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A VALIDATED SECOND DERIVATIVE VISIBLE SPECTROPHOTOMETRIC PROCEDURE FOR THE ASSAY OF CIPROFLOXACIN IN PHARMACEUTICAL DOSAGE FORMS USING FE (III) IN BUFFER MEDIA

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Abstract: Fe(III) and ciprofloxacin form a yellow colored complex at pH 2.5. The second derivative spectrum of the complex shows a maximum at 510 nm and a valley at 445 nm and zero point at 478 nm. The derivative amplitude of the complex at 445 nm is proportional to the amount of ciprofloxacin. A plot between the amount of ciprofloxacin and the derivative amplitude at 445 nm is linear which obeys the equation A_{445} = 0.0086 C + 0.0003. The linear plot shows that Beer's law is obeyed in the range 1.0-50.0 µg/ml of ciprofloxacin. The standard deviation of the method for ten determinations of 10 µg/ml of ciprofloxacin is 0.0046. The correlation coefficient (γ) is 0.9999. The effect of excipients that are generally associated with ciprofloxacin in pharmaceutical dosage forms is investigated. The proposed visible second derivative spectrophotomeric method was validated as per ICH specifications. The validation parameters such as, linearity, accuracy, precision, LOD, LOQ and robustness were investigated. The method is simple, rapid, precise, selective and accurate. The present method is applied for the determination of ciprofloxacin in pharmaceutical dosage forms.

Keywords: Ciprofloxacin, Fe (III), Second derivative visible Spectrophotometry, Method validation.

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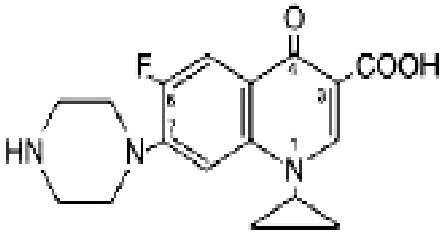
INTRODUCTION

Ciprofloxacin is 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid. Its empirical formula is $C_{17}H_{18}FN_3O_3$. 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 wider 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.

Several analytical methods are reported in literature for the assay of ciprofloxacin hydrochloride using high performance liquid chromatography[1-5]. Marilyn J. Schineider *et al*., determined simultaneously fluoroquinolones and tetracyclines in chicken muscle using HPLC with fluorescence detection[6]. Ciprofloxacin is determined by differential electrolytic potentiometric titration method[7] developed based on complexation reaction between iron (III) and ciprofloxacin in a ratio of 1: 3, respectively, in sulphuric acid media of 0.09 mol. Dm^3. HPLC and other techniques are expensive and time consuming.

Because of this reason spectrophotometry is an useful alternative for the determination of several drugs in pharmaceutical dosage forms and in biological samples because of its simplicity in operation, speed, accuracy, precision, sensitivity and low cast over HPLC method.

Several visible spectrophotometric[8-38] or UV spectrophotometric methods[39-41] are reported in the literature for the assay of ciprofloxacin. But these procedures involve costly reagents and required time for the production of color. There are reports of visible spectrophotometric

methods for the determination of ciprofloxacin using iron(III)[13,18,19,42]. In these methods, color of the complex between Fe(III) and ciprofloxacin is unstable beyond one hour. We have reported a visible spectrophotometric method[43] for the determination of ciprofloxacin using Fe(III) in HCl – sodium acetate buffer mediam which is more sensitive.

Derivative spectrophotometry (DS) is an analytical technique of great utility for extracting both qualitative and quantitative information from spectral curves composed of unresolved bands. DS enhances the resolution of spectral bands allowing the detection and location of the wavelength of poorly resolved components of complex spectra and reducing the effect of spectral background interferences. Because of these characteristics, the process of isolation of active components, usually require in qualitative and quantitative spectrophotometric procedures, applied in the analysis of complex system is completely avoided. The simplicity, relatively quick and easy realization, increased selectivity and sensitivity in the analysis of minor components are the important reasons, why the interest in DS is constantly growing for practical application. DS have been used in pharmaceutical analysis , environmental analysis and finger print analysis of proteins but few data have been published on the determination of inorganic ions [44]. Methods for the determination of organic substances by the DS technique have been develop mainly for application in the analysis of pharmaceuticals and clinically and biochemically interesting systems. The interference of formulation excepients or other UV – absorbing component, such as co- formulated drugs and degradation products can be successfully eliminated by the DS technique.

A survey of literature shows that only a first derivative spectrophotometric method for the determination of ciprofloxacin using iron (III) is reported[45]. However the color stability of the complex is less than one hour and is also less sensitive. In continuation of our work on the development of derivative spectrophotometric methods in the visible region for the assay of drugs, we report a validated second derivative spectrophotometric procedure for the determination of ciprofloxacin based on its reaction with Fe(III) in sodium acetate – HCl buffer medium in which the color of the complex is highly stable and sensitive.

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_2SO_4 in 100 ml volumetric flask and standardized[46] .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[47] 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 $\pm$ 0.01 pH.

EXPERIMENTAL PROCEDURES:

Preparation of Pharmaceutical sample solution

Known number of tablets are weighed and powdered 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.

Second derivative spectrum:

The second derivative spectrum of experimental solution containing Fe (III) solution, ciprofloxacin solution and buffer solution of pH 2.5 in the wavelength region 360 - 600 nm at 9 degrees of freedom is recorded and is shown in Fig:1. The spectrum in fig.1 shows that the complex has a peak at 510 nm and valley at 445 nm. It shows at zero point at 476 nm. Different known amounts of ciprofloxacin are transferred into a series of 10 ml volumetric flask containing 5 ml of buffer solution of pH 2.5 and Fe(III) solution. The second derivative spectra for all the solutions are recorded. The amplitude at 445 nm measured in each case. A plot is made between the amount of ciprofloxacin and derivative amplitude at 445 nm.

Assay of ciprofloxacin

The present method for the determination ciprofloxacin is applied for its determination in a pharmaceutical dosage form. A known aliquot of pharmaceutical sample solution containing ciprofloxacin is added to a 10ml volumetric flask containing 5 ml of buffer solution of pH 2.5 and 1ml of Fe(III) [5 x 10^{-3}M] solution. The contents are made up to the mark with distilled water. The second derivative spectrum is recorded and the amplitude is measured at 445 nm against Fe (III) blank. The amount of ciprofloxacin is then computed from the predetermined calibration plot prepared as described above.

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 derivative amplitude is measured under optimal conditions. The concentration (µg/ml) at which various excipients do not cause an error of more than $\pm$ 4% in derivative amplitude 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, Fe (III) react in the pH range 1.0-5.0 forming a yellow coloured complex solution. The second derivative spectrum in fig.1 shows that the complex has a peak at 510 nm and valley at 445 nm. The amplitude of the valley at 445 nm is found to be proportional to the amount of ciprofloxacin. Hence analytical studies carried out at 445 nm. The derivative amplitude 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 derivative amplitude attains a maximum value instantaneously.

The derivative amplitude of the complex is stable for 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 derivative amplitude. A study of the influence of surfactants on the derivative amplitude of the complex solution showed that none of the surfactants studied (TritionX-100, SDS, CPC etc) had any effect on the derivative amplitude of the complex. The derivative amplitude varied linearly with the concentration of ciprofloxacin. Beer's law is obeyed in the range 1.0-50.0 µg/ml of ciprofloxacin. The linear plot obeys the equation A_{445} = 0.0086C + 0.003. 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.

Method Validation and Statistical Analysis

The present method was validated duly following the official specifications of ICH[48].

Selectivity

The second derivative spectral studies show that excipients did not interfere even in large quantities making the method highly sensitive.

Precision

Intraday and interday decision of the analytical method was evaluated and the data is shown in table-4. The data in table -4 conform that the method is highly precise.

Accuracy

The accuracy of the method was confirmed by determining the average recoveries from the samples following the method of standard addition. The results are shown in table-5. The data indicate the method is highly accurate.

Robustness:

The robustness method was evaluated by comparing the results obtained by two different analysts. The data is shown in table-6. The data indicate the method is highly robust.

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.

CONCLUSION:

The present method for the determination of ciprofloxacin is a highly sensitive, rapid, stable and selective visible second derivative spectrophotometric procedure. The method is easily accessible to normal clinical laboratories. The linearity parameters and the corresponding regression data indicate excellent linear relationship (γ =0.9999) of the calibration plot. A literature survey did not show any report of a simple, sensitive, selective second derivative visible spectrophotomeric procedure for the assay of ciprofloxacin in pharmaceutical dosage forms. The first derivative spectrophotometric method reported by Edith C. L. cazedey et al[42] in unbuffered medium is less sensitive and color is highly unstable. 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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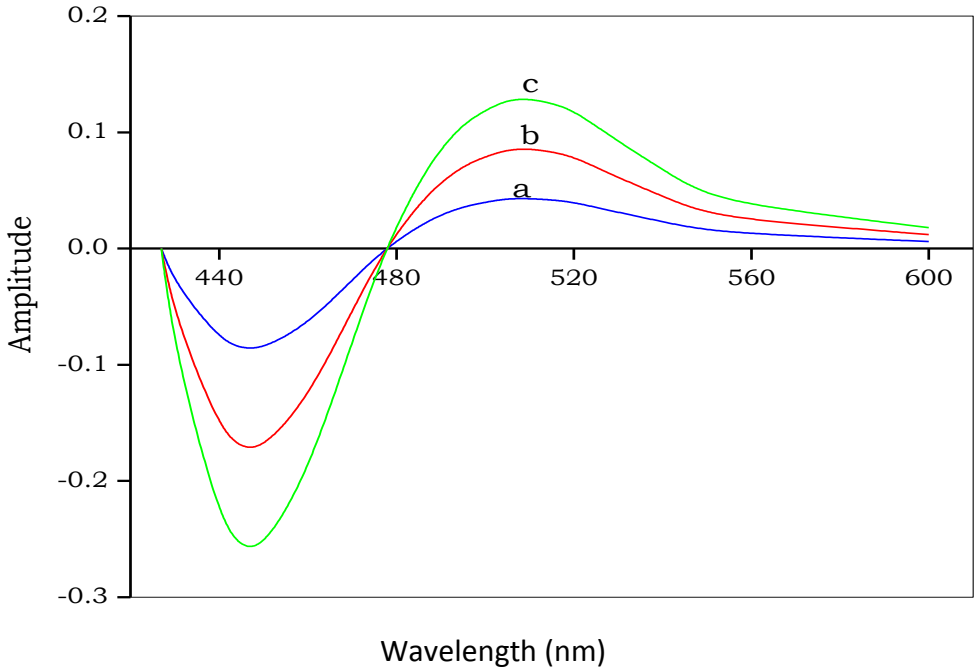

Fig.1 Second derivative spectra of CFN – Fe(III) vs. metal blank

[CFN] = (a) 3 x 10^{-5}M (b) 6 x 10^{-5}M (c) 9 x 10^{-5}M

Table – 1 Tolerance limit of excipients Amount of Ciprofloxacin = 10 µg/ml pH = 2.5

Excipient	Tolerance limit (µg/ml)
Fructose	11400
Glucose	12600
Sucrose	27400
Lactose	15500
Gelatin	8000
Starch	14100
Sodium Alginate	2600
Boric Acid	15600
Magnesium stearate	2000

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

Parameter	Ciprofloxacin
Analytical wavelength (nm)	445
Beer's law limits (µg/ml)	1.0 – 50.0
Limits of detection (µg/ml)	0.10
Limits of quantization (µg/ml)	0.38
Regression equation (y= a + b x)	
Slope (b)	0.0086
Intercept (a)	0.0003
Correlation coefficient (γ)	0.9999
Standard deviation (Sd)	0.0011

Table – 3 Assay of ciprofloxacin in pharmaceutical formulation

Sample (Manufacturer – Formulation)	Label Claim (mg)	Amount found * (mg)	Error (%)
CIPLOX (Cipla Ltd., – Tablet)	250.0	251.2	0.48
CIPROLET (Dr. Reddy's Laboratories – Tablet)	250.0	249.6	-0.16

* Average of seven determinations

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

Con(µg/ml)	Mean amplitude		%RSD		t-value
	Day-1	Day-2	Day-1	Day-2	
5	0.043	0.041	2.33	1.39	0.116
10	0.085	0.084	1.18	1.19	0.116
15	0.132	0.131	0.76	0.44	0.116

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	30.18	101.20
	15	20	34.83	99.51
	15	25	40.46	101.15
Brand-II (Ciprolet)	20	15	35.18	100.51
	20	20	40.21	100.52
	20	25	44.96	99.91

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	250.8	100.32	250.4	**100.16**
BRAND- II	**250.0**	**249.6**	**99.84**	**250.3**	**100.12**

*Average of Seven determination

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