International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 10, Issue 3, 2018

Original Article

DEVELOPMENT AND VALIDATION OF A SIMPLE UV-SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF CIPROFLOXACIN HCL PRESENT IN TASTE MASKED DRUG RESIN COMPLEX

AYYA RAJENDRA PRASAD[a*], JAYANTHI VIJAYA RATNA[a]

[a]Department of Pharmaceutical Technology, A. U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India

Email: dr.ayyarajendraprasad@gmail.com

Received: 09 Dec 2017, Revised and Accepted: 10 Mar 2018

ABSTRACT

Objective: The objective of this study was to develop and validate a novel, specific, precise and simple UV-spectrophotometric method for the estimation of ciprofloxacin HCl present in taste-masked drug-resin complex.

Methods: UV-spectrophotometric determination was performed with ELICO SL 1500 UV-Vis spectrophotometer using 0.1 N HCl as a medium. The spectrum of the standard solution was run from 200-400 nm range for the determination of absorption maximum (λ max). λ max of ciprofloxacin HCl was found at 276 nm. The absorbance of standard solutions of 1, 2, 3, 4, 5 and 6 µg/ml of drug solution was measured at an absorption maximum at 276 nm against the blank. Then a graph was plotted by taking concentration on X-axis and absorbance on Y-axis which gave a straight line. Validation parameters such as linearity and range, selectivity and specificity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision and robustness were evaluated as per ICH guidelines.

Results: Linearity for the UV-spectrophotometric method was noted over a concentration range of 1-6 µg/ml with a correlation coefficient of 0.9995. The limit of detection (LOD) and limit of quantification (LOQ) for ciprofloxacin HCl was found at 0.46 µg/ml and 1.38 µg/ml respectively. Accuracy was in between 98.89 and 99.27%. % RSD for repeatability, intraday precision and interday precision were found to be 1.271, in between 0.351 and 0.659 and in between 1.067 and 1.769 respectively. The proposed UV spectrophotometric method is found to be robust.

Conclusion: The proposed UV-spectrophotometric method was validated according to the ICH guidelines and results and statistical parameters demonstrated that the developed method is sensitive, precise, reliable and simple for the estimation of ciprofloxacin HCl present in taste-masked drug-resin complex.

Keywords: UV spectroscopy, Validation, Analytical Method Development, Ciprofloxacin HCl, Indion 414, Indion 254, Drug resin complex, Resinate, 0.1 N HCl

DOI: http://dx.doi.org/10.22159/ijap.2018v10i3.24199

INTRODUCTION

Ciprofloxacin is an antibiotic used to treat a number of bacterial infections [1]. It is a second-generation fluoroquinolone with a broad spectrum of activity that usually results in the death of the bacteria [2-3]. Chemically it is the monohydrochloride monohydrate salt of 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid. It is a faintly yellowish to light yellow crystalline substance [4]. It is soluble in water, slightly soluble in methanol, very slightly soluble in ethanol, practically insoluble in acetone, in ethyl acetate and in methylene chloride [5]. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV [6], enzymes necessary to separate bacterial DNA, thereby inhibiting cell division. Although human cells do not contain DNA gyrase, they do contain a topoisomerase enzyme that functions in the same manner. This mammalian enzyme is not affected by bactericidal concentrations of quinolones. It is unclear how inhibition of DNA gyrase leads to bacterial cell death. Both rapid and slow growing organisms are inhibited by fluoroquinolones. In addition, fluoroquinolones exhibit a prolonged post-antibiotic effect (PAE). Organisms may not resume growth for 2-6 h after exposure to ciprofloxacin HCl, despite undetectable drug levels.

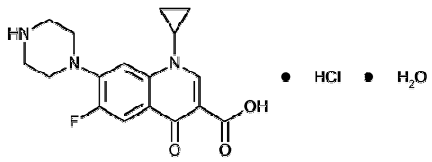

Fig. 1: Chemical structure of ciprofloxacin HCl

The literature survey reveals that various analytical methods have been developed such as HPLC [9-14], and UV spectroscopy methods [15] for the estimation of ciprofloxacin HCl present in bulk, single or combinational pharmaceutical formulations. Most of the reported methods are using several solvents, expensive reagents and often time-consuming. Because of simplicity of UV spectrophotometry and also precise, reliable, minimum solvent usage and requires less analysis time, it is widely used for the estimation of drug content in bulk and pharmaceutical products.

A detailed review of the literature regarding the existing methods revealed that there is a need for the development of the spectrophotometric method, which is simple for the estimation of ciprofloxacin HCl present in taste-masked drug-resin complex which are prepared by batch technique or column technique [16]. An effort was made in the present method to develop a simple, sensitive, accurate, reliable and reproducible with minimum Relative Standard Deviation (RSD) values for the estimation of ciprofloxacin HCl present in taste-masked drug-resin complex.

MATERIALS AND METHODS

Materials

Ciprofloxacin HCl is gifted by Darvin private Laboratories Ltd, vijayawada. Indion 414 and Indion 254 were received as a gift sample from Ion Exchange India Limited. All other chemicals used were of analytical reagent grade.

Equipment used

Spectroscopic analysis was carried out using ELICO SL 1500 double beam UV-Visible spectrophotometer with 10 mm path length quartz cells was used for the analytical purpose.

Preparation of stock and standard solution for the calibration curve

For the method development, a stock solution of ciprofloxacin HCl was prepared by dissolving 100 mg of the drug in 100 ml of 0.1 N HCl so as to obtain a final concentration of 1 mg/ml. From this stock solution, subsequent dilutions were made with 0.1 N HCl to obtain the series of standard solutions containing 1, 2, 3, 4, 5 and 6 µg/ml of solution. The spectrum of the standard solutions was run from 200-400 nm range for the determination of absorption maximum (λ max). λ max of ciprofloxacin HCl was found at 276 nm. The absorbance of above dilutions was measured at 276 nm by using 0.1 N HCl as a blank. A graph was plotted by taking concentration on X-axis and absorbance on Y-axis which gave a straight line.

Analysis of ciprofloxacin HCl in drug-resin complex

Drug-resin complex equivalent to 100 mg of ciprofloxacin HCl has weighed accurately and was transferred into 100 ml of volumetric flask. 100 ml of 0.1 N HCl was added to this volumetric flask and was stirred continuously for 1 hour on a magnetic stirrer. After stirring, this solution was filtered through whattman filter paper. Filtered sample was suitably diluted with 0.1 N HCl and analyzed spectrophotometrically at 276 nm using the proposed method.

Validation of the developed analytical method

This developed method for estimation of ciprofloxacin HCl was validated as per ICH guidelines [17].

Linearity and range

Linearity is the ability of a method to elicit test results that are directly proportional to the analyte concentration within a given range. The range is the interval between the upper and lower levels of analytes that have been demonstrated to be determined with precision, accuracy, and linearity using the method as described. 1, 2, 3, 4, 5 and 6 µg/ml of standard solutions were selected for assessing linearity range. The calibration curve was plotted using concentration of the standard solution versus absorbance and the regression equation was calculated. The least squares method was used to calculate the slope, intercept and correlation coefficient.

Selectivity and specificity

The ability to measure accurately and specifically the analyte of interest in the presence of other components present in the drug-resin complex was analyzed. Placebo solution was prepared by adding Indion 414 and Indion 254. The standard, placebo, placebo along with analyte and test preparations were analyzed as per the method to identify interference of placebo with the absorbance of ciprofloxacin HCl.

Detection and quantification limits

Limit of detection (LOD) represents the lowest amount of analyte in the sample which can be detected. Limit of quantification (LOQ) represents the lowest amount of analyte, which can be quantitatively determined. LOD and LOQ are calculated based on the standard deviation of the response and the slope of the calibration curve.

$LOD = 3.3\sigma/S$

$LOQ = 10\sigma/S$

Accuracy

The accuracy of the proposed method was assessed by recovery studies at three different levels i.e., 80%, 100% and 120%. The recovery studies were carried out by adding a known amount of standard solution of ciprofloxacin HCl to a pre-analyzed drug-resin complex solution. The resulting solutions were then re-analyzed by the proposed method. The total amount of drug found and percentage recovery was calculated.

Precision

The precision of the method was performed at three different levels: repeatability, intra-day precision and inter-day precision at different concentration levels of analyte covering the concentration range. In repeatability, the absorbance of the 3 µg/ml ciprofloxacin HCl concentration solution was measured six times and % RSD was calculated. The intra-day precision was performed by analyzing six replicate standard solutions on the same day, and inter-day precision was performed by analyzing a series of standard solutions for 3 consecutive days using the proposed UV-spectrophotometric method.

Robustness

The evaluation of robustness should be considered during the development phase and depends on the type of procedure deliberate variations in method parameters. If measurements are susceptible to variation in analytical conditions, the analytical condition should be suitably controlled or a precautionary statement should be included in the procedure. In this present work absorption maxima was decreased and increased by 1 nm and the process was carried for 4µg/ml standard solution. The % RSD was calculated.

RESULTS AND DISCUSSION

The proposed method was found to be simple, sensitive, accurate, precise, economical and rapid for the estimation of ciprofloxacin HCl present in drug-resin complex. The method was validated as per ICH guidelines (Q2 (R1).

Linearity and range

A linear correlation was found between absorbance at λ_{max} and concentration of ciprofloxacin HCl. The graph is described by the regression equation: Y= a+bX (where Y=absorbance of drug solution; a =intercept; b =slope and X=concentration of drug in µg/ml). The slope (b), intercept (a) and correlation coefficient (r) were evaluated by using the method of least squares. Good linear correlations were obtained between absorbance and standard drug concentration in the selected range of 1-6 µg/ml. Characteristic parameters such as slope is 0.094, the intercept is 0.004, and the correlation coefficient is 0.9995.

Specificity in the presence of resins

The selectivity and specificity of the proposed method were tested by studying the effect of resins used in the preparation of drug-resin complexes. In the analysis of placebo, the absorbance value was nearly the same as that for solvent suggesting the non-interference by the resins added to prepare the placebo solution.

Table 1: Calibration curve for the estimation of ciprofloxacin HCl in 0.1 N HCl

Concentration (µg/ml)	Absorbance (mean±SD*)(n=6)
0	0
1	0.088±0.007
2	0.185±0.008
3	0.279±0.004
4	0.361±0.006
5	0.465±0.004
6	0.571±0.003

*SD-standard deviation

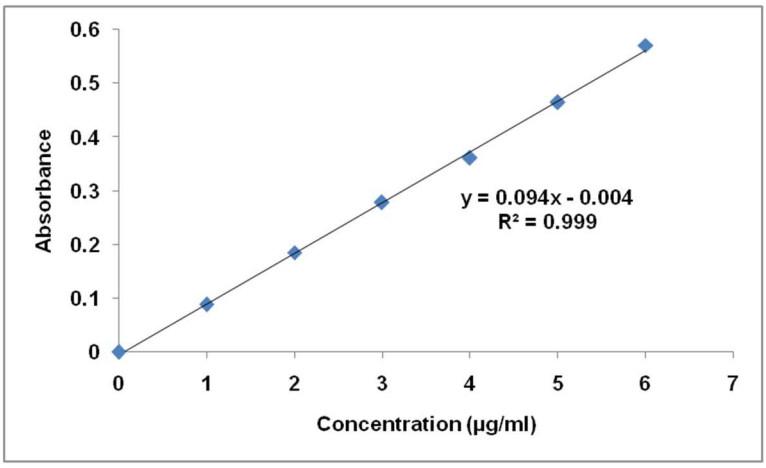

Fig. 2: Calibration curve of ciprofloxacin HCl in 0.1 N HCl

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of ciprofloxacin HCl were determined by using the standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines. The limit of detection (LOD) and limit of quantification (LOQ) for ciprofloxacin HCl were found to be 0.46 µg/ml and 1.38 µg/ml respectively, indicating that the proposed UV method is highly sensitive. Results of regression and analytical parameters are reported in table 2.

Table 2: Regression and analytical parameters

Parameter	Value
$\lambda_{max,}$ nm	276
Beer's law limits (µg/ml)	1-5
Regression equation, Y *	Y = 0.094X+0.004
Intercept, (a)	0.004
Slope, (b)	0.094
Correlation coefficient (r)	0.9995
Standard deviation of intercept	0.012981125
Limit of detection(µg/ml)	0.46
Limit of quantification (µg/ml)	1.38

*Y=a+bX, where Y is the absorbance, a is the intercept, b is the slope and X is the concentration in µg/ml.

Accuracy

The accuracy of an analytical method is the closeness of test results to a true value. The accuracy of the method was evaluated by standard addition method at 3 levels. A standard quantity equivalent to 80%, 100% and 120% is to be added to the sample. Results within the range of 98.89-99.27% ensure an accurate method as well as indicate of non-interference with the resins.

Results of recovery studies are reported in table 3.

Table 3: Accuracy/recovery studies for three different concentrations of ciprofloxacin HCl by the proposed method

% Drugadded	Amount of drug added to preanalysed sample(µg/ml)	Amount found(µg/ml)	% recovery	% Average recovery(n=3), (mean±SD*)	% RSD**
80	3.2	3.17	99.06	99.27±0.180	0.182
80	3.2	3.18	99.37		
80	3.2	3.18	99.37		
100	4	3.98	99.50	99.17±0.382	0.385
100	4	3.95	98.75		
100	4	3.97	99.25		
120	4.8	4.78	99.58	98.89±0.732	0.740
120	4.8	4.75	98.96		
120	4.8	4.71	98.13		

*SD-standard deviation, RSD**-relative standard deviation

Precision

% RSD for repeatability, intraday precision and interday precision were found to be 1.271, in between 0.351 and 0.659 and in between 1.067 and 1.769 respectively. The results showed an excellent repeatability, intra-day precision and inter-day precision of the proposed method. The results obtained were presented from table 4-6.

Robustness

The robustness of this method was tested in terms of variation in wavelength change. Experimental findings proved that the change since % RSD values was found to be less than 0.8% (less than the acceptable theoretical limit of<2% RSD). The proposed UV-spectrophotometric method was found to be robust. The results obtained were presented in table 7.

Table 4: Repeatability studies

Concentration(µg/ml)	Absorbance	mean±SD*(n=6)	% RSD**
3	0.282	0.279±0.004	1.271
3	0. 275		
3	0. 282		
3	0. 277		
3	0. 275		
3	0. 282		

*SD-standard deviation, RSD**-relative standard deviation

Table 5: Intra-day precision for five different concentrations of ciprofloxacin HCl

Concentration (µg/ml)	Time			% RSD**
	10:30 a. m.	1:0 p. m.	4:30 p. m.	
1	0.088	0.088	0.087	0.659
2	0.185	0.184	0.183	0.543
3	0.279	0.278	0.276	0.550
4	0.361	0.359	0.357	0.557
5	0.465	0.463	0.461	0.432
6	0.571	0.569	0.567	0.351

RSD**-relative standard deviation,

Table 6: Inter-day precision for five different concentrations of ciprofloxacin HCl

Concentration (µg/ml)	Day			% RSD**
	1	2	3	
1	0.088	0.086	0.085	1.769
2	0.185	0.182	0.179	1.648
3	0.279	0.274	0.270	1.644
4	0.361	0.356	0.352	1.265
5	0.465	0.460	0.455	1.087
6	0.571	0.564	0.559	1.067

RSD**-relative standard deviation,

Table 7: Effect of different wavelengths of light on a 3 µg/ml solution of ciprofloxacin HCl in 0.1N HCl

Wavelength (nm)	Concentration (µg/ml)	Absorbance	mean±SD* (n=3)	%RSD**
275	4	0.360	0.358±0.003	0.581
		0.359		
		0.356		
276	4	0.363	0.361±0.003	0.733
		0.362		
		0.358		
277	4	0.360	0.358±0.003	0.426
		0.358		
		0.357		

*SD-standard deviation, RSD**-relative standard deviation

CONCLUSION

This developed method is found to be specific as there was no interference of any resin present in the taste-masked drug-resin complex in the analysis of ciprofloxacin HCl. The results and the statistical parameters demonstrated that the proposed UV spectrophotometric method is simple, rapid, reliable, accurate and precise. Hence, it can be used conveniently for the determination of drug present in taste-masked drug-resin complex.

ACKNOWLEDGEMENT

The authors like to acknowledge the staff and management of the KVSR Siddhartha College of pharmaceutical sciences, Vijayawada, Andhra Pradesh, India for providing necessary facilities to carry out the research work.

AUTHORS CONTRIBUTIONS

The author, Ayya Rajendra Prasad is a Ph. D. student of Dr. Smt. Jayanthi Vijaya Ratna. The work relates to Ph. D. work of author under the guidance of Dr. Smt. Jayanthi Vijaya Ratna.

CONFLICTS OF INTERESTS

The authors confirm that this article content has no conflict of interest.

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