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RESEARCH ARTICLE

A Validated Visible Spectrophotometric Method for the Assay of Moxifloxacin Tablet Dosage Forms Using Fe (III) in Buffer Media

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ABSTRACT

Moxifloxacin reacts with Fe(III) in the pH range 1.0-4.0 to form a yellow colored complex solution. Its absorption spectrum shows maximum absorbance at 440 nm. The absorbance is constant and maximum in the pH range 2.0-3.0. pH 2.5 is selected for analytical studies. The absorbance of the complex solution varied linearly with the amount of moxifloxacin. A plot of the amount of moxifloxacin and the absorbance at 440 nm is linear and obeys the equation A_{440} = 0.0317 C + 0.0004. The linear plot shows that Beer's law is obeyed in the range 2.0-32.0 µg/ml of moxifloxacin. The molar absorptivity is 1.286 x 10^4 l mol^{-1} cm^{-1}. The Sandell's sensitivity is 0.0314 µg cm^{-1}. The standard deviation of the method for ten determinations of 10 µg/ml of moxifloxacin is 0.0017. The correlation coefficient (γ) is 0.9999. The effect of excipients that are generally associated with moxifloxacin in pharmaceutical dosage forms is investigated. The proposed visible spectrophotometric 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 moxifloxacin in its tablet dosage forms.

KEYWORDS

Moxifloxacin, Fe (III), Visible Spectrophotometry, Method Validation, Buffer Media

INTRODUCTION

Moxifloxacin hydrochloride is1-Cyclopropyl-6-fluoro-1,4-dihydro-8- methoxy-7-[(4aS,7aS)-octahydro-6H-pyrrolol[3,4-b]pyridin-6-yl]-4-oxo-3-quinoline carboxylic acid monohydro-chloride. Moxifloxacin hydrochloride is a slightly yellow to yellow crystalline powder. The molecular formula is $C_{21}H_{24}FN_3O_4$.HCl, molecular weight is 437.9 and the molecular structure is as follows. Moxifloxacin is a new 8-methoxy quinolone derivative of fluoroquinoline with enhanced activity in vitro against gram positive bacteria and maintenance of activity against gram negativity bacteria[1-3].

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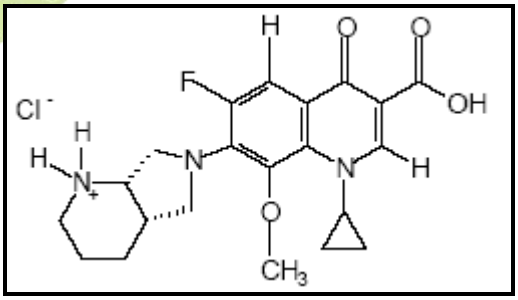

The drug is rapidly absorbed, reaching maximum plasma concentration between 1and 4 hours after oral administration. Its half life of 11-15 hours allows a daily administration.[4] Moxifloxacin is administrated to patients in 400mg daily doses being that the final concentration in serum and urine for the treated patients are of 2.00- 5.00 and 30.00-60.00µg/ml respectively.[5]

Square – wave adsorptive voltammetric determination of moxifloxacin[6] in tablets and spiked urine human samples is reported based in the electrochemical reduction at hanging mercury drop electrode (HMDE). The support electrolyte that provided a more defined and intense peak current for moxifloxacin determination is the phosphate buffer (pH 8.0). Simultaneous determination of cefepime and the quinolones generoxacin, moxifloxacin and levofloxacin in human urine using HPLC – UV is reported by J. A. Ocana Gonzalez *et al*[7]

The above survey of literature shows no direct visible spectrophotometric procedure for the determination of moxifloxacin using Fe(III) in buffer media. As a part of our ongoing studies on the development of validated simple direct and derivative spectrophotometric methods[8] for the assay of drugs in dosage forms, report a validated visible spectrophotometric procedure for the determination of moxifloxacin in buffer media using Fe(III).

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

Gatifloxacin Solution

100 mg of moxifloxacin 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[10] 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

UV-Visible Recording Spectrophotometer (UV – 160A)

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

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

10 tablets of moxifloxacin are weighed and powdered. A suitable quantity of the powder containing 100 mg of the active component is accurately weighed into a 100 ml volumetric flask, 60 ml 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 moxifloxacin solution in buffer solution of pH 2.5 and that of the experimental solution containing solutions of the Fe (III), moxifloxacin 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 maximum absorbance at 440 nm over other constituents. Neither Fe (III) nor moxifloxacin have significant absorbance at 440 nm. Hence, analytical studies are carried out at 440 nm.

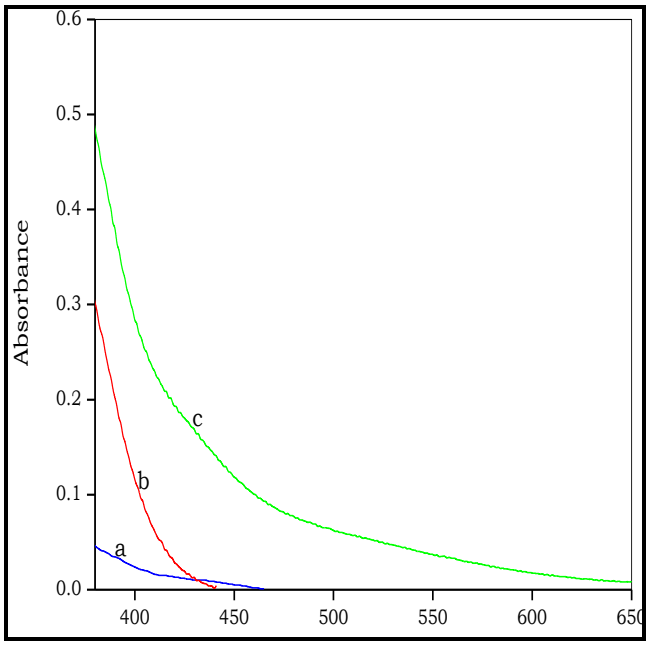

Figure 1: Absorption spectra of a) Fe(III) vs. buffer blank b) MFN vs. buffer blank; c) Fe (III) – MFN vs. buffer blank [Fe(III)] = 5.0 x 10^{-4} M; [MFN] = 1.0 x 10^{-5} M

Assay of Moxifloxacin

The proposed visible spectrophotometric method for the determination moxifloxacin is applied for its determination in tablet dosage forms. A known aliquot of pharmaceutical sample solution of moxifloxacin 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 440 nm against the Fe (III) blank. The amount of moxifloxacin is then computed from the predetermined calibration plot at 440 nm.

Effect of Excipients

Various amounts of excipients that are generally associated with moxifloxacin in its pharmaceutical formulations are added to a known amount of moxifloxacin (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.

Table 1: Tolerance limit of excipients Amount of Moxifloxacin = 10 µg/ml pH = 2.5

Excipient	Tolerance limit (µg/ml)
Fructose	12238
Glucose	13230
Sucrose	30068
Lactose	16961
Gelatin	8481
Starch	15384
Sodium Alginate	2839
Boric Acid	16961
Magnesium stearate	1820

The data in Table 1 reveal that various excipients that are normally associated with moxifloxacin in its dosage forms do not interfere even in large quantities in the determination of moxifloxacin making the method highly selective.

RESULTS AND DISCUSSION

Moxifloxacin 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 is shown in Fig-1 along with the spectra of Fe(III) solution and that of moxifloxacin solution alone. The spectra in Fig-1 show that the absorbance difference between the spectrum due to the complex (Fig-1.c) and that of the moxifloxacin (Fig-1.b) is maximum at 440 nm. Hence, analytical studies are made at 440 nm. The absorbance of the complex solution is found to be maximum and constant 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 20 hours. The change in the order of mixing of various components of the reaction mixture [Buffer, Fe(III) solution and moxifloxacin solution] did not show any effect

on the maximum absorbance. A study of the effect of surfactants on the maximum absorbance of the complex solution showed that none of the surfactants tried (TritionX-100, SDS, CPC etc) had any effect on the maximum absorbance of the complex. The absorbance varied proportionally with the concentration of moxifloxacin. Beer's law is obeyed in the range 2.0-32.0 µg/ml of moxifloxacin. The linear plot obeys the equation A_{440} = 0.0317 C + 0.0004. Optical characteristics and regression data are presented in Table 2. The method was applied successfully for the determination of moxifloxacin in pharmaceutical tablet dosage forms. The data are presented in Table 3. The data show that the method is highly sensitive.

Table 2: Optical and regression data of the Proposed method for moxifloxacin

Parameter	Moxifloxacin
λ_{max} (nm)	440
Beer's law limits (µg/ml)	2.0 – 32.0
Limits of detection (µg/ml)	0.1788
Limits of quantization (µg/ml)	0.5365
Molar absorptivity ($l.mo1^{-1}cm^{-1}$)	1.286 x 10^4
Sandell's Sensitivity ($\mu g/cm^2$)	0.0314
Regression equation (y= a + b x)	
Slope (b)	0.0317
Intercept (a)	0.0004
Correlation coefficient (γ)	0.9999
Standard deviation (Sd)	0.0017

Method Validation and Statistical Analysis

The present method was validated duly following the official specifications of ICH[11]. 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.

Table 3: Assay of moxifloxacin in pharmaceutical formulation

Sample (Manufacturer – Formulation)	Label Claim (mg)	Amount found * (mg)	Error (%)
MOXICIP (Cipla Ltd., – Tablet)	400.0	399.4	-0.15
NUMMOX	400.0	400.8	0.20

* Average of seven determinations

Repeatability of the method was verified by intraday 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 moxifloxacin 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 moxifloxacin in pharmaceutical dosage forms. UV spectrophotometric methods suffer from interference from excipients. Other methods reported in the literature for its determination use costly and sophisticated instrumentation which require expertise in operation. The method is more sensitive than the one reported by El-Hawary et al.[11]

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

Concentration(µg/ml)	Mean absorbance		%RSD		t-value
	Day-1	Day-2	Day-1	Day-2	
10	0.326	0.324	0.47	0.47	0.099
15	0.477	0.476	0.44	0.32	0.539
20	0.640	0.636	0.24	0.33	0.055

Table 5: Recovery studies for moxifloxacin in tablets

Tablet	Amount of Sample(µg/ml)	Amount of Drug added(µg/ml)	Amount Recovered(µg/ml)	% of Recovery
Brand—I (Moxicip)	15	10	25.14	100.56
	15	15	30.41	101.36
	15	20	34.53	98.65
Brand-II (Nummox)	20	10	30.19	100.63
	20	15	34.63	98.94
	20	20	39.72	99.30

Table 6: Ruggedness studies for the moxifloxacin in tablets

Tablet	Analyst- I			Analyst- II	
	Label Claim(mg)	Amount found*(mg)	(%)Recovery	Amount found *(mg)	(%)Recovery
MOXICIP	400.0	400.9	100.2	399.3	99.8
NUMMOX	400.0	400.4	100.1	400.7	100.2

*****Average of Seven determination

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