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Short communication

Stability indicating reversed-phase liquid chromatographic determination of ciprofloxacin as bulk drug and in pharmaceutical formulations

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1. Introduction

Ciprofloxacin (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-(1-piperazinyl)-3-quinolinecarboxylic acid) (Fig. 1) is a broad spectrum fluoroquinolone antibacterial agent used for the treatment of urinary tract infections, bacterial gastroenteritis, enteric fever, gonorrhea, osteomyelitis, and infections of the bone, joints and respiratory tract [1]. LC [2–7], Spectrophotometric, [8], HPTLC [9] and spectrofluorometric [10] methods have been reported for the determination in pharmaceuticals as well as in biological fluids. Spectrophotometric assays, although simple, are not stability indicating and cannot be used for analysis of stability batches.

An ideal stability indicating LC method would quantify the drug per se and also resolve its degradation products. An accurate, specific and reproducible method is described for the determination of ciprofloxacin in presence of its degradation products for the assessment of the stability of the bulk drug and of pharmaceutical dosage forms containing the analytes.

2. Experimental

2.1. Materials

Ciprofloxacin hydrochloride was a gift from Dr Reddy's laboratories. Organic solvents for chromatography were of HPLC grade (Ranbaxy Laboratories) and double distilled water was used. All other chemicals used were of analytical grade and were purchased from Ranbaxy chemicals, India.

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2.2. HPLC instrumentation

The liquid chromatograph consisted of a Jasco-PV 980 pump (Jasco, Japan) coupled with a Jasco U-975 UV/VIS intelligent detector. Data integration was done using Borwin software package Version 1.21 for LC peak integration.

2.3. Chromatography

Chromatography was performed in the reversed-phase (RP) mode. The column was constructed of stainless steel (250 × 4 mm i.d.) and prepacked with Lichrospher 100 RP-18 (10 μm). Manual injections were carried out using a Rheodyne model 7725 injector with a 20 μl sample loop. The mobile phase consisted of water-acetonitrile-triethylamine (80:20:0.6, v/v/v) adjusted with *o*-phosphoric acid to a pH* of 3.0. The mobile phase was filtered through Nylon 66 membrane filters (47 mm, 0.45 μm) and degassed by sonication. A flow rate of 1.5 ml/min was maintained.

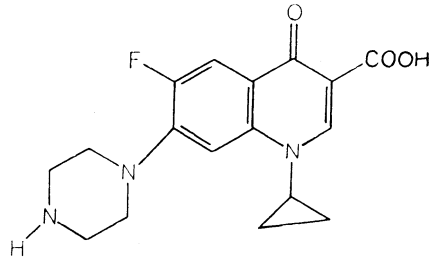

Fig. 1. Structure of ciprofloxacin.

Table 1
Accuracy and precision of LC assay of ciprofloxacin[a]

Tested concentration (ng/ml)	S.d. of areas	RSD (%)
Accuracy		
800	894.6	1.3
1200	322.8	0.3
20	216.1	1.9
Precision		
800	503.2	0.7
1200	515.2	0.5
20	323.4	2.5

[a] $n = 6$.

2.4. Preparation of standard solution

A stock solution of ciprofloxacin hydrochloride (calculated as free base) (1 mg/ml) was prepared in double distilled water and diluted further with mobile phase to obtain a standard of 10 μg/ml.

2.5. Calibration curve of ciprofloxacin

Appropriate dilutions of the standard solution (10 μg/ml) were made with the mobile phase to obtain solutions of concentrations of 200, 400, 600, 800, 1000, 1200, ng/ml of ciprofloxacin. A correlation between peak area and the concentration of ciprofloxacin was established and the calibration curves were obtained. Linearity was also checked over the range of 20–200 ng/ml. The limit of detection and quantitation based on the instrumental parameters were also determined.

2.6. Accuracy and precision of the assay

The accuracy and precision of the assay were tested at 800 and 1200 ng/ml of ciprofloxacin. The intra-day variation was evaluated in the range of 200–1200 ng/ml three times a day. The inter-day variation was similarly evaluated over a period of 3 days. The accuracy and precision was also tested at 20 ng/ml (Table 1).

2.7. Sample preparation

To determine the content of ciprofloxacin of conventional tablets (label claim: 100 mg/tablet), samples were prepared by a reported method [3]. The tablets contained ciprofloxacin, 50%; lactose, 31%; dicalcium phosphate, 12%; starch, 5%; magnesium stearate, 1%; and talc, 1%; as excipients. Ophthalmic solutions (label claim: 0.03% w/v) were filtered through 0.45 μm membrane filter and directly injected after appropriate dilution. The eye-drops were prepared in acetate buffer pH 5.0 containing 0.01% of benzalkonium chloride as a preservative.

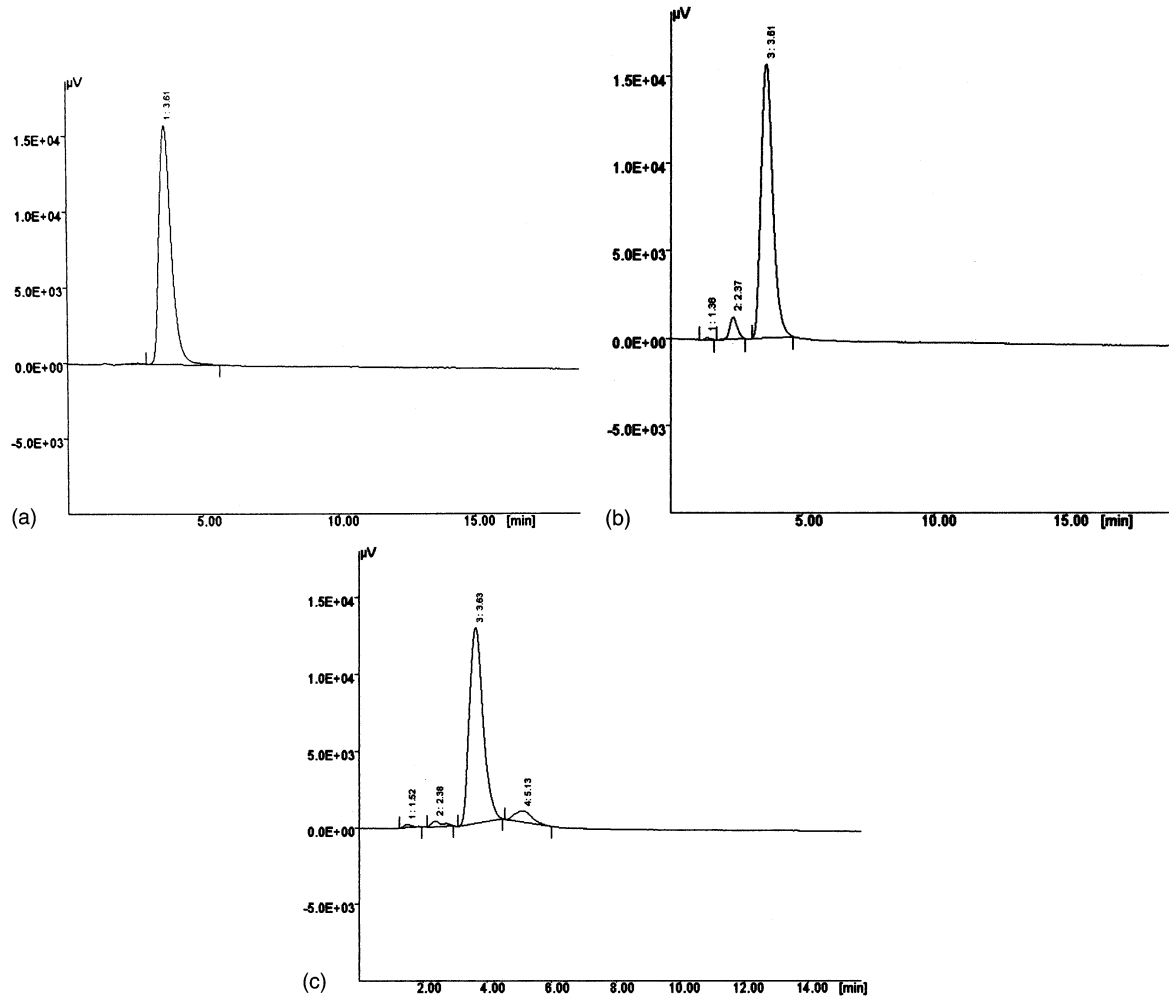

Fig. 2. (a) Chromatogram of 5 μg/ml ciprofloxacin: peak 1 (RT: 3.61 min) is of ciprofloxacin; (b) chromatogram of 5 μg/ml ciprofloxacin degraded with acid: peak-1 (RT: 1.36 min) and peak-2 (RT: 2.36 min) are of acid degraded products. Peak-3 (RT: 3.61 min) is of ciprofloxacin. (c) Chromatogram of 5 μg/ml ciprofloxacin degraded with base: peak-1 (RT: 1.36 min), peak-2 (RT: 2.36 min) and peak-4 (RT: 5.13) are of base degraded products. Peak-3 (RT: 3.63 min) is of ciprofloxacin.

2.8. Recovery studies

The analysed samples were spiked with extra 50, 100 and 150% of the standard ciprofloxacin hydrochloride and the mixtures were reanalysed by the proposed method ($n = 3$). This was done to check for the recovery of the drug at different levels in the formulations.

2.9. Degradation of ciprofloxacin hydrochloride

The effect of pH, buffer type and drug concentration on the photodegradation of ciprofloxacin has been reported [11]. Photodegradation of fluoroquinolones using a continous-flow photochemical reaction unit has been reported [12]. In an attempt to develop a stability-indicating assay

Table 2
Intra-day and inter-day variation — comparison of slopes and coefficient of regression[a]

	Slope (mean ± S.D.)	Coefficient of regression (mean ± S.D.)
Intra-day variation	85.9 ± 0.4	0.9997 ± 0.0001
Inter-day variation	85.4 ± 0.4	0.9996 ± 0.0001

[a] $n = 3$.

method, the drug was degraded in solution by the following procedure: 5 ml of methanolic 2 M HCl was added to a 0.1% w/v solution of ciprofloxacin hydrochloride and refluxed for 1 h. Similar procedure was followed with 2 M NaOH solution. This solution after appropriate dilution with the mobile phase was injected into the chromatographic system.

3. Results and discussion

3.1. Optimization of the LC procedure

The LC procedure was optimised with a veiw to develop a stability indicating method. The mobile phase was modified from that reported in the USP [13] so as to resolve the degraded products from the drug. The mobile phase consisting of water-acetonitrile-triethylamine (80:20:0.6, v/v/v) of pH 3.0 adjusted with ortho-phosphoric acid resulted in a retention time of 3.61 min for ciprofloxacin (Fig. 2a). The chromatogram of the acid degraded sample (Fig. 2b) showed two additional peaks at 1.36 and 2.36 min. The chromatogram of the base degraded sample (Fig. 2c) showed three additional peaks at 1.55, 2.87, and 5.33 min. The peaks of the degraded products were well resolved from the ciprofloxacin peak (3.61 min).

Table 4
Applicability of the proposed LC method to the analysis of marketed formulations[a]

Sample (label claim)	Drug content (%)	RSD (%)
Tablet (100 mg)	98.4	0.01
Eye-drops (0.03% w/v)	99.6	0.18

[a] $n = 6$.

3.2. Validation of the method

3.2.1. Accuracy and precision of the assay

The results in Table 1 revealed excellent accuracy and high precision of the assay method.

3.2.2. Ruggedness of the method

The intra-day and inter-day variation was evaluated by comparing the slopes as depicted in Table 2. It is evident that there was no significant variation in the slope values (ANOVA; $P > 0.05$).

3.2.3. Calibration curve of ciprofloxacin

The polynomial regression data for the calibration plots ($n = 3$) showed a good linear relationship over a concentration range of 200–1200 ng/ml. No significant difference was observed in the slopes of standard curves (ANOVA; $P > 0.05$). The mean values (± S.D.) of correlation coefficient, slopes and intercept were 0.99971 ± 0.000101, 85.8896 ± 0.3986 and 959.97 ± 1.538, respectively, with %RSD of less than 1. The calibration curve in the range of 20–200 ng/ml

Table 3
Recovery studies[a]

Excess drug added to the analyte (%)	Theoretical content (ng/ml)	Recovery (%)	RSD (%)
0	500	98.4	0.01
50	750	99.6	0.18
100	1000	101.2	0.63
150	1250	100.0	0.53

[a] $n = 6$.

showed the mean values ($\pm$S.D.) of correlation coefficient, slopes and intercept as 0.99549 ± 0.00121, 61.14699 ± 0.539 and 1954.97 ± 2.538, respectively, with %RSD of less than 2.5.

3.2.4. LOD and LOQ

The limit of detection, with a signal to noise ratio of 3:1, was found to be 5 ng/ml. Here the noise (peak to peak) was 20 units and the signal was 60 units. The limit of quantitation, with a signal to noise ratio of 10:1, was found to be 20 ng/ml where the signal was 200 units.

3.2.5. Recovery studies

The proposed method when used for extraction and subsequent estimation of ciprofloxacin from pharmaceutical dosage forms afforded recovery of 98–100% as listed in Table 3.

3.2.6. Analysis of the pharmaceutical formulations

A single peak at 3.61 min was observed in the chromatogram of the drug samples extracted from the conventional tablets and eye-drops and there was no interference from the excipients commonly present in the conventional tablets and eye-drops. It may therefore be inferred that degradation of ciprofloxacin has not occurred in the marketed formulations, which were analysed by this method (Table 4).

4. Conclusion

The developed LC technique is precise, specific, accurate and stability-indicating. The statistical analysis proves that the method is reproducible and selective for the analysis of ciprofloxacin as bulk drug and in pharmaceutical formulations. The run time of less than 8 min was found to be practicably advantageous for use of this method in routine analysis. It may be extended to study the degradation kinetics of ciprofloxacin and also for its estimation in plasma and other biological fluids.

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