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Research Article

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RP-HPLC method development and validation for the determination of ciprofloxacin from marketed tablet dosage forms

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ABSTRACT

The present study deals with the development and validation of a simple, rapid, precise and economical reverse phase liquid chromatographic technique for the determination of Ciprofloxacin form marketed tablet dosage forms. Chromatographic analysis was carried out using C18 Xterra Column (150 × 4.6 mm, 5µm) and mobile phase consisting of a mixture of 5.3mM phosphate buffer (pH = 3.5) and acetonitrile in the ratio 60:40 at a flow rate of 0.5ml per minute and the detection was done at 275nm. The retention time of analyte peak was 2.29 min. The linearity was obtained in the concentration range of 0.01 to 0.50 µg/ml with correlation coefficient 0.99. The analysis results of ciprofloxacin were validated statistically and by recovery studies and the mean recovery was found to be 100.14%. The limit of detection (LOD) and limit of quantification (LOQ) for ciprofloxacin was found to be 0.06 µg/ml and 0.20 µg/ml, respectively. The proposed procedure was found to be accurate and precise for the estimation of ciprofloxacin from marketed formulations and can be used for the regular analysis of this drug in quality control laboratories.

Keywords: Ciprofloxacin; RP-HPLC; Chromatography; Validation.

INTRODUCTION

The fluoroquinolones presents a popular class of synthetic antibacterial agents having a broad spectrum of applications. Ciprofloxacin is a second generation class II fluoroquinolone [1] used for the treatment of complicated urinary tract infections, gastroenteritis with severe diarrhoea, protatitis, nosocomial infection, sexually transmitted diseases, lower respiratory tract infections [2], community acquired abdominal infections, prosthetic joint infections and management of resistant gram-negative infections. Ciprofloxacin was found effective over trimethoprime - sulfamethoxazole in case of urinary tract infections however, found ineffective in many cases of respiratory tract diseases. Chemically ciprofloxacin is 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid as presented in Fig. 1.

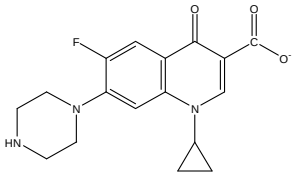

Fig. 1 Chemical structure of Ciprofloxacin

Ciprofloxacin is soluble in 0.1M hydrochloric acid solution and practically insoluble in ethanol. The biological half-life is 5.37± 0.82hrs with C_{max} 1.5±0.43µg/ml/70kg. The molecule well distributed throughout the body as with other fluoroquinolones. Initially, microbiological methods of analysis [3] were developed but these were found to be slow and suffered from poor precision and specificity. Extensive literature survey presents fewer analytical methods for the estimation of ciprofloxacin from oral dosage forms. Most of the methods reported the estimation of the blood from different body fluids [4-8] and from edible animal tissues and poultry samples [9, 10]. Only a very few methods have been reported that presents the estimation of the drug from formulations [11-15]. The current method is much simpler, precise and less time consuming compared to the presented analytical techniques.

EXPERIMENTAL SECTION

Apparatus: The chromatography was performed on a Waters (USA) Alliance e2695 instrument equipped with Waters 2489 dual λ-absorbance detector. A C_{18} X terra column (150 × 4.6 mm i.d., 5 µm particle sizes) was used as stationary phase. To deliver the mobile phase an auto sampler (e2695, Waters, USA) with 10 µl loop was used in this study. Aurium 611 UV water purification system of Sartorius, Germany was used to prepare HPLC grade water.

Reagents and materials: The reference standard of ciprofloxacin was supplied by Central Drug Laboratory, Kolkata. The tablet formulation Cipgen [(Cadila (Genvista)] having of label claim 500 mg ciprofloxacin was procured from from local pharmacy. HPLC grade acetonitrile, orthophosphoric acid and AR grade monobasic and dibasic potassium phosphate were purchased from Merck Ltd., Mumbai.

Selection of mobile phase and wavelength: During the development of the proposed method various mobile phase composition were tried to get a good resolution and sharp peaks and the chosen mobile phase was a mixture of 60 volumes of 5.3 mM phosphate buffer adjusted to pH 3.5 ± 0.1 with orthophosphoric acid and 40 volumes of acetonitrile. The standard stock solution prepared by using mobile phase was scanned over the range of 200-400 nm and spectrum was overlain. It was observed that 275 nm was λ_{max} of ciprofloxacin and was preferred as suitable wavelength for detection.

Chromatographic conditions of the proposed method: The mobile phase was filtered by passing through 0.45 µm membrane filter (Millipore). Chromatographic analysis was carried out at ambient temperature. The compounds were separated isocratically with a mobile phase at a flow rate of 0.5 ml /min. The effluent was monitored spectrophotometrically at a wavelength of 275 nm and all data were analysed by using Empower 3 software.

Preparation of standard stock solution: An accurately weighed quantity of standard ciprofloxacin (12.5 mg) was transferred to a 25 ml volumetric flask. 10 ml of HPLC grade water and one drop of conc. HCl was added to it. The drug was allowed to dissolve by sonication for 15 minutes and the final volume was made by mobile phase. Final working standard solution containing 0.02 mg/ml was prepared from the prepared stock solution diluting with mobile phase. The standard solutions were filtered using 0.45 µm syringe filter before making injection.

Preparation of sample solution: Twenty tablets of ciprofloxacin were weighed. Their mean weight was determined and they were crushed in a mortar. Tablet powder equivalent to 12.5 mg of ciprofloxacin was accurately weighed and transferred to a 25 ml volumetric flask. 10 ml HPLC grade water and one drop of conc. HCl were inserted and sonicated for ~15 min to dissolve the drug. Then the volume was made up to the mark and filtered with 0.45 µm filter. From this stock solution 1 ml is transferred to the 25 ml volumetric flask and volume was made up to the mark with the mobile phase to prepare 0.02 mg/ml concentration. The content of sample solution was filtered through 0.45 µm syringe filter before making injection.

Assay of tablet formulation: With the optimized chromatographic conditions the standard solutions were injected (six replicates) and the chromatograms were recorded (Fig. 2). This procedure was repeated (two replicates) for the sample solution obtained from the formulation. The retention time was 2.143 min. The amount of the drug in tablet formulation was calculated by comparing area of the sample solution with that of the standard solution. The result was given in Table 1.

Table 1: Assay parameters

Tablet Formulation	Drug	Amount of Drug (mg/tab)		% of Label Claim	% RSD
		Labelled	Estimated		
Cipgen Tab [Cadila(Genvista)]	Ciprofloxacin	250	251.09	100.44	0.89

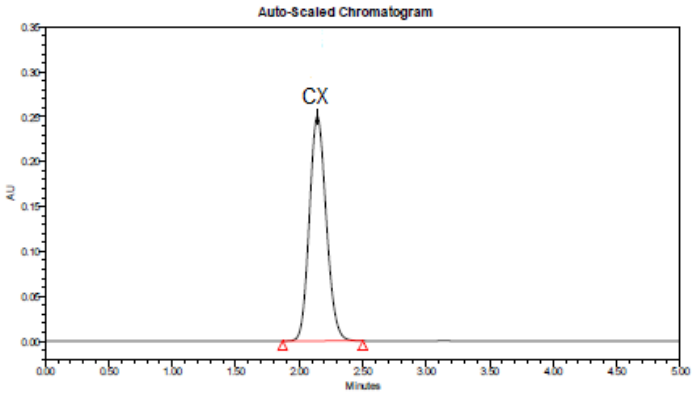

Fig. 2 Representative chromatogram for Ciprofloxacin (retention time = 2.143)

Validation of the proposed method: The system suitability parameters, linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ were studied systematically to validate the optimized chromatographic method as per recommendation of USP and ICH guidelines [16, 17].

System suitability parameters: To ensure the validity of the proposed analytical procedure a system suitability test was established. Data from six replicate injection of 10 µl of the standard solution of ciprofloxacin were used for the evaluation of the system suitability parameters like wavelength, theoretical plates, tailing factor and retention time. The results obtained were shown in Table 2.

Table 2: System suitability parameter

Parameters	Ciprofloxacin
Wavelength of max absorbance (nm)	275
Retention time (min)	2.143
Tailing factor	1.09
Theoretical factor	10314
LOD (µg/ml)	0.06
LOQ (µg/ml)	0.20

Linearity (calibration curve): The solution for linearity was prepared at five concentration levels ranging from 0.01 µg/ml to 0.50 µg/ml of the target concentration. Each experiment was performed in triplicate according to optimized chromatographic conditions. The peak areas of the chromatograms were plotted against the concentrations of the ciprofloxacin (Fig. 3) and correlation co-efficient, slope and intercept of the calibration curve was determined. These results were presented in Table 3.

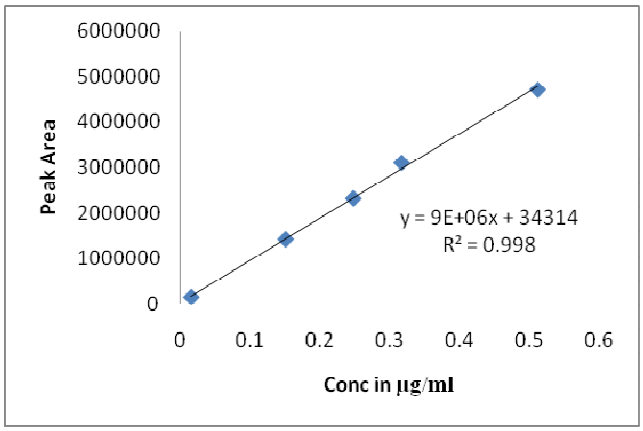

Fig. 3 Calibration curve (Plot of peak areas *versus* concentrations)

Precision of the proposed method: The precision of the method was demonstrated by repeatability and intermediate precision i.e. by intraday and interday variation studies respectively. The intraday and interday variability were determined by making six repeated injections of standard solution on the same day as well as on three consecutive days respectively. The percentage RSD with respect to the peak area, peak retention time and the amount were calculated for each case and presented in Table 4.

Table 3: Linearity parameters of the proposed method

Parameters	Ciprofloxacin
Linearity range (µg/ml)	0.01 to 0.50
Regression coefficient	0.99
Slope	1987
Intercept	1511

Table 4: Precision parameters

Parameters	Intra-day	% RSD	Inter-day			
			Day1	Day2	Day3	% RSD
Peak Area	2323956	0.52	2323871	2323859	2322129	0.04
Peak RT	2.15	0.09	2.14	2.15	2.14	0.26
Amount (mg/Tab)	250.89	0.53	250.88	250.87	250.69	0.04

Accuracy (recovery study): Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. A known amount of standard solutions (90, 110 and 120%) were added to the pre-analyzed sample solution and these mixtures were determined by the proposed method in triplicates. Recovery (%) and RSD (%) were calculated for each concentration and results were given in Table 5.

Table 5: Accuracy parameters (recovery)

Tablet Formulation	Drug	LabeledAmt of Drug (mg/tab)	Amount mg/tab found	% label claim (n =6)	Recovery Studies (n = 3)				
					Total Amt. after spiking (mg)	Amt recovered (mg) Mean ± SD	% Recovery	% Mean Recover	% RSD
Cipgen Tab [Cadila(Genvista)]	CX	250	251.09	100.44	225 275 300	225.99±3.00 274.87±1.99 300.09±4.91	100.44 99.95 100.03	100.14	0.51

Ruggedness and robustness: In order to demonstrate the ruggedness of the proposed method, different instruments like Waters HPLC (515 pump, 600 pump, 2487 dual λ absorbance detector), Merck Hitachi HPLC (La Chrome pump L-7100, L-7400, UV detector) were used to carry out the experiment by different operators using different columns of similar type in different days.

In order to evaluate the robustness of the proposed method deliberate change in chromatographic condition i.e. change in flow rate, change in pH of the mobile phase and temperature were carried out.

Limit of detection and limit of quantification: Limit of detection (LOD) and limit of quantification (LOQ) were determined by using the formula based on the standard deviation of the response and the slope of the calibration curve. LOD and LOQ were calculated by using the equations LOD = 3.3σ/s and LOQ = 10σ/s, where σ is the standard deviation of response and s is the slope of the calibration curve. The values of LOD and LOQ were given in Table 1.

RESULTS AND DISCUSSION

In order to develop an effective method for the analysis of ciprofloxacin in pharmaceutical formulations, preliminary tests were performed in order to select adequate and optimum conditions. A satisfactory separation and good peak symmetry was obtained with a C_{18} X terra (150 mm × 4.6 mm, 5 µm) column and a mobile phase consisting of a mixture of 60 volumes of 5.3 mM phosphate buffer solution adjusted to pH 3.5 ± 0.1 with orthophosphoric acid and 40 volumes of acetonitrile at a flow rate of 0.5 ml/min. Quantification was achieved with UV detection at 275 nm based on peak area. The retention time was found to be 2.143 min (Fig. 2). The developed method was validated as per USP and ICH guidelines [16, 17]. The system suitability was evaluated by making six replicate injections of the standard preparation and the tests revealed that number of theoretical plates was above 2000 and tailing factor is less than 2. The peak areas of the different concentrations of ciprofloxacin were determined to obtain the calibration curve. The calibration curve showed good linearity over a concentration range of 0.01 µg/ml to 0.5 µg/ml for the drug with correlation coefficient of 0.99 (Table 3). Repeatability and intermediate precision values (Table 4) were within the acceptable limits. This indicates that the method was precise. The % recovery was found to be within limits of the acceptance criteria with recovery range 99.95 % - 100.44 % (Table 5) for ciprofloxacin, which showed that there was no interference with excepients. The limit of detection (LOD) and limit of quantification (LOQ) were

separately determined based on standard deviation of response of calibration curve. The values were 0.06 µg/ml and 0.20 µg/ml respectively (Table 2). The method was also satisfactory with respect to ruggedness and robustness.

CONCLUSION

The results of the study indicate that the developed method was found to be simple, reliable, accurate, linear, sensitive, reproducible and have short run time which makes the method rapid and economical. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine analysis of ciprofloxacin in bulk drug and in pharmaceutical dosage forms.

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