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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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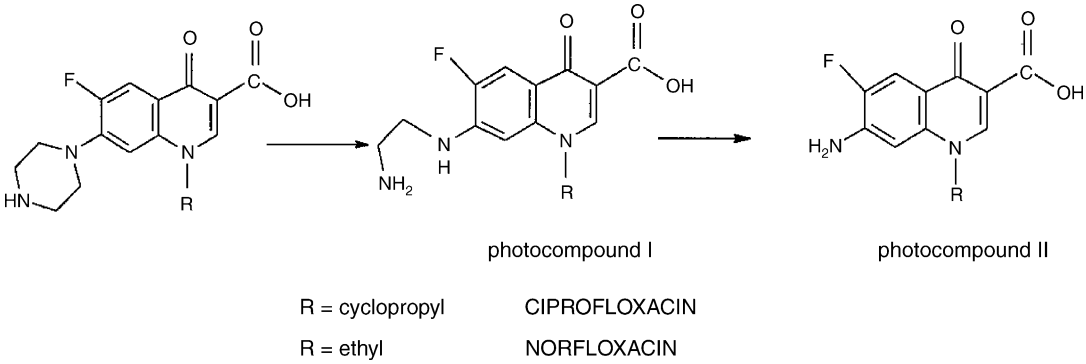


Scheme 1. Pathway photodegradation for CIPRO and NOR.

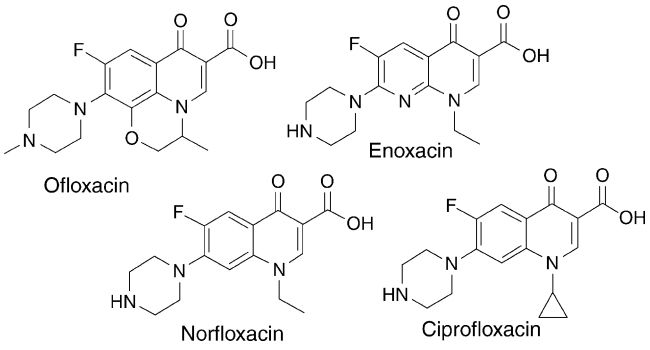
The concentration of the compound and the pH affect the photoreaction yield, and the 7-amine-1-cyclopropil-6-fluoro-1,4-dihydro-4-oxo-3-one carboxylic acid has been proposed as the most probable photoproduct from ciprofloxacin.

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.

The cells were placed in an optical bench at 30 cm from the mercury lamp. The solutions were magnetically stirred during the UV irradiation.

2.2. Reagents and solutions

All solvents used were gradient grade for liquid chromatography (Merck, Spain). Enoxacin, ofloxacin and norfloxacin were purchased from Aldrich (Sigma–Aldrich Química, SA, Spain). Ciprofloxacin was purchased from Fluka (Sigma–Aldrich Química, SA, Spain). Standard solutions of each compound (100 $\mu g\,mL^{-1}$) were prepared by dilution in ethanol (avoiding exposure to direct light and maintaining the solution stored at 4 °C).

2.2.1. Mobile phase

The mobile phase was formed by a mixture of phosphate buffer and tetrahydrofurane (THF, Merck). To prepare the buffer solution, di-potassium hydrogenphosphate trihydrate (Merck) was dissolved in ultrapure water, which was obtained from a Millipore Milli-Q system. The acidity was fixed using HCl (Merck) at pH 3.0. The buffer concentration was 30.0 mmol/L.

The final mobile phase composition was 96% of buffer solution and 4% of THF to determine the fluoroquinoles in urine, and 97% of buffer solution and 3% of THF in serum.

2.2.2. General procedure: calibration curves

Calibration samples were prepared in the concentration range up to 160 $ng\,mL^{-1}$ for each fluoroquinolone. In 10 mL volumetric flasks, appropriate volumes of stock standard solutions of fluoroquinolones, 0.5 mL of pH 7.05 Tris/HCl (0.015 M) buffer solution, ethanol to obtain a final amount of 50%, and ultrapure water to the mark were added. Then, the samples were transferred to 10 mL quartz cells and irradiated during 10 min under constant magnetic stirring. In a 10 mL volumetric flask, 5 mL of the irradiated solution and pH 3.0 phosphate buffer (0.030 M) solution up to the mark were added. These standard samples were filtered before injection through a Millipore syringe adapter, containing a 0.45 μm regenerated cellulose membrane filter, and injected in the chromatographic system.

2.2.3. Procedure to analyze ENO, NOR, CIPRO and OFLO in urine

Freshly urine samples were fortified with the fluoroquinolones by addition of 50 $\mu g\,mL^{-1}$ of each fluoroquinolone, and then waiting at least 30 min, before the analysis of the samples. An aliquot of 1.00 mL of this solution was diluted to 25 mL with ultrapure water. Then, an aliquot of 0.5 mL was placed in a 10 mL volumetric flask, and the general procedure above mentioned was applied. The standard addition method was used.

2.2.4. Procedure to analyze ENO, NOR, CIPRO and OFLO in serum

Serum samples conserved at −20 °C were unfrozen and immediately spiked with the fluoroquinolones (1 $\mu g\,mL^{-1}$ of each) and analyzed according to the following procedure. An aliquot of 1.00 mL of fortified serum, 0.5 mL of pH 7.05 Tris/HCl (0.015 M) buffer solution, ethanol to obtain a final percentage of 50%, and ultrapure water were added to the mark, in a 10 mL volumetric flask. Then, the sample was transferred to a 10 mL quartz cell and irradiated during 10 min, under constant magnetic stirring. In a 10 mL volumetric flask, 5 mL of the irradiated serum solution, and pH 3.0 phosphate buffer (0.030 M) solution up to the mark, were added. Then, the standard samples were centrifuged at 4000 rpm during 5 min at room temperature. The clear supernatant was filtered before injection through a Millipore syringe adapter, containing a 0.45 μm regenerated cellulose membrane filter. The standard addition method was applied.

3. Results and discussion

3.1. Photoinduced fluorescence reaction

The irradiation of ethanolic solutions of fluoroquinolones during a few minutes, by using a high power UV lamp, drastically increases the fluorescence quantum yield of ENO, NOR and CIPRO, and decreases the native fluorescence of OFLO. The photodegradation process for each fluoroquinolone depends on the irradiation time, solvent composition, and on the initial pH of the fluoroquinolone solutions. Different ethanolic water mixtures were investigated to establish the optimum irradiation conditions, and the best signals were obtained when a (50–50, v/v), aqueous–ethanolic mixture, was used. In only about 10 min, ENO, NOR and CIPRO gave rise to stable fluorescent photoproducts. However, in the studied media, OFLO was only slightly affected by the irradiation.

3.2. Acid–base characteristic of the fluoroquinolones and photoproducts

With the aim to establish the acid–base behaviour of the photoproducts, the p*K* values of these have been studied in a (50%) ethanolic–water medium. Samples containing the individual fluoroquinolones, at different pH values, were irradiated during about 10 min, and then the fluorescent spectra record for each pH value. In all cases, the fluorescence drastically increases, preferably at pH about 6–7. Also, the pH of samples containing individual fluoroquinolones, previously irradiated during 10 min at a fixed pH 6.5, was modified between 4 and 10 and then the fluorescence spectra was obtained (Fig. 1). In all cases, the highest fluorescence yield appeared for pH < 5, and a pH 3 was selected as optimum for the fluorimetric detection.

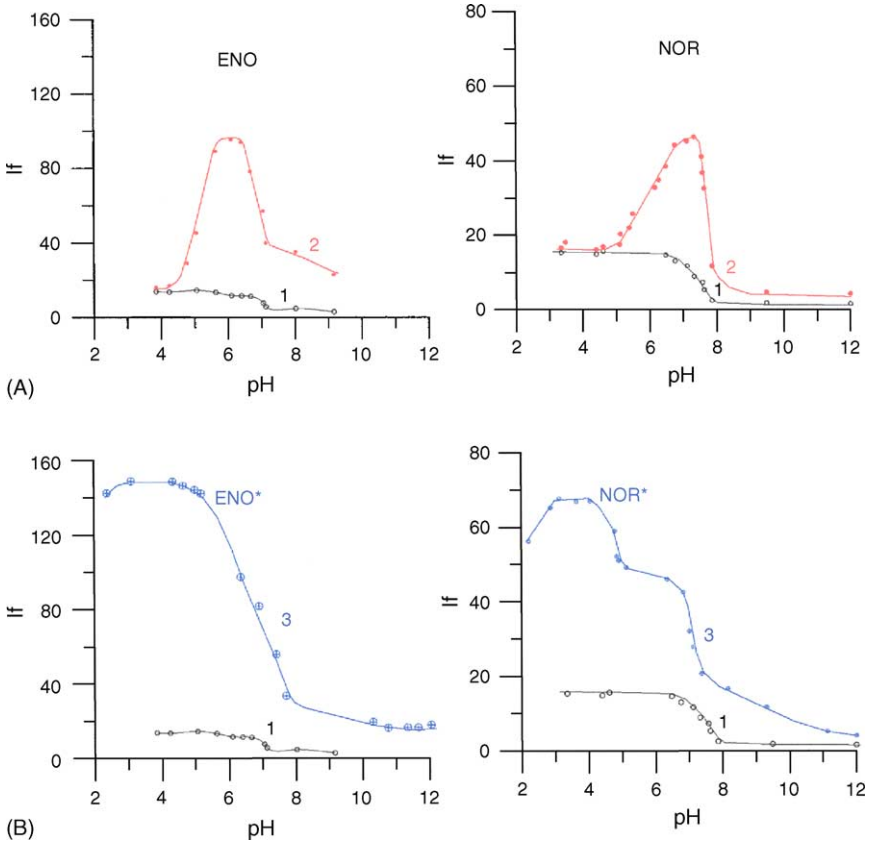


Fig. 1. Influence of the pH in the fluorescence intensity of (A) ENO and NOR and in (B) ENO* and NOR*. (1) Influence of the pH in the fluorescence intensity of water–ethanolic solutions of ENO and NOR. (2) Variation of the fluorescence intensity of water–ethanolic solutions of ENO and NOR, when are irradiated during 10 min at different pH values. (3) Influence of the pH in the fluorescence intensity of water–ethanolic solutions of the photoproducts (ENO* and NOR*), once irradiated during 10 min at pH 6.5.

The p*K* values corresponding to the photoproducts are summarized in Table 1. These values were in corcordance with the photocompound I for NOR, and photocompound II for ENO and CIPRO, as in the two cases, the deprotonation of the aromatic primary amine groups were not observed. In the established conditions photoreaction for OFLO was not observed.

Table 1
p*K* values for the studied fluoroquinolones and the generated photoproducts

Parent fluoroquinolone	p*K*[a]	p*K*[b]	p*K*[c]
ENO	6.0	7.0	6.85
	9.6	10.5	
NOR	5.9	6.6	4.7
	8.8		7.1
OFLO	5.6	6.4	6.4
	9.0		
CIPRO	6.0	7.5	7.15
	8.6		

[a] p*K* value for fluoroquinolones in water.
[b] p*K* value for fluoroquinolones in a 50% ethanolic–water medium.
[c] p*K* values for the photoproducts in a 50% ethanolic–water medium.

3.3. Selection of the chromatographic parameters

The excitation and emission spectra of the four fluoroquinolones, once irradiated in the optimized conditions, are shown in Fig. 2. The excitation was fixed at 277 nm, as

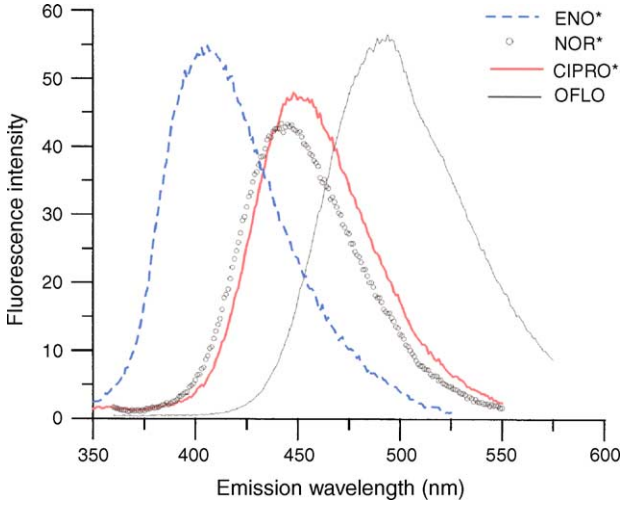


Fig. 2. Emission spectra of ENO, NOR, CIPRO and OFLO exciting at 277 nm, before the irradiation process.

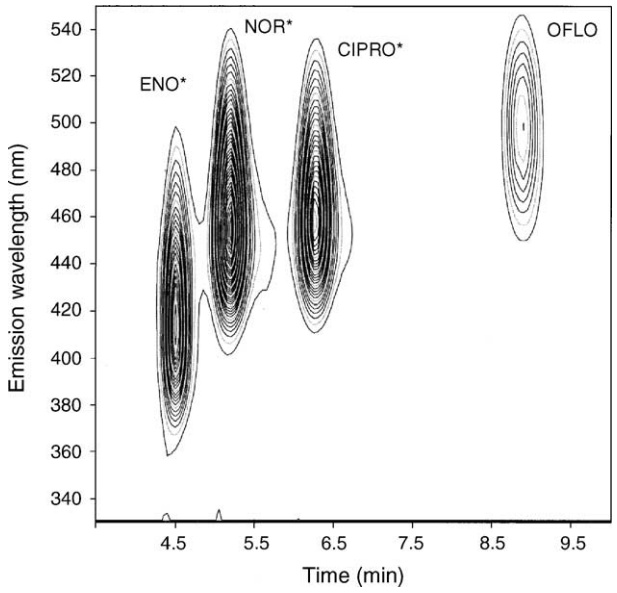


Fig. 3. Bidimensional proyection, as contour plots, of the intensity of fluorescence, emission wavelength and retention time, exciting at 277 nm, in the optimized chromatographic conditions.

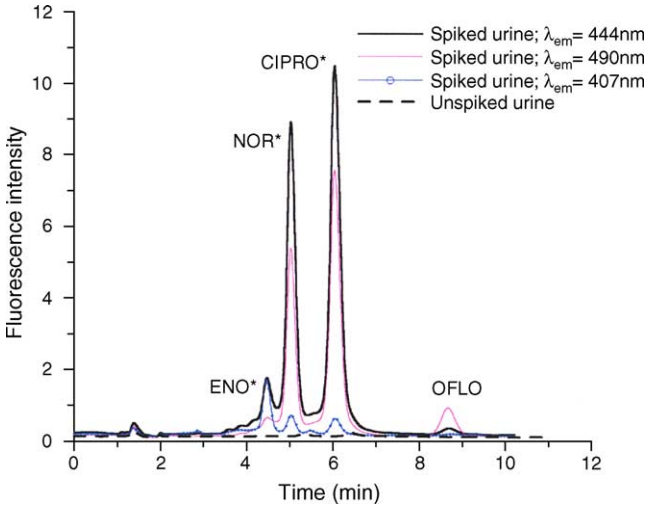


Fig. 4. Chromatogram of unspiked and spiked urine samples containing ENO, NOR, CIPRO and OFLO, at different detection emission wavelengths (λ_{ex} = 277 nm).

adequate for the simultaneous detection of the four fluoroquinolones. The optima emission maxima were 409 nm for ENO, 444 nm for both NOR and ENRO, and 490 nm for OFLO. The possibility of using a multiemission detection scan, allows us to select the appropriate emission wavelength and increases the sensitivity for each component.

To choose the mobile phase, different organic solvents were tested. Acetonitrile, methanol and THF were used to select the best one. The best results were obtained when THF was used as organic modifier. The medium acidity was studied with phosphate buffer, and the best resolutions were obtained when an acid medium was assayed. A pH 3 was fixed with phosphate buffer to obtain the best resolution and highest sensitivity. Diverse percentages of THF and buffer were tested and the best results were obtained when the mobile phase composition was 94% buffer and 6% THF. The presence of THF in the mobile phase provided tighter and well defined peaks.

In the optimized conditions for the mobile phase, the resolution of all peaks was to the baseline, and all the compounds were eluted in about 8 min. In Fig. 3, a bidimensional emission wavelength–time fluorescence projection is shown. The good chromatographic profile obtained in the established conditions is shown in Fig. 4. The fluorescence intensity of NOR* and CIPRO* is very low at 407 nm, however, this is the most adequate emission wavelength for ENO*. The highest emission for OFLO is obtained when measuring at 490 nm.

3.4. Optimization of the irradiation time

The optimum irradiation time was selected by testing the variation of the chromatographic peak area for the parent fluoroquinolones, and also for the generated photoproducts, in function of the irradiation time. For ENO, NOR and CIPRO, the peak area is diminishing when the irradiation time is increasing, and only one new peak is generated when the irradiation time is increased. For OFLO, a slightly diminishing of the peak area is observed, but any new peak appears. The maximum peak area is obtained in about 6 min for ENO*, however, a higher irradiation time is necessary for NOR*. A compromise irradiation time of 10 min was selected for all the fluoroquinolones. The stability of the photoproducts was tested for successive injections, resulting stable during at least 3 h.

3.5. Analytical parameters

Under the selected conditions, calibration graphs were obtained by preparing standard samples of the mixture of the four compounds by triplicate, with increasing concentrations of each analyte. The standard preparation was performed in two steps. First, a standard was prepared in an ethanol–water medium (pH 6.5), then, the sample was irradiated during 10 min. With this irradiation time, all the initial products were transformed to the corresponding photoproducts. In a second step, 5.0 mL were diluted with the phosphate buffer (pH 3) to 10 mL, according to the proposed method. The study was performed with concentrations of each drug between 40 and 120 ng mL^{-1}, for ENO and NOR, and between 20 and 120 ng mL^{-1} for CIPRO and OFLO, respectively. The results obtained are summarized in Table 2. In addition, the detection limit value (LOD), according with Long and Winefordner [24] and Clayton criterium [25], linearity and analytical sensitivity, were also calculated [26]. The repeatability was studied by performing 10 successive injections. The results are shown in Table 3.

Table 2
Statistical parameters for the regression of ENO, NOR, ENRO and OFLO

	ENO	NOR	CIPRO	OFLO
Calibration sensitivity[a] (±S.D.)	0.146 (0.003)	0.729 (0.011)	1.059 (0.017)	0.109 (0.003)
Regression coefficient (R)	0.998	0.999	0.999	0.997
Linearity (%)[b]	98.1	98.5	98.4	97.0
Limit of detection[c] ($K = 3$)	4.9	4.0	4.0	10.0
Limit of detection[d] ($\alpha = \beta = 0{\cdot}05$)	7.3	6.0	6.3	14.5
Analytical sensitivity[e] ($1/\gamma$)	2.2	1.8	2.1	3.7

[a] FU (fluorescence units) (s mL ng^{-1}).
[b] According with [26].
[c] Expressed as ng mL^{-1} according to [24].
[d] Expressed as ng mL^{-1} according to [25].
[e] Expressed as ng mL^{-1}; S.D., standard deviation.

Table 3
Repeatability of the chromatographic separation

	ENO[a]	NOR[b]	CIPRO[b]	OFLO[b]
Calculated mean concentration (±S.D.) (ng mL^{-1})	70.8 ± 5.8	103.6 ± 5.1	94.8 ± 4.3	103.4 ± 5.7
R.S.D. (%)	8.2	5.0	4.6	5.5
t_R (min) (±S.D.)	4.7 ± 0.1	5.3 ± 0.1	6.4 ± 0.1	8.9 ± 0.1
$W_{1/2}$ (min) (±S.D.)	0.21 ± 0.01	0.24 ± 0.01	0.11 ± 0.01	0.40 ± 0.02
Resolution (±S.D.)	–	1.30 ± 0.02	3.17 ± 0.04	4.95 ± 0.12

S.D., standard deviation for $n = 10$; R.S.D., relative standard deviation.
[a] Repeatability for injection of 60 ng mL^{-1} of ENO.
[b] Repeatability for injection of 100 ng mL^{-1} of NOR, CIPRO and OFLO.

3.6. Analysis of urine samples

The proposed method was applied to the determination of the cited fluoroquinolones in urine samples. Urines belonging to healthy people were appropriated fortified with ENO, NOR, CIPRO and OFLO (50 μg mL^{-1}), simulating biological concentrations. The total dilution factor applied to the urine samples was 1000-fold. In Fig. 4, chromatograms of unspiked and spiked urine samples are shown. In this figure, it can be observed that ENO is adequately measured at 407 nm, NOR and CIPRO exhibit the highest sensitivity at 444 nm, and OFLO at 490 nm. No peak from the urine matrix was observed in the chromatogram. The addition standard methodology was applied and the results are summarized in Table 4. Recovery values were between 95 and 102% for ENO, 93 and 104% for NOR, 94 and 104% for CIPRO, and 96 and 107% for OFLO.

3.7. Analysis of serum samples

The proposed method was also applied to the determination of the cited fluoroquinolones in serum samples. Serum belonging to healthy people were appropriated fortified with ENO, CIPRO, NOR and OFLO (1 μg mL^{-1}), simulating biological concentrations. The total dilution factor applied to the samples, before measurement, was 20-fold. After introducing the serum samples into the chromatographic system, the samples were centrifuged during 5 min, with the object of separating the insoluble matter (proteins and others) generated (in the ethanolic–water medium), when the sample was acidified (pH 3). In Fig. 5, a chromatogram of spiked serum is shown. The standard addition method was applied and recoveries were between 96 and 104% for ENO, 91 and 108% for NOR, 97 and 102% for CIPRO and 99 and 105% for OFLO. In Table 5, these results are summarized.

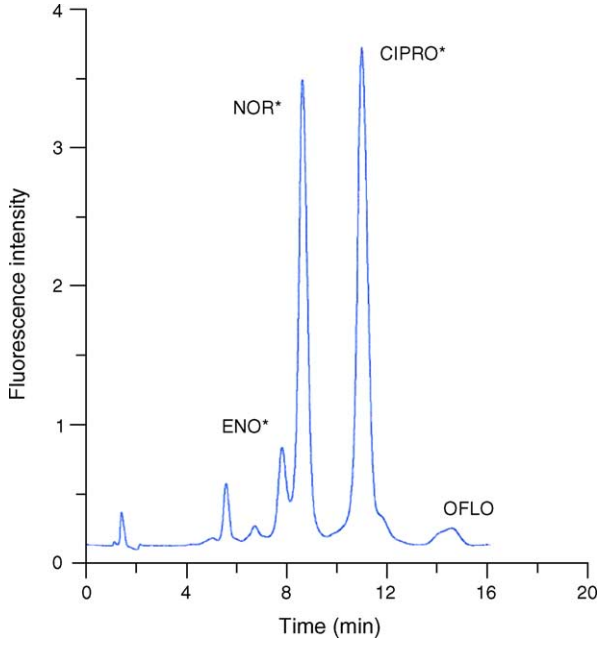


Fig. 5. Chromatogram of a serum sample spiked with ENO, NOR, CIPRO and OFLO, exciting at 277 nm (emission wavelength of 444 nm).

Table 4
Determination of ENO, NOR, CIPRO and OFLO, in fortified human urine samples (50 μg mL^{-1}), using the standard addition method

Added (ng mL^{-1})	Found (ng mL^{-1})	Recovery (%)	Regression found vs. added
ENO			
0	58	–	$[ENO]_{found} = 0.990\ [ENO]_{added}$, $R^2 = 0.9966$
60	119	102	
80	134	95	
100	159	101	
Found: 58.0 ng mL^{-1} (corresponding to 58.0 μg mL^{-1} in the original fortified urine)			
NOR			
0	59	–	$[NOR]_{found} = 0.975\ [NOR]_{added}$, $R^2 = 0.9895$
60	121	102	
80	143	104	
100	153	93	
Found: 59.7 ng mL^{-1} (corresponding to 59.7 μg mL^{-1} in the original fortified urine)			
CIPRO			
0	49	–	$[CIPRO]_{found} = 0.969\ [CIPRO]_{added}$, $R^2 = 0.9920$
40	92	104	
60	111	103	
80	125	94	
Found: 50.2 ng mL^{-1} (corresponding to 50.2 μg mL^{-1} in the original fortified urine)			
OFLO			
0	47	–	$[OFLO]_{found} = 0.996\ [OFLO]_{added}$, $R^2 = 0.9923$
60	88	100	
80	112	107	
100	125	96	
Found: 48.0 ng mL^{-1} (corresponding to 48.0 μg mL^{-1} in the original fortified urine)			

Table 5
Determination of ENO, NOR, CIPRO and OFLO in fortified human serum samples (1 μg mL^{-1}) using the standard addition method

Added (ng mL^{-1})	Found (ng mL^{-1})	Recovery (%)	Regression found vs. added
ENO			
0	51	–	$[ENO]_{found} = 0.982\ [ENO]_{added}$, $R^2 = 0.9969$
60	115	104	
80	129	96	
100	150	98	
Found: 51.7 ng mL^{-1} (corresponding to 1.03 μg mL^{-1} in the original fortified serum)			
NOR			
0	53	–	$[NOR]_{found} = 1.020\ [NOR]_{added}$, $R^2 = 0.9839$
60	119	108	
80	127	91	
100	159	104	
Found: 54.5 ng mL^{-1} (corresponding to 1.09 μg mL^{-1} in the original fortified serum)			
CIPRO			
0	46	–	$[CIPRO]_{found} = 1.010\ [CIPRO]_{added}$, $R^2 = 0.9980$
40	106	100	
60	124	97	
80	148	102	
Found: 46.3 ng mL^{-1} (corresponding to 0.93 μg mL^{-1} in the original fortified serum)			
OFLO			
0	52	–	$[OFLO]_{found} = 1.033\ [OFLO]_{added}$, $R^2 = 0.9982$
60	113	101	
80	132	99	
100	157	105	
Found: 52.2 ng mL^{-1} (corresponding to 1.04 μg mL^{-1} in the original fortified serum)			

Table 6
Bibliographic data for fluoroquinolones determination methods

Compound	Analytical technique	Analytical signal	Concentration range/ type of sample	Total retention time or total migration time	Limit of detection ($ng\,mL^{-1}$)	Reference
ENO	HPLC	Photoinduced fluorescence (PIF)	40–120 $ng\,mL^{-1}$	Urine: 9 min serum: 16 min	5.0	Proposed method
NOR		Irradiation time = 10 min			4.0	
CIPRO		(λ_{exc} = 277 nm)	Urine and serum		4.5	
OFLO		(λ_{em} = 407, 444 and 490 nm)			10	
NOR	LC	Electrospray ionization (ESI)-mass spectrometry (MS)	10–1000 $ng\,mL^{-1}$	9 min	13	21
CIPRO					17	
OFLO					21	
ENRO			Urine		–	
GATI	HPLC with column switching	Native fluorescence	160–5000 $ng\,mL^{-1}$	15 min	120	
MOXI		(λ_{exc} = 296 nm)	125–4000 $ng\,mL^{-1}$		35	22
LEVO		(λ_{em} = 504 nm)	Serum		60	
NOR	CZE	Native fluorescence	100–500 $ng\,mL^{-1}$	25 min		23
CIPRO		Excitation band = 200–400 nm			6	
OFLO		Emission filter = 435 nm			7.5	
Other six			Blood		3.5	

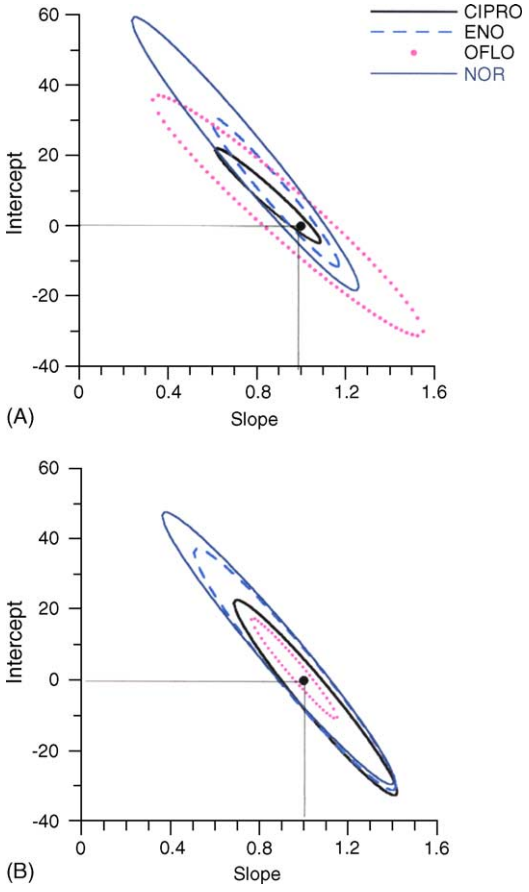


Fig. 6. Elliptical joint confidence regions for the slope and intercept, corresponding to regression of predicted vs. actual concentrations of ENO, NOR, CIPRO and OFLO, applying the proposed chromatographic method in the analysis of (A) urine and (B) serum samples.

3.8. Comparative analysis

In order to get further insight into the accuracy and precision of the different methods herein developed, linear regression analysis of the nominal versus found concentration values was applied. The estimated intercept and slope were compared with their ideal values 0 and 1, using the elliptical joint confidence region (EJCR) test [27]. Any point which lies inside the EJCR is compatible with the data at the chosen confidence level. If the point (0, 1) lies inside the EJCR, then bias is absent and, consequently, the recovery may be taken as unity (or 100% in percentile scale). Fig. 6 shows the EJCR plots for the proposed chromatographic method, in urine and serum samples. As can be seen, all the ellipses contain the theoretical (0, 1 point), although the corresponding to NOR, in both urine and serum, shows a larger size. On the other hand, the determination of ENO in urine and OFLO in serum, produce the highest precision results.

Comparative data about the irradiation conditions, analytical characteristics and figures of merit of related methods of fluoroquinolones analysis have been summarized in Table 6.

4. Conclusions

The proposed LC method is simple, and a short time, less than 10 min, is necessary for all compounds elution. An acceptable chromatographic resolution is obtained for the four analytes. The proposed method is applicable to ENO/NOR/CIPRO/OFLO analysis, in serum and urine samples. It is necessary to take into account that, in biological fluids, CIPRO is the main metabolite of ENRO, and

then, the proposed method could be an alternative method for ENO/NOR/ENRO/OFLO determination. This possibility will be later analyzed. An acidic water–ethanolic medium is optimum to obtain the highest quantum fluorescence yield of the photoproducts. In these conditions, proteins are efficiently eliminated by centrifugation. Hence, the method can be applied to the analysis of urine and serum samples, without pre-treatment. The limits of detection are better and the chromatographic retention times are shorter than those recently reported in the bibliography.

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