant bacteria, cause potential adverse side effects in humans, such as allergic reactions in hypersensitive individuals, or can be potential carcinogens [7].

Numerous methods have been developed for identification and quantification of antibiotics in complex aqueous media (e.g., wastewater) and in different solid matrices

Received July 3, 2012; accepted May 21, 2013

E-mail: xujian@craes.org.cn

(e.g., sludge, sediment, soil, biota) at trace levels. Extraction methods of these compounds from aqueous samples include liquid-liquid extraction [8], solid-phase extraction (SPE) [9], and solid-phase microextraction (SPME) [10], among others. In the case of solid samples, matrix solid-phase dispersion (MSPD) [11], ultrasonic solvent extraction (USE) [6,12], microwave assisted solvent extraction (MASE) [13], pressurized liquid extraction (PLE) [14], and supercritical fluid extraction (SFE) [15] are employed. Quantification of contaminants in the extracts might be achieved by employing gas chromatography–mass spectrometry (GC–MS), gas chromatography–tandem mass spectrometry (GC–MS/MS), liquid chromatography–mass spectrometry (LC–MS), or liquid chromatography–tandem mass spectrometry (LC–MS/MS) techniques [16,17]. In complex environmental matrices, MS is the only generally applicable quantification technique for the simultaneous determination of a variety of organic compounds present at very low concentrations.

However, although antibiotics are known to exist in the environment, there is no telling, for environmental monitoring, which antibiotic compound is actually present and in what level each compound occurs. While numerous literature reported the analysis of a certain class of antibiotics in one medium, only a few investigated the determination of multiple classes of antibiotics in the environment [7,18,19]. Therefore, it necessitates the development of methods capable of determining several classes of antibiotics in environmental samples simultaneously. In this study, we tested effective analytical protocols that simultaneously detected multiple classes of antibiotics in environmental media, including water, sediments, and fish tissues at environmentally relevant levels. Specifically, seven sulphonamides (sulphamethizole, sulphacetamide, sulphathiazole, sulphachloropyridazine, sulphamethoxazole, sulphisoxazole, and sulphadimethoxine), four fluoroquinolones (norfloxacin, ofloxacin, ciprofloxacin, and enrofloxacin), two macrolides (roxithromycin and erythromycin), three tetracyclines (oxytetracycline, tetracycline, and chlortetracycline), and trimethoprim were selected as target chemicals. The proposed protocols were applied to real environmental samples taken from Dianchi Lake to evaluate the antibiotic contamination status.

2 Materials and methods

2.1 Chemicals

Sulphacetamide (SCT), sulphathiazole (STZ), trimethoprim (TMP), norfloxacin (NOR), ofloxacin (OFL), oxytetracycline (OTC), ciprofloxacin (CIP), tetracycline (TC), sulphamethizole (SMZ), chlortracycline (CTC), sulphachloropyridazine (SCP), sulphamethoxazole (SMX), sulphisoxazole (SIA), sulphadimethoxine (SDX), enrofloxacin (ENR), erythromycin (ERM), and roxithromycin (ROM) were purchased from Sigma-Aldrich Co. (Shanghai, China). Surrogate $^{13}C_3$-caffeine was obtained from Cambridge Isotope Laboratories (USA). Acetonitrile (ACN) and methanol (high performance liquid chromatography (HPLC) grade) were obtained from Fisher Scientific (Houston, TX, USA). Ultra-pure water (18.3 $M\Omega \cdot cm^{-1}$) used in this study was purified from a Milli-Q system. Analytical grade formic acid, disodium ethylenediamine tetraacetate (Na_2EDTA), acetone, and other chemicals were all purchased from Beijing Chemical Reagents Company (Beijing, China). Chemical Abstracts Service (CAS) registry numbers, molecular weights, and chemical structures of the compounds are shown in Table 1.

The stock solutions of antibiotics were prepared by dissolving each compound in methanol at a concentration of 100 $mg \cdot L^{-1}$, with the exception of fluoroquinolone antibiotics, which were dissolved in methanol containing 0.1% 1 $mol \cdot L^{-1}$ formic acid. Solutions were stored at −18°C.

2.2 Sample collection

Dianchi Lake (24°28′ – 25°28′ N, 102°30′ – 103°00′ E) is located in the middle of the Yunnan-Guizhou Plateau in south-west China. The entire basin has a total area of approximately 2920 km^2, including part of Kunming City (the capital of Yunnan Province), and Songming, Chenggong, Jinning, and Xishan Counties. The lake serves many social and economic purposes, with 2.68 million residents in the Dianchi Basin, and yearly burden of 216×10^6 m^3 of household wastewater and 47.6×10^6 m^3 of industrial wastewater. A total of 27 samples (water and sediments) were collected in October 2010 (Fig. 1). All water samples were collected 1 m below the surface with a water grab sampler and stored in pre-cleaned brown glass bottles. Surface sediment samples (0–5 cm) were collected with a stainless steel grab sampler and placed in polypropylene bags. Samples were transported to the laboratory on ice for further treatment.

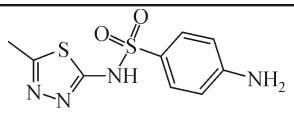
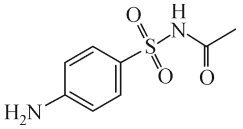
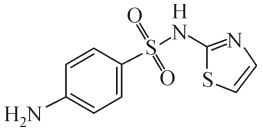
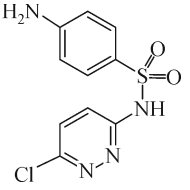
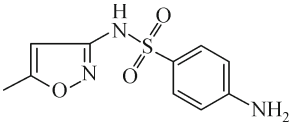
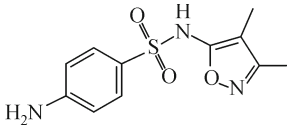
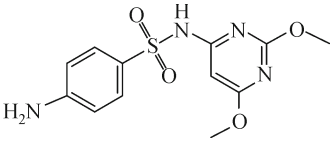
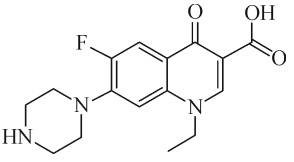
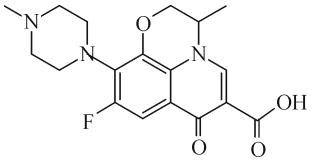
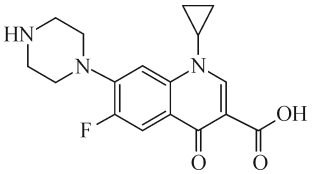
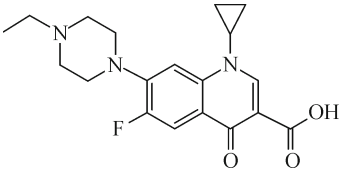
Thirteen fish samples from the lake were obtained from the local market and analyzed for target compounds. All tissue samples were homogenized and stored at −20°C until analysis.

2.3 Sample treatment

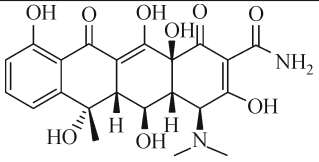
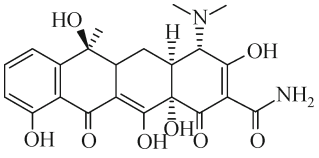
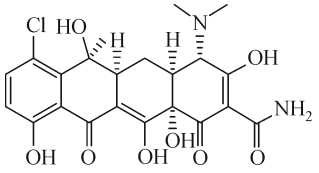
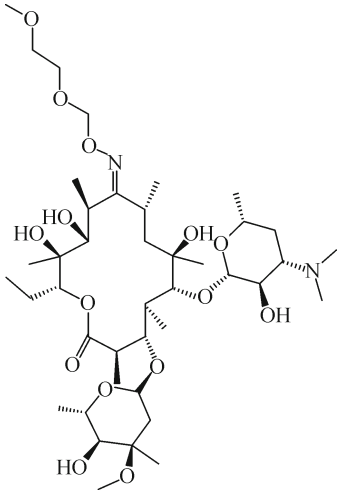
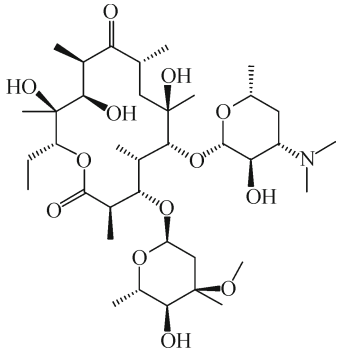
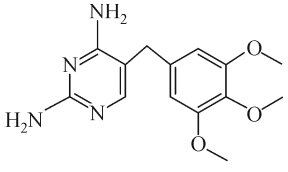
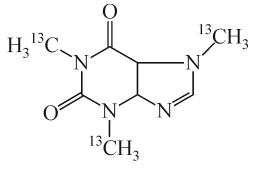
2.3.1 Extraction of water samples

Lake water from Miyun Reservoir (drinking water source for Beijing, China) was spiked with a mixture of antibiotics at two levels of 10 $ng \cdot L^{-1}$ and 100 $ng \cdot L^{-1}$. One thousand milliliters (1000 mL) of water samples were filtered through glass microfiber filters (GF/B, Whatman), and 100 ng $^{13}C_3$-caffeine was added as a surrogate. The pH

Table 1 CAS registry numbers, molecular weights, and chemical structures of selected antibiotics

analyte (class)	compound	CAS number	molecular weight	structure
sulphonamides	sulphamethizole	144-82-1	270.34	
	sulphacetamide	144-80-9	214.24	
	sulphathiazole	72-14-0	255.32	
	sulphachloropyridazine	80-32-0	284.73	
	sulphamethoxazole	723-46-6	253.28	
	sulphisoxazole	127-69-5	267.31	
	sulphadimethoxine	122-11-2	310.33	
fluoroquinolones	norfloxacin	70458-96-7	319.33	
	ofloxacin	82419-36-1	361.37	
	ciprofloxacin	85721-33-1	331.34	
	enrofloxacin	93106-60-6	359.39	

(*Continued*)

analyte (class)	compound	CAS number	molecular weight	structure
tetracyclines	oxytetracycline	79-57-2	460.44	
	tetracycline	60-54-8	444.44	
	chlortracycline	57-62-5	478.89	
macrolides	roxithromycin	80214-83-1	837.05	
	erythromycin	114-07-8	733.93	
bacteriostatic antibiotic	trimethoprim	738-70-5	290.32	
surrogate	$^{13}C_3$-caffeine	78072-66-9	197.18	

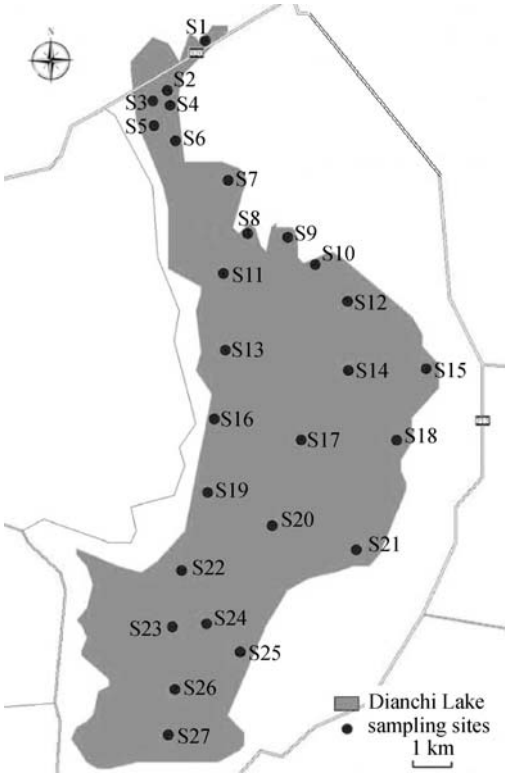


Fig. 1 Sampling locations in Dianchi Lake, China

value was adjusted to 3 with concentrated sulfuric acid. Na_2EDTA (0.2 g) was added, and samples were loaded at a flow rate of 10 mL·min^{-1} on Waters Oasis hydrophilic lipophilic balanced (HLB) cartridges (500 mg, 6 mL), which were pre-conditioned sequentially with 5 mL of methanol, 5 mL of water, and 5 mL of 10 mmol·L^{-1} Na_2EDTA (pH 3.0) solution. The cartridges were rinsed with 5 mL of 5% methanol aqueous solution and 5 mL of ultra-pure water, and dried over a vacuum. Antibiotics were eluted with 10 mL of methanol, and the eluant was concentrated under gentle nitrogen to near dryness. The extract was brought to 1.0 mL with methanol and ready for analysis.

2.3.2 Extraction of sediment samples

Sediment samples for extraction and recovery tests were taken from Miyun Reservoir, freeze-dried, and passed through a sieve with 0.5 mm openings. Aliquots of the chemicals in methanol solution were mixed with 10 g of sediment. To ensure the even distribution of chemicals in the sediments, adequate acetone was added and the sediments were stirred [17]. The solvents in the spiked sediments were allowed to evaporate at room temperature in a darkened fume hood for 12 h. The spiked sediments were then thoroughly mixed with a certain amount of untreated sediment to attain the desired concentration. Sodium azide was added to inhibit microbial activities. During the method development, different experimental conditions were tested to optimize the extraction of the target antibiotics from spiked sediments (100 μg·kg^{-1}), including pH value of extraction buffer (3, 5, and 7) and different volume ratios of extraction buffer and organic solvent. A mixture of citric buffer (pH 5) and methanol (1:1, v/v) was finally selected as the extraction solvent mixture for the developed method.

The $^{13}C_3$-caffeine surrogate (100 ng) was added to 2 g of sediment, and 30 mL of extraction buffer (pH = 5) consisting of 15 mL of methanol, 5 mL of 0.1 mol·L^{-1} Na_2EDTA, and 10 mL of citrate buffer was added. Each sample was vortexed at 300 r·min^{-1} for 20 min, ultrasonic extracted for 15 min, and centrifuged at 4000 r·min^{-1} for 5 min. The supernatant was decanted into a brown glass bottle. The extraction was repeated two more times and the supernatants were combined. Rotary evaporation was applied to remove organic solvent from the supernatant, and the residue was brought to 500 mL with ultra-pure water. The strong anion exchange (SAX) cartridge (3 mL, 200 mg, Thermo, USA) and Waters Oasis HLB cartridge were tandem connected to extract antibiotics from the solution. The diluted extracted solution was passed through the cartridges at a flow rate of 10 mL·min^{-1}. After the sample loading, the SAX cartridge was discarded, and the HLB cartridge was washed with 5 mL of deionized water, vacuum dried for 1.5 h, and eluted with 10 mL of methanol. The procedures were the same as that for water sample treatment.

2.3.3 Extraction of fish samples

The extraction procedure of fish muscle samples used in the present study was modified according to the method described by Juan-Garcia et al. [20], where they used a mixture of ACN and citric buffer (80:20, v/v) solution as extractant. The extraction consisted of two steps: extraction of the antibiotics from the fish muscles by vortex shaking and sonication, followed with enrichment and clean-up of the extract by SPE. The crucial step in the multi-residue antibiotic analysis is the extraction and clean-up. The pH values of the buffers (citric buffer) and extractant volumes were tested for the optimization of the extraction method.

After being homogenized with a high-speed blender, fish muscle samples (2.5 g) were added into a 50-mL polypropylene centrifuge tube. The 100 ng of $^{13}C_3$-caffeine surrogate was spiked into the tubes and the samples were allowed to stand for 15 min in darkness. One milliliter of Na_2EDTA (0.1 mol·L^{-1}) solution and a certain amount of extractant mixture (ACN + citric buffer (80:20, v/v)) were added into each centrifuge tube. Extraction was carried out using a vortex mixer at 300 r·min^{-1} for 10 min and sonicated for 15 min. After 5 min centrifugation at 4500 r·min^{-1} at 4°C, the supernatant was transferred to a 200 mL Florence flask. The extraction was performed two

more times and supernatants were decanted to the same flask. Prior to the SPE purification, organic solvent in the supernatants was rotary evaporated, and the extract was brought to 500 mL with ultra-pure water. The treatment was the same as extraction for water samples.

2.4 LC–MS/MS analysis

The target antibiotics were analyzed by HPLC–MS/MS. The HPLC separation was performed using an Agilent 1200 series (Palo Alto, CA, USA) equipped with an Agilent Zorbax Eclipse XDB-C18 column (4.6 mm × 150 mm, 5 μm). The column was maintained at 30°C during the sample analysis. The mobile phase consisted of eluent A (acetonitrile) and eluent B (0.1% formic acid in ultra-pure water). The flow rate was kept at 0.3 mL·min^{-1} and the injection volume was 10 μL. The separation of antibiotics was achieved with a gradient program as follows: 0–8 min: 15% A; 8–16 min: 15%–50% A; 16–24 min: 50%–60% A; 24–26 min: 15%–60% A; 26–28 min: 15% A. The system was re-equilibrated for 10 min between runs.

Mass spectrometric analyses were performed in an Agilent 6410 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source that operated in the positive ionization (PI) mode. The nebulizer pressure was set to 40 psi and the flow rate of drying gas was 3 L·min^{-1}. The capillary and nozzle voltages were 4000 and 0 V, respectively. The flow rate and temperature of the sheath gas were 8 L·min^{-1} and 350°C, respectively. Sample acquisition was performed in the multiple reaction monitoring (MRM) mode.

2.5 Quantification and method validation

The calibration curves for detection of the analytes were obtained by performing a linear regression analysis on spiked samples. Concentrations in the samples were calculated by external standard method based on the peak area of the monitored product ion. The linearity obtained for all analytes was good in the investigated ranges from all three matrices, with correlation coefficients higher than 0.98.

Recoveries of the antibiotics were tested with the water, sediment, and fish samples from Miyun Reservoir. Water was spiked at two levels (10 and 100 ng·L^{-1}) and sediment and fish were spiked at three levels (50, 100, and 200 μg·kg^{-1}). The $^{13}C_3$-caffeine was spiked to the samples as a surrogate prior to extraction. Limit of detection (LOD) and limit of quantification (LOQ) of the antibiotics were calculated with signal/noise ratios (S/N) of 3 and 10, respectively. The S/N ratios were obtained using the software Masshunter (Agilent) by processing the results of the recovery tests done at a concentration of 5 μg·kg^{-1} for sulphonamides and macrolides and 10 μg·kg^{-1} for fluoroquinolones and tetracyclines.

3 Results and discussion

3.1 HPLC–MS/MS analysis

ESI is an excellent and mild ionization method and suitable for ionizing polar and non-polar compounds [21]. ESI–MS conditions in this work were optimized by direct infusion of a standard solution of 1 mg·L^{-1} of each target compound. Retention time and MRM transitions were determined for each compound with direct infusion of pure reference standards into the MS/MS compartment. Table 2 shows the optimal conditions for the identification and quantification of target analytes. Chromatographic conditions were optimized to provide an overall optimum peak shape and resolution. Thus, the mobile phase composition was investigated to maximize the method sensitivity and resolution, obtaining the best results when acetonitrile was used as an organic modifier and an aqueous solution of 0.1% formic acid was employed. Low pH buffer by formic acid was reported to enhance protonation and help in eluting the analytes completely without tailing, which assisted in proper quantification of analyte peaks [22]. For mass spectrometric analysis, operating conditions such as collision energy, fragmentor voltage, precursor ion, and product ion for each antibiotic were determined with the Agilent software, Optimizer. MS acquisition was performed in the MRM mode, with the ESI operating in the positive mode. Figure 2 shows a total ion chromatogram (TIC) of the antibiotic standards from sediment extraction, indicating that the optimized chromatographic conditions gave good outcomes for the separation and resolution of target compounds. Extracted ion chromatograms of MRM for the antibiotics are shown in Fig. 3, from which it can be seen that the peak shapes for each compound were very good.

3.2 Extraction and method validation

During the extraction of antibiotics from real environmental matrices, the interference of heavy metal ions has to be avoided, because antibiotics are prone to chelating to metal ions, making the extraction difficult. In this study, Na_2EDTA, an excellent chelating agent, was added into the extractant to diminish the interference of heavy metals. The addition of Na_2EDTA was reported to enhance the recovery of TCs by 20% [4].

3.2.1 Water samples

Spiking experiments are generally used to determine the recovery rates and extraction yields for the developed analytical methods. Our experiments for water extraction were conducted under pH 3 at two spiking concentrations (10 and 100 ng·L^{-1}). Lake water from Miyun Reservoir was used, in which no antibiotics were detected.

Table 2 Optimal LC–MS/MS parameters for multiple reaction monitoring (MRM) acquisition conditions of each antibiotic for the MS detector

compound	retention time /min	fragmentor voltage	MRM transitions /(m/z)	collision energy /eV
sulphacetamide	9.90	90	215 → 156[a)]	5
		90	215 → 108[b)]	18
$^{13}C_3$-caffeine[c)]	10.32	120	198 → 120[a)]	15
sulphathiazole	10.79	110	256 → 156[a)]	10
		110	256 → 108[b)]	20
trimethoprim	12.08	110	291 → 230[a)]	25
		110	291 → 261[b)]	25
norfloxacin	13.31	90	320 → 302[a)]	18
		90	320 → 276[b)]	18
ofloxacin	13.65	110	362 → 318[a)]	15
		110	362 → 261[b)]	25
oxytetracycline	13.97	120	461 → 426[a)]	15
		120	461 → 444[b)]	10
ciprofloxacin	14.95	110	332 → 314[a)]	20
		110	332 → 231[b)]	35
tetracycline	16.67	120	445 → 410[a)]	15
		120	445 → 427[b)]	10
enrofloxacin	16.85	130	360 → 316[a)]	15
		130	360 → 342[b)]	15
sulphamethizole	17.19	100	271 → 156[a)]	12
		100	271 → 92[b)]	25
chlortetracycline	18.60	130	479 → 444[a)]	18
		130	479 → 462[b)]	13
sulphachloropyridazine	19.53	100	285 → 156[a)]	10
		100	285 → 92[b)]	25
erythromycin	19.99	90	734.5 → 158[a)]	35
		90	734.5 → 576[b)]	15
sulphamethoxazole	20.31	100	254 → 156[a)]	10
		100	254 → 92[b)]	25
sulphisoxazole	20.79	100	268 → 156[a)]	8
		100	268 → 92[b)]	25
roxithromycin	21.61	130	837.5 → 679[a)]	15
		130	837.5 → 158[b)]	35
sulphadimethoxine	21.76	100	311 → 156[a)]	18
		100	311 → 92[b)]	35

Notes: a) product ion used for quantification; b) product ion used for identification; c) surrogate standard

Many studies have investigated the optimal pH values for antibiotic extraction using the solid-phase extraction procedure, and almost all literature reported that acidic conditions gave better recoveries for antibiotics. Some antibiotics have amphoteric properties: for instance, fluoroquinolone has carboxylic acid (pKa 5) and one or more amine functional groups (pKa 8–9), and sulphonamides show either characteristics of weak alkalinity due to anilinic nitrogen, or characteristics of weak acids due to the N-H bond of the sulphonamidic group [4]. Besides, some antibiotics are prone to hydrolysis under neutral or basic conditions. For example, the hydrolysis rate for oxytetracycline increases with increasing pH value [19]. Under acidic conditions, these chemicals are in their undissociated forms. Thus, pH at 3 was chosen as the extraction condition. Results in Table 3 show that recoveries of all analytes from the lake water at two spiking levels (10 and 100 $ng \cdot L^{-1}$) were within acceptable ranges from 61%–89% and 60%–95%, respectively, with standard deviation for each triplicate within 10%. The recovery of surrogate from water was between 79.9%–96.4%. LOD and LOQ were from 0.56 to 6.52 $ng \cdot L^{-1}$ and 1.18 to 18.52 $ng \cdot L^{-1}$, respectively.

3.2.2 Sediment samples

In this work, the combination of the SAX and HLB solid-phase extraction cartridges was employed as both clean-up

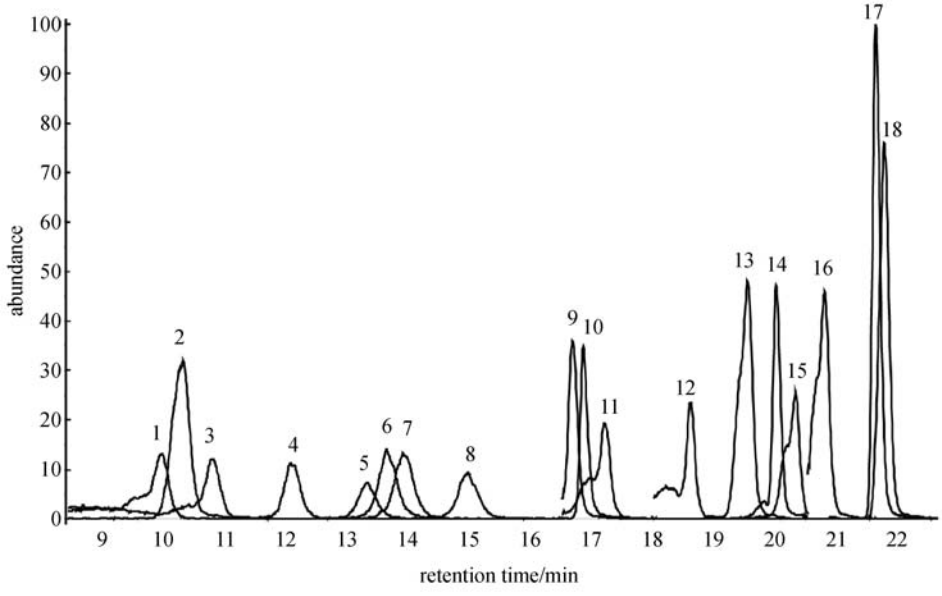


Fig. 2 Total ion chromatogram (TIC) of the antibiotic extract of a spiked sediment (100 μg·kg^{-1} each) from Miyun Reservoir (1: SCT, 2: $^{13}C_3$-caffeine, 3: STZ, 4: TMP, 5: NOR, 6: OFL, 7: OTC, 8: CIP, 9: TC, 10: ENR, 11: SMZ, 12: CTC, 13: SCP, 14: ERM, 15: SMX, 16: SIA, 17: ROM, 18: SDX)

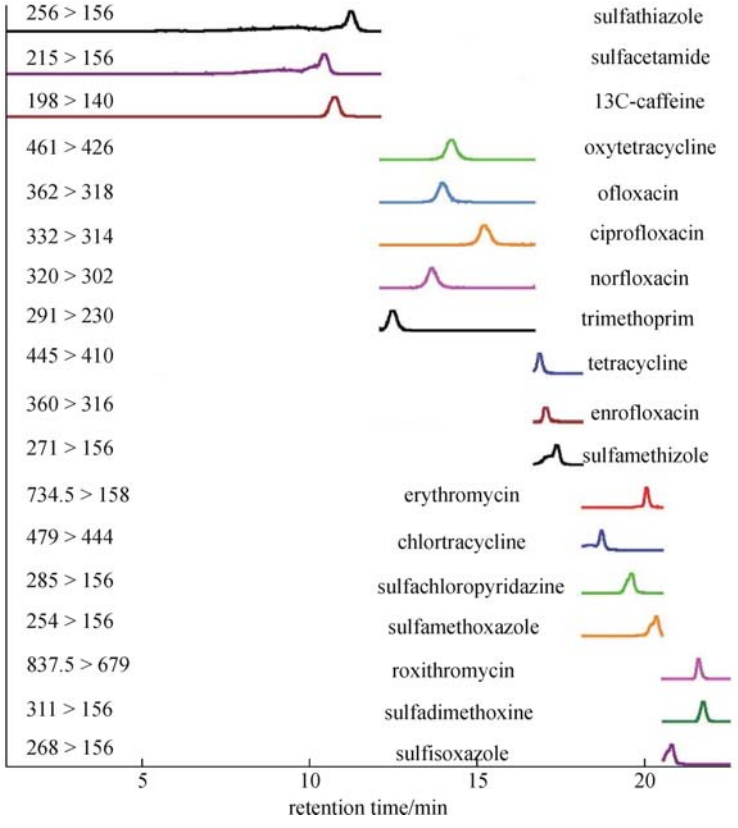


Fig. 3 Extracted ion chromatograms (XIC) of the multiple reaction monitoring (MRM) chromatograms of the antibiotics (100 μg·kg^{-1} each in sediment)

Table 3 Extraction recoveries (n = 3) for the selected antibiotics at different spiking concentrations in water from Miyun Reservoir ($ng \cdot L^{-1}$)

compound	spike concentration		LOD[a)]	LOQ[b)]	linearity (r^2)[c)]
	10 $ng \cdot L^{-1}$	100 $ng \cdot L^{-1}$	/($ng \cdot L^{-1}$)	/($ng \cdot L^{-1}$)	
sulphacetamide	88±0.8	63±4.1	0.36	0.92	0.9984
sulphathiazole	67±2.0	89±2.7	1.04	3.34	0.9959
trimethoprim	82±4.2	89±3.9	3.05	10.10	0.9955
norfloxacin	83±2.9	91±3.5	0.56	1.18	0.9988
ofloxacin	72±3.1	94±4.3	3.11	10.20	0.9982
oxytetracycline	89±3.7	86±4.8	4.17	12.66	0.9959
ciprofloxacin	72±4.8	93±2.3	3.10	10.64	0.9980
tetracycline	82±3.1	84±4.3	2.52	9.52	0.9987
enrofloxacin	82±2.3	95±4.7	2.60	8.70	0.9969
sulphamethizole	65±8.7	60±9.3	0.81	2.70	0.9955
chlortetracycline	68±9.2	71±8.3	4.15	12.63	0.9991
sulphachloropyridazine	81±3.5	71±2.9	1.02	5.12	0.9973
erythromycin	77±1.9	79±3.1	4.28	12.44	0.9923
sulphamethoxazole	61±7.7	66±8.2	1.20	4.10	0.9976
sulphisoxazole	67±6.3	68±5.8	0.86	2.93	0.9928
roxithromycin	83±2.4	85±2.2	3.52	11.14	0.9932
sulphadimethoxine	75±1.9	78±2.4	0.62	2.08	0.9970

Notes: a) limit of detection; b) limit of quantification; c) calibration curves (5–200 $\mu g \cdot L^{-1}$ for each compound)

and pre-concentration for sediment extracts. The SAX cartridge reduces matrix interferences by adsorbing anionic humic materials from the sediment extracts, avoiding contamination, and blocking and overloading the HLB sorbent [18]. Studies have shown a mixture of citric buffer and methanol to be efficient for the extraction of antibiotics from biota and sediment samples [3,14]. Effects of different pHs of the buffer solution were examined for optimizing the extraction buffer of antibiotics; results are summarized in Fig. 4(a). In general, the solution at pH 5 gave better recoveries and lower standard deviations, as some antibiotics were prone to hydrolysis under neutral or basic conditions [19]. The TC recovery ratio showed the highest variation depending on the compound, and the measured SAs showed little variation under three different pH values. Acetonitrile and McIlvaine buffer solution were also tested in previous studies, however, the use of acetonitrile and McIlvaine buffer was not sufficient to efficiently extract fluoroquinolones [3,19]. The citric buffer solution was determined in this study. Meanwhile, at a buffer pH of 5, the antibiotics were overall neutral or cations and therefore not retained on the SAX cartridge, while the polymer-based HLB cartridge simultaneously retained neutral polar and non-polar compounds, including the studied antibiotics.

Different ratios of citric buffer (pH 5) to methanol were also tested for sediment extraction (Fig. 4(b)). Using a mixed solution of methanol and citric buffer (50:50, v/v, solvent C), the recoveries of the antibiotics from the spiked sediment were above 60% for most analytes. In general, the measured recovery ratio of TCs (17.9%–78.6%) and FQs (7.9%–74.8%) showed higher variability than SAs (28.7%–106.9%) and MCs (20.7%–83.8%) under the five different conditions. These results showed a better performance of mixed citric acid and methanol (solvents C, D, and E in Fig. 4) in the extraction of antibiotics than citric acid buffer (solvent A in Fig. 4) or organic solvent methanol (solvent B) only. Use of citric buffer (solvent B) enhanced recoveries for TCs rather than methanol (solvent A), indicating that citric acid was strong enough to desorb the antibiotics from the sediment particles, as they tended to complexate with divalent metal ions (e.g., Mg^{2+} and Ca^{2+}) and to adsorb strongly onto sediment [19]. As shown in Fig. 4(b), solvents A, B, D, and E had some low recoveries for FQ or TC antibiotics (below 30%). Solvent C was suitable to simultaneously extract four classes of antibiotics from the sediment. Since all analytes were recovered with an acceptable recovery at pH 5 and mixed solution of methanol and citric buffer, the optimized pH of the citric solution was determined to be pH 5 and mixed solution ratio of 50:50 (v/v).

Sediments with three spiking levels (50, 100, and 200 $\mu g \cdot kg^{-1}$) of antibiotics were tested for the recovery rates and extraction yields. As shown in Table 4, recoveries from three spiking levels ranged between 55.9%–104.2%, 48.1%–90.9%, and 67.1%–105.3%, respectively. It is notable that except for tetracycline, all other analytes had recoveries above 65%. Tetracycline had the lowest

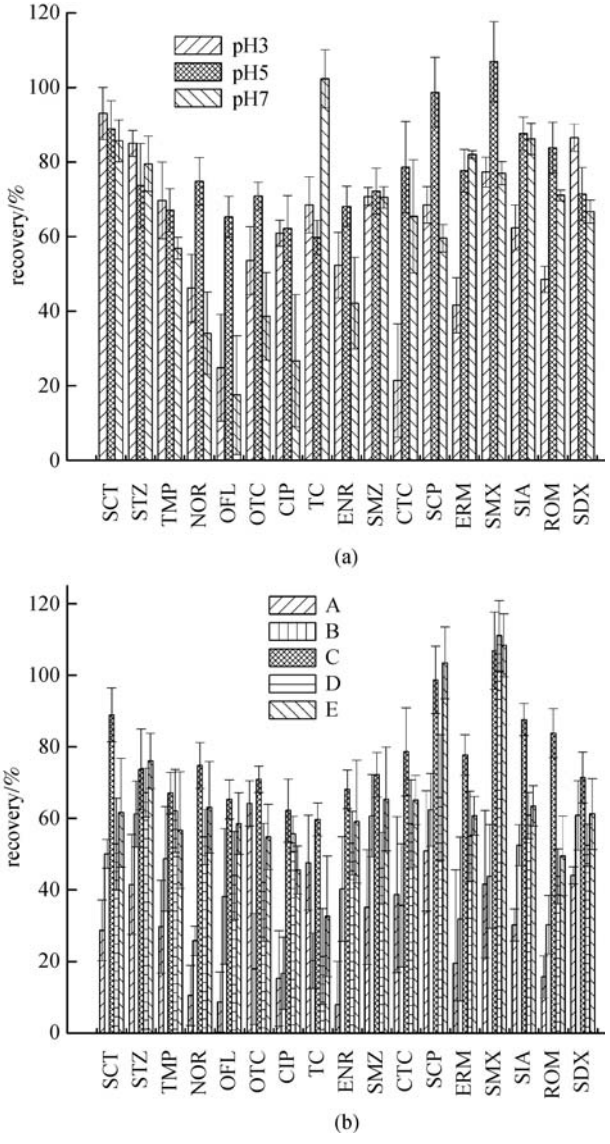


Fig. 4 (a) Extraction recoveries of the antibiotics in sediment using methanol and citric buffer with different pH values; (b) Extraction recoveries of the antibiotics in sediment using different extraction solvents. A: methanol; B: citric buffer; C: methanol + citric buffer (50:50 v/v); D: methanol + citric buffer (20:80 v/v); E: methanol + citric buffer (80:20 v/v)

recovery rates under employed extraction conditions at all three spiking levels. This lowest recovery should be ascribed to the strong adsorption of tetracycline to sediment particles [16,23]. Jacobsen et al. [18] used a 1:1 (v/v) mixture of methanol and 0.2 mol·L^{-1} citric acid buffer as the extractant to extract tetracyclines from soils, and obtained a recovery rate between 50%–70%, which is consistent with our results. To simultaneously determine several groups of antibiotics, including a wide range of different physico-chemical properties, the method has to be a compromise to accommodate different properties. Even for tetracycline, the recovery rates from 48.1%–67.1% were acceptable for its quantification from sediments with the developed method. Values obtained for LODs and LOQs for the antibiotics were in the range from 0.1 to 4.9 μg·kg^{-1} and 0.2 to 12.6 μg·kg^{-1}, respectively, as shown in Table 4. The recovery of surrogate from sediment samples was between 66.8%–85.9%.

3.2.3 Fish tissues

The optimization of the method was carried out by analyzing antibiotic-free fish edible muscles spiked with the chemicals. The effect of extractant pH was evaluated by extracting the antibiotics from fish samples at 100 μg·kg^{-1} spiking level with mixed solution of ACN and citric buffer at three different pH values (3, 5, and 7). As illustrated in Fig. 5, the highest recoveries were obtained at pH 5; note that recovery decreased when extraction pH increased to 7 or decreased to 3. Romero-González et al. [21] tested two different pH solutions (pH 4 and 6) for the optimization of extraction procedures for several veterinary antibiotics (quinolones, tetracyclines, sulphonamides, and trimethoprim), and found that the solution at pH 4 had a better performance than the solution at pH 6. To simultaneously extract several groups of antibiotics with different properties from fish tissues, a recovery range of 71.4%–103.4% by extractant at pH 5 in this study was good for the chemical quantification.

The volume of the extractant solution of ACN and citric buffer (5, 10, and 25 mL) was studied. The results in Fig. 5 indicated that extraction of fish samples by 5 or 10 mL of extractant only obtained recovery rates as low as 40%–70% for most selected antibiotics, while more than 80% of target chemicals could be extracted by 25 mL of extractant from spiked fish tissues. For 2.5 g of fish muscle sample in this work, at least 25 mL of extractant was necessary to give a satisfactory recovery rates for all selected antibiotics in a single run.

Antibiotic-free fish samples were spiked at three levels, i.e., 50, 100, and 200 μg·kg^{-1}. Their recovery rates are summarized in Table 4. From fish samples spiked at 50, 100, and 200 μg·kg^{-1} of target antibiotics, the recoveries were in the range of 59.8%–89.5%, 71.4%–103.4%, and 71.8%–101.5%, respectively. The precision was also satisfactory with standard deviation lower than 17%. LOD and LOQ ranged between 0.2 and 4.3 μg·kg^{-1} and 0.9–14.6 μg·kg^{-1}, respectively. The recovery of surrogate from fish samples was between 61.3%–88.4%.

3.3 Occurrence of antibiotics in Dianchi Lake

Antibiotic levels in the surface water and sediments in Dianchi Lake are shown in Fig. 6. Of the 17 target antibiotics, 13 were detected in water and 8 were detected in sediment samples. It is notable that the total concentration of antibiotics in the northern lake (S1–S8) was higher than in the south part of the lake (Fig. 6). For each class of antibiotics, their highest total concentrations were mainly

Table 4 Recovery results obtained for the selected antibiotics with the optimized extraction method in spiked sediment and fish

compound	sediment				fish			
	amount /(μg·kg^{-1})	recovery /%	LOD /(μg·kg^{-1})	LOQ /(μg·kg^{-1})	amount /(μg·kg^{-1})	recovery /%	LOD /(μg·kg^{-1})	LOQ /(μg·kg^{-1})
sulphacetamide	50	97.6±13.7	1.4	4.6	50	89.5±7.8	1.2	4.0
	100	90.9±16.6			100	86.7±13.7		
	200	105.3±7.3			200	91.1±5.6		
sulphathiazole	50	79.8±17.2	3.4	11.2	50	80.2±6.9	4.3	14.6
	100	72.6±6.9			100	90.9±7.4		
	200	73.2±12.6			200	90.7±3.6		
trimethoprim	50	83.8±8.4	0.2	1.1	50	77.5±7.4	0.8	2.4
	100	82.9±10.6			100	88.9±7.9		
	200	87.0±10.4			200	90.4±11.5		
norfloxacin	50	69.1±10.8	3.8	11.9	50	67.9±13.6	4.1	12.5
	100	65.8±4.7			100	82.9±6.3		
	200	68.0±6.6			200	79.3±8.2		
ofloxacin	50	69.4±5.8	0.6	1.9	50	82.9±7.0	1.8	5.1
	100	70.5±5.4			100	91.8±4.5		
	200	73.7±6.9			200	88.9±6.6		
oxytetracycline	50	86.3±7.1	0.9	3.0	50	83.3±4.0	1.1	4.2
	100	83.0±4.8			100	86.3±11.8		
	200	87.5±11.2			200	90.3±10.2		
ciprofloxacin	50	73.3±14.3	0.9	3.0	50	75.4±16.3	0.8	2.7
	100	75.8±13.1			100	87±12.4		
	200	77.1±9.5			200	86.2±12.9		
tetracycline	50	55.9±8.3	1.9	6.2	50	59.8±15.5	3.3	10.2
	100	48.1±10.6			100	86.5±8.1		
	200	67.1±9.8			200	80.2±11.2		
enrofloxacin	50	65.5±1.1	4.9	12.6	50	64.2±17.8	3.4	11.1
	100	77.6±6.3			100	74.8±15.7		
	200	69.4±1.7			200	77.3±12.5		
sulphamethizole	50	65.8±5.1	0.3	1.1	50	75.4±4.1	0.2	0.9
	100	77.2±8.3			100	83.8±7.4		
	200	76.7±9.1			200	101.5±6.9		
chlortetracycline	50	79.3±16.8	0.4	1.3	50	81.2±7.5	1.0	3.2
	100	83.2±19.4			100	83.8±12.9		
	200	85.5±19.9			200	94.6±14.3		
sulphachloropyridazine	50	75.8±4.9	0.2	0.6	50	65.3±5.5	0.3	2.1
	100	80.0±7.5			100	71.44±5.1		
	200	79.7±6.0			200	77.3±9.2		
erythromycin	50	70.3±7.1	0.3	1.1	50	88.6±3.8	0.5	1.5
	100	74.7±11.7			100	103.4±2.4		
	200	75.8±11.5			200	91.2±2.6		
sulphamethoxazole	50	104.2±5.2	0.1	0.4	50	68.5±12.0	0.8	2.4
	100	88.1±11.9			100	72.2±16.8		
	200	97.6±8.6			200	71.8±11.7		
sulphisoxazole	50	69.9±11.3	0.1	0.2	50	65.4±12.8	0.6	1.9
	100	76.4±5.8			100	88.6±11.2		
	200	74.5±12			200	93.4±14.5		
roxithromycin	50	70.1±2.2	0.1	0.5	50	79.1±16.3	0.3	1.7
	100	73.5±4.1			100	85.6±10.9		
	200	81.8±6.1			200	82.4±15.2		
sulphadimethoxine	50	79.1±4.1	0.1	0.4	50	70.5±16.2	0.2	1.2
	100	84.2±1.7			100	83.8±9.4		
	200	91.7±3.3			200	90.2±6.2		

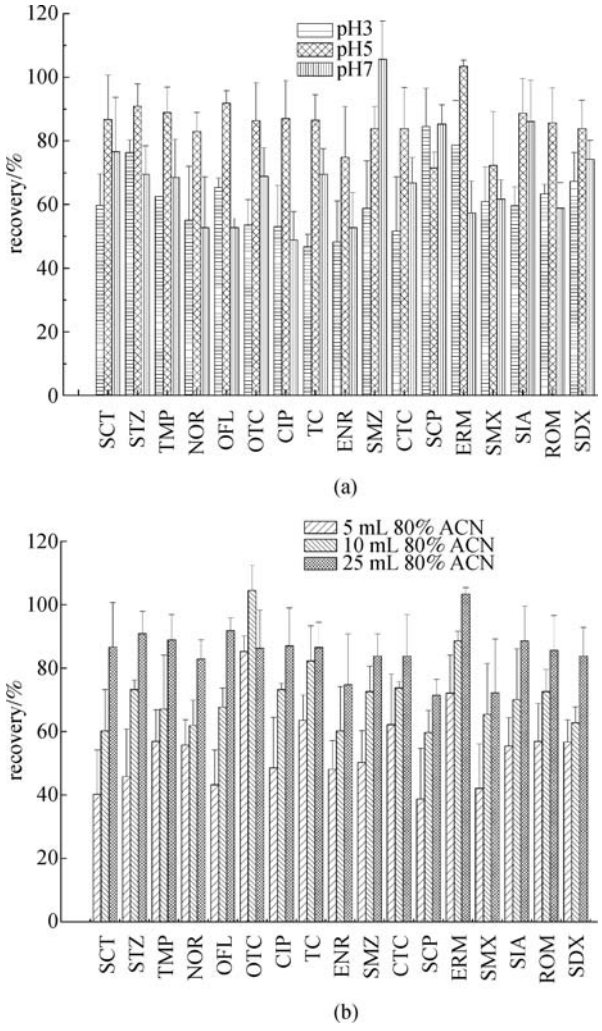


Fig. 5 Recoveries of antibiotics from fish tissues under different conditions: (a) pH effects and (b) extractant volumes

observed at sampling sites located within the suburban area (S1–S12), e.g., S8 for NOR, OFL, and ROM in water (Fig. 6(a)), and S1 and S3 for OTC, TC, and ROM in sediments (Fig. 6(b)). The north part of the lake, which is surrounded by Kunming City, receives direct discharge of effluents from municipal sewage treatment plants and river flows containing pharmaceutical wastewater, which may be the reason for its relatively high levels of antibiotics in this area [24].

In water samples, high levels of sulphonamide, fluoroquinolone, and macrolide antibiotic contamination were detected in the suburban area. In addition, OFL showed the highest concentration among the antibiotics at most of the sampling locations (not detectable to 713.6 ng $\cdot L^{-1}$), which was similar to the value reported in the Yellow River in China, with concentrations ranging from n.d. to 264 ng $\cdot L^{-1}$ [25], and relatively lower than those reported in Bohai Bay (n.d. to 5100 ng $\cdot L^{-1}$) and Victoria Harbour (8.1–1140 ng $\cdot L^{-1}$), Hong Kong, China [26,27]. SMX was detectable in 13 sampling sites, with concentrations ranging from 17.6 to 499.2 ng $\cdot L^{-1}$, which were slightly higher than those found in the Seine River in France with concentrations from 40 to 140 ng $\cdot L^{-1}$ [28], Elbe River in Germany with concentrations from 30 to 70 ng $\cdot L^{-1}$, and Haihe River and its tributaries in China (n.d. to 171 ng $\cdot L^{-1}$) [26,29]. Levels of ROM in water varied from 0.9 to 314.2 ng $\cdot L^{-1}$, which was similar to levels in the Seine River in France with n.d. to 305 ng $\cdot L^{-1}$ [28], relatively lower than those reported in Bohai Bay of China, with concentrations ranging from 10 to 630 ng $\cdot L^{-1}$ [26], and much higher than those in the Elbe River in Germany (n.d. to 40 ng $\cdot L^{-1}$) and Victoria Harbour, Hong Kong, China (5.5–47 ng $\cdot L^{-1}$) [27,29]. Among the detected antibiotics, much lower levels of tetracyclines were found in Dianchi Lake, probably due to their strong binding capacity to particulate matter and interaction with cations [30,31].

The occurrence of antibiotics in Dianchi sediments was different from their occurrence in surface water samples. Overall, fluoroquinolones were detected in the sediments of Dianchi Lake with high concentrations and detection frequencies, and tetracyclines were also found in some sites with high concentrations, however, sulfonamides were often less detected and at low concentrations. The antibiotic levels in the sediments are illustrated in Fig. 6(b). Three (NOR, CIP, and OFL) out of four target fluoroquinolones were detected, and the concentrations ranged from n.d. to 55.2 ng $\cdot g^{-1}$ for NOR, from n.d. to 75.8 ng $\cdot g^{-1}$ for CIP, and from n.d. to 108.9 ng $\cdot g^{-1}$ for OFL. The OFL concentrations in sediment were comparable to those in the Hai River (mean level of 10.3 ng $\cdot g^{-1}$ dry weight (dw), ranging from n.d. to 653 ng $\cdot g^{-1}$ dw) and the Liao River (mean level of 3.56 ng $\cdot g^{-1}$ dw, ranging from n.d. to 50.5 ng $\cdot g^{-1}$ dw) in China [32], but much lower than the Pearl River with the mean concentration of 156 (n.d. to 1560) ng $\cdot g^{-1}$ dw [19]. As for NOR, its sediment concentrations were comparable to those in the Yellow River and Liao River, with mean concentrations of 8.34 (n.d. to 141) and 3.32 (n.d. to 176) ng $\cdot g^{-1}$ dw, respectively [32], but much lower than the Pearl River with the mean concentration of 88 (n.d. to 1120) ng $\cdot g^{-1}$ dw [19]. All three TCs were detected in the sediments. The concentrations ranged from n.d. to 64.8 ng $\cdot g^{-1}$ for OTC, from n.d. to 50.2 ng $\cdot g^{-1}$ for TC, and from n.d. to 92.1 ng $\cdot g^{-1}$ for CTC. Compared with the concentrations of TCs in other places of the world, the TC levels in the sediments of Dianchi Lake were much lower. For instance, TC, OTC, and CTC were found in the sediment in Jiulongjiang River in south China, with the highest concentrations up to 713, 10 363, and 14 666 ng $\cdot g^{-1}$, respectively [33]. In Pearl River sediments, TC and OTC were found at concentrations of 81 and 232 ng $\cdot g^{-1}$, respectively [19]. In the sediments of Cache La Poudre River (northern Colorado, USA), the highest concentrations of TC and OTC were 102 and 56 ng $\cdot g^{-1}$, respectively [34]. Results from previous studies and the present study suggested that sediment was an important sink for tetracycline and fluoroquinolone antibiotics in the aquatic

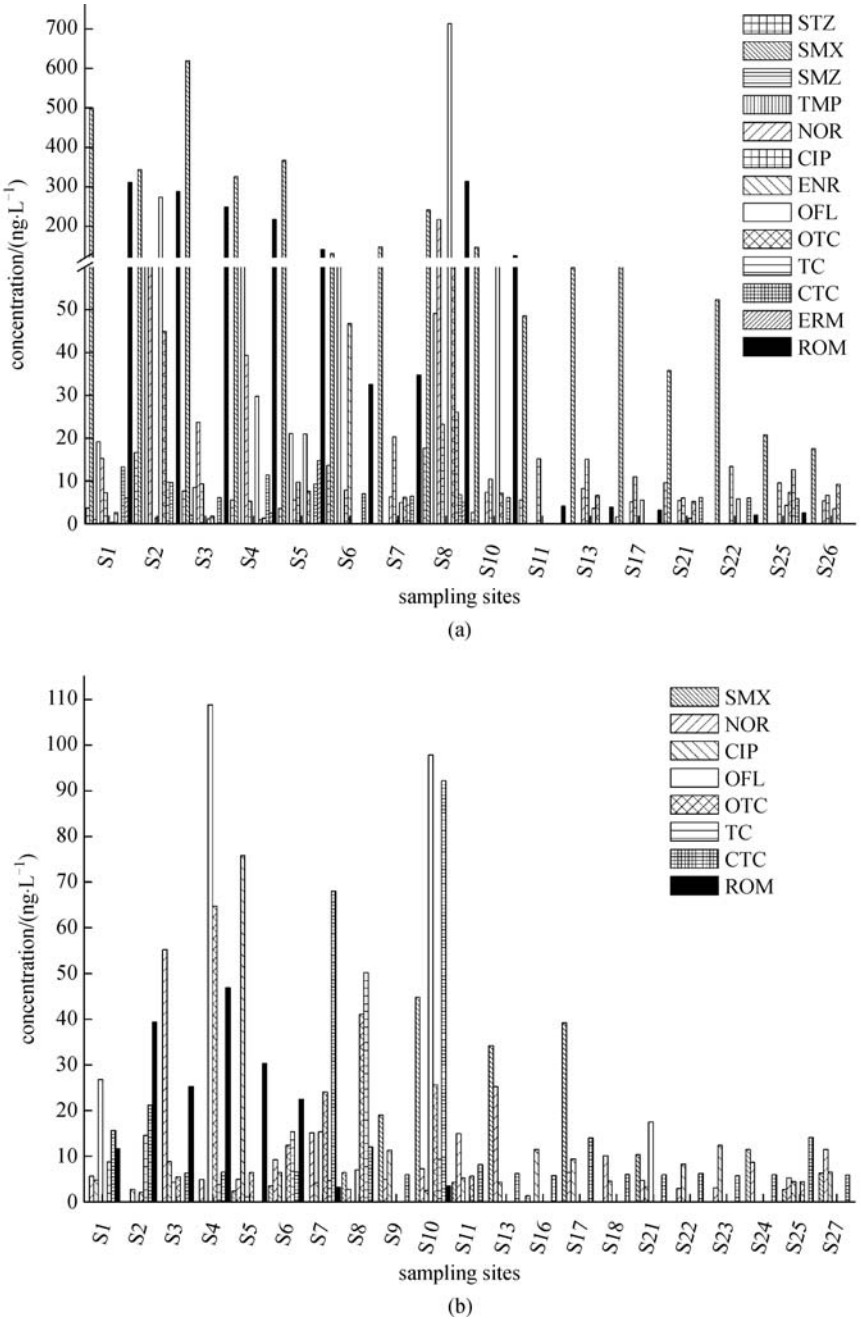


Fig. 6 Spatial distribution of antibiotics detected in different sites in (a) surface water and (b) sediment from Dianchi Lake

environment due to their strong adsorption onto particles and sediments.

Table 5 shows the antibiotic concentrations in the fish muscle samples from Dianchi Lake. It can be seen that every class of antibiotics was detected in fish muscles, with individual antibiotic levels from n.d. to 38.5 $\mu g \cdot kg^{-1}$. SMX and NOR were the two drugs with the highest detection frequencies of 30.7%, and the highest concentration of 38.5 $\mu g \cdot kg^{-1}$ was for NOR found in one fish muscle sample. Out of 13 fish, ROM and ERM were only detected in one sample, and TC and OTC were individually detected in the muscles of two fish. In addition to their low detection frequencies, their levels in fish were also low. However, although the amounts of these antibiotic compounds in Dianchi Lake were several magnitudes lower compared to those amounts applied in medicine, their chronic effects to the environment caused by low environmental concentrations should not be overlooked.

4 Conclusions

Potential ecological and human health risks associated

Table 5 Antibiotic concentrations in fish muscles from Dianchi Lake ($\mu g \cdot kg^{-1}$)

sample	sulfonamides			fluoroquinolones			tetracyclines		macrolides	
	SMX	SDX	STZ	NOR	OFL	CIP	TC	OTC	ROM	ERM
1	0.9	0.4	–	2.8	–	–	–	–	–	–
2	–[a)]	–	–	–	–	3.9	–	2.8	0.4	–
3	1.2	–	6.1	–	1.9	–	–	–	–	–
4	–	–	–	38.5	–	–	–	–	–	–
5	–	–	–	–	–	–	3.7	–	–	–
6	–	–	–	–	–	2.5	–	–	–	–
7	3.4	5.3	–	–	–	–	–	–	–	–
8	–	–	–	11.2	2.4	–	–	–	–	–
9	–	–	2.7	–	–	–	–	–	–	0.7
10	–	–	–	–	–	–	–	1.5	–	–
11	–	–	–	–	–	–	4.2	–	–	–
12	2.3	–	3.2	–	4.5	–	–	–	–	–
13	–	–	–	5.7	–	1.7	–	–	–	–

Notes: a) below LOD

with the presence of human and veterinary antibiotics in environmental matrices necessitate the development of rapid, sensitive, and direct analytical methods to support the research on their occurrence and environmental behavior. In this work, a strategy was developed and validated to rapidly and simultaneously determine several classes of antibiotics from water, sediment, and fish samples. The methods involved SPE extraction from water samples, ultrasonic-assisted solvent extraction from sediment followed by SAX-HLB clean-up, and solvent extraction followed with SPE clean-up from fish muscles. The methods were able to detect seven sulphonamides (sulphamethizole, sulphacetamide, sulphathiazole, sulphachloropyridazine, sulphamethoxazole, sulphisoxazole, and sulphadimethoxine), four fluoroquinolones (norfloxacin, ofloxacin, ciprofloxacin, and enrofloxacin), two macrolides (roxithromycin and erythromycin), three tetracyclines (oxytetracycline, tetracycline, and chlortetracycline), and trimethoprim from environmental matrices in a single run. Recoveries, LOD, and LOQ for extraction procedures were satisfactory, and the methods were employed to extract antibiotics from water, sediment, and fish samples collected from Dianchi Lake. Results showed that 13 and 8 out of the 17 target antibiotics were detected in water and sediment samples from Dianchi Lake, respectively, with antibiotic concentrations up to $mg \cdot kg^{-1}$ levels. In fish muscles, individual antibiotic compounds were also detected, with the highest level up to 38.5 $\mu g \cdot kg^{-1}$. The successful application of the proposed method to real environmental samples allows for sufficient detection limits and quantification of antibiotics for the screening of environmental samples.

Acknowledgements This work was financially supported by the National Natural Science Foundation of China (Grant No. 51178438) and the National Basic Research Program of China (No. 2008CB418201).

References

1. Ingerslev F, Halling-Sørensen B. Biodegradability properties of sulfonamides in activated sludge. Environmental Toxicology and Chemistry, 2000, 19(10): 2467–2473
2. Holm J V, Ruegge K, Bjerg P L, Christensen T H. Occurrence and distribution of pharmaceutical organic compounds in the groundwater downgradient of a landfill (grindsted, denmark). Environmental Science & Technology, 1995, 29(5): 1415–1420
3. Luo Y, Xu L, Rysz M, Wang Y, Zhang H, Alvarez P J J. Occurrence and transport of tetracycline, sulfonamide, quinolone, and macrolide antibiotics in the Haihe River Basin, China. Environmental Science & Technology, 2011, 45(5): 1827–1833
4. Tong L, Li P, Wang Y, Zhu K. Analysis of veterinary antibiotic residues in swine wastewater and environmental water samples using optimized SPE-LC/MS/MS. Chemosphere, 2009, 74(8): 1090–1097
5. Halling-Sørensen B, Nors Nielsen S, Lanzky P F, Ingerslev F, Holten Lützhøft H C, Jørgensen S E. Occurrence, fate and effects of pharmaceutical substances in the environment: a review. Chemosphere, 1998, 36(2): 357–393
6. Boxall A B A, Blackwell P, Cavallo R, Kay P, Tolls J. The sorption and transport of a sulphonamide antibiotic in soil systems. Toxicology Letters, 2002, 131(1–2): 19–28
7. Dasenaki M E, Thomaidis N S. Multi-residue determination of seventeen sulfonamides and five tetracyclines in fish tissue using a multi-stage LC-ESI-MS/MS approach based on advanced mass spectrometric techniques. Analytica Chimica Acta, 2010, 672(1–2): 93–102
8. Soliman M A, Pedersen J A, Suffet I H. Rapid gas chromatography-

mass spectrometry screening method for human pharmaceuticals, hormones, antioxidants and plasticizers in water. Journal of Chromatography. A, 2004, 1029(1–2): 223–237

9. Lindsey M E, Meyer T M, Thurman E M. Analysis of trace levels of sulfonamide and tetracycline antimicrobials in groundwater and surface water using solid-phase extraction and liquid chromatography/mass spectrometry. Analytical Chemistry, 2001, 73(19): 4640–4646
10. Wen X, Tu C, Lee H K. Two-step liquid-liquid-liquid microextraction of nonsteroidal antiinflammatory drugs in wastewater. Analytical Chemistry, 2004, 76(1): 228–232
11. Bogialli S, Curini R, Di Corcia A, Nazzari M, Samperi R. A liquid chromatography-mass spectrometry assay for analyzing sulfonamide antibacterials in cattle and fish muscle tissues. Analytical Chemistry, 2003, 75(8): 1798–1804
12. Tolls J. Sorption of veterinary pharmaceuticals in soils: a review. Environmental Science & Technology, 2001, 35(17): 3397–3406
13. Liu R, Zhou J L, Wilding A. Microwave-assisted extraction followed by gas chromatography-mass spectrometry for the determination of endocrine disrupting chemicals in river sediments. Journal of Chromatography. A, 2004, 1038(1–2): 19–26
14. Li W, Shi Y, Gao L, Liu J, Cai Y. Investigation of antibiotics in mollusks from coastal waters in the Bohai Sea of China. Environmental Pollution, 2012, 162: 56–62
15. Yamini Y, Asghari-Khiavi M, Bahramifar N. Effects of different parameters on supercritical fluid extraction of steroid drugs, from spiked matrices and tablets. Talanta, 2002, 58(5): 1003–1010
16. de Liguoro M, Cibin V, Capolongo F, Halling-Sørensen B, Montesissa C. Use of oxytetracycline and tylosin in intensive calf farming: evaluation of transfer to manure and soil. Chemosphere, 2003, 52(1): 203–212
17. Xu J, Wu L, Chen W, Chang A C. Simultaneous determination of pharmaceuticals, endocrine disrupting compounds and hormone in soils by gas chromatography-mass spectrometry. Journal of Chromatography. A, 2008, 1202(2): 189–195
18. Jacobsen A M, Halling-Sørensen B, Ingerslev F, Hansen S H. Simultaneous extraction of tetracycline, macrolide and sulfonamide antibiotics from agricultural soils using pressurised liquid extraction, followed by solid-phase extraction and liquid chromatography-tandem mass spectrometry. Journal of Chromatography. A, 2004, 1038(1–2): 157–170
19. Yang J F, Ying G G, Zhao J L, Tao R, Su H C, Chen F. Simultaneous determination of four classes of antibiotics in sediments of the Pearl Rivers using RRLC-MS/MS. Science of the Total Environment, 2010, 408(16): 3424–3432
20. Juan-García A, Font G, Picó Y. Determination of quinolone residues in chicken and fish by capillary electrophoresis-mass spectrometry. Electrophoresis, 2006, 27(11): 2240–2249
21. Romero-González R, López-Martínez J C, Gómez-Milán E, Garrido-Frenich A, Martínez-Vidal J L. Simultaneous determination of selected veterinary antibiotics in gilthead seabream (*Sparus Aurata*) by liquid chromatography-mass spectrometry. Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences, 2007, 857(1): 142–148
22. Mistri H N, Jangid A G, Pudage A, Shah A, Shrivastav P S. Simultaneous determination of sulfamethoxazole and trimethoprim in microgram quantities from low plasma volume by liquid chromatography–tandem mass spectrometry. Microchemical Journal, 2010, 94(2): 130–138
23. Loke M L, Tjørnelund J, Halling-Sørensen B. Determination of the distribution coefficient (log K_d) of oxytetracycline, tylosin A, olaquindox and metronidazole in manure. Chemosphere, 2002, 48 (3): 351–361
24. Wang B, Huang B, Jin W, Zhao S, Li F, Hu P, Pan X. Occurrence, distribution, and sources of six phenolic endocrine disrupting chemicals in the 22 river estuaries around Dianchi Lake in China. Environmental Science and Pollution Research International, 2013, 20(5): 3185–3194
25. Xu W, Zhang G, Zou S, Ling Z, Wang G, Yan W. A preliminary investigation on the occurrence and distribution of antibiotics in the Yellow River and its tributaries, China. Water Environment Research, 2009, 81(3): 248–254
26. Zou S, Xu W, Zhang R, Tang J, Chen Y, Zhang G. Occurrence and distribution of antibiotics in coastal water of the Bohai Bay, China: impacts of river discharge and aquaculture activities. Environmental Pollution, 2011, 159(10): 2913–2920
27. Gulkowska A, He Y, So M K, Yeung L W Y, Leung H W, Giesy J P, Lam P K S, Martin M, Richardson B J. The occurrence of selected antibiotics in Hong Kong coastal waters. Marine Pollution Bulletin, 2007, 54(8): 1287–1293
28. Tamtam F, Mercier F, Le Bot B, Eurin J, Tuc Dinh Q, Clément M, Chevreuil M. Occurrence and fate of antibiotics in the Seine River in various hydrological conditions. Science of the Total Environment, 2008, 393(1): 84–95
29. Wiegel S, Aulinger A, Brockmeyer R, Harms H, Löffler J, Reincke H, Schmidt R, Stachel B, von Tümpling W, Wanke A. Pharmaceuticals in the river Elbe and its tributaries. Chemosphere, 2004, 57(2): 107–126
30. Figueroa R A, MacKay A A. Sorption of oxytetracycline to iron oxides and iron oxide-rich soils. Environmental Science & Technology, 2005, 39(17): 6664–6671
31. MacKay A A, Canterbury B. Oxytetracycline sorption to organic matter by metal-bridging. Journal of Environmental Quality, 2005, 34(6): 1964–1971
32. Zhou L J, Ying G G, Zhao J L, Yang J F, Wang L, Yang B, Liu S. Trends in the occurrence of human and veterinary antibiotics in the sediments of the Yellow River, Hai River and Liao River in northern China. Environmental Pollution, 2011, 159(7): 1877–1885
33. Zhang D, Lin L, Luo Z, Yan C, Zhang X. Occurrence of selected antibiotics in Jiulongjiang River in various seasons, South China. Journal of Environmental Monitoring, 2011, 13(7): 1953–1960
34. Meija J, Bisenieks J. Solution to half-titration challenge. Analytical and Bioanalytical Chemistry, 2007, 389(5): 1301–1302