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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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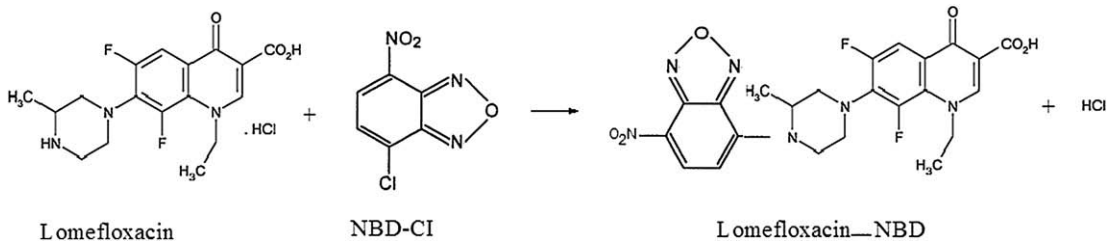

Fig. 1. The reaction between LOM and NBD-CI.

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