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Simple high-performance liquid chromatographic assay for the determination of ciprofloxacin in human plasma with ultraviolet detection

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Abstract

A simple high-performance liquid chromatographic method is described for the quantitative analysis of ciprofloxacin in human plasma. Following protein precipitation from plasma by means of 6% perchloric acid, the upper layer which contains the analyte and the internal standard lomefloxacin, was analysed on a reverse phase column LiChrospher® 60 RP-select B (5 μm) (EcoCART® 125-3) with ultraviolet detection at 280 nm. The mobile phase was acetic acid 5%-methanol–acetonitrile (90:5:5, v/v/v). The assay was linear for ciprofloxacin over the concentration range of 0.050 to 6.00 μg ml^{-1}. The limit of quantification (LOQ) was 0.050 μg ml^{-1}. The method was successfully applied to a bioavailability study with five different ciprofloxacin formulations.

Keywords: Ciprofloxacin

1. Introduction

Ciprofloxacin, 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl) 3-quinolone carboxylic acid (Fig. 1), is a fluoroquinolone that has been shown to exhibit an extensive antibacterial spectrum.

Ciprofloxacin provides an effective treatment of some infections, especially for gram-negative organisms such as *Pseudomonas aeruginosa*.

Currently, high-performance liquid chromatography with ultraviolet or fluorescence detection is the analytical method utilized for the quantitative determination of ciprofloxacin in human plasma, urine and/or saliva [1–7].

In general, the extraction procedures utilized for the sample preparations are slow and elaborate, involve more than one extraction step, and are time consuming.

The analytical procedure described here was developed to be applied to a bioavailability study with five different ciprofloxacin oral formulations, administered as tablets on the dosage forms of 250, 500 and 750 mg. Thus, since a large number of samples were expected, as well low plasma concentrations values for ciprofloxacin during the absorption and elimination phases, a rapid, accurate and sensitive

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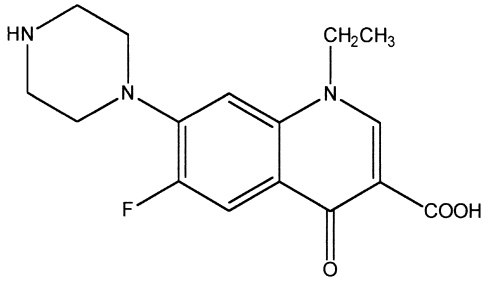
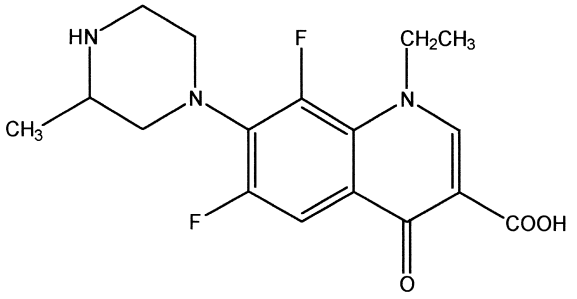


Ciprofloxacin



Lomefloxacin

Fig. 1. Structures of ciprofloxacin and lomefloxacin (the internal standard).

method was developed for the determination of ciprofloxacin in the human plasma. Moreover, multiple blood samples were collected over a long sampling time, one of the main goals was to find a balance between a low volume of the biological sample (100 μl) and the sensitivity of the analytical method.

2. Experimental

2.1. Chromatography

A Merck–Hitachi liquid chromatograph equipped with a UV–VIS detector L4200 and an Auto Sampler L-7200 Merck–Hitachi (both from Merck Hitachi, Darmstadt, Germany) were used, with an EcoCART® 125-3 LiChrospher® 60 RP-select B (5 μm) column (Merck, Darmstadt, Germany) mantained at 50°C on a Column Thermostat L-5052 (Merck–Hitachi). A guard column of LiChrospher® 60 RP-select B (5 μm), LiChroCART 4-4 (Merck) was used to reduce contamination of the analytical column.

The mobile phase was a 5% acetic acid solution, modified with methanol and acetonitrile (90:5:5, v/v/v), delivered at a flow-rate of 0.5 ml min^{-1}. Ciprofloxacin was monitored at 280 nm.

Peaks were recorded with a 3395 HP-Integrator (Hewlett-Packard, Avondale, PA, USA).

2.2. Reagents and materials

Ciprofloxacin hydrochloride monohydrate and the internal standard (I.S.) lomefloxacin (Fig. 1) were supplied by Tecnimede (Lisboa, Portugal). Acetonitrile and methanol were liquid chromatography grade and acetic acid 100% and perchloric acid 70–72% were analytical grade. All these reagents were purchased from Merck. Freshly distilled, deionised water was used throughout the procedure (Barnstead Thermolyne, Iowa, USA).

2.3. Stock and working solutions

Stock solutions: Ciprofloxacin and lomefloxacin were prepared at 1 mg ml^{-1} in methanol:destilled water (1:10). Solutions were kept at 4°C. Fresh stock solutions were prepared every month.

Working solutions: Ciprofloxacin solutions at 0.50, 1.0, 10 and 20 μg ml^{-1} in methanol–destilled water (1:10). Lomefloxacin at 5.0 μg ml^{-1} in methanol-destilled water (1:10). Solutions were kept at 4°C. Fresh working solutions were prepared every week.

2.4. Sample preparation

To an aliquot of 100 μl of plasma (either unknown plasma samples or plasma spiked with varying amounts of ciprofloxacin reference substance), in a 1.5 ml Eppendorf tube, 20 μl of a lomefloxacin solution at 5 μg ml^{-1} was added. Protein precipitation was carried out by adding 100 μl of 6% perchloric acid.

Eppendorf tubes were vortex-mixed for 30 s and after standing for 10 min, the tubes were centrifuged

at 17 320 *g* for 10 min. The upper layer (150 μl) was transferred into a polypropylene insert and 15 or 30 μl aliquots were injected onto the HPLC system.

3. Results and discussion

The analytical method described was developed and validated to be applied to a comparative bioavailability study of five different ciprofloxacin formulations. As an oral dose of 500 or 750 mg of ciprofloxacin was administered to the volunteers, plasma concentration levels between 0.100 and 4.00 $\mu g\,ml^{-1}$ were expected for the active substance. Consequently, for validation, a range from 0.050 to 6.00 $\mu g\,ml^{-1}$ of ciprofloxacin was therefore chosen.

To test the specificity of the method, six different independent sources of the biological matrix, obtained from healthy blood donors, were analysed. Fig. 2A and B shows typical chromatograms obtained from drug-free plasma and drug-free plasma spiked with ciprofloxacin plus internal standard. The chromatograms show that the separation from matrix constituents is sufficient for reliable quantitation in that no endogenous components interfered with the analyte and internal standard peaks.

Chromatograms obtained from a healthy volunteer 1.5 and 3.0 h after oral administration of 500 mg of ciprofloxacin are presented in Fig. 3A and B respectively. The retention times for ciprofloxacin and the I.S. were 9 and 11 min, respectively.

Calibration curves were generated by least-squares linear regression analysis. Good linearity was observed for all calibration curves over the range of 0.050, 0.10, 0.50, 1.0, 2.0, 4.0 and 6.0 $\mu g\,ml^{-1}$ of ciprofloxacin. Typical calibration curves are presented in Table 1 and the mean linear regression equation was $y=0.0160x+1.00$ and the coefficient of correlation (r) was 0.999.

The assay was precise and accurate. Results of the intra- and inter-assay precision and accuracy are summarised in Tables 2 and 3, respectively.

The values obtained for the precision (RSD%) and accuracy (bias %) of the method were well within the range of 15–20%, an acceptable value as indicated in the conference report on the analytical methods validation for bioavailability, bioequivalence and pharmacokinetic studies [8].

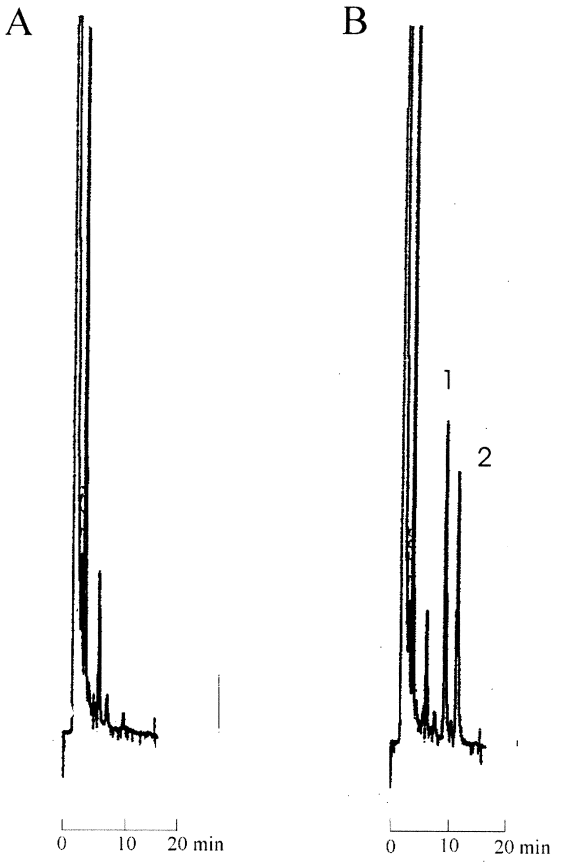


Fig. 2. Chromatogram of blank plasma (A), and blank plasma spiked with ciprofloxacin (1) and the internal standard (2) (B).

The limit of quantification (LOQ) was calculated on the basis of the analysis of six replicates of different concentrations of ciprofloxacin, taking as the LOQ the lowest concentration value for which an RSD of less than 20% was found (Table 2). When 100 μl of the sample was used, the LOQ was 0.050 $\mu g\,ml^{-1}$. The relative standard deviation was 8.24%.

Since the preparation of the plasma samples prior to injection only involved the precipitation of plasma proteins with 6% perchloric acid (no extraction step was required), it was assumed that the recovery would be 100%.

On-machine stability of the analyte was assessed by re-analysing samples after they were left in the auto sampler at ambient temperature for the expected time required to process a batch of trial samples. The results are presented in Table 4 and correspond to

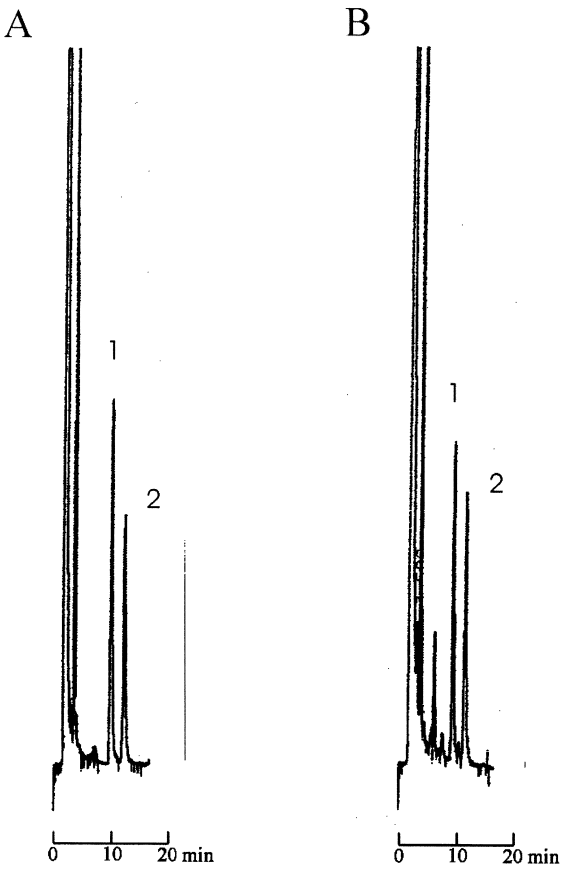


Fig. 3. Chromatograms obtained from one healthy volunteer after the oral administration of 500 mg of ciprofloxacin at two different collection times: (A)1.5 h and 3.0 h (B). Peak 1, Ciprofloxacin; peak 2, internal standard (lomefloxacin).

Table 2
Intra-assay precision and accuracy (n=6)

Nominal concentration ($\mu g\ ml^{-1}$)	Actual concentration (mean±SD) ($\mu g\ ml^{-1}$)	Precision (RSD) (%)	Accuracy (bias) (%)
0.050	0.053±0.004	8.24	6.33
0.100	0.100±0.003	3.02	−0.410
0.500	0.475±0.011	2.37	−4.92
2.00	1.89±0.035	1.88	−5.57
6.00	5.96±0.11	1.84	−0.624

Table 3
Inter-assay precision and accuracy (n=6)

Nominal concentration ($\mu g\ ml^{-1}$)	Actual concentration (mean±SD) ($\mu g\ ml^{-1}$)	Precision (RSD) (%)	Accuracy (bias) (%)
0.050	0.05±0.005	8.48	10.0
0.100	0.105±0.007	6.56	4.83
0.500	0.506±0.031	6.11	1.27
2.00	2.04±0.142	6.94	2.07
6.00	6.56±0.497	7.58	9.28

two experience days. Each point concentration was prepared in duplicate. No relevant degradation could be observed.

In conclusion, the chromatographic assay reported shows good characteristics of selectivity, simplicity, linearity, sensitivity and precision, allowing for numerous samples to be processed in a short period of time. As demonstrated by the plasma concentration–time curves presented in Fig. 4, the method

Table 1
Typical calibration curves for ciprofloxacin in human plasma

Day	Nominal concentration ($\mu g\ ml^{-1}$)						
	0.050	0.100	0.500	1.00	2.00	4.00	6.00
	Actual concentration ($\mu g\ ml^{-1}$)						
1	0.057	0.117	0.536	1.01	1.97	3.99	5.86
	0.051	0.107	0.513	0.960	1.93	4.04	6.15
2	0.034	0.093	0.532	1.01	2.01	3.92	5.91
	0.050	0.098	0.554	0.985	1.95	[a]	6.15
3	0.062	0.104	0.510	0.959	1.89	3.78	5.93
	0.054	0.105	0.5 02	0.994	1.89	3.91	6.37
Mean	0.051	0.104	0.525	0.988	1.94	3.93	6.06
SD	0.010	0.008	0.020	0.024	0.047	0.101	0.196
Precision (RSD%)	18.6	7.88	3.72	2.47	2.44	2.56	3.23
Accuracy (bias %)	2.67	4.00	4.90	−1.25	−3.06	−1.76	0.994
n	6	6	6	6	6	5	6

[a] Missing value; SD=standard deviation; n=number of observations.

Table 4
On-machine stability

Nominal concentration ($\mu g\ ml^{-1}$)	Actual concentration ($\mu g\ ml^{-1}$)			
	Day 1 Time (h)		Day 2 Time (h)	
	0	15	0	15
0.050	0.062	0.057	0.057	0.059
	0.054	0.065	0.051	0.053
0.100	0.104	0.109	0.117	0.117
	0.105	0.108	0.107	0.112
0.500	0.510	0.531	0.536	0.530
	0.502	0.519	0.513	0.515
1.00	0.959	1.02	1.01	1.03
	0.994	1.02	0.960	0.960
2.00	1.89	2.00	1.97	1.94
	1.89	1.98	1.93	1.94
4.00	3.78	4.12	3.99	4.04
	3.91	3.96	4.04	4.01
6.00	5.93	6.04	5.86	5.91
	6.37	[a]	6.15	6.28

[a] Missing value.

is adequate for the evaluation of ciprofloxacin in human pharmacokinetic studies and can be used to clearly define the absorption and elimination phases of ciprofloxacin. Moreover, the fact that a small biological sample (100 μl) is required for the entire analytical process, makes the assay particularly advantageous for use in studies with children.

Acknowledgements

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References

[1] P.T. Isavadharm, D. Keeratithakul, G. Watt, H.K. Webster, M.D. Edstein, Ther. Drug Monit. 13 (1991) 263.
[2] J.D. Davis, L. Aarons, J.B. Houston, J. Chromatogr. 621 (1993) 105.
[3] S. Zhai, M.R. Korrapati, X. Wei, S. Muppalla, R.E. Vestal, J. Chromatogr. 669 (1995) 372.
[4] A. Mizuno, T. Uematsu, M. Nakashima, J. Chromatogr. 653 (1994) 187.
[5] G.J. Krol, G.W. Beck, T. Benham, J. Pharm. Biomed Anal. 14 (1995) 181.
[6] M. Kamberi, K. Tsutsumi, T. Kotegawa, K. Nakamura, S. Nakano, Clin. Chem. 44 (1998) 1251.
[7] M. Kamberri, N. Hajime, P. Kamberri, N. Uemura, K. Nakamura, S. Nakano, Ther. Drug Monit. 21 (1999) 335.
[8] V.P. Shah et al., J. Pharm. Sci. 81 (1992) 309.

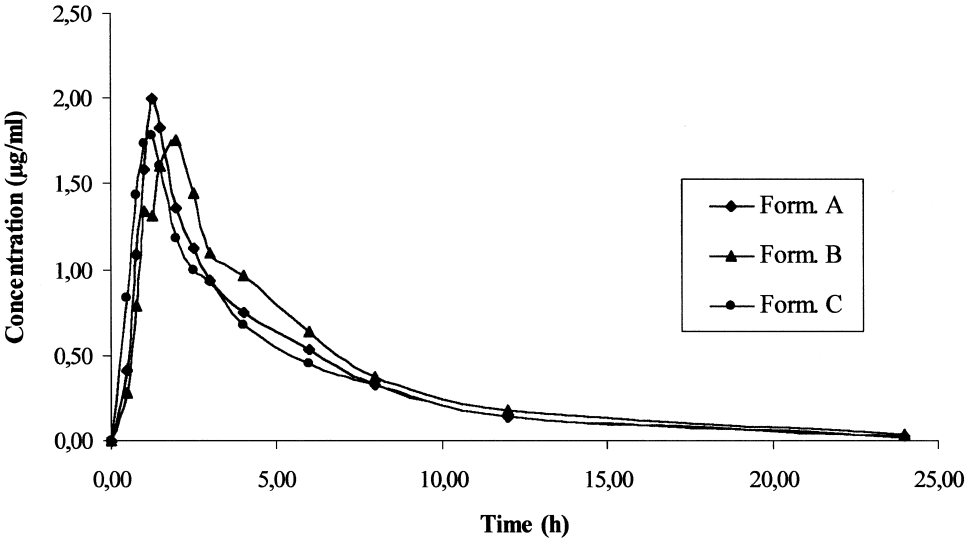

Fig. 4. Concentration vs. time curve of ciprofloxacin of a healthy volunteer who was given a single oral dose of 500 mg of ciprofloxacin as three different formulations (A; B; C).