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Determination of ofloxacin in human serum by high-performance liquid chromatography with column switching

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ABSTRACT

The chromatographic behaviour of ofloxacin on various sorbents, including ODS, C_8, C_1, nitril, phenyl and *tert.*-butyl, as stationary phases was investigated and a high-performance liquid chromatography (HPLC) assay was developed for the determination of ofloxacin in serum. The serum samples were directly introduced onto an HPLC column after filtering through a Morcut II membrane filter to remove proteins. The filtrate was concentrated on a pre-column using a phenyl stationary phase and was then introduced to an analytical column with an ODS stationary phase by column switching. Ofloxacin and enoxacin as an internal standard were detected by ultraviolet absorbance at 300 nm. Determination was possible for ofloxacin over the concentration range 50–2000 ng/ml; the limit of detection was 20 ng/ml. The recovery of ofloxacin added to serum was 88.8–101.7% with a coefficient of variation of less than 5.2%. This method is applicable to pharmacokinetic studies of patients after treatment with ofloxacin.

INTRODUCTION

Ofloxacin, 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*] - 1,4 - benzoxazine - 6 - carboxylic acid (Fig. 1), is a fluoroquinolone that shows a good activity against Gram-negative and Gram-positive bacteria *in vivo* and *in vitro* [1]. The mechanism of the effect is based on the inhibition of the DNA-gyrase of the bacteria. A microbiological assay of ofloxacin has been described previously [2]. Assay methods such as this have the disadvantage of a poor limit of detection and lack selectivity. Therefore, several workers have reported high-performance liquid chromatography (HPLC) methods for the determination of ofloxacin in body fluids [3–7].

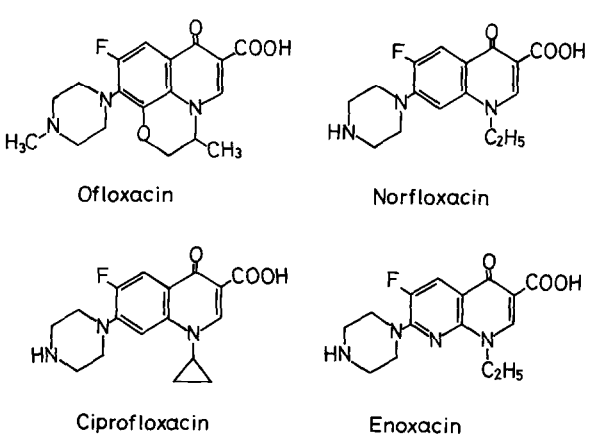

Fig. 1. Structures of ofloxacin, enoxacin, ciprofloxacin and norfloxacin.

Many attempts to separate ofloxacin have been made using reversed-phase HPLC with various mobile phases. There are a few reports describing HPLC methods for ofloxacin using an ODS stationary phase with an alkylamine [3,4] and sodium laurylsulphate [5] containing mobile phase. Ion-paired chromatographic methods cause problems in column maintenance and reproducibility. Therefore, it is preferable that the

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HPLC determination of quinolone derivatives is achieved by reversed-phase HPLC with a non-ion-paired mobile phase.

In previous studies, the HPLC determination of quinolone derivatives with non-ion-paired mobile phases was not examined. In addition, in most of the previously published methods, the extraction yield was not sufficient and the extraction procedures tedious [6,7]. Okazaki *et al.* [8] reported an HPLC method for the determination of (*S*)-(−)ofloxacin in serum and urine. This method was an improvement of the procedure using solid-phase extraction of ofloxacin from biological samples. A direct injection method using column switching without extraction has also been described [9]. This method is very useful for the determination of a compound with a low recovery and unstable compounds in biological samples.

This paper describes HPLC on ODS, C_8, C_1, CN, Ph and *tert.*-butyl columns using a non-ion-paired mobile phase with column switching for the determination of ofloxacin in serum. The analysis of serum samples from clinical patients was carried out using this method.

EXPERIMENTAL

Chemicals and materials

Ofloxacin, enoxacin, ciprofloxacin and norfloxacin (Fig. 1) were extracted from commercially available tablets in these laboratories. The chemical structure and the purity were checked by thin-layer chromatography, IR, NMR and mass spectrometry. A Molcut II membrane filter was obtained from Millipore (Bedford, MA, USA). All other solvents used were of HPLC grade (Wako Pure Chemicals Industries, Tokyo, Japan). All other reagents and chemicals were purchased from Wako Pure Chemicals Industries or Nakarai Tesque (Kyoto, Japan) and were purified by recrystallization or distillation prior to use.

Apparatus

The apparatus used for HPLC was a Jasco Model PU-880 chromatography pump (Jasco, Tokyo, Japan) equipped with a UVIDEC 100-VI ultraviolet detector (Jasco). Test samples were injected using a Model 7125 injector (Rheodyne, Cotati, CA, USA) with an effective volume of 500 μl. The HPLC column used Develosil ODS-5, Develosil C_8-5, Develosil Ph-5 and Develosil CN-5 stationary phases (5 μm, Nomura Chemical, Seto, Japan); dimethyl *tert.*-butyl and methyl bonded stationary phases were synthesised in our laboratories using Fine SIL 100-5 (5 μm) (Jasco).

Stainless-steel analytical columns (150 mm × 4.6 mm I.D.) were packed in these laboratories by a conventional high-pressure slurry-packing procedure. Precolumns (50 mm × 4.6 mm I.D.) were packed by a low-pressure packing technique. The mobile phase consisted of 0.5% sodium acetate–acetonitrile (87:13, v/v). Before mixing, the pH of the mobile phase was adjusted with 50% phosphoric acid and it was degassed ultrasonically.

Determination of ofloxacin

Enoxacin (900 ng) in methanol (10 μl) was added to the serum samples (250–500 μl) as an internal standard and then 100 μl of acetonitrile were added. The mixture was filtered through a Molcut II membrane filter for deproteinization. The filtrate (50–100 μl) was then loaded onto the precolumn for the elimination of interfering substances in the serum sample. After washing for 2.1 min, ofloxacin and enoxacin were eluted from the precolumn and then led to the analytical column by a column-switching technique using 0.5% sodium acetate (pH 2.5)–acetonitrile (87:13, v/v) as the mobile phase (Fig. 2). The procedure was carried out on-line with one pump and one isocratic mobile phase.

Recovery test for ofloxacin

The spiked samples were prepared by adding 200, 500 and 1000 ng/ml ofloxacin to serum samples from patients treated with ofloxacin (100 mg, orally) and 400, 1000, 2000 ng/ml ofloxacin to serum samples from patients treated with ofloxacin (200 mg, orally). Pretreatment and the subsequent column-switching HPLC technique were carried out as described.

Calibration graphs

Known amounts of ofloxacin in the range 50–

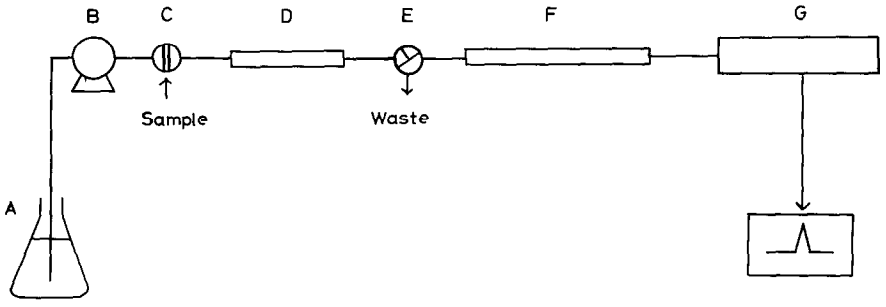

Fig. 2. Schematic diagram of the column-switching HPLC system. A = Carrier solution; B = HPLC pump; C = injector; D = precolumn; E = three-way valve; F = analytical column; G = UV detector.

2000 ng/ml were added to blank serum samples. There serum samples were treated according to the described determination procedure. The peak-height ratios of ofloxacin to enoxacin were measured and plotted against the concentration of ofloxacin in serum.

Drug administration and sampling

Ofloxacin (100 or 200 mg) was given orally to four patients. Blood samples (2 ml) were collected by venous puncture 1, 2, 3, 4, 5 and 6 h after treatment. Serum samples were separated by centrifugation at 1900 *g* for 15 min and stored at −45°C until analysis.

RESULTS AND DISCUSSION

Initially, efforts were directed towards devel-

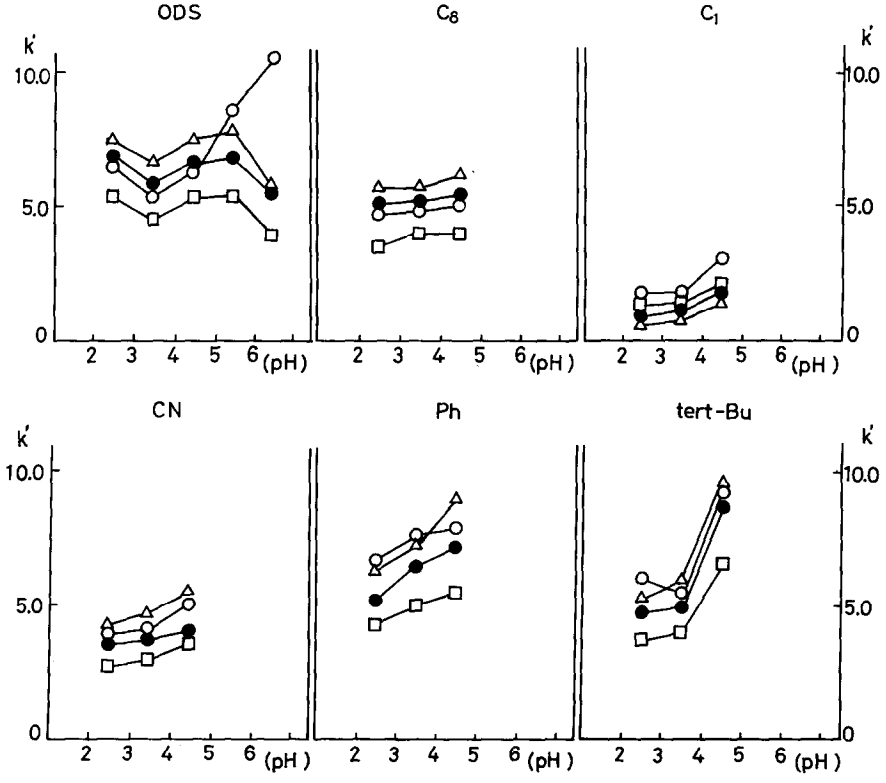

Fig. 3. Effect of mobile phase pH on the k' values of the quinolone derivatives on the ODS, C_8, C_1, CN, Ph and *tert.*-butyl stationary phases. Conditions: mobile phase, sodium acetate buffer–acetonitrile (87:13, v/v); flow-rate, 1.0 ml/min. (○) ofloxacin; (□) enoxacin; (△) ciprofloxacin; (●) norfloxacin.

oping an efficient chromatographic system for the direct determination of serum ofloxacin using column-switching techniques. The effects of the pH of the mobile phase on the capacity factors (k') of ofloxacin, norfloxacin, ciprofloxacin and enoxacin were studied using six kinds of stationary phase. Analytical methods for the determination of ofloxacin have been described previously using octadecyl [3–5] stationary phase columns. Ion-paired chromatography has been applied to those analytical methods using triethylamine [3], tetrabutylammonium [4] and sodium laurylsulphate [5] containing mobile phases on an octadecyl column. Ion-paired chromatographic methods have problems with column maintenance and reproducibility. This work therefore examined the characterisation of ofloxacin and other quinolone derivatives on several stationary-phase columns using a number of non-ion-paired mobile phases. In all these compounds, the k' values increased with increasing pH in the range 3.5–4.5 with 0.5% sodium acetate–acetonitrile (87:13, v/v) (Fig. 3). However, sufficient resolution was not obtained at pH 4.5 as a result of peak broadening and tailing.

These quinolone derivatives were retained more strongly on phenyl, *tert.*-butyl and octadecyl stationary phases. Enoxacin could be easily separated with these mobile phases over the entire pH range. Sufficient resolution was obtained with these stationary phases. Enoxacin is therefore suitable as an internal standard. Under these chromatographic conditions, the retention times of ofloxacin and enoxacin on the phenyl, *tert.*-butyl and octadecyl columns were 6.0–10.5 and 3.5–5.5 min, respectively, with a single column (150 mm × 4.6 mm I.D.). As the time of analysis at a mobile phase pH of 2.5 was shorter than that at higher pH, pH 2.5 was selected for the mobile phase.

Several papers describe the determination of ofloxacin in body fluids using extraction and purification steps. However, these involve tedious extraction procedures and low extraction yields. This work was therefore directed towards the determination of ofloxacin in biological fluids by direct injection using column-switching techniques [9].

A combination of a precolumn and an analytical column was selected so that interfering endogenous substances and the quinolone derivatives had different interactions with the two columns. The separation of endogenous substances and the quinolone derivatives was satisfactory using stationary phases of phenyl on the precolumn and octadecyl on the analytical column. A mobile phase of 0.5% sodium acetate (pH 2.5)–acetonitrile (87:13, v/v) was used for the two columns. Fig. 4 shows representative chromatograms for a blank serum sample and a serum sample obtained from a patient treated with ofloxacin. No significant interfering peaks derived from biological substances were seen on the chromatogram.

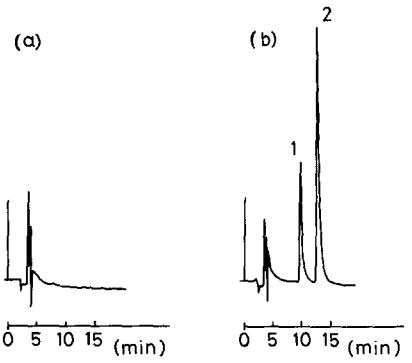

Fig. 4. Chromatograms of (a) blank serum sample and (b) a serum sample 2 h after treatment by mouth with 200 mg of ofloxacin and with added enoxacin (I.S.). Peaks: 1 = enoxacin (I.S.); 2 = ofloxacin.

Calibration graphs for ofloxacin in human se-

TABLE I

RECOVERY OF OFLOXACIN ADDED TO SERUM FROM A PATIENT RECEIVING 100 AND 200 mg OF OFLOXACIN BY MOUTH

Added (ng/ml)	Found (mean ± S.D.) (ng/ml)	Recovery (mean ± S.D.) (%)
100-mg dose (n=6)		
0	1012 ± 9	–
200	1210 ± 10	98.9 ± 1.9
500	1519 ± 8	101.1 ± 1.9
1000	1982 ± 9	97.0 ± 0.6
200-mg dose (n=8)		
0	1657 ± 13	–
400	2052 ± 23	98.6 ± 5.2
1000	2675 ± 29	101.7 ± 3.2
2000	3434 ± 14	88.8 ± 0.6

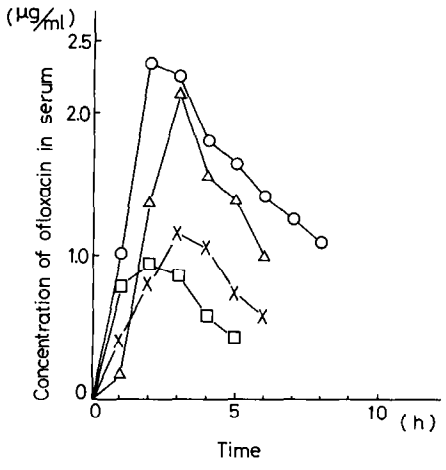

Fig. 5. Concentration of ofloxacin in serum from four clinical patients receiving ofloxacin by mouth. Doses: 100 mg (□ and ×); 200 mg (○ and △).

rum were linear in the range 50–2000 ng/ml. The limit of detection for ofloxacin was 20 ng/ml. The results of recovery studies are given in Table I. The recovery of ofloxacin was determined by adding the following six known levels to samples: 200, 500 and 1000 ng/ml to serum from one patient receiving 100 mg of ofloxacin, and 400, 1000 and 2000 ng/ml to serum from one patient receiving 200 mg of ofloxacin. The recovery values for ofloxacin were 88.8–101.7% at 200–2000 ng/ml Coefficients of variation were less than 5.2% at 200–2000 ng/ml. These results show that the proposed method is satisfactory with respect to accuracy and precision.

The concentrations of ofloxacin in serum samples from four patients receiving 100 and 200 mg of ofloxacin by mouth were determined by the proposed method (Fig. 5). The time required to reach the maximum concentrations was 2–3 h, and the concentrations were 930–1150 and 2120–2330 ng/ml for doses of 100 and 200 mg, respectively. It was shown that the proposed method for the determination of ofloxacin in clinical samples was satisfactory with respect to feasibility, simplicity and rapidity.

In conclusion, a sensitive, precise and accurate HPLC method has been developed for the determination of ofloxacin in human serum without prior extraction. This method is useful for pharmacokinetic studies in patients receiving ofloxacin clinically. Further applications to therapeutic drug monitoring for pharmaceutical studies of ofloxacin in several different dosage forms are being conducted in these laboratories and the results will be published elsewhere [10].

REFERENCES

1 K. Sato, Y. Matsuura, M. Inoue, T. Ueno, Y. Osada, H. Ogawa and M. Mitsuhashi, *Antimicrob. Agents Chemother.*, 22 (1982) 548.
2 M. R. Lockley, R. Wise and J. Dent, *J. Antimicrob. Chemother.*, 14 (1984) 647.
3 A. Le Coguic, R. Bidault, R. Frarinotti and A. Dauphin, *J. Chromatogr.*, 434 (1988) 320.
4 A. Mignot and M. A. Lefebvre, *J. Chromatogr.*, 430 (1988) 192.
5 Y. Katagiri, K. Naora, N. Ichikawa, M. Hayashibara and K. Iwamoto, *J. Chromatogr.*, 431 (1988) 135.
6 K. H. Lehr and P. Damm, *J. Chromatogr.*, 425 (1988) 153.
7 D. J. Griggs and R. Wise, *J. Antimicrob. Chemother.*, 24 (1989) 437.
8 O. Okazaki, H. Aoki and H. Hakusui, *J. Chromatogr.*, 563 (1991) 313.
9 T. Ohkubo and T. Nambara, *J. Chromatogr.*, 527 (1990) 441.
10 T. Ohkubo, M. Kudo and K. Sugawara, in preparation.