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In vitro Protein Binding Study of Ciprofloxacin by New UV - Spectrophotometric Method

A.Karunasree*,K.Thejomoorthy, M.Jaffer, Y.PadmanabhaReddy and P.Ramalingam

Department of Pharmaceutical Analysis and Quality Assurance, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur Dt, Andhra Pradesh ,India – 515 721.

Corres. author:karun.pharma@gmail.com
Phone no: 9642472203, 9440727715

Abstract: A rapid, reliable and sensitive UV - spectrophotometeric method has been developed for the determination of ciprofloxacin in tablet formulations and for its *in vitro* protein binding studies. The estimation of ciprofloxacin hydrochloride was carried out at 271.6 nm at Beer's law concentration range of 2-10 µg/ml. The developed method has been validated statistically as per USP. The Beers limit was found to be 1 – 20 µg/ml with %RSD 0.2078 for precision LOD and LOQ were found to be 0.054 and 0.165 respectively. The developed method was employed *in vitro* protein binding studies using semi permeable membrane and the results were significant.
Keywords: Ciprofloxacin, protein binding, UV method.

1. Introduction

Ciprofloxacin hydrochloride, 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxilic acid, is a broad spectrum fluoroquinolone antibacterial agent used in the treatment of various bacterial infections caused by gram-positive and gram-negative microorganisms[1-2]. Ciprofloxacin is official in IP, USP and BP and their monograph revealed that RP- HPLC method (IP and USP) and non-aqueous titrimetric method (BP) were described for its estimation[3-5]. Literature survey reveals that few RP- HPLC, simultaneous equation methods and vast colorimetric methods were reported, herein efforts were made on development of simple UV spectrophotometric method in phosphate buffer pH 6.0 owing to its tubular secretion in urinary system as a urinary antiseptic [6-8]. Ciprofloxacin is being reported to possess about 40% protein binding to retard secretion, the present method may be reliable to estimate at urinary pH and release kinetic from protein bindings.

2. Experimental

2.1 Materials and Methods

A Systronics UV-Visible Spectrophotometer-117 with 1 cm matched quartz cells were used for all spectral measurements. All chemicals used were obtained from Lobha Chemie and Merck Pvt. Ltd, Mumbai. Sample Standard of ciprofloxacin Hcl was obtained from Natco Pharmaceuticals pvt.Ltd, and was systematically authenticated for its standard and identity.

2.2 Preparation of standard stock solution

100mg of accurately weighed ciprofloxacin Hcl was dissolved in small quantitiy of phosphate buffer pH 6.0 and the final volume was made up to 100ml with same solvent to get 1mg / ml concentration of standard stock solution. Various working concentrations were made by further dilution with same medium.

2.3 Assay

20 tablets of various trades from community pharmacy were taken, finely powdered, and weighed an equivalent weight of 100mg of ciprofloxacin and

was transferred into a 100ml volumetric flask and successive extraction was carried out with 20 ml of methanol followed by phosphate buffer pH 6.0. The resulting solution was filtered and volume was made up to 100 ml with phosphate buffer pH6.0. The solution was suitably diluted and absorbance was measured at 271.6nm. The total content of tablets was determined from the standard plot and was cross-examined on regression equation. The results were interpreted for the method accuracy and were shown in Table – 2.

3. Method Validation

3.1 Linearity

The method was validated according to ICH Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy of the analyte. Five point calibration curves were generated with appropriate volumes of the working standard solutions for UV methods. The linearity and regression equation was shown in Table-1 and Figure.1

3.2 Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. Both Inter - day precision and Intra - day precision were carried out as per the statistical requirement to support reproducibility of the method. The results were shown in Table -2 and Figure -2.

3.3 LOD and LOQ

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations LOD=3s/m;LOQ=10s/m

Where s, the noise of estimate, is the standard deviation of the absorbance of the sample and m is the slope of the related calibration graphs

The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision, and variability. The values of LOD and LOQ were given in table1.

3.4 Recovery study

Recovery of the analyte of interest from a given matrix can be used as a measure of the accuracy or the bias of the method. The same range of concentrations as employed in the linearity studies was used. Recovery studies was carried out on standard addition of 80%, 100% and 120% of labeled dose and the total amounts were determined by the method and the % recovery reports were shown in Table.3.

4. In vitro protein Binding Studies

The protein binding studies was performed on semi permeable membrane using sac content of 10 mg ciprofloxacin Hcl in 10ml distilled water as control and sac content of 10mg ciprofloxacin Hcl in 10ml of 1% protein solution as test. The phosphate buffer pH 6.0 100ml was taken as simulated fluid and samples were collected for every five minutes time interval and suitably diluted with same buffer for the measure of absorbance. The drug release kinetics was studied by plotting % Drug release Vs Time and was shown in Figure.3.

5. Results and Discussion

The ciprofloxacin HCl shows λ max at 271.6 nm and the linearity plot yielded a correlation coefficient (R^2) of 0.9991 over the Beers range of 1 – 20 μg/ml. The regression equation was found to be Y=0.0977x + 0.0153. The molar absorptivity (lit/mol.cm) was found to be 3.280365 x 10^4 with Sandells sensitivity (μg/cm^2/0.001) of 0.00908 μg/cm^2 revealed the strong UV absorbing species nature under the experimental condition. The results of analysis of marketed formulation and % Recovery were good and shown in Table.3. The reproducibility and accuracy of the method was found to be good and was evidenced by low standard deviation. The % recovery values indicated non-interference from excipients of formulation. The results of protein binding study were shown about 40 -50 % protein binding and it was good agreement with reported pharmacokinetic data and shown in Table. 3 and drug release kinetics was shown in fig1. The proposed UV method was found to be simple, sensitive, selective, accurate, precise and economical and can be used in the determination of ciprofloxacin Hcl in bulk and its pharmaceutical preparations, in a routine manner.

Table 1. Regression data for the method

S.No	Parameter	Optimized for Ciprofloxacin Hcl
1	λmax(nm)	271.6
2	Beers law(µg/ml)	1-20
3	Regression equation	Y=0.0977x + 0.0153
	a.Slope b.Intercept c.Correlation co-efficient	0.0934 0.0525 0.9996
4	Molar extinction co-efficient(lit/mol.cm)	3.280365×10^4
5	Sandells sensitivity(µg/cm^2/0.001)	0.00908.
6	Limit of detection (LOD, µg/ml)	0.054
7	Limit of quantification (LOQ, µg/ml)	0.165

Table 2: Assay of marketed tablets

Formulation	Labelled claim	Amount found	Assay	% RSD
Trade 1	500 mg	492.50±0.01306*	98.5%	0.223
Trade 2	500 mg	496.72±0.01211*	99.34%	0.209

* Values are replicate of five determinations and reported as Mean ±SD (n = 5)

Table 3. Results of protein binding study by the Proposed method

S. No.	Time (min)	% Drugrelease for control*	%Drug release for test*
1	0	0	0
2	5	22	7.5
3	10	26	9
4	15	30	14
5	20	34	17
6	25	39	22
7	30	40	24
8	35	42.5	30
9	40	43	31
10	45	43	32
11	50	44	34
12	55	45	34.5

*values are mean of three replicates

Table 3. Recovery studies

Dosage form	Labelled claim	Amount added	% Recovered	%RSD
Ciprolet ™ DS	500 mg	80% 100% 120%	102.44±0.0039 98.97±0.0134 99.86±0.0130	0.212 0.398 0.331

(n=5)

Table 4. Precision

S.No	Concentration (µg/ml) Ciprofloxacin Hcl	Absorbances	%RSD
1	10	0.999	0.2078
2	10	0.998	
3	10	0.996	
4	10	0.997	
5	10	0.999	
6	10	0.999	

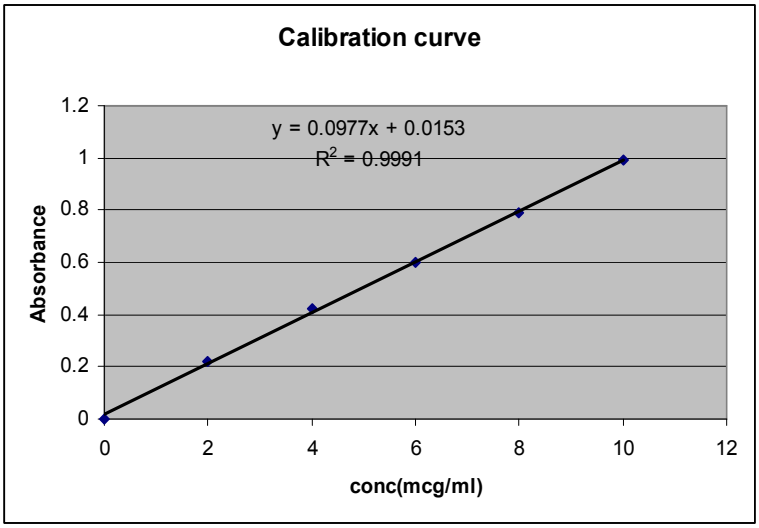

Figure1: Calibration curve

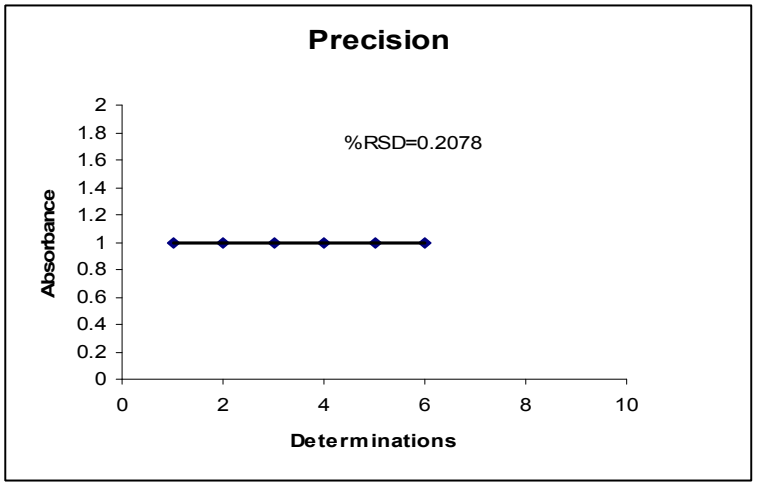

Figure 2: Precision of absorbencies

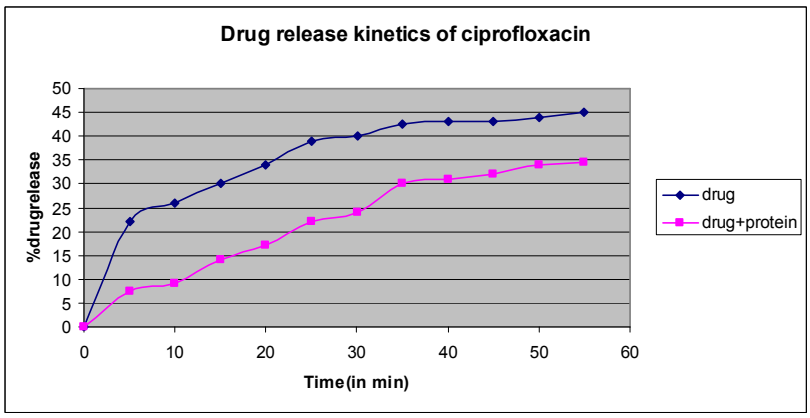

Figure 3: Drug release kinetics of ciprofloxacin Hcl

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