

Determination of ciprofloxacin in plasma and urine by HPLC with ultraviolet detection

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A simple, sensitive isocratic method for the detection and quantification of ciprofloxacin in plasma and urine has been developed. The assay consisted of reversed-phase HPLC with ultraviolet detection. Plasma proteins were removed by a fast and efficient procedure. For the urine samples, the only required sample preparation was dilution. Separation was achieved on a C₁₈ reversed-phase column. The quantification limit was 0.01 mg/L in plasma and 0.5 mg/L in urine. This method was sufficiently sensitive for pharmacokinetic studies.

Ciprofloxacin, 1-cyclopropyl-6 fluoro-1,4-dihydro-4-oxo-7-(1 piperazinyl)-3 quinolone carboxylic acid, is a relatively new quinolone carboxylic acid derivative with an extensive antibacterial spectrum (1). Several HPLC methods have been reported for the analysis of ciprofloxacin in biological fluids (2-9). Some of these methods use ultraviolet (UV) detection (2-5), whereas others use expensive fluorescence detection (6-9), a method that is not commonly available in every laboratory. Most of these methods do not include an internal standard (IS), which is crucial because the sample preparation methods involve more than one extraction step (2, 3, 5, 7).

We describe a rapid, accurate, and specific method that is not completely different from those described earlier but that combines a simple procedure of sample preparation, an isocratic eluent of very simple composition, and UV detection. The method has been used in pharmacokinetic studies in healthy volunteers (unpublished results).

Materials and Methods

CHEMICALS

All chemicals were analytical grade. Ciprofloxacin was obtained from Bayer Pharmaceuticals. Lomefloxacin,

used as an IS, was obtained from Shionogi Pharmaceutical. All solvents were HPLC grade. Acetonitrile, acetic acid, and methanol were from Wako Pure Chemical Industries.

INSTRUMENTATION AND CHROMATOGRAPHY

Chromatography was performed with a high-performance liquid chromatograph LC-6A (Shimadzu, Analytical Instruments Division) and an UV-8010 spectrophotometer (TOSOH, Scientific Instrument Division) set at 280 nm. The output of the detector was monitored with a chromatocorder 12 (SIC System Instruments). A stainless steel column packed with YMC pack A-312 (octadecylsilane; bead size, 5 μ m; 150 mm \times 6 mm i.d., Yamamura Chemical Laboratory) was used. The column was protected with a pre-column (Guard-PakTM) filled with a μ BondapakTM C₁₈ cartridge (Merck kGaA).

STOCK SOLUTIONS AND STANDARDS

Ciprofloxacin and lomefloxacin (IS) were made up as 1 g/L stock solutions in methanol and distilled water (1:10, by volume). Ciprofloxacin was diluted with distilled water to make additional working stocks of 10 mg/L for the plasma assay. Lomefloxacin was diluted with mobile phase to make a single working IS stock solution of 5 mg/L. Plasma calibrators (0.01 to 2.5 mg/L) for the calibration curve were prepared in drug-free control plasma; urine calibrators (0.5 to 500 mg/L) were prepared in drug-free control urine. The working solutions were used to supplement the drug-free matrices.

SAMPLE PREPARATION

Plasma. In a 5-mL Eppendorf vessel, 2 mL of acetonitrile was added to 1 mL of plasma, plasma blank, or plasma calibrator. The mixture was agitated for 30 s with a mechanical shaker and centrifuged for 5 min at 10 000g. A 2.5-mL volume of clear supernatant was transferred into a glass tube (75 \times 12 mm). The liquid phase was evaporated to dryness under nitrogen in a dry block bath at 50 $^{\circ}$ C. The residue was then reconstituted in 50 μ L of IS and 200 μ L

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of 50 mL/L acetic acid. The final solution was transferred into the automatic sampler vessels, and 20 μ L was injected into the HPLC system.

Urine. The urine samples were diluted 1:10 (by volume) with distilled water. In a microcentrifuge tube, 30 μ L of the working solution of lomefloxacin was added to 50 μ L of the diluted urine. The mixture was vortex-mixed, and 20 μ L was injected directly into the HPLC system.

CHROMATOGRAPHIC CONDITIONS

Separation of ciprofloxacin was achieved at 50 °C, using an isocratic mode. The mobile phase consisted of a mixture of 900 mL of 50 mL/L acetic acid, 50 mL of acetonitrile, and 50 mL of methanol per liter. The UV detector was set at 280 nm, and the sensitivity was set at 0.02 absorbance units full scale. The chart speed was 2 cm/min. The flow rate was 1 mL/min.

Results

Typical chromatograms are shown in Fig. 1. The retention times for ciprofloxacin and lomefloxacin were ~12 and 16 min, respectively. No interference from endogenous components or ciprofloxacin metabolites was observed in plasma or urine from volunteers. The baseline was relatively free from drift. Validation of the method consisted of two distinct phases: (a) the development phase, in which the assay was defined, and (b) the application phase, in which the method was applied to the actual analysis of samples from a single 200-mg oral-dose ciprofloxacin pharmacokinetic study. Six concentrations (excluding blank values) defined the calibration curves. The linearity of the calibration curves was verified from 0.01 to 2.5 mg/L for ciprofloxacin in plasma and from 0.5 to 500 mg/L for ciprofloxacin in urine. The correlation coefficients between the peak-area ratio of the drug to the IS and to concentration were >0.999. The limit of quantitation was 0.01 mg/L for plasma and 0.5 mg/L for urine. The relation between response and concentration was demonstrated to be continuous and reproducible. A calibration curve was generated for each analytical run and was used to calculate the concentration of ciprofloxacin in the unknown samples assayed with that analytical run. The calibration curves covered the entire range of expected concentrations. The specificity of the assay was established with nine independent sources of the same matrix. The accuracy and precision were determined with five determinations per concentration. Within- and between-day accuracy and precision values are given in Tables 1 and 2. Recovery of ciprofloxacin from plasma was 96.1%, 98.8%, and 98.4% at 0.01, 0.5, and 2.5 mg/L, respectively; the recovery from urine was 95.2%, 99.9%, and 99.96% for ciprofloxacin at 0.5, 50, and 500 mg/L, respectively. Recovery for the IS was 90.8% at 5 mg/L from plasma and 92.5% at 50 mg/L from urine.

Discussion

The present study describes a highly sensitive, accurate, and reproducible HPLC method for the determination of ciprofloxacin in human plasma and urine. This method has several advantages over the previously reported methods (2–9). Sample preparation is simpler, and the chromatographic column and IS used are available commercially. The procedure for sample preparation is rapid and inexpensive. Because the IS and samples containing unknown concentrations are handled simultaneously, errors of manipulation are taken into account. The very low quantification limit obtained with a UV detector allowed us to avoid using fluorometric detection, which requires more expensive equipment, and makes this method particularly useful for pharmacokinetic studies. On the other hand, UV detectors give more reproducible and stable responses than fluorometric detectors (10).

Another advantage of our method is the use of an isocratic mobile phase of very simple composition, which gives the column a longer lifetime and lowers the risk of protein precipitates associated with the use of tetrabutylammonium salts in the solvent system (11). Between 900 and 1200 separations, depending on the dilution and matrix, can be performed without loss of separation capacity.

Our method has been used extensively for measuring ciprofloxacin in the plasma and urine of healthy volunteers in a single-dose pharmacokinetic study. The concentration-time profile for ciprofloxacin in plasma after administration of a single 200-mg oral dose to a healthy volunteer is shown in Fig. 2. The increased sensitivity of the present assay should prove advantageous and will also be useful in pharmacokinetic studies involving administration of single doses of ciprofloxacin to humans and animals, in which concentrations of the drug are expected to be much lower than those observed at steady-state.

Small concentrations of four ciprofloxacin metabolites have been reported: desethyleneciprofloxacin, sulfociprofloxacin, oxociprofloxacin, and formylciprofloxacin (1). All of the metabolites have some antibacterial activity, but the activity is less than that of ciprofloxacin. Although formylciprofloxacin is the most active, it is only a very minor metabolite of ciprofloxacin. Only a few reports have been published for the determination of ciprofloxacin and its metabolites in human specimens (11–14). With the exception of the study by H. Scholl et al. (11), current methods have been unable to determine formylciprofloxacin. To separate the metabolites, some of the present methods include the use of two different mobile phases (11–13) or a gradient system (14). The only HPLC method in which all four known metabolites can be determined with the same sensitivity and selectivity as ciprofloxacin requires an additional postcolumn derivatization by successive thermolysis and photolysis (11). The additional procedure converts the metabolites into intensely fluorescent secondary products that are easily distinguished

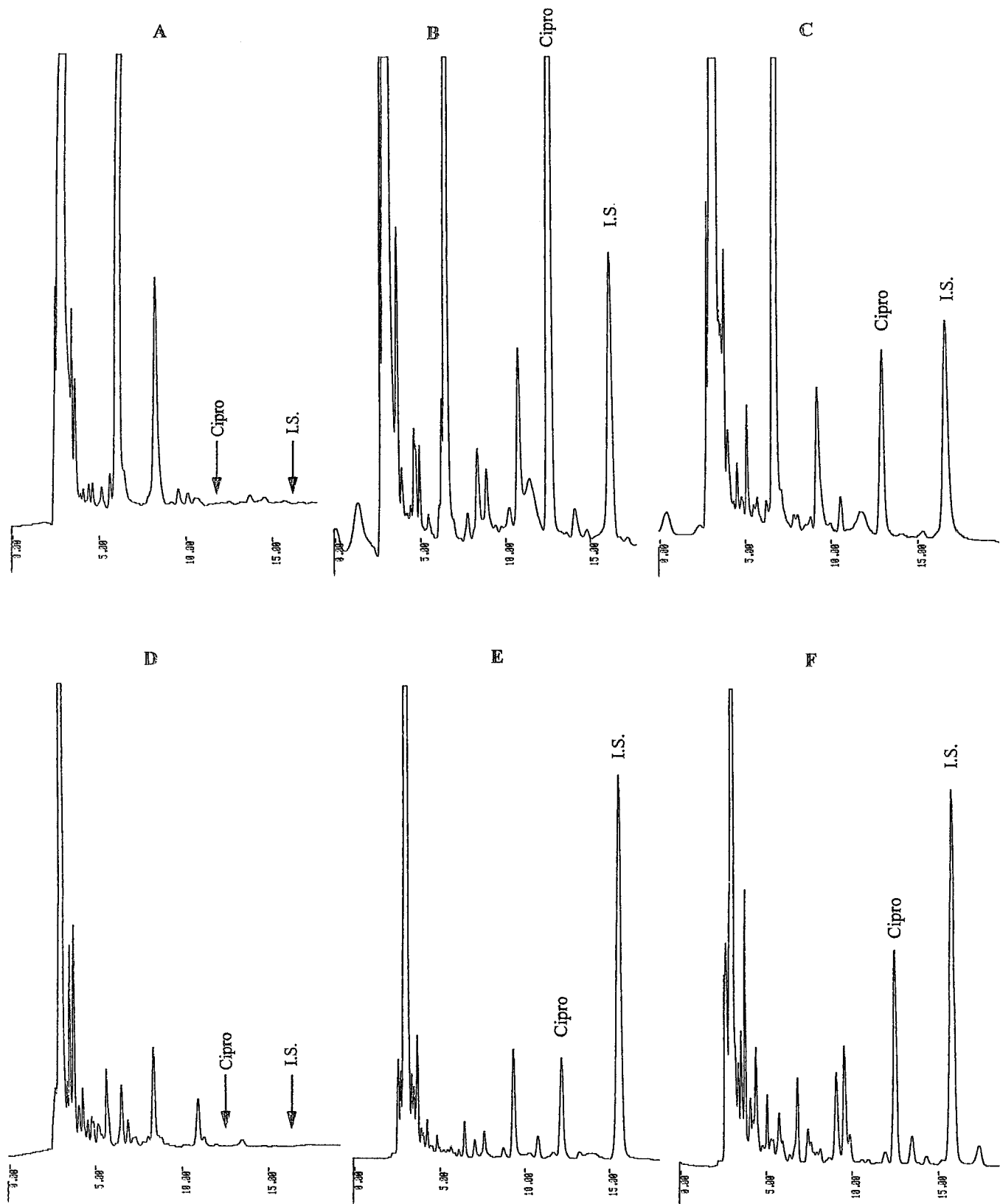


Fig. 1. Representative chromatograms of (A) plasma blank, (B) plasma standard (0.5 mg/L), (C) plasma sample collected 6 h after a 200-mg oral dose of ciprofloxacin, (D) urine blank, (E) urine standard (5 mg/L), and (F) urine sample collected 24 h after a 200-mg oral dose of ciprofloxacin. Peaks: *Cipro*, ciprofloxacin; *I.S.*, lomefloxacin.

Table 1. Within-day precision of ciprofloxacin determination in plasma and urine.^a

Theoretical concentration, mg/L	Concentration found, mg/L	CV, %
Plasma		
0.01	0.0086 ± 0.00023 ^b	1.04
0.5	0.48 ± 0.013	0.50
2.5	2.53 ± 0.052	2.06
Urine		
0.5	0.50 ± 0.005	1.05
50.0	49.84 ± 0.33	0.66
500.0	499.8 ± 1.04	0.21

^a Analysis was done five times in plasma and urine. Analysis condition as in Fig. 1. Quantitation was performed by a weighted linear calibration curve of peak-area ratios of ciprofloxacin/lomefloxacin vs concentration over the range of 0.01–2.5 mg/L of plasma and 0.05–500 mg/L of urine.

^b Mean ± SD.

from the matrix components and quantified. In our method, which has a comparable sensitivity to those mentioned above (13,14), no detectable quantities of metabolites appeared in the plasma samples obtained from healthy volunteers after a single 200-mg oral dose of ciprofloxacin. On the other hand, the urine samples appeared to contain detectable quantities of oxociprofloxacin, which is the major urinary metabolite. However, this method has not been evaluated for metabolites. Because of the very low concentrations of ciprofloxacin metabolites found in human serum and urine and because we wanted to optimize and simplify the chromatographic procedure, we developed the method reported here to measure only the parent drug in human serum and urine.

Table 2. Between-day precision of ciprofloxacin determination in plasma and urine.^a

Theoretical concentration, mg/L	Concentration found, mg/L	CV, %
Plasma		
0.01	0.0082 ± 0.0001 ^b	0.78
0.05	0.0575 ± 0.0003	0.44
0.1	0.1054 ± 0.0006	0.61
0.5	0.4653 ± 0.0013	0.28
1.0	1.0295 ± 0.0049	0.47
2.5	2.4999 ± 0.0037	0.15
Urine		
0.5	0.5307 ± 0.0139	2.62
5.0	4.8148 ± 0.1334	2.77
10.0	9.8226 ± 0.0197	0.20
50.0	50.1978 ± 0.020	0.04
100.0	99.1347 ± 0.0591	0.06
500.0	501.0248 ± 0.1015	0.02

^a Analysis was done five times in plasma and urine. Analysis condition as in Fig. 1. Quantitation was performed by a weighted linear calibration curve of peak area ratios of ciprofloxacin/lomefloxacin vs concentration over the range of 0.01–2.5 mg/L of plasma and 0.05–500 mg/L of urine.

^b Mean ± SD.

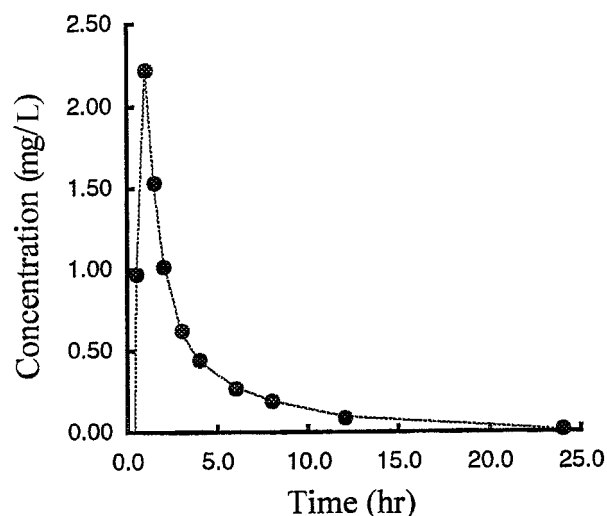


Fig. 2. Plasma concentrations of ciprofloxacin vs time after administration of a 200-mg oral dose to a healthy volunteer.

The plasma concentrations of the drug were measured using the method described in *Materials and Methods*.

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