

# Determination of fluoroquinolones in environmental waters by in-tube solid-phase microextraction coupled with liquid chromatography–tandem mass spectrometry

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Received 4 December 2005; received in revised form 17 January 2006; accepted 18 January 2006

Available online 21 February 2006

## Abstract

We developed a sensitive and useful method for the determination of five fluoroquinolones (FQs), enoxacin, ofloxacin, ciprofloxacin, norfloxacin, and lomefloxacin in environmental waters, using a fully automated method consisting of in-tube solid-phase microextraction (SPME) coupled with liquid chromatography–tandem mass spectrometry (LC/MS/MS). These compounds were analysed within 7 min by high-performance liquid chromatography (HPLC) using a CAPCELL PAK C8 column and aqueous ammonium formate (pH 3.0, 5 mM)/acetonitrile (85/15, v/v) at a flow rate of 0.2 mL/min. Electrospray ionization conditions in the positive ion mode were optimized for MS/MS detection. In order to optimize the extraction of FQs, several in-tube SPME parameters were examined. The optimum in-tube SPME conditions were 20 draw/eject cycles of 40  $\mu$ L of sample at a flow-rate of 150  $\mu$ L/min, using a Carboxen 1010 PLOT capillary column as an extraction device. The extracted compounds were easily desorbed from the capillary by passage of the mobile phase. Using the in-tube SPME LC/MS/MS method, good linearity of the calibration curve ( $r \geq 0.997$ ) was obtained in the concentration range from 0.1 to 10 ng/mL for all compounds examined. The limits of detection (S/N = 3) of the five FQs ranged from 7 to 29 pg/mL. The in-tube SPME method showed 60–94-fold higher sensitivity than the direct injection method (5  $\mu$ L injection). This method was applied successfully to the analysis of environmental water samples without any other pretreatment and interference peaks. Several surface waters and wastewaters were collected from the area around Asahi River, and ofloxacin was detected in wastewater samples of a sewage treatment plant and other two hospitals at 17.5–186.2 pg/mL. The recoveries of FQs spiked into river water were above 81% for a 0.1 or 0.2 ng/mL spiking concentration, and the relative standard deviations were below 1.9–8.6%.

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**Keywords:** In-tube solid-phase microextraction; Automated sample preparation; Liquid chromatography–tandem mass spectrometry; Fluoroquinolone antibacterial agents; Environmental water samples

## 1. Introduction

Fluoroquinolones (FQs) are antibacterial agents widely used for various infections because of characteristic of their broad activity spectrum and good oral absorption. Fluoroquinolones are applied as both human and veterinary medicine, and used at sub-therapeutic levels to promote growth for animals. Generally, FQs are prescribed 300–600 mg/day to the patient for therapeutic treatment. The FQs administered to humans or animals are almost excreted as unchanged compounds in urine, and are mainly effluent from the wastewater treatment plants

(WWTP) [1], but these compounds are not carried out monitoring on WWTP.

Recently, several studies have indicated the presence of antibiotic residues at a pg/mL level in environmental water including municipal wastewater effluents and surface waters [2–9]. The literature for analysis of FQs in environmental waters, however, is very few. Several analytical methods by fluorescence or mass spectrometry detectors have reported to detect FQs in surface water, groundwater, and WWTP. Hospital wastewater is one of main source of contamination. In fact, some studies have demonstrated that bacterial resistance against FQs in the hospital wastewater is higher than that in the sewage treatment plant wastewater [10].

These antibiotics are rather resistant to microbial degradation [11–13], and these compounds may be persisting within

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environmental waters because of their strong sorption properties. On the other hand, degradation of antibiotics, including photolysis [14–17] and chemical oxidation [18–20] may be significant on their environmental ecosystem. Effluence of FQs into the environmental waters occurs mainly as the parent compounds and as a consequence of inadequate treatment of human and animal excreta. It is apprehensive that bacteria exposed with antibiotics in environmental waters may acquire resistances against antibiotics. Additionally, it is important to perform more efficient and reliable environmental monitoring in order to know the stability of the FQs in the water samples. Therefore, it is necessary to develop a simple and sensitive analytical method for enabling the determination of these antibiotics at naturally occurring levels.

Analysis of FQs for the drug monitoring in human serum or urine and the determination in aqueous samples have been carried out mainly by high-performance liquid chromatography (HPLC) with UV [2,4] and fluorescence detector [7,9], liquid chromatography/mass spectrometry (LC/MS) [3,7,5], or LC–tandem mass spectrometry (LC/MS/MS) [6]. These methods are coupled with off-line or on-line solid-phase extraction (SPE) techniques for extraction and concentration of FQs in environmental water samples [2–9]. Most of these techniques, however, require large sample volumes. The in-tube solid-phase microextraction (SPME) technique [21], using an open tubular fused-silica capillary with an inner surface coating as extraction device, is simple and can be easily coupled on-line with HPLC, LC/MS and LC/MS/MS. In-tube SPME allows convenient automation of the extraction process, which not only reduces the analysis time, but also provides better accuracy, precision, and sensitivity than off-line manual techniques. We have reported development of the in-tube SPME method for the determination of various compounds, such as drugs and endocrine disruptors, by coupling with HPLC [22–24], LC/MS [25,26] and LC/MS/MS [27]. The details of the in-tube SPME technique

and its applications have also been summarized in a number of reviews [28–30].

In the present study, we developed an automated on-line in-tube SPME LC/MS/MS method for the determination of the five FQs in environmental waters to achieve high throughput analysis. Target FQs are ciprofloxacin, norfloxacin, ofloxacin, lomefloxacin, and enoxacin. Using this method, we applied to the determination of FQs in several environmental water samples.

## 2. Experimental

### 2.1. Materials

Fig. 1 shows the structures of the five FQs examined in this study. Enoxacin, ofloxacin, ciprofloxacin, norfloxacin, and lomefloxacin were purchased from Sigma–Aldrich (Saint Quentin Fallavier, France). Ofloxacin is the racemic mixture of the chiral compounds, and the biologically active enantiomer is called levofloxacin. These FQs were in >98% purity. Each compound was dissolved in methanol to make a stock solution at a concentration of 1 mg/mL. The solutions were stored at 4 °C and diluted to the required concentrations with pure water prior to use. Acetonitrile and water (HPLC grade) as a mobile phase, and ammonium formate were purchased from Nacalai Tesque (Kyoto, Japan). All other chemicals were of analytical grade.

### 2.2. Sample collection and preparation

Surface water and wastewater samples were collected in 1 L amber glass bottles with Teflon-lined caps, and filtrated through 0.2- $\mu$ m nylon syringe filters 13 mm in diameter (Tosoh, Tokyo, Japan) if necessary. Sampling was performed twice at five sites in the area around Asahi River and Sasagase River in Okayama City including wastewaters from hospital and WWTP in May

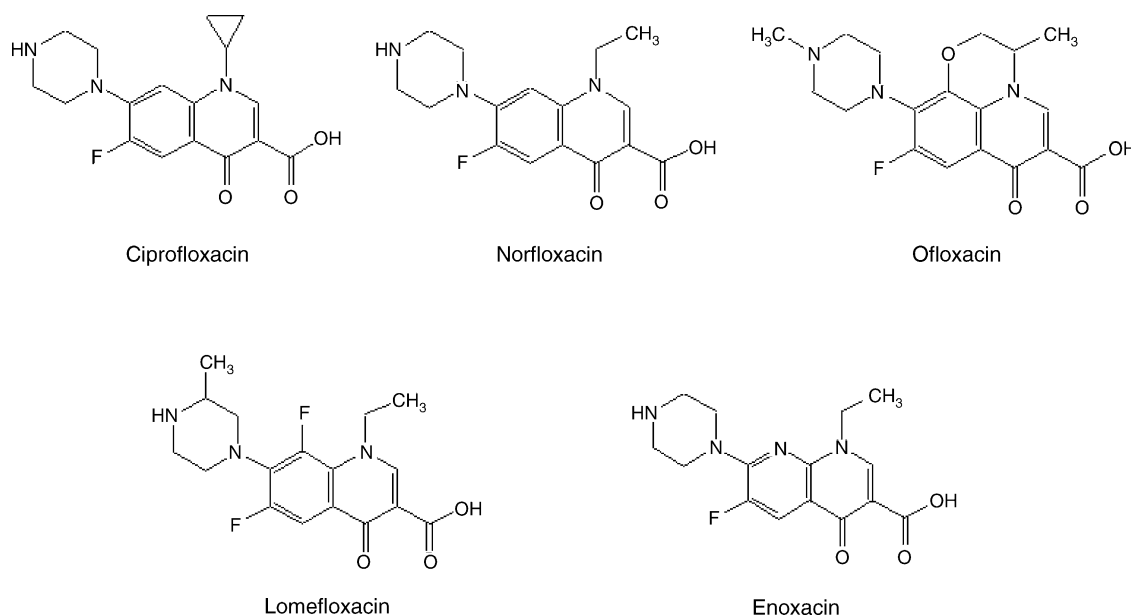


Fig. 1. Chemical structures of fluoroquinolones used in this study.

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