Journal of Chromatography, 431 (1988) 461–464
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4321

Note

Method for the determination of ofloxacin, a quinolone carboxylic acid antimicrobial, by high-performance liquid chromatography

LIDIA J. NOTARIANNI*

School of Pharmacy and Pharmacology, University of Bath, Bath BA2 7AY (U.K.)

and

ROY W. JONES

Research Institute for the Care of the Elderly, St. Martin's Hospital, Bath BA2 5RP (U.K.)

(First received April 7th, 1988; revised manuscript received May 30th, 1988)

Ofloxacin (Fig. 1) is a new orally acting quinolone carboxylic acid derivative which shows a broad antimicrobial spectrum [1]. Ofloxacin is excreted largely unchanged in urine with 70–80% of the drug being excreted in 24 h [2]. It is currently in clinical use on the continent of Europe and is under clinical trials in the U.K. As part of a study of the pharmacokinetics of ofloxacin in the elderly, a sensitive assay was devised for routine use. Since the concentrations of ofloxacin in plasma were at least an order of magnitude smaller than those detected in urine, two methods of detection, fluorescence and ultraviolet (UV), were employed.

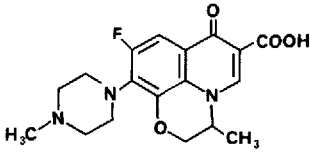

Fig. 1. Chemical structure of ofloxacin.

EXPERIMENTAL

Samples

Heparinized blood (10 ml) samples were collected over 72 h by venepuncture from volunteers who had taken a single oral dose of ofloxacin (200 mg). Plasma

0378-4347/88/$03.50

was obtained immediately on collection of the blood by centrifugation at 4000 *g* and stored at −20 °C prior to analysis. Urine aliquots were stored at −20 °C prior to analysis.

Reagents and chemicals

Ofloxacin was supplied by Hoechst U.K. (Hounslow, U.K.). Flufenamic acid was purchased from Sigma (Poole, U.K.). Organic solvents were of HPLC grade and purchased from Fisons (Loughborough, U.K.).

Procedure

Plasma. To 1 ml of plasma were added 2 ml of phosphate buffer (0.1 *M*, pH 7.0), 0.1 ml of 1 mg/ml flufenamic acid in phosphate buffer as internal standard and 6 ml of chloroform. The tubes were stoppered with a PTFE-lined top and agitated on a rotary mixer for 15 min. The tubes were subsequently centrifuged at 4000 *g* for 5 min at 4 °C to separate the layers and the aqueous phase was removed by suction using a water pump. The chloroform phase was decanted into a clean tube and evaporated to dryness under nitrogen at 37 °C. The residue was subsequently suspended in 0.1 ml of phosphate buffer (0.1 *M*, pH 7.0) by whirlimixing for 45 s. The residue was transferred into a 1-ml microfuge tube and spun at 9000 *g* for 2 min. The supernatant was taken for HPLC.

Urine. Acetonitrile (1 ml) was added to 1 ml urine to denature any protein present. The tubes were centrifuged at 4000 *g* for 15 min and the supernatant was taken for HPLC.

High-performance liquid chromatography

Plasma. Analysis was performed at 17 °C using a Constametric III pump (LDC, Stone, U.K.) combined with a fluorometric detector (LDC), equipped with an excitation filter of 370 nm and an emission filter of 400–700 nm, and a Rheodyne 7125 injection valve fitted with a 0.02-ml loop. The analytical column used was 15 cm × 4.5 mm I.D. packed with Hypersil ODS 5 μm (Shandon Southern Products, Runcorn, U.K.). The mobile phase was acetonitrile–0.04 *M* phosphoric acid (45:55). The final pH of the mobile phase was between 2.56 and 2.59. The flow-rate employed was 2 ml/min giving a column pressure of 8.2 MPa. Under these conditions the following retention times were obtained: ofloxacin, 3.83 min; flufenamic acid, 2.67 min. Concentration of ofloxacin was obtained using the peak-height ratio ofloxacin/flufenamic acid.

Urine. Analysis was performed at 23 °C using an SP8100 liquid chromatograph (Spectra-Physics, St. Albans, U.K.) equipped with an automatic injector and UV detection set at 254 nm. The column, mobile phase and flow-rate were identical to those stated above for plasma, and the injection volume was 0.01 ml. The retention time obtained on this system for ofloxacin was 4.63 min. Concentration of ofloxacin was obtained utilising the area under the peak which was obtained using an SP4200 integrator (Spectra-Physics).

RESULTS AND DISCUSSION

Typical chromatographic traces of ofloxacin assayed in plasma and urine are presented in Fig. 2. The plasma sample, representing 1416 ng/ml (1.416 mg/l), was taken from a volunteer 4 h after a single oral dose of ofloxacin (200 mg), and the urine sample, representing 228.3 mg/l, was taken collected 2–4 h post dosing. The difference in sensitivity required for the analysis of plasma and urine samples necessitated the use of two detection systems. Urine was assayed without the need for the extraction and/or concentration using UV detection. Calibration curves using area of peak were linear over the range 10–5000 mg/l. The coefficients of variation ($n=10$) were 9.9% (10 mg/l), 9.4% (25 mg/l) and 3.5% (200 mg/l). This sensitivity was adequate for the study of urinary excretion of ofloxacin where the mean excretion of unchanged drug ($n=12$) was 138 mg in 24 h in a volume of less than 2 l. Since no extraction was required, the need for an internal standard was eliminated. Following extraction and concentration of plasma sam-

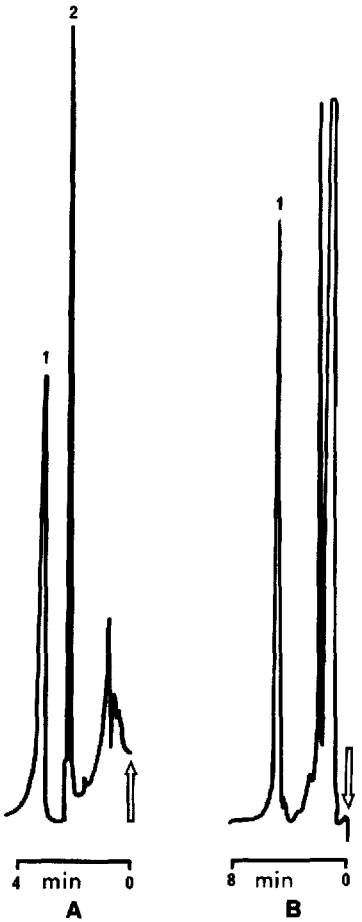

Fig. 2. Typical chromatograms of (A) plasma sample using fluorescence detection and (B) urine sample using UV detection as described in the text. Peaks: 1 = ofloxacin; 2 = flufenamic acid.

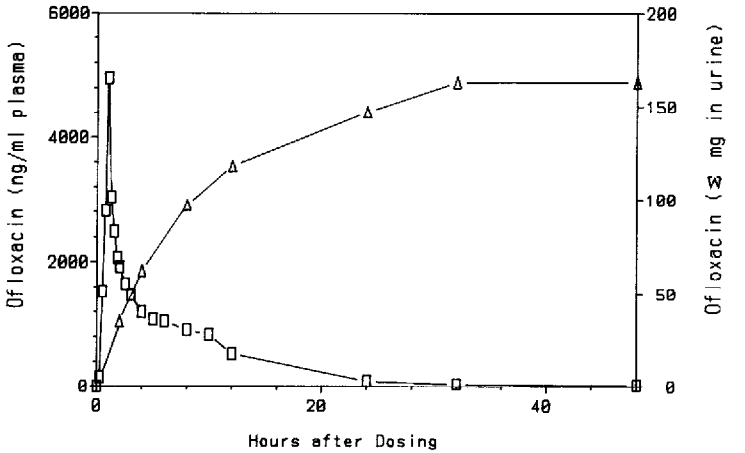

Fig. 3. Pharmacokinetic profile of ofloxacin in plasma (□) and urine (△) following a single oral dose of ofloxacin (200 mg) in a human volunteer.

ples, the UV method permitted concentrations as low as 1000 ng/ml to be assayed, but this was not sufficiently sensitive for the plasma samples. Calibration curves for plasma utilising fluorescence detection was linear over the range 2.5–1000 ng/ml and 1000–5000 ng/ml. The coefficients of variation ($n=10$) were 8.8% (2.5 ng/ml), 4.45% (20 ng/ml), 2.4% (200 ng/ml) and 4.7% (2000 ng/ml). Peak plasma concentration of ofloxacin in our study of elderly volunteers was 2880 ng/ml (mean; $n=12$) reducing with a half-life of 8.92 h to 224.7 ng/ml at 24 h and 37.6 ng/ml at 48 h. A typical pharmacokinetic profile and urinary excretion of this drug in a volunteer following a single 200-mg oral dose is given in Fig. 3.

These methods afford rapid and sensitive assay for the measurement of ofloxacin in plasma and urine.

REFERENCES

1 H.C. Neu, Lancet, ii (1987) 1319–1322.
2 J.P. Monk and D.M. Lampoli-Richards, Drugs, 33 (1987) 346–391.