A Simple UV Spectrophotometric Method Development and Validation for Estimation of Ciprofloxacin Hydrochloride in Bulk and Tablet Dosage Form

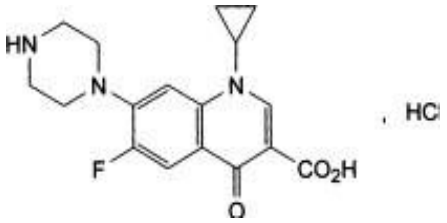
Rallabandi Lakshmi Pavani, Malik. Hasan, Yarra.Uma Venkata Lakshmi, Pasupuleti. Ravi Teja
Department of Pharmaceutical Analysis, St Ann's college of Pharmacy, Chirala.

***Abstract*-A novel, simple, sensitive and rapid UV Spectrophotometric method was developed for the estimation of Ciprofloxacin hydrochloride in bulk and its pharmaceutical tablet dosage form. Ciprofloxacin hydrochloride exhibiting maximum absorbance at 274 nm in distilled water and obeyed linearity in the concentration range of 2-10 ppm. The proposed method has been applied successfully for the analysis of Ciprofloxacin hydrochloride either in bulk or pharmaceutical tablet dosage form with good accuracy and precision. The method herein described can be employed for quality control and routine analysis of Ciprofloxacin hydrochloride in pharmaceutical tablet dosage form.**

Key Words: **Ciprofloxacin hydrochloride, UV Spectrophotometry, maximum absorbance, bulk**

I. INTRODUCTION

Ciprofloxacin hydrochloride is, chemically, 1-Cyclopropyl- 6-fluoro-4-oxo-7-(piperazin-1-yl)-1, 4-dihydroquinoline-3- carboxylic acid hydrochloride. The molecular formula is $C_{17}H_{18}FN_3O_3$.HCl, (fig. 1) representing a molecular weight of 367.8 and belongs to the group of synthetic fluoroquinolone antibiotics with broad antimicrobial activity [1], and is structurally related to nalidixic acid. It is believed that the mode of action of this family of drugs is through binding DNA-gyrase enzyme [2]. It is also reported that there is a direct correlation of fluoroquinolone bonding with inhibition of DNA gyrase enzyme activity and induction of DNA breakage. Because of this special mechanism of action, fluoroquinolones are considered to be the most effective gram-positive-gram-negative pathogens to combat infections caused by micro organisms that are resistant to other microbial, such as tetracycline. The drug is official in British Pharmacopoeia [3] and United States Pharmacopoeia [4] which describes a high performance liquid chromatographic (HPLC) method for its assay.



Ciprofloxacin hydrochloride is a pale yellow, crystalline powder, slightly hygroscopic that is freely Soluble in water, slightly soluble in methanol, very slightly soluble in ethanol, practically insoluble in acetone, in ethyl acetate and in methylene chloride . Literature survey revealed that various analytical methods such as LC, HPLC, HPTLC and capillary electrophoresis methods are used for estimation of Ciprofloxacin hydrochloride in single as well as in combination with various drugs. Hence, an attempt has been made to develop new simple, sensitive UV spectrophotometry method for its estimation either in bulk or pharmaceutical tablet dosage form with good accuracy, simplicity, precision and economy.

II. EXPERIMENTAL ANALYSIS

Instrumentation: Spectral and absorbance measurements were made on UV spectrophotometer. Digital balance (1 mg sensitivity) Sansui was used for weighing the samples. Commercially available tablets of Ciprofloxacin hydrochloride were procured from the local market and estimated.

Chemicals and reagents: -

1. Distill Water In-house
2. Ciprofloxacin hydrochloride (99%) Shreya Life Sciences Pvt. Ltd., Roorkee

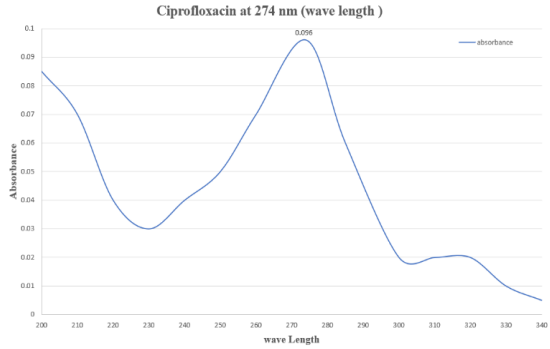
List of Equipments :-

- UV Spectrophotometer

- Micro pipette
- Digital Balance

Optimization

Scanning and determination of maximum wavelength (λ_{max}): In order to ascertain the wavelength of maximum absorption (λ_{max}) of the drug (10 ppm) in distill water was scanned using UV spectrophotometer within the wavelength region of 220 – 800 nm against distill water as blank. The resulting spectrum And the absorption curve showed characteristic absorption maxima at 274 nm for Ciprofloxacin hydrochloride.



Preparation of standard stock solutions: Standard stock solution was prepared by dissolving 100 mg of Ciprofloxacin hydrochloride in distill water and volume made up to 100 ml with distill water to get concentration of 1mg/ml (1000 ppm) solution.

Preparation of working standard solutions and construction of standard graph: The prepared stock solution was further diluted with distill water to get working standard solutions of 2, 4, 6, 8, and 10 ppm of Ciprofloxacin hydrochloride to construct Beer's law plot for pure drug, the absorbance was measured at λ_{max} 274 nm, against distill water as blank. The results are shown in table. The standard graph was plotted by taking concentration of drug on X-axis and absorbance on Y-axis and is shown in fig (3). The drug has obeyed Beer's law in the concentration range of 2-10 ppm. The linearity curve data is shown in table

Preparation of sample stock solutions and working sample solutions:

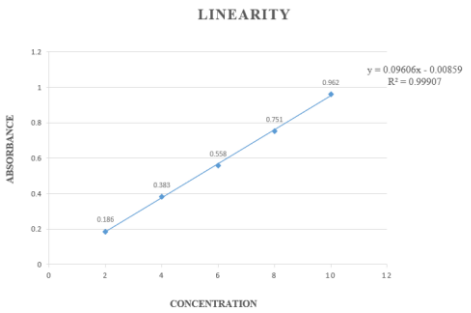
Ten tablets were accurately weighed and average was calculated. The tablets were then crushed to obtain fine powder. An accurately weighed quantity of tablet powder equivalent to about 100 mg of Ciprofloxacin hydrochloride was transferred to 100 ml volumetric flask, add 50 ml of distill water and shaken for 1 hour. The volume was made up to the mark with distill water and filtered through Whatman filter paper and required dilutions were made from sample stock solution. The spectrum of sample is shown in fig. 4 and recovery study from formulation is shown in table (3).

LINEARITY:

The linearity of an analytical method is its ability to produce test results that are directly proportional to the concentration of analyte in sample. Linearity methods were determined by taking absorbance by spectrophotometry and percent relative transmittance by of working solutions prepared using different solvents. Finally, the linear equation and regression coefficient were calculated.

A calibration curve was plotted using concentration (on x-axis) against absorbance at 274 nm (on y-axis) from the graph linearity regression co-efficient y intercept was calculated.

Concentration	Absorbance
2	0.186
4	0.383
6	0.558
8	0.751
10	0.962



The linearity regression co-efficient was more than 0.99 and hence the method was said to obey Beer's law and there is a linear and proportional relationship exists between concentration and absorbance.

Optimum Conditions	Uv Method
Beer's Law Limit (ppm)	2-10
Correlation Coefficient (R^2)	0.99907
Regression equation (y*)	y = 0.09606x – 0.00859
Slope (m)	0.09606
Y- Intercept (c)	0.00859
Chi Square	0.00055
Standard error of estimate	0.01073
RSD	0.00929

ACCURACY

The accuracy of the proposed methods was assessed by recovery studies at three different levels. Recovery studies were carried out by standard addition method. It was performed by adding known amount of ciprofloxacin solution of the pure drug to pre-analyzed tablet solutions. The resulting solutions were then reanalyzed by proposed methods.

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of bulk samples of Ciprofloxacin hydrochloride within the linearity range were taken and added to the preanalyzed formulation of concentration 5 ppm.
According to ICH guidelines the % RSD value should not exceeded upto 2%.
The formula for calculating the %RSD is %RSD = SD/Mean x 100

SLNO	Concentration	Sample	Absorbance	% Recovery	Stastical Analysis
1.	80 %	5	0.834	97.46	Mean = 97.54
		5	0.834	97.46	SD = 0.133
		5	0.836	97.69	% RSD = 0.136
2.	100 %	5	0.930	97.71	Mean = 97.74
		5	0.930	97.71	SD = 0.058
		5	0.931	97.81	% RSD = 0.059
3.	120 %	5	1.045	99.71	Mean = 99.65
		5	1.045	99.71	SD = 0.11
		5	1.043	99.52	% RSD = 0.111

PRECISION

The precision is measure of the degree of reproducibility or repeatability of an analytical method. It provides an indication random error. The precision of an analytical method is usually expressed as standard deviation, relative standard deviation or coefficient of variance of a series of measurements.From the above prepared standard stock solution, 10 ml of the solution was diluted to 100 ml using distilled water to get a concentration of 100 µg/ml. From the EXPERIMENTAL WORK above solution 6 ml of solutions were pipetted out into 6 different 100 ml volumetric flasks and the volume was made with distilled water to get the final concentrations of 6 µg/ml. The calculated percentage relative standard deviation (% RSD) of the results were used to evaluate the method precision.

According to ICH guidelines, the %RSD should not be exceeded upto 2%.

Concentration	Absorbance	Stastical Analysis
6	0.578	
6	0.579	Mean = 0.573
6	0.577	SD = 0.0075
6	0.562	%RSD = 1.31
6	0.575	
6	0.564	

Limit of detection (LOD):
The limit of detection (LOD) was determined based on standard deviation of calibration curve. The standard deviation of absorbance of calibration curve and slope of calibration curve were used. According to formula LOD was calculated and found to be
LOD=3.3×SD/S
Where,
S=slope of calibration curve
SD=standard deviation of calibration curve
LOD of Ciprofloxacin :
LOD=3.3×SD/S
=3.3×0.007/0.096
=0.32 ppm

Limit of Quantification (LOQ):
The limit of quantification (LOQ) as determined based on standard deviation and slope of calibration curve/According to the formula LOQ was calculated and found to be mg/ml.
LOQ=10×standard deviation/slope
Where,
S=slope of calibration curve
SD=Standard deviation of calibration curve
LOQ of Ciprofloxacin :
LOQ=10×sd/s
=10×0.007/0.096
=0.97 ppm
LOQ = 0.97 ppm

Molar Absorptivity:
Molar Absorption coefficient is a spectrophotometric unit indicating the light a substance absorbs with respect to length, usually centimeters and concentration usually moles per liter. Molar absorptivity is particularly useful in spectrometry for measuring the concentration of chemical solutions.

Molar absorptivity is calculated by using the following formula:

Molar absorptivity = Slope / Pathlength

Where,

Slope = log (y2-y1 / x2-x1)

MOLAR ABSORPITIVITY OF CIPROFLOXACIN :

Molar absorptivity = Slope / Pathlength

= 0.09606 / 1 = 0.09606 mol-1 cm-1

III. RESULTS AND DISCUSSION

CIPROFLOXACIN HYDROCHLORIDE IN BULK AND PHARMACEUTICAL TABLET DOSAGE FORM:

The method was developed and validated as per ICH guidelines. The method was validated in terms of Linearity, Precision, Accuracy, LOD, LOQ and Molar absorptivity. Detection wavelength was selected at 274 nm. Linearity in response was observed on 2– 10µg/ml. The linearity equation was found to be y = 0.09606x – 0.00859, R^2 = 0.99907 . The precision results showed a % RSD = 0.00929% at each level, clearly indicating that the method was precise enough for the analysis of Ciprofloxacin. The accuracy of the method was checked by recovery studies. The LOD = 0.32 ppm and LOQ = 0.97 ppm indicate sensitivity of the method. The molar absorptivity was found to be 0.09606 mol-1cm-1.

Characteristic parameters	**Ciprofloxacin in Bulk and Pharmaceutical Tablet dosage Form**		
λ Max	274 nm		
Beer's Law limit (µg/ml)	2-10 µg/ ml		
Linearity			
Correlation Coefficient (R^2)	0.99907		
Regression Equation	y = 0.09606x- 0.00859		
Slope (m)	0.09606		
Intercept ©	0.00859		
Accuracy			
Concentration (µg/ml)	80%	100%	120%
%RSD	0.136	0.059	0.11
Precision	% RSD= 1.31		
LOD	0.32 ppm		
LOQ	0.97 ppm		
Molar absorptivity	0.09606 mol-1 cm-1		

IV. CONCLUSION

The simple, sensitive, easily accessible and reliable and economical uv-spectrophotometric method is developed for the routine estimation of ciprofloxacin hydrochloride in bulk and pharmaceutical tablet dosage form. In this proposed method the linearity was Observed in the concentration range of 2-10µg/ml with correlation coefficient R2-0.999 for ciprofloxacin at 274 nm. These methods are validated in terms of linearity, accuracy, precision, LOD, LOQ, Percentage recovery. The results are obtained with use of water solvent. Results are developed methods were as per analytical Parameters and statically expressed. It was observed that all parameters were with in standard limit. Thus, the developed methods are simple, precise, rapid, specific and accurate that can be used to estimate ciprofloxacin hydrochloride. This method was validated as per ICH guidelines.

V. REFERENCES

[1] M.I. Andersson, A.P. MacGowan, Development of the quinolones, J. Antimicrob. Chemother. 51 (Suppl. 1) (2003) 1–11.

[2] P.C. Sharma, A. Jain, S. Jain, Fluoroquinolone antibacterials: a review on chem istry, microbiology and therapeutic prospects, Acta Pol. Pharm. 66 (2009) 587–604.

[3] M. Muller, M. Brunner, U. Hollenstein, C. Joukhadar, R. Schmid, E. Minar, Pen etration of ciprofloxacin into the interstitial space of inflamed foot lesions in non-insulin-dependent diabetes mellitus patients, Antimicrob. Agents Chemother. 43 (1999) 2056–2058.

[4] L.A. Lavery, D.G. Armstrong, R.P. Wunderlich, M.J. Mohler, C.S. Wendel, B.A. Lipsky, Risk factors for foot infections in individuals with diabetes, Diabetes Care 29 (2006) 1288–1293.

[5] A.S. Powlson, A.P. Coll, The treatment of diabetic foot infections, J. Antimicrob. Chemother. 65 (Suppl. 3) (2009) iii3–iii9.

[6] G. Carlucci, Analysis of fluoroquinolones in biological fluids by high performance liquid chromatography, J. Chromatogr. A. 812 (1998) 343–367.

[7] R.F. Venn, Principles and Practice of Bioanalysis, second ed., Taylor and Francis, London, 2005.

[8] S.N. Muchohi, N. Thuo, J. Karisa, A. Muturi, G.O. Kokwaro, K. Maitland, Determination of ciprofloxacin in human plasma using high-performance liq uid chromatography coupled with fluorescence detection: application to a

population pharmacokinetics study in children with severe malnutrition, J. Chromatogr. B. 879 (2011) 146–152.

[9] S. Imre, M.T. Dogaru, C.E. Vari, T. Muntean, L. Kelemen, Validation of an HPLC method for the determination of ciprofloxacin in human plasma, J. Pharm. Biomed. Anal. 33 (2003) 25–30.

[10] S. Watabe, Y. Yokoyama, K. Nakazawa, K. Shinozaki, R. Hiraoka, K. Takeshita, Y. Suzuki, Simultaneous measurement of pazufloxacin, ciprofloxacin, and lev ofloxacin in human serum by high-performance liquid chromatography with fluorescence detection, J. Chromatogr. B 878 (2010) 1555–1561.

[11] M. Khalid Khan, M. Farid Khan, Extraction and separation of ciprofloxacin by HPLC from human plasma, Int. Chem. Pharm. Med. 2 (2005) 267–270.

[12] W. Lertpaitoonpan, S.K. Ong, T.B. Moorman, Effect of organic soil and pH on soil sorption of sulfamethazine, Chemosphere 76 (2009) 558–564.

[13] J. Tolls, Sorption of veterinary pharmaceuticals in soils: a review, Environ. Sci. Technol. 35 (2001) 3397–3406.

[14] ICH Q2(R1), Harmonised tripartite guideline: validation of analytical pro cedures: methodology, in: Proceedings of the International Conference on Harmonization, Geneva, 2005.

[15] J.C. Garrelts, G. Jost, S.F. Kowalsky, G.J. Krol, J.T. Lettieri, Ciprofloxacin phar macokinetics in burn patients, Antimicrob. Agents Chemother. 40 (1996) 1153–1156.