Visible Spectrophtometric Estimation of Moxifloxacin in Bulk and its Pharmaceutical Formulations

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Abstract: A simple and sensitive visible spectrophotometric method has been developed and subsequently validated for the determination of Moxifloxacin in bulk and pharmaceutical formulations. The method is based on the formation of yellow colored complex of the drug with sodium nitroprusside and hydroxylamine hydrochloride in alkaline medium. Quantitative measurements were made at the maximum absorption of 361 nm. The method was validated over the range of 30 to 100 µg/mL with correlation coefficient r = 0.9998. The method was shown to be accurate and precise with inter-day and intra-day percent relative standard deviation values in the range of 0.05 to 0.106 and 0.047 to 0.113. The percent recoveries of Moxifloxacin were found to be 99.36 to 100.12. The limit of detection (LOD) was 0.172 µg/mL and limit of quantitation was 0.573 µg/mL. The method has been successfully utilized to determine the Moxifloxacin in tablets and can be extended for the routine analysis in bulk drugs.

Keywords: Moxifloxacin, Spectrophotometer, Method validation.

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Moxifloxacin, 1-cyclopropyl-7-[(S, S)-2, 8-diazabicyclo [4.3.0] non-8-yl]-6-fluoro-8-methoxy-1, 4-dihydro-4-oxo-3 quinoline carboxylic acid.[1] Moxifloxacin is advanced new generation synthetic fluoroquinolone derivative. It has a wide range antimicrobial activity in-vitro against aerobic gram positive and aerobic gram-negative bacteria. Moxifloxacin is an oral 8-methoxyquinolone antimicrobial approved in December 1999 for use in the treatment of acute bacterial sinusitis, acute bacterial exacerbations of chronic bronchitis, and community- acquired pneumonia.[2]Moxifloxacin differs structurally from other fluoroquinolones in the methoxy function at the 8-position and an S,S-configured diazabicyclononyl ring moiety at the 7-position. It is thought that the diazabicyclononyl ring, because of its large size, may contribute to decreased bacterial resistance by reducing moxifloxacin efflux from bacterial cell walls. The cyclopropyl group at the N-1 position and the fluorine group at the 6-position enhance the antimicrobial activity of moxifloxacin.

Several analytical methods, such as High performance liquid chromatography [HPLC] [3], Liquid chromatography mass spectrometry (LC/MS)[4], Capillary electrophoresis[5] spectrofluorimetry[6] Reverse phase high performance liquid chromatography [RP-HPLC][7], High performance thin layer chromatography [HPTLC][8] and Simultaneous spectrophotometric method[9] of Moxifloxacin in bulk and pharmaceutical formulation have been reported.

Results and discussion

The present study was carried out to develop a simple, accurate and sensitive visible spectrophotometric method for the determination of Moxifloxacin in tablets. Linearity was observed in the range 30-100 µg/ml. From the optical characteristics of these proposed methods, it was found that the drugs obey linearity within the concentration range of 30-100µg/ml in visible region. From the results shown in precision Table-3, it was found that % RSD is less than 2%; which indicates that these proposed method have good reproducibility. From the results shown in accuracy Table-4, it was found that the percentage recovery values of pure drug from the analyzed formulation were in between 99.36-100.12 %. The assay values for the marketed formulation were found to be within limit as listed in table 2. All validation parameters are incorporated in table 7. These indicate that this method is accurate and the commonly used

excipients and additives present in the formulations were not interfering the proposed method. The system suitability parameters also reveal that the values were within the specified limits.

Conclusions

The proposed method was found to be simple, precise, accurate and sensitive. High percentage recovery showed that the method was free from interference of excipients used in the formulation. Values of LOD and LOQ showed that the proposed method was sensitive enough to analyze the drug in bulk as well as in its pharmaceutical formulation. Hence the proposed method renders suitable for routine analysis in quality-control laboratories.

Experimental

Instruments

Spectrophotometric analysis was carried out on a Systronics 2101 double beam spectrophotometer with a fixed slit width (2 cm) using a pair of 1 cm matched quartz cells. All weighing were performed on an electronic single pan balance (Citizen). Calibrated borosilicate glass wares were used in the study. Pure sample Moxifloxacin was kindly provided by Torrent Pharma (Baddi, India). Moxifloxacin tablets, Moxifloxacin (Formulation I, Torrent Pharmaceutical Industries Ltd,Baddi) and Staxom (Formulation II, Stancare, Delhi) were procured from local drug stores. Other chemicals and solvents were of analytical grade.

Reagents used

Methanol, Sodium nitroprusside, Hydroxyl amine hydrochloride, Sodium carbonate, Double distilled water, Chloroform**.**

Preparation of Stock Solutions

100mg of standard Moxifloxacin was transferred into a separator with 10ml distilled water. The separator was shaken to disperse the material and the contents were extracted with 3x25ml portions of chloroform. The total chloroform extracts were collected in a 100ml volumetric flask

and then diluted to the mark with same solvent to obtain stock solution (1mg mL^{-1}) in chloroform.

20ml of the above stock solution (1mg mL^{-1}) was taken and chloroform portion was evaporated to dryness and the residue was dissolved initially in 5ml methanol and diluted to 40mL with the same solvent.

Preparation of Working Standard Solutions and construction of standard graph

To construct Beer's law plot for Moxifloxacin, different aliquots of standard MOX (1.5-5.0mL, 500µgmL^{-1}) were transferred into a series of 25ml calibrated tubes and the volume in each tube was brought to 5.0mL with distilled water. 1mL each of ($1.678x10^{-2}$ M) sodium nitroprusside and ($7.195x10^{-2}$M) hydroxyl amine hydrochloride solution were successively added to each test tube and shaken for 2 minutes. Then 1.0mL of ($9.43x10^{-2}$M) Na_2CO_3 solution was added and further shaken for 15 minutes. The contents were diluted to the mark with distilled water and the absorbance was measured at 361nm against a reagent blank.

The standard graphs for Moxifloxacin were plotted by taking concentration of drug on x-axis and absorbance on y-axis and was shown in fig-1. The drug has obeyed Beer's law in the concentration range of 30-100µg/ml. The results were shown in table1.

Estimation of Moxifloxacin in tablets

For analysis of commercial formulations, twenty tablets were taken and powdered. Tablet powder equivalent to 100mg of formulation were transferred into a separator with 10ml distilled water. The separator was shaken to disperse the material and the contents were extracted with 3x25ml portions of chloroform. The total chloroform extracts were collected in a 100ml volumetric flask and then diluted to the mark with same solvent to obtain stock solution (1mg mL^{-1}) in chloroform.

20ml of the above stock solution (1mg mL^{-1}) was taken and chloroform portion was evaporated to dryness and the residue was dissolved initially in 5ml methanol and diluted to 40ml with the same solvent to get 500µgmL^{-1}. Different aliquots of Moxifloxacin (1.5-5.0mL, 500µg mL^{-1}) were transferred into a series of 25mL calibrated tubes and the volume in

each tube was brought to 5.0ml with distilled water. 1mL each of ($1.678x10^{-2}$ M) sodium nitroprusside and ($7.195x10^{-2}$M) hydroxyl amine hydrochloride solution were successively added to each test tube and shaken for 2 minutes. Then 1.0mL of ($9.43x10^{-2}$M) Na_2CO_3 solution was added and further shaken for 15 minutes. The contents were diluted to the mark with distilled water and the absorbances were measured at 361nm against a reagent blank and the drug content was estimated. The results were shown in table 2.

Validation criteria

Precision

The precision of the proposed method was ascertained by actual determination of eight replicates of fixed concentration of the drug within the Beer's range and finding out the absorbance by the proposed method. From this absorbance Mean, Standard deviation, % RSD and percentage range of errors (at 0.05 and 0.01 confidence limits) was calculated. The readings were shown in Table 3.

Accuracy

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 MOXI within the linearity range were taken and added to the pre-analyzed formulation of concentration 6µg/mL for MOXI. From that percentage recovery values were calculated.

Repeatability

Repeatability is given by inter-day and intra-day precision. Intra-day precision was determined by analyzing the three different concentration of drug for three times in the same day. Inter-day precision was determined by analyzing the three different concentration of drug for three days in a week; results are presented in table -5 .Fro m the data % RSD was determined.

Ruggedness

Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot in different laboratories using similar operational and environmental condition.

Fig. 1 Calibration curve of Moxifloxacin

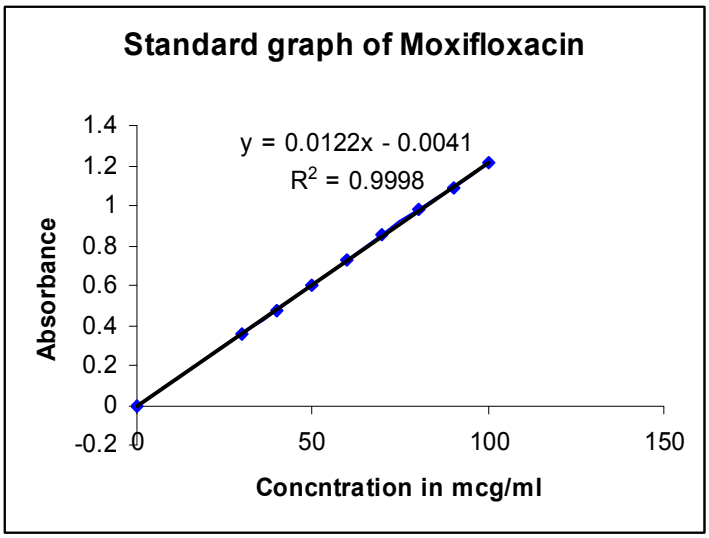

Table 1 Linearity table of Moxifloxacin in Working Standard

Conc. (µg/mL)	Absorbance
30	0.36
40	0.478
50	0.6
60	0.724
70	0.855
80	0.982
90	1.09
100	1.21

Table 2 Analysis of tablets in commercial formulations

Formulation	Labelled Amount (mg.)	Amount obtained (mg)	%Drug present	%RSD
Moxif	400	395.86±1.543	98.97	1.56
Staxom	400	398.39±0.3258	99.60	0.0818

(Each value is average of three determinations ± standard deviation

Table 3 Precision Readings

Conc. of Moxifloxacin (µg/mL)	Absorbance	Statistical analysis
50	0.60	
50	0.598	
50	0.598	MOXI
50	0.6	Mean:0.599
50	0.599	S.D: 0.0007
50	0.6	%R.S.D:.0.116
50	0.6	
50	0.599	

Table 4 Accuracy

Sample ID	Conc. of Moxifloxacin: µg/mL		Absorbance of pure drug + formulation	% Recovery of pure drug	Statistical Analysis	
	Pure drug	Formulation				
S_1 : 80 %	40	50	0.602	99.45	Mean	99.46
S_2 : 80 %	40	50	0.602	99.58	SD	0.0636
S_3 : 80 %	40	50	0.601	99.36	% RSD	0.0639
S_4 : 100%	50	50	0.604	99.72	Mean	99.73
S_5 : 100%	50	50	0.603	99.62	SD	0.0919
S_6 : 100%	50	50	0.604	99.85	% RSD	0.0921
S_7 : 120%	60	50	0.606	100.12	Mean	99.89
S_8 : 120%	60	50	0.604	99.83	SD	0.282
S_9 : 120%	60	50	0.604	99.72	% RSD	0.283

Table 5 Results for Repeatability studies

Amount taken (μg/mL)	Inter-day		Intra-day	
	Amount found (μg/mL)	%RSD	Amount found (μg/mL)	%RSD
50	49.89	0.098	49.88	0.113
50	49.94		49.95	
50	49.96		49.96	
60	59.88	0.106	59.89	0.047
60	59.95		59.97	
60	59.97		59.93	
70	69.93	0.05	69.96	0.05
70	69.98		69.89	
70	69.96		69.91	

Table 6 Ruggedness data

Amount taken (µg/mL)	ELICO INDIA 2101		SYSTRONICS DOUBLE BEAM UV VISIBLE SPECTROPHOTOMETER-2201.	
	Amount found (µg/mL)	%RSD	Amount found (µg/mL)	%RSD
60	59.88		59.97	
60	59.92	0.011	59.94	0.023
60	59.87		59.99	

Table 7 Validation Parameters

Parameters	*Results*
Beer's law limit (µg/mL)	30-100
Sand ell's sensitivity (µg/cm^2/0.001)	0.083
Absorptivity (1mole^{-1},cms^{-1})	0.012×10^4
% Relative standard deviation	0.116
% Range of error	
0.05 confidence limits	0.048
0.01 confidence limits	0.064
Limit of detection	0.172
Limit of quantitation	0.573
Correlation coefficient	0.9998
Regression equations (Y*)	
Slope (a)	0.0122
Intercept (b)	-0.0041

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