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Original article

Highly sensitive spectrofluorimetric determination of lomefloxacin in spiked human plasma, urine and pharmaceutical preparations

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ABSTRACT

A sensitive, simple and selective spectrofluorimetric method was developed for the determination of lomefloxacin in biological fluids and pharmaceutical preparations.

The method is based on the reaction between the drug and 4-chloro-7-nitrobenzodioxazole in borate buffer of pH 8.5 to yield a highly fluorescent derivative that is measured at 533 nm after excitation at 433 nm. The calibration curves were linear over the concentration ranges of 12.5–625, 15–1500 and 20–2000 ng/mL for plasma, urine and standard solution, respectively.

The limits of detection were 4.0 ng/mL in plasma, 5.0 ng/mL in urine and 7.0 ng/mL in standard solution. The intra-assay accuracy and precision in plasma ranged from 0.032 to 2.40% and 0.23 to 0.36%, respectively, while inter-assay accuracy and precision ranged from 0.45 to 2.10% and 0.25 to 0.38%, respectively. The intra-assay accuracy and precision estimated on spiked samples in urine ranged from 1.27 to 4.20% and 0.12 to 0.24%, respectively, while inter-assay accuracy and precision ranged from 1.60 to 4.00% and 0.14 to 0.25%, respectively. The mean recovery of lomefloxacin from plasma and urine was 98.34 and 98.43%, respectively. The method was successfully applied to the determination of lomefloxacin in pharmaceuticals and biological fluids.

1. Introduction

Lomefloxacin HCl (LOM), a difluoroquinolone, is the monohydrochloride salt of (±)-1-ethyl-6,8-difluoro-1,4-dihydro-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid (Fig. 1) [1].

Lomefloxacin is a third-generation fluoroquinolone available in Brazil for systemic administration since 1993. Lomefloxacin is nearly completely absorbed when taken orally and is slowly eliminated, having a half-life of seven to eight hours [2]. Similar to other fluoroquinolones, lomefloxacin has a broad spectrum of action, including Gram-positive and Gram-negative microorganisms. As a third-generation quinolone, it also has the advantage of being effective against some anaerobic bacteria [3–8].

The antibacterial activity of fluoroquinolones, such as lomefloxacin, is mediated through inhibition of the bacterial enzyme DNA gyrase, resulting in failure to synthesize bacterial DNA. As a consequence, fluoroquinolones are bactericidal [1,9,10].

Several types of analytical procedures have been employed for the analysis of LOM in pharmaceutical formulations and biological samples. Among techniques used in several procedures most are based on fluorimetry [11–15], derivative spectrophotometry [16] and high performance liquid chromatography [17,18].

Few analytical methods have been used for the determination of LOM in biological fluids. Determination of LOM in human urine and serum by differential-pulse adsorptive stripping voltammetric method has also been described [19]. Capillary electrophoresis method has also been reported for determination of LOM in plasma [20].

Recently, Tieli et al. [21] described a photochemical fluorimetry method for LOM in body fluids. Wei et al. [22] developed a spectrofluorimetry method for the assay of LOM in biological samples.

Garcia et al. [23] have used an HPLC method with fluorescence detection for the assay of LOM in plasma samples. Shah et al. [24] have used an HPTLC method for the assay of plasma and urine samples collected for bioequivalence study of lomefloxacin tablets.

In this study, a sensitive spectrofluorimetric method for the assay of LOM in human plasma, urine and eye drops by means of the derivative formed with NBD-CI, which is a specific reagent in the analysis of primary and secondary aliphatic amines. In literature research, LOM, for the first time has been derivatized by a reagent and has been determined using a spectrofluorimetric method.

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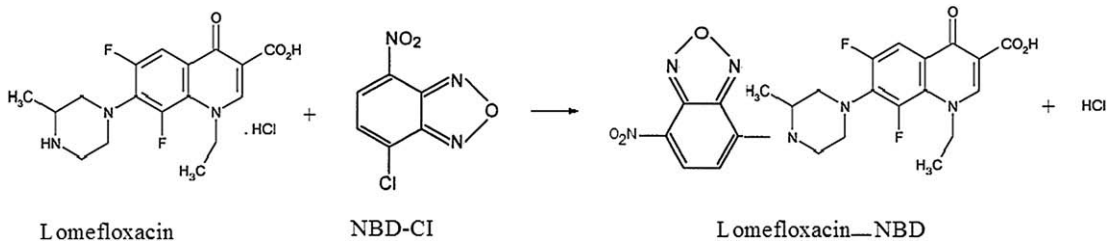

Fig. 1. The reaction between LOM and NBD-CI.

2. Materials and methods

2.1. Chemicals and reagents

Pure powder of LOM was obtained from Sigma (St. Louis, MO, USA). Okacin (3 mg/mL, Novartis, Istanbul, Turkey) eye drops were obtained from pharmacy. NBD-CI was purchased from Merck (Darmstadt, Germany). All chemicals were of analytical grade. Plasma and urine samples were obtained from healthy volunteers. Venous blood samples were collected into ethylenediaminetetraacetic acid (EDTA) and centrifuged (4500 rpm for 15 min). The plasma was immediately collected and stored at −20 °C until it was analyzed as described above.

2.2. Apparatus

The fluorescence intensities were measured using a RF 1501 Model (Shimadzu, Japan) spectrofluorimeter equipped with Xenon lamp and a 10 mm quartz cell. The excitation and emission wavelength bandpasses were both set at 10 nm. All the assays were performed at room temperature excitation and emission wavelengths were set at 433 and 533 nm.

2.3. Solutions

Stock standard LOM solution of 1.0 mg/mL and working standard solution of 5.0 μg/mL were prepared by dissolving them in methanol. The solutions were stable for at least one month if kept in the refrigerator at 4 °C.

Borate buffer solution (pH 8.5) was prepared by adding the appropriate volume of the 0.1 M boric acid to water and adjusting the pH with 0.1 M sodium hydroxide.

NBD-CI was prepared as a 0.2% w/v solution of methanol.

2.4. Procedures

2.4.1. Preparation of calibration graphs

In a 12 mL glass tube, different aliquots (20–2000 μL) ml of the working drug solution 5 μg/mL in methanol were successively added (an aliquot of 20–2000 μL of the LOM then evaporated to dryness at 45 °C), then 100 μL borate buffer solution (pH 8.5) and 100 μL NBD-CI. The sample was vortex-mixed for a few seconds. The solutions were allowed to stay for 15 min in a water bath at 70 °C. Then the mixture was cooled to room temperature and 200 μL of 0.1 N hydrochloric acid solutions were added. The mixture was extracted three times with 1.5 mL ethylacetate. Organic phases were transferred into a 5 mL volumetric flask. The relative fluorescence intensity was measured spectrofluorimetrically at $\lambda_{ex} = 433$ nm and $\lambda_{em} = 533$ nm against blank treated similarly.

2.4.2. Procedure for the plasma and urine

A working standard solution containing 5.0 μg/mL of LOM was prepared. Control samples of plasma and urine were spiked with different quantities of LOM to give a final drug concentration cited in Table 1. To 200 μL of plasma and urine (1:100) samples and 1.0 mL of acetonitrile were added, vortex was mixed for 1–2 min and centrifuged at 4500 rpm for 20 min.

The resulting supernatant was evaporated to dryness under nitrogen at ambient temperature.

Then 100 μL of borate buffer and 100 μL of NBD-CI solution were added and mixed. The mixture was heated in a water bath at 70 °C for 15 min. The tubes were then cooled and 200 μL of 0.1 M HCl solution was added. The solution was then extracted with 3 × 1.5 mL of ethylacetate. Organic layers were transferred into a 5 mL volumetric flask. The relative fluorescence intensity was measured spectrofluorimetrically at $\lambda_{ex} = 433$ nm and $\lambda_{em} = 533$ nm against blank treated similarly.

2.4.3. Procedure for the eye drops

The proposed procedure for the determination of LOM was applied to the direct determination in one pharmaceutical formulation (Okacin® eye drops). A volume of 50 μL of each commercial eye drop (3 mg/mL) was transferred into a volumetric flask and diluted to volume with methanol (6 μg/mL). The LOM content in eye drops was calculated from the regression equation of the calibration curve prepared from **standard LOM** in the concentration range 20–2000 ng/mL.

2.5. Method validation

2.5.1. Linearity

The calibration curves ($I_f = ax + b$) were constructed by the plots of the fluorescence intensities (I_f) of the analyte of the concentrations (x) of the calibration standards. A linear least-square regression analysis was performed for the analyte and the calibration curve was repeated if the correlation coefficient was below 0.999. The concentrations of the analyte in unknown samples were determined by interpolation from the calibration curve.

2.5.2. Accuracy and precision

Accuracy, intra- and inter-day precisions of the method were determined. Five replicate spiked plasma and urine samples were assayed intra- and inter-day at four different concentrations for each analyte. Accuracy was calculated as deviation of the mean from the nominal concentration. Intra- and inter-day precision was expressed as the relative standard deviation of each calculated concentration.

Table 1
Statistical parameters for derivatives of LOM with NBD-CI.

Parameters	Plasma	Urine	Standard solution
Linear range (ng/mL)	12.5–625	15–1500	20–2000
LOD (ng/mL)	4.0	5.0	7.0
LOQ (ng/mL)	12.0	15.0	21.0
Slope (b)	1.217	1.217	5.730
Intercept (a)	1.897	0.111	0.017
Correlation coefficients (r)	0.9997	0.9999	0.9999

Table 2
Recovery of LOM from plasma, urine and standard samples ($n = 5$).

Added amount (ng/mL)	Found (ng/mL)	% Recovery
Plasma		
625	618.25	98.92
500	493.50	98.70
100	97.95	97.95
12.5	12.23	97.84
Urine		
1500	1479.0	98.60
500	493.0	98.60
250	246.30	98.52
15	14.70	98.00
Standard solution		
2000	1998.68	99.93
500	497.67	99.53
250	248.30	99.32
20	19.71	98.55

2.5.3. Recovery

The percentage recovery of LOM in human plasma and urine, based on the average of three replicate measurements, is listed in Table 2.

2.5.4. Specificity

The specificity of the method was investigated by observing any interference encountered from the common eye drop excepients, such as sodium hydroxide, glycerin, disodium ethylenediaminetetraacetic acid.

3. Results and discussion

3.1. Method development

As the derivatization reaction is the typical nucleophilic reaction, the pH of derivatization is an important factor in affecting the derivatization efficiency.

The fluorimetric reaction of LOM with NBD-CI exhibits its highest fluorescence intensity at λ_{ex} of 433 nm and λ_{em} of 533 nm (Fig. 2).

The reaction of NBD-CI with LOM has been shown to be strongly pH-dependent. Thus, the pH of the reaction medium was varied to observe the relative fluorescence of LOM. First, the pH was varied over the whole pH range (7–10) in phosphate and borate buffers. It was noticed that the fluorescence is developed only in alkaline medium and disappears completely in acid media, therefore, the study of the pH was restricted to the range 8–10 using borate buffer. As shown in Fig. 3, the highest fluorescence readings for LOM was obtained at pH 8.5. This is, probably, because the hydrolysis of NBD-CI to NBD-OH was much slower.

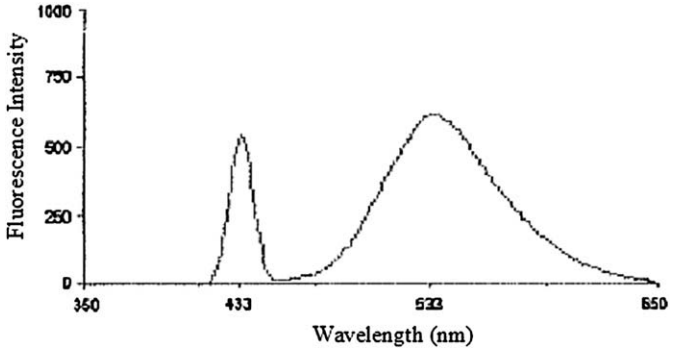

Fig. 2. Fluorescence spectrum of LOM-NBD derivative.

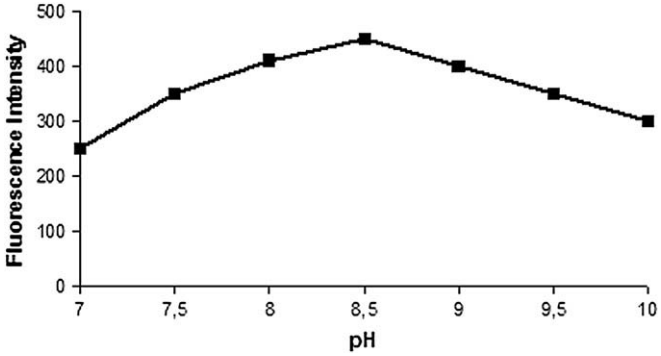

Fig. 3. Effect of pH on fluorescence intensity of lomefloxacin-NBD derivative.

The influence of different heating temperatures and times was studied using a water bath. The effect of the reaction time on the reaction course was studied by measuring the corresponding fluorescence at different temperature for different periods of time. The variation of fluorescence intensity with heating time and heating temperature is shown in Fig. 4. Heating at 70 °C for 15 min gave the highest fluorescence intensity.

In the pretreatment procedure of human blood, sodium EDTA and organic solvent, acetonitrile (1 mL), were used for hemolysis and the following deproteinization, respectively. The recovery of LOM was in proportion to the amount of sodium EDTA. Using the proposed method, 200 μL of plasma and urine was sufficient for the determination of LOM in one analysis. The advantage should allow analyses of small samples.

3.2. Method validation

3.2.1. Linearity

Calibration curves of LOM were linear over the concentration range of 12.5–625 ng/mL for plasma samples and 15–1500 ng/mL for urine samples and 20–2000 ng/mL for standard solution, which is as good as that reported in literature [13,14,16,21].

Correlation coefficients of 0.999 or higher were obtained from the relationship between fluorescence intensities and the corresponding calibration concentration.

3.2.2. Sensitivity

The calculated detection limit (LOD = 3.3SDa/b, where SDa is the standard deviation of intercept and b the slope of the regression line) for standard solution was 7.0 ng/mL. In plasma and urine, the experimental limit of detection was 4.0 and 5.0 ng/mL, respectively. The quantification limit (LOQ = 10 SDa/b, where SDa is the standard

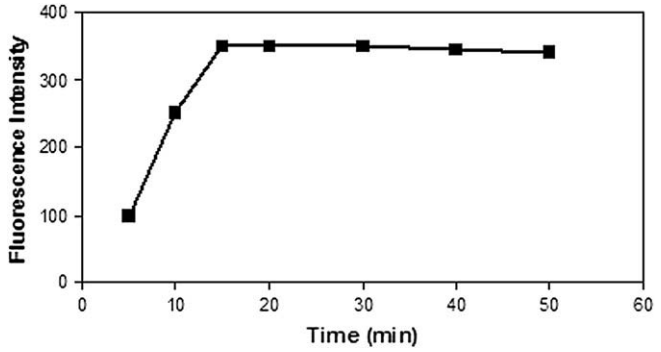

Fig. 4. Effect of time on the reaction of lomefloxacin with NBD-CI (70 °C).

Table 3
Analysis of LOM (Okacin drops 3 mg/mL) by the proposed and reference method [13].

Recovery[a] (%) ± SD		*t* value[b]	*F* value[b]
Proposed method	Reference method [13]		
99.12 ± 0.95	98.95 ± 1.32	1.05	1.93

[a] Mean ± standard deviation.
[b] Theoretical value for six determinations: Student's *t* test, $t = 2.23$; variance ratio test, $F = 5.05$.

deviation of intercept and *b* the slope of the regression line) for standard solution was 21 ng/mL. For the determination of LOM in plasma and urine the LOQ was 12 and 15 ng/mL, respectively (Table 1). The LOD obtained with this method was better than those obtained in other papers [12–14,16,20,23].

3.2.3. Accuracy and precision

The intra- and inter-day precision (relative standard deviation, RSD) in measurement of LOM in plasma ranged from 0.23 to 0.36% and 0.25 to 0.38%, respectively. The intra- and inter-day accuracy (relative mean error, RME) in measurement of LOM in plasma ranged from 0.032 to 2.40% and 0.45 to 2.10%, respectively. The intra-day accuracy ranged from 1.27 to 4.20% for the same concentration range, the inter-day accuracy ranged from 1.60 to 5.80%. The obtained results of intermediate precision also indicated a good method precision, which is as good as that reported in other paper [24].

3.3. Recovery

The mean extraction recoveries of LOM determined at low, medium and high concentrations in plasma and urine samples are shown in Table 2. The RSD of the recoveries were 0.12, 0.17, 0.21 and 0.31% in 625, 500, 100 and 12.5 ng/mL plasma standards, respectively. The RSD of the recoveries were 0.19, 0.22, 0.26 and 0.38% in 1500, 500, 250 and 15 ng/mL urine standards, respectively.

3.4. Specificity

There was no interference from commonly used excipients such as glycerin, sodium hydroxide, disodium ethylenediaminetetraacetic acid.

3.5. Determination of LOM in eye drops

The proposed spectrofluorimetric method was applied for the analysis of Okacid® eye drops. The results were compared statistically with those obtained by the spectrofluorimetric method (reference method [13]) using *t*- and *F*-tests. The results showed that the *t*- and *F*-values were less than the critical value indicating that there was no significant difference between the proposed and reference method (Table 3).

4. Conclusion

A highly selective, sensitive and rapid method for the determination of lomefloxacin in human plasma, urine and eye drops is reported using spectrofluorimetry. The method requires only 200 μL of plasma and urine samples and allows high sample throughput due to a simple sample preparation procedure. The proposed method proved to be simpler, easier and less time consuming comparing to previously reported bioanalytical methods.

Distinct advantages of the present method include the simplicity and rapidity of sample preparation and fluorescence spectra, good sensitivity and the requirement of only common instruments. The assay has been validated, and the results of validation show the method is reproducible and accurate.

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