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DETERMINATION OF FLUOROQUINOLONE ANTIBIOTICS IN WASTEWATER EFFULUENTS BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY AND FLUORESCENCE DETECTION

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Introduction

In recent years, public concerns about the environmental occurrence of pharmaceuticals including antibiotics and personal care products (PPCPs) have been increasing. It is evidenced that numerous PPCPs and antibiotics have been detected in waste and natural water resources[1,2]. Fluoroquinolones (FQs) are one of important antibiotics, which have been widely used for the last 15-20 years in human & veterinary medicine and in aquaculture in Europe and the United States[3]. They are active against many Gram-negative and Gram-positive bacteria, and function by inhibiting the DNA gyrase[4], a key enzyme in DNA replication.

Investigations of the occurrence of FQs in wastewater effluents and natural waters have been conducted in several European countries, such as Switzerland[5], France, Italy, Sweden and Greece[6]. These studies have reported FQ levels in the environment and have evaluated the adverse effects on microbial activity in wastewater treatment plants (WWTPs), algae, *Daphnia* and fish[5]. However, little information is available on the occurrence of FQs in WWTP and natural waters in the United States. In this study, a method was developed to determine nine FQs: pipemidic acid (PIP), ofloxacin (OFL), norfloxacin (NOR), ciprofloxacin (CIP), lomefloxacin (LOM), enrofloxacin (ENR), difloxacin (DIF), sarafloxacin (SAR) and tosufloxacin (TOS) in wastewater effluents and river/lake waters in the US and Canada using a liquid chromatograph interfaced with a mass spectrometer (LC-MS) and fluorescence detector (LC-FLD). Further, concentrations of FQs were measured, to allow an understanding of the status of contamination.

Materials and Methods

Samples

Secondary and tertiary treatment waters and final effluent waters were collected from a WWTP in East Lansing, Michigan. The WWTP uses four processes to treat wastewater: ‘preliminary’, ‘settling’, ‘secondary’ and ‘tertiary’ treatments. The average influent volume is 12.6 million gallons

per day (MGD) in this WWTP. Nine samples of river and lake waters were collected from Detroit, Lansing and Petoskey in Michigan and from western Lake Ontario in Canada. All samples were obtained during August and October of 2002.

Analytical Methods

FQs were determined by following the methods described previously[7] with some modifications. Briefly, 150-500 ml of water samples were collected in 1 L plastic bottles and filtered through 0.45 μm cellulose nitrate membrane filters. Samples were adjusted to pH 3 by formic acid to reduce biological activity. The analytes were concentrated from water samples by solid-phase extraction using mixed-phase cation exchange (MPC) disk cartridges (3M Empore). After extraction, the disk cartridges were eluted with 4 mL of 5% ammonia solution in methanol. The eluted solvents were concentrated and adjusted to 2 mL by 5% NH_4OH/15% MeOH/water. Determination of FQs was performed on a LC (HP-1100, Hewlett Packard) equipped with MS with electrospray ionization (VG Platform, Fisons Instruments). The mobile phases, A and B were a mixture of water and acetonitrile (98:2, pH: 3.0) and acetonitrile, respectively. The elution started with 5:95 (A:B) and programmed to 45:65 (A:B) in 25 min (flow speed: 0.2 mL/min). A parent ion (MH^+) of each FQs was monitored (PIP: 304, OFL: 362, NOR: 320, CIP: 332, LOM: 352, ENR: 360, SAR: 386, DIF: 400, TOS: 405). In this study, LC-FLD was also used for determination of FQs for further confirmation. The FLD excitation wavelength was 278 nm and the emission wavelength was 445 nm, except for ofloxacin (500 nm). The mobile phase and gradient of the LC-FLD were similar to those of LC-MS. The LC columns, YMC ODS-AQ S-3 (4.0 x 50 mm, Waters) and Discovery RP-Amide C16 (4.0 x 50 mm, Supelco) were used for the separation of FQs prior to detection by LC-MS and LC-FLD, respectively.

Quality Control:

Quality control samples included spike recovery tests through the entire analytical procedure. Two hundred ml of river water that did not contain any FQs was spiked with 200 ng of a standard mixture containing eight FQs. Three replicate analyses were performed and the recoveries of FQs ranged from 78 to 108 %.

Results and Discussion

OFL (Fig. 1) was found in secondary and final effluents of WWTP at concentrations of 204 and 100 ng/L, respectively. The LC-MS chromatograms of FQ standards and a wastewater sample are shown in Figure 2. OFL is marketed in the US for oral treatment of various infections caused by susceptible microorganisms, and is the second most used quinolone with approximately $900 million on a global scale in 1999[8].

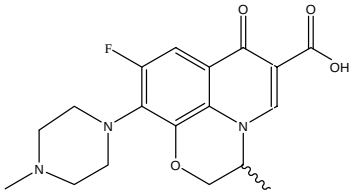

Fig. 1 Chemical structure of ofloxacin

Because the LC-MS used in this study could monitor only one ion (parent MH^+) with SIM mode, the LC-FLD was used as an additional identification method for confirmation purposes.

The retention time of the OFL standard peak was in agreement with that of samples (Fig. 3) that contained OFL.

The OFL concentration in final effluent was approximately 50 % less than the concentration in the secondary treatment effluent. Although the number of samples was limited, this indicates a partial removal of OFL during the wastewater treatment processes. The reduction of FQ concentrations during the treatment processes was also reported in a previous study[5]. The mass flow calculation, based on the OFL concentration in the final effluent (100 ng/L) and the average influent volume of WWTP (12.6 MGD), estimated that the amount of discharge of OFL to the river is 4.8 g/day. However, FQs were not detected in river and lake waters in this study, which may due to dilution and the higher detection limits of these compounds than those of previous reports [5,6]. An earlier study conducted in Switzerland calculated that CIP and NOR were discharged at levels ranging from 1.3 to less than 0.1 g/day.

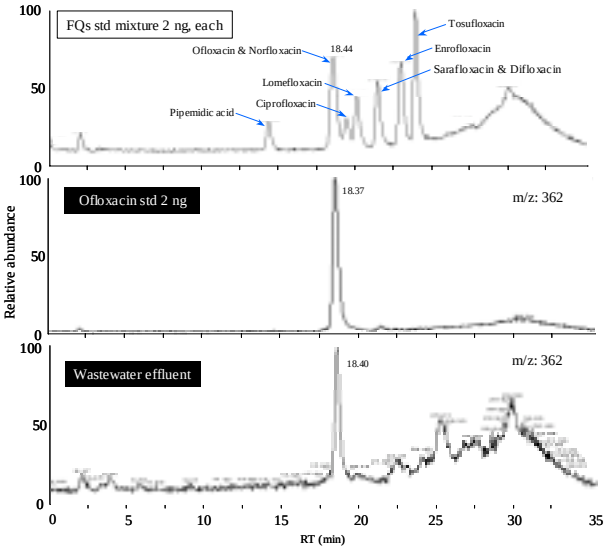

Fig. 2 LC-MS chromatogram of FQ mixtures (total) and ofloxacin standard and wastewater sample (SIM mode, m/z: 362)

The greater concentrations of OFL were found in the effluents from sewage treatment plants in European countries, such as France (330-510 ng/L), Italy (290-580 ng/L) and Greece (460 ng/L) (Table 1). The OFL concentrations measured in our study are comparable to or less than those observed in previous studies. CIP and NOR were detected in the effluents of WWTPs in Switzerland[5] and several European countries, although they were not detected in this study. An earlier study found that CIP was a less frequently detected compound in US streams (2.6 %)[1]. Such geographical differences might be due to the varying patterns of FQ usage among countries and regions, as well as the relatively higher detection limit of FQ that was used in this study.

Table 1 Concentrations of fluoroquinolone antibiotics (ng/L) found in wastewater effluents from the US and from European countries

	USA	France[6)]	Italy[6)]	Sweden[6)]	Greece[6)]	Switzerland[5)]
Ofloxacin	100-204	330-510	290-580	120	460	< 1
Lomefloxacin	< 70	180-190	180-320	130	290	< 1
Norfloxacin	< 70	50-80	60-70	30	70	50-120
Ciprofloxacin	< 70	60	40-70	30	70	50-110

The EC_{50} values for FQs have been calculated with an inhibition assay using a marine bacterium, *Vibrio fischeri* as the test organism[9]. OFL was highly toxic to the test bacteria, and the EC_{50} was 14,000 ng/L. This value is approximately two orders of magnitude greater than the levels determined in wastewaters in this study. However, greater concentrations of CIP and NOR were detected in sewage sludges from WWTP (1.4 to 2.4 mg/kg of dry wt.) in Switzerland[3]. Monitoring studies of FQs in water and sewage from WWTPs and in sediment/soil near aquaculture facilities and livestock farms will be necessary for the evaluation of their environmental distribution and risks.

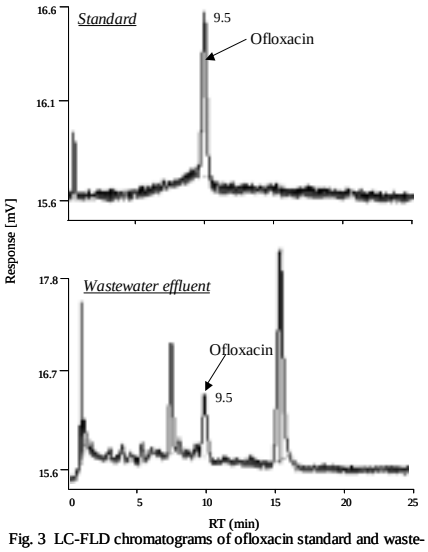

Fig. 3 LC-FLD chromatograms of ofloxacin standard and wastewater sample (Excitation: 278 nm, Emission: 500 nm)

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