A preliminary study on the occurrence and behavior of sulfonamides, ofloxacin and chloramphenicol antimicrobials in wastewaters of two sewage treatment plants in Guangzhou, China

Xianzhi Peng a,⁎, Zhendi Wang b, Wenxing Kuang b, Jianhua Tan a, Ken Li b

a State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, 510640, PR China
b Environmental Technology Centre, Environment Canada, Ottawa, Canada K1A0H3

Received 30 November 2005; received in revised form 22 June 2006; accepted 2 July 2006
Available online 8 August 2006

Abstract

Wastewater samples collected from two sewage treatment plants (STPs) in Guangzhou, China were acidified, solid-phase extracted (SPE) with Oasis HLB cartridges, followed by instrumental measurement by high performance liquid chromatography (HPLC) coupled with a diode array UV detector (DAD) and a fluorescence detector (FLD) for the occurrence and fate of antimicrobial compounds sulfadiazine (SDZ), sulfamethoxazole (SMX), ofloxacin (OFX) and chloramphenicol (CAP). Antimicrobials have been detected at 5.10–5.15, 5.45–7.91, 3.52–5.56 and 1.73–2.43 μg L⁻¹ for SDZ, SMX, OFX and CAP in the raw sewages of the two STPs, respectively. The concentrations of antimicrobials do not show substantial changes after preliminary mechanical sedimentation. No quantifiable sulfonamides and chloramphenicol have been identified, and >85% of ofloxacin has been removed in the effluents after activated sludge treatment in the two STPs, indicating that activated sludge treatment is effective and necessary to remove antimicrobial substances in municipal sewage.

© 2006 Elsevier B.V. All rights reserved.

Keywords: Antimicrobials; Wastewater; Sewage treatment plants (STPs); Solid-phase extraction (SPE); HPLC-DAD-FLD; China

1. Introduction

The occurrence of antimicrobial compounds in aquatic environment and the potential ecological risks have drawn increasing concerns (Halling-Sorensen et al., 1998; Daughton and Ternes, 1999; Erickson, 2002; Heberer, 2003). The recognized negative effects of antimicrobials in environment to date include development of antibacterial resistance among organism (Halling-Sorensen et al., 1998, 2002; Baguer et al., 2000; Wollenberger et al., 2000; Golet et al., 2002) and some direct toxicity to micro-organisms (Kummerer et al., 2000; Halling-Sorensen et al., 2003). Antimicrobials are widely used to prevent and to treat bacterial infections by human and animals, as well as promote growth in agriculture and aquaculture. These compounds and/or their metabolites find their way to environment via a variety of routes after they have been used and subsequently excreted by human and animals. Antimicrobial compounds have been detected widely in raw sewage, sewage treatment plants

⁎ Corresponding author. Tel.: +86 20 85290191; fax: +86 20 85290706.
E-mail address: pengx@gig.ac.cn (X. Peng).
(STPs) effluents, surface water and even groundwater in Europe and North America (Holm et al., 1995; Ternes, 1998; Ternes et al., 2001; Sacher et al., 2001; Kolpin et al., 2002, 2004; McArdell et al., 2003; Gobel et al., 2004, 2005; Miao et al., 2004; Thomas and Hilton, 2004; Yang and Carlson, 2004). Most antimicrobials are nonvolatile, hydrophilic and polar compounds. They present in aquatic environment usually at concentrations of microgram and/or submicrograms per liter. Therefore, it is not an easy work to determine antimicrobial compounds in waters, especially in wastewaters that are very complex matrices. Up to now, a number of methods have been available for the analysis of antibiotics in environmental samples, most of them involved of liquid chromatography–mass spectrometry (LC/MS) and liquid chromatography–tandem mass spectrometry (LC/MS/MS) (Lindsey et al., 2001; Ternes et al., 2001; Miao et al., 2004; Gobel et al., 2004; Yang and Carlson, 2004; Díaz-Cruz and Barcelo, 2005). These methods have been successfully used to investigate the occurrence and behavior of antibiotics in waters in Europe and North America (Gobel et al., 2004; Miao et al., 2004; Thomas and Hilton, 2004; Yang and Carlson, 2004). HPLC coupled with UV and/or fluorescence detections is also a sensitive alternative approach to quantify trace levels of antibiotics that respond well to UV and/or fluorescence detections (Golet et al., 2001; Prat et al., 2004; Hermo et al., 2006; Garcia-Mayor et al., 2006). Furthermore, it is relatively simple, less expensive and currently much more easily accessible in developing countries like China.

Sulfonamides (SAs) and fluoroquinolones (FQs) are widely used antimicrobials by both human and animals. SAs are known to have a high potential to resist degradation and are hydrophilic enough to be transferred into the aquatic environment (Holm et al., 1995; Kummerer, 2001; Miao et al., 2004), posing an especially potential hazard to the health of aquatic organism and human. Detailed knowledge of the environmental occurrence and behavior of these antimicrobials must be helpful to properly assess their ecological risks. To achieve this aim, investigations on the occurrence and fate of antimicrobial compounds in municipal wastewaters become essential because municipal wastewaters represent a significant contribution to the presence of antimicrobials in various aquatic environment as they are primary pathways of continuous entry of antibiotics into environment due to potentially incomplete elimination during treatments in

![Molecular structures of the selected antimicrobial compounds in this study.](image-url)
Abuse of antimicrobial pharmaceuticals has become a serious concern in China (http://www.39.net/nursing/antibiotic/, in Chinese) in recent years although there are not accurate statistic figures yet. It has been reported that the expenditure on antimicrobials has been leading in both surgeries and routine sickness treatments, accounting for 30–40% of the total cost on pharmaceutical consumption in major cities in recent years (http://www.china.com.cn/chinese/2002/Dec/241753.htm, in Chinese). In addition, it was said that among 15 top sale pharmaceuticals in China, 10 are antibiotics (http://health.enorth.com.cn/system/2004/06/21/000805764.shtml, in Chinese). It was reported by the Chinese Monitoring Center of Adverse Effects of Pharmaceuticals that 1/3 of the adverse pharmaceuticals effects were caused by antibiotics application in China. However, there have been scarce data so far on occurrence and behavior of antibiotic compounds in aquatic environment in China.

Guangzhou is the biggest city in south China with a population of about 10 million. Antibiotics abuse is significant indicated by the higher degree of anti-bacterial resistance to antibacterial pharmaceuticals among residents in this city (17% versus the national average 15%) due to the warm and humid sub-tropic weather. All STPs running in Guangzhou include at least one-time biological treatment step with the total treatment capability of around 1,453,000 m³ day⁻¹. The annual municipal wastewater amount is around 670,000,000 m³. That is to say that more than 70% urban residential wastewater has been treated in STPs before discharge into receiving waters. As a pilot study, four antibiotic compounds including sulfadiazine (SDZ), sulfamethoxazole (SMX) and sulfamerazine (SMR) were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). HPLC grade methanol (MeOH) is product of Fisher Scientific Inc. (New Jersey, USA). HPLC grade acetonitrile (ACN) is from Merck Inc. (Darmstadt, Germany). Acetic acid and ammonium acetate were bought from Tedia Company (www.tedia.com, USA). Ethyl acetate and acetone were purchased from Xilong Solvent Company, China, and was redistilled prior to use.

2. Experimental

2.1. Chemicals

Antimicrobial standards ofloxacin (OFX), chloramphenicol (CAP), sulfadiazine (SDZ), sulfamethoxazole (SMX) and sulfamerazine (SMR) were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). HPLC grade methanol (MeOH) is product of Fisher Scientific Inc. (New Jersey, USA). HPLC grade acetonitrile (ACN) is from Merck Inc. (Darmstadt, Germany). Acetic acid and ammonium acetate were bought from Tedia Company (www.tedia.com, USA). Ethyl acetate and acetone were purchased from Xilong Solvent Company, China, and was redistilled prior to use.

![Fig. 2. HPLC-DAD-MS chromatograms of the investigated antibiotics extracted from spiked river water, Ottawa. On column injection of 10 ng. Peaks: 1, sulfadiazine (SDZ); 2, ofloxacin (OFX); 3, sulfapyridine (SPD); 4, sulfamerazine (SMR); 5, sulfamethoxazole (SMX). SPD was not included in wastewater analysis.](image)
Stock solutions of standards were prepared by dissolving each compound in methanol and milli-Q water mixture (1:1, v/v) at a concentration of 1 mg mL$^{-1}$ and stored in amber vials in a freezer (−20 °C). Fresh stock solutions were made every month. Working standard solutions (0.1, 0.5, 1, 2.5, 5, 20 and 50 ppm) were made by serially diluting the stock solutions with methanol/milli-Q water mixture (1:3, v/v) every time just before use and stored at 4 °C in amber vials.

2.2. Wastewater sampling and preparation

Wastewater samples were collected from two STPs in Guangzhou. The first STP (GZ-STP1), located in the industrial area of the city, whose designed capacity is 30,000 m$^3$ day$^{-1}$, has a mixed input of industrial and residential wastewater (about 4:6). The major industrial wastewater comes from food, chemical and personal care products manufacturers, most of which has been primarily treated by the manufacturers prior to discharge into GZ-STP1. The plant has to work overloaded sometimes due to the increase of sewage caused by the rapid increase of population and industry. The second one (GZ-STP2), located in the central urban area of Guangzhou city, treats mainly residential sewage (around 90%) with a treatment capability of 440,000 m$^3$ day$^{-1}$. The sewage drainage system in Guangzhou is actually a mixture of underground trunk sewers that are adopted in newly developed residential districts, septic tanks and man-made streams that usually originate from artificial lakes or reservoirs, run through mainly from north to south of the city and finally link to the Pearl River by waterlocks. Water in the streams is quiet a complexity of lake water, rainwater, surface runoff, directly discharged wastewater, leakage from nearby sewers or septic tanks, river water from the Pearl River during tide, and other waters which connected with the streams. Trunk pipelines are usually set somewhere along the streams to collect and transfer the water to STPs. Some wastewater in GZ-STP2 is introduced through these man-built streams. The critical parameters of the two STPs are summarized in Table 2.

Raw sewages, effluents of each treatment step, were collected as grab samples in amber glass bottles (2.5 L) that had been pre-cleaned successively with detergent, water, distilled water and MeOH, and finally dried. Every bottle was pre-rinsed with sample for three times prior to sample collection. Drops of HCl (12 N) and appropriate amount of sodium azide (10 mg L$^{-1}$) were added into each bottle to prevent potential biodegradation. Samples were stored in a fridge (<4 °C) till they were processed within 2 days.

Wastewater samples were centrifuged in glass centrifuge tubes at 1500 rpm for 15 min. The supernatants were then filtered with glass microfiber filters (0.7 μm, 47 mm i.d., Whatman, Maidstone, England) before extracted with HLB cartridges. The microfiber filters had been pre-baked at 450 °C for >4 h prior to use. The aqueous samples were then adjusted to pH 3.0 with addition of HCl just prior to SPE. All samples were prepared in duplicate. Due to the lack of suitable surrogate standards, recovery samples spiked at 2 and 5 μg L$^{-1}$ of each target were also set for each sample to evaluate the recoveries. Recoveries were calculated by subtracting amounts of analytes measured in non-spiked samples from those in the recovery samples.

2.3. Solid-phase extraction

A variety of sorbents were used in order to optimize extraction efficiency including Oasis HLB (a hydrophilic and lipophilic balance cartridge, 500 mg 6 mL$^{-1}$, Waters, Mississauga, Ontario, Canada), C$_{18}$ (500 mg 6 mL$^{-1}$, Varian, Canada), C$_8$ (500 mg 6 mL$^{-1}$, Varian, Canada), C$_3$ (500 mg 6 mL$^{-1}$, Varian, Canada), SAX (1 g 6 mL$^{-1}$, Varian, Canada) and CN (1 g 6 mL$^{-1}$, Varian, Canada) cartridges. Solid-phase extraction was performed on a 12-fold vacuum box (Supeco, USA). Cartridge was preconditioned subsequently with 3 × 2 mL of mixture of MeOH and ethyl acetate (1:1), 3 × 2 mL of MeOH and 3 × 2 mL of redistilled water (pH 3.0). Sample was passed through the cartridge at a flow rate of around 5 mL min$^{-1}$ after its pH was adjusted to ~3.0 by addition of HCl. After percolation, the cartridge was washed with 2 mL of redistilled water and the

Table 2  
Operational parameters of the selected STPs in Guangzhou, China  

<table>
<thead>
<tr>
<th>STP</th>
<th>Population coverage</th>
<th>1st treatment</th>
<th>2nd treatment</th>
<th>3rd treatment</th>
<th>Designed capability (m$^3$ day$^{-1}$)</th>
<th>Wastewater sources</th>
<th>Sampling date (dd/mm/yy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GZ-STP1</td>
<td>~150,000</td>
<td>Sedimentation</td>
<td>Activated sludge</td>
<td>Filtration</td>
<td>30,000</td>
<td>Industrial + residential (4:6)</td>
<td>25/08/2005 9:00–11:30</td>
</tr>
<tr>
<td>GZ-STP2</td>
<td>195,000</td>
<td>Activated sludge</td>
<td>Filtration</td>
<td>Chlorination</td>
<td>440,000</td>
<td>Residential (~90%)</td>
<td>07/09/2005 14:30–16:00</td>
</tr>
</tbody>
</table>
eluents was discarded. The cartridge was then vacuum dried for about 30 min and was finally eluted with $3 \times 1.5 \text{ mL}$ of MeOH into a graduated glass centrifuge tube. The eluent was concentrated to about 0.1 mL under a gentle flow of high purity nitrogen and thereafter reconstituted into redistilled water containing $5 \mu g \text{ mL}^{-1}$ internal standard in an amber HPLC vial and stored at 0 °C prior to instrumental analysis.

2.4. Instrumentation

2.4.1. High performance liquid chromatography

An Agilent 1100 HPLC coupled with a diode UV array detector (DAD) and a fluorescence detector (FLD) was used to quantify the target analytes. In DAD detection, the UV wavelength was monitored at 240, 256 and 280 nm. The FLD excitation wavelength was set at 278 nm and emission wavelength at 500 nm, which is optimal for OFX detection (Golet et al., 2001). A ZORBAX SB-C$_{18}$ column (2.1 × 100 mm, particle size 3.5 μm, Agilent) was installed when analyzing spiked samples of Milli-Q water, tap water, groundwater and river water. The column was set at room temperature. Milli-Q water acidified with 0.1% of acetic acid and ACN with 0.1% of acetic acid were employed as mobile phases A and B, respectively. The elution gradient was programmed as starting at a flow rate of 0.2 mL min$^{-1}$ from 0% B for 3 min, to 10% B by 5 min, 50% by 10 min, 65% by 16.5 min and 100% by 20 min. The post time was set at 5 min to allow the re-equilibrium of the column for the next run. Investigated antibiotics in wastewater samples were separated on a Zorbax Eclipse XDB-C$_{18}$ column (3.0 × 150 mm, 3.5 μm, Agilent) connected with a guard column of the same type from Phenomenex (2.0 × 4 mm) at room temperature using 50 mM buffer of acetic acid and ammonium acetate (pH 4.5) and ACN as mobile phases. The binary gradient was programmed as starting at 10% B for 3 min, to 55% B by 10 min, 75% B by 15 min, to 90% B by 18 min, held for 3 min and finally back to initial state at 25 min. A post time of 5 min was set for column re-equilibrium. The flow rate was set at 0.25 mL min$^{-1}$. Sample aliquot of 2 μL was injected.

2.4.2. Mass spectrometry

Antimicrobial compounds were confirmed using Agilent 1100 LC-MS equipped with electrospray ionization by their protonated ions ([M+H]$^+$), Table 1. Analyses were performed in the positive mode. Nitrogen was used as nebulizer and drying gas. The parameters were set as: capillary voltage, 3500 V; dry gas temperature, 350 °C; and drying gas flow rate of 12 L min$^{-1}$.

2.5. Compound identification and quantification

Peaks in the spiked Milli-Q water, tap-, ground- and river-water samples (including original and spiked samples) were identified using both protonated ions and retention times by comparing their chromatograms with that of standard calibration solutions (Fig. 2). Quantification has been performed using HP ChemStations acquired by HPLC-DAD. A six-point calibration curve in the range of 0.5 to 50 ppm acquired at the UV wavelength of 280 nm using SMR as the internal standard was established for SDZ, SMX and CAP on the basis of their good response to UV detection (Fig. 2), and the fact that no quantifiable SMR was detected in wastewaters after enriching reasonable volume of samples. In analyzing antibiotics in wastewater samples, standard addition method (Lindsey et al., 2001) was applied to help compound identification. Standard solution containing the investigated antibiotics was spiked at 0.2 and 0.5 μg L$^{-1}$ into the extract of each wastewater sample. Chromatograms of the original wastewaters and spiked recovery samples were compared with those the corresponding standard-addition samples and standard solutions. The deviation of retention time was defined within 0.1 min for each compound. In addition, chromatograms obtained at UV wavelengths 240 and 254 nm were also monitored to assure the compound identification. For OFX, an external calibration curve was constructed in the range of 0.5–50 ppm acquired by FLD. The correlation coefficients ($R^2$) for these calibration curves were in the range of 0.992 to 0.997. Concentrations of target antimicrobials in samples were calculated based on the calibration curves.

2.6. QA/QC

A solvent blank (MeOH), an instrumental blank (redistilled water) and a mid-range calibration standard solution were injected in duplicate every time before running environmental samples to check the potential analytical interference and the sensitivity of the instrumental performance. The 1-day RSD of the calibration solution must be less than 20%. Procedural blanks consisting of redistilled water were analyzed as a control of procedural contamination. Duplicates and spiked recovery samples (spiked at 2 and 5 μg L$^{-1}$) were set for each wastewater sample. Laborious and time-cost standard-addition method was used to assure the identification of the investigated antibiotics in wastewater samples. An injection of MeOH was run after each sample of potentially high level of the targets to avoid possible cross contamination. A mid-range
calibration standard solution was run at the beginning, in the middle and at the end of each sequence to monitor the instrumental sensitivity and reproducibility. For routine determination, samples were performed in duplicate and the mean values were adopted.

3. Results and discussion

3.1. Method validation

The SPE extraction efficiency was tested by spiking known amounts of standard solutions containing all selected antimicrobials (0.2 to 50 μg of each compound) into 100 mL of milli-Q water, groundwater and river water (Rideau River, Ottawa, Canada) that had been confirmed to be free of antimicrobials by enrichment a volume of 100 mL respectively to compare the recoveries for each analyte. The results are shown in Table 3. In general, satisfactory recoveries were obtained for the selected antimicrobials when extracted with the C_{18}, C_{8} and Oasis HLB cartridges. Both CN and SAX cartridges gave relatively lower recoveries. Oasis HLB cartridges thereafter was chosen to extract wastewater samples from the two STPs of Guangzhou in this work provided its compromisingly optimal multi-residue extract efficiency.

Breakthrough of analytes on the SPE cartridge was checked by stacking a C_{18} cartridge (500 mg 6 mL⁻¹) under an Oasis HLB cartridge (500 mg 6 mL⁻¹) cartridge. Standard calibration solution containing 50 μg of each analyte was spiked into 250 mL of groundwater (Ottawa, Canada) and filtered raw sewage (Guangzhou, China), respectively. The spiked waters were percolated through the stacked cartridges. After passage, each cartridge was vacuum dried and eluted with 3×2 mL MeOH separately. Complete elution was verified by eluting the already MeOH eluted HLB cartridges for the second time with 2 mL of acetone. No quantifiable target analytes were detected in the eluents of the second cartridges and acetone eluates, which suggests that no breakthrough was occurring during SPE with the Oasis HLB cartridge at quite high level of target analytes in a reasonable volume of water samples, and 3×2 mL MeOH is effective enough for elution.

Linearity range between 0.2 and 100 ng of injection was tested for the analytes except OFX (0.2–50 ng on column). MQLs were calculated for groundwater and river-water samples that contained no target analytes by spiking the lowest concentration of the investigated analytes in various aquatic matrices. MQLs were roughly estimated from the chromatograms for S/N of 6, combinedly taking into consideration of recovery for each analyte. The MQLs were at 0.08–0.3 μg L⁻¹ for groundwater and river-water samples, and 3×2 mL MeOH is effective enough for elution.

Table 3
<table>
<thead>
<tr>
<th>Cartridges</th>
<th>SDZ</th>
<th>SMX</th>
<th>OFX</th>
<th>CAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLB</td>
<td>64 ± 9</td>
<td>90 ± 5</td>
<td>90 ± 3</td>
<td>99 ± 2</td>
</tr>
<tr>
<td>C_{18}</td>
<td>82 ± 6</td>
<td>119 ± 8</td>
<td>na</td>
<td>94 ± 5</td>
</tr>
<tr>
<td>C_{8}</td>
<td>83 ± 10</td>
<td>96 (3)</td>
<td>93b</td>
<td>81b</td>
</tr>
<tr>
<td>CN</td>
<td>81b</td>
<td>55 ± 12</td>
<td>65 ± 9</td>
<td>na</td>
</tr>
<tr>
<td>SAX</td>
<td>45 ± 15</td>
<td>66 ± 14</td>
<td>82 ± 20</td>
<td>87 ± 6</td>
</tr>
</tbody>
</table>

* 0.2 to 50 μg of each target was spiked in most cases unless specified; groundwater and river water were collected in Ottawa, Canada; na, not analysed.

b Only one analyse, recovery from river water; no concentration dependence was observed for the recovery of any of the analytes.

detected in the eluents of the second cartridges and acetone eluates, which suggests that no breakthrough was occurring during SPE with the Oasis HLB cartridge at quite high level of target analytes in a reasonable volume of water samples, and 3×2 mL MeOH is effective enough for elution.

Linearity range between 0.2 and 100 ng of injection was tested for the analytes except OFX (0.2–50 ng on column). MQLs were calculated for groundwater and river-water samples that contained no target analytes by spiking the lowest concentration of the investigated analytes in various aquatic matrices. MQLs were roughly estimated from the chromatograms for S/N of 6, combinedly taking into consideration of recovery for each analyte. The MQLs were at 0.08–0.15 μg L⁻¹ and 0.3–0.5 μg L⁻¹ for STP final effluents and raw sewage respectively due to the matrix interferences (Table 4).

Recovery was calculated by subtracting the measured amounts of analytes in original environmental samples from the spiked recovery samples, divided by the spiked amounts and then multiplied by 100. Recoveries of 64–99% were obtained for the investigated antibiotics in tap water, groundwater and river water (Table 3). In the case of urban wastewaters, the recoveries were in the range of 52–132%, showing larger variations and uncertainties, especially for the raw sewages (Table 5).

3.2. Occurrence and behavior of SDZ, SMX, OFX and CAP in municipal wastewater in Guangzhou, China

Wastewater samples of 250 mL were acidified and extracted and analyzed by HPLC-DAD/FLD as described above. All samples were run in duplicate and the mean values were adopted. The deviations of duplicate...
samples varied within 25%. The reported results have been corrected by recoveries generated from the corresponding recovery samples for each analyte.

Antimicrobials have been detected in raw sewages in Guangzhou at 5.10–5.15, 5.45–7.91, 3.52–5.56 and 1.73–2.43 μg L⁻¹ for SDZ, SMX, OFX and CAP, respectively (Table 6). The concentrations of SDZ and CAP in GZ-STP1 might be overestimated due to the co-elution of matrix components. The consumption data (such as amount and pattern) is helpful to better explicate the occurrence of antibiotics in sewages because the presence in municipal wastewater usually closely related with the use of these substances. Unfortunately, there is not published related information so far in China. To our knowledge, the selected antibiotics are used for both human and veterinary medicines. For example, SMX (usually prescribed in combination with trimethoprim) and OFX (and its enantiomer levofloxacin) are commonly used to treat intestinal infection, and infections of respiratory tract and urinary tract. SDZ are mainly used to cure epidemic meningitis. Sulfadiazine silver is used to heal burn injuries. CAP has been less frequently used for human medicine due to the severely adverse effect. However, it is applied to cure typhoid and paratyphoid fever. CAP is also prescribed in combination with penicillin to treat meningitis. The high concentrations of SDZ and CAP in wastewaters in Guangzhou might be related with the occurrence of epidemic meningitis in the city (http://www.ynet.com/view.jsp?oid=4663047, in Chinese).

In spite of the difference in the size and population served between the two STPs, levels of antimicrobials in their raw sewages are quite similar. Considering a portion of wastewater is conveyed to GZ-STP2 by some man-made streams, a dilution effect caused by other-originated waters, such as lake (or reservoir) water and the Pearl River water, and/or photodegradation of antimicrobials might have occurred in streams on their way to GZ-STP2, resulting in the relative lower level of antimicrobials in raw sewage in GZ-STP2. In addition, raw sewage samples in GZ-STP1 were collected as “fresh” wastewater which was pouring right from the inlet. While, samples in GZ-STP2 were collected in a huge tank in which raw sewage stores. The different protocol adopted during sampling might impact the results between the samples from the two STPs.

The concentrations of antimicrobial compounds are 4.18, 9.49, 6.13 and 1.46 μg L⁻¹ for SDZ, SMX, OFX and CAP respectively in the effluent of GZ-STP1 after preliminary mechanical sedimentation (Table 6), showing no obvious reduction compared with those in the raw sewage. Pharmaceuticals including antimicrobials are eliminated during STP treatments mainly via sorption and biodegradation (Ingerslev and Halling-Sørensen, 2000; Ternes et al., 2004; Urase and Kikuta, 2005). The results demonstrate that no important sorption or coagulation onto particulate matter has occurred for the investigated antibiotics during the preliminary sedimentation in GZ-STP1. That is to say that the mechanical sedimentation has minor effect on removal of these antimicrobials present in sewage.

SMX has been frequently detected in primary sewage effluents in Europe, at concentrations of 2, 1.45, 0.58 and 0.145 μg L⁻¹ in Germany, Switzerland, Spain and Austria, respectively (Hirsch et al., 1999; Hartig et al., 1999; Gobel et al., 2004, 2005; Carballa et al., 2004; Clara et al., 2005). SDZ was found at 10–100 ng L⁻¹ in Germany primary effluents (Hartig et al., 1999). CAP was detected at 0.56 μg L⁻¹ in Germany (Hirsch et al., 1999). The detected concentrations of antimicrobials in STP primary effluents (1.46–9.49 μg L⁻¹) in GZ-STP1 appear higher than those reported in Europe. The higher levels of antimicrobials in wastewater possibly reflect

### Table 5
Recoveries (% ± S.D.) of selected antimicrobials from STP wastewater, Guangzhou, China

<table>
<thead>
<tr>
<th>Compounds</th>
<th>SDZ</th>
<th>SMX</th>
<th>OFX</th>
<th>CAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infl</td>
<td>114±7</td>
<td>117±3</td>
<td>132±25</td>
<td>52±18</td>
</tr>
<tr>
<td>Effl#3</td>
<td>73±8</td>
<td>85±4</td>
<td>115±13</td>
<td>112±12</td>
</tr>
</tbody>
</table>

a Values of recovery are average of 4 replicate analysis by spiking 2 and 5 μg L⁻¹ of each analyte into wastewaters, respectively.

### Table 6
Selected antimicrobials (μg L⁻¹) detected in STP wastewaters, Guangzhou, China

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>GZ-STP1</th>
<th>GZ-STP2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infl</td>
<td>Effl#1b</td>
</tr>
<tr>
<td>SDZ</td>
<td>5.15</td>
<td>4.18</td>
</tr>
<tr>
<td>SMX</td>
<td>7.91</td>
<td>9.46</td>
</tr>
<tr>
<td>OFX</td>
<td>5.56</td>
<td>5.70</td>
</tr>
<tr>
<td>CAP</td>
<td>2.43</td>
<td>1.46</td>
</tr>
</tbody>
</table>

a Infl: raw sewage influent.

b Effl#1: the first effluent; Effl#2: the secondary effluent; Effl#3: the tertiary effluent, see in Table 1; nq: not quantifiable; nd: not detected.
been detected in the range of 1.73 to 7.91μg L⁻¹ in the secondary and tertiary effluents of GZ-STP1 and <MQLs in effluents of GZ-STP2 respectively (Table 6), showing that >85% of OFX has been removed from the sewages before they are discharged into ambient water. This result is higher than those reports in USA (Renew and Huang, 2004; Nakata et al., 2005), Canada (Miao et al., 2004), Switzerland (Gobel et al., 2004) and Italy (Castiglioni et al., 2005). However, it can be suggested from our results that the activated sludge treatment is effective and necessary to eliminate the antimicrobials in urban wastewaters. This work is only a preliminary study by grab samples. More work is needed to be done in detail in order to better illustrate the occurrence and fate of antibiotics in wastewaters and during STP treatments in China.

4. Conclusions

Solid-phase extraction combined with HPLC-DAD-FLD were employed to develop a feasible and simple method to analyze sulfonamides, fluoroquinolones and chloramphenicol in a variety of water matrices, spanning from the complex raw municipal sewage to groundwater and tap water. Although this method has only been illustrated with two SAs, one FQ and CAP, it can be applied to the other members of these antimicrobial groups as well.

Antimicrobials SDZ, SMX, OFX and CAP have been detected in the range of 1.73 to 7.91μg L⁻¹ in the raw municipal sewages in Guangzhou, China. The results show that the mechanical sedimentation has minor effects on removal of these antimicrobials in wastewater. Activated sludge treatment is effective and necessary to eliminate the antimicrobial compounds present in sewages, thus reducing the potentially ecological risks posed by discharges of STP effluents into ambient natural waters.

Acknowledgement

This work was financially supported by the National Basic Research Program of China (No. 2003CB415002) and GDSF Project (No. 021428). The authors want to acknowledge the generous support from the Environmental Technology Centre (ETC), Environment Canada (Ottawa, Canada). We thank Dr. Zhiqiang Yu in the State Key Laboratory of Organic Geochemistry (SKLOG), Chinese Academy of Sciences for the help in the instrumental analysis and valuable discussion in preparation of this paper. We also want to express our appreciation to Dr. Fanzhong Chen and Mr. Yanqing Sheng in SKLOG and STPs staff Mrs. Yuan, Mr. Sheng and Mr. Li for their assistance in wastewater sampling. The anonymous reviewers are sincerely appreciated for their valuable comments and recommendations that have greatly improved this paper.

References

Erickson BE. Analyzing the ignored environmental contaminants. Environ Sci Technol 2002;36:140A–5A.
Gobel A, McArdell CS, Suter MJ, Giger W. Trace determination of microlide and sulfonamide antimicrobials, a human sulfonamide metabolite, and trimethoprim in wastewater using liquid