JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Occurrence and Risk Assessment of Four Typical Fluoroquinolone Antibiotics in Raw and Treated Sewage and in Receiving Waters in Hangzhou, China

Changlun Tong,*[,†,‡] Xiajun Zhuo,[†] and Yun Guo[†]

[†]Key Laboratory of Environmental Remediation and Ecological Health, Ministry of Education, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, China

[‡]Zhejiang Provincial Key Laboratory of Organic Pollution Process and Control, Hangzhou 310058, China

Supporting Information

ABSTRACT: A sensitive liquid chromatography-fluorescence detection method, combined with one-step solid-phase extraction, was established for detecting the residual levels of the four typical fluoroquinolone antibiotics (ofloxacin, norfloxacin, ciprofloxacin, and enrofloxacin) in influent, effluent, and surface waters from Hangzhou, China. For the various environmental water matrices, the overall recoveries were from 76.8 to 122%, and no obvious interferences of matrix effect were observed. The limit of quantitation of this method was estimated to be 17 ng/L for ciprofloxacin and norfloxacin, 20 ng/L for ofloxacin, and 27 ng/L for enrofloxacin. All of the four typical fluoroquinolone antibiotics were found in the wastewaters and surface waters. The residual contents of the four typical fluoroquinolone antibiotics in influent, effluent, and surface water samples are 108−1405, 54−429, and 7.0−51.6 ng/L, respectively. The removal rates of the selected fluoroquinolone antibiotics were 69.5 (ofloxacin), 61.3 (norfloxacin), and 50% (enrofloxacin), indicating that activated sludge treatment is effective except for ciprofloxacin and necessary to remove these fluoroquinolone antibiotics in municipal sewage. The risk to the aquatic environment was estimated by a ratio of measured environmental concentration and predicted no-effect concentration. At the concentrations, these fluoroquinolone antibiotics were found in influent, effluent, and surface waters, and they should not pose a risk for the aquatic environment.

KEYWORDS: Fluoroquinolone antibiotics, HPLC, fluorescence detection, wastewater, surface water, environmental risk assessment

INTRODUCTION

In recent years, the occurrence, fate, and potential toxic effects of pharmaceuticals have become one of the emerging research areas in the environmental field. Fluoroquinolone antibiotics are probably the most important class of synthetic antibiotics in human and veterinary medicines because of their broad activity spectrum and good oral absorption. A large quantity of fluoroquinolone antibiotics are produced and consumed in China as human and veterinary medicines as well as growth promoters each year.[1] Among these fluoroquinolone antibiotics, ofloxacin, norfloxacin, ciprofloxacin, and enrofloxacin (Figure 1) are the main species.[2,3]

A major source of contamination by fluoroquinolone antibiotics is human and animal excretion. Because of the extensive use of fluoroquinolone antibiotics in urban centers and the fact that they are largely excreted unchanged,[4,5] a quantity of these antibiotics can be found in municipal wastewaters[6,7] as well as in surface waters[8,9] and even groundwaters[10] of the surrounding areas. Although the concentrations of fluoroquinolone antibiotics in the ecosystem are very low, usually at ng/L to μg/L levels in water, the presence of fluoroquinolone antibiotics in the environment is potentially linked to their resistant bacteria, which may result in disturbed aquatic ecosystems and make humans and animals more susceptible to antibiotic-resistant microbes.[11] With their continuous input into the environment, together with their relative persistence,[12] the study of their presence, fate, and adverse effects has attracted increasing attention. Therefore, it is necessary to develop a sensitive, reliable, and rapid method to detect fluoroquinolone antibiotics in different water samples.

Because of the high sensitivity and specificity, liquid chromatography coupled to mass spectrometry or tandem mass spectrometry (LC-MS/MS) has been used extensively to monitor fluoroquinolone antibiotics in the environment during the past few years.[1,5,8,9,13,14] Fluoroquinolone antibiotics are present in the wastewaters, surface waters, and other environments at trace levels or ultra trace levels. Thus, a preconcentration step is generally required for the determination of these pollutants at trace levels in the environment. Sample preparation is traditionally carried out by solid-phase extraction (SPE) techniques. In previous studies, the hydrophilic−lipophilic balance (HLB) cartridge has been employed to extract trace fluoroquinolone antibiotics from water samples without further cleanup procedures.[13−15] Ion-exchange cartridges such as the Oasis WCX cartridge[5,16] are also used for

Received: April 7, 2011
Accepted: June 1, 2011
Revised: June 1, 2011
Published: June 01, 2011

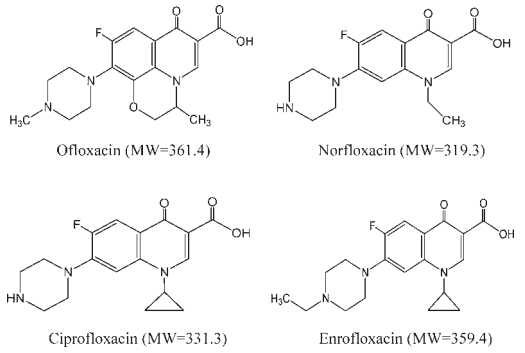

Figure 1. Molecular structures of ofloxacin, norfloxacin, ciprofloxacin, and enrofloxacin.

extraction with a potential cleanup purpose. In addition, solid-phase microextraction is also used for the extraction of trace fluoroquinolone antibiotics from water samples.[17] Recently, a novel high-performance liquid chromatography (HPLC) technology named ultraperformance liquid chromatography (UPLC) has been developed. UPLC performs much better in terms of resolution, sensitivity, and separation efficiency with a significant reduction in sample analysis time and mobile phase solvent consumption.[1,18] For conventional detection, UV detection can provide a very good stability signal; its shortcoming is short of selectivity. Electrochemical detection is more sensitive than UV detection. However, their performances are highly dependent on the types of samples analyzed. Components from dirty samples are deposited on the electrochemical cell, and the detector's sensitivity is rapidly decreased.[19] Fluorescence detection can provide not only high sensitivity but also good selectivity. So, fluorescence detection as a conventional detection method is the primary selection for the determination of fluoroquinolone antibiotics, just because these compounds can emit highly strong native fluorescence. HPLC coupled with fluorescence[20] or chemiluminescence[21] detection is a sensitive alternative approach to quantify trace levels of fluoroquinolone antibiotics. Furthermore, it is relatively simple, less expensive, and currently much more easily accessible in developing countries like China. The LC-MS/MS or UPLC-MS/MS method has good specificity and high sensitivity. However, the instruments are too expensive, and the costs are also very high. The method is not suitable for the general laboratories to carry out the relevant studies on the fluoroquinolone antibiotics in the environment. Although HPLC coupled with fluorescence detection has been widely used to analyze some fluoroquinolone antibiotics in the wastewaters,[22,23] food, and biological fluids,[24] its applications in surface water or groundwater are very few. Moreover, the sample preconcentration procedure is comparably complicated, usually using two-step extraction.

The objective of this study is to develop a simple, sensitive, and specific method for trace analysis of four typical fluoroquinolone antibiotics to meet the demands of the study on the occurrence, fate, and potential toxic effects of these antibiotics in the environment. Another objective of this work is to determine residues of the four typical fluoroquinolone antibiotics in STP and its receiving river samples from Hangzhou, China. On the basis of the above environmental investigation, the risk to the aquatic environment was estimated by a ratio of measured environmental concentration (MEC) and predicted no-effect concentration (PNEC).

MATERIALS AND METHODS

Materials and Reagents. All chemicals and reagents are of analytical reagent grade, unless stated otherwise. Ofloxacin, norfloxacin, ciprofloxacin, and enrofloxacin were supplied from Hangzhou Institute for Drug Control (Hangzhou, China). Methanol was purchased from Tianjin Shield Co. (Tianjin, China). Methanol was HPLC grade, and it was used without further purification. Standard samples of ofloxacin, norfloxacin, ciprofloxacin, and enrofloxacin were respectively dissolved in water as a stock solution (1.0×10^{-3} mol/L). These stock solutions were stored in the dark at room temperature. Standard sample solutions were provided daily at different concentrations by diluting the stock standard solution with water. All water used in the experiments was obtained from a ULUPURE pure water machine (Shanghai Youpu Industry Co. Ltd., Shanghai, China).

Sample Collection. Twenty-four hour composite influent and effluent samples were collected during a 1 week period on July 30 to August 9, 2007, and July 15 to July 21, 2009, from Sibao Sewage Treatment Plant (STP) (Hangzhou, China). Hangzhou is a big city in southern China with a population of about 6 million. This STP mainly (about 60−70%) receives domestic water from Hangzhou city, and its processing capacity is 600 000 m^3/day. The treatment processes at the STP are in the following order: influent water first through thick-case-bar and thin-case-bar, then through aerated grit chamber, via primary and secondary treatment processes, effluent water. Surface water samples were collected from Qiantang River on the same day, which receives the effluent from Hangzhou Sibao STP. These samples were stored in 10 L amber glass bottles that had been washed with methanol and purified water and were carried immediately to the laboratory. After delivery to the laboratory, samples were filtered through a medium qualitative filter paper and then filtered through a 0.45 μm micropore filter under a slight vacuum to remove suspended matter and stored in the dark at 4 °C until extraction within 24 h.

SPE. Water samples were added with Na_2EDTA (0.2%, w/v) and adjusted to pH 4.0 with acetic acid and then were extracted by LC-18 cartridges (500 mg, 3 mL, Supelco, United States). The LC-18 cartridge was preconditioned by 3 mL of CH_3OH and 5 mL of 0.2% (w/v) Na_2EDTA solution. The influent (0.2 L), effluent (0.2 L), and river water (1 L) samples were extracted through the LC-18 cartridges, which were placed in a SPE manifold, and the samples were siphoned through the cartridges at a flow rate of 3−6 mL/min. After the cartridges were rinsed with 5 mL of 0.2% (w/v) Na_2EDTA solution, they were kept in the vacuum for 20 min and then eluted with 3 × 1 mL of methanol containing 6% ammonia−water (v/v). The eluates were blown down to 200 μL and then reconstituted to 1.0 mL with pure water for LC-FLD analysis.

High-Performance Liquid Chromatography with Fluorescence Detection (LC-FLD). LC analysis was performed by using an Agilent 1200 series LC system consisting of a solvent degasser (G1322A), a quaternary pump (G1354A), a manual injection valve 7725i equipped with a 20 μL injection loop, a thermostatted column compartment (G1316A), and a LS-55 fluorescence spectrophotometer (PerkinElmer, United States) combined with HPLC by a LC flow cell as a fluorescence detector. Separation of the four fluoroquinolone antibiotics was achieved by a 150 mm × 4.6 mm i.d., 5.0 μm, Eclipse XDB-C18 (Agilent, United States) at 30 °C under isocratic conditions at a flow rate of 1.0 mL min^{-1}. The mobile phase was a mixture of methanol/phosphoric acid (0.1%, v/v)−triethylamine buffer (pH 3.0) 20/80 (v/v). The fluorescence detection was operated at an excitation wavelength of 281 nm and an emission wavelength of 460 nm. The spectroscopic bandwidth was 10 nm for both excitation and emission. The results were recorded by FL WinLab Software.

Compound Identification by LC-ESI-MS. The selected fluoroquinolone antibiotics in the environmental samples (sewage and

surface water samples) were confirmed using Agilent 1100 LC/MSD SL equipped with electrospray ionization (ESI) by their protonated molecular ions ($[M + H]^+$). The detector was operated in the ESI^+ mode using nitrogen as a drying gas at a flow rate of 13 L/min and a temperature of 350 °C. The nebulizer pressure was 50 psi. Operating parameters such as capillary voltage and fragmentor were 4000 and 100 V, respectively. In analyzing the selected fluoroquinolone antibiotics in the sewage and surface water samples, standard addition method was applied to help compound identification. A standard solution containing the selected fluoroquinolone antibiotics was spiked at 1.0 μmol/L into the extract of each sewage and surface water sample. Peaks in the spiked sewage and surface water samples (including original and spiked samples) were identified using both protonated molecular ions and retention times by comparing their chromatograms with those of standard calibration solutions. Chromatograms of the original sewage and surface water samples and their spiked samples were compared with those of the corresponding standard addition samples and standard solutions. The deviation of retention time was defined within 0.1 min for each compound. The results indicated that around the retention times of the selected fluoroquinolone antibiotics in the different types of real environmental samples, no interference was detected in any of the samples analyzed by HPLC-FLD and LC-MS. Chromatograms of the four typical fluoroquinolone antibiotics in the spiked effluent sample detected by LC-MS are shown in Figure 2.

RESULTS AND DISCUSSION

Optimization of SPE. Previous studies show that a single cleanup through a C18 cartridge is not always sufficient for analysis of fluoroquinolone antibiotic residues in the wastewater samples,[25] because organic matter existing in the wastewaters can reduce the extraction efficiency and interfere with detection.[26] Because HLB cartridges have great potential to concentrate both hydrophilic and lipophilic compounds, both objective compounds and matrix interferences can be accumulated and therefore cause signal interference in LC-MS/MS.[27] Therefore, tandem SPE methods using a strong anion-exchange (SAX) cartridge[28] or a weak cation-exchange (WCX) cartridge[16] combined with an HBL cartridge were used in the sample cleanup. The anion exchange cartridges can reduce matrix interferences by adsorbing negatively charged humic material and other highly negatively charged natural organic matter from the wastewater samples; thus, this can prevent contamination, blockage, and overload of the HLB sorbent.[20] Although the two-step cleanup procedure can separate fluoroquinolone antibiotics from impurities present in wastewater samples, the extracted objective compounds from the wastewater samples, that is, extraction efficiency will be reduced. Moreover, the signal suppression for LC-MS/MS was still observed in the wastewater extracts.[26]

Because fluorescence detection has high selectivity, some interference matrix existing in the coextracts probably does not emit fluorescence or emits very weak fluorescence at the selected excitation and emission wavelengths. This means that a simple one-step SPE procedure can eliminate the interference from the matrix. The four fluoroquinolone antibiotics have a lower polarity and are hydrophobic compounds. They may have three types of potential interactions (hydrophobic, ion-exchange, and hydrogen bondings) with the end-capped silanols of the LC-18 reversed phase cartridges. The main interaction might be hydrophobic.

Because of the acid−base properties of the fluoroquinolone antibiotics, SPE of the analytes is expected to be strong dependent on pH. During the optimization stage, different buffers for cartridge conditioning and different sample pH values were evaluated. Samples were evaluated at pH 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0, and cartridges were conditioned with methanol and 0.2% (w/v) Na_2EDTA solution. Acidification of the sample with a weak acid (acetic acid) at pH 4.0 was selected, because the fluoroquinolone antibiotics, with a piperazinyl moiety, are fully protonated at low pH. Na_2EDTA is added as a chelating reagent to prevent fluoroquinolone antibiotics coordinating with metal ions and to ensure high extraction efficiency. These metal ions such as Ca^{2+}, Mg^{2+}, Al^{3+}, and rare earth ions can easily bind to carbonyl and carboxyl groups of the fluoroquinolone antibiotics. Accordingly, a complex compound with a six-membered ring is formed.

After sample percolation, the LC-18 cartridge was washed with 5 mL of 0.2% (w/v) Na_2EDTA solution and dried under vacuum for 20 min. Fluoroquinolone antibiotics were eluted from the LC-18 cartridge with 3 × 1 mL of methanol containing 6% ammonia−water (v/v). To examine whether the interfering substances around the retention times of the selected fluoroquinolone antibiotics exist or not, different types of wastewater and surface water samples were analyzed. No interference was detected in any of the samples analyzed, and the overall recoveries of the selected fluoroquinolone antibiotics in the sewage and surface water samples were in the range of 76.8−122%. So, the one-step SPE procedure not only has a high extraction efficiency (all recoveries more than 75%) but also meets the demands of fluorescence detection.

Optimization of HPLC Separation. The effect of the pH of the mobile phase on the resolution of the analytes was examined based on the acid−base properties of fluoroquinolone antibiotics. When the pH of the mobile phase was more than 4.0 or lower than 2.3, the best results in terms of resolution and selectivity could not be achieved. When the pH was within 2.3−4.0, no obvious effect on the resolution was observed. A pH of 3.0 for the mobile phase was thus selected. Phosphoric acid was used to adjust the pH of the mobile phase to 3.0, which could protonate the amino groups of the fluoroquinolone antibiotics and the residual silanol groups of the stationary phase so that their interaction could take place; thus, peak asymmetry was reduced. To reduce tailing in the LC separation of the selected fluoroquinolone antibiotics, triethylamine as an additive was added to the phosphoric acid buffer.

Excitation and emission scans were performed with a fluorescence spectrophotometer to establish optimum excitation and emission wavelengths. The optimal excitation and emission wavelengths obtained for the different fluoroquinolones were as follows: For norfloxacin, ciprofloxacin, and enrofloxacin, their excitation and emission wavelengths are very close, about λ_{ex} = 277 nm, and λ_{em} = 445 nm;[29] for ofloxacin, λ_{ex} = 285 nm, and λ_{em} = 485 nm.[30] In previous reports, an emission wavelength of 450 nm was usually selected. However, at this emission wavelength, the fluorescences of norfloxacin, ciprofloxacin, and enrofloxacin are very strong, while the fluorescence of ofloxacin is not strong, which will influence its sensitivity. Chromatographic detection was therefore performed at excitation and emission wavelengths of 281 and 460 nm, respectively.

Method Validation. Calibration curves for the selected fluoroquinolone antibiotics were tested in a concentration range of 5−20000 μg/L. The results indicated that calibration curves of norfloxacin and ciprofloxacin showed good linearity in the range of 5.0−16000 μg/L with good correlation coefficients ($r^2 > 0.9970$), while for ofloxacin and enrofloxacin, their linear relationships

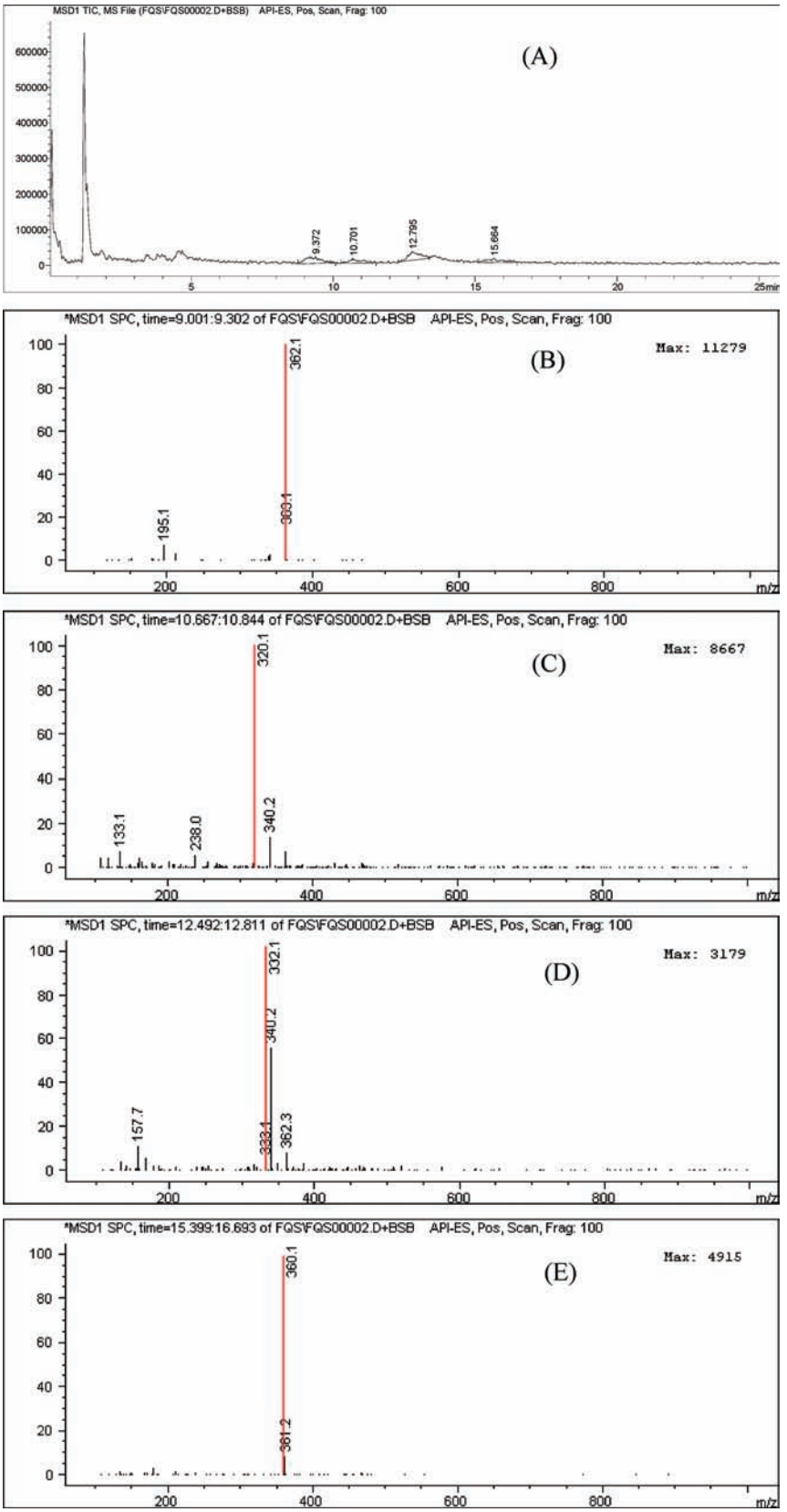

Figure 2. Chromatograms of the four typical fluoroquinolone antibiotics in the spiked effluent sample detected by LC-ESI-MS. (A) TIC, (B) ofloxacin, (C) norfloxacin, (D) ciprofloxacin, and (E) enrofloxacin.

Figure 3. Sampling sites of receiving river and location of STPs.

were in the ranges of 6−18000 and 8−18000 μg/L, respectively, and with good correlation coefficients ($r^2 > 0.9993$).

The limit of quantitation (LOQ) for the selected fluoroquinolone antibiotics was determined by using a 1 L surface water sample free of these analytes, spiked at a low concentration of 0.1 nmol/L, extracted with the above optimal conditions of SPE, detected with LC-FLD, and evaluated by the criterion that the signal-to-noise ratio (S/N) should be more than 10 for quantitation purposes. The LOQ was estimated to be 17 ng/L for ciprofloxacin and norfloxacin, 20 ng/L for ofloxacin, and 27 ng/L for enrofloxacin.

The accuracy and precision of the method were examined by determining the spiked sewage and surface water samples at 0.01 μmol/L. The mean recoveries of the selected fluoroquinolone antibiotics in the sewage water samples were in the range of 76.8−122% with a RSD less than 7%, while for the surface water samples their recoveries were in the range of 83.9−89.2% with a RSD less than 3%. Within-day accuracy was evaluated by continuously performing five replicates within a day for the determination of the same spiked distilled water sample. The results indicated that within-day RSD of retention times on the basis of the selected fluoroquinolone antibiotics was between 0.6 and 0.7%, while for fluorescence intensities, its RSD was between 0.1 and 2.6%. Day-to-day accuracy was also evaluated by performing determination of the same spiked distilled water sample each day on five consecutive days. The day-to-day RSD of retention times on the basis of the selected fluoroquinolone antibiotics was between 2.9 and 3.2%, while for fluorescence intensities its RSD was between 4.3 and 12.5%.

Occurrence and Behavior of Four Typical Fluoroquinolone Antibiotics in Municipal Wastewater and Receiving River in Hangzhou, China. Influent and final effluent samples of 100 mL and surface water samples of 1 L were acidified and extracted using the present SPE procedure and analyzed by the HPLC-FLD method established in this study. All samples were run in three replicates, and the mean values were adopted. The deviations of three replicates varied within 20%. The reported results have been corrected by recoveries generated from the corresponding recovery samples for each analyte. The levels of ofloxacin, norfloxacin, ciprofloxacin, and enrofloxacin found in the influent and final effluent samples collected from Hangzhou Sibao Sewage Treatment Plants, and surface water samples of the two sites from the receiving river of the STP, Qiantang River (Figure 3), are listed in Table 1. Chromatograms of the standard solution and the extracts from effluent and surface water samples are shown in Figure 4.

Table 1. Concentrations of the Four Typical Fluoroquinolone Antibiotics Detected in the Sewage Water and Surface Water Samples

	Sibao STP (ng/L)		surface water (ng/L)		
compounds	influent	effluent	site 1	site 2	average
ofloxacin	1405	429	51.6	45.7	48.7
norfloxacin	248	96	7.0	12.9	10.0
ciprofloxacin	268	199	9.3	11.0	10.2
enrofloxacin	108	54	10.5	18.7	14.6
total FQs	2029	778	78.4	88.3	83.5

For the residuals of fluoroquinolone antibiotic in all water samples, ofloxacin was dominant with concentrations of 429 ng/L in effluent and 1405 ng/L in influent, which was consistent with the previous reports of 503 ng/L ofloxacin in effluent and 1208 ng/L in influent in Beijing, China.[16] As compared with previous reports in other countries, 94 ng/L ofloxacin in effluent in Canada[13] and ofloxacin not detected in Portugal,[20] levels of ofloxacin either influent or effluent in China are relatively high, indicating the different consumption style in China. Whereas ciprofloxacin (199 ng/L in effluent and 268 ng/L in influent) and norfloxacin (96 ng/L in effluent and 248 ng/L in influent) were comparable with previous reports of 118 ng/L ciprofloxacin and 50 ng/L norfloxacin in effluent in Canada,[13] 100.8 ng/L ciprofloxacin and 35 ng/L norfloxacin was found in effluent in Portugal.[20] Norfloxacin (54 ng/L in effluent and 108 ng/L in influent), used only in veterinary medicine, was detected in our study in municipal wastewaters. The same case is also found in the previous reports that levels of enrofloxacin are 53.7 ng/L in effluent and 121.8 ng/L in influent in Portugal,[20] probably because of agricultural sources, such as manure dispersion and animal excretion on to soils.

Comparing the concentrations in the influent with that in the effluent, we found that the reduction rates of the selected fluoroquinolone antibiotics were 69.5 (ofloxacin), 61.3 (norfloxacin), 50 (enrofloxacin), and 25.8% (ciprofloxacin). As compared with the reported results of ofloxacin (58.4%), norfloxacin (74.9%), and ciprofloxacin (66.3%) from Gao Beidian STP (Beijing, China),[16] the reduction percentages of ofloxacin and norfloxacin are comparable; however, the reduction percentages of ciprofloxacin are significantly reduced. Other literature on the reduction of ofloxacin, norfloxacin, and ciprofloxacin indicates more than 40% during the treatment process in STP in Canada.[5] The concentrations of ciprofloxacin in STP effluents are different according to the efficiency of treatment (22.2−100%), which depends on the secondary treatment process used. Use of an oxidation ditch and activated sludge reduction of ciprofloxacin was 22.2 and 71.4%, respectively.[31] In addition, it should be noted that the reduction percentages of fluoroquinolone antibiotics are related to seasons. This is consistent with a study from Switzerland,[32] where fluoroquinolone antibiotics are significantly reduced in the STP process during the summer period than that in winter. This is another reason why in this study the percentages of reduction detected in summer are comparatively lower than those of the previous reports.

In the surface water samples, all of the selected fluoroquinolone antibiotics (ofloxacin, norfloxacin, ciprofloxacin, and enrofloxacin)

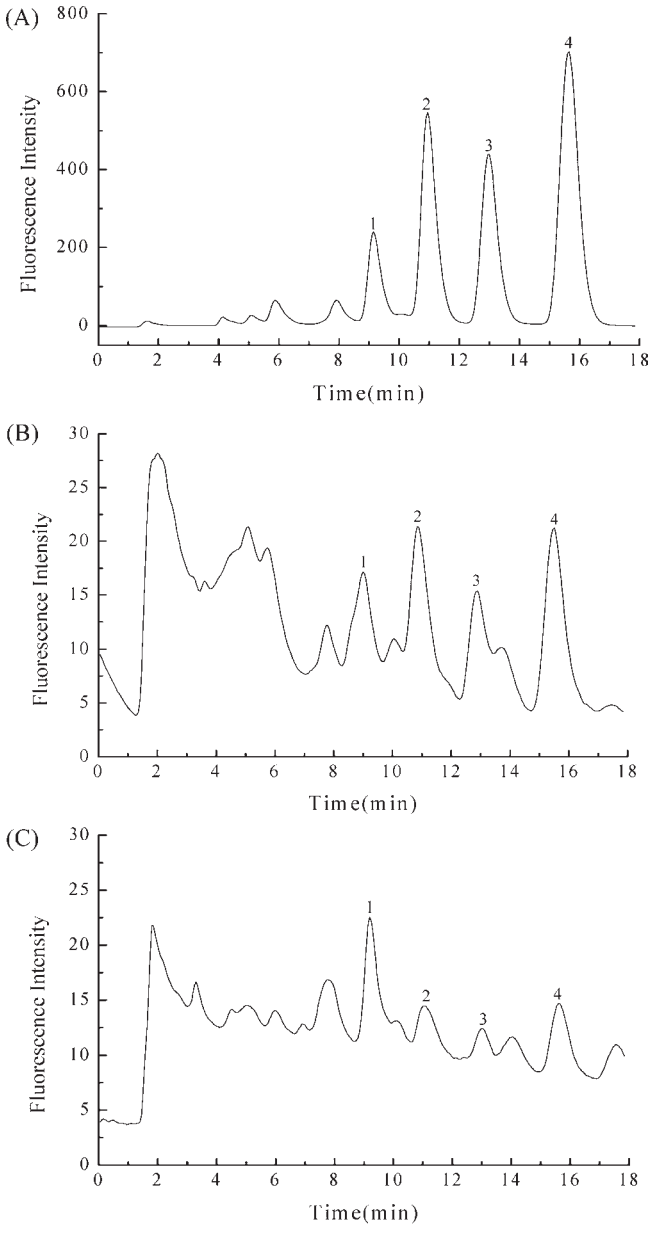

Figure 4. Chromatograms of standard solution (A), effluent water sample from Hangzhou Sibao STP (B), and surface water sample from Qiantang River (C). Peak identification: 1, ofloxacin; 2, norfloxacin; 3, ciprofloxacin; and 4, enrofloxacin.

were detected, with the concentrations ranging from 7.0 (norfloxacin) to 51.6 ng/L (ofloxacin), which was similar to those in the effluent samples. This work is only a preliminary study by grab samples. More work is needed to be done in detail to better illustrate the occurrence and fate of antibiotics in wastewaters, surface waters, and during STP treatments in China.

Risk to the Aquatic Environment. Considering the lack of field data, the calculation of predicted environmental concentrations (PECs) is a generally accepted approach for environmental risk assessment. Herein, we aim to assess environmental risk using the actual MECs. The environmental risk assessment (ERA) for surface waters was based on the Draft Discussion Paper proposed by the European Agency for the Evaluation of Medical Products (EMEA). The exposure data of ciprofloxacin for final effluents and river water were related to acute toxicity for aquatic organisms. Following the recommendations of the European guidelines and draft documents, a PNEC in surface waters of 3 μg/L using EC_{50} (growth inhibition) data to the algae *Selenastrum capricornutum* was obtained after applying a safety factor of 1000 to the lowest EC_{50} value.[33] A PNEC in Wastewater Treatment Plants (WWTPs) of 8 μg/L using EC_{50} data of ciprofloxacin to a relevant bacterial population of *Pseudomonas putida*[34] was obtained after applying a safety factor of 10 for interspecies variability. These values are comparable to the lowest found minimum inhibition concentration (MICs) for ciprofloxacin and norfloxacin (MIC90 $\geq$10 μg/L) without applying further safety factors[32] or with a MIC of 1 μg/L when applying a safety factor of 10, which should account for uncertainties derived from intraspecies variability. As shown in Table 2, all of the calculated values of MEC/PNEC are less than 1 (risk quotient MEC/PNEC > 1). Even if the total FQs of the four typical fluoroquinolones in the receive river is 0.08 μg/L, this concentration is still far less than 3 μg/L. According to the above calculated values of MEC/PNEC, it could be concluded that at the concentration level found in the studied river, the individual pharmaceuticals do not pose an environmental risk. However, we should point out that conventional ERA is usually limited to one compound per assessment. Because FQs are very much related structurally as well as in their mode of action, the total FQ concentration should be considered to demonstrate the potential additive toxicity of FQs. For a more advanced and more sophisticated risk characterization, data on mixture toxicities as well on subinhibitory effects would be needed.[33] In addition, chronic effects of these pharmaceuticals, effects of their metabolites, and synergetic effects are not known.[35] Thus, a direct answer to the question of whether these pharmaceuticals pose a risk in the aquatic environment cannot be given.

Table 2. Calculated Values of MEC/PNEC

sampling points	ofloxacin	norfloxacin	ciprofloxacin	enrofloxacin	total FQs
influent	0.176	0.031	0.034	0.036	0.254
effluent	0.054	0.012	0.025	0.018	0.097
surface water	0.016	0.003	0.003	0.005	0.028

■ ASSOCIATED CONTENT

Supporting Information. Tables of characters of the selected fluoroquinolone antibiotics and analytical parameters of the HPLC-FLD method for the determination of the four typical fluoroquinolone antibiotics. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +86-571-86968415. Fax: +86-571-86968415. E-mail: cltong@zju.edu.cn.

Funding Sources

This work was supported by the National Natural Science Foundation of China (No. 20877067) and Zhejiang Provincial Natural Science Foundation of China (No. R507212).

■ REFERENCES

(1) Li, B.; Zhang, T.; Xu, Z. Y.; Fang, H. H. P. Rapid analysis of 21 antibiotics of multiple classes in municipal wastewater using ultra

performance liquid chromatography-tandem mass spectrometry. *Anal. Chim. Acta* **2009**, *645*, 64–72.

(2) Tong, C. L.; Xiang, G. H. Sensitive determination of norfloxacin by the fluorescence probe of Tb^{3+}-SDBS and its luminescence mechanism. *J. Fluoresc.* **2006**, *16*, 831–837.

(3) Tong, C. L.; Xiang, G. H. Sensitive determination of enoxacin by its enhancement effect on the fluorescence of terbium (III)−sodium dodecylbenzene sulfonate and its luminescence mechanism. *J. Lumin.* **2007**, *126*, 575–580.

(4) Hartmann, A.; Alder, A. C.; Koller, T.; Widmer, R. M. Identification of fluoroquinolone antibiotics as the main source of *umuC* genotoxicity in native hospital wastewater. *Environ. Toxicol. Chem.* **1998**, *17*, 377–382.

(5) Lee, H. B.; Peart, T. E.; Svoboda, M. L. Determination of ofloxacin, norfloxacin, and ciprofloxacin in sewage by selective solid-phase extraction, liquid chromato- graphy with fluorescence detection, and liquid chromatography−tandem mass spectrometry. *J. Chromatogr. A* **2007**, *1139*, 45–52.

(6) Ternes, T. A. Occurrence of drugs in Germany sewage treatment plants and rivers. *Water Res.* **1998**, *32*, 3245–3260.

(7) Ternes, T.; Bonerz, M.; Schmidt, T. Determination of neutral pharmaceuticals in wastewater and rivers by liquid chromatography−electrospray tandem mass spectrometry. *J. Chromatogr. A* **2001**, *938*, 175–185.

(8) Vieno, N. M.; Tuhkanen, T.; Kronberg, L. Analysis of neutral and basic pharmaceuticals in sewage treatment plants and in recipient rivers using solid phase extraction and liquid chromatography−tandem mass spectrometry detection. *J. Chromatogr. A* **2006**, *1134*, 101–111.

(9) Conley, J. M.; Symes, S. J.; Kindelberger, S. A.; Richards, S. M. Rapid liquid chromatography−tandem mass spectrometry method for the determination of a broad mixture of pharmaceuticals in surface water. *J. Chromatogr. A* **2008**, *1185*, 206–215.

(10) Holm, J. V.; Rugge, K.; Bierg, P. L.; Christensen, T. H. Occurrence and distribution of pharmaceutical organic compounds in the groundwater downgradient of a landfill (Grindsted, Denmark). *Environ. Sci. Technol.* **1995**, *29*, 1415–1420.

(11) Daughton, C. G.; Ternes, T. A. Pharmaceuticals and personal care products in the environment: Agents of subtle change. *Environ. Health Perspect.* **1999**, *107* (Suppl. 6), 907–938.

(12) Samuelsen, O. B.; Lunestad, B. T.; Ervik, A.; Fjelde, S. Stability of antibacterial agents in an artificial marine aquaculture sediment studied under laboratory conditions. *Aquaculture* **1994**, *126*, 283–290.

(13) Miao, X. S.; Bishay, F.; Chen, M.; Metcalfe, C. D. Occurrence of antimicrobials in the final effluents of wastewater treatment plants in Canada. *Environ. Sci. Technol.* **2004**, *38*, 3533–3541.

(14) Reverté, S.; Borrull, F.; Pocurull, E.; Marcé, R. M. Determination of antibiotic compounds in water by solid-phase extraction−high-performance liquid chromatography−(electrospray) mass spectrometry. *J. Chromatogr. A* **2003**, *1010*, 225–232.

(15) Castiglioni, S.; Bagnati, R.; Fanelli, R.; Pomati, F.; Calamari, D.; Zuccato, E. Removal of pharmaceuticals in sewage treatment plants in Italy. *Environ. Sci. Technol.* **2006**, *40*, 357–363.

(16) Xiao, Y.; Chang, H.; Jia, A.; Hu, J. Y. Trace analysis of quinolone and fluoroquinolone antibiotics from wastewaters by liquid chromatography−electrospray tandem mass spectrometry. *J. Chromatogr. A* **2008**, *1214*, 100–108.

(17) Mitani, K.; Kataoka, H. Determination of fluoroquinolones in environmental waters by in-tube solid-phase microextraction coupled with liquid chromatography−tandem mass spectrometry. *Anal. Chim. Acta* **2006**, *562*, 16–22.

(18) Ibáñez, M.; Guerrero, C.; Sancho, J. V.; Hernández, F. Screening of antibiotics in surface and wastewater samples by ultra-high pressure liquid chromatography coupled to hybrid quadrupole time-of-flight mass spectrometry. *J. Chromatogr. A* **2009**, *1216*, 2529–2539.

(19) Lacorte, S.; Fraisse, D.; Barceló, D. Efficient solid-phase extraction procedures for trace enrichment of priority phenols from industrial effluents with high total organic carbon content. *J. Chromatogr. A* **1999**, *857*, 97–106.

(20) Seifrtová, M.; Pena, A.; Lino, C. M.; Solich, P. Determination of fluoroquinolone antibiotics in hospital and municipal wastewaters in Coimbra by liquid chromatography with a monolithic column and fluorescence detection. *Anal. Bioanal. Chem.* **2008**, *391*, 799–805.

(21) Wan, G. H.; Cui, H.; Pan, Y. L.; Zheng, P.; Liu, L. J. Determination of quinolones residues in prawn using high-performance liquid chromatography with Ce(IV)−$Ru(bpy)_3^{2+}$−HNO_3 chemiluminescence detection. *J. Chromatogr. B* **2006**, *843*, 1–9.

(22) Golet, E. M.; Alder, A. C.; Hartmann, A.; Ternes, T. A.; Giger, W. Trace determination of fluoroquinolone antibacterial agents in urban wastewater by solid-phase extraction and liquid chromatography with fluorescence detection. *Anal. Chem.* **2001**, *73*, 3632–3638.

(23) Golet, E. M.; Strehler, A.; Alder, A. C.; Giger, W. Determination of fluoroquinolone antibacterial agents in sewage sludge and sludge-treated soil using accelerated solvent extraction followed by solid-phase extraction. *Anal. Chem.* **2002**, *74*, 5455–5462.

(24) Schneider, M. J.; Darwish, A. M.; Freeman, D. W. Simultaneous multiresidue determination of tetracyclines and fluoroquinolones in catfish muscle using high performance liquid chromatography with fluorescence detection. *Anal. Chim. Acta* **2007**, *586*, 269–274.

(25) Turiel, E.; Bordin, G.; Rodríguez, A. R. Determination of quinolones and fluoroquinolones in hospital sewage water by off-line and on-line solid-phase extraction procedures coupled to HPLC-UV. *J. Sep. Sci.* **2005**, *28*, 257–267.

(26) Renew, J. E.; Huang, C. H. Simultaneous determination of fluoroquinolone, sulfonamide, and trimethoprim antibiotics in wastewater using tandem solid phase extraction and liquid chromatography-electrospray mass spectrometry. *J. Chromatogr. A* **2004**, *1042*, 113–121.

(27) Ye, Z. Q.; Weinberg, H. S.; Meyer, M. T. Trace analysis of trimethoprim and sulfonamide, macrolide, quinolone, and tetracycline antibiotics in chlorinated drinking water using liquid chromatography electrospray tandem mass spectrometry. *Anal. Chem.* **2007**, *79*, 1135–1144.

(28) Jacobsen, A. M.; Halling-Sorensen, 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. *J. Chromatogr. A* **2004**, *1038*, 157–170.

(29) Tong, C. L.; Xiang, G. H.; Liu, W. P. Study on the determination of ciprofloxacin by the terbium (III) ion fluorescence probe sensitized by the surfactant. *Spectrosc. Spect. Anal.* **2005**, *25*, 2061–2064.

(30) Tong, C. L.; Xiang, G. H.; Huang, D. J.; Liu, W. P. Determination of ofloxacin by the terbium (III) ion fluorescence probe sensitized by the surfactant. *Chin. J. Anal. Chem.* **2004**, *32*, 619–621.

(31) Karthikeyan, K. G.; Meyer, M. T. Occurrence of antibiotics in wastewater treatment facilities in Wisconsin, USA. *Sci. Total Environ.* **2006**, *361*, 196–207.

(32) Golet, E. M.; Alder, A. C.; Giger, W. Environmental exposure and risk assessment of fluoroquinolone antibacterial agents in wastewater and river water of the Glatt Valley Watershed, Switzerland. *Environ. Sci. Technol.* **2002**, *36*, 3645–3651.

(33) Giger, W.; Alder, A. C.; Golet, E. M.; Kohler, H. P. E.; McArdell, C. S.; Molnar, E.; Siegrist, H.; Suter, M. J. F. Occurrence and fate of antibiotics as trace contaminants in wastewaters, sewage sludges, and surface waters. *Chimia* **2003**, *57*, 485–491.

(34) Kümmerer, K.; Al-Ahmad, A.; Mersch-Sundermann, V. Biodegradability of some antibiotics, elimination of the genotoxicity and affection of wastewater bacteria in a simple test. *Chemosphere* **2000**, *40*, 701–710.

(35) Lindqvist, N.; Tuhkanen, T.; Kronberg, L. Occurrence of acidic pharmaceuticals in raw and treated sewages and in receiving waters. *Water Res.* **2005**, *39*, 2219–2228.