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Sensitive LC determination of ciprofloxacin in pharmaceutical preparations and biological fluids with fluorescence detection

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Abstract

A simple and highly sensitive isocratic reversed-phase high-performance liquid chromatographic method (RP-HPLC) has been developed for the determination of ciprofloxacin. Separation of ciprofloxacin and anthranilic acid (internal standard) was achieved on a Kromasil 100, C_{18}, 5 μm (250 × 4.6 mm i.d.) reversed-phase column, using fluorescence detection with $\lambda_{exc} = 300$ nm and $\lambda_{emi} = 458$ nm. The mobile phase consisted of acetonitrile–methanol–acetate buffer (pH 3.60; 0.05 M) (10:30:60 v/v/v) containing 1% v/v acetic acid. The analysis was performed in less than 9 min, with a flow rate of 0.8 ml min^{-1}. A rectilinear relationship was observed for concentrations between 0.005 and 1.0 μg ml^{-1} of ciprofloxacin in aqueous standard solutions and serum and the detection limit was 20 pg injected on-column. The intra- and inter-day relative standard deviation ($n = 8$) ranged from 1.6 to 2.6% and from 1.9 to 4.8%, respectively, calculated at three concentration levels of standard solutions. Direct measurements of ciprofloxacin in pharmaceutical preparations and in serum, after precipitation of proteins, were performed with high precision and accuracy. The application of the method to urine samples involved a solid-phase extraction treatment of the samples using C_{18} cartridges. The linear working range in urine extended from 0.05 to 2.0 μg ml^{-1} and the detection limit was 0.2 ng injected on-column. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Ciprofloxacin; Reversed-phase liquid chromatography; Fluorescence detection; Pharmaceutical preparations; Biological fluids

1. Introduction

Ciprofloxacin, 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolone carboxylic acid (see Fig. 1) is a relatively new, second-generation fluoroquinolone antibiotic with an extended antibacterial spectrum, which includes Gram-negative and Gram-positive bacteria [1–4]. Stability studies [5,6] performed in ciprofloxacin, utilizing HPLC with UV detection, have revealed that the molecule is heat- and humidityproof, but it is sensitive to photodegrada-

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tion at slightly basic pH, where the drug is in the zwitterionic form. The stability increases considerably when the pH is lowered towards 3–4. The main degradation product identified is the desethyleneciprofloxacin. The mechanism of action of ciprofloxacin is the inhibition of DNA gyrase, an enzyme that is critical to bacterial chromosome replication. Ciprofloxacin, like enoxacin, ofloxacin, norfloxacin, pefloxacin and cinoxacin are all quinoline derivatives that are structurally related to nalidixic acid and although they have a parallel spectrum of activity, ciprofloxacin seems to be more potent than the other quinoline derivatives [7]. Ciprofloxacin is used in a wide range of infections of the respiratory, urinary and gastrointestinal tract, as well as in skin and soft tissue infections [8]. Recently there has been interest in the administration of a single drug, substituting drug combinations, for the treatment of occular infections [9]. Ciprofloxacin exhibits a fast onset of action and lacks crossreactivity with penicillins, cephalosporins and aminoglycosides [10].

Treatment with ciprofloxacin includes intravenous infusions containing 100–200 mg of the drug over a period of 30–60 min or tablets containing 250–750 mg. Initial intravenous administration may be followed by treatment with oral ciprofloxacin [11]. Usually, after a single oral dose of 250 mg ciprofloxacin, the mean concentration of the drug in plasma is approximately 2 $\mu g\ ml^{-1}$, while after the administration of a 3 $mg\ ml^{-1}$ ophthalmic solution of ciprofloxacin, its concentration levels in plasma range up to 5 $ng\ ml^{-1}$.

Several RP-HPLC methods have been reported for the analysis of ciprofloxacin in biological fluids. Some of these methods have employed UV detection [7,8,12–15], while the others have employed fluorescence detection [16–22]. A number of the above publications suggest the use of ion-pair HPLC [7,12,17–19]. In several of these works, the simultaneous determination of ciprofloxacin and one or more of its metabolites is reported [8,16–18,20]. To our knowledge, no HPLC method has been reported so far for the determination of ciprofloxacin in pharmaceutical preparations.

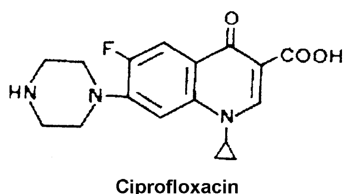

Fig. 1. Structure of ciprofloxacin.

In this paper, a highly sensitive and simple RP-HPLC method is described, which presents for the first time in the literature a direct and selective determination of ciprofloxacin in pharmaceutical preparations. The method was proved very selective in serum matrices as well and was therefore applied directly to the analysis of serum samples after protein precipitation. The determination of ciprofloxacin in non-diluted urine samples, after a solid-phase extraction (SPE) pretreatment, as a clean-up step, yielded very satisfactory recoveries of the drug. To our knowledge, no SPE treatment of undiluted urine samples has been reported previously in the literature for the determination of ciprofloxacin. Most of the published procedures make use of tedious and time consuming liquid–liquid extractions. The procedure described in this paper combines the novelty of the SPE treatment of urine and the use of undiluted samples, which increases the sensitivity of the assay, and presents for the first time the analysis of pharmaceutical preparations, making use of a simple mobile phase with no use of ion-pairing agents.

2. Experimental

2.1. Reagents and materials

Ciprofloxacin hydrochloride was purchased from Sigma (St. Louis, MO, USA). Anthranilic acid (the internal standard), acetic acid 100%, sodium hydroxide Titrisol 1M and HPLC-grade methanol and acetonitrile were all obtained from Merck (Darmstadt, Germany). Stock solutions of ciprofloxacin and internal standard were prepared in double-deionized water and kept refrigerated. The acetate buffer (pH 3.6; 0.05 M) was prepared by mixing 650 ml of 1 M acetic acid solution and 50 ml of 1 M sodium hydroxide solution and

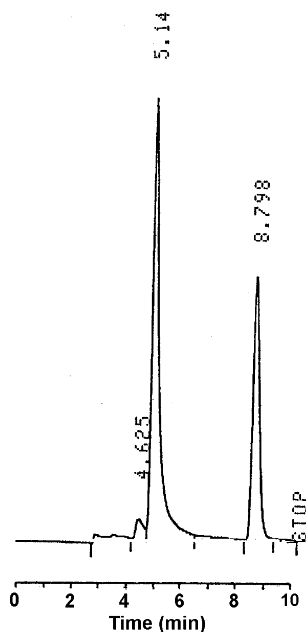

Fig. 2. Chromatogram of a standard 0.212 μg ml^{-1} ciprofloxacin solution (5.14 min) with the internal standard (8.798 min); conditions as described in Section 2; chart speed = 5 mm min^{-1}; attenuation = 7.

diluted to 1 l with double-deionized water. All the reagents used were of analytical reagent grade.

The RP-18 (200 mg per 3 ml) SPE Lichrolut cartridges, used for the treatment of urine samples, were also obtained from Merck. Membrane 0.2 μm filters, used for the filtration of buffer and samples, were obtained from Schleicher and Schuell (Dassel, Germany).

2.2. Instrumentation

The HPLC system consisted of a Shimadzu LC-9A isocratic pump (Kyoto, Japan), equipped with a Rheodyne 7725 injection valve (California, USA) with a 20-μl loop, a Shimadzu RF-551 spectrofluorometric detector and a Shimadzu Chromatopac C-R6A integrator. The analytical column was a reversed-phase Kromasil 100, C_{18}, 5 μm (250 × 4.6 mm i.d.) obtained from MZ Analysentechnik (Mainz, Germany).

A glass vacuum solvent-filtration apparatus, obtained from Altech Associates Inc. (Deerfield, IL, USA) and a stainless steel syringe filter-holder (model FM 025/0) obtained from Schleicher and Schuell were used for the filtration of the buffer solution and the samples, respectively, using 0.2 μm filters of appropriate diameter.

A Glass-col, Terre Haute in 47802 small vortexer and a Hermle centrifuge, model Z230 (B. Hermle AG, Gosheim, Germany) were used for the sample pretreatment.

The SPE of urine samples was performed on a Vac-Elut system, having a ten-position capacity, obtained from Analytichem International, a Division of Varian (Harbor City, USA) and the evaporation of solvents was carried out in a waterbath under nitrogen, by means of a model 18780 Reacti-Vap device (Pierce, Rackford, IL, USA).

2.3. Chromatographic conditions

The analysis was performed at ambient temperatures under isocratic conditions and ciprofloxacin was detected fluorometrically ($\lambda_{exc} = 300$ nm and $\lambda_{emi} = 458$ nm) with a gain setting of × 1. The mobile phase consisted of a mixture of acetonitrile–methanol–acetate buffer (pH 3.6; 0.05 M) (10:30:60 v/v/v) containing 1% v/v acetic acid and was delivered at a flow-rate of 0.8 ml min^{-1}. Under these conditions, the approximate retention times of standard ciprofloxacin and the internal standard were 5.1 and 8.8 min, respectively, as shown in the chromatogram of Fig. 2.

2.4. Establishment of calibration curves

2.4.1. In standard solutions

Working aqueous solutions of ciprofloxacin–HCl at concentrations of 0.00530, 0.0106, 0.0530, 0.106, 0.212, 0.530 and 1.06 μg ml^{-1}, containing the internal standard (anthranilic acid) at a fixed concentration of 0.1 μg ml^{-1} were prepared by serially diluting the stock 106 μg ml^{-1} ciprofloxacin–HCl solution in 50-ml volumetric flasks and adding a 5-ml volume of anthranilic acid (1.0 μg ml^{-1}) to all working solutions.

Six replicate injections of each working standard were made into the chromatograph and the mean values of the peak area ratios of ciprofloxacin relative to the internal standard were plotted against concentration.

2.4.2. In serum

Standard serum samples of 0.00505, 0.0101, 0.0505, 0.101, 0.202, 0.505 and 1.01 $\mu g\,ml^{-1}$ in ciprofloxacin were prepared by spiking aliquots of drug-free pooled serum with aqueous standard ciprofloxacin–HCl solutions. A blank sample was also prepared. Treatment of samples was performed as described below. The standard additions' calibration curve was constructed by plotting the reported peak area ratios (means of six determinations per standard) against ciprofloxacin concentrations.

2.4.3. In urine

Standard urine samples of 0.0505, 0.101, 0.202, 0.505, 1.01 and 2.02 $\mu g\,ml^{-1}$ in ciprofloxacin were prepared by spiking aliquots of drug-free pooled urine with aqueous standard ciprofloxacin–HCl solutions. A blank sample was also prepared and the samples were treated as described below. The standard additions' calibration curve was constructed by plotting the reported peak area ratios (means of six determinations per standard) versus ciprofloxacin concentration.

2.5. Sample treatment

2.5.1. Pharmaceutical preparations

2.5.1.1. Tablets. Ten tablets of 500 mg (labeled amount) in ciproflocaxin (as a base) were weighed and their mean weight was found to be 0.7620 g. The tablets were finely powdered and a portion of the tablet composite equal to the mean weight was quantitatively transferred to a 250-ml volumetric flask and diluted to volume with a 1:1 mixture of methanol–water. The resulting solution, having a labeled concentration of 2000 $\mu g\,ml^{-1}$ in ciprofloxacin base, was serially diluted in 50-ml volumetric flasks with double-deionized water. Three solutions of 0.080, 0.120 and 0.240 $\mu g\,ml^{-1}$ (labeled concentration) in ciprofloxacin base, all containing the same fixed concentration of anthranilic acid (0.1 $\mu g\,ml^{-1}$) were prepared. The mean values of measured peak area ratios (six injections per concentration) of ciprofloxacin relative to the internal standard were employed for quantitation, based on the calibration curve for standard solutions. In order to evaluate the accuracy of the method, the recovery of ciprofloxacin from spiked mixtures was calculated. Aliquots of a 0.120 $\mu g\,ml^{-1}$ synthetic tablet solution were spiked with known quantities of standard ciprofloxacin at amounts equivalent to 50, 100 and 150% of the nominal content. The resulting solutions containing 0.180, 0.240 and 0.300$\mu g\,ml^{-1}$ of ciprofloxacin were then analyzed by the method with six repetitions each.

2.5.1.2. Ophthalmic solution. A 1-ml volume of the ophthalmic solution 3 $mg\,ml^{-1}$ (or 0.3% labeled concentration) in ciprofloxacin base was quantitatively transferred to a 100-ml volumetric flask and diluted to volume with double-deionized water. The resulting solution, having a labeled concentration of 30 $\mu g\,ml^{-1}$ in ciprofloxacin base was diluted with double-deionized water by a factor of ten and from this dilute solution, three working solutions of 0.060, 0.150 and 0.240 $\mu g\,ml^{-1}$ (labeled concentration) in ciprofloxacin base, all containing the same fixed concentration of internal standard (0.1 $\mu g\,ml^{-1}$) were prepared in 50-ml volumetric flasks by proper dilutions with double-deionized water. The mean values of the reported peak area ratios (six HPLC measurements per solution) were employed for quantitation, based on the calibration curve for standard solutions. For the recovery study, aliquots of a 0.150 $\mu g\,ml^{-1}$ synthetic ophthalmic solution were spiked with known quantities of standard ciprofloxacin at amounts equivalent to 50, 100 and 150% of the nominal content. The resulting solutions containing 0.225, 0.300 and 0.375 $\mu g\,ml^{-1}$ of ciprofloxacin were then analyzed by the method six repeated times each.

2.5.2. Serum

Aliquots (40 μl) of drug-free pooled serum were spiked with aqueous standard ciprofloxacin–HCl solutions (100 μl) and acetonitrile (200 μl) was added to precipitate the proteins. The samples were vortex-mixed and the proteins were removed by centrifugation at 3500 rpm for 15 min and subsequent transfer of supernatants to clean eppendorf vials. The supernatants were evaporated

to dryness under a stream of nitrogen in a water-bath at 45 °C and the residues were reconstituted with a 100-μl volume of an aqueous 0.1 $\mu g\,ml^{-1}$ solution of anthranilic acid. Repetitive analyses ($n = 6$) of the resulting solutions at each concentration level were performed.

2.5.3. Urine

Aliquots (100 μl) of drug-free pooled urine were spiked with aqueous standard ciprofloxacin–HCl solutions (100 μl) and acetonitrile (200 μl) was added. The samples were vortex-mixed and subsequently centrifuged at 3500 rpm for 15 min. The supernatants were quantitatively transferred to clean eppendorf vials and the organic solvents were evaporated at 45 °C under nitrogen. The remaining aqueous supernatants were then applied to C_{18} SPE cartridges, preconditioned with consecutive one volume of methanol and one volume of double-deionized water. The wash step was carried out by means of two volumes of double-deionized water under a slight vacuum. Ciprofloxacin was eluted by means of one volume of methanol and the eluates were evaporated to dryness under nitrogen at 45 °C. The residues were reconstituted with a 100-μl volume of an aqueous 0.1 $\mu g\,ml^{-1}$ solution of anthranilic acid and the resulting solutions were repeatedly analyzed ($n = 6$) at each concentration level.

Table 1
Regression analysis equations for ciprofloxacin

Samples	Regression equation $Y = (a \pm SD_a) + (b \pm SD_b)X$	Correlation coefficient
Standard solutions	$Y = (0.2445 \pm 0.0521) + (6.683 \pm 0.114)X$	0.9993
Serum	$Y = (0.0317 \pm 0.0648) + (10.84 \pm 0.149)X$	0.9995
Urine	$Y = (0.3340 \pm 0.0506) + (5.742 \pm 0.053)X$	0.9998

Y = peak area ratio of ciprofloxacin to internal standard; X = concentration in $\mu g\,ml^{-1}$; a = intercept, b = slope, SD_a, SD_b = standard deviations of intercept and slope, respectively.

3. Results and discussion

3.1. Linearity of the method and limits of detection and quantitation

Ciprofloxacin standard curves were linear in the 0.005–1.0 $\mu g\,ml^{-1}$ range in standard solutions and serum and in the 0.05–2.0 $\mu g\,ml^{-1}$ range in urine. The straight-line regression equations, describing these curves, were treated statistically and are presented with their correlation coefficients in Table 1. The limits of detection (LOD), defined as those quantities producing a signal-to-noise ratio of 2:1, were 20 pg for reference standard solutions and serum and 0.2 ng for urine, injected on-column, with a 20-μl loop. The limits of quantitation (LOQ) defined as those quantities producing a signal-to-noise ratio of approximately 10 and a standard deviation (SD) $\leq 3\%$, for a set of six measurements, were 0.1 ng for pharmaceutical preparations and serum and 1.0 ng for urine samples injected on-column with a 20-μl loop.

3.2. Precision and accuracy

In order to assess the within-day precision of the assay procedure in standard solutions, eight replicate measurements of three standard solutions at different concentration levels were performed on the same day. The between-day precision of the assay was assessed by the repeated analysis of ciprofloxacin standard solutions over a period of eight consecutive days. Each day's representative peak area ratio, for the three concentration levels tested, was the mean value of six replicate injections. The results are given in Table 2.

The precision of the assay in pharmaceutical preparations was assessed by repeatedly analyzing three solutions of different labeled concentrations, prepared separately from the tablet-composite and the ophthalmic solution and calculating the mean found concentrations with their SDs and RSDs. The results, summarized in Table 3, show a good agreement between labeled and found amounts and a high degree of precision, as can be concluded by the satisfactory RSD values. The accuracy of the method with respect to pharma-

Table 2
Within-day and between-day precision of assay for determination of ciprofloxacin in standard solutions

Injected quantity (ng)	Mean measured quantity ± SD (ng) [a]		RSD (%)	
	WD	BD	WD	BD
1.06	0.962 ± 0.025	1.04 ± 0.05	2.6	4.8
4.24	4.13 ± 0.10	4.15 ± 0.08	2.4	1.9
10.6	11.4 ± 0.18	10.7 ± 0.37	1.6	3.5

SD = standard deviation; RSD = relative standard deviation; WD = within-day; BD = between-day.
[a] Means of values calculated from the regression straight-line equation for eight determinations within a day (WD) and six determinations per day over a period of eight days (BD).

Table 3
Precision of Assay for determination of ciprofloxacin [a] in pharmaceutical preparations

Therapeutic form (labeled amount)	Injected labeled quantity (ng)	Mean measured quantity ± SD (ng) [b]	Quantity found in preparations ± SD [c]	RSD (%)
Tablets (500 mg)	1.60	1.59 ± 0.009	495.8 ± 3.0	0.60
	2.40	2.43 ± 0.05	506.7 ± 9.7	1.9
	4.80	4.79 ± 0.04	498.5 ± 4.4	0.88
Ophthalmic solution ($3\ mg\ ml^{-1}$)	1.20	1.19 ± 0.02	2.97 ± 0.06	2.0
	3.00	2.97 ± 0.06	2.97 ± 0.06	2.0
	4.80	4.78 ± 0.19	2.99 ± 0.12	4.0

[a] As a base.
[b] Values (means of six determinations) obtained from the regression equation for ciprofloxacin in standard solutions; other abbreviations as in Table 2.
[c] mg in tablets; $mg\ ml^{-1}$ in ophthalmic solution.

ceutical preparations was evaluated by calculating the recovery of ciprofloxacin from synthetic pharmaceutical mixtures in spiked form. Dosage–formulation assays (tablet and ophthalmic solutions) spiked with known concentrations of the active substance (at levels of 50, 100 and 150% of the target level in the preparation) were considered sufficient to represent synthetic samples. The procedure was carried out in a manner identical to that for the real sample. With previous knowledge of the amounts added, the recovery of the sample preparation was calculated. The results are presented in Table 4.

The within-day and between-day precision of the assay in serum and urine samples was assessed by running drug-free serum and urine samples repeatedly, spiked with three different concentrations of the analyte, and calculating the mean measured amounts along with their SD and RSD values. The within-day results were based on eight replicate measurements for each sample on the same day and the between-day data was assessed for a period of eight consecutive days, employing the mean peak area ratios from six injections per sample and per day. The recovery of ciprofloxacin from the spiked matrices was calculated as the ratio of measured versus injected (spiked) amount of the substance. The results are presented in Tables 5 and 6.

3.3. Selectivity

The high selectivity of the present method in serum and pharmaceutical preparations permitted their direct analysis. Chromatograms resulting from the analysis of pharmaceutical preparations

Table 4
Accuracy of assay for determination of ciprofloxacin [a] in spiked pharmaceutical preparations

Therapeutic form (synthetic mixture)	Initial quantity (ng)	Mean measured quantity ± SD (ng) [b]	Added quantity (ng)	Total quantity injected (ng)	Corresponding quantity in preparations [c]	Mean measured total quantity ± SD (ng) [b]	Quantity found in preparations ± SD [c]	RSD (%)	Recovery (%)
Tablet-solution	2.40	2.43 ± 0.05	1.20	3.60	750	3.64 ± 0.06	758.3 ± 12.5	1.6	101.1
			2.40	4.80	1000	4.84 ± 0.07	1008.3 ± 14.9	1.5	100.8
			3.60	6.00	1250	6.03 ± 0.08	1256.2 ± 16.8	1.3	100.5
Ophthalmic solution	3.00	2.97 ± 0.06	1.50	4.50	3.00	4.48 ± 0.05	4.48 ± 0.05	1.1	99.6
			3.00	6.00	6.00	5.97 ± 0.07	5.97 ± 0.07	1.2	99.5
			4.50	7.50	7.50	7.47 ± 0.08	7.47 ± 0.08	1.1	99.6

[a] As a base.
[b] Values (means of six determinations) obtained from the regression equation for ciprofloxacin in standard solutions; other abbreviations as in Table 2.
[c] mg in tablets; mg ml^{-1} in ophthalmic solution.

Table 5
Within-day and between-day precision and recovery data for ciprofloxacin from serum

Injected quantity (ng)	Mean measured quantity ± SD (ng) [a]		RSD (%)		Recovery (%)	
	WD	BD	WD	BD	WD	BD
0.202	0.218 ± 0.003	0.216 ± 0.004	1.4	1.8	107.9	106.9
4.04	3.95 ± 0.08	3.94 ± 0.04	2.0	1.0	97.8	97.5
10.1	10.1 ± 0.08	10.1 ± 0.05	0.79	0.49	100.0	100.0

[a] Means of values calculated from the regression straight line equation for ciprofloxacin in serum; other abbreviations as in Table 2.

Table 6
Within-day and between-day precision and recovery data for ciprofloxacin from urine

Injected quantity (ng)	Mean measured quantity ± SD (ng) [a]		RSD (%)		Recovery (%)	
	WD	BD	WD	BD	WD	BD
2.02	1.92 ± 0.01	1.92 ± 0.02	0.52	1.0	95.0	95.0
10.1	9.76 ± 0.16	9.56 ± 0.09	1.6	0.94	96.6	94.6
20.2	19.9 ± 0.59	19.4 ± 0.22	2.9	1.1	98.5	96.0

[a] Means of values calculated from the regression straight line equation for ciprofloxacin in urine; other abbreviations as in Table 2.

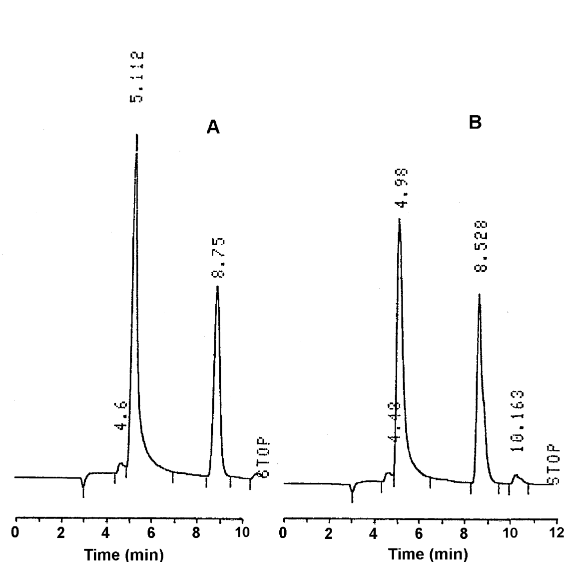

Fig. 3. Chromatograms of: (A) a tablet composite solution containing a 0.240 μg ml^{-1} labeled concentration of ciprofloxacin (5.112 min) and the internal standard (8.75 min); and (B) an ophthalmic solution containing a 0.240 μg ml^{-1} labeled concentration of ciprofloxacin (4.98 min) and the internal standard (8.528 min); conditions as described in Section 2; chart speed = 5 mm min^{-1}; attenuation = 7.

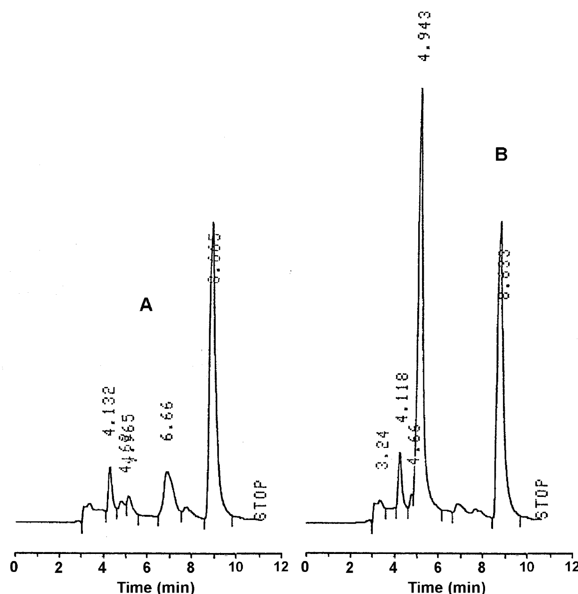

Fig. 4. Chromatograms of: (A) a blank drug-free pooled serum sample, after protein precipitation, containing the internal standard (8.665 min); and (B) a patient serum, after protein precipitation, having a 0.101 μg ml^{-1} ciprofloxacin concentration (4.943 min) and the internal standard (8.633 min); conditions as described in Section 2; chart speed = 5 mm min^{-1}; attenuation = 6.

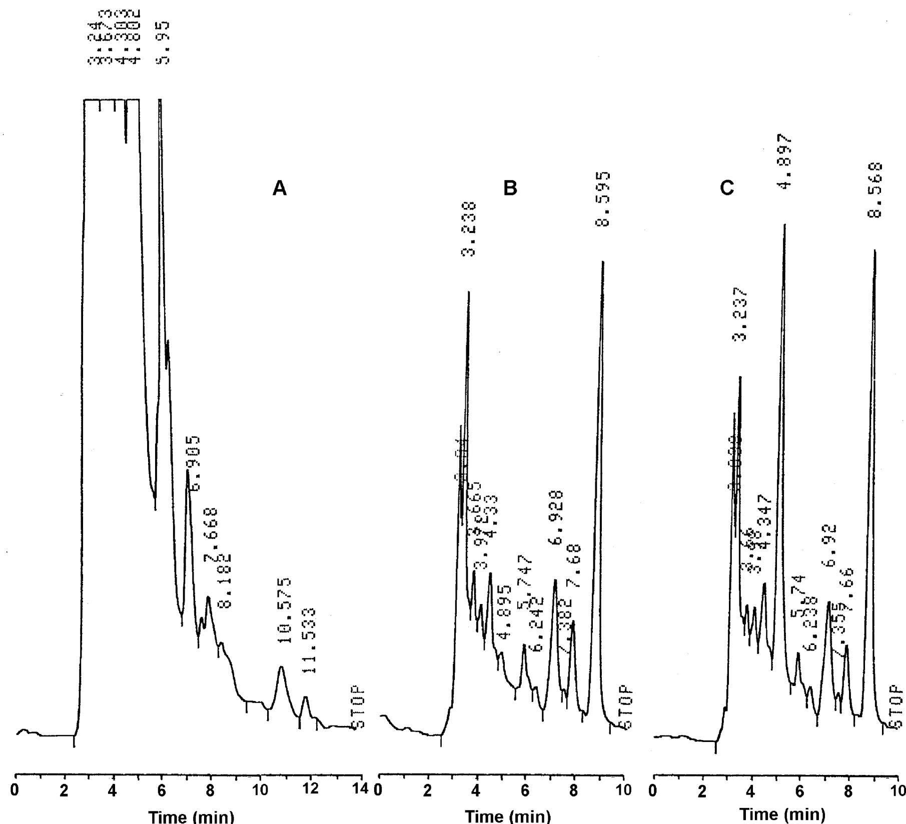

Fig. 5. Chromatograms of: (A) a blank drug-free pooled urine sample before any treatment; (B) blank urine sample subjected to SPE, containing the internal standard (8.595 min); and (C) urine spiked with 0.505 μg ml^{-1} of ciprofloxacin (4.897 min) and subjected to SPE, containing the internal standard (8.568 min); conditions as described in Section 2; chart speed = 5 mm min^{-1}; attenuation = 6.

(tablets and ophthalmic solution) are shown in Fig. 3(A) and (B), respectively. Fig. 4(A) and (B) illustrates representative chromatograms of blank drug-free pooled serum and of a serum sample, obtained from a subject given ciprofloxacin, after protein precipitation. As can be seen, no interfering peaks were detected in either of the above matrices.

In non-diluted urine samples severe interferences from endogenous compounds were observed at the retention time of ciprofloxacin, as can be seen in the chromatogram in Fig. 5(A); therefore, an SPE pretreatment of the samples was used as a clean-up step. The applied SPE treatment yielded an extraction recovery of 86%, as calculated by comparing the resulting peak areas from blank urine samples, spiked with ciprofloxacin and subjected to SPE, with the peak areas obtained by injecting standard solutions at the same concentration, not subjected to SPE treatment. The SPE treatment resulted in a sufficiently clean sample, free from interfering peaks at the retention time of ciprofloxacin (see Fig. 5(B)).

3.4. Robustness and ruggedness

Typically, the reagents employed have been prepared with limits of ±5% of the nominal composition, the buffer pH varied within ±0.5 pH units, the column temperature varied from ±1 to 5 °C (the separation was carried out at ambient temperatures). The method proved to be robust enough with respect to these critical parameters. No adverse effect was observed because of the slight changes in separation parameters. On the

other hand, the ruggedness of the method was tested by varying some operating conditions, including different operators in the laboratory, changing the source of reagents and solvents and changing to a new column (of the same type and manufacturer). The method proved to be rugged enough to allow routine laboratory use.

4. Conclusions

The described fluorometric RP-HPLC procedure for ciprofloxacin is highly sensitive, simple, precise, accurate and selective in pharmaceutical preparations and serum (after precipitation of proteins), thus permitting their direct analysis. The procedure is also suitable for the analysis of undiluted urine samples, after an SPE clean-up step through C_{18} cartridges. Minute amounts of biological samples are required. The analysis of ciprofloxacin in pharmaceutical preparations and the use of SPE treatment in non-diluted urine samples are presented for the first time in the literature.

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