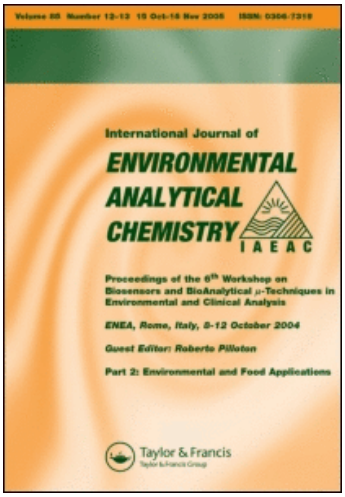

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Development of an analytical method for eight fluoroquinolones using solid-phase extraction and liquid chromatography with fluorescence detection

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Development of an analytical method for eight fluoroquinolones using solid-phase extraction and liquid chromatography with fluorescence detection

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In this study, a practicable and effective analytical method based on solid-phase-extraction and reversed-phase liquid chromatography with fluorescence detection (SPE-LC-FLD) was developed and partially validated for routine analysis of eight FQs in wastewater at the trace level. Different SPE materials, pH conditions and eluents were modified to find an economic and effective SPE conditions. In our work, it is the first time that well-known commercially available SPE sorbent are compared to 'generic' cheap SPE sorbent. Aqueous samples (pH 2–3) were extracted using Anpel^{TM} MEP cartridges where they were subsequently eluted by 6 mL of 2% formic acid in MeOH. The aqueous extracts were analysed by gradient elution LC-FLD, whose initial mobile phase was composed of ACN and 10 mmol L^{-1} tetrabutyl ammonium bromide (4/96, v/v, pH 3). The LODs and LOQs of the wastewater were as low as 0.32–2.12 ng L^{-1} and 1.07–7.07 ng L^{-1}, respectively. The precisions of the overall method (RSD, $n = 3$) using wastewater were below 10%. The method was used to quantify FQs in influents and effluents of several typical sewage treatment plants (STPs) in Shanghai. The extraction recoveries of 100 mL influent, 500 mL effluent and 500 mL of river water samples were between 88.6 and 102.6%, 79.2 and 109.2%, 80.0 and 105.5% and 87.4 and 99.4%, respectively. FQs of interest except sarafloxacin were identified in the influents, effluents and river waters with concentrations varying from 0.012–1.163 μg L^{-1}, 0.003–0.291 μg L^{-1}, and 0.002–0.040 μg L^{-1}, respectively. The method can serve as a tool to obtain detailed information on occurrence, behaviour and fate of FQs in the aquatic environment. Occurrence of FQs detected in summer is higher than in spring at STPs, and those detected in the suburban area are less than those in the urban area. Complete removal of FQs is not achieved from the STPs, indicating domestic wastewater and STP discharge is the source of FQs in the surface water.

Keywords: fluoroquinolones; antibacterial; solid-phase extraction; liquid chromatography; fluorescence detection

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1. Introduction

During the last decades, the occurrence and the fate of pharmaceuticals and personal care products (PPCPs) in the environment has been recognised as one of the prevailing problems in ecological risk assessment and chemistry, and articles about the emerging contaminants in water, soil and sludge have been on the increase [1–4]. Among various pharmaceuticals, fluoroquinolone antibacterial agents (FQs) are of particular concern. Fluoroquinolones belongs to the family of gyrase inhibitors; they show broad activity against Gram-negative Gram-positive bacilli, lower toxicity and longer life [5]. FQs have been used in human and veterinary medicines for over a decade; their entry to the environment has been continuous [6]. Due to the extensive use of FQs in urban centres and the fact that they are largely excreted unchanged [7], significant quantities of these antibacterial agents can be found in municipal wastewaters as well as in surface waters of the surrounding areas; for instance, Norfloxacin and Ciprofloxacin occurred in primary and tertiary treated sewage at levels from 27 to 489 ng L^{-1} [6,8].

Most methods have been developed for analysing FQs in food and biological matrices [9,10], and did not suit for the analysis of FQs in the environment. The low concentration levels and the complexity of environment matrices make it necessary to use highly sensitive and selective methods of FQs' determination. To our knowledge, there are several methods available to analyse trace amounts of FQs in environmental samples [11–17]. Most of these methods use solid-phase extraction (SPE) for extraction and concentration of FQs, which then are analysed by liquid chromatography with either fluorescence detection (LC-FLD) [13,18–20], ultraviolet detection (LC-UV) [11], mass spectrometric (LC-MS) [15] or tandem mass spectrometric detection (LC-MS–MS) [16]. However, the LC-UV method does not have enough sensitivity for analysing environmental water samples, and the LC-MS and LC-MS–MS methods are used for confirmatory analysis due to the complexity of the analytical system and comparatively high costs involved. Therefore these methods are not suitable for routine analysis. Since FQs are polar compounds and most of them are highly fluorescent, reversed-phase liquid chromatography with fluorescence detection should be a cheaper option/alternative for quantitative analysis of trace FQs.

One difficulty in the analysis of FQs involves interaction of these compounds with residual silanol groups and metal ions on the LC column, often resulting in severe peak tailing and variable analyte recoveries. Given the very low concentrations of FQs detected in aqueous samples reported by previous studies [9–13], it is essential to develop and modify a robust multi-residue method for determining them at the trace level.

Because of FQs' low concentrations occurring in the environment, enrichment and sample clean-up are difficult. SPE is frequently used for concentrating and purifying antibacterial extracted from animal tissues and food samples, and is highly suited for extracting aqueous samples. However, sufficient information is not available on which SPE method is more suitable for determining FQs in a wide variety of matrices. The selection of SPE materials and eluent are key factors affecting the recovery. In previous studies, various materials including C18, HLB, MPC, WCX were applied for extracting FQs from environmental samples [8–10,16]; however, the extraction conditions were different as well as the recovery efficiencies. When high-throughput of samples is required the cost of SPE cartridges can become a drawback, at least in developing countries. Therefore, it is necessary to establish an applicable, economic and stable extraction system.

The objectives of this study were to develop and validate a specific and sensitive LC-FLD method for analysis of multiple FQs in wastewater samples. We present

a sensitive and simple method developed in this study, focusing on (a) the development of the solid-phase extraction enrichment procedures, according to the different acid-base properties of the selected FQ compounds, (b) develop and validate a sensitive LC-FLD method, and (c) how to use the method to analyse eight FQs in trace water samples. In our work, it is the first time that well-known commercially available SPE sorbent are compared to 'generic' cheap SPE sorbent.

2. Experimental

2.1 *Chemicals and reagents*

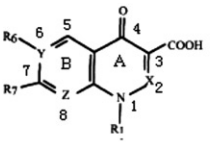
Reference compounds of FOs (structure shown in Figure 1) were purchased from Sigma-Aldrich (St. Louis, USA), including Norfloxacin (NOR, CAS. 110871-86-8), Pefloxacin mesylate dehydrate (PEF, CAS. 70458-95-6), Ciprofloxacin (CIP, CAS. 85721-33-1), Lomexacin (LOM, CAS. 98079-52-8), Danofloxacin (DANO, CAS. 112398-08-0), Enrofloxacin (ENRO, CAS. 93106-60-6), Difloxacin (DIF, CAS. 91296-86-5) and Sarafloxacin (SAR, CAS. 91296-87-6). All of the solvents were of the reagent grade or higher in quality. Acetonitrile (ACN), methanol (MeOH) and dichloromethane (DCM) were purchased from Sigma-Aldrich. *Ortho*-phosphoric acid (*o*-H_3PO_4) 85%, hydrochloric acid (HCl) 32%, formic acid 85%, and tetra-butyl ammonium bromide (TBAB) were obtained from Fluka AG (Buchs, Switzerland). The water for the experiment was deionised water and passed though 0.45 μm nylon filter membrane before use.

The extraction materials applied in this study (shown in Table 1) were as follows: (1) silica based C18 sorbent, including LC-18 (Supelco, 500 mg, 3 cc, Park Bellefonte, PA, USA) and ENVI-18 (Supelco, 500 mg, 3 cc, Park Bellefonte, PA, USA), (2) polymeric sorbent, including MEP (Anpel^{TM}, 60 mg, 3 cc, Shanghai Anpel Scientific Instrument, China), ENVI Chrom P (Supelco, 200 mg, 3 cc, Park Bellefonte, PA, USA), and Oasis HLB (Waters, 30 mg, 3 cc, Milford, MA, USA), (3) ion exchanger sorbent, including Oasis MAX (Waters, 30 mg, 3 cc, Milford, MA, USA), and Oasis MCX (Waters, 30 mg, 3 cc, Milford, MA, USA).

Individual stock solutions of 200 mg L^{-1} of each of the eight FQs were prepared in a water : ACN mixture (1 : 1) containing 0.2% (v/v) hydrochloric acid and stored at −20°C and renewed monthly. Working standard mixtures of eight FQs of interest with concentrations of 1 to 10 μg mL^{-1} were prepared in ACN for spiking purposes. Instrument calibration standards were prepared by dilution of the above mixtures in the initial mobile phase consisting of ACN/10 mmol L^{-1} tetra-butyl ammonium bromide (TBAB) solution (4/96, v/v, TBAB solution were acidified to pH 3.0), and stored at +4°C, and re-prepared weekly.

2.2 *Wastewater sample collection*

Water samples were collected from six different STPs (namely STP-A, STP-B, STP-C, STP-D, STP-E and STP-F) and three rivers (Suzhou River, Nanhengyin River and Huangpu River) in Shanghai, China. Figure 2 shows the distribution of sampling points. The description of the STPs and rivers were shown in Tables 2 and 3. Influents were collected after screen; effluents were collected after secondary sedimentation tank. River samples were collected in the middle of the river. Grab samples were collected every 6 h for making 24-h composite samples. Samples were collected in amber glass bottles and immediately adjusted to pH < 3 using a 1% *ortho*-phosphoric acid solution to reduce



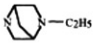
FQs	Structure of substitue					
	R1	X	Y	R6	R7	Z
NOR	$-C_2H_5$	CH	C	F		CH
CIP		CH	C	F		CH
PEF	$-CH_2CH_2F$	CH	C	F		CH
LOM	$-C_2H_5$	CH	C	F		CH
ENRO		CH	C	F		CH
DANO		CH	C	F		CH
SAR		CH	C	F		CH
DIF		CH	C	F		CH

Figure 1. Structure and substitute of selected fluoroquinlones.

Table 1. Composition of selected SPE material.

SPE material type		Composition	Extraction mechanism
C18	LC-18	Silica based octadecyl	Reverse phase, adsorption
	ENVI-18	Silica based octane	Reverse phase, adsorption
Polymeric	Oasis HLB	Polystyrene/divinylbenzene	Adsorption
	MEP	Polystyrene/divinylbenzene	Adsorption
	ENVI Chromp	Composite resin, particular size 80–160 μm	Adsorption
Ion exchanger	Oasis MAX	Vinylpyrrolidone and a strong anion exchanger dimethyl butylamine	Reverse phase, anion ion change
	Oasis MCX	Vinylpyrrolidone and a strong cation exchanger benzenesulfonate	Reverse phase, cation ion change

biological activity, and filtered through 0.45 um glass fibrefilters to remove suspended matter and then stored in the dark at +4°C until extraction.

2.3 *Extraction procedure*

To develop a proper SPE method, a variety of extraction materials were tested, including MEP, LC-18, ENVI-18, ENVI Chrom P, Oasis HLB, Oasis MAX, and Oasis MCX

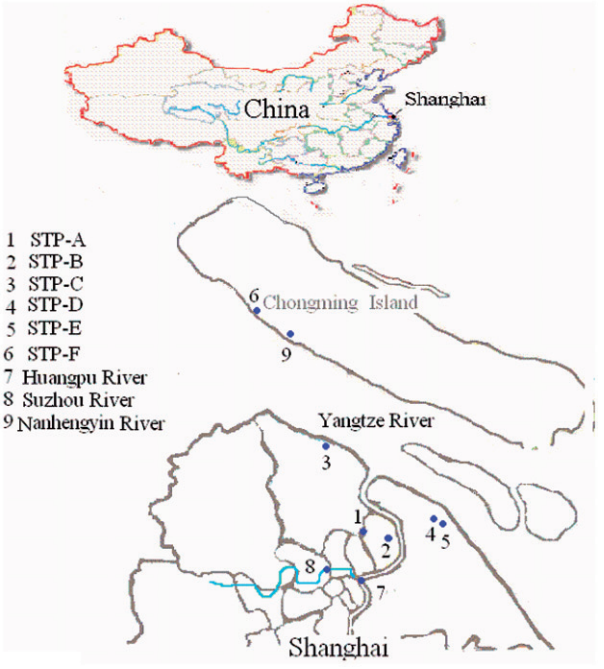


Figure 2. Distribution of sampling points in Shanghai, China.

Table 2. Information about the sewage treatment plants investigated.

STPs	District of location	Average daily flow ($*10^4$ m^3 D^{-1})	Treatment process	Sewage source	Effluent discharge
A	Yangpu	5.67	A/A/O[a] or MBR[b]	Domestic sewage	Surface water
B	Hongkou	3.11	CAS[c]	Domestic sewage	Surface water
C	Baoshan	34.43	UNITANK[d]	Domestic and industrial sewage	Surface water
D	Pudong	170	CBF[e]	Domestic and industrial sewage	Surface water
E	Pudong	50	A/O[f]	Domestic and industrial sewage	Surface water
F	Chongming	5	A/O	Domestic sewage	Surface water

Notes: [a]Anaerobic/anoxic/aerobic; [b]Membrane biological reactor; [c]Conventional activated sludge; [d]UNITANK = Integrated activated sludge processing; [e]Chemical and biological flocculation; and [f]Anoxic/aerobic.

as cartridges. FQs were extracted from wastewaters using a selected cartridge (e.g. MEP SPE) that was preconditioned with 5 mL of DCM, 5 mL of MeOH and 10 mL of water either a 1% (v/v) H_3PO_4 solution (pH 3.0) or a 1 mmol L^{-1} NaOH solution (pH 10). To test which cartridge would result in the best extraction effect, 100 mL deionised water at pH 3 or pH 10 spiked with 1 ng mL^{-1} of FQs standard mixtures were percolated through the preconditioned cartridges at a flow rate of 1 mL min^{-1} using a vacuum manifold (Supelco, Park Bellefonte, PA, USA). To extract FQs in wastewaters, wastewater samples

Table 3. Information about the surface water investigated.

River	Source	Length (km)	Area (km^2)	Main function
Huangpu River	Tai lake basin	80	2.38	Domestic and industuial water supply, shipping
Suzhou River	Yangtze river basin	125	855	Domestic and industuial water supply
Nanhengyin River	Chongming Island inland river	74.8	150	Domestic supply, navigation

(100 mL of influents, 500 mL of effluents and 500 mL of river water samples) at pH 3 (pre-adjusted for sample preservation) were percolated through the cartridges at a flow rate of 1 mL min^{-1} using the same vacuum manifold, afterwards the cartridges were washed with 10 mL of deionised water (pH 2–3) containing 5% MeOH. After extraction, the 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 MeOH. The eluent was evaporated to dryness under a gentle flow of high pure nitrogen and was then reconstituted by 1 mL of initial mobile phase mentioned above (see Section 2.1 above) and stored in the dark at +4°C for analysis within one week.

Once MEP cartridges were found to be the best for extraction, they were used to evaluate the effect of sample pH on the recovery of FQs. In these tests, the water used for preconditioning varied with the sample pHs (which were adjusted from 2 to 12).

2.4 *LC-FLD conditions*

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 445 nm. The LC column was Kromasil ODS C18 (250 mm × 4.6 mm, 5 μm). Eluent A was ACN, and eluent B was a 10 mmol L^{-1} tetra-butyl 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% A, followed by a 10 min isocratic elution, and a 5 min linear gradient to 25% A, 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^{-1} and temperature of 30°C, with a sample injection volume of 20 μL.

3. Results and discussion

3.1 *Chromatographic separations*

Because the inherent fluorescence of the FQs enables very sensitive and specific detection, fluorescence detection (FLD) was selected as the main identification and quantification technique. Exciting-emission scans were performed to establish optimum exciting and emission wavelength. The maximum wavelengths for the different FQs were: for NOR Exciting/emission wavelength ($\lambda e_x/\lambda e_m$) 278/445 nm, for PEF 278/445 nm, for CIP 278/445 nm, for DANO 290/459 nm, for LOM 278/451 nm, for ENRO 289/453 nm, for SAR

278/450 nm and for DIF 285/450 nm. Chromatographic detection was therefore performed at $\lambda e_x/\lambda e_m$ of 278/445 nm [19].

Based on several reported cases of chromatographic separation of FQs using the C18 stationary phase, we initiated our work with a Kormosil C18 column. In an effort to reduce the tailing in the LC column of FQs, the pH of the mobile phase was set to values below the pK_as of the analytes by adding acids. Phosphoric acid, phosphate buffer, acetic acid, citric acid and formic acid were used to protonate the amino groups of the FQs and the residual silanol groups of the stationary phase, so that their interactions, and thus, peak asymmetry could be reduced. Although mobile phases containing acid or buffers were commonly used to separate FQs, they could not separate some FQs with similar property, such as NOR from PEF, and LOM from DANO. Therefore, counter ions or an ion pair was added to the mobile phase to restrain the disassembly of the FQs' molecule. In this study, we used tetra-butyl ammonium bromide (TBAB) as a counter ion or ion-pair reagent, which can change the retention factor of the FQs to the C18 stationary phase and result in good separations of these FQs.

Different concentrations of the TBAB ion-pair reagent were studied. PEF and CIP did not obtain good resolution when the concentrations of the TBAB ion pair reagent were lower than 10 mmol L^{-1}. Some of the FQs (such as NOR, PEF and CIP) perform peak tailing when the pH value of the aqueous phase was higher than 3. Considering high salt concentration and low pH would damage the LC column and the system, the mass concentration of the TBAB reagent was set to 10 mmol L^{-1}, and the pH value of the aqueous phase was adjusted to 3.0 using acetic acid. The gradient elution system was carried out to make a better separation. FQs selected in this study were polar, thus the elution system started with a low organic proportion (4% ACN), by applying a flat gradient from 4% to 15% ACN, most FQs were efficiently separated, then a rising from 15% to 25% ACN was needed to elute the more retained SAR and DIF. Under the conditions that we tested with the C18 column, good resolution for the eight FQs could be achieved (Figure 3(I)).

3.2 *Selection of SPE materials*

In this study, a variety of other extraction materials were tested including LC-18, ENVI-18, MEP, ENVI Chrom P, Oasis HLB, Oasis MAX and Oasis MCX. The recovery tests were carried out with an extraction condition at pH 3 and 10 (shown in Table 4, specific date of each FQs shown in our previous study [22]). At pH 3, copolymer materials showed good recovery efficiencies (such as Chrom P (composite resin) 69.6–83.3%, HLB (polystyrene/divinylbenzene) 64.1–80.7% and MEP (polystyrene/divinylbenzene) 87.4–101.9%), while the C18 SPE cartridge offered recovery efficiencies ($<61\%$), as compared with polymeric materials. This fact is not surprising since it is known that copolymer materials offer higher affinity for polar analytes because aromatic rings in the polymeric matrix produce π-π^* intense interaction [11]. MAX at pH 10 and MCX at pH 3 showed poor recovery efficiency (MCX 6–15% and MAX 16–25%, respectively), while higher efficiencies (MCX at pH 10, 61–71% and MAX at pH 3, 87–106%, respectively) were achieved. The ion-changer material in the study were mixed mode sorbent (see Table 1), which might result in complicated SPE mechanisms such as adsorption and ion changer. Those may be explained by the structure change of FQs in different acid to base conditions. At pH 3, the FQs were in cation form, which can change with the cation

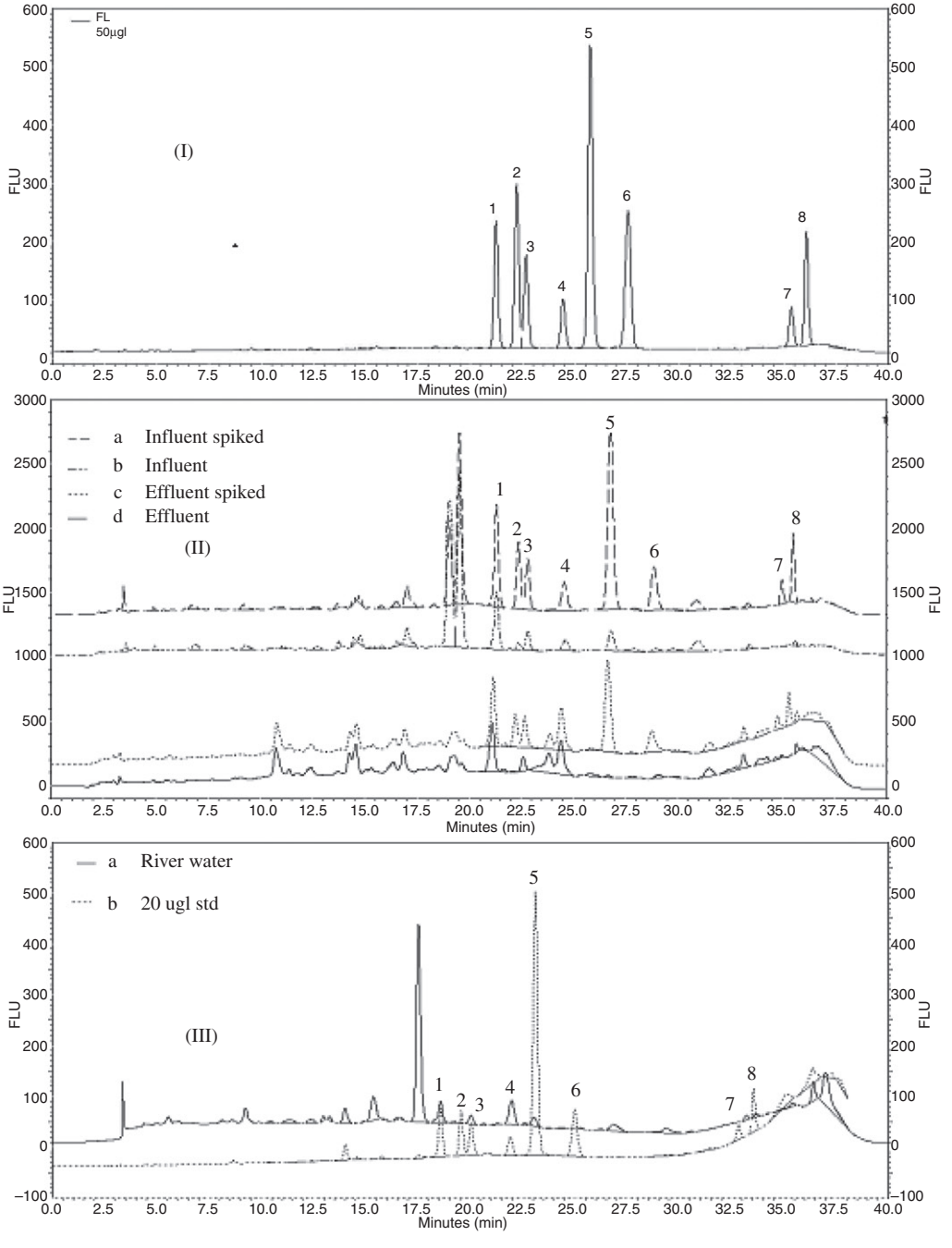


Figure 3. Liquid chromatogram of FQs, i.e. 1 = NOR; 2 = PEF; 3 = CIP; 4 = LOM; 5 = DANO; 6 = ENRO; 7 = SAR; and 8 = DIF. Mobile phase: A ACN; and B 10 mmol L^{-1} tetrabutyl ammonium bromide.
(I) Chromatograms of standard solution of the mixture of 8 FQs (50 ng mL^{-1}).
(II) Chromatograms obtained from wastewater samples (a) 100 mL influent spiked with 1 ng mL^{-1} FQs, (b) 100 mL influent, (c) 500 mL effluent spiked with 100 pg mL^{-1} FQs, (d) 500mL effluent.
(III) Chromatograms obtained from river water samples (a) 500 mL surface water of Suzhou River (b) Chromatograms of standard solution of the mixture of 8 FQs (20 ng mL^{-1}).

Table 4. Recoveries of different types of SPE cartridges.

Recovery range of FQs (%)	LC-18	ENVI-18	CHROMP	HLB	MEP	MAX	MCX
pH 3	31.3–60.9	39.9–52.7	69.6–83.3	64.1–80.7	87.4–101.9	27.9–56.2	12.8–24.9
pH 10	26.0–60.8	45.6–68.4	18.7–40.5	55.3–81.2	84.7–106.3	4.7–14.8	61.5–70.6

changer of MCX, and vice versa, the anion form changes with anion changer of MAX at pH 10. As many factors might affect the recoveries of ion-changer materials and previous studies achieved different results, for 32% [21] or >60% [11], the further study on these ion-changer materials was not carried out.

In comparison to C18 materials, copolymer materials showed good results due to their specific structure which results in a good combination for extraction of a group of FQs. Taking economic factors into consideration, the MEP cartridges were half the price of the HLB cartridges in the Chinese market, thus the MEP cartridge was chosen to be the SPE material in this study.

3.3 *Other factors of SPE*

Because of the acid-base characteristics of FQs, SPE of the analytes is expected to be strongly pH dependent and thus, the effect of the pH of the water samples on FQs recoveries was evaluated. At pH 2–3, high and stable recovery efficiencies (70–94%) of the eight FQs were acquired. Due to their acid-base properties, FQs has two pK_a, the pK_a values for the carboxylic group are between 5.9 and 6.3 and that for the amino groups on the piperazine moiety, between 7.9 and 10.2. It is important to adjust the sample pH to a proper value, typically, to very acidic values, far from the pK_a of the molecules to restrain the decomposing of the FQs' molecules. Therefore, FQs could be retained in the SPE cartridge and later eluted by proper solvents.

To choose a proper eluent for the retained FQs, several solvent mixtures were tested for elution of the FQs from MEP cartridges (Table 5). Adding ammonia or KOH to the eluting solvent strongly improved the recovery rate, and satisfactory results were achieved when using 2.5 mL of NH_4OH/MeOH/water (v/v/v, 5/15/80) or 2.5 mL of 10 mmol L^{-1} KOH solution/ACN (v/v, 75/25). Although aqueous solutions showed better recovery efficiencies than organic solvents, more than 25% organic solvent in the eluting solution without evaporation was not favourable for the chromatographic separation, since the excess organic solvent resulted in peak broadening and losses in response. Due to the difficulty in evaporation of aqueous solution, a variety of organic solutions containing either ammonia, or formic acid, or acetic acid at different concentrations (1% and 5%) were evaluated. Finally, a 6 mL 2% formic acid in MeOH was selected as the best eluent.

3.4 *Stability, linearity range, calibration parameters, and method precision*

The stability of standards and of sample extracts was evaluated. Stock standards solutions were stored at −20°C and analysed weekly and working standards solutions were stored at 4°C in the dark and analysed every other day during a week period. The results showed

Table 5. Recovery of FQs of different eluents.

FQs	2.5 mL 10 mmol L^{-1} KOH/ACN 75/25 (%)	2.5 mL NH_4OH/MeOH/water 5/15/80 (%)	6 mL 1% acetic acid in ACN (%)	6 mL 5% NH_4OH in MeOH (%)	6 mL 2% fomic acid in MeOH (%)*
NOR	100.1	87.2	74.6	74.9	85.0
PEF	93.0	82.3	68.0	76.3	83.6
CIP	97.0	88.8	70.9	71.0	80.8
LOM	88.0	92.7	85.5	75.9	84.5
DANO	108.8	82.0	57.0	70.4	85.9
ENRO	99.0	83.6	57.8	73.0	83.7
SAR	93.3	80.5	70.5	68.3	77.5
DIF	89.3	85.9	68.5	70.2	80.8

Table 6. Linearity range and calibration parameters.

FQs	Linearity range (injected to HPLC, ng mL^{-1})	IDL/IQL (ng mL^{-1})	RSD of injection ($n = 6$, %)	LOD/LOQ (ng L^{-1})
NOR	0.5–250	0.35/1.15	3.20	0.70/2.33
PEF	0.5–250	1.06/3.54	4.28	2.12/7.07
CIP	1.0–250	1.01/3.37	2.76	2.02/6.73
LOM	2.5–250	0.75/2.50	2.05	1.50/5.00
DANO	0.25–100	0.16/0.54	4.12	0.32/1.07
ENRO	1.0–250	0.19/0.62	4.43	0.38/1.27
SAR	1.0–250	0.88/2.92	4.80	1.76/5.87
DIF	2.5–250	0.29/0.96	3.55	0.58/1.93

Note: LOD and LOQ were calculated for effluent with the pre-concentration factor of 500 times.

that, stock standards solutions and the sample extracts were stable when stored at −20°C in a month or at 4°C in the dark during a week, respectively.

Working standards in initial mobile phase of 0.5–250 ng mL^{-1} of each FQ were injected in sequence. Calibration curves were prepared by plotting the peak area versus the analyte concentration. Good linearity was observed over 1–2 orders of magnitude of concentrations (e.g. 0.5–250 ng mL^{-1}, shown in (Table 6) with correlation factors of $R^2 > 0.995$.

In this study, instrument detection limits (IDLs) and limits of quantification (IQLs) were calculated as 3 and 10 times standard deviation of low concentrations (in this work, 10 pg mL), respectively. The precision of injection is calculated by relative standard deviation (RSD, $n = 6$) of the analysis of a FQ standard mixture of 50 μg L^{-1}. Limit of detection (LOD) and limit of quantification (LOQ) were calculated for effluent with the pre-concentration factor of 500 times. Three replicate analyses were performed for the RSD test to acquire an overall precision of the method, in which 1 μg mL^{-1}, 100 ng mL^{-1} and 100 ng mL^{-1} of FQs standard mixtures were spiked into influents, effluents and river samples (100 mL, 500 mL and 500 mL, respectively) in the recovery tests. The IDLs and IQLs were found to be 0.16–1.06 ng mL^{-1} and 0.54–3.54 ng mL^{-1}, respectively. The LODs and LOQs were found to be 0.32–2.12 ng L^{-1} and 1.07–7.07 ng L^{-1}, respectively (Table 6). The recovery of influent, effluents and river samples were ranged from 79.2 to 109.2%,

Table 7. Recovery and precision of FQs obtained from different spiked samples.

	100 mL influent spiked with 1 ng mL^{-1} FQs (%)		500 mL effluent spiked with 100 pg mL^{-1} FQs (%)		500 mL river water spiked with 100 pg mL^{-1} FQs (%)	
FQs	Recovery (%)	RSD ($n=3$)	Recovery (%)	RSD ($n=3$)	Recovery (%)	RSD ($n=3$)
NOR	100.2	105.5	4.04	6.82	6.92	98.5
PEF	97.7	87.6	2.52	6.52	5.68	86.7
CIP	93.2	100.3	8.39	5.34	9.08	99.4
LOM	109.2	84.0	9.25	5.58	9.82	85.9
DANO	97.9	90.2	6.30	8.83	9.71	96.7
ENRO	97.4	96.5	7.38	7.16	9.46	97.5
SAR	79.2	96.2	9.37	5.06	9.60	87.4
DIF	85.9	80.0	9.57	3.85	9.01	89.0

80.0 to 105.5%, and 87.4 to 99.4%, respectively, and the precisions of the overall method (RSD, $n=3$) were below 10% (Table 7).

3.5 *Analysis of water samples*

To verify the aforementioned method for quantitatively detecting FQs in real wastewater, we used the method to determine the FQs concentrations in different STPs and rivers around Shanghai, China. Figures 3(II) and 3(III) show a typical LC-FLD chromatogram obtained from extracted influent, effluent sample and river sample, indicating the methods developed in this study is good for determination of FQs in environmental water samples.

As shown in Figure 3(II) and Table 8, seven FQs, including NOR, CIP, LOM, PEF, DANO, ENRO and DIF, were detected in STPs, while SAR did not occur in any of the STPs. Determination of FQs in extracts influents and effluents ranged from 0.007 to 1.540 μg L^{-1}, 0.003 to 0.405 μg L^{-1}. Commonly consumed FQs, namely NOR, CIP and LOM, were the most frequently detected, and could be identified in sewage by the method developed in this study, with concentrations that were 0.022–1.540 μg L^{-1}, 0.014–0.559 μg L^{-1} and 0.024–0.735 μg L^{-1}, respectively. Some of the veterinarian-use FQs, such as ENRO, DANO, and DIF, were also detected.

Among the six STPs, STP-F got the lowest concentrations of FQs. One reason for this is the location of STP-F, which in the suburban area, Chongming Island, far from the Shanghai urban area. STP-A and STP-B, located in the urban area, had higher concentrations, which means FQs in the sewage mainly come from the domestic wastewaters. Dates of occurrences of FQs achieved in summer were higher than in spring, perhaps caused by the adsorption and de-sorption property of FQs from solid phase to the aquatic phase, Due to our lab-scale study, higher temperature is helpful to the de-sorption. Therefore, special attention should be paid to the transportation and fate of the FQs in the solid phase.

These results are consistent with those previously reported in the USA (19–45 ng L^{-1}) [20], Portugal (29–10,962 ng L^{-1}) [18], Switzerland (36–106 ng L^{-1}) [8], Sweden (30–130 ng L^{-1}) [17,23], Greece (30–460 ng L^{-1}) [24], France (330–510 ng L^{-1}) [21], Italy (290–580 ng L^{-1}) [21,24], Canada (30–460 ng L^{-1}) [25], and China (Beijing (16–111 ng L^{-1}) [26],

Table 8. Occurrence of FQs in the STPs and rivers in Shanghai, China. ($\mu g\,L^{-1}$).

Sampling date	Water samples		NOR	PEF	CIP	LOM	DANO	ENRO	SAR	DIF
Apr, 2008	STP-A	Influent	0.663	0.055	0.155	0.735	0.025	n.d.	n.d.	0.032
		Effluent 1	0.256	0.005	0.094	0.291	0.004	n.d.	n.d.	<LOD
		Effluent 2	0.265	0.01	0.084	0.207	0.012	n.d.	n.d.	n.d.
Aug, 2008		Influent	1.498	0.065	0.288	0.477	0.102	0.048	n.d.	0.053
		Effluent 1	0.246	0.006	0.064	0.099	0.147	0.009	n.d.	n.d.
Apr, 2008	STP-B	Influent	1.163	0.093	0.444	0.712	0.095	0.069	n.d.	0.082
		Effluent	0.216	0.004	0.064	0.405	0.003	<LOD	n.d.	<LOD
Aug, 2008		Influent	1.540	0.166	0.559	0.413	0.150	0.187	n.d.	0.036
		Effluent	0.340	0.030	0.014	0.083	0.026	<LOD	n.d.	0.014
Apr, 2008	STP-C	Influent	0.232	0.009	0.042	0.262	0.011	0.020	n.d.	0.016
		Effluent	0.121	0.008	0.018	0.032	0.003	<LOD	n.d.	0.006
Apr, 2008	STP-D	Influent	0.279	0.021	0.07	0.271	0.012	n.d.	n.d.	0.052
		Effluent1	0.097	<LOD	0.014	0.036	0.002	n.d.	n.d.	0.018
Apr, 2008	STP-E	Influent	0.133	0.008	0.032	0.305	0.007	0.007	n.d.	0.024
		Effluent	0.056	0.003	0.020	0.024	0.003	0.007	n.d.	0.005
Apr, 2008	STP-F	Influent	0.047	<LOD	0.039	0.198	0.007	0.011	n.d.	0.017
		Effluent	0.022	<LOD	0.017	0.038	0.005	0.003	n.d.	<LOD
Aug, 2008	Huangpu River		0.010	<LOD	0.007	0.029	0.005	0.006	n.d.	n.d.
Aug, 2008	Nanhenyin River		0.007	<LOD	<LOD	0.011	0.002	n.d.	n.d.	n.d.
Aug, 2008	Suzhou River		0.016	<LOD	0.016	0.040	0.003	0.003	n.d.	n.d.

Notes: n.d.: not detected. <LOD: below the limit of detection.
Effluent 1 was the discharge of the A/A/O process, and Effluent 2 was the discharge of the MBR process.

PRD (9–2054 $ng\,L^{-1}$) [27], Guangzhou (3520–5560 $ng\,L^{-1}$) [28], Shenzhen and Hong Kong [85–320 $ng\,L^{-1}$] [29]).

Estimated removal rates of FQs in the STPs varied between 70 and 100%. These results are in accordance with the scientific literature reported: from Switzerland [8], the reduction in concentrations of NOR and CIP was reported to be in the range 79–87%, in Sweden [17,23] the efficiency reported was 87%, 86% and 87% for NOR, CIP and ENRO, respectively, and in Portugal [18], the efficiency reported was 85% and 92% for NOR, 86% for 54% and 56% for CIP, and 53 and 56% for ENRO. These findings clearly show that the load of FQs in wastewater is reduced considerably during wastewater treatment, but complete removal is not achieved. Due to high adsorption of FQs onto the biosolids, considerable amounts of FQs' residues still can escape from the STPs, and therefore, are discharged into the receiving water bodies.

As shown in Figure 3(III) and Table 8, five FQs (including NOR, CIP, LOM, DANO and ENRO) were detected in river samples. Determination of FQs in extracted river samples still showed the trace level of FQs, with concentrations less than 40 $ng\,L^{-1}$. The occurrence of FQs in surface water was in the same range of countries, Spain (5.5 to 9.1 $ng\,L^{-1}$) [30], USA (<44 $ng\,L^{-1}$) [20], and Beijing, China (6.5 to 66 $ng\,L^{-1}$) [26], but in Japan, FQs were not detected [4].

Among them, Suzhou River flows through living area around the urban area, the concentration and number of FQs is more than other rivers, which further verifies that FQs in the water comes from the domestic wastewaters. Trace levels of FQs also detected

in Nanhengyin River, which is the receiving body of STP-F, indicating STP discharge is the point source of FQs' occurrence in the surface water.

4. Conclusion

The results of this study demonstrate that solid-phase extraction followed by reversed-phase chromatography with fluorescence detection is a sensitive, reproducible and specific instrumental method that can be used to quantitatively determine trace amounts of a wide variety of FQs antibacterial agents in water samples for routine analysis. Enrichment using the MEP cartridges, as compared to the other materials used in this study, is an appropriate SPE procedure for extracting FQs in wastewaters. Sensitivity can be enhanced and interferences minimised by concentrating these compounds using SPE. High and stable recovery efficiencies of eight FQs can be achieved with the method developed in this study at the working pH of 2–3. Out of the eight FQs investigated, seven FQs were detected in the effluent of the six STPs and three rivers studied with the concentration of CIP, NOR and LOM being much higher than other FQs. While the overall estimated removal of FQs in conventional STPs is relatively efficient (i.e. 70–100%), trace amounts of FQs still can escape from the STPs, and, thus, are discharged into the receiving waters.

The method is convenient to use and is ready for environmental applications, because the sample enrichment procedure is simple, fast and available, the separation is reliable, and the detection is highly sensitive. The analytical method developed in this study was found to fulfil validation requirements of precision and selectivity for FQs determination in aquatic (sewage and river water) samples, and may be used for partially routine analysis because LC-FLD is an inexpensive analytical technique compared with LC–MS. Therefore, the analytical procedure of the method may be used for the in-depth studies of occurrence, behaviour and fate of FQs in the aquatic environment.

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