Occurrence and Removal of Fluoroquinolone Antibiotics in a Sewage Treatment Plant in Shanghai, China

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Abstract - In this paper, the occurrence and removal of eight fluoroquinolone antibiotics were investigated at a sewage treatment plant (STP) in Shanghai, China. The most frequently detected fluoroquinolone antibiotics in the present study were norfloxacin, ciprofloxacin and lomefloxacin. The concentrations of these compounds in water samples at the STP ranged from 103 to 1786 ng L⁻¹, 14 to 1406 ng L⁻¹ and 21 to 2246 ng L⁻¹, respectively. The other fluoroquinolone antibiotics were detected only in a few samples from the STP. FQs in aquatic phase could not be eliminated completely from the STP, with the removal efficiencies from 70 % to 100 %. The concentrations of FQs in water detected in summer were higher than in other seasons, due to the more desorption from sludge to aquatic phase under higher temperature.

Keywords- PPCPs; Fluoroquinolones; Antibiotics; Sewage treatment plant; Occurrence; Removal

I. INTRODUCTION

The occurrence of pharmaceuticals and personal care products (PPCPs) in the environment has been recognized as one of emerging issues in environmental field[1, 2]. Recent work has reported the presence of a large variety of PPCPs in STP effluents and surface waters, with concentrations up to several mg L⁻¹. In fact, more than 70 PPCPs have been detected during the last years in different environmental samples, due to the continuous improvement of the analytical techniques[3]. Many of these samples have been taken from wastewater[4, 5], but also from surface or groundwater[6].

Sewage treatment plants (STPs) play an important role in the life cycle of antibiotics in modern society. Modern STPs can effectively accomplish carbon and nitrogen removal, while trace polluting compounds may be only partially eliminated. However studies focusing on sewage treatment systems for the occurrence and removal of these micro pollutants are somewhat limited. More data are available from Europe. The concentrations of antibiotics in the influent/effluent from the STPs range from several hundred ng L⁻¹ to several mg L⁻¹[7, 8].

Fluoroquinolone antibiotics agents are powerful and effective groups of synthetic antibiotics in human and veterinary medicines worldwide. In Lindberg’s study[9, 10] of Umea STP in Sweden, FQs were quantified in the raw sewage water entering the plant, its final effluent, and the digested dewatered sludge. The results from that study suggested that the fluoroquinolones were sorbed to sludge. The major goal of this study was, therefore, to determine the presence of fluoroquinolone antibiotics in the influent and effluent from a sewage treatment plant in Shanghai, China, and to investigate the behavior of FQs along the different units. The removal efficiencies from the aquatic phase of FQs in each particular unit were also determined.

II. MATERIALS AND METHODS

A. Chemicals

FQs studied were purchased from Sigma-Aldrich (St. Louis, USA), including Norofloxacin (NOR, CAS.110871-86-8), Pefloxacin mesylate dehydrate (PEF, CAS.70458-95-6), Ciprofloxacin (CIP, CAS. 85721-33-1), Lomoxacin (LOM, CAS. 98079-52-8), Danofloxacin (DANO, CAS. 112398-08-0), Enrofloxacin (ENRO, CAS. 93106-60-6), Difloxacin (DIF, CAS. 91296-86-5) and Sarafloxacin (SAR, CAS. 91296-87-6).

B. Sewage treatment plant

The sewage treatment plant studied in this work located in Shanghai, China, has a nominal handling capacity of 75,000 m³ D⁻¹ and corresponds to a service area and a population of approximately 450 hm² and 200,000 inhabitants. The plant includes four main sections: primary treatment, biological treatment, secondary treatment and biofilter (Figure 1.). After the pumping of the inlet wastewaters, coarse screening and fine screening are carried for grit and fat removal. The primary treatment is carried out in circular sedimentation tanks. Finally, after processed by secondary sedimentation and biofilter, the effluent discharges into the receiving water.

The sampling points for analysis were the following (Figure 1): (i) inlet to the grit removal unit (Sin); (ii) outlet to grit removal and the primary sedimentation tank (Sps); (iii)
outlet to the activated sludge reactor (Sas); (iv) outlet to the secondary sedimentation tank (Sss) and; (v) outlet of the biofilter (Sef).

Four analytical campaigns, during 1 year, were carried out, that was in Dec, 2007, Apr, 2008, Aug, 2008 and Oct, 2008.

Grab samples were collected in amber glass bottles and immediately adjusted to pH 2-3 using a 10% sulfuric acid solution to reduce biological activity, and then stored in the dark at +4°C until analysis.

C. Sample preparation and analysis

FQs were extracted from wastewaters using MEP (Anpel™, 60 mg, 3 cc, Shanghai Anpel Scientific Instrument Co., Ltd, P.R.C) SPE disk cartridges preconditioned with 5 mL of methanol and 10 mL of water (pH 2-3, adjusted with 10% sulfuric acid). Sewage Samples (100 mL of Sin and Sps; 500 mL of Sas, Sss and Sef) at pH 2-3 were then percolated through the disk cartridge using a vacuum manifold (Supelco;USA), then the disk cartridges were washed with 10mL of 5% methanol in water (pH 2-3). After extraction, the disk cartridges were vacuum-dried for 30 min to remove the residual water in the cartridges. Compounds were then eluted using 6 mL of 2 % formic acid in methanol; the eluent was evaporated under a gentle flow of gentle nitrogen and was then reconstructed by 1mL of mobile phase and was stored at 4°C until analysis.

Separation was performed with a HITACHI high-performance liquid chromatograph (HPLC) equipped with a HITACHI L-2485 fluorescence detector (FLD) and an Ezchrom Elite workstation. The FLD excitation wavelength was 278 nm and the emission wavelength was 445 nm. The LC column was Kromasil ODS C18 (250 mm × 4.6 mm, 5 μm). Eluent A was acetonitrile, and eluent B was a 10 mM tetrabutyl ammonium bromide (TBAB) solution (pH 3.0). Elution started with 4% A, followed by an 8-min isocratic elution, and an 8-min linear gradient to 15% B, followed by a 10-min isocratic elution, and a 5-min linear gradient to 25% B, then decreased to the initial condition in 5 min, followed by an equilibration time of 6 min. Analyses were performed at a flow rate of 1 mL/min and temperature of 30°C. The sample injection volume was 20 μL.

Accuracy of the method was determined by recovery studies by spiking wastewater samples with known concentrations of FQs. Working standards of 0.5-250 μg L⁻¹ of each FQ were injected in sequence. Calibration curves were prepared by plotting the peak area versus the analyte concentration. In this study, limits of detection (LOD) and limits of quantification (LOQ) were calculated as 3 and 10 times the signal-noise ratio of the FQs measurements, respectively. Six replicate analyses were performed for the RSD test, in which 1 μg L⁻¹ and 100 ng L⁻¹ of FQs standard mixtures were spiked into influent and effluent samples (100 mL and 500 mL, respectively) in the recovery tests.

D. Calculations

Removal efficiencies from the aqueous phase for all FQs were calculated, taking into account the measured concentration at the inlet of the plant (Sin), outlet of the biological reactor (Sas) and the final effluent (Sef). The percentage related to the primary treatment was then calculated as (Sin-Sps)/Sin*100, the percentage related to the activated sludge reactor was obtained using (Sps-Sas)/Sin*100, and the percentage related to the second sedimentation tank and the biofilter was obtained using (Sas-Sss)/Sin*100 and (Sss-Sef)/Sin*100, and the overall efficiency using (Sin-Sef)/Sin*100. In all cases, Sin was used as the reference in order to be able to compare and to add the partial percentages and obtain the overall one.

III. RESULTS AND DISCUSSION

A. Recoveries and Quality Control

Recoveries of the FQs in 100 mL spiked influent samples and 500 mL tertiary effluent samples (Table 1) ranged from 79 to 109% and from 80 to 105%, respectively. Good linearity was observed over 1-2 orders of magnitude of concentrations (e.g., 0.5-250 μg L⁻¹), with correlation factors of $R^2 > 0.995$ (Table 1). In this study, the LOD and LOQ were found to be 0.11-1.06 μg L⁻¹ and 0.35-3.54 μg L⁻¹, respectively. The RSDs were generally below 5%.
B. Occurrence of FQs in Sewage treatment Plant

The concentrations of the selected FQs in sewage waters at the STP were presented in Table 2. All the FQs selected were detected in STP to different extent. NOR, CIP and LOM which were commonly consumed human-use FQs, were detected in all the samples in this study. Determination of FQs in sewage samples showed trace level of NOR, CIP and LOM, with concentrations from 103 to 1786 ng L\(^{-1}\), 14 to 1406 ng L\(^{-1}\) and 21 to 2246 ng L\(^{-1}\), respectively (Table 2). These results were consistent with those previously reported in the USA (e.g., NOR: <45 ng L\(^{-1}\), LOM: <41 ng L\(^{-1}\) and CIP: <19 ng L\(^{-1}\)) [11], Switzerland (NOR: 26-553 ng L\(^{-1}\), LOM: <5.0 ng L\(^{-1}\) and CIP: 62-568 ng L\(^{-1}\)) [12], France (NOR: 50-70 ng L\(^{-1}\), LOM: 180-290 ng L\(^{-1}\) and CIP: 60 ng L\(^{-1}\)), Sweden (NOR: 30 ng L\(^{-1}\), LOM: 130 ng L\(^{-1}\) and CIP: 30 ng L\(^{-1}\)), and Italy (NOR: 60-70 ng L\(^{-1}\), LOM: 180-320 ng L\(^{-1}\) and CIP: 40-20 ng L\(^{-1}\)) [13]. Weihai Xu et al. [14] also reported the concentrations of FQs in the sludge samples at four STPs in China, with the concentrations of NOR in the influents ranged from 54 to 263 ng L\(^{-1}\).

Some of the veterinarian-use FQs, such as DANO and DIF, were also detected in the influents and effluents at the trace level. The concentration of ENRO and SAR were below 100 ng L\(^{-1}\) or below the LOD in the majority of samples.

### TABLE 1. Method validation parameters and recovery

<table>
<thead>
<tr>
<th>FQs</th>
<th>Linearity range (μg L(^{-1}))</th>
<th>R(^2)</th>
<th>LOD (μg L(^{-1}))</th>
<th>LOQ (μg L(^{-1}))</th>
<th>RSD(%) n=6</th>
<th>Recovery of FQs (%)</th>
</tr>
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<tbody>
<tr>
<td>NOR</td>
<td>0.5-250</td>
<td>0.9999</td>
<td>0.35</td>
<td>1.15</td>
<td>3.2</td>
<td>100.2</td>
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<tr>
<td>PEF</td>
<td>0.5-250</td>
<td>0.998</td>
<td>1.06</td>
<td>3.54</td>
<td>4.28</td>
<td>97.7</td>
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<tr>
<td>CIP</td>
<td>1.0-250</td>
<td>0.9998</td>
<td>0.11</td>
<td>0.35</td>
<td>2.76</td>
<td>93.2</td>
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<tr>
<td>LOM</td>
<td>2.5-250</td>
<td>0.9811</td>
<td>0.15</td>
<td>0.49</td>
<td>2.05</td>
<td>109.2</td>
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<tr>
<td>DANO</td>
<td>0.2-100</td>
<td>0.9977</td>
<td>0.16</td>
<td>0.34</td>
<td>4.12</td>
<td>97.9</td>
</tr>
<tr>
<td>ENRO</td>
<td>1.0-250</td>
<td>0.9979</td>
<td>0.19</td>
<td>0.62</td>
<td>4.43</td>
<td>97.4</td>
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<tr>
<td>SAR</td>
<td>1.0-250</td>
<td>0.999</td>
<td>0.88</td>
<td>2.92</td>
<td>4.8</td>
<td>79.2</td>
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<tr>
<td>DIF</td>
<td>2.5-250</td>
<td>0.9811</td>
<td>0.29</td>
<td>0.96</td>
<td>3.55</td>
<td>85.9</td>
</tr>
</tbody>
</table>

### TABLE 2. Profiles of selected FQs in the influent and effluent at the STP (μg L\(^{-1}\))

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>NOR</td>
<td>Sin 0.336</td>
<td>Sef 0.103</td>
<td>Sin 1.498</td>
<td>Sef 0.246</td>
</tr>
<tr>
<td>PEF</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>0.065</td>
<td>0.006</td>
</tr>
<tr>
<td>CIP</td>
<td>0.007</td>
<td>0.015</td>
<td>0.288</td>
<td>0.064</td>
</tr>
<tr>
<td>LOM</td>
<td>0.802</td>
<td>0.021</td>
<td>0.477</td>
<td>0.099</td>
</tr>
<tr>
<td>DANO</td>
<td>0.026</td>
<td>&lt;LOD</td>
<td>0.102</td>
<td>0.147</td>
</tr>
<tr>
<td>ENRO</td>
<td>0.009</td>
<td>0.044</td>
<td>0.048</td>
<td>0.009</td>
</tr>
<tr>
<td>SAR</td>
<td>nd</td>
<td>nd</td>
<td>0.034</td>
<td>nd</td>
</tr>
<tr>
<td>DIF</td>
<td>nd</td>
<td>nd</td>
<td>0.053</td>
<td>nd</td>
</tr>
</tbody>
</table>

C. Behavior of FQs along the STP

Figure 2. showed the removal efficiencies calculated along the STP, including the removal efficiencies of primary treatment, biotreatment, secondary sedimentation and biofilter. The overall efficiencies achieved for CODcr, BOD5, TP, TN, NH\(_3\)-N and TSS along the entire STP were 80-94%, 90-94%, 72-80%, 70-75%, 91-95% and 88-93%, respectively, and the total removal efficiencies of FQs ranged from 70% to 100%. During the primary treatment, approximate half of the FQs in the aquatic phase were removed. Besides, their good adsorption onto solid surfaces allowed an important removal in the primary sedimentation to be obtained. During the secondary treatment (activated sludge process), all the FQs detected were partially removed. The secondary sedimentation tank and the biofilter played minor points to the FQs removal. Weihai Xu et al. [14] reported the mean and maximum removal rates of NOR were 66% and 81%. In Switzerland, the mean removal rate of NOR at the largest STP was 88% [15]. In Lindberg’s investigation in Sweden [9], the mean removal degrees of NOR was estimated to be 87%. It should be noted that sorption onto the sludge and biodegradation function were simultaneously carried out during the treatment process at the plant.

Two mechanisms could be used to explain this removal: the degradation of the compound and the adsorption onto primary and secondary sludge. Consequently, it was necessary to consider the treatment of the excess sludge under the complete management of the pollution associated with these particular compounds, which requires a further study.

D. Seasonal variation of FQs at the STP

Seasonal influences on the frequency of detection of the FQs were illustrated in Figure 3. The occurrence of NOR, CIP and LOM were evident, especially in the samples collected in summer, and the detection of other FQs also performed the same results. It could be explained by the influences of temperature to the sorption and desorption of FQs onto sludge. Due to our lab-scale study, higher temperature is helpful to FQs desorption from the solid phase to the aquatic phase. Thereby higher temperature might lead to higher concentrations...
of FQs in water detected in summer than in other seasons. Therefore, special attention should be paid to the transportation and fate of the FQs in the solid phase.

![Figure 2. Removal efficiencies along the STP](image)

![Figure 3. Seasonal variation of FQs at the STP](image)

IV. CONCLUSIONS

All the fluoroquinolones antibiotics selected were detected in the influents and effluents at the STP in Shanghai, China, with the concentrations ranging from 15 to 2246 ngL⁻¹. Norfloxacin, ciprofloxacin and lomefloxacin were the most frequently detected antibiotics in the wastewaters. The total removal efficiencies of FQs ranged from 70% to 100%, the removal of FQs at the STP was generally incomplete. FQs were easily removed from the aquatic phase, but more easily adsorbed to sludge during the treatment process. The concentrations of FQs in water detected in summer were higher than that in other seasons, due to the more desorption from sludge phase to aquatic phase under higher temperature.

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