Journal of Pharmaceutical & Biomedical Analysis
Vol. 6, No. 3, pp. 221–228, 1988
Printed in Great Britain

0731–7085/88 $3.00 + 0.00
Pergamon Press plc

High-performance liquid chromatographic analysis of norfloxacin in human tissues and plasma with fluorescence detection

A. LAGANÀ,*[1] A. MARINO,[1] M. ROTATORI,[1] R. CURINI,[2] G. D'ASCENZO[2] and L. MIANO[3]

[1] *Dipartimento di Chimica, Università degli Studi di Roma, Roma, Italy*
[2] *Dipartimento di Scienze Chimiche, Università di Camerino, Camerino, Italy*
[3] *Clinica Urologia, Università dell'Aquila, Aquila, Italy*

Abstract: A high-performance liquid chromatographic analysis with fluorimetric detection is described for the quantitative determination of norfloxacin in renal, prostatic tissues and in plasma. The analytical procedure in the tissue pretreatment, consists of purification of the obtained sample by a solid state extraction and quantitation by HPLC. The samples were chromatographed on a C_8 reversed-phase column. Analytical recoveries ranged from 95.2 to 97.6%. Within and between day precision were assessed by analysing serum containing 50 and 500 ng/ml norfloxacin. At each concentration, within day precision was $\leq$3.6% (relative standard deviation, n = 10) and day-to-day precision was $\leq$5.3% (n = 10). Limit of detection was *ca* 1 ng/ml.

Keywords: *Reversed-phase high-performance liquid chromatography; fluorimetric detection; antibacterial agents; tissue and plasma drug levels.*

Introduction

Norfloxacin (1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7 (1 piperazinyl)-3-quinidine carboxylic acid) is a new synthetic broad spectrum antibacterial agent structurally related to nalidixic and oxolinic acids [1]. Norfloxacin exhibits antibacterial activity against both gram-positive and gram-negative bacteria greater than other nalidixic acid analogues and also exhibits greater activity than gentamicin against *Pseudomonas aeruginosa* [2, 3].

The *in vitro* activity of norfloxacin against *Neisseria gonorrhoee* is comparable to that of Rosaxacin [3]. Norfloxacin is moderately well absorbed after oral administration in a percentage ranging from 15 to 30%, depending on the animal species [4, 5]. The potential usefulness of norfloxacin in the treatment of urinary tract infections and especially in the infections of the prostate (as is well known this is a very critical tissue for antibiotic treatment) justifies in-depth studies into its tissue concentrations and pharmacokinetics.

This report describes a rapid and specific method for the analysis of norfloxacin in renal, prostatic tissues and plasma.

*To whom correspondence should be addressed.

The analytical procedure in the pretreatment of tissues, consists of purification of the samples obtained by solid-state extraction and quantitation by HPLC equipped with a fluorimetric detector.

Materials and Methods

Principle

The renal and prostatic tissues were previously extracted by homogenization in methanol. The extracts obtained or the plasma were then purified by adsorption on a liquid–solid column extraction. The organic phase was evaporated and reconstituted with the mobile phase, and a portion of this solution injected into the chromatograph. The concentration of the drug in the specimen was calculated by comparing the peak area produced by norfloxacin in the sample with that of a standard.

Instrumentation

A series 3 liquid chromatograph was used equipped with a Model LS-3 fluorescence detector and a Rheodyne Model 7125 injector with a 20-μl loop (all from Perkin–Elmer, Norwalk, CT 06856). A Partisil PXS 10/25 C_8 column (Whatman Inc., Clifton, NJ 07014) was used.

As the mobile phase, a mixture of acetonitrile–methanol–phosphate buffer (pH = 2.5) (19:3:78; v/v) was used at a flow rate of 12 ml min^{-1}. Norfloxacin was monitored by setting the excitation and emission wavelengths of the detector at 300 and 420 nm, respectively, with 10-nm slit widths.

Reagents and materials

All chemicals used were of reagent grade. Chromatography grade methanol and acetonitrile were obtained from Carlo Erba, Milan, Italy. Phosphate buffer (pH = 2.5) was prepared by dissolving 2.72 g of KH_2PO_4 in 1 l of fresh distilled water and adjusting the pH to 2.5 with phosphoric acid. Carbopack B 80–120 mesh was obtained from Supelco (Supelco Inc., Bellofonte, USA).

Standards

Stock norfloxacin standard (Merck Sharp & Dohme Research Laboratories, West Point, PA, USA) was prepared in 0.05 M sodium hydroxide and was stable at 4°C. The working standard was prepared by diluting the stock solution with methanol.

Procedures: Collection of specimens.

Prostatic specimens. Eight subjects with a prostatic adenoma were submitted for transvesical prostatectomy. The subjects were treated with 400 mg of norfloxacin 12 h before the operation and with a further 400 mg about 90 min before the collection.

The adenoma was washed many times by physiological solution, and by sterile surgical instruments, incised to obtain a sample of uncontaminated tissue. This method avoids any contamination from the urine. Simultaneously a blood sample was collected. The serum obtained was frozen together with the prostatic tissue.

Renal specimens. Seven subjects, 3 males and 4 females, subject to urological operation and with good renal functionality were used. The patients were treated with 400 mg of norfloxacin 12 h before the operation and with another 400 mg about 90 min

before the collection of a sample of renal parenchyma. A blood sample was collected at the same time. The serum obtained was frozen together with the renal tissue.

Extraction column. Prepare the Carbopack B 80–120 mesh bed by pouring 200 mg of adsorbent into water; eliminate floating Carbopack B by decantating and introduce the suspension into a 15 × 0.5 cm glass column with a small plug of glass wool in the bottom. Pack the adsorbent by tapping gently for a short time while eluting the column with water. To treat the solution obtained after tissue homogenization, the adsorbent was suspended in methanol.

Sample pretreatment and extraction

Tissues. Samples of tissues (masses ranged between 50–500 mg) were homogenized for 2 min in 10 ml of methanol by means of a Politron homogenizer. The mixture obtained was then centrifuged at 6000 r.p.m. for 10 min and the supernatant was passed through the Carbopack column.

The column was then washed with 10 ml of a solution A (0.02 mol l^{-1} of HCOOH in methanol) and then with 5 ml of a mixture of chloroform–solution A (20:80, v/v). Finally the norfloxacin was eluted by a mixture of chloroform–solution A (80:20, v/v) to obtain an eluate volume of 8 ml.

The sample obtained was evaporated under a stream of nitrogen at 50°C. The residue was dissolved by adding 100 μl of mobile phase; greater volumes (500 μl) were used when a high level of norfloxacin was expected. An aliquot of 20 μl of the solution obtained was injected into the chromatograph.

Serum. 100 μl were diluted with 5 ml of distilled water. The solution was percolated through the Carbopack column. The column was washed with 5 ml of distilled water, then with 10 ml of solution A and with 5 ml of a mixture of chloroform–solution A (20:80, v/v).

The elution of the norfloxacin from the column, the extraction and the analysis were then carried out as for the final step described for the tissues. Under the experimental conditions used, the flow rate of the solvents percolating through the Carbopack column was 2 ml min^{-1}.

Results and Discussion

Optimization of the method

To optimize the chromatographic conditions the composition, pH and flow rate of the mobile phase were systematically varied. Different mixtures such as acetonitrile–phosphate buffer, methanol–phosphate buffer or acetonitrile–methanol–phosphate buffer were evaluated at different ratios. The mobile phase and the flow rate specified above were selected because the interference from endogenous and exogenous compounds was minimized at this composition. The retention time for norfloxacin was found to be 5.6 min.

Homogenization and extraction

Evaluation of the recovery from the tissues was quite impossible because spiking with known quantities of drug does not reproduce the real chemico-physical condition of the antibiotic in the tissue.

To evaluate the extraction it is better to carry out the process by means of different media to choose the extraction solvent that gives the highest efficiency and precision. Different media such as $HClO_4$ (0.4 M), CH_3OH and CH_3OH–$CHCl_3$ (1:1, v/v) were tested. Organic non-polar solvents were not considered because of their limited penetration through the membrane. Perchloric acid and methanol showed the highest and comparable extraction efficiency for norfloxacin. Methanol was selected as the operational system because it minimized the level endogenous interferences.

Recovery from the purification column

The recovery from the Carbopack column was evaluated in distilled water, methanol or plasma (drug-free), to which known quantities of norfloxacin had been added to achieve the concentrations referred to in Table 1.

Table 1
Analytical recoveries of norfloxacin from serum

Added (ng ml^{-1})	Found (mean ng ml^{-1} ± s.d.)	RSD (%)	Recovery (%)	*n*
10	9.76 ± 0.23	2.5	97.6	6
50	47.6 ± 1.6	3.4	95.2	6
100	96.3 ± 3.0	3.1	96.3	6
500	478 ± 12	2.5	95.6	6
1000	970 ± 26	2.7	97.0	6
1500	1451 ± 35	2.4	96.7	6

It is interesting to note that the omission of the acid washing, as reported in the purification scheme, allows a low recovery to be obtained. The phenomenon can be ascribed to the formation of chemical complexes between anions and impurities at the Carbopack B surface [6]. The complexes, however, readily hydrolyze by washing Carbopack B with an acid solution.

The analytical recovery of the norfloxacin was the same from water, methanol and plasma. Average recovery results in the concentration range considered were 96.4%.

Linearity

The peak-heights and concentrations varied linearly over the range investigated (10–500 ng ml^{-1}).

Sensitivity

The smallest concentration measurable by this method was 1 ng ml^{-1} of norfloxacin. This concentration gave a chromatographic peak that was 3 times the height of the baseline noise.

Precision

Within-run and day-to-day precision of the method were determined by assaying the spiked serum with, respectively, 50 and 500 ng ml^{-1} (Table 2).

Table 2
Precision of the assay

Within day (n = 10)		
Mean ($ng\ ml^{-1}$)	s.d. ($ng\ ml^{-1}$)	RSD (%)
47.7	1.6	3.6
480	12.5	2.6
Day-to-day (n = 10)		
Mean ($ng\ ml^{-1}$)	s.d. ($ng\ ml^{-1}$)	RSD (%)
46.0	2.4	5.3
463	23.1	5.0

Background

More than 20 different drug-free tissues and plasma samples were processed to ascertain background peak interference at the elution times corresponding to the norfloxacin. The background from these samples did not interfere with the analyses.

Clinical applications

Pharmacokinetic studies. Figure 1 shows the plasma as a function of time after a single oral dose of 400 mg of norfloxacin. As is possible to see, the maximum drug concentration (C max) occurs 1 h after the administration. This behaviour agrees with the data referred to by other authors [7, 8]. According to these results the tissues were samples between 60 and 90 min after the drug administration. In Fig. 2 the chromatograms of a drug-free serum (a) and of a serum of a patient after a single dose administration of 400 mg of norfloxacin (b) are referred to.

Prostatic and renal tissues. The concentration values of norfloxacin in the kidney and prostate (Tables 3 and 4) compared with the *in vitro* minimal inhibitory concentrations (MIC 90%) [9, 10] show that tissue levels reach quite high values, which are capable of combatting the most common bacterial urological infections.

Table 3
Concentration of norafloxacin in prostatic tissues

Patient	ng of norfloxacin/g of tissue
A	420
B	169
C	391
D	40
E	210
F	321
G	518
H	793

Table 4
Concentration of norfloxacin in renal tissues

Patient	ng of norfloxacin/g of tissue
A	3665
B	1012
C	1689
D	787
E	1531
F	3909
G	830

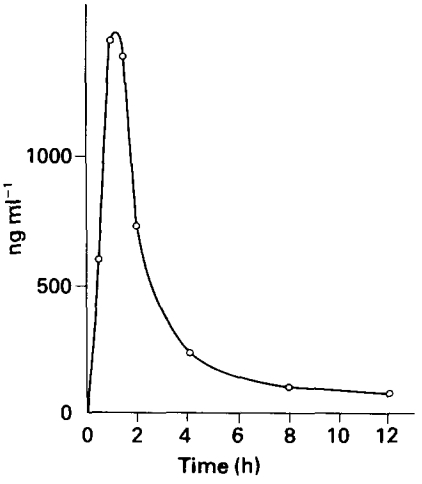

Figure 1
Plasma concentration–time curve, after a single oral dose of 400 mg of norfloxacin.

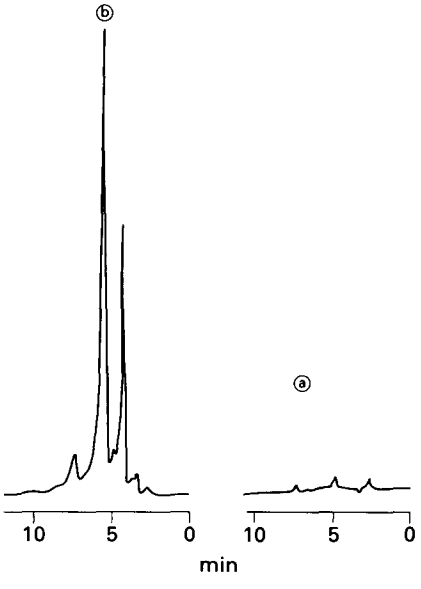

Figure 2
Chromatograms of a drug-free serum (a) and of a serum of a patient treated with norfloxacin (b). The level of norfloxacin was 162 ng ml^{-1}.

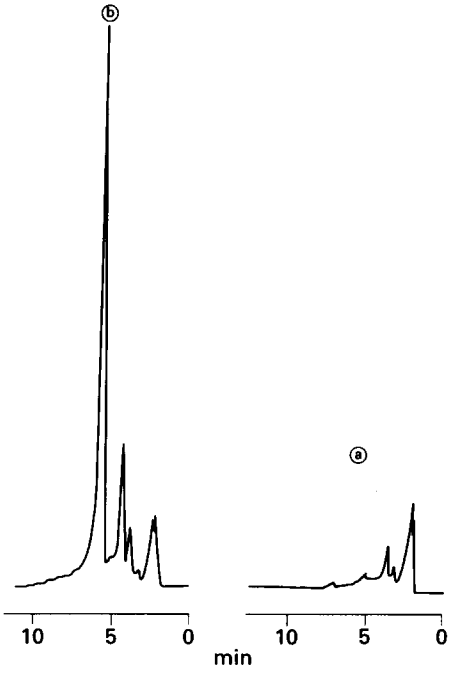

Figure 3
Representative chromatograms of blank renal tissue (a), and of renal tissue of a patient treated with norfloxacin (b). The level of norfloxacin was 787 ng g^{-1}.

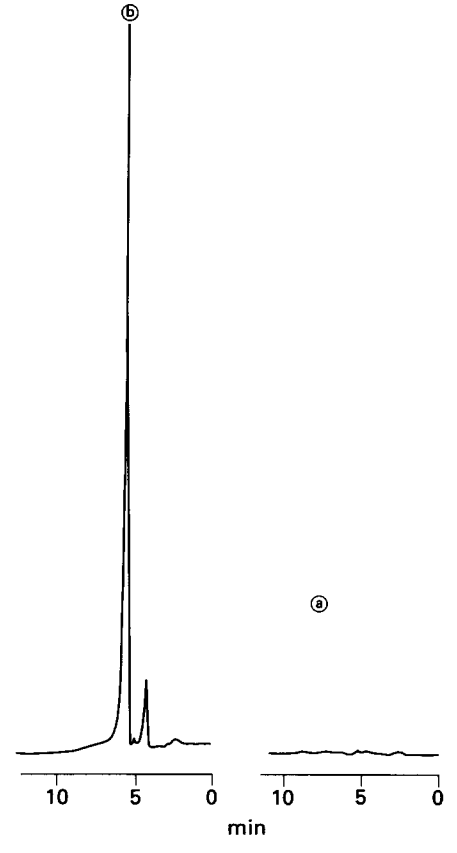

Figure 4
Representative chromatograms of blank prostate tissue (a), and of prostate tissue of a patient treated with norfloxacin (b). The level of norfloxacin was 518 ng g^{-1}.

Conclusions

Norfloxacin is a recently developed antibiotic which is useful in the therapy of prostatic and urological infections.

An assay has been developed to quantify the concentration of the norfloxacin in the renal and prostatic tissues and in the plasma.

The combination of the purification system and the quantification by HPLC using a fluorescence detector allows adequate sensitivity coupled with good precision and accuracy to be reached. The analytical and the pharmacokinetic studies show that the concentrations reach a therapeutic level both in renal and prostatic tissues in particular, the concentration is quite high in the renal tissues.

References

[1] H. H. Gadebusch, L. R. Koupal, E. Celozzi, D. L. Shungu, J. Bland, B. Weissberger, B. A. Pelak, E. Fisch, S. K. Chung and J. Huber, in *Current Chemotherapy*, Vol. 1, pp. 351–353. American Society for Microbiology (1982).
[2] K. Hirai, A. Ito, Y. Abe, S. Suzue, T. Irikura, M. Inoue and S. Mitsumashi, *Antimicrob. Agents Chemother.* **19**, 188–189 (1981).
[3] A. Ito, K. Irai, M. Hinoue, H. Koga, S. Suzue, T. Irikura and S. Mitsumashi, *Antimicrob. Agents Chemother.* **17**, 103–108 (1980).
[4] S. Murayama, K. Hirai, A. Ito, Y. Abe and T. Irikura, *Chemotherapy (Tokyo)* Supplement **4**, 98–104 (1981).
[5] Y. Nagatsu, K. Endo and T. Irikura, *Chemotherapy (Tokyo)* Supplement **4**, 105–118 (1981).
[6] L. Campanella, A. di Corcia, R. Samperi and A. Gambacorta, *Mater. Chem.* **7**, 429–438 (1982).
[7] V. K. Boppana and B. N. Swanson, *Antimicrob. Agents Chemother.* **21**, 808–810 (1982).
[8] M. Eandi, I. Viano, F. di Nola, L. Leone and E. Gennazzani, *Eur. J. Clin. Microbiol.* **2**, 253–259 (1983).
[9] M. Y. Khan, R. P. Gruninger, S. M. Nelson and R. E. Klicker, *Antimicrob. Agents Chemother.* **21**, 848–851 (1982).
[10] S. W. B. Newsom, *Eur. J. Chemother. Antiobiot.* **3**, 9–14 (1982).