Journal of Chromatography, 563 (1991) 211–215
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5621

Note

Determination of ciprofloxacin in human serum by liquid chromatography

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(First received July 10th, 1990; revised manuscript received August 28th, 1990)

Ciprofloxacin and norfloxacin are a new generation of quinolones that show greater potency, low toxicity and a broader antibacterial spectrum [1,2]. The main difference between ciprofloxacin and other antibiotics is that it can be administered both parenterally and orally [3]. Because of this, ciprofloxacin represents and alternative to the systemic broad-spectrum antibiotics. Prospects for the therapeutic use of ciprofloxacin are good, but more data on clinical assays are necessary.

Although liquid chromatographic (LC) assays have been developed for ciprofloxacin [4,5], and ciprofloxacin and its metabolites [6,7], all these methods involve conventional columns (100–200 mm length) and some of them require expensive fluorometric detectors [5–7].

This paper describes an isocratic reversed-phase high-performance liquid chromatographic (HPLC) method with a 30-mm-long column which results in a reduction of the chromatographic analysis time. The UV detection provides adequate sensitivity for routine use. The procedure was evaluated in a clinical setting to determine its usefulness in monitoring serum levels in patients receiving ciprofloxacin treatment.

EXPERIMENTAL

Standards and reagents

Ciprofloxacin (844 μg/mg) and eprofloxacin were kindly supplied by Bayer, Pharma Research Center (Wuppertal, F.R.G.). Acetonitrile was purchased from Scharlau (Barcelona, Spain). Potassium dihydrogenphosphate (KH_2PO_4), tetrabutylammonium bromide (TBA), methanol and water were obtained from Merck (Darmstadt, F.R.G.). Methylene chloride and 85% phosphoric acid were from Carlo-Erba (Milan, Italy). All chromatographic solvents were of HPLC grade, and all other chemicals were of analytical grade.

0378-4347/91/$03.50

Apparatus

The LC analyses were performed on a Perkin-Elmer (Norwalk, CT, U.S.A.) Model Series 4 equipped with a Rheodyne syringe-loading sample inject Model 7105 with a 20-μl loop, a Perkin-Elmer LC-85 B UV absorption variable-wavelength detector and a Perkin-Elmer Sigma-15 integrator.

Chromatographic conditions

A column (30 mm × 4.6 mm I.D.) packed with Nucleosil C_{18}, particle size 3 μm, was used. The mobile phase was 0.05 *M* KH_2PO_4 (pH 3.0)–water–acetonitrile–0.1 *M* TBA (pH 3.0) (60:30:5:5, v/v). The flow-rate was 2.0 ml/min, and the detector wavelength was set at 277 nm.

Drug reference standards

A stock solution of ciprofloxacin (500 mg/l) in methanol and an internal standard (I.S.) solution of eprofloxacin (20 mg/l) in KH_2PO_4 (pH 3.0) were prepared. All the solutions were stable for several weeks when stored at −20°C.

Sample preparation

A modification of the method described by Jehl *et al.* [8] was used. In a 10-ml glass screw-capped culture tube, 1 ml of serum, 100 μl of the I.S. and 3.5 ml of methylene chloride were added. The contents were gently shaken for 10 min by rotation on a mixer (Coulter Electronics, North Well Drives, U.K.) and then centrifuged for 10 min at 1000 *g*. The upper aqueous layer was removed by aspiration. The organic phase was transferred to a second culture tube and evaporated under nitrogen at room temperature. To the dry residue, 1 ml of methylene chloride and 150 μl of phosphoric acid (pH 2.0) were added and mixed for 30 min. The tube was centrifuged at 1000 *g* for 15 min, and 20 μl of the upper aqueous layer were directly injected in duplicate onto the column.

Calibration graphs

Working standard solutions containing 0.25–5 mg/l ciprofloxacin were prepared in drug-free human serum by dilution of the stock solution. Extraction was performed as described above. The ratio of the peak area of the drug to that of the I.S. was plotted against the concentration of the drug.

Patients

Twenty-four samples were obtained from seventeen patients treated with ciprofloxacin, 250 mg intravenously every 12 h ($n = 22$) or 750 mg orally every 12 h ($n = 2$). Serum samples were taken 60 min after administration of the drug (peak level) and immediately before subsequent dosing (trough level). Serum samples were stored at −20°C until assayed.

Analytical recovery and precision

The extraction efficiency was determined by comparison of the peak area of ciprofloxacin from extracts of standard solutions with those of non-extracted aqueous calibration standards.

Within-day variation was determined by assaying each serum sample of a known concentration (0.5, 2 and 5 mg/l) ten times in a single day. Day-to-day variation was calculated by assaying serum samples once a day for ten days.

RESULTS AND DISCUSSION

Fig. 1 shows a typical chromatogram obtained with this procedure. The retention times for ciprofloxacin and eprofloxacin were 2.9 and 4.2 min, respectively.

The linearity was verified from 0.1 to 40 mg/l. The limit of detection at a signal-to-noise ratio of 5 was 0.1 mg/l. The regression equation and the correlation coefficient between the peak-area ratio of the drug to the I.S. (y) and the

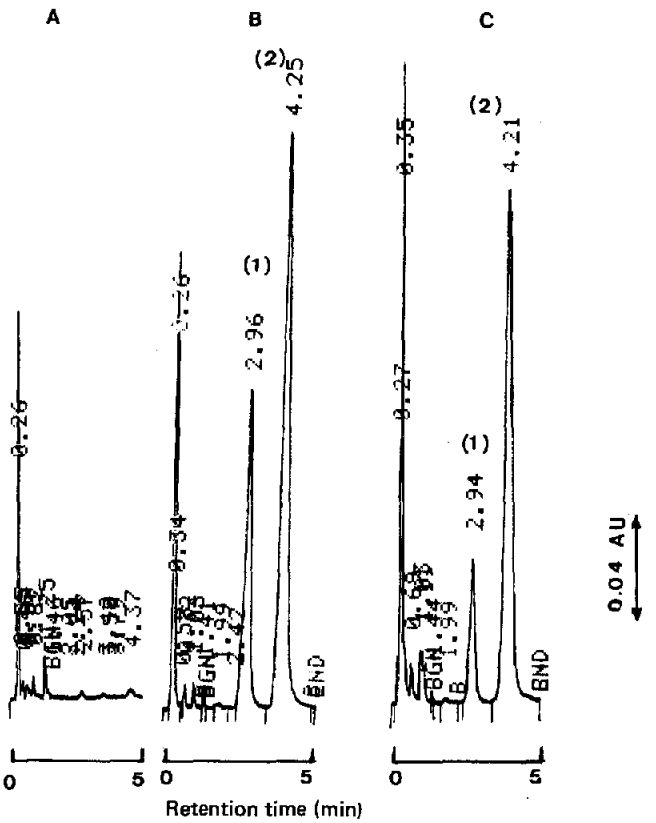

Fig. 1. Sample chromatograms from drug-free serum (A), and serum from patients containing 2.3 mg/l (B) and 1.1 mg/l (C) ciprofloxacin. Peaks: 1 = ciprofloxacin; 2 = I.S.

TABLE I

ACCURACY AND PRECISION OF THE HPLC ASSAY OF CIPROFLOXACIN ($n = 10$)

Concentration added (mg/l)	Concentration found (mg/l)		C.V. (%)
	Mean ± S.D.	Range	
Within-day			
0.5	0.47 ± 0.01	0.44–0.49	2.99
2.0	1.85 ± 0.08	1.72–1.96	4.59
5.0	5.10 ± 0.19	4.83–5.33	3.86
Day-to-day			
0.5	0.49 ± 0.06	0.41–0.65	13.26
2.0	2.00 ± 0.14	1.80–2.13	7.08
5.0	4.94 ± 0.21	4.60–5.37	4.25

concentration (x) were $y = 0.429x - 0.0026$; $r = 0.999$ ($n = 8$). The within-day and day-to-day coefficients of variation (C.V.) are presented in Table I. Mean recoveries from the extraction procedure were: 52.63 ± 4.83% at 0.5 mg/l; 49.01 ± 2.75% at 2 mg/l and 50.88 ± 2.08% at 5 mg/l ($n = 9$).

Table II shows the mean serum peak and trough concentrations of ciprofloxacin at the steady state.

All the chromatographic methods previously described for the determination of ciprofloxacin in biological fluids have utilized conventional columns [4,5,8]. The use of short columns (30 mm × 4.6 mm I.D.) has several advantages; fast equilibrium, shorter retention time and lower consumption of the mobile phase. These factors are important in evaluating the practicability of the method.

Although the sensitivity of our UV detector was lower than that reported for fluorimetric detectors [5–7], the serum levels we found in patients treated with ciprofloxacin remained within the range of detection and the linearity of the method.

Bergan *et al.* [9] found that serum concentrations of the ciprofloxacin metabolites were less than 10% of the ciprofloxacin levels. Work is currently being undertaken to obtain them and to assess their chromatographic behaviour.

TABLE II

SERUM PEAK AND TROUGH CONCENTRATIONS OF CIPROFLOXACIN IN PATIENTS

	Concentration (mg/l)		n
	Mean ± S.D.	Range	
Peak	3.43 ± 3.80	0.82–13.82	24
Trough	1.32 ± 2.70	0.10–11.80	24

Our results indicate that the short-colum reversed-phase HPLC analysis of ciprofloxacin provides a fast, inexpensive and accurate method for therapeutic monitoring in clinical laboratories.

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