Original Research

Ciprofloxacin method development and validation in solid dosage form

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Abstract

Objective: This study aimed to develop and validate an high-performance liquid chromatography method coupled with an ultraviolet detection for determination of ciprofloxacin .The chromatographic separation was achieved on an RP-C18 column (Lachrom Hitachi, 250×4.6 mm, 5 µm), utilizing a mobile phase of phosphate buffer/acetonitrile (77:23, v/v, pH 3.0±0.1) at a flow rate of 1.0 ml/minutes. Detection is carried out at 288.6 nm using a spectrophotometer. The developed method is statistically validated for the linearity, accuracy, limit of detection (LOD), limit of quantitation, precision, and specificity. The LOD and limit of quantification (LOQ) were 0.06 µg/ml and 0.16 µg/ml, respectively. The regression curve of the standard was linear (R>0.999) over a range concentration of 0.02-3.20 µg/ml. The mean recovery of the method ranged between 96.27% and 107.45%. Both intra- and inter-day precision data showed reproducibility (relative standard deviation ≤8.0, n=9).

Keywords: Ciprofloxacin,Validation, High-performance liquid chromatography,Plasma, Bioequivalence

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Introduction

Ciprofloxacin is one of a fluoroquinolone class of antibiotics that are widely used in the treatment of several bacterial infections. Ciprofloxacin exhibits a broad spectrum antimicrobial activity against Gram-negative and Gram-positive bacteria. Ciprofloxacin has low toxicity and potential for used as oral therapy in urinary tract as well as skin and soft infections .[1-3]

Various methods have been developed for the determination of ciprofloxacin individually using spectrophotometer, spectrofluorometer, flow injection analysis, or highperformance liquid chromatography (HPLC) method that can be found in British Pharmacopeia, United States Pharmacopeia, and Indonesian Pharmacopeia.[4-7] In these pharmacopeias, HPLC methods were officially method for the determination of ciprofloxacin as alone. Many applications of HPLC method have been reported in the literature for determination ciprofloxacin in pharmaceuticals preparations, human plasma, and other biological fluids such as simultaneous analysis of ciprofloxacin and phenazopyridine in solid dosage form using isocratic RP-HPLC that reported Pola and Sankar. In addition, Sachan et al. (2010) have reported simultaneous estimation of ciprofloxacin hydrochloride and ofloxacin by reverse phase-HPLC (RP-HPLC). Some of HPLC methods that involved the use ion pair reagent to get better peak shape have been also reported in the literature .[8-10]

The current study was devised to develop and validate the HPLC method for ciprofloxacin determination in human plasma. The developed method is expected tobe simpler, faster, and more reliable than previously published methods. Validation will be conducted in accordance with ICH harmonization for validation of analytical procedure ICH Q2 (R1) .[11]
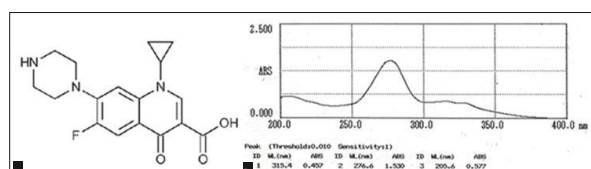

Fig. 1: Structure of ciprofloxacin (a); Ultraviolet spectra of ciprofloxacin (b)

MATERIALS AND METHODS

Drug samples ciprofloxacin hydrochloride were obtained as gift samples from the Gracure pharmaceuticals Pvt. Ltd., Bhiwadi (Rajasthan).

Chemicals and reagents: Orthophosphoric acid (Analytical grade), Acetonitrile (HPLC grade), Methanol (HPLC grade), Water (HPLC grade), were purchased from Merck Co. Mumbai and S.D. fine chemicals, Mumbai, respectively. All the reagents and chemicals used for analysis were of analytical grade and HPLC grade.

Experimental conditions: A high performance liquid chromatography (Waters e2695), variable wavelength programmable PDA detector, with empower software was used. The chromatography column used was reverse phase an RP-C18 column (Lachrom Hitachi, 250×4.6 mm, 5 µm). A mixture of phosphate buffer/acetonitrile (77:23, v/v, pH 3.0±0.1) was used as mobile phase and was filtered through 0.45µ Millipore membrane filter. The flow rate of mobile phase was maintained at 1.0 ml /min. Detection was carried out at 288.6 nm at room temperature. Standard solution of ciprofloxacin was prepared in diluents. A quantity of powder equivalent to about 50mg of USP ciprofloxacin hydrochloride was weighed and transferred to 100ml volumetric flask containing 60 ml of mobile phase and

The mixture was sonicated .The volume was make up to mark (100ml) with mobile phase. The contents were filter through whatmann filter paper. Further dilutions were made to get a concentration of 50µg/ml of Ciprofloxacin .Twenty tablets, each containing 500mg of ciprofloxacin were weighed and powdered. A quantity of powder equivalent to 250 mg of ciprofloxacin was weighed and transferred to 200ml volumetric flask containing 120ml of mobile phase. The mixture was sonicated for 30 min. The volume was made upto 200ml with mobile phase. The contents were filter through whatmann filter paper. Further dilutions were made to get a concentration of 50µg/ml of Ciprofloxacin. Twenty microliters of the test and standard solutions were injected separately and chromatograms were recorded upto to 15 minutes.

Table 1: Result of the phosphate buffer/acetonitrile (77:23, v/v, pH 3.0±0.1) at a flow rate of 1.0 ml/minutes.

Flow rate (ml/minute)	Retention time (minute)	Theoretical plate number (N)	Tailing factor (Tf)	Height of equivalent of theoretical plate/HETP (mm)	Peak area (miliarea unit)	Note
1	12.58	11460	1.34	0.002182	6273454	
1.5	7.19	12979	1.31	0.001926	6404905	Optimum
2	2.48	4453	2.54	0.005614	3416720	

HETP: Height equivalent of theoretical plate

Table 2: Result of optimization of mobile phase composition phosphate buffer/acetonitrile (77:23, v/v, pH 3.0±0.1) at a flow rate of 1.0 ml/minutes.

Option	Mobile phase composition (phosphate buffer: acetonitrile)	Retention time (minute)	Theoretical plate number (N)	Tf	HETP (mm)	Peak area (miliarea unit)
1	80:20	2.89	4546	1.95	0.0055	5192543
2	83:17	3.57	6632	1.79	0.0037	5868978
3	85:15	4.92	8847	1.63	0.0028	5835432
4	86:14	5.55	9864	1.52	0.0025	5781028
5	87:13	7.19	11460	1.31	0.0022	5915811

RESULTS AND DISCUSSION

Ciprofloxacin was analyzed by using RP-HPLC method. The present investigation was aimed at developing a simple, precise and accurate HPLC method to estimate ciprofloxacin in tablet dosage form. Several trials are carried out for selection of column and mobile phase for the method development. The chromatographic separation was achieved on an RP-C18 column (Lachrom Hitachi, 250×4.6 mm, 5 µm), utilizing a mobile phase of phosphate buffer/acetonitrile (77:23, v/v, pH 3.0±0.1) at a flow rate of 1.0 ml/minutes. Detection is carried out at 288.6 nm using a spectrophotometer. With the above mentioned composition of mobile phase a good analysis of ciprofloxacin was achieved. The retention time of ciprofloxacin was found to be 10.82 min, respectively. Run time was 16 minutes and injection volume was 20µl. The peak shape of drugs were symmetrical and asymmetric factor was lesser than 2.0. The response factor of the standard and test solution was calculated. The proposed method was validated as per ICH guidelines. Each of Samples was injected 6 times and the retention time was observed in all the cases. Precision of proposed method (RSD) for Repeatability was found to be 0.29% for ciprofloxacin and for intermediate precision was found to be 0.39% for ciprofloxacin .The low RSD value indicated that proposed method has good precision. Linearity experiments were performed at five different concentrations, thrice for both the compounds and the response was found to be linear in the range of 27.5 - 82.5µg/ml for ciprofloxacin.

Linearity of ciprofloxacin was plotted by a graph of response factor versus concentration. The correlation coefficient (r) value for ciprofloxacin was 0.9999, respectively. Accuracy of the method was calculated by recovery studies at three levels. Amount of drug recovered at each level was calculated. Percent recovery study at each level was calculated. The average recovery of ciprofloxacin were 99.80%. The sample recovery in the formulation was in good agreement with the label claim. High percentage recovery showed that the method was free from interferences of excipients used in the formulations. The results of study indicate that proposal method is simple, precise, highly accurate and specific
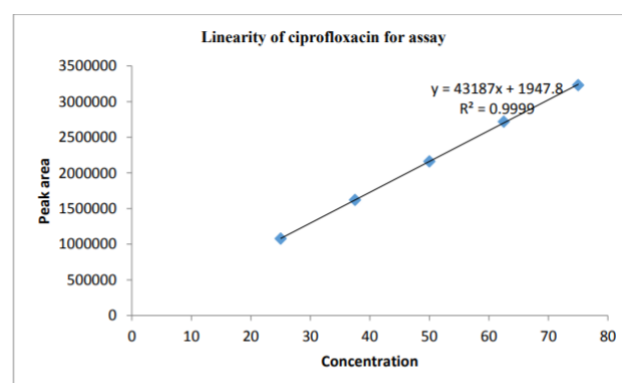

Figure 2: Calibration curve of ciprofloxacin
Table 3: Result of Recovery Study ciprofloxacin

DRUG	AMOUNT ADDED	AMOUNT RECOVE-RED	RECO-VERY (%)	AVERAGE RECOVERY (%)
CIPROFL-OXACIN	126.2	125.6	99.52	99.80
	251.2	250.4	99.68	
	372.4	373.2	100.21	

Result of System Suitability Parameter are given in table 4, Result of Recovery Study ciprofloxacin are given in table 3 and Calibration curve of ciprofloxacin is given in figure 2.

Parameters	Ciprofloxacin
Tailing factor	1.36
Theoretical plates	9841
Calibration range	27.5 - 82.5µg/ml

Table 4: Result of System Suitability Parameter

Conclusion: According to the validation result, the method was rapid, simple, and reliable. It can be used for routine analysis of ciprofloxacin in solid dosage form.The developed method is statistically validated for the linearity, accuracy, limit of detection (LOD), limit of quantitation, precision, and specificity. The LOD and limit of quantification (LOQ) were 0.06 µg/ml and 0.16 µg/ml, respectively. The regression curve of the standard was linear (R>0.999) over a range concentration of 0.02-3.20 µg/ml. The mean recovery of the method ranged between 96.27% and 107.45%.

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