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Research Article

A Visible Spectrophotometric Validated Procedure for the Assay of ciprofloxacin in Pharmaceutical Formulation using Fe(III) in buffer media

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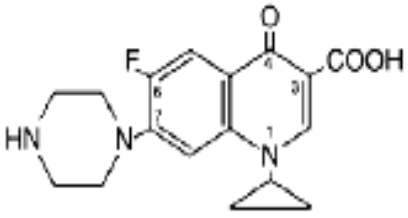
ABSTRACT

Fe(III) and ciprofloxacin react in the pH range 1.0-4.0 to form a yellow colored complex solution. The absorption spectrum of the complex solution shows maximum absorbance at 430 nm. The absorbance is maximum in the pH range 2.0-3.0. pH 2.5 is selected for analytical studies. The absorbance of the complex solution is proportional to the amount of ciprofloxacin. A graph between the amount of ciprofloxacin and the absorbance at 430 nm is linear which obeys the equation A_{430} = 0.0271 C + 0.0024. The linear plot shows that Beer's law is obeyed in the range 2.5-45.0 µg/ml of ciprofloxacin. The molar absorptivity is 9.083 x 10^3 l mol^{-1} cm^{-1} and Sandell's sensitivity is 0.0364 µg cm^{-1}. The standard deviation of the method for ten determinations of 10 µg/ml of ciprofloxacin is 0.0019. The correlation coefficient (γ) is 0.9999. The effect of excipients that are generally associated with ciprofloxacin in pharmaceutical formulations is investigated. The proposed visible spectrophotomeric method was validated as per ICH guidelines. The validation parameters such as, linearity, accuracy, precision, LOD, LOQ and ruggedness were investigated. The method is simple, rapid, precise, selective and accurate. The present method was applied for the determination of ciprofloxacin in pharmaceutical formulations.

Key words: Ciprofloxacin, Fe (III), Visible Spectrophotometry, Method validation.

INTRODUCTION

Ciprofloxacin is 1 - cyclopropyl - 6 - fluoro - 1, 4 - dihydro - 4 - oxo - 7 - (1-piperazinyl) - 3 - quinoline carboxylic acid. Its empirical formula is $C_{17}H_{18}FN_3O_3$ and its molecular weight is 331.4. It is a faintly yellowish to light yellow crystalline substance and its chemical structure is



Ciprofloxacin belongs to the second generation of quinolone analogues of nalidix acid that has greater potency, lower toxicity and a broader antibacterial spectrum. The main difference between ciprofloxacin and other antibiotics is that it can be administrated both parenterally and orally. It is well absorbed and widely distributed into various body tissues and fluids. It is used in a wide variety of infections of the urinary tract and gastrointestinal tract as well as skin and soft tissue infections.

Recently ciprofloxacin has been approved by the Food and Drug Administration (FDA) for prophylaxis of in hold bacillus antrocin infections. After the recent bioterrorist attacks, recommendations for anthrax prophylaxis include ciprofloxacin, doxycycline or amoxicillin in certain cases to avoid potential toxicity of quinolones and tetracycline's. The combination of antibiotic therapy during the initial phase of the illness and aggressive supportive care may improve the survival rate.

A simple flow injection UV spectrophotometric sensing device developed for the determination of ciprofloxacin is based on its transient retention and concentration on sephadex SPC-25 cation –exchange

gel beads packed in the flow cell and continuous monitoring of its negative absorbance on the solid phase at 277nm[1].

Marilyn J. Schineider *et al*., determined simultaneously fluoroquinolones and tetracyclines in chicken muscle using HPLC with fluorescence detection[2]. Determination of ciprofloxacin in tablets and in solutions for infusion by visible light spectrophotometry using 1% iron (III) nitrate in 1% nitric at 435nm is reported by Lorena et al[3]. Spectrophotometric methods for the determination of three fluoroquinolones (levofloxacin, norfloxacin and ciprofloxacin) have been performed either in pure form or in their tablets through charge transfer and ion-pair complexation reactions[4].

Ciprofloxacin is determined by differential electrolytic potentiometric titration method[5] developed based on complexation reaction between iron (III) and ciprofloxacin in a ratio of 1: 3, respectively, in sulphuric media of 0.09 mol. dm^3.

Sensitive spectrophotometric methods are developed for the determination of amoxycilin, ciprofloxacin and piroxicam in pure and pharmaceutical formulations by B.S. Nagaralli *et al*.,[6]. iprofloxacin is determined using solid – phase spectrophotometry, intrinsic absorbance of ciprofloxacin fixed on a dextran-type cation – exchange resin, sephadex SPC-25, was measured directly at 277 and 380 nm after packing the gel beads in a 1-mm cell[7].Direct determination of four fluoroquinolones, enoxacin, norfloxiacin, ofloxacin and ciprofloxacin, in pharmaceuticals and blood serum using HPLC is carried out by V.F. Samnidou *et al*.,[8].A multi – residue method[9] is described for assaying 13 quinolones in feeds. The samples are extracted by a metaphosphoric/acetonitrile mixture at pH 2.6 and automatically purified onto OASIS HLB catridges (ASPECXL).

The above survey of literature shows no report of a validated direct visible spectrophotometric method for the assay of ciprofloxacin in buffer medium. In continuation of our studies on developing simple visible spectrophotometric methods[10] for the assay of drugs, we now report a validated simple, rapid, stable, visible spectrophotometric procedure for the determination of ciprofloxacin in pharmaceutical formulations.

MATERIALS AND METHODS

All chemicals and solvents used were of analytical reagent grade.

Solutions:

Iron (III) solution

Stock solution ($1.0x10^{-2}$M) of ammonium ferric sulphate (A.R.BDH) is prepared by dissolving 0.4822 gm in double distilled water containing few drops of $H_2 SO_4$ in 100 ml volumetric flask and standardized[11] .Working concentrations are prepared by suitably diluting the stock solution.

Ciprofloxacin Solution

100 mg of ciprofloxacin is transferred in to a 100 ml volumetric flask and 5 ml of 0.1 N HCl solutions are added. The contents are made up to the mark with distilled water. This solution is suitably diluted to get the required concentrations

Buffer solutions:

Buffer solutions are prepared by standard procedures reported in the literature[12] using 1M sodium acetate and 1M hydrochloric acid (pH 0.5 – 3.0) and 0.2 M sodium acetate and 0.2 M acetic acid (pH 3.0 – 6.0)

Instruments employed:

a) **UV-Visible recording spectrophotometer (UV – 160A):**

UV-Visible recording spectrophotometer (UV-160A) supplied by Shimadzo, Japan was used for absorbance measurements.

b) **ELICO digital pH meter:**

ELICO digital pH meter manufactured by M/s ELICO Private Limited, Hyderabad, India was used for pH measurements of buffer solutions. The instrument has a temperature compensate arrangement. The reproducibility of measurements is within ± 0.01 pH .

EXPERIMENTAL PROCEDURES:

Preparation of Pharmaceutical sample solution

Known number of tablets are weighed and made to a fine powder in a mortar. A suitable quantity of the powder containing 100 mg of the active component is accurately weighed into a 100 ml volumetric flask, 60ml of distilled water are added and shaken thoroughly for about 20 minutes to extract the drug. The contents are diluted to the mark, mixed well and filtered using quantitative filter paper to remove the insoluble residue. The filtrate is diluted to get required concentration of drug.

Absorption spectrum:

The absorption spectra of the Fe (III) solution and ciprofloxacin solution in buffer solution of pH 2.5 and that of the experimental solution containing solutions of the Fe (III) , ciprofloxacin and the buffer (pH 2.5) against the buffer blank are recorded in the wavelength range 300-600nm. The spectra are presented in fig.1.The spectra in fig.1 show that the complex has an absorption maximum at 430 nm. Neither Fe (III) nor ciprofloxacin have significant

absorbance at 430 nm. Hence, analytical studies are made at 430 nm.

Assay of ciprofloxacin

The present method for the determination ciprofloxacin is applied for its determination in a pharmaceutical formulation. A known aliquot of pharmaceutical sample solution of ciprofloxacin is added to a 10ml volumetric flask containing 5 ml of buffer solution of pH 2.5 and 1ml of Fe(III) [5×10^{-3}M] solution. The contents are made upto the mark with distilled water. The absorbance is measured at 430 nm against the Fe (III) blank. The amount of ciprofloxacin is then computed from the predetermined calibration plot at 430 nm.

Effect of excipients

Various amounts of excipients that are generally associated with ciprofloxacin in its pharmaceutical formulations are added to a known amount of ciprofloxacin (10µg/ml) solution and the absorbance is measured under optimal conditions. The concentration (µg/ml) at which various excipients do not cause an error of more than ± 4% in absorbance of the complex solution is taken as the tolerance limit. The results are summarized in Table -1

The data in Table-1 reveal that various excipients that are associated with ciprofloxacin in pharmaceutical formulations do not interfere even in large quantities in the determination of ciprofloxacin making the method highly selective.

RESULTS AND DISCUSSION

Ciprofloxacin and Fe (III) react in the pH range 1.0-5.0 forming a yellow coloured complex solution. The absorption spectrum of the yellow colored complex shows (Fig-1) an absorption maximum at 430 nm. At this wavelength both Fe (III) and have no significant absorbance. The colour intensity of the complex is found to be maximum in the pH range 2.0-3.0. Hence, pH 2.5 which is midway between 2.0 - 3.0 is chosen for analytical studies. The color intensity attains a maximum value instantaneously.

The absorbance of the complex remains stable for more than 30 hours. The order of mixing of various components of the reaction mixture (buffer, Fe (III) solution and ciprofloxacin solution) did not show any effect on the maximum absorbance. A study of the influence of surfactants on the maximum absorbance of the complex solution showed that none of the surfactants studied (TritionX-100, SDS, CPC etc) had any effect on the maximum absorbance of the complex. The absorbance varied proportionally with the concentration of ciprofloxacin. Beer's law is obeyed in the range 2.5-45.0 µg/ml of ciprofloxacin. The linear plot obeys the equation A = 0.0271 C + 0.0024. Optical characteristics and regression data are presented in Table-2.The method was applied successfully for the determination of ciprofloxacin in pharmaceutical tablets. The data are presented in Table-3. The data show that the method is highly sensitive.

Method Validation and Statistical Analysis

The present method was validated duly following the official specifications of ICH[13]. The validation parameters indicate that the method is accurate and precise. Statistical results are expressed in terms of, mean ± SD, %RSD and student t-test values are calculated with the aid of Excel-2007. Differences were considered significant at the 95% confidence interval. Repeatability of the method was verified by intra day and inter day precision studies and the data are presented in Table-4. Accuracy of the method was studied by employing recovery procedure and the results are given in Table-5, Ruggedness studies were carried out by changing the analyst and the results are reported in Table-6.

CONCLUSION

The present method for the determination of ciprofloxacin is a highly sensitive, rapid, stable and selective visible spectrophotometric procedure. The method is not only, precise and sensitive but also is within the reach of an ordinary clinical laboratory. The linearity parameters and the corresponding regression data indicate excellent linear relationship (γ =0.9999) and the method to be highly sensitive and selective. A literature survey did not show any report of a simple, sensitive, selective direct visible spectrophotometric procedure for the assay of ciprofloxacin in pharmaceutical formulations. The method reported by Lorena et al[3] is less sensitive, unstable and was not validated following ICH norms. Other methods reported in the literature for its determination either use costly and sophisticated instrumentation or suffer from interference from various excipients.

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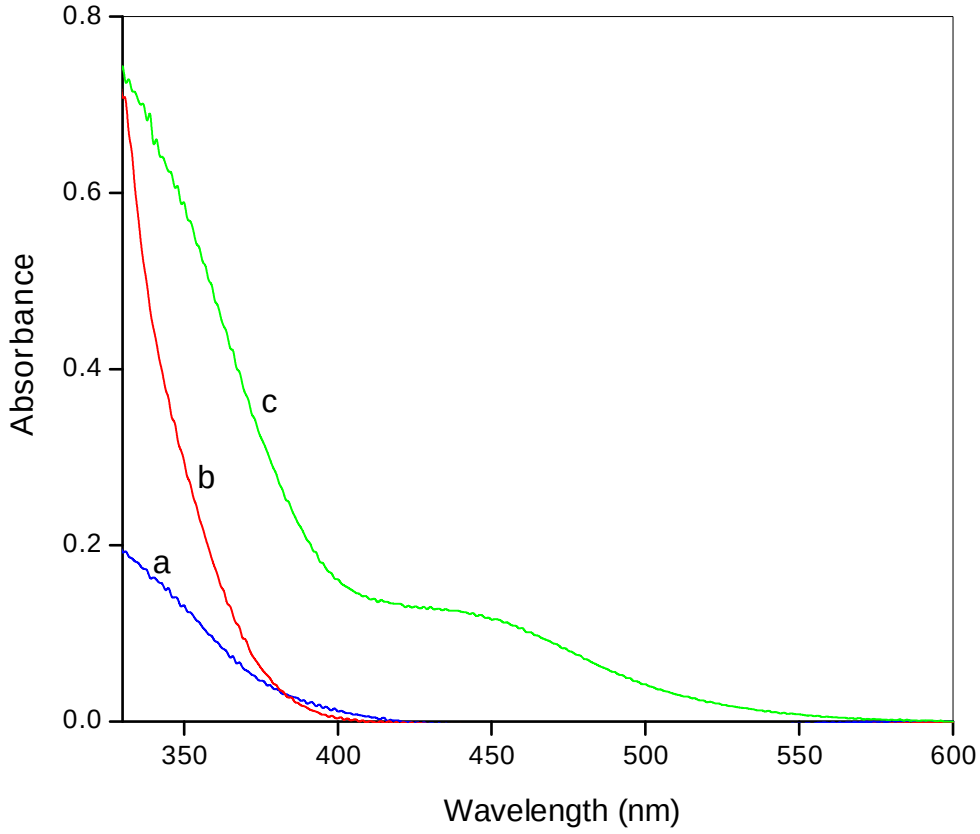

Fig.1
Absorption spectra of
a) Fe(III) vs. buffer blank
b) CFN vs. buffer blank;
c) Fe (III) – NFN vs. buffer blank
[Fe(III)] = 5.0 x 10^{-4} M; [CFN] = 2.0 x 10^{-5} M

Table 1
Tolerance limit of excipients
Amount of CFN = 15 µg/ml pH = 2.5

Excipient	Tolerance limit (µg/ml)
Fructose	11100
Glucose	12000
Sucrose	27272
Lactose	15384
Gelatin	7692
Starch	13953
Sodium Alginate	2575
Boric Acid	15384
Magnesium stearate	1650

Table 2
Optical and regression data of the Proposed method for ciprofloxacin

Parameter	Ciprofloxacin
λ_{max}(nm)	430
Beer's law limits (µg/ml)	2.5 – 45.0
Limits of detection (µg/ml)	0.2324
Limits of quantization (µg/ml)	0.6974
Molar absorptivity ($l.mo1^{-1}cm^{-1}$)	9.083×10^3
Sandell's Sensitivity ($\mu g/cm^2$)	0.0364
Regression equation (y= a + b x)	
Slope (b)	0.0271
Intercept (a)	0.0024
Correlation coefficient (γ)	0.9999
Standard deviation (Sd)	0.0019

Table – 3
Assay of ciprofloxacin in pharmaceutical formulation

Sample (Manufacturer – Formulation)	Label Claim (mg)	Amount found * (mg)	Error (%)
CIPLOX (Cipla Ltd., – Tablet)	250.0	251.6	0.64
CIPROLET (Dr. Reddy's Laboratories – Tablet)	250.0	249.5	-0.2

* Average of seven determinations

Table-4
Intra- and Inter- day precision studies of ciprofloxacin (n=3, p=0.05)

Con(µg/ml)	Mean absorbance		%RSD		t-value
	Day-1	Day-2	Day-1	Day-2	
10	0.272	0.267	0.92	1.20	0.118
15	0.410	0.406	0.49	0.65	0.104
20	0.546	0.541	0.38	0.59	0.087

Table -5
Recovery studies for ciprofloxacin in tablets

Tablet	Amount of Sample(µg/ml)	Amount of Drug added(µg/ml)	Amount Recovered(µg/ml)	% of Recovery
Brand—I (Ciplox)	15	15	30.28	100.9
	15	20	34.61	98.88
	15	25	40.56	101.4
Brand-II (Ciprolet)	20	15	35.39	101.1
	20	20	40.31	100.7
	20	25	44.88	99.7

Table-6
Ruggedness studies for the ciprofloxacin in tablets

Tablet	Analyst- I			Analyst- II	
	Label Claim(mg)	Amount found*(mg)	(%)Recovery	Amount found *(mg)	(%)Recovery
BRAND-I	250.0	250.9	100.36	249.8	99.92
BRAND- II	250.0	250.6	100.24	250.7	100.28

*Average of Seven determination

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