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HPLC determination of enoxacin, ciprofloxacin, norfloxacin and ofloxacin with photoinduced fluorimetric (PIF) detection and multiemission scanning
Application to urine and serum

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Abstract

The fluorescence emission of the fluoroquinolones enoxacin (ENO), ciprofloxacin (CIPRO), norfloxacin (NOR) and ofloxacin (OFLO) notably increased by UV irradiation during few minutes, in ethanolic–water medium. An HPLC method has been developed, for the determination of these fluoroquinolones, based in the separation of the formed irradiation photoproducts. Optimization of the analytical wavelengths has been carried out by fast multiemission scanning fluorescence detection. The highest sensitivity has been found when measuring at emission wavelengths of 407 and 490 nm, for ENO and OFLO, respectively, and at 444 nm for both NOR and CIPRO (exciting at 277 nm). According to the criterium of Clayton, using 0.05 as false positive and false negative error assurance probabilities, detection limits of 7.3, 6.0, 6.3 and 14.5 ng/mL, for ENO, NOR, CIPRO and OFLO, respectively, have been found. Urine and serum samples have been successfully analyzed, with recovery values ranging among 99–97% and 98–103%, for urine and serum, respectively.

Keywords: Photoinduced fluorescence (PIF); Fluoroquinolones; HPLC; Serum; Urine

1. Introduction

Quinolones are an important group of synthetic antibiotics with antibacterial action. These compounds have a carboxylic acid group in position 4, and are frequently referred to as 4-quinolones. Their antibacterial activity increases by the addition of 6-fluoro- and 7-piperazinyl groups to the molecule. The introduction of the fluorinated quinolones represents an important therapeutic advantage because this group of quinolones shows higher antibacterial activity than the parent compounds [1]. They are widely used to treat human and veterinary diseases and also to prevent diseases in animals [2–4]. Their main excretion pathway is urinary, and low amounts are found in plasma being in the order of 5 mg L^{-1} for the fluoroquinolones herein studied [5]. On the other hand, there is concern about the possibility of exposure to low levels of these compounds, resulting in the development of resistance of human pathogens to antibiotics [6].

It is known that the fluoroquinolones suffer degradation processes by UV irradiation. Depending of the chemical and environmental conditions, such as the instrumental irradiation parameters and irradiation time, different structural photoproducts may be generated.

In the bibliography several papers are reported, related to the photodegradation of fluoroquinolones, but the applications of this methodology to the analysis of these compounds is very scarce. In general, the photodecomposition of fluoroquinones generate photoproducts presenting the acid–base properties of the carboxilic group of the original molecule. Hence, the decomposition of the piperazinic group is proposed as the most probable via of decomposition via [7].

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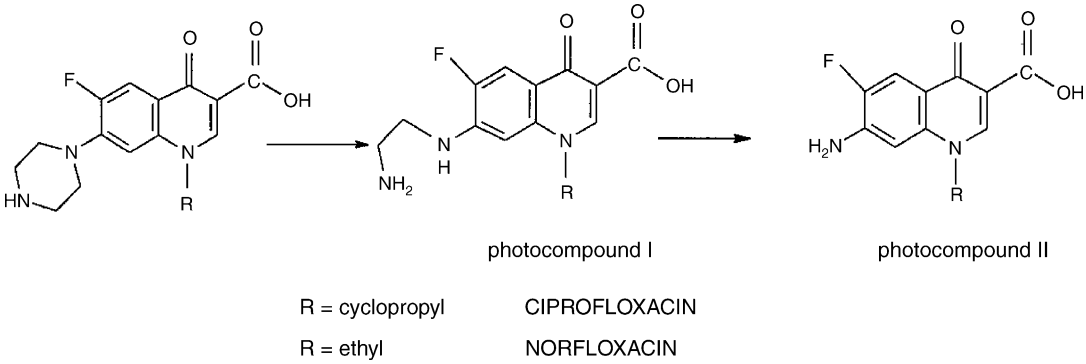


Scheme 1. Pathway photodegradation for CIPRO and NOR.

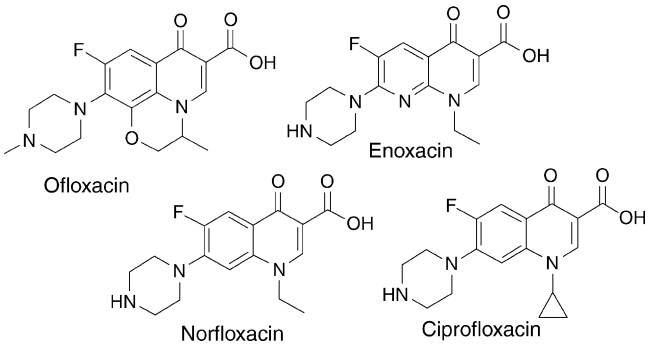
The concentration of the compound and the pH affect the photoreaction yield, and the 7-amine-1-cyclopropil-6-fluoro-1,4-dihydro-4-oxo-3-one carboxylic acid has been proposed as the most probable photoproduct from ciprofloxacin.

The degradation processes of enrofloxacin, ciprofloxacin, norfloxacin and danofloxacin have been studied by HPLC and mass spectrometric detection [8,9]. A pathway for degradation was proposed for norfloxacin and ciprofloxacin (Scheme 1), and depending of the irradiation conditions, the photocompounds I or II may be formed.

The photolability of enrofloxacin and other fluoroquinolones was studied by capillary electrophoresis, and the p*K* values of the parent fluoroquinolones and photoproducts generated were calculated [10]. No data about the photodecomposition of enoxacin has been reported to date.

The analysis of fluoroquinolones has traditionally been performed using microbiological methods [11,12]. However, these techniques are slow and suffer from poor precision and specificity. In the past decade, multivariate techniques have been incorporated to the analytical protocols [13]. Chemometric methodologies have been employed for the simultaneous determination of fluoroquinolones [14]. In particular, full spectrum multivariate calibration methods offer the advantage of their speed, as the separation steps may be avoided [15]. However, for chemometric modelling, some spectral differences are needed among the analytes. High performance liquid chromatography has become an important tool for the routine determination of antimicrobial agents in body fluids, with specific emphasis on fluoroquinolones [16,17]. In the literature, there are some references about the determination of these four fluoroquinolones [18,19]. Although the references about the determination of fluoroquinolones in biological fluids using HPLC are numerous, the most recent reported methods exhibit higher limits of detection values (LODs), than the calculated by the method proposed in this work [20–22]. Recently, a capillary electrophoretic separation of nine fluoroquinolones with fluorescence detection has been reported for biological samples [23].

In the present work an HPLC method for the determination of enoxacin (ENO), ciprofloxacin (CIPRO), norfloxacin (NOR) and ofloxacin (OFLO) (Scheme 2), based in the separation of the fluorescent products generated in a photoinduced process is reported. Previously, the optima conditions for the irradiation process have been established. In serum and in urine samples, the four fluoroquinolones can be determined in a single-run analysis. The compounds are determined using multiemission scan fluorimetric detection of the ENO, NOR and CIPRO photoproducts and for undegraded OFLO.



Scheme 2. Chemical structures of the ENO, NOR, CIPRO and OFLO.

2. Experimental

2.1. Apparatus

The chromatographic studies were performed on a Hewlett-Packard Mod. 1100 LC instrument, equipped with degasser, quaternary pump, manual six-way injection valve, containing a 20 μL loop, fast-scanning fluorimetric detector, and CHEMSTATION software package to control the instrument, data acquisition and data analysis. An analytical column Nova-Pak C_{18} (150 mm × 3.9 mm, Waters Millipore) was used.

An Osram 200 W HBO high-pressure mercury lamp, with an Oriel model 8500 power supply (Spectra-Physics, Newport, USA), was used for the photoreaction of the fluoroquinolones. The photochemical set-up included a light-box consisting of a fan, a mercury lamp and a quartz lens. Both, 3 and 10 mL quartz cells, were used in the irradiation process.