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Short Communication

Analytical procedure for the determination of rufloxacin, a new pyridobenzothiazine, in human serum and urine by high-performance liquid chromatography

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ABSTRACT

A high-performance liquid chromatographic method for the quantification of rufloxacin in human serum and urine has been developed and validated. The compounds, rufloxacin and internal standard, are extracted from buffered serum and urine using dichloromethane. They are then separated on an anion-exchange column using 0.05 *M* phosphate buffer–acetonitrile (80:20, v/v). The eluate is quantified by measuring the ultraviolet absorbance at 296 nm. The lower limit of detection for the analyte is 0.1 μg/ml in serum and 0.05 μg/ml in urine. The method is linear from 0.3 to 10 μg/ml for serum and 0.1 to 10 μg/ml for urine. The method has been applied in a pharmacokinetic study in volunteers.

INTRODUCTION

A new potent, orally active antimicrobial agent, rufloxacin or 9-fluoro-10-(4-methyl-1-piperazinyl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-[1,4]-benzothiazine-6-carboxylic acid hydrochloride (Fig. 1), is currently under preclinical and clinical investigation [1–3]. In support of these studies, a highly

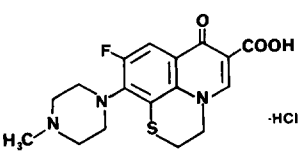

Fig. 1. Molecular structure of rufloxacin.

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specific and sensitive method for the determination of the drug in biological fluids was required. This paper decribes a high-performance liquid chromatographic (HPLC) assay for analysis of rufloxacin in human serum and urine, involving liquid–liquid extraction for sample purification. The method has been applied for pharmacokinetic studies.

EXPERIMENTAL

Chemicals

HPLC-grade acetonitrile, dichloromethane and all other analytical-grade reagents (sodium hydroxide, sodium hydrogenphosphate and potassium dihydrogenphosphate) were obtained from Farmitalia Carlo Erba (Milan, Italy). HPLC-grade water was filtered through WCN 0.45-μm filters, and acetonitrile and dichloromethane were filtered through WTP 0.5-μm filters (Whatman, Maidstone, U.K.). Rufloxacin was supplied by Mediolanum Farmaceutici (Milan, Italy). The internal standard (I.S.), ofloxacin, was obtained from Sigma-Tau (Pomezia, Italy).

Standard solutions

Stock solutions (10 mg/ml) of rufloxacin and the I.S. were prepared in 0.5 *M* sodium hydroxide. Standard solutions containing 0.1–10 μg/ml were prepared by diluting the stock solution with 0.5 *M* sodium hydroxide. The I.S. solution was diluted with 0.5 *M* sodium hydroxide to a final concentration of 5.0 μg/ml. These solutions could be stored for over one month with no evidence of decomposition. Human serum and urine standards for calibration curves were prepared by diluting the stock solutions with serum and urine, respectively.

Chromatographic system and conditions

The liquid chromatographic system consisted of an M6000A pump, a U6K injector and a Lambda Max Model 481 LC–UV detector (Waters Assoc., Milford, MA, U.S.A.) connected to a Model CC-12 computing integrator (Perkin-Elmer, Norwalk, CT, U.S.A.). The analysis was performed using a Vydac anion-exchange column (25 cm × 4.6 mm I.D., 10 μm particle size) (Separations Group, Hesperia, CA, U.S.A.) connected to a disposable AXGU anion-exchange precolumn (20 mm × 4.6 mm I.D., 10 μm particle size) (Rainin Instruments, Woburn, CA, U.S.A.). The mobile phase, 0.05 *M* phosphate buffer (pH 7)–acetonitrile (80:20, v/v), was prepared daily and delivered at a flow-rate of 1.8 ml/min.

Sample preparation

Serum and urine samples were stored frozen (−20°C) until required for assay. Samples were thawed just before extraction.

Serum (1 ml) samples were mixed with 1.0 ml of 0.1 *M* phosphate buffer (pH 7) and 100 μl of the I.S. (5 μg/ml) in a 10-ml plastic centrifuge tube (Falcon Plastic,

Oxnard, CA, U.S.A.). Dichloromethane (2.5 ml) was added to all the samples, and the tubes were shaken for 10 min. Separation of the two phases was achieved by centrifugation at 1500 *g* for 10 min, and *ca.* 2 ml of the organic phase were transferred to a second tube. Fresh dichloromethane (2.5 ml) was added to the first tube, and the extraction procedure was repeated twice. The organic phases collected from the three extractions of the same sample were pooled and evaporated to dryness with nitrogen under vacuum. The samples were then reconstituted with 200 μl of mobile phase and mixed on a vortex agitator. Aliquots of each sample (20 μl) were chromatographed using the same mobile phase. The column eluate was monitored at 296 nm.

Urine

The extraction procedure was identical with that described for serum, except that the amount of the I.S. solution added was 150 μl.

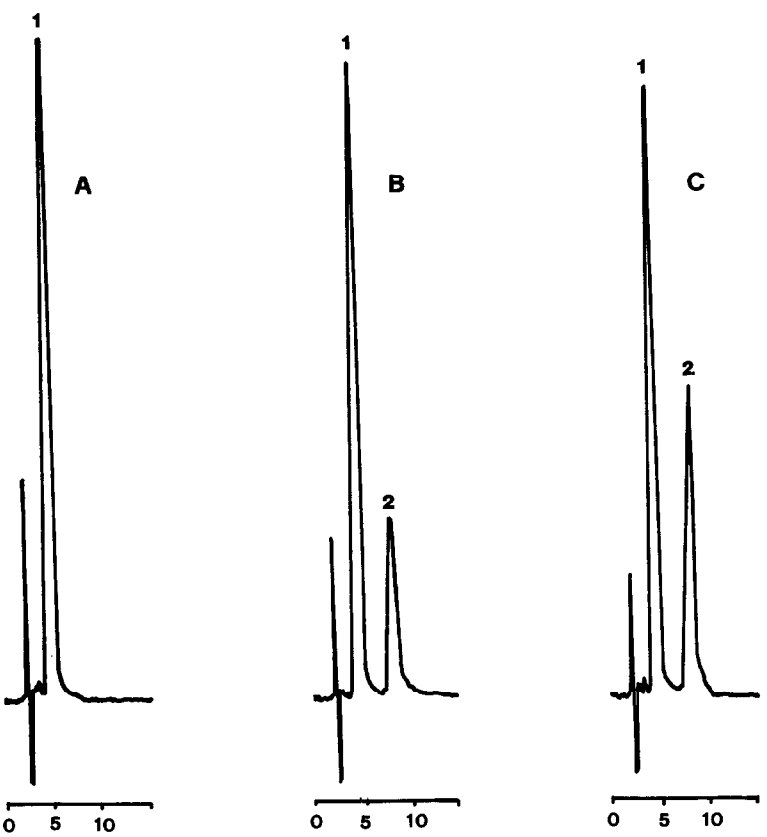

Fig. 2. Chromatograms from human serum extracts. (A) A blank serum spiked with the I.S. (2.5 μg/ml); (B) a blank serum spiked with 2.5 μg/ml I.S. (1) and 1.5 μg/ml rufloxacin (2); (C) a serum sample collected 3 h after a dose of 400 mg of rufloxacin. All concentrations refer to sample extracts. Horizontal axis, retention time (min).

Data analysis

The pharmacokinetic parameters of rufloxacin in volunteers were estimated using a one-compartment model with first-order elimination and first-order absorption. The parameters for rufloxacin were obtained using the SIPHAR (Creteil, France) computer program. The area under 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 esti-

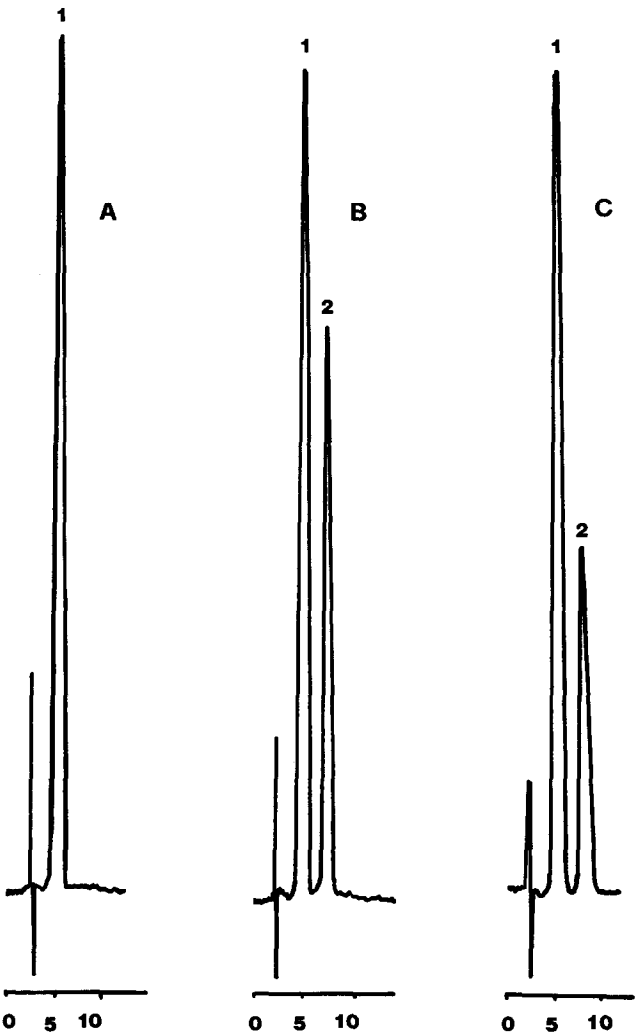

Fig. 3. Chromatograms from human urine extracts. (A) A blank urine spiked with the I.S. (3.75 μg/ml); (B) a blank urine spiked with 3.75 μg/ml I.S. (1) and 3.0 μg/ml rufloxacin (2); (C) a urine sample collected 2.5 h after a dose of 400 mg of rufloxacin (2.7 μg/ml). All concentrations refer to sample extracts. Horizontal axis retention time (min).

mated 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

Fig. 2 illustrates typical chromatograms from blank human serum spiked with the I.S. (A), serum spiked with the I.S. and rufloxacin (B) and serum of a volunteer treated with rufloxacin (C). Fig. 3 illustrates chromatograms from blank urine spiked with the I.S. (A), urine spiked with the I.S. and rufloxacin (B) and urine of a volunteer treated with rufloxacin (C). No endogenous components or metabolites were observed near the retention time corresponding to rufloxacin or the I.S. The retention times for rufloxacin and the I.S. were 7.5 and 5.6 min, respectively. The assay was validated by analysing seven rufloxacin standards (serum and urine) five-fold for each concentration. The best-fit line were determined daily by least-squares regression analysis using a weighting factor of $1/(\text{concentration})^2$ [4]. The results of a typical regression line were: for serum, peak-height ratio $= 0.674x + 0.003$ ($r = 0.999$); for urine, peak-height ratio $= 0.564x + 0.004$ ($r = 0.999$); where x is the rufloxacin concentration. The mean slope and

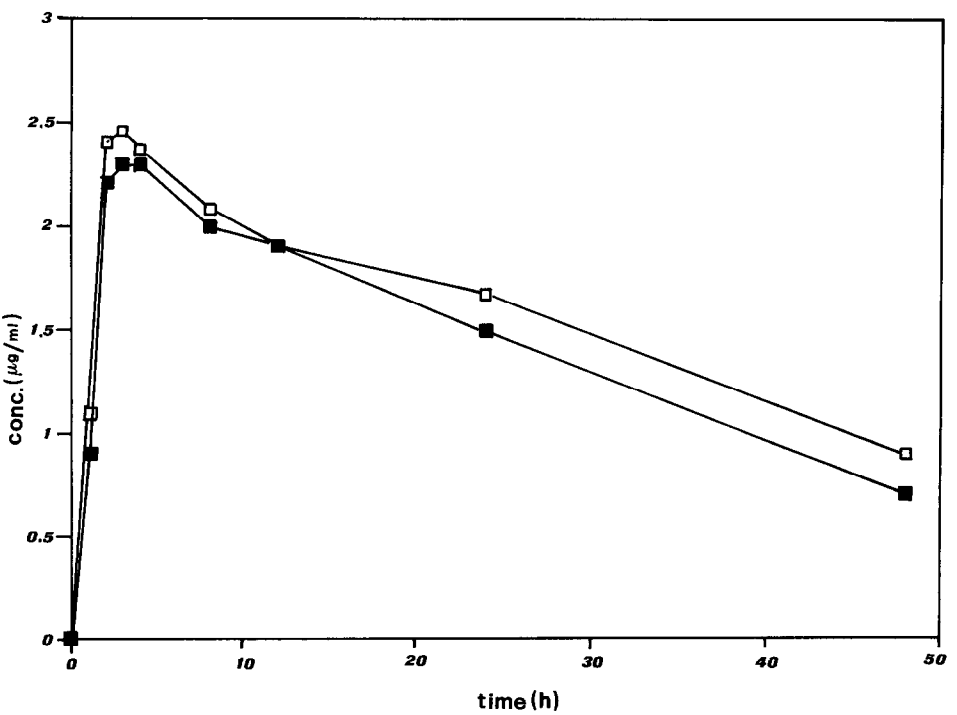

Fig. 4. Concentration–time curves after oral administration of 400 mg of rufloxacin to two volunteers.

TABLE I

PRELIMINARY PHARMACOKINETIC DATA FOR RUFLOXACIN

Parameter	Volunteer 1	Volunteer 2
C_{max} (mg/l)	2.3	2.5
T_{max} (h)	4.2	3.6
$t_{1/2}$ elimination (h)	40.2	38.6
$AUC_{0-\infty}$ (mg h/l)	152.0	184.0
V_d (l)	160.0	148.0
Cl (ml/min)	43.8	36.2

intercept data from a three-day period were 0.674 (± 0.022) and 0.0027 (± 0.002) for serum and 0.564 (± 0.019) and 0.004 (± 0.001) for urine.

The accuracy and precision of the calibration curves were determined from the variation of the standards from the regression line. The precision for the serum calibration standards ranged from 2.6 to 5.4% (R.S.D.) with relative errors of 2.9–4.0%. The precision for urine calibration standards ranged from 1.5 to 3.4% with relative errors of 2.2–3.2%. Based on these results, the method is linear from 0.3 to 10 μg/ml for serum and 0.1 to 10 μg/ml for urine. The detection limits for serum and urine were 0.1 and 0.05 μg/ml, respectively. The extraction efficiency of the I.S. was 97.4% for the concentration used for serum sample.

Fig. 4 shows the semilogarithmic plot of rufloxacin serum concentration after a single dose (400 mg) of drug. Table I lists the preliminary pharmacokinetic data from two volunteers.

The method has been used extensively for measuring rufloxacin in patients' serum and urine, for assessing patient compliance in adhering to prescribed rufloxacin regimens, and for examining the relationship between rufloxacin concentration in serum and antimicrobial effects in clinical trials.

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REFERENCES

1 G. C. Crumplin, J. M. Midgley and J. T. Smith, *Top. Antibiot. Chem.*, 8 (1980) 9.
2 G. Segre, D. Cerretani, D. Cerri and L. Moltoni, *Drug Exp. Clin. Res.*, 14 (1988) 747.
3 V. Cecchetti, S. Dominici, A. Fravolini and F. Schiaffella, *Eur. J. Med. Chem.*, 19 (1984) 29.
4 P. R. Bevington, *Data Reduction and Error Analysis for the Physical Sciences*, McGraw Hill, New York, 1969, p. 105.