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VALIDATED, VISIBLE SPECTROPHOTOMETRIC METHOD FOR THE ASSAY OF NORFLOXACIN IN PHARMACEUTICAL FORMULATIONS

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Abstract: A visible spectrophotometric method for the assay of norfloxacin was developed based on the yellow coloured reaction between Fe(III) and norfloxacin in the pH range 2.0-3.5. The absorption spectrum of the complex solution shows maximum absorbance at 430 nm. pH 2.5 is selected for analytical studies. The absorbance of the complex solution varied linearly with the amount of norfloxacin. A plot between the amount of norfloxacin and the absorbance at 430 nm is linear and obeys the equation A_{430} = 0.0394 C + 0.0004. The linear plot shows that Beer's law is obeyed in the range 2.0-30.0 µg/ml of norfloxacin. The molar absorptivity is 1.253 x 10^4 l mol^{-1} cm^{-1} and Sandell's sensitivity is 0.0254 µg cm^{-1}. The standard deviation of the method for ten determinations of 10 µg/ml of norfloxacin is 0.0016. The correlation coefficient (γ) is 0.9999. The proposed spectrophotmetric method was validated as per ICH specifications. The validation parameters such as, linearity, accuracy, precision, LOD, LOQ and ruggedness were investigated. The present method for the assay of norfloxacin was successfully applied for its assay in pharmaceutical formulations.

Keywords: Norfloxacin, Fe (III), Visible Spectrophotometry, Method validation.

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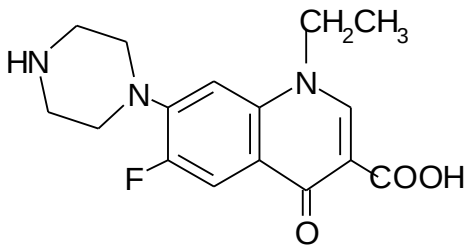
INTRODUCTION

Norfloxacin, a fluoroquinolone, is 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid. Its empirical formula is $C_{16}H_{18}FN_3O_3$ and the structural formula is



Norfloxacin is a white to pale yellow crystalline powder with a molecular weight of 319.34 and a melting point of about 221^0 C. It is freely soluble in diluted HCl and very slightly soluble in ethanol, methanol and water.

Norfloxacin is the third generation quinoline synthetic antibiotic, which has the characteristic of broad bacterium contradicting, small side effects and cross resistance with other drugs. So, has been widely used for the clinic. It works by entering the bacterial cell and inhibiting a chemical called DNA gyrase, which is involved in the production of genetic material (DNA). This therefore prevents the bacteria from reproducing. Norfloxacin reaches high level in the urine and so it is used in the treatment of urinary tract infections. Norfloxacin is a synthetic, broad-spectrum antibacterial agent for oral administration.

Norfloxacin drug has powerful broad spectrum. The mode of action is thought to be through blocking bacterial DNA replication and transcription by inhibiting DNA gyrase ultimately giving rise to cellysis. Because of these special mechanism of action, they are considered to be the most active broad spectrum, antibiotics effective against gram positive and gram negative pathogens to combat injection caused by microorganism, that are resistant or multi resistant to other antimicrobials such as aminoglycosides, tetracycline's of beta lactums.

Charge transfer complexes of norfloxacin with m – dinitrobenzene, picric acid, 3,5, - dinitro benzoic acid and 3, 5 – dinitrosalicylic acid have been synthesized and characterized by elemental analysis as well as by different spectroscopic techniques. The potentiality of the electron acceptors as new chromophoric reagents for the spectrophotometric determination of norfloxacin is studied by extensive investigation of optimum conditions favoring the formation of the coloured complexes[1]. Sonia Z. El Khateeb et al[2] reported the stability – indicating methods for the spectrophotometric determination of norfloxacin. The first method depends upon measurement of the pH – induced absorbance difference of the drug solution between 0.1 N HCl and 0.1 N NaOH at 280 nm. The second method involves chelation of the intact drug with iron(II) in acetate buffer solution (pH 5.7 ± 0.1) to form a yellow – coloured chelate which absorbs at 358 nm.

Fluorescence spectroscopic determination of ciprofloxacin, norfloxacin, lomefloxacin and flerofloxacin (electron donars) based on charge transfer reaction with chloranilic acid (electron acceptor) is reported by Du Liming et al[3]. Spectrophotometric and spectrofluorimetric estimation of ciprofloxacin and norfloxacin by ternary complex formation with eosin and palladium(II) is reported by Abdel Fattah M. El Walily et al[4].

Li Ming Du et al[5] reported the spectrofluorimetric determination of ciprofloxacin, norfloxacin, perfloxacin and fleroxacin (electron donors) based on charge transfer complexation reaction with 7,7,8,8 – tetracyanoquinodimethane (electron acceptors). Charge transfer complexation reaction between 7,7,8,8 – tetracyanoquinodimethane (TCNQ) as electron acceptor and cinnarizine, analgin, norfloxacin as electron donors have been studied using spectrophotometric method by Zhao Feng – lin et al.[6] Spectrofluorimetric method based on formation of yttrium complexes has been developed for the determination of norfloxacin by Yunxiao Han et al.[7] Atomic absorption spectroscopic, conductometric and colorimetric methods for determination of norfloxacin, ciprofloxacin, ofloxacin and enrofloxacin are reported. The methods depend upon the reaction of ammonium reineckate with the studied drugs to form stable precipitate of ion – pair complexes, which is dissolved in acetone is reported by Gamal H. Ragab et al.[8] A spectrophotometric method is developed for assay of norfloxacin using 2,4 – dinitro fluorobenzene by Abdel Fattah M El Walily et al[9].

The above survey of literature shows no report of a direct visible spectrophotometric method for the assay of norfloxacin using Fe (III). In continuation of our work on development of simple visible spectrophotometric methods[10] for the assay drugs in pharmaceutical formulations, we are now reporting a validated, simple visible spectrophotmetric procedure for the assay of norfloxacin .

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[11]. Working concentrations are prepared by suitably diluting the stock solution.

Norfloxacin Solution

100 mg of norfloxacin is transferred in to a 100 ml volumetric flask and 5 ml of 0. 1 N HCl solution are added the contents are made upto the mark with distilled water. This solution is suitably diluted to get the required concentrations

Buffer solutions:

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

Instruments employed:

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

Shimadzo UV-Visible recording spectrophotometer (UV-160A) 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 measuring the pH 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

A known number of tablets are weighed and ground to a fine powder. A portion 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 norfloxacin solution in buffer solution of pH 2.5 and that of the experimental solution containing solutions of the Fe (III) , norfloxacin 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 presented in fig.1 show that the complex shows an absorption maximum at 430 nm. Both Fe (III) and norfloxacin have insignificant absorbance at 430 nm. Hence, analytical studies are made at 430 nm.

Assay of norfloxacin

The present method for the determination norfloxacin is applied for its determination in a pharmaceutical formulation. A known aliquot of pharmaceutical sample solution of norfloxacin 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 upto the mark with distilled water. The absorbance is measured at 430 nm against the Fe (III) blank. The amount of norfloxacin is then computed from the predetermined calibration plot at 430 nm.

Effect of excipients

Various amounts of excipients that are generally associated with norfloxacin in its pharmaceutical formulations are added to a fixed amount of norfloxacin (10µg/ml) solution and the absorbance measurements are made 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 norfloxacin in pharmaceutical formulations do not interfere even in large quantities in the determination of norfloxacin making the method highly selective.

RESULTS AND DISCUSSION

Norfloxacin 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 norfloxacin have no significant absorbance. The colour intensity of the complex is found to be maximum in the pH range 2.0-3.5. Hence studies were carried at pH 2.5, where the interference due to excipients or diverse ions is negligible. 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 norfloxacin solution) did not show any effect on the maximum absorbance. A study of the influence of surfactants on the absorbance of the complex 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 linearly with the concentration of norfloxacin. Beer's law is obeyed in the range 2.0-30.0 µg/ml of norfloxacin. The straight line plot obeys the equation A = 0.0394 C + 0.0004. Optical characteristics and regression data are presented in Table-2.The method was applied successfully for the determination of norfloxacin in pharmaceutical tablets. The data are presented in Table-3. The results show that the method is highly sensitive.

Method Validation and Statistical Analysis

The present method was validated 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 intraday and interday precision studies (Table-4). Accuracy of the method was studied by employing recovery procedure and the results are presented in Table-5, Ruggedness studies were carried out by changing the analyst and the results are given in Table-6.

CONCLUSION:

The present method for the determination of norfloxacin is a highly sensitive and selective visible spectrophotometric procedure. The method is not only, precise and sensitive but also is within the ambit 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 norfloxacin in pharmaceutical formulations. 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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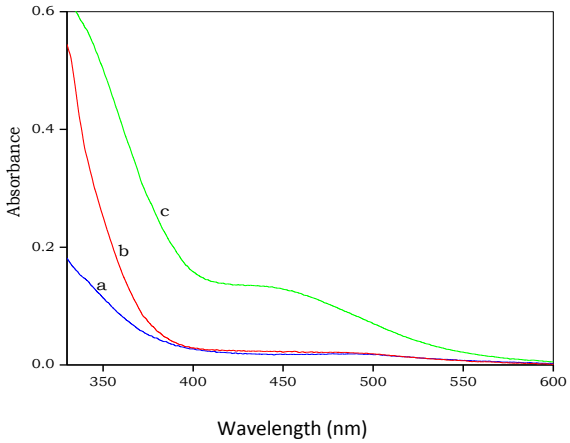

Fig 1 Absorption spectra of a) Fe(III) vs. buffer blank b) NFN vs. buffer blank; c) Fe (III) – NFN vs. buffer blank [Fe(III)] = 5.0 x 10^{-4} M; [NFN] = 1.0 x 10^{-5} M

Table – 1

Tolerance limit of excipients Amount of NFN = 10 μg/ml pH = 2.5

Excipient	Tolerance limit (μg/ml)
Fructose	10402
Glucose	11246
Sucrose	25558
Lactose	14417
Gelatin	7209
Starch	13076
Sodium Alginate	2413
Boric Acid	14417
Magnesium stearate	1547

Table – 2

Optical and regression data of the Proposed method for norfloxacin

Parameter	Norfloxacin
λ_{max} (nm)	430
Beer's law limits (μg/ml)	2.0 – 30.0
Limits of detection (μg/ml)	0.1371
Limits of quantization (μg/ml)	0.4113
Molar absorptivity (l.mo1^{-1}cm^{-1})	1.253 x 10^4
Sandell's Sensitivity (μg/cm^2)	0.0254
Regression equation (y= a + b x)	
Slope (b)	0.0394
Intercept (a)	0.0004
Correlation coefficient (γ)	0.9999
Standard deviation (Sd)	0.0011

Table – 3

Assay of norfloxacin in pharmaceutical formulation

Sample (Manufacturer – Formulation)	Label Claim (mg)	Amount found * (mg)	Error (%)
NORILET (Dr. Reddy's Laboratories Ltd.,– Tablet)	200.0	198.6	-0.70
NORFLOX(Cipla Ltd.Tab)	200.0	201.2	+0.60

*** Average of seven determinations**

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

Con(µg/ml)	Mean absorbance		%RSD		t-value
	Day-1	Day-2	Day-1	Day-2	
5	0.200	0.198	0.50	0.77	0.091
10	0.400	0.396	0.38	0.76	0.089
15	0.599	0.596	0.35	0.34	0.116

Table -5
Recovery studies for norfloxacin in tablets

Tablet	Amount of Sample(µg/ml)	Amount of Drug added(µg/ml)	Amount Recovered(µg/ml)	% of Recovery
Brand—I (Norilet)	20	20	40.19	100.48
	20	30	49.56	99.12
	20	40	60.81	101.35
Brand-II (Norflox)	30	20	49.60	99.20
	30	30	60.61	101.01
	30	40	69.12	98.74

Table-6
Ruggedness studies for the norfloxacin in tablets

Tablet	Analyst- I			Analyst- II	
	Label Claim(mg)	Amount found*(mg)	(%)Recovery	Amount found *(mg)	(%)Recovery
BRAND-I	200.0	200.15	100.08	199.85	99.92
BRAND- II	200.0	199.66	99.83	199.73	99.87

***Average of Seven determination**

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