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# Determination of lomefloxacin in human plasma by solid-phase extraction and high-performance liquid chromatography with UV detection*

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**Abstract**: A high-performance liquid chromatographic method for the determination of lomefloxacin in human plasma has been developed and validated. A solid-phase extraction procedure was used to isolate lomefloxacin from the biological matrix prior to the quantitative analysis. The compound was separated on a Vydac anion-exchange column using acetonitrile–phosphate buffer (pH 7.0) as the mobile phase and quantified by measuring its UV absorbance at 280 nm. The lower limit of detection for the analyte was 0.05 $\mu g\ ml^{-1}$. Enoxacin was used as the internal standard. The calibration graph of the method was linear from 0.1 to 10 $\mu g\ ml^{-1}$ of lomefloxacin in human plasma. This procedure is suitable for pharmacological and pharmacokinetic studies of lomefloxacin.

**Keywords**: *Lomefloxacin; human plasma; solid-phase extraction; high-performance liquid chromatography.*

## Introduction

Lomefloxacin is a difluorinated quinolone with a broad spectrum of activity against clinically important Gram-positive and Gram-negative bacteria [1–3], including bacteria resistant to beta-lactam antibiotics and aminoglycosides [4]. The antibacterial activity of lomefloxacin is related to its ability to inhibit DNA-gyrase.

Its chemical name is 1-ethyl-6,8-difluoro-1,4-dihydro-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid hydrochloride; its formula is shown in Fig. 1.

Several useful methods for the determination of lomefloxacin in biological fluids are currently available. Some of these are based on microbiological methods [5–7], others on HPLC [8–11]. Most of the HPLC methods use a liquid–liquid extraction procedure for sample enrichment and preparation.

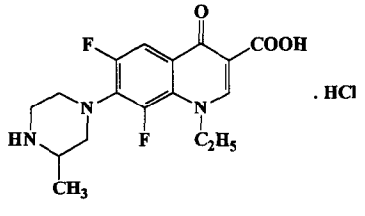


**Figure 1**
Chemical structure of lomefloxacin.

This paper describes the development of a HPLC method with UV detection using a solid-phase extraction (SPE) for the determination of lomefloxacin in human plasma. The method has been applied in a pharmacokinetic study in volunteers.

## Experimental

### *Chemicals and materials*

HPLC-grade acetonitrile and sodium hydroxide (analytical grade) were obtained from Farmitalia-Carlo Erba (Milan, Italy). Enoxacin, the internal standard, was purchased from Sigma Chemical Company (St Louis, MO, USA). Sodium hydrogen phosphate and potassium dihydrogen phosphate (analytical grade) were obtained from Fluka Chemika-BioChemika (Buchs, Switzerland). Lomefloxacin was extracted from 400-mg tablets. Water (HPLC-grade) was obtained by distillation in glass and passage through a Milli-Q water purification system (Millipore Corporation, Bedford, MA, USA). The extraction apparatus was a Supelco solid-phase extraction manifold equipped with a drying attachment

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(Supelco, Bellefonte, PA, USA). Bakerbond $C_{18}$ cartridges (100 mg) were purchased from B.H. Schilling (Società Chimica, Milan, Italy).

*Chromatographic system and conditions*

The chromatographic analysis was performed with a Waters-Millipore (Waters Associates, Milford, MA, USA) model 590 pump and a Lambda Max model 481 LC variable wavelength detector connected to a model HP-3396-II integrator (Hewlett-Packard, Rome, Italy). A model 7125 sample injector Rheodyne (Cotati, CA, USA) equipped with a 20-μl loop was used. The separation was performed on a 250 × 4.6 mm i.d. column packed with 10-μm anion-exchange Vydac (Separations Group, CA, USA) and protected by a 2-cm disposable 40-μm Pelliguard column (Supelco Inc., Bellefonte, CA, USA). The mobile phase, phosphate buffer (pH 7.0; 0.05 M)–acetonitrile (90:10, v/v), was prepared daily, filtered, ultrasonicated before use, and delivered at a flow rate of 1.5 ml $min^{-1}$. Phosphate buffer was filtered through WCN 0.45-μm filters and acetonitrile was filtered through WTP 0.5-μm filters (Whatman Ltd, Maidstone, UK). The detector wavelength was set at 280 nm.

*Standard solutions*

Stock solutions of lomefloxacin and enoxacin (internal standard) were prepared by dissolving 10 mg of each compound in 10 ml of 0.5 M sodium hydroxide. These solutions could be stored at −20°C for over 1 month with no evidence of decomposition. Standard solutions, containing lomefloxacin in the concentration range 0.1–10 μg $ml^{-1}$ and the internal standard at a constant level of 10 μg $ml^{-1}$, were prepared by diluting the stock solutions with control human plasma. A calibration curve was obtained by plotting the peak-height ratio of the drug to internal standard against the drug concentration.

*Biological samples*

Healthy volunteers, from whom informed consent had been obtained, were treated with a single 400-mg tablet of lomefloxacin. Plasma samples were collected at various time intervals afterwards and extracted prior to HPLC analysis.

*Sample preparation*

Heparinized blood samples from various volunteers were centrifuged and the plasma was collected and frozen at −20°C until required for assay. Samples were thawed just before the extraction procedure, thoroughly agitated and centrifuged at 3000*g* for 10 min. The Bakerbond cartridges were placed in a Luer syringe that fitted the top of the Supelco vacuum manifold, which may be loaded with up to 12 cartridges. A vacuum of 250–500 torr was applied to the manifold to carry out the various steps of the extraction. A 1.0-ml rinse of acetonitrile followed by 2 ml of HPLC-grade water served to desorb any organic impurities from the cartridge and to wet the ODS packing prior to introduction of the plasma sample. Then 1.0 ml of plasma added with 100 μl of the internal standard (10 μg $ml^{-1}$ in 0.5 M NaOH) was passed through the cartridge, followed by 1.0 ml of water. The effluent was discarded; 2 ml of acetonitrile was then applied to the cartridge and the eluate collected. This fraction was finally centrifuged (1000*g* for 10 min), filtered through a WTP 0.5-μm filter, evaporated to dryness with a nitrogen stream under vacuum utilizing the Supelco drying attachment. The sample was then reconstituted to 100 μl with 0.02 M sodium hydroxide and mixed with a Vortex agitator. Aliquots of each sample (20 μl) were chromatographed using the apparatus described previously.

*Pharmacokinetic data analysis*

The pharmacokinetic parameters of lomefloxacin in volunteers were estimated using a one-compartment model with first-order elimination and first-order absorption. The parameters for lomefloxacin were obtained using the SIPHAR (Creteil, France) computer program. The area under the curve (AUC) was calculated by the trapezoidal rule to the last sampling time, and extrapolated to infinite time using the terminal elimination rate constant. The total body clearance was estimated as the dose administered divided by the AUC. The volume of distribution was calculated by dividing the total body clearance by the terminal elimination rate constant.

## Results and Discussion

Under the chromatographic conditions described, the lomefloxacin and internal standard peaks are well resolved. Figure 2 shows typical chromatograms of blank plasma spiked

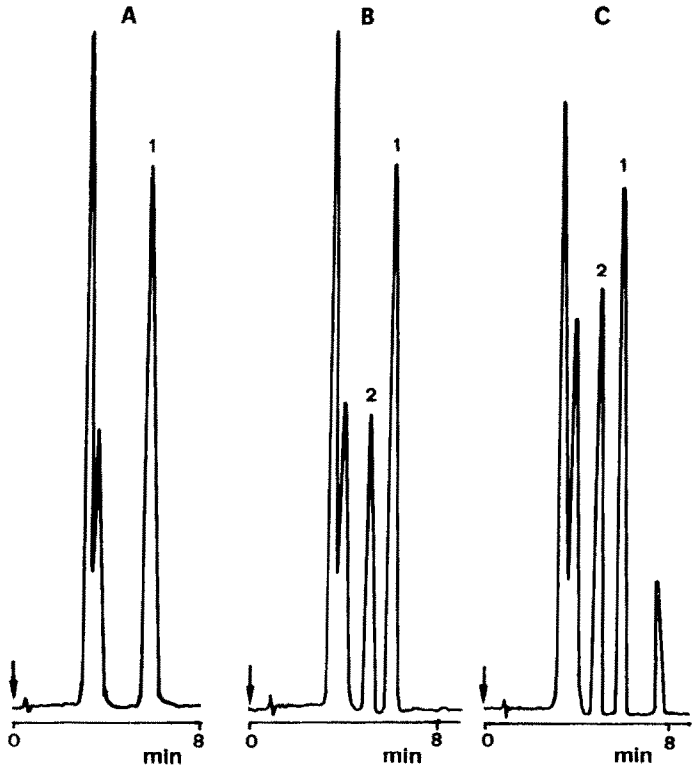


**Figure 2**
Chromatograms of blank plasma spiked with internal standard (10 μg $ml^{-1}$). (A) Blank plasma spiked with internal standard (10 μg $ml^{-1}$) and lomefloxacin (2.0 μg $ml^{-1}$). (B) Plasma of a volunteer treated with 400 mg of lomefloxacin (3.4 μg $ml^{-1}$). (C) 1, internal standard; and 2, lomefloxacin.

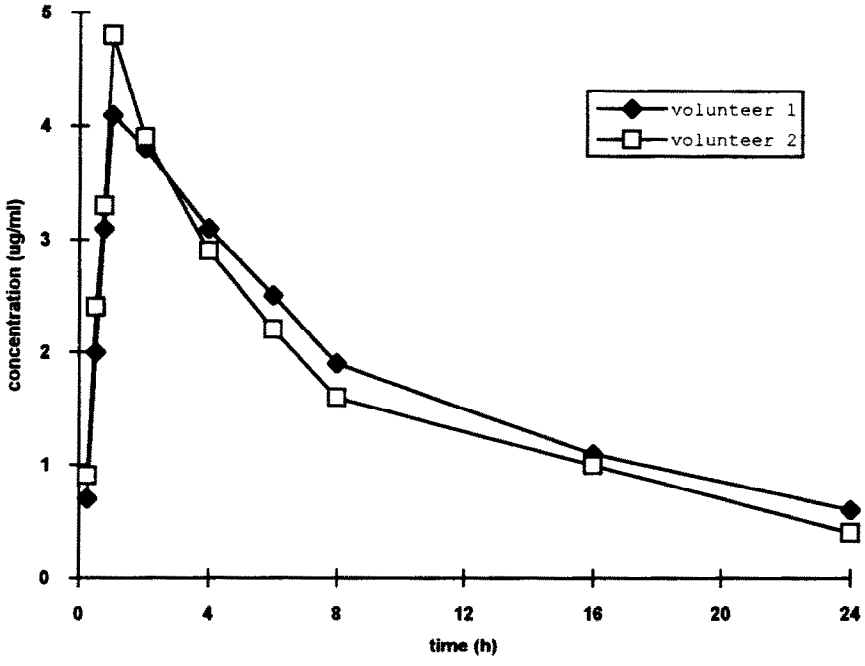


**Figure 3**
Concentration–time curves after oral administration of 400 mg of lomefloxacin to two volunteers.

with the internal standard (A), blank plasma spiked with lomefloxacin and internal standard (B), and plasma of a volunteer treated with lomefloxacin (C). No endogenous component or metabolite was observed near the retention times corresponding to lomefloxacin or the internal standard. The retention times for lomefloxacin and the internal standard were 4.4 and 5.6 min, respectively. The assay was validated by analysing seven lomefloxacin standards. Each data set represents the mean of a minimum number of five replicates. The equation obtained by regression analysis of data for the above standard solutions was $y = -2.9 \times 10^4 + 4.6 \times 10^5 x$ (correlation coefficient $r = 0.999$), where $y$ = peak-height ratio and $x$ = lomefloxacin concentration (μg $ml^{-1}$). Using a signal-to-noise ratio of 3, the lower detection limit of lomefloxacin in human plasma was 0.05 μg $ml^{-1}$. The precision (RSD) for the lomefloxacin calibration standard ranged from 2.5 to 4.1% with relative errors of 2.8–3.7%. Based on these results, the calibration graph for the method is linear from 0.1 to 10 μg $ml^{-1}$. The lomefloxacin extraction efficiency, determined by comparing peak-heights of plasma extracts versus the corresponding aqueous standards, was approximately 85%. Figure 3 shows the semilogarithmic plot of lomefloxacin concentration in plasma after a single dose (400 mg) of drug. Table 1 lists the preliminary pharmacokinetic data from two volunteers.

**Table 1**
Preliminary pharmacokinetic data for lomefloxacin

| Parameter | Volunteer 1 | Volunteer 2 |
|---|---|---|
| $c_{max}$ (mg $l^{-1}$) | 4.1 | 4.8 |
| $T_{max}$ (h) | 1.0 | 1.0 |
| $t_{½}$ elimination (h) | 10.9 | 7.9 |
| $AUC_{0-\infty}$ (mg h $l^{-1}$) | 51.1 | 42.4 |
| $V_d$ (l) | 123.2 | 108.6 |
| $Cl$ (ml $min^{-1}$) | 7.8 | 9.4 |

This simple HPLC method which uses a simple solid-phase extraction technique should be of value for monitoring the lomefloxacin concentration in plasma in patients, for assessing patient compliance in following prescribed lomefloxacin regimens and for examining the relationship between lomefloxacin concentration in plasma and its antimicrobial effect. The HPLC method is rapid, sensitive, and yields accurate and precise results. Therefore, the procedure is suitable for examining a large number of samples.

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