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BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTIFICATION OF CIPROFLOXACILIN HCL IN PLASMA BY HPLC.

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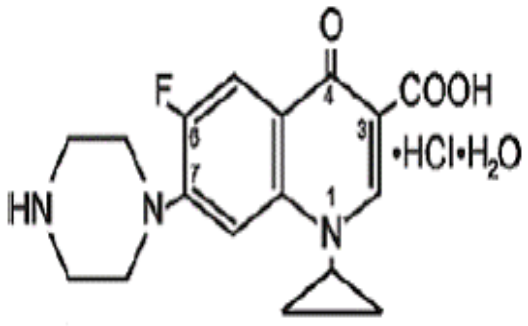
ABSTRACT

The development of sound bioanalytical method is an important paramount process in drug discovery, development and in pharmacokinetics of drug. In the present investigation the HPLC bioanalytical method of liquid- liquid extraction of drug from plasma is established and the linearity study of Ciprofloxacilin HCl was found in range of 0.5 µg/ml to 4 µg/ml, the regression equation was found to be y = 1326.3X + 7.4819. The method checked for Pression and % RSD was found to be 1.45 % and the accuracy was in the range 101.0% to 102.40%. The limit of detection and the limit of quantification were calculated by the non instrumental that is by equation mentioned in the methodology. The LOD was found to be 0.039 µg/ml and LOQ was found to be 0.11 µg/ml. Thus the developed bioanalytical HPLC is selective and quantity the Ciprofloxacilin HCL in plasma and supports the pharmacokinetics of colon targetd Ciprofloxacilin HCl matrix tablets.

KEYWORDS: Ciproloxacillin HCl, Leveoloxacillin, Linearity, Precession, Relative standard deviation, Plasma drug concentration.

INTRODUCTION

Ciprofloxacin, structurally (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[1-piperazinyl]-3- quinolinecarboxylic acid), was as a potent floroquinolone chemotherapeutic of the second- generation group of nalidixic acid derivatives first commercially introduced in the 1980s.



Due to the broad spectrum effect and systemic matter of action, it is widely used both in human and veterinary medicine to treat infectious diseases, caused particularly by Gram- negative and some Gram-positive bacteria. The target of highly selective action of ciprofloxacin is bacterial DNA gyrase, a type of topoisomerase II. After peroral administration in human ciprofloxacin is rapidly absorbed from GIT into the systemic circulation and reaches the maximal concentration in 1–2 h. The bioavailability is 56–79%, about 65% of unchanged ciprofloxacin and 10–15% of metabolites is excreted in the urine and about 15% in feces .[1] It is well known that the pharmacokinetic processes (absorption, distribution, metabolism and excretion) can change due to physiological alterations in critically ill patients. These alterations necessitate rational adjustment of the dosage of the drug, especially of antibiotics.

Consequently, to investigate the pharmacokinetics of the Ciprofloxacilin was needed. Therefore, there is a need to develop a single bio-analytical method for the quantification of the Ciprofloxacilin in plasma. Numerous methods for ciprofloxacin determination in biological samples have been referred and reviews have been published. High-performance liquid chromatography (HPLC) with UV or fluorescence detectionwas the technique most used. These particular assays describe the quantification of a fluoroquinolone in human plasma. In addition, several papers have reported the separation and/or simultaneous quantification of two or more fluoroquinolones.

Chan et al. described the simultaneous analysis of ofloxacin and moxifloxacin in human aqueous and vitreous humor using HPLC with fluorescence detection. Liang et al. developed a method for the separation of six fluoroquinolones including ciprofloxacin and moxifloxacin.[2] Under the scope of this view, the present investigation was to develop the more precise, accurate, rugged and reliable method of ciprofloxacin determination in plasma samples for application in

pharmacokinetics study and great number of samples needed to be analyside in the relatively short time.

MATERIALS AND METHODS

Gift sample of Ciprofloxacilin HCl and Levofloxacillin was obtained from Aurobindo Pharma Ltd, Hyderabad. Ammonium acetate phosphate, Acetonitrile, potassium die hydrogen orthophosphate and sodium hydroxide was received from S.d.Fine chemicals, Bangalore.

Instrumentation[3]

The chromatographic technique performed on a Shimadzu LC20-AT Liquid chromatography with SPD-20A prominence UV-visible detector and Spinchrom software , reversed phase C18 column (Phenomenex C18 column (4.6 , 3.5µm)) as stationary phase, Electron corporation double beam UV-visible spectrophotometer (vision pro-software), Ultrasonic cleaner, Shimadzu analytical balance AY-220, Vacuum micro filtration unit with 0.45µ membrane filter was used in the study.

HPLC conditions[3]

The contents of the mobile phase of phosphate buffer pH 4.5 and Acetonitrile at ratio of(3:7) is used for Ciprofloxacin HCl Before using, the mobile phases were filtered through 0.45- µm membrane filter and degassed with a helium spurge for 15 minutes. The components of the mobile phase were pumped from the respective solvent reservoirs in to the column at a flow rate of 1.5 ml/min which yielded a column back pressure of 120 – 130 kg/cm^2. The run time was set at 20 min and the column temperature was maintained at 40 ^{o} C. The volume of the injection loop was 10 µL. Prior to the injection of the sample solutions the column was equilibrated for at least 30 min with the mobile phase flowing through the systems. The eluents were monitored at the wavelength of 277 nm for Ciprofloxacin HCl .The data were acquired, stored and analyzed with the software Class – VP series version 5.03 (Shimadzu).

Preparation of Stock Solution

Ciprofloxacin HCL stock solution and Levofloxacin internal standard stock solution: Accurately weighed and transferred 10mg of Ciprofloxacin sample in a 100 ml volumetric flask then dissolved by sonication and made the volume up to the mark with mobile phase , similarly the internal standard levofloxacin stock solution was prepared by transferring the 10 mg of accurately weighed drug into 100 ml volumetric flask and then dissolved by sonication and made the volume up to the mark with mobile phase.

From above stock solution of 10 µg/mL of Ciprofloxacin and 10 µg/mL of Internal standard is prepared by diluting 1 ml of standard stock solution and 1mL of internal standard stock solution to 10ml with mobile phase. The resulting solutions are scanned for wavelength at their respective peaks.

Extraction and sample preparation[4]

Transferred 0.2mL of plasma which was previously taken from the rabbit animal in a conical shape test tube, added 1ml of sample to the plasma and samples were vortexed briefly for proper mixing. Added 3mL of Chilled methanol and then also added 1mL of internal standard solution, swirl it. Made the volume up to 10mL with methanol. Centrifuge the mixture and collected the supernatant.

The above established HPLC method was further studied for the following parameter like linearity, precision, accuracy, LOD, LOQ.

Linearity[5]

The linearity of the proposed HPLC method was determined in terms of correlation coefficient between concentration of the drug and its respective peak area. The data were subjected to regression analysis using least square method.

Precision[6]

The precision of the analytical procedure express the closeness of agreement between series of measurement obtained from the multiple sampling of same homogeneous solution. Ciprofloxacin HCL solution of 100% concentration at six determinations is injected into the column, the peak area and the retention time is recorded and tabulated.

The precision is expressed in terms of coefficient of variation (CV) which is calculated by multiplying the ratio of standard deviation to the mean with 100 (C.V=SD/meanX100).

Accuracy[5]

The accuracy of an analytical method is the closeness of test results to the true value. The accuracy is expressed in terms % recovery, which is calculated by multiplying the ratio of measured % drug concentration to the expected % drug concentration with 100 so as to give the percent recovery.

Limit of detection[6]

The limit of detection is the lowest amount of analyte in a sample that can be detected , but not necessicerly quantiated under the stated experimental conditions and it is calculated by.

$$LOD = \frac{\text{Standard deveiation}}{\text{Slope}} \times 3.3$$

Limit of quantification[6]

The limit of quantification is the lowest amount of analyte in a sample that can be quantified with the acceptable accuracy and precision under the stated experimental conditions and it is calculated by.

$$LOD = \frac{\text{Standard deveiation}}{\text{Slope}} \times 10.0$$

RESULTS

Table No 01. Spectrophotometric data of ciprofloxacin HCl

Concentration (µg/ml)	AVG. Peak Area	SD	%RSD
0	0	0	0
0.5	656.1667	8.024269	1.222901
1	1327.333	14.29063	1.076642
2	2628.00	18.8326	0.716613
3	3948.333	24.00463	0.607969
4	5321.333	13.59739	0.255526

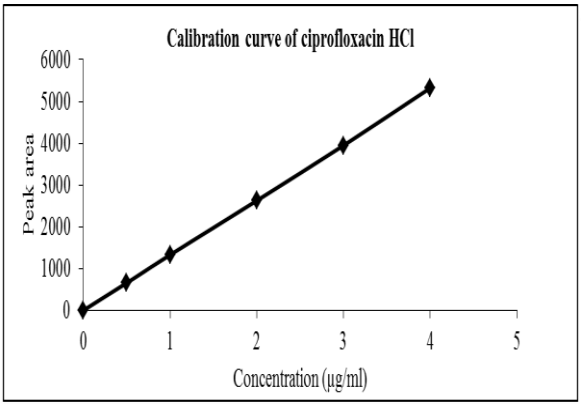


Fig No 01. Calibration curve of Ciprofloxacin HCl

Table No 02. Linearity study of Ciprofloxacin HCl

Concentration (µg/ml)	AVG.Area	SD	% RSD	Regression equation Data
0	000000	0000000	0000000	
0.5	656.1667	8.024269	1.222901	M = 1326.3 C= 7.4819 R^2 = 0.9999
1	1327.333	14.29063	1.076642	
2	2628.00	18.8326	0.716613	
3	3948.333	24.00463	0.607969	
4	5321.333	13.59739	0.255526	

M = slope, C = intercept on y axis, R = correlation coefficient

Table No 03. Precision study of Ciprofloxacin HCl

Inj.No	Timings (hrs)	Peak area
1	7.30 AM	5518
2	10.30 AM	5620
3	1.30 PM	5500
4	4.30 PM	5642
5	7.30 PM	5700
6	9.30 PM	5713
Average	-	5615.5
SD	-	81.86931
%RSD	-	1.45

Table No 04. Accuracy study of Ciprofloxacin HCl

Standard solution		Test sample solution				% con founded	% Con recovery
Trial	Peak area	% Con Added	Peak area	AVG. Peak area	SD		
1 2 3	5424 5302 5205	50	2702 2710 2650	2687.3	26.39	50.6	101.20
AVG	5310.3	100	5485 5326 5500	5437	78.72	100.2	102.40
SD	89.60	150	8000 8100 8025	8041.6	42.49	151.4	101.00

DISCUSSION

To estimate the plasma drug concentration, the simple, selective bioanalytical HPLC method has been established and studied the following parameters like, linearity, accuracy, precision and LOD and LOQ. The linearity of ciprofloxacin HCl was found in the range of 0.5 µg/ml to 4 µg/ml and it showed the linear relationship with concentration and peak area .The correlation of coefficient was found to be 0.9999 indicating the greater dependence between concentration and peak area and the slope was found to 1326.3. The regression equation obtained as y = 1326.3X + 7.4819 .The developed methods were checked for precision by spiking the same concentration at six determinations. The results observed from table no 02 indicates that peak area is consistent and % RSD was found to be 1.45,

which is within the acceptable limit. Accuracy measures the closeness of the test sample value to true value. It is performed at three different concentration level and the results of % recovery was found to be 101.0% to 102.40%, for ciprofloxacin HCl, which is within the acceptable limit. The limit of detection and the limit of quantification were calculated by the non instrumental that is by equation mentioned in the methodology. The LOD and LOQ was found to be 0.039µg/ml and 0.11µg/ml respectively.

CONCLUSION

The established bioanalytical HPLC methods were found to be simple and specific in separating the peak area of internal standard and sample peak area. Accurate and precise in estimating the drug in plasma, the above HPLC methods can be used for quantifying the plasma drug concentration of Ciprofloxacin HCl colon targeted tablets.

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