Preliminary occurrence studies of antibiotic residues in Hong Kong and Pearl River Delta

YuMei Chen · Kelvin Sze-Yin Leung ·
Jonathan Woon-Chung Wong ·
Ammaiyappan Selvam

Abstract An improved liquid chromatography–tandem mass spectrometry (LC-MS/MS) method was developed and validated to investigate the occurrence of selected antibiotic residues in Hong Kong and Pearl River Delta. LC and MS parameters were optimized to produce the maximum analytical responses for each compound. The established method targeted five groups of antibiotics, namely, macrolides, sulfonamides, tetracyclines, quinolones, and amphenicols. The method was validated for three types of environmental water matrices, namely, reservoir, river, and estuarine waters. Low detection limits of 0.17–0.18 ng/L for macrolides, 0.22–0.34 ng/L for quinolones, 0.67–1.65 ng/L for tetracyclines, and 0.27–0.56 ng/L for sulfonamides were obtained. No apparent interferences were observed in the chromatograms of all antibiotics groups. The developed method was preliminarily used to analyze water samples collected from Yuen Long River in New Territories, Hong Kong and four sites along the Pearl River Delta. Results showed that high level of tetracycline was found among the groups tested in the local river samples. In Pearl River Delta, we identified significant levels of erythromycin, roxithromycin, and sulfadiazine in Shenzhen Reservoir.

Keywords Liquid chromatography–tandem mass spectrometry · Method validation · Antibiotics in environment

K. S.-Y. Leung (✉) · J. W.-C. Wong (✉) · A. Selvam
Sino-Forest Applied Research Centre for Pearl River Delta Environment,
Hong Kong, People's Republic of China
e-mail: s9362284@hkbu.edu.hk
e-mail: jwcwong@hkbu.edu.hk

J. W.-C. Wong · A. Selvam
Department of Biology, Hong Kong Baptist University,
Kowloon Tong, Hong Kong SAR, People's Republic of China

Y. Chen · K. S.-Y. Leung
Department of Chemistry, Hong Kong Baptist University,
Kowloon Tong, Hong Kong SAR, People's Republic of China

Introduction

Antibiotics are a class of water contaminants that are raising increasing concern, just as they are rising in importance (Khetan and Collins 2007; Richardson and Ternes 2005; Richardson 2007, 2009, 2010). Human and veterinary antibiotics are continuously being released into the environment at low concentrations as a result of manufacturing processes, disposal of unused or expired products, and excretion (Kim et al. 2011; Zuccato et al. 2010). The continuous environmental input of trace amount of antibiotics may lead to long-term accumulation, with unknown impacts on human and animal health, and/or to the development of antibiotics resistance genes in the bacteria in the environment (Chopra and Roberts 2001; Roberts and Thomas 2006)

With the rising awareness of potential threats from antibiotic residues in the environment, methodology for their precise determination in complex environmental matrices is needed. Although some such analytical methods have been reported in the literature, they have focused on food-related matrices such as milk products or meats (Link et al. 2007; Núñez et al. 2005; Mottier et al. 2003; Samanidou and Nisyriou 2008; Kantiani et al. 2009). For environmental-related studies, relevant works are limited (Lei et al. 2009; Pailler et al. 2009; Gros et al. 2006; Grujić et al. 2009; Jelić et al. 2009). Among these previous works, most dealt with tetracyclines or quinolones, such as tetracycline, oxytetracycline, and chlorotetracycline, in sewage, surface, or drinking water (Pena et al. 2007; Verma et al. 2007). Only a few methods have been published on macrolides and, sulfonamides, as well as the tetracycline and quinolone groups (Hoff and Kist 2009; Heller and Nochetto 2004; Önal 2011; Hernández-Arteseros et al. 2002). Three parameters constrain the development of an appropriate method: First, the method must be sensitive enough to cope with the extremely low concentrations of target chemicals; second, the equipment must be robust enough to handle high-throughput of a diverse range of water matrices; and third, it should be relatively easy to operate and inexpensive. We have developed a method that meets these requirements. Therefore, in the present work, a method is described that comprises a solid phase extraction procedure followed by liquid chromatography–tandem mass spectrometry (LC-MS/MS) for assessing these groups of antibiotics in three types of environmental waters. The developed method was fully validated and preliminarily applied in an antibiotic residues monitoring program in Hong Kong and Pearl River Delta.

Materials and methods

Chemicals and reagents

Standards of antibiotics groups of macrolides (MLs), quinolones (Qs), sulfonamides (SAs), tetracyclines (TCs), amphenicols (AMs) namely erythromycin (ERY), roxithromycin (ROX), ciprofloxacin (CIP), norfloxacin (NOR), ofloxacin (OFX), chlorotetracycline (CTC), oxytetracycline (OTC), tetracycline (TC), sulfadiazine (SDZ), sulfamethoxazole (SMX),

and chloramphenicol (CAP) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Riedel-de haën (Hanover, Germany). Individual stock solutions of the analytes were initially prepared at 1 mg/mL in methanol. Erythromycin–H_2O, major degradation of erythromycin, was obtained by acidification from erythromycin using the method described by McArdell et al. (2003). Standard mixtures of the antibiotics were then prepared in methanol at different concentrations by appropriate dilution of the individual stock solutions and stored at 4 °C. Methanol and acetonitrile (high-performance liquid chromatography grade) were obtained from Merck (Darmstadt, Germany). Ultrapure water was prepared from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Ethylene diaminetetracetic acid disodium salt dehydrate and citric acid were purchased from Eluka and UNI-CHEM, respectively. Simatone was used as the internal standard (Accustandards, New Haven, CT, USA).

Divinylbenzene DVB cartridge and Oasis HLB SPE cartridge were purchased from Alltech Biotechnology (Victoria, Australia) and Waters (Milford, MA, USA), respectively. The purity of nitrogen for drying was 99.995 %.

Sample collections

Local water sampling in Yuen Long River was conducted in June 2010. Altogether, 40 samples were collected from Heyuan, Huizhou, Dongguan and Shenzhen River in Jan 2010. All samples were collected about 1 m below the surface using a 2.0-L water grab sampler and stored in a precleaned water container and kept in the dark at 4 °C in a cold storage room.

Analytical method

Water samples of three matrices were spiked with different levels of antibiotics (see "Results and discussion"). The target analytes were extracted with an Oasis HLB cartridge (200 mg, 6 mL, Waters) previously conditioned with 10 mL methanol and 6 mL Milli-Q water. Five hundred milligrams of Na_2EDTA was added to each 500 mL sample to stabilize the target antibiotics. The pH values of the samples were adjusted to pH 4.0±0.05 using 0.5 M citric acid. The 500-mL spiked water sample was then loaded to the SPE cartridge with a flow rate of about 5 mL/min. After loading of the 500-mL sample,

the cartridge was washed with 10 mL Milli-Q water and dried for 5 min under vacuum.

The elution of the target analytes from the loaded SPE cartridge was performed by passing a small amount of 0.1 % formic acid in methanol (10 mL) through the cartridge. The eluant was then concentrated by N_2 stripping in a 40 °C water bath. The concentrated eluant was reconstituted to 2.0 mL using 10 % aqueous acetonitrile solution. Twenty microliters was injected for LC-ESI-MS/MS analysis.

Chromatographic separation was performed on an Agilent 1100 series (Agilent, Palo Alto, CA, USA) incorporating a pump, autosampler, reversed-phase Phenomenex C18 analytical column (150×2.0 mm i.d., 3 μm) at room temperature. A flow rate of 0.2 mL/min was maintained. The gradient program is shown in Table 1. An API-4000Q tandem mass spectrometer (Applied Biosystems/MDS SCIEX, Concord, ON, Canada) equipped with an electrospray ionization (ESI) source was used for the mass analysis. Analyst software (Applied Biosystems/MDS SCIEX, version 1.3) was used for data acquisition and processing. ESIs in the positive and negative ion mode were used in all experiments. The detector was operated in the multiple reaction monitoring (MRM) mode using the protonated molecular ion to produce ion transitions. MS parameters were optimized by infusing a standard solution of analyte and internal standard into the mass spectrometer using a syringe pump. Table 2 summarizes the MS conditions and MRM parameters for all eleven antibiotics.

For elemental analysis, an Elan DRC II ICP-MS instrument (PerkinElmer, Waltman, MA, USA) with a Meinhard nebulizer and a cyclonic spray chamber was used. The sample solution was directly aspirated

through a sampling tube (0.55 mm i.d.). The elemental contents were calculated using TotalQuant elemental analysis. The operation parameters are summarized in Table 3.

Method validation

The method validation procedure was based on the German standard method DIN 32645 with the following analytical parameters: linearity, recovery, repeatability, reproducibility, matrix effects, and limit of detection.

For quantitative analysis, a 5-point matrix-matched calibration curve was established with spiked water samples in three types of water matrices, namely, reservoir water and low- and high-concentration waters (depict nature waters already containing high levels of target analytes), with concentration levels in the range of 10–500 ng/L. For low concentration analytes, another calibration curve of 0.1–10 ng/L was used. From the calibration curves, their regression coefficients were calculated. The limit of detection was determined by S/N=3 in matrix-matched spiked samples.

For repeatability study, three spiked water samples at concentration levels of 100, 200, and 500 ng/L were analyzed. From the results, the standard deviations were calculated. With spiked waters, a comparison of the resulting peak areas to the peak areas of a direct injection at the same concentration yields the recoveries. A comparison of the data for three different types of water matrix gives information on how the type of matrix influences the results of the procedure.

The extracts were analyzed by LC-ESI-MS/MS. Electrospray ionization was performed in the positive–negative–positive (PI-NI-PI) mode for Qs, MLs, SAs, TCs (PI), and AMs (NI). By optimizing the analytical conditions of LC-ESI-MS/MS, a single chromatographic run was adequate for each water spiked sample.

Results and discussion

Method development

Our present work aims to develop a single solid phase extraction cleanup procedure for all the target antibiotics under groups 1 and 2 in EPA Method 1694 (Method 1694) and applicable to three major

Table 1 Gradient conditions for LC-ESI-MS/MS analysis

Time (min)	Flow rate	A (%)	B (%)
0.0	0.2	95	5
2.0	0.2	95	5
12.5	0.2	12	88
13.0	0.2	0	100
16.0	0.2	0	100
16.5	0.2	95	5
35.0	0.2	95	5

Mobile phase A: 0.1 % ammonium acetate, pH=2.5; mobile phase B: acetonitrile/methanol, 1:1

Table 2 Summary of MRM and MS conditions

Antibiotics	Q1 Mass (Da)	Q3 mass (Da)	Polarity	DP (V)	CE (V)	MRL (ng/L)[a]
MRM parameters						
Chloramphenicol	322.9	152.1	−ve	−50	−30	1.11
Chlortetracycline	479.2	444.1	+ve	51	31	3.33
Ciprofloxacin	332.2	314.3	+ve	66	29	0.75
Erythromycin	716.4	158.2	+ve	66	45	0.51
Ofloxacin	362.2	318.3	+ve	66	27	0.66
Oxytetracycline	461.1	426.2	+ve	51	29	4.95
Roxithromycin	837.5	679.6	+ve	91	31	0.54
Sulfadiazine	251.1	156.3	+ve	50	20	0.81
Sulfamethoxazole	254.1	156.1	+ve	56	23	1.68
Norfloxacin	320.2	302.3	+ve	71	29	1.02
Tetracycline	445.2	410.1	+ve	66	18	2.01
MS conditions						
For all antibiotics except chloramphenicol			Chloramphenicol			
Curtain gas (CUR)	35		Curtain gas (CUR)	35		
Collision gas (CAD)	Medium		Collision gas (CAD)	Medium		
Ion spray voltage (IS)	5,000 V		Ion spray voltage (IS)	−4,500 V		
Temperature(TEM)	500 °C		Temperature (TEM)	500 °C		
Ion source gas 1(GS1)	40 U		Ion source gas 1(GS1)	40 U		
Ion source gas 2(GS2)	30 U		Ion source gas 2(GS2)	30 U		

[a] Method reporting limit (MRL) is defined as three times of the respective limit of detections

environmental water matrices. The intention is that this established method will then be applied to a large-scale environmental monitoring program to be conducted in the Hong Kong and Pearl River Delta. One of the missions is to identify the regional occurrence profiles, migration, and probable transformation of these antibiotics residues among the three water matrices. Preliminary works have shown that complex water matrices, in particular for those samples containing considerably antibiotics residues, rendered precise determination difficult. In this context, we collected water samples from a variety of sources in local rivers and estuarine, and first screened on their antibiotics levels using alternative literature methods. Those samples containing one or more target analytes with concentrations >200 ng/L were classified as "high-concentration" matrix. These high-concentration matrices, together with low-concentration counterparts, were spiked with various levels of antibiotics, and we then proceeded with method validation. The aim was to validate the method performance in the presence of different antibiotics levels. Typical physical parameters

of water collected from a reservoir (Shek Pik Reservoir) and river (Yuen Long River) were found to be within normal ranges (Table 4).

The recovery of each compound by variation on the solid phase materials, sample pH, matrix properties, extraction buffer properties and concentrations, sample loading flow rate, and elution solvent were evaluated. The validation of the method was performed using Milli-Q water and also matrix-matched environmental waters.

Solid phase properties of SPE cartridge

Considering the nature of the 11 targeted analytes, co-polymeric and divinylbenzene cartridges would be an appropriate choice for cleanup. Therefore, DVB and Oasis HLB cartridges were evaluated for their selective retention of target antibiotics. Comparable recoveries were obtained for both types of cartridges for all antibiotics. However, Oasis HLB cartridge gives a better precision (<3 %) over DVB counterpart (~5 %) in seven runs. Due to its better reproducibility, the Oasis HLB

Table 3 Operation parameters for ICP-MS

Parameter	Setting
ICP-MS	PerkinElmer ELAN DRC II
RF power	1,175 W
Plasma gas flow	15 L min^{-1}
Auxiliary gas flow	1 L min^{-1}
Nebulizer gas flow	0.98 L min^{-1}
Cones	Pt and Ni
AutoLens	On

cartridge was chosen for our method. In fact, HLB cartridge is widely used for the extraction of antibiotics in various analytical matrices (Önal 2011; Hernández-Arteseros et al. 2002).

Elution solvent

A selective elution solvent can effectively improve the detection and the recovery of the analytes by eliminating the coextraction of interfering compounds and selectively concentrating the target analytes into the extract. The use of methanol, hexane, and acetonitrile has been reported previously (Batt and Aga 2005; Xu et al. 2007; Martínez-Carballo et al. 2007). The elution performance of three different elution solvents, namely, methanol, 0.1 % formic acid in methanol, and acetonitrile, were investigated. The addition of a minimal amount of 0.1 % formic acid in methanol improved the elution of the 11 target antibiotics. A typical total ion chromatogram is given in Fig. 1.

Sample loading flow rate

Previous works demonstrated that a flow rate below 10 mL/min (Erbs et al. 2007; Koh et al. 2007) onto the SPE cartridge can result in lower recoveries. Our

investigations were performed with 500 mL spiked water samples at 5, 10, and 15 mL/min flow rates. Although using a relatively lower flow rate (i.e. 5 mL/min), our work showed a promising and reproducible recovery.

pH effect on extraction process

The pH of environmental water samples may vary considerably and affect the extraction efficiency and even impair the recovery due to degradation of analytes. It is important to evaluate the extent of pH influence on the extraction of analytes. Poor reproducibility of the extraction process on spiked water samples was obtained in our preliminary study, which also resulted in varied final pH values. An optimization run was performed using spiked Milli-Q water (100 ng/L of target analytes) at pH 2.0, 3.0, 4.0, and 5.0. The recovery values of ERY, ROX, SDZ, and CAP are comparatively higher at pH 4.0, at which pH longer retention of the target analytes in the extraction column can be achieved. However, tetracyclines are not stable in extreme acidic conditions (i.e., pH~2) and thus cannot be recovered from the water samples at those pHs (Halling-Sørensen et al. 2002). As a result, the sample was adjusted to pH 4.0 after the addition of Na$_4$EDTA and prior to solid phase extraction.

### Influence of iron and copper

ICP-MS is used for determining the contents of elemental profiles in three types of water matrices. In general, reservoir water contains minimal elemental contents (<10 ng/mL). From river water samples, it is found that they contain significant levels of iron and copper (~100 ng/mL). The presence of these trace elements in reservoir water could likely compete for the binding of EDTA. In this context, river water with

Table 4 Water monitoring parameters of Yuen Long River (YLR) and Shek Pik Reservoir (SPR)

Sampling location	pH	EC (mS/cm)	Salinity	TSS (mg/L)	TOC (mg/L)
YLR-Upper	7.33±0.04	0.42±0.00	0.20±0.00	7.5±2.6	3.69±1.22
YLR-Middle	7.27±0.03	0.41±0.02	0.20±0.00	6.1±4.6	3.66±1.17
YLR-Down	7.18±0.01	0.40±0.02	0.20±0.00	16.2±2.8	2.24±3.33
SPR	7.81±0.09	0.05±0.01	0.00	5.43±1.74	11.29±1.08

Results are expressed as mean ± SD (n=3)

EC electrical conductivity, TSS total suspended solids, TOC total organic carbon
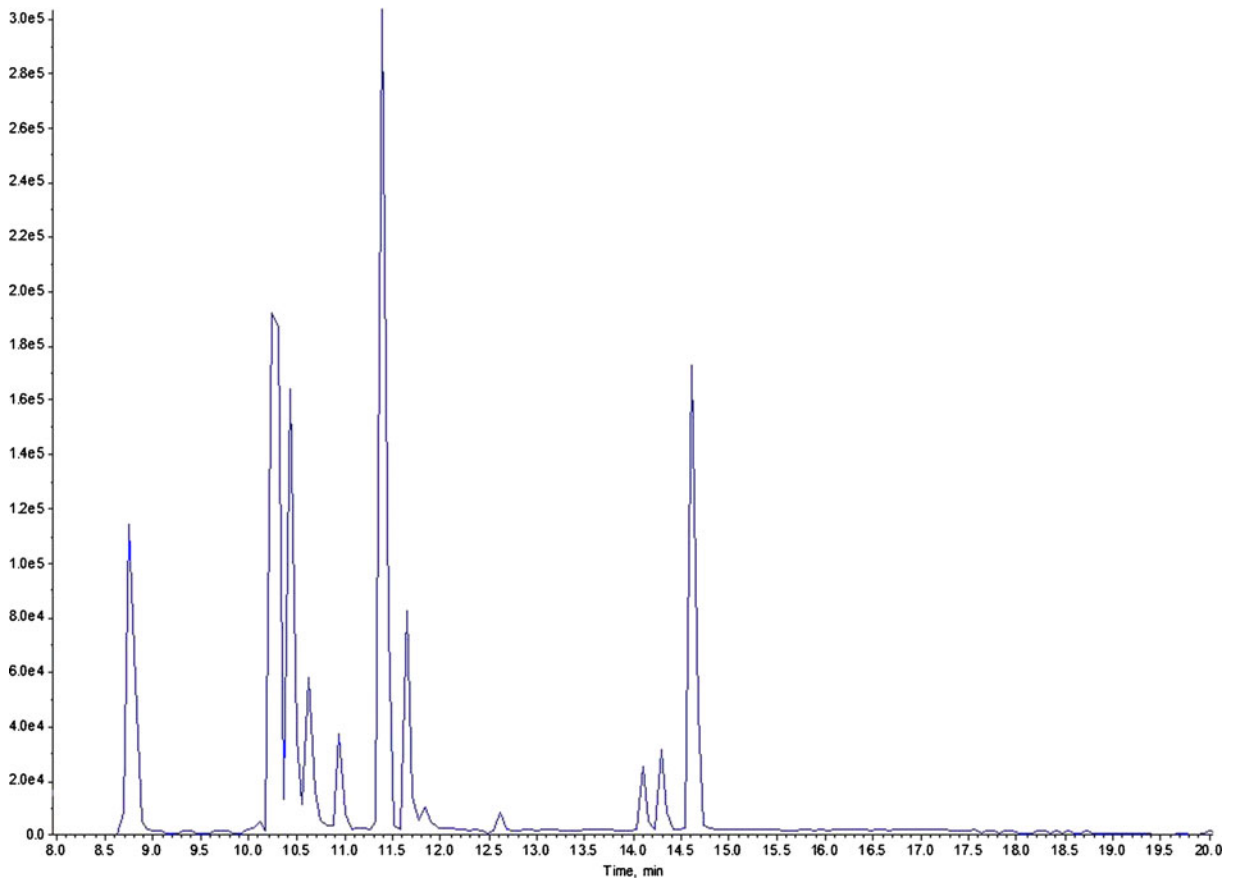
Fig. 1 Typical total ion chromatogram of 11 target antibiotics at 10 ng/L in river water matrix. Retention time of SDZ, 8.75 min; OFX, 10.23 min; NOR, 10.30 min; CIP, 10.43 min; OTC; 10.43 min; TC, 10.62 min; SMX, 11.65 min; CTC, 11.80 min; CAP, 12.70 min; ERY, 14.09 min; ROX, 14.61 min; internal standard, 11.39 min

high levels of iron and copper (both at ~100 ng/L) are spiked together with target analytes and investigated on their recoveries at pH 3.0, 4.0, and 5.0. The results are shown in Fig. 2. It is found that, under an optimized pH condition (i.e., pH 4.0), most of the analytes give satisfactory recoveries. Our findings also indicate that the addition of EDTA is essential for a satisfactory extraction of analytes from the three types of water matrices.

Matrix properties

The components and properties of environmental water samples may vary significantly, which may induce various extents of degradation. As a direct measurement, the recovery study best illustrates the extent of matrix influence. The recovery values of the 11 selected antibiotics were determined from three matrix types,

namely, reservoir water, low-concentration river water, and high-concentration river water. Figure 3 shows the low-concentration spikes (i.e., 100 ng/L) of our recovery study ($n=4$). After the above procedures optimization, the mean recoveries of the three different water matrices ranged from 82 to 110 % for the reservoir water, 60 to 112 % for the low-concentration river water, and 96 to 104 % for the high-concentration river water. It is worth noting that, in general, recoveries of both ROX and SDZ in low concentration study were found to be relatively lower. A similar phenomenon was also reported by Babić et al. (2010). It is postulated that the group of target analytes, which varied in polarity, compete for binding sites on the solid phase material. Among all antibiotics, ROX is the most hydrophobic with a bulky chemical structure. It is strongly retained and could obstruct the retention of other compounds (e.g., SDZ).
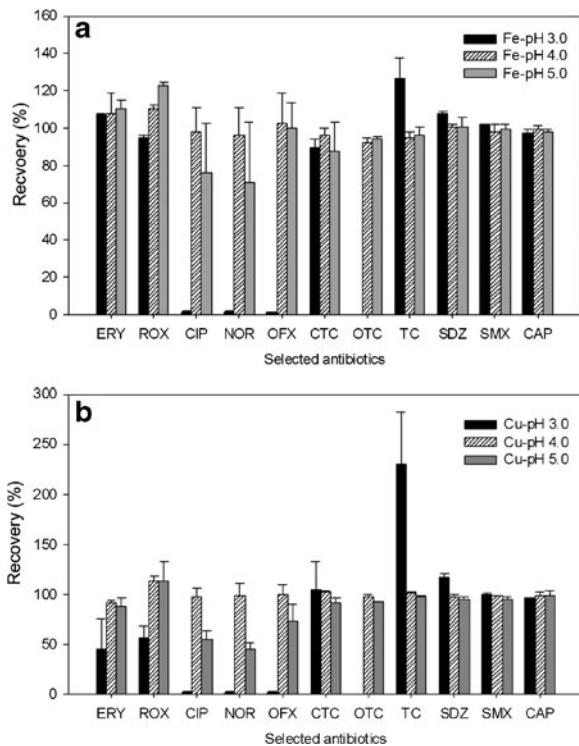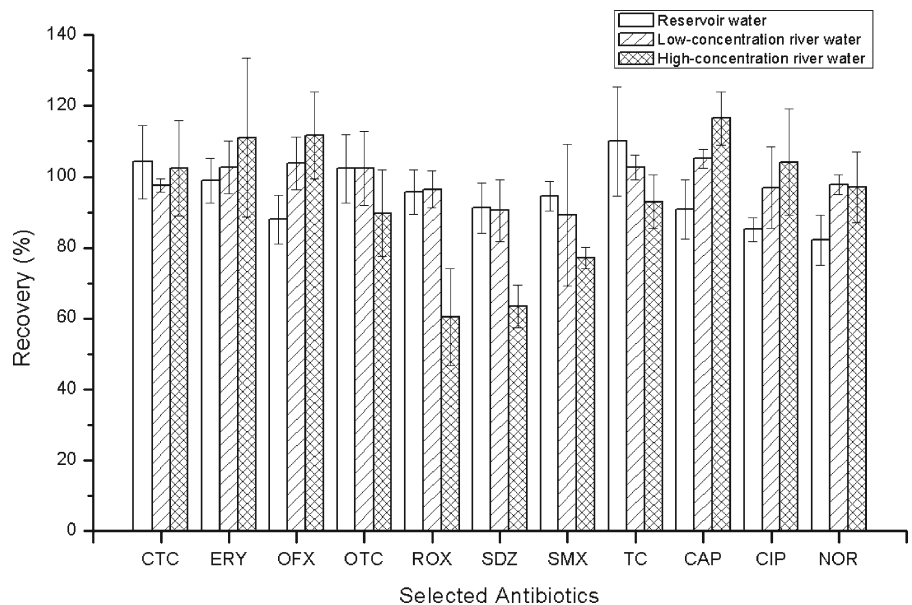
Fig. 2 a Effect of elemental iron (100 ng/mL) on the recovery of antibiotics in waters. b Effect of elemental copper (100 ng/mL) on the recovery of antibiotics in waters

The above results represent a satisfactory recovery of the five major groups of antibiotics from various matrices of low and high concentrations.

Method specificity

The validation data shows that the performance specificities for all of the target antibiotics under investigation are satisfactory. Regression coefficients are generally >0.999, indicating good linearity of the calibrations in the concentration ranges of 0.1–10 and 10–500 ng/L. For repeatability and reproducibility, their standard deviations were found to be <1 and 2 %, respectively. Limits of detection are satisfactory for all antibiotics (0.17–0.18 ng/L for macrolides, 0.22–0.34 ng/L for quinolones, 0.67–1.65 ng/L for tetracyclines, and 0.27–0.56 ng/L for sulfonamides) in various matrices under investigation, underlining the good analytical performance. These values are comparable to those in literature (Link et al. 2007; Mottier et al. 2003; Hernández-Arteseros et al. 2002). In summary, the validation data proved that the method established for the water monitoring program is well suited for the precise determination of 11 antibiotics in three types of water matrices. The method is particularly useful for water laboratories that require a high sample throughput and simplified analytical procedures with promising analytical performance for antibiotics analysis.

Application on real samples

The developed method was preliminarily used to analyze water samples collected from Yuen Long River

Fig. 3 Recovery of eleven antibiotics from three types of water matrices at pH 4.0

(YLR) in Yuen Long, New Territories, Hong Kong. Before 1980s, the region was occupied by majority of poultry activities. To date, Yuen Long has partly been urbanized with both human and livestock activities. In the YLR, tetracyclines (TCs) were the highest among the groups tested. Among the TCs, concentrations of TC were significantly higher than OTC and CTC. TC concentrations were the highest in upstream, followed by middle and down streams indicating the dilution of the TCs along the river with probable natural attenuation (e.g., degradation). Similar to TCs, sulfonamides (SAs) including SDZ and SMX were also found higher in concentrations in upstream, followed by middle and down streams. Among the Qs, NOR was not detected, whereas OFX and CAP were detected. ERY dominated the MLs, and the trend of its concentration follows the Qs, indicating that both Qs and MLs were influenced by the activities other than livestock in both middle and down streams (Table 5).

For Pearl River Delta, the sampling spots are located in Heyuan, Huizhou, Dongguan, and Shenzhen Reservoir. In dry season (Jan 2010), we identified the presence of all except CTC residues in these four locations. In Heyuan (i.e., the most upstream source of Dongjiang water), Huizhou, and Dongguan, they reported with relatively lower levels of residues among the four locations. However, significant amount of ERY and ROX are observed in Shenzhen Reservoir, followed by SDZ. Our on-site observations showed that these high levels of veterinary antibiotics residues are related to the associated poultry activities. Although lethal levels of antibiotics to invertebrates and fishes lie in the high milligrams per liter range, little is known about the chronic effects from long-term, low level exposures of these residues. The synergistic effects derived from multiply residues remains unknown. Table 5 shows the occurrence of these residues in four sampling sites. An extensive occurrence study of antibiotic residues in water, sediment, and soil together with their temporal variations are now underway. The information will be used to devise their migration mechanisms.

Conclusion

A single LC-MS/MS method for multiclass antibiotics, applicable to various water matrices, has been developed and fully validated. Most targeted analytes

Table 5 Contents of antibiotics residues in Yuen Long River [results are expressed as mean ± SD ($n=3$)] and Pearl River Delta [results are expressed as mean ± SD ($n=10$)] (ng/L)

	CTC	OTC	TC	SDZ	SMX	CAP	NOR	OFX	ERY	ROX	CHL
YLR-Upper	79.4±13.9	29.3±3.0	468.2±25.6	37.9±10.3	107.6±26.3	20.6±3.4	N.D.	67.2±11.1	16.5±2.4	0.95±0.10	N.D.
YLR-Middle	69.6±19.0	16.1±1.9	284.0±30.8	3.3±0.4	59.4±19.3	30.2±1.8	N.D.	91.2±20.9	33.7±9.0	8.4±0.5	1.6±0.3
YLR-Down	34.8±5.6	8.4±1.0	128.3±29.1	3.1±0.2	18.0±1.3	62.8±7.5	N.D.	118.9±6.7	36.7±2.8	12.3±0.3	3.5±0.4
Heyuan	N.D.	N.D.	2.8±0.3	3.0±2.2	10.9±0.9	N.D.	N.D.	N.D.	13.6±1.0	N.D.	N.D.
Dongguan	N.D.	N.D.	N.D.	4.2±0.1	22.6±1.6	N.D.	N.D.	N.D.	47.2±2.2	N.D.	4.8±1.2
Huizhou	N.D.	N.D.	N.D.	1.2±0.1	10.5±0.1	N.D.	N.D.	N.D.	28.4±0.4	N.D.	0.87±0.1
Shenzhen	N.D.	18.8±0.2	16.4±0.2	286.6±8.7	12.5±0.2	28.5±0.3	38.5±0.4	52.4±0.9	890.5±11.2	348.5±10.8	26.7±0.4

N.D. not detected or less than respective detection limit

determined by this method show satisfactory analytical performance in three environmental water matrices. This study has shown, for the first time, antibiotic residues present in water sources from Dongjiang to Hong Kong. In particular, high levels of residues were detected in Shenzhen Reservoir. The detected high concentrations of antibiotics are comparable to those reported in Pearl River during wet season. A large-scale environmental monitoring program for antibiotics residues in these sites are urgently needed for ecological assessment.

References

Babić, S., Pavlović, D. M., Ašperger, D., Periša, M., Zrnčić, M., Horvat, A. J. M., et al. (2010). Determination of multi-class pharmaceuticals in wastewater by liquid chromatography-tandem mass spectrometry (LC-MS-MS). *Analytical and Bioanalytical Chemistry, 398*(3), 1185–1194.

Batt, A. L., & Aga, D. S. (2005). Simultaneous analysis of multiple classes of antibiotics by ion trap LC/MS/MS for assessing surface water and groundwater contamination. *Analytical Chemistry, 77*(9), 2940–2947.

Chopra, I., & Roberts, M. (2001). Tetracycline antibiotics: Mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Review, 65*(2), 232–260.

Erbs, M., Hoerger, C. C., Hartmann, N., & Bucheli, T. D. (2007). Quantification of six phytoestrogens at the nanogram per liter level in aqueous environmental samples using C-13(3)-labeled internal standards. *Journal of Agricultral and Food Chemistry, 55*(21), 8339–8345.

Gros, M., Petrović, M., & Barceló, D. (2006). Development of a multi-residue analytical methodology based on liquid chromatography–tandem mass spectrometry (LC–MS/MS) for screening and trace level determination of pharmaceuticals in surface and wastewaters. *Talanta, 70*(4), 678–690.

Grujić, S., Vasiljević, T., & Laušević, M. (2009). Determination of multiple pharmaceutical classes in surface and ground waters by liquid chromatography–ion trap–tandem mass spectrometry. *Journal of Chromatography. A, 1216*(25), 4989–5000.

Halling-Sørensen, B., Sengeløv, G., & Tjørnelund, J. (2002). Toxicity of tetracyclines and tetracycline degradation products to environmentally relevant bacteria, including selected tetracycline-resistant bacteria. *Archives of Environmental Contamination and Toxicology, 42*(3), 263–271.

Heller, D. N., & Nochetto, C. B. (2004). Development of multi-class methods for drug residues in eggs: Silica SPE cleanup and LC-MS/MS analysis of ionophore and macrolide residues. *Journal of Agricultral and Food Chemistry, 52*(23), 6848–6856.

Hernández-Arteseros, J. A., Barbosa, J., Compañó, R., & Prat, M. D. (2002). Analysis of quinolone residues in edible animal products. *Journal of Chromatography. A, 945*(1–2), 1–24.

Hoff, R., & Kist, T. B. L. (2009). Analysis of sulfonamides by capillary electrophoresis. *Journal of Separation Science, 32*(5–6), 854–866.

Jelić, A., Petrović, M., & Barcelóm, D. (2009). Multi-residue method for trace level determination of pharmaceuticals in solid samples using pressurized liquid extraction followed by liquid chromatography/quadrupole-linear ion trap mass spectrometry. *Talanta, 80*(1), 363–371.

Kantiani, L., Farré, M., & Barceló, D. (2009). Analytical methodologies for the detection of β-lactam antibiotics in milk and feed samples. *Trends in Analytical Chemistry, 28*(6), 729–744.

Khetan, S. K., & Collins, T. J. (2007). Human pharmaceuticals in the aquatic environment: A challenge to green chemistry. *Chemistry Reviews, 107*(6), 2319–2364.

Kim, K. R., Owens, G., Kwon, S. I., So, K. H., Lee, D. B., & Ok, Y. S. (2011). Occurrence and environmental fate of veterinary antibiotics in the terrestrial environment. *Water, Air, & Soil Pollution, 214*(1–4), 163–174.

Koh, Y. K. K., Chiu, T. Y., Boobis, A., Cartmell, E., Lester, J. N., & Scrimshaw, M. D. (2007). Determination of steroid estrogens in wastewater by high performance liquid chromatography–tandem mass spectrometry. *Journal of Chromatography. A, 1173*(1–2), 81–87.

Lei, T., Li, P., Wang, Y. X., & Zhu, K. Z. (2009). Analysis of veterinary antibiotic residues in swine wastewater and environmental water samples using optimized SPE-LC/MS/MS. *Chemosphere, 74*(8), 1090–1097.

Link, N., Weber, W., & Fussenegger, M. (2007). A novel generic dipstick-based technology for rapid and precise detection of tetracycline, streptogramin and macrolide antibiotics in food samples. *Journal of Biotechnology, 128*(3), 668–680.

Martínez-Carballo, E., González-Barreiro, C., Scharf, S., & Gans, O. (2007). Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Austria. *Environmental Pollution, 148*(2), 570–579.

McArdell, C. S., Molnar, E., Suter, M. J. F., & Giger, W. (2003). Occurrence and fate of macrolide antibiotics in wastewater treatment plants and in the Glatt Valley Watershed, Switzerland. *Environmental Science and Technology, 37*(24), 5479–5486.

Method 1694: Pharmaceuticals and personal care products in water, soil, sediment, and biosolids by HPLC/MS/MS, EPA-821-R-08-002.

Mottier, P., Parisod, V., Gremaud, E., Guy, P. A., & Stadler, R. H. (2003). Determination of the antibiotic chloramphenicol in meat and seafood products by liquid chromatography–electrospray ionization tandem mass spectrometry. *Journal of Chromatography. A, 994*(1–2), 75–84.

Núñez, O., Moyano, E., & Galceran, M. T. (2005). LC–MS/MS analysis of organic toxics in food. *Trends in Analytical Chemistry, 24*(7), 683–709.

Önal, A. (2011). Overview on liquid chromatographic analysis of tetracycline residues in food matrices. *Food Chemistry, 127*(1), 197–203.

Pailler, J. Y., Pfister, K. L., Hoffmann, L., & Guignard, C. (2009). Solid phase extraction coupled to liquid chromatography-tandem mass spectrometry analysis of sulfonamides, tetracyclines, analgesics and hormones in surface water and wastewater in Luxembourg. *Science of the Total Environment, 407* (16), 4736–4743.

Pena, A., Chmielova, D., Lino, C. M., & Solich, P. (2007). Determination of fluoroquino one antibiotics in surface waters from Mondego River by high performance liquid chromatography using a monolithic column. *Journal of Separation Science, 30*(17), 2924–2928.

Richardson, S. D. (2007). Water analysis: Emerging contaminants and current issues. *Analytical Chemistry, 79*(12), 4295–4324.

Richardson, S. D. (2009). Water analysis: Emerging contaminants and current issues. *Analytical Chemistry, 81*(12), 4645–4677.

Richardson, S. D. (2010). Water analysis: Emerging contaminants and current issues. *Analytical Chemistry, 82*(12), 4742–4774.

Richardson, S. D., & Ternes, T. A. (2005). Water analysis: Emerging contaminants and current issues. *Analytical Chemistry, 77*(12), 3807–3838.

Roberts, P. H., & Thomas, K. V. (2006). The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. *Science of the Total Environment, 356*(1–3), 143–153.

Samanidou, V., & Nisyriou, S. (2008). Multi-residue methods for confirmatory determination of antibiotics in milk. *Journal of Separation Science, 31*(11), 2068–2090.

Verma, B., Headley, J. V., & Robarts, R. D. (2007). Behaviour and fate of tetracycline in river and wetland waters on the Canadian Northern Great Plains. *Journal of Environmental Science and Health Part A-Toxic/Hazardous Substances & Environmental, 42*(2), 109–117.

Xu, W. H., Zhang, G., Zou, S. C., Li, X. D., & Liu, Y. U. (2007). Determination of selected antibiotics in the Victoria Harbour and the Pearl River, South China using high-performance liquid chromatography–electrospray ionization tandem mass spectrometry. *Environmental Pollution, 145*(3), 672–679.

Zuccato, E., Castiglioni, S., Bagnati, R., Melis, M., & Fanelli, R. (2010). Source, occurrence and fate of antibiotics in the Italian aquatic environment. *Journal of Hazardous Materials, 179*(1–3), 1042–1048.