Journal of Applied Pharmaceutical Science
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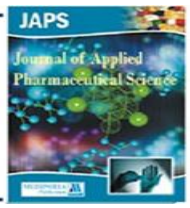

ISSN: 2231-3354
Received on: 25-09-2011
Revised on: 08:10:2011
Accepted on: 15-10-2011

High Performance Liquid Chromatography (HPLC) Method Development and Validation Indicating Assay for Ciprofloxacin Hydrochloride

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ABSTRACT

A new simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid chromatography assay has been developed for the estimation of Ciprofloxacin Hydrochloride in tablet formulation. The separation was achieved by using C-18 column (LichroCART® 125x4mm, 5µm) coupled with a guard column of silica in mobile phase methanol: buffer (0.025M Orthophosphoric acid with the pH adjusted to 3.0±0.1 with triethylamine) (40:60v/v). The flow rate was 2.0ml/min and the drug was detected using UV detector at the wavelength of 278nm. The retention time was within 1.753 – 1.757 minutes. The method was validated as per ICH guidelines. The proposed method was found to be accurate, repeatability and consistent. It was successfully applied for the analysis of the drug in marketed formulation and could be effectively used for the routine analysis of formulation containing the drug without any alteration in the chromatography conditions.

Keywords: Ciprofloxacin, HPLC, Liquid Chromatography.

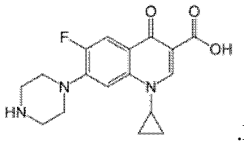
INTRODUCTION

Ciprofloxacin Hydrochloride **is a** pale yellow, slightly hygroscopic, crystalline powder, soluble in water, very slightly soluble in dehydrated alcohol, practically insoluble in acetone, in dichloromethane, and in ethyl acetate and slightly soluble in methyl alcohol. A 2.5 % solution in water has a pH of 3.5 to 4.5. Store in airtight containers. Protect from light (Martindale, 2009).

Drug profile

3-quinoline carboxylic acids, I cyclopropyl -6- fluro -1,4-dilydro-4-oxo-7-(1-piperazinyl)-monohydrochloride.

Chemical structure



.HCl

Molecular weight: 367.82g/mol
Molecular Formular: $C_{17}H_{18}FN_3O_3$.HCl

Uses

Ciprofloxacin is the most commonly used fluoroquinolones and it is a broad-spectrum antibiotic, effective against both gram positive and gram negative organisms (Rang, et al 2008). It is particularly active

against gram negative bacteria, including Salmonella, Shigella, Campylobacter, Neisseria and Pseudomonas. Ciprofloxacin has only moderate activity against gram positive bacteria such as Streptococcus, Pneumoniae and Enterococcus faecalis, it should not be used for pneumococcal pneumonia (BNF, 2010). Also use for Chronic bacterial prostatitis (Dimitrakov, etal,2009) to Lower respiratory tract infection (this two are not recommended as a first-line antibiotic choice) (Vardakas, etal,2008) for treatment of tuberculosis (Thomas, et al, 2008).

Availability

Ciprofloxacin is available for oral administration in 250mg, 500mg and 750mg tablets, parental administration in 2mg/ml, ciprofloxacin ophthalmic solution in 0.3% as base.

Several papers have been described for the determination of ciprofloxacin in biological fluids by HPLC with UV (Gladys, 1992) or fluorescence (El-Yazigi A. and Al- Rawithy S., 1990) detector or by microbiological methods (Jehl et al, 1985). Poor reproducibility and accuracy for the last method has been reported (Jehl et al, 1990). However, HPLC is the analytical method of choice for measuring and assaying ciprofloxacin (Teja-Isavadharm et al, 1991). Literature survey has also revealed a few HPLC methods for the estimation of Ciprofloxacin HCl and Tinidazole (George et al, 2000; Garcia and Albero, 2001). Also some HPLC methods are available for the determination of Ofloxacin and Ornidazole (Bakshi et al, 2001; Natrajan and Raman, 2005). Similarly some HPLC methods are reported for the estimation of Ofloxacin and Tinidazole (Panzade and Mahadik, 2000; Amini et al, 2005; Salomies and Salo, 2005; Behl et al, 2005). HPLC method for simultaneous estimation of Ciprofloxacin HCl, Tinidazole, Ofloxacin and Ornidazole was also carried out (Ranjit et al, 2009).

METHODOLOGY

Method that was carried out using the template and the sketch below as a guiding principle. The rationale behind the use of this method is that Ciprofloxacin HCl has a molecular weight less than 1000, soluble in water, and ion forming, thus the use of Reversed phase chromatography. Methanol was thought of to replace acetonitrie because of its greater polarity in silica column.

MATERIALS

The Chromatographic system consisted of HITACHI L-2130 pump connected to an autosampler L-2200 syringe loading sample injector valve fitted with a 20µl sample loop of 200vials, a variable wavelength UV-VIS detector L-2420. A column oven L-2300, packed with silica C18, 5µm particle size, an organizer and diode Arrary Detector L-2455. Standard Ciprofloxacin HCl was obtained from NAFDAC Drug Laboratory, Maiduguri. Ciprofloxacin tablets were obtained from pharmacy outlets and patent medicine stores within Maiduguri metropolitan of Borno State.

DETECTION OF WAVELENGTH

In setting up the conditions for development of the assay method, the choice of a detection wavelength was based on the scanned absorption spectrum for Ciprofloxacin HCl. The spectrum was scanned over the range of 200-400nm and was obtained by measuring the absorption of 20µg/ml solution of Ciprofloxacin HCl in mobile phase, prepared from a stock solution. The spectrum was obtained by using a 1cm silica cell and the reference cell contained mobile phase; as a result a wavelength of 278nm was chosen which correspond with USP standard.

OPTIMIZATION OF CHROMATOGRAPHIC CONDITION

Spectroscopic analysis of Ciprofloxacin HCl showed that it has a maximum UV absorbance at 278nm. Therefore, the chromatographic detection was performed at 278nm using a UV-Visible detector. It was observed that when Ciprofloxacin HCl was injected at the mobile phase composition ACN: buffer (13:87) (USP) as shown in fig I, it indicates non specificity in activity.

PREPARATION OF MOBILE PHASE

Preparation of Buffer

1.6ml of 15.7M of Orthophosphoric acid was dissolved in 1000ml distilled water and final concentration made up to 0.025M. Triethylamine was used to adjust the pH to 3.0 ± 0.1.

Mobile Phase

Put 400ml of methanol into a 1000ml beaker, to this add 600ml of the buffer (Orthophosphoric acid + Triethylamine), it was sonicated for 10minutes using the sonicator and filtered using the vacuum pump.

Mobile Phase Ratio

Methanol: Buffer (Orthophosphoric acid + Triethylamine) = 40:60

Standard Preparation

25mg of the standard Ciprofloxacin HCl was dissolved in 50ml of the mobile phase. Dilution (1:25) from this solution was made to obtain a concentration which contains 0.02mg/ml of the standard solution and 20µl was injected to obtain the peak area as shown in fig IV.

Sample Preparation

20 intact tablets (Ciprofloxacin) were weighed accurately to obtain the average tablet weight; the tablets were then crushed and triturated in a mortar until a fine powder was obtained. An amount of the powder equivalent to one tablet (25mg) was weighed accurately and taken into a 50ml volumetric flask. The powder was dissolved in 50ml of the mobile phase. Dilution (1:25) from this solution was made and the clear solutions were then ready for injection (20µl was injected).

CONDITIONS OF CHROMATOGRAPHIC METHOD

Chromatographic separation was performed using an isocratic elution at 40^{o}c on C-18 column (5µm particle size) parked with silica. The mobile phase was composed of Methanol: Buffer (40:60v/v) and pH of mobile phase was adjusted to 3.0 ± 0.1 with triethylamine. The flow rate is 2.0ml/min and the injection volume

was 20µl. UV measurement was made at wavelength of 278nm. The retention time was between 1.750 to 1.753minutes for samples.

Table I: Uniformity weight of tablets.

S. No.	Sample	Average weight 0f tablets (mg) ± SD	Amount weighed (mg)
1	A	919.2 ± 0.01	45.96
2	B	681.2 ± 0.03	34.06
3	C	766.9 ± 0.01	38.35
4	D	740.4 ± 0.01	37.02
5	E	833.6 ± 0.02	41.68

RESULTS

METHOD VALIDATION

The method was validated in terms of the following parameters; linearity, specificity, accuracy, precision and system suitability (ICH).

Calculations

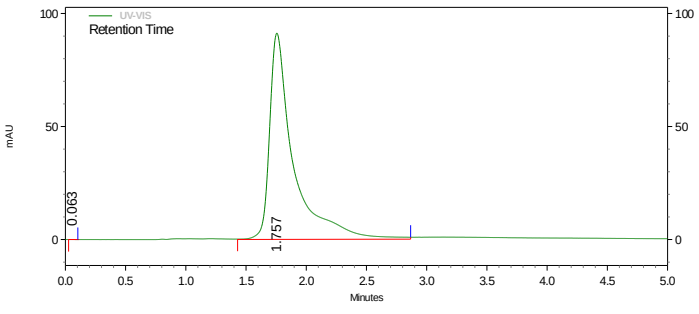
$$\text{Mg/tab} = \frac{A_{sa} \times M_{std} \times P \times AVem}{A_{std} \times M_{sa} \times 100}$$

A_{sa} = Area of sample peak
A_{std} = Area of standard peak
M_{std} = Mass of standard weighed (in mg)
M_{sa} = Mass of sample weighed (in mg)
P = Potency of standard in %
AVem = Average mass of tablets (in mg)

Sample A

Sample Name : A – *20 µg/ml*
System : HPLC
Detector : UV – VIS
Type of Analysis : Peak Area
Peak area : 4724281.3
Retention time : 1.757 minutes

Fig VA

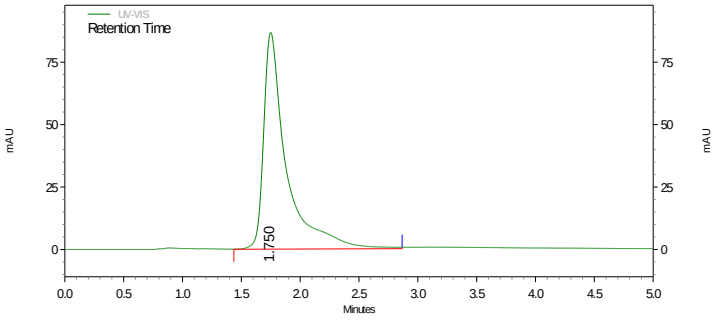


Mg/tab = $\frac{4724281.3 \times 25 \times 99.7 \times 919.2}{4368440.7 \times 45.96 \times 100}$ = 539.11mg (107.8%)

Sample B

Sample Name : B – *20 µg/ml*
System : HPLC
Detector : UV – VIS
Type of Analysis : Peak Area
Peak area : 4628085.7
Retention time : 1.750 minutes

Fig VB

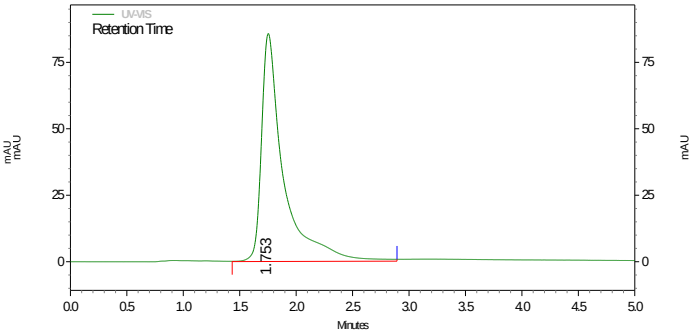


Mg/tab = $\frac{4628085.7 \times 25 \times 99.7 \times 681.2}{4368440.7 \times 34.06 \times 100}$ = 528.13mg (105.6%)

Sample C

Sample Name : C – *20 µg/ml*
System : HPLC
Detector : UV – VIS
Type of Analysis : Peak Area
Peak area : 4646783
Retention time : 1.753 minutes

Fig VC

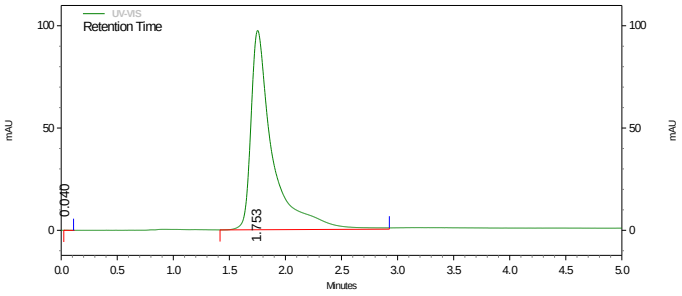


Mg/tab = $\frac{4611585.7 \times 25 \times 99.7 \times 766.9}{4368440.7 \times 38.35 \times 100}$ = 526.18mg (105.2%)

Sample D

Sample Name	: D – *20 µg/ml*
System	: HPLC
Detector	: UV – VIS
Type of Analysis	: Peak Area
Peak area	: 4987905
Retention time	: 1.753 minutes

Fig VD

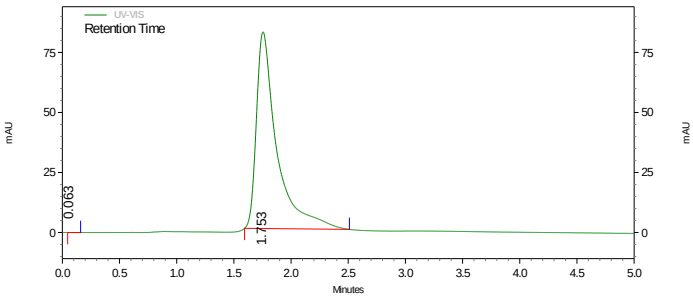


$$\text{Mg/tab} = \frac{4987905 \times 25 \times 99.7 \times 740.4}{4368440.7 \times 37.02 \times 100} = 569.19\text{mg}\ (113.8\%)$$

Sample E

Sample Name	: E – *20 µg/ml*
System	: HPLC
Detector	: UV – VIS
Type of Analysis	: Peak Area
Peak area	: 4152365.7
Retention time	: 1.753 minutes

Fig VE



$$\text{Mg/tab} = \frac{4152365.7 \times 25 \times 99.7 \times 833.6}{4368440.7 \times 41.68 \times 100} = 473.84\text{mg}\ (94.8\%)$$

An RP-HPLC method for the determination of Ciprofloxacin HCl was developed and validated. Results of analysis of the formulations are tabulated in table I. The amounts obtained by the proposed method are between 94.8% and 113.8%, within the acceptance level of 90% to 110% (USP) except for sample D which falls out of the range. The reason for the variation could be as result of less potency of the standard Ciprofloxacin HCl obtained.

Table II: Evaluation of Ciprofloxacin in pharmaceutical formulations.

S.No	Sample	Labelled amount (mg)	Amount obtained by proposed method	Percentage recovery*
1	A	500	539.11	107.8%
2	B	500	528.13	105.6%
3	C	500	526.18	105.2%
4	D	500	569.19	113.8%
5	E	500	473.84	94.8 %

*Average of three determinations

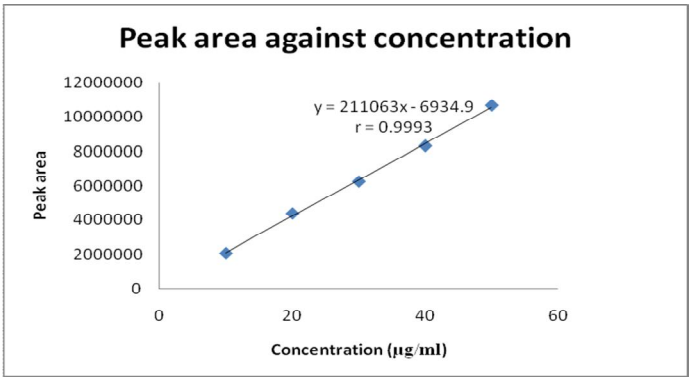
METHOD VALIDATION FOR CIPROFLOXACIN HYDROCHLORIDE

Linearity

Five different concentrations (10-50µg/ml) of Ciprofloxacin HCl were prepared for linearity studies. The responses were measured as peak areas as shown in fig VI-X. The calibration curve obtained by plotting peak area against concentration showed linearity in accordance to Beer's law over this range and the linearity equation was y=211063x – 6934.9 and the regression coefficient was r=0.9993 (n=5).

Table III: Linearity of Ciprofloxacin Hydrochloride.

S. No.	Concentration (µg/ml)	Peak area
1	10	2071855
2	20	4361218
3	30	6234483
4	40	8303177
5	50	10654017



Graph I: Linearity curve of Ciprofloxacin Hydrochloride.

Table IV: Specificity of method (retention time of standard is 1.753).

Sample	Retention time
A	1.757
B	1.750
C	1.753
D	1.753
E	1.753

SPECIFICITY

The specificity of the method was ascertained by analyzing standard drug and sample. The retention time (RT) of Ciprofloxacin HCl confirmed by comparing the RT with that of the standard at 20µg/ml.

REPEATABILITY

Three 20µl injections from a standard solution were injected on to the analytical column and the peak areas data obtained were used in assessing system suitability and quality of analysis by calculating the %relative standard deviation (%RSD=0.12 n=3) at 20µg/ml. The lower %RSD indicates that there are less variation and there are high precision in the values.

Table V: Repeatability of method.

No of injection	Values of peak areas	Mean of values	SD	%RSD	%CV
3	4361218, 4372202, 4371902	4368441	5108.665	0.12	0.12

ACCURACY AND PRECISION

The method was established by carrying out analysis of the analyte (standard Ciprofloxacin HCl) using proposed method. The low value of % CV showed that the method is precise within the acceptance limit of 2%. The results are shown in Table V.

CONCLUSION

An RP-HPLC method for the determination of Ciprofloxacin HCl was developed and validated. Results of analysis of the formulations are tabulated in table I. The amounts obtained by the proposed method are between 94.8% and 113.8%, within the acceptance level of 90% to 110% (USP) except for sample D which falls out of the range.

The results obtained indicate that the proposed method is simple, rapid, accurate, and specific. Linearity was observed over a concentration range of 10 to 50µg/ml for the standard Ciprofloxacin Hydrochloride. This project, therefore describes an isocratic HPLC method using UV detection, which provides adequate sensitivity for routine use and diminishing the time of sampling and chromatographic analysis.

RECOMMENDATION

It can be used for routine analysis of formulations containing Ciprofloxacin Hydrochloride without any alteration in the assay. The main advantage of the method is the simple chromatographic conditions adopted. Therefore, the proposed method reduces the retention time and as such saves time and quantity of mobile phase used.

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