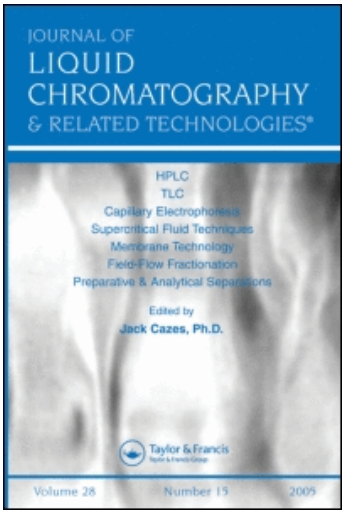

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A Validated Ion-Pairing High Performance Liquid Chromatographic Method for the Determination of Enoxacin and its Metabolite Oxo-Enoxacin in Plasma and Urine

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A VALIDATED ION-PAIRING HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF ENOXACIN AND ITS METABOLITE OXO-ENOXACIN IN PLASMA AND URINE

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ABSTRACT

A rapid, sensitive, and specific determination of enoxacin and its principal metabolite, oxo-enoxacin, in plasma and urine is described. The method, which employs the structurally related compound ciprofloxacin as internal standard, involves a protein precipitation step for plasma and solid-phase extraction for urine. Liquid chromatographic analysis is carried out on a C-18 bonded silica column; the mobile phase consists of 0.1 M citric-acid/acetonitrile employing ammonium perchlorate and tetrabutyl-ammonium hydroxide as ion-pairing agents. Quantitation is performed by UV-detection at 340 nm.

The analytical method was validated by examining the performance characteristics specificity, linearity, precision, accuracy, sensitivity, and recovery. Enoxacin calibration curves were linear between 0.02 and 3.2 µg/ml of plasma and from 0.5 to 125 µg/ml of urine. Limits of quantitation in plasma and urine were 0.01 and 0.5 µg/ml, respectively. For oxo-enoxacin, linear

calibration curves were obtained in the range 0.05 to 1.6 µg/ml (plasma) and 1 to 50 µg/ml (urine); the respective quantitation limits were approximately 0.02 and 1 µg/ml.

The present assay procedure has been applied to monitoring plasma and urine concentrations in several pharmacokinetic studies in humans and different animal species.

INTRODUCTION

Enoxacin, 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1.8-naphthyridine-3-carboxylic acid (Fig. 1), is a member of a new class of antibacterial agents, the fluorinated 4-quinolones (1-2). It has a broad spectrum of antibacterial activity and is particularly potent against Gram-negative organisms and staphylococci (3). Enoxacin is excreted mainly in urine as unchanged drug. Its primary metabolite, oxo-enoxacin, accounts for 10-15% of the administered dose and has about 10% of the antibacterial activity of the parent compound (3-6).

Several HPLC methods have already been developed for the determination of enoxacin in plasma, employing protein precipitation (5,7,8) or liquid/liquid extraction with and without derivatization (9-11). However, the published procedures either involve time-consuming clean-up steps, require rather large sample vol-

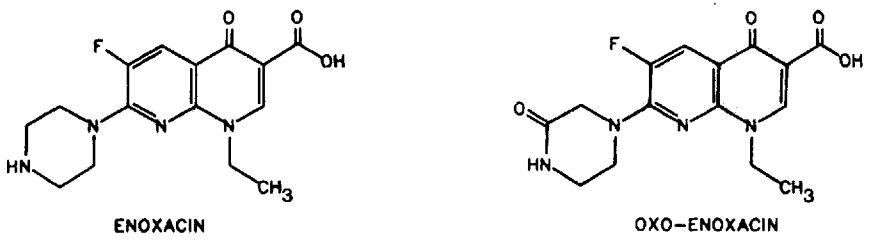

FIGURE 1: Chemical structures of enoxacin and oxo-enoxacin

umes, do not allow a simultaneous determination of the 4-oxo-metabolite, or are not sensitive enough. Moreover, these assay methods more or less lack of comprehensive validation procedures mandated by most regulatory agencies for Good Laboratory Practice (GLP), i.e. careful evaluation of the method characteristics specificity, linearity, precision, accuracy, sensitivity, and recovery [12,13]. Therefore, a rapid, specific, reproducible and sensitive ion-pairing liquid chromatographic method was developed and validated for the simultaneous determination of enoxacin and oxo-enoxacin in plasma and urine.

MATERIALS AND METHODS

Chemicals and Reagents

Enoxacin (CI-919, PD-107,779) and oxo-enoxacin (PD-112,475) were supplied by Warner-Lambert (Ann Arbor, Mich., USA), the internal standard ciprofloxacin (BAY o 9867) was obtained from Bayer (Leverkusen, FRG). Sodium phosphate monobasic and dibasic, citric acid, perchloric acid (70%, D = 1.67), and citrate buffer solution pH 6 were of analytical grade and purchased from Merck (Darmstadt, FRG). Additional chemicals used were Acetonitrile (HPLC grade S, Zinsser Analytic, Frankfurt, FRG), ammonium perchlorate (99.8%, Ega-Chemie, Steinheim, FRG), and tetrabutylammonium hydroxide (TBAH, 20% in water, reagent grade, Merck-Schuchardt, Hohenbrunn, FRG). Disposible Bond-Elut SCX cation-exchange cartridges (500 mg sorbent) were obtained from ICT (Frankfurt, FRG), and Ultrafree- MC filter units (0.22 µm, for centrifugal filtration) were supplied by Millipore, (Neu-Isenburg, FRG).

An 0.2 M phosphate buffer solution (pH 7.4) was prepared by mixing 95 ml 0.4 M phosphate (27.6 g $NaH_2PO_4 \cdot H_2O$ in 500 ml water) and 405 ml of 0.4 M phosphate (71.6 g $NaH_2PO_4 \cdot 12\ H_2O$ in 500 ml water) and diluting this solution with water to 1000 ml.

Standard Solutions

(a) Plasma: 4.34 mg enoxacin sesquihydrate (= 4 mg parent compound) and 2 mg oxo-enoxacin were dissolved in 1 ml methanol and 50 µl 1 N NaOH using ultrasonication and brought to a final volume of 25 ml with 0.2 M phosphate buffer pH 7.4. This stock solution was diluted with control plasma to obtain a series of plasma standards in the concentration range 0.02 to 3.2 µg/ml for enoxacin and 0.05 to 1.6 µg/ml for the oxo-metabolite. The internal standard solution was prepared by dissolving 1 mg ciprofloxacin (= 0.85 mg parent compound) in 20 ml methanol.

(b) Urine: 5.43 mg enoxacin sesquihydrate (= 5 mg parent compound and 2 mg oxo-enoxacin were dissolved in 1 ml methanol and 50 µl 1 N NaOH using ultrasonication and brought to a final volume of 20 ml with control urine. Further dilutions with urine were made to provide urine standards in the concentration range 0.5 to 125 µg/ml (enoxacin) and 1 to 50 µg/ml (oxo-enoxacin). The internal standard solution consisted of 5 mg ciprofloxacin (= 4.25 mg parent compound) dissolved in 10 ml methanol.

Plasma and urine standards were stored frozen at -20°C until assayed. Methanolic internal standard solutions were kept at +4°C and renewed in weekly intervals.

Quality Control Samples

Independent of calibration standards, three sets of quality control samples were prepared in plasma and urine. Concentrations of enoxacin/oxo-enoxacin in plasma were 2/1, 0.5/0.25, and 0.1/0.05 µg/ml, and concentrations in urine were 100/40, 20/8 and 5/2 µg/ml, respectively. Control samples were divided into suitable aliquots, stored frozen at -20°C, and used for both prestudy validation and sample analysis.

In addition, three sets of control samples in the low concentration range were prepared to bracket the limit of quantita-

tion in plasma. Concentrations were 0.035/0.050, 0.015/0.025 and 0.01/0.0175 µg/ml for enoxacin and oxo-enoxacin, respectively.

Assay Procedure

(a) Plasma: 0.2 ml plasma was mixed with 20 µl internal standard solution, and 50 µl of a freshly prepared mixture of acetonitrile/perchloric acid (4+1) was added dropwise, with intermittent vortex-mixing. Vortex-mixing was continued for 30 seconds at high speed and followed by centifugation for 2 min at 10,000 x g. The clear supernate was removed, filtered through a 0.22 µm Ultrafree-MC filter unit, and a 50 µl aliquot was injected onto the column.

(b) Urine: A simple dilution of urine with water (1:50 or 1:100) is often sufficient for sample pretreatment before directly injecting aliquots onto the analytical column. However, when there are low drug concentrations and, moreover, concentrated urines, a further sample clean-up proved to be necessary. Thus, 500 µl of a 1:2 dilution of citrate buffer pH 6, 250 µl urine, and 50 µl internal standard solution were applied to Bond-Elut SCX cartridges (500 mg, 2.8 ml), which were preconditioned with one column volume of methanol and one column volume of water. The sorbent was washed with 2 x 1 ml water and drugs were eluted with 3 x 1 ml of a mixture of ammonium perchlorate (1 M in water)/acetonitrile (4:1.5). Following dilution with equal amounts of water (to adjust the fraction of acetonitrile to mobile phase conditions), 50-100 µl aliquots were injected into the chromatographic system.

Chromatographic System

The following system was used for HPLC: Series 10 liquid chromatograph (Perkin-Elmer, Überlingen, FRG) fitted with a model 231 autosampler (Gilson/Abimed, Langenfeld, FRG); Spectroflow 773 UV detector (Kratos, Karlsruhe, FRG), which was operated at

340 nm; PE Nelson Analytical Chromatography Workstation 2600 with Nelson Analytical Interface 970 (Nelson / ESWE, Sinsheim, FRG); 125 x 4.6 mm stainless steel column filled with Nucleosil C-18, 5µ (Grom, Herrenberg, FRG).

The mobile phase consisted of the following components: 860 ml 0.1 M citric acid, 140 ml acetonitrile, 6.5 ml tetrabutylammonium hydroxide (20% in water), and 4.5 g ammonium perchlorate. The flow rate was 1ml/min at ambient temperature.

Validation Procedure and Data Analysis

Three sets of calibration standards, quality controls, and low concentration standards were run in triplicate on three separate days. Calibration curves were constructed by plotting peak height ratios of the calibration samples against concentration. The best-fit straight line was determined by least-squares linear regression analysis of the calibration data using a weighting factor of 1/concentration squared. Concentrations of drug and metabolite in quality controls and unknown samples were calculated using the regression parameters.

Relative standard deviations (RSD) and relative errors (RE) of the mean values were determined to assess the reliability and overall performance of the method. A limit of 10% was established for these parameters as the basis for acceptance of the validation data.

RESULTS

The analytical method was validated by examining specificity, linearity, precision, accuracy, sensitivity and recovery.

Specificity

Typical chromatograms of human plasma samples obtained from blank plasma, spiked plasma (0.8 µg/ml enoxacin, 0.4 µg/ml oxo-

enoxacin and internal standard), and from a sample taken 9 hours after administration of a single oral 400-mg dose of enoxacin are shown in Figure 2. Chromatograms of blank urine (with and without sample clean-up), spiked urine (25 µg/ml enoxacin and 10 µg/ml oxo-enoxacin), and a sample from a 12-24 hour urine fraction of a subject having received a single 400-mg dose of enoxacin are illustrated in Figure 3.

The retention times for enoxacin, ciprofloxacin (internal standard), and oxo-enoxacin were approximately 3.2, 4.9, and 6.7 min, respectively. No significant interferences were detected in plasma; interferences from endogenous compounds observed in concentrated urins could be minimized by the clean-up procedure described above (Figure 3a).

The quinolones norfloxacin, rosoxacin, ofloxacin, pefloxacin and cinoxacin, the retention times of which are compiled in Table 1, were also tested for possible interferences. Some of these substances elute close to the internal standard ciprofloxacin, whereas enoxacin and its oxo-metabolite are well separated. Therefore, ciprofloxacin as internal standard may easily be replaced by any of these compounds, if necessary, and it should also be possible to quantitate other quinolones using the described method.

Linearity

The response of the amount of enoxacin and oxo-enoxacin added to blank plasma and urine was found to be linear for plasma concentrations ranging from 0.02 to 3.2 and 0.05 to 1.6 µg/ml and for urine concentrations ranging from 0.5 to 125 and 1 to 50 µg/ml, respectively (Tables 2 and 3). The best-fit line was obtained using linear regression analysis with weighting factors of $1/\text{concentration}^2$. The results of a typical regression analysis for enoxacin and oxo-enoxacin in plasma were $y = 0.8548x + 0.0015$ ($r = 0.9999$) and $y = 0.2917x + 0.0002$ ($r = 0.9998$). In-

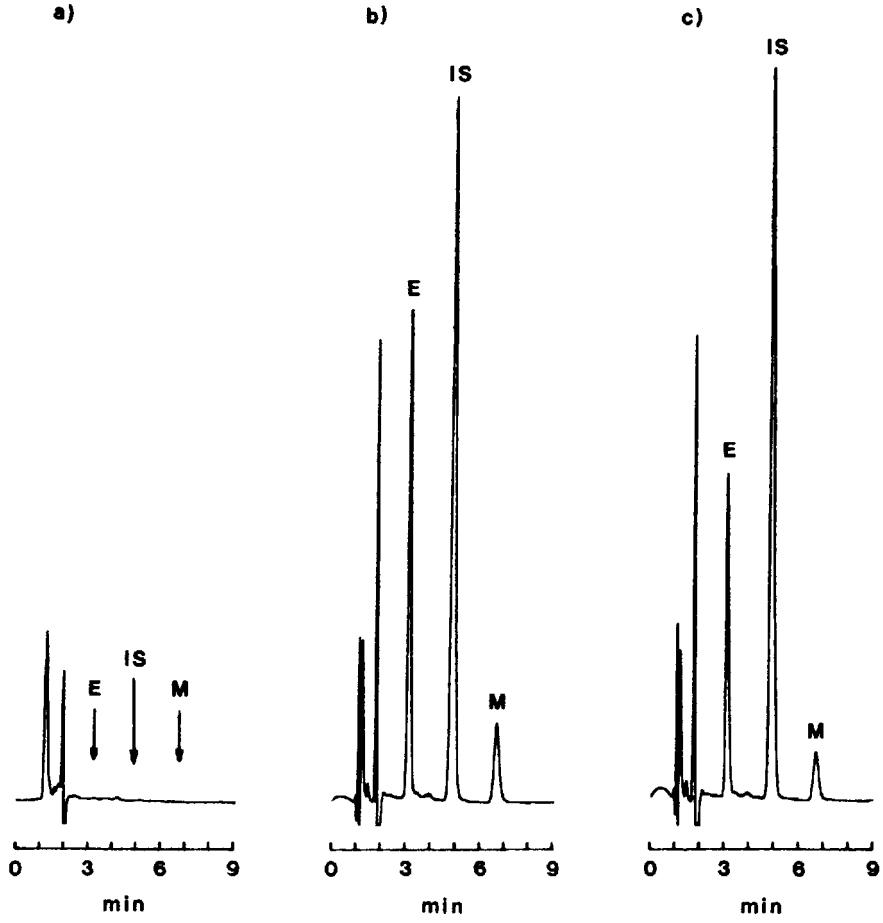

FIGURE 2: Representative chromatograms of enoxacin (E), its oxo-metabolite (M) and the internal standard ciprofloxacin (IS) in plasma: a) human blank plasma, b) 0.8 µg/ml enoxacin and 0.4 µg/ml oxo-enoxacin plasma calibration standard and c) plasma sample of a subject 9 h after oral administration of 400-mg single dose of enoxacin, containing 0.51 µg/ml enoxacin and 0.23 µg/ml oxo-enoxacin

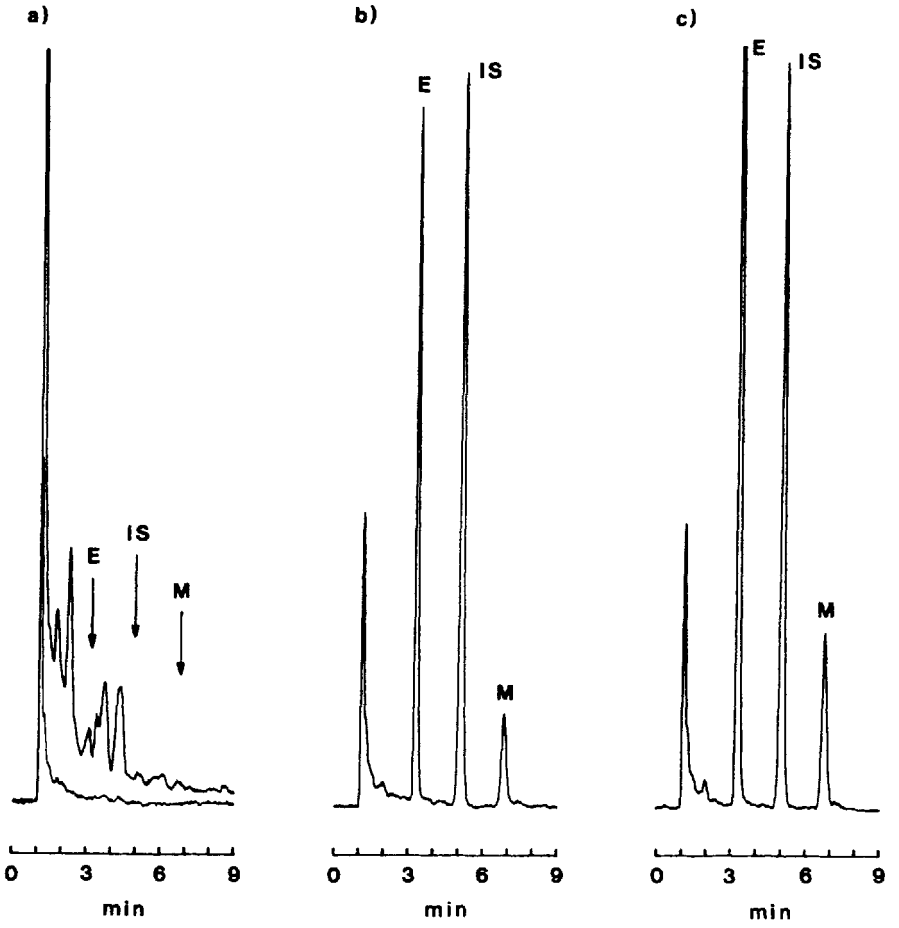

FIGURE 3: Representative chromatograms of enoxacin (E), its oxo-metabolite (M) and the internal standard ciprofloxacin (IS) in urine: a) human blank urine, injected after 1:50 dilution with water (1), and after solid-phase clean-up (2), b) 25 µg/ml enoxacin and 10 µg/ml oxo-enoxacin plasma calibration standard and c) 12-24 h urine sample of a subject after receiving a single 400-mg dose of enoxacin; peaks amount to a concentration of 87.5 µg/ml enoxacin and 18.5 µg/ml oxo-metabolite

TABLE 1

Quinolones Tested for Possible Interferences with Enoxacin and Oxo-enoxacin

Quinolone	Retention Time (min)
Enoxacin	3.2
Norfloxacin	4.1
Rosoxacin	4.4
Ofloxacin	4.5
Ciprofloxacin	4.9
Pefloxacin	5.2
Oxo-Enoxacin	7.3
Cinoxacin	11.5

tercepts were not significantly different from zero. The results of the analysis in urine were similar.

Precision and Accuracy

Calibration curve precision (represented by the relative standard deviation of the mean of replicate analyses) and accuracy (relative difference between experimental and theoretical concentration) were assessed by assaying triplicates of each plasma and urine calibration standard on each of three consecutive days. Mean values of the backcalculated concentrations of the calibration standards are summarized in Table 2 (plasma) and Table 3 (urine); relative standard deviations were below 5% and 3% and relative errors were less than 2% and 3% for the plasma and urine assay, respectively.

To evaluate method precision and accuracy, three sets of quality control samples in plasma and urine were analyzed in triplicate over a three-day period. Drug concentrations were then calculated using the regression parameters of the corresponding calibration curves. Mean enoxacin and oxo-enoxacin plas-

TABLE 2

Plasma Calibration Curve Precision and Accuracy

	Enoxacin			Oxo-enoxacin		
Added [μg/ml]	Found* [μg/ml]	R.S.D. [%]	R.E. [%]	Found* [μg/ml]	R.S.D. [%]	R.E. [%]
0.02	0.020	4.95	-0.16			
0.05	0.051	3.09	+1.37	0.050	4.65	+0.53
0.10	0.099	1.98	-0.78	0.099	4.11	-1.48
0.20	0.199	3.00	-0.46	0.202	2.45	+0.87
0.40	0.398	2.17	-0.42	0.398	2.28	-0.43
0.80	0.800	1.58	-0.02	0.814	2.48	+1.74
1.60	1.63	2.50	+1.71	1.58	2.07	-1.16
3.20	3.16	1.26	-1.31			

* mean of 9 replicate analyses performed over a three-day period
R.S.D. = relative standard deviation, R.E. = relative error

TABLE 3

Urine Calibration Curve Precision and Accuracy

	Enoxacin			Oxo-enoxacin		
Added [μg/ml]	Found* [μg/ml]	R.S.D. [%]	R.E. [%]	Found* [μg/ml]	R.S.D. [%]	R.E. [%]
0.500	0.507	1.52	+1.35			
1.00	0.987	1.83	-1.33	1.01	0.13	+0.45
2.00				1.99	0.64	-0.68
2.50	2.44	0.94	-2.28			
5.00	4.94	1.08	-1.16	4.94	2.20	-1.30
10.0				10.1	1.99	+0.91
12.5	12.4	2.18	-0.41			
20.0				20.2	2.33	+1.07
25.0	25.2	1.31	+0.67			
50.0	51.0	1.23	+2.06	50.1	1.57	+0.12
125.0	128.1	1.67	+2.50			

* mean of 9 replicate analyses performed over a three-day period
R.S.D. = relative standard deviation, R.E. = relative error

TABLE 4

Method Precision and Accuracy
for Enoxacin and Oxo-enoxacin in Plasma

	Enoxacin			Oxo-enoxacin		
Added [µg/ml]	Found* [µg/ml]	R.S.D. [%]	R.E. [%]	Found* [µg/ml]	R.S.D. [%]	R.E. [%]
0.05				0.049	6.87	-1.86
0.10	0.101	3.08	+1.01			
0.25				0.249	2.98	-0.24
0.50	0.503	2.20	+0.68			
1.00				0.960	2.66	-4.38
2.00	2.03	2.41	+1.26			

* mean of 9 replicate analyses performed over a three-day period
R.S.D. = relative standard deviation, R.E. = relative error

TABLE 5

Method Precision and Accuracy
for Enoxacin and Oxo-enoxacin in Urine

	Enoxacin			Oxo-enoxacin		
Added [µg/ml]	Found* [µg/ml]	R.S.D. [%]	R.E. [%]	Found* [µg/ml]	R.S.D. [%]	R.E. [%]
2.00				2.05	3.11	+2.49
5.00	4.99	1.76	-0.28			
8.00				8.25	3.06	+3.18
20.0	20.1	1.09	+0.49			
40.0				41.6	1.98	+4.01
100.0	103.2	0.55	+3.19			

* mean of 9 replicate analyses performed over a three-day period
R.S.D. = relative standard deviation, R.E. = relative error

TABLE 6

Evaluation of the Limit of Quantitation in Plasma
Method Precision and Accuracy in the Low Concentration Range

	Enoxacin			Oxo-enoxacin		
Added [µg/ml]	Found* [µg/ml]	R.S.D. [%]	R.E. [%]	Found* [µg/ml]	R.S.D. [%]	R.E. [%]
0.010	0.0105	9.51	+5.12			
0.015	0.0144	6.47	-4.29			
0.0175				0.0177	9.29	+1.04
0.025				0.0253	8.31	+1.10
0.035	0.0344	3.61	-1.68			
0.050				0.0491	6.87	-1.86

* mean of 9 replicate analyses performed over a three-day period
R.S.D. = relative standard deviation, R.E. = relative error

ma and urine concentrations are given in Tables 4 and 5. Overall relative standard deviations were less than 7% and 4% with relative errors below 5% for plasma and urine, respectively.

Sensitivity

Three sets of control plasma samples in the low concentration range were assayed in triplicate on three different days to determine the limit of quantitation, defined as the concentration at which replicate analysis meets a specified precision and accuracy of 10% or less. Using injection volumes of 50µl, the limits of quantitation for enoxacin and oxo-enoxacin in plasma were 0.01 and 0.0175 µg/ml, respectively (Table 6).

The limit of quantitation in urine is somewhat dependent on the purity of the urine, even after clean-up by solid-phase extraction. Therefore, the concentration of the lowest calibration

standard (0.5 µg/ml for enoxacin and 1 µg/ml for oxo-enoxacin) was used as the limit of quantitation in urine.

Recovery

Analytical recoveries of enoxacin, oxo-enoxacin, and the internal standard ciprofloxacin from plasma and urine were determined by assaying plasma and urine quality control samples using the same procedure as described above, but additionally adding norfloxacin as internal standard after the clean-up procedure. Six determinations were made at each concentration. Resulting peak height ratios were then compared with those obtained after direct injection of aqueous standard solutions of the same concentration according to the following equation:

$$\text{Recovery} = \frac{\text{peak height ratio drug/norflox. in plasma/urine}}{\text{peak height ratio drug/norflox. in aqueous std.}} \times 100$$

Recoveries of enoxacin, oxo-enoxacin, and the internal standard ciprofloxacin from plasma were 92.9±2.3, 62.3±2.7, and 76±2.1%, respectively, and recoveries from urine were 79±2.0, 99±1.1, and 78±1.8%, respectively. Plasma and urine recoveries were independent of drug concentration.

Applicability of the Method

The new HPLC method has been applied to numerous pharmacokinetic studies of enoxacin in various animal species (rat, dog, rabbit) as well as in human volunteers and patients. A typical plasma concentration versus time profile of both enoxacin and oxo-enoxacin following administration of a single oral 400-mg dose of enoxacin to a human volunteer is shown in Figure 4.

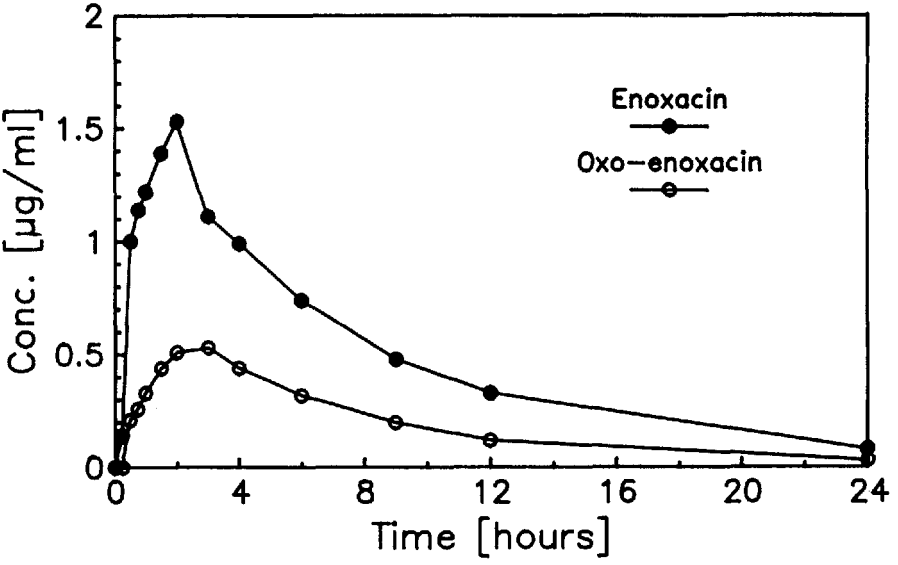

FIGURE 4: Plasma concentration versus time profile of enoxacin (●) and oxo-enoxacin (o) following administration of a single 400-mg oral dose of enoxacin to a human subject

DISCUSSION

This paper describes a simple, reproducible, and sensitive HPLC assay method for the simultaneous determination of enoxacin and oxo-enoxacin in biological fluids.

As has already been demonstrated for different quinolone plasma assays [5,7,8,14,15], a simple protein precipitation step is sufficient for sample pretreatment. The perchloric acid/acetonitrile mixture used as precipitating agent in this assay was found to be superior to perchloric acid, acetonitrile, or methanol alone. Perchloric acid alone was very efficient in removing plasma proteins, but drug recovery was lower due to increased co-precipitation. By contrast, relatively high amounts of the

organic solvents are required to completely remove plasma proteins (at least 1.5-3 ml per ml of plasma [16]), resulting in an appreciable dilution of the sample. Therefore, an additional evaporation step often becomes necessary before sample injection in order to increase assay sensitivity and/or to adapt the solvent composition to mobile phase conditions.

Ion-pairing chromatography using tetrabutylammonium ions has already been reported to significantly improve peak shape and resolution of ciprofloxacin [17,18]. This effect was also observed for enoxacin, whereas oxo-enoxacin continued to elute comparatively late with bad peak tailing. Both problems were overcome by adding perchlorate to the mobile phase. The oxo-metabolite was then eluted as a sharp, symmetrical peak at a retention time close to that of enoxacin and the internal standard ciprofloxacin.

The chromatographic performance, especially peak shape and height of the oxo-metabolite, was also dependent on the stationary phase used. Best resolutions were obtained with Nucleosil C18, whereas Lichrosorb RP18 and Spherisorb ODS 2 in that order gave less satisfactory results.

The mobile phase was prepared in 2-L batches and recycled, thus providing a stable, cost-effective system which not only economizes on solvents, but also prolongs column life [19]. The mobile phase was discarded when peak-broadening occurred, which was usually after 4-6 weeks of operation, depending on the number of samples processed.

The enoxacin and oxo-enoxacin calibration curves in urine do not cover the entire concentration range observed following therapeutic doses. Since oxo-enoxacin in particular is poorly soluble in aqueous solutions, recrystallization occurred from urine calibration standards containing more than 175 and 70 µg/ml enoxacin and oxo-enoxacin, respectively. (The solubility

of oxo-enoxacin alone is even lower, but is increased in the presence of enoxacin.) By contrast, crystallization has not been observed in urine samples from patients and volunteers, even at concentrations as high as 300 µg/ml of enoxacin and 100 µg/ml of oxo-enoxacin. Therefore, samples with drug concentrations above that of the highest calibration standard were diluted with blank biomatrix to fit in with the calibration curve and reassayed.

Since the quality of pharmacokinetic data evaluation and interpretation is strongly dependent on the quality of the analytical method employed, most regulatory agencies have mandated Good Laboratory Practice (GLP) regulations also for the validation of chromatographic methods [13]. Therefore, great importance has been attached to carefully determining the performance characteristics and limitations of the method, i.e. evaluation of specificity, linearity, precision, accuracy, sensitivity and recovery. The validation procedure is performed by each analyst on a given instrument before sample analysis is undertaken (prestudy validation) and the same pools of quality controls are assayed during sample analysis to document the quality of the final analytical results, applying the same acceptance criteria (sample analysis validation).

In conclusion, the assay described proved to be well suited for the simultaneous determination of enoxacin and its metabolite oxo-enoxacin in plasma and urine samples derived from pharmacokinetic studies. The method should also be useful for the analysis of other quinolones with similar structures.

REFERENCES

1. Hooper DC, Wolfson JS: The fluoroquinolones: Pharmacology, clinical uses, and toxicities in humans. Antimicrob. Agents Chemother. 28, 716 (1985)

2. Janknegt R: Fluorinated quinolones: A review of their mode of action, antimicrobial activity, pharmacokinetics and clinical efficacy. Pharm. Weekbl. [Sci] 8, 1 (1986)

3. Henwood JM, Monk JP: Enoxacin. A review of its antibacterial activity, pharmacokinetic properties and therapeutic use. Drugs 36, 32 (1988)

4. Yamaguchi T, Suzuki R, Sekine Y: Pharmacokinetics of a new antibacterial agent AT-2266 III. Plasma levels and urinary excretion of AT-2266 and its metabolites in man. Chemotherapy 32/3, 109 (1984)

5. Vree TB, Baars AM, Wijnands WJA: High-performance liquid chromatography and preliminary pharmacokinetics of enoxacin and its 4-oxo metabolite in human plasma, urine and saliva. J. Chromatogr. 343, 449 (1985)

6. Wise R, Lockley R, Webberly M, Adhami ZN: The pharmacokinetics and tissue penetration of enoxacin and norfloxacin. J. Antimicrob. Chemother. 14/C, 75 (1984)

7. Manoharan M, Jaehde U, Koch HU, Malter U, Naber K, Sörgel F: High-performance liquid chromatographic assays for gyrase inhibitors in plasma, urine, several body fluids and tissues Rev. Infec. Dis. 10/1, 98 (1988)

8. Rogge MC, Solomon WR, Sedman AJ, Welling PG, Toothaker RD, Wagner JG: The theophylline-enoxacin interaction: I. Effect of enoxacin dose size on theophylline disposition. Clin. Pharmacol. Ther. 44, 579 (1988)

9. Nakamura R, Yamaguchi T, Sekine Y, Hashimoto M: Determination of a new antibacterial agent (AT-2266) and its metabolites in plasma and urine by high-performance liquid chromatography. J. Chromatogr. 278, 321 (1983)

10. Van der Auwera P, Martin A: High-performance liquid chromatography for the assay of enoxacin in serum samples and exsudates from burned patients. Rev. Infec. Dis. 10/1, 100 (1988)

11. White LO, Bowyer HM, McMullin CH, Desai K: Assay of enoxacin in human serum and prostatic tissue by HPLC. J. Antimicrob. Chemother. 21/4, 512 (1988)

12. Dadgar D, Smyth MR: Validation of chromatographic methods of analysis of drugs in biological samples. Trends Anal. Chem. 5, 115 (1986)

13. Pachla LA, Wright DS, Reynolds D: Bioanalytic considerations for pharmacokinetic and biopharmaceutic studies. J. Clin. Pharmacol. 26, 332 (1986)

14. Nix DE, De Vito JM, Schentag JJ: Liquid-chromatographic determination of ciprofloxacin in serum and urine.
Clin. Chem. 31/5, 684 (1985)

15. Borner K, Lode H, Höffken G, Prinzing C, Glatzel P, Wiley R: Liquid chromatographic determination of ciprofloxacin and some metabolites in human body fluids.
J. Clin. Chem. Clin. Biochem. 24, 325 (1986)

16. Blanchard J: Evaluation of the relative efficacy of various techniques for deproteinizing plasma samples prior to high-performance liquid chromatographic analysis.
J. Chromatogr. 226, 455 (1981)

17. Jehl F, Gallion C, Debs J, Brogard JM, Monteil H, Minck R: High-performance liquid chromatographic method for determination of ciprofloxacin in biological fluids.
J. Chromatogr. 339, 347 (1985)

18. Gau W, Ploschke HJ, Schmidt K, Weber B: Determination of ciprofloxacin (Bay o 9867) in biological fluids by high-performance liquid chromatography.
J. Liquid Chromatogr. 8/3, 485 (1985)

19. Lensmeyer GL, Evenson MA: Stabilized analysis of antidepressant drugs by solvent-recycled liquid chromatography: Procedure and proposed resolution mechanisms for chromatography.
Clin. Chem. 30/11, 1774 (1984)