Spectrophotometric Estimation of Moxifloxacin in Bulk and its Pharmaceutical Formulations

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Summary

A simple and sensitive UV spectrophotometric method has been developed and subsequently validated for the determination of Moxifloxacin in bulk and pharmaceutical formulations. 0.01N HCL was used as a solvent in the present investigation. Quantitative measurements were made at the maximum absorption of 294.4 nm. The method was validated over the range of 2 to 8 µg/mL with correlation coefficient r = 0.9999. The method was shown to be accurate and precise with inter-day and intra-day percent relative standard deviation values in the range of 0.176 to 0.356 and 0.448 to 1. 07. The percent recoveries of Moxifloxacin were found to be 99.31 to 100.13. The limit of detection was 0.011 µg/mL and limit of quantification was 0.038 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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Introduction:

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. Various solvents were employed for different methods however; these solvents are suffering from one or other disadvantages like accuracy, stability, sensitivity and specificity**.**

Hence it is thought worthwhile to develop a simple and precise spectrophotometric method using 0.01N HCL as a solvent for the estimation of Moxifloxacin in bulk and its pharmaceutical formulation.

Experimental

Instruments and reagents

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, Moxif (Formulation I, Torrent Pharmaceutical Industries Ltd, and Baddi) and Staxom (Formulation II, Stancare, and Delhi) were procured from local drug stores. Other chemicals and solvents were of analytical grade.

Selection of Solvent

Different solvents were tested to establish absorption maxima of Moxifloxacin at 5 μg/mL concentration. The absorbance intensities followed the order 0.01N HCL > 0.1N HCL> 1N HCL>Distilled water (corrected by the corresponding blank). Accordingly, 0.01N HCL (λ_{max} 294.4 and absorbance intensity 0.912) was selected the best solvent. Results are presented in Table 1.

Among the three solvents, 0.01N HCL showed greater absorbance 0.912 nm at λ_{max} 294.4 and selected as a solvent for the estimation of Moxifloxacin.

Table 1 Selection of solvents for moxifloxacin

Concentration (μg/mL)	Solvent	λ_{max}	Absorbance
5	Distilled water	295	0.396
5	1N HCL	296.6	0.820
5	0.1N HCL	296.0	0.902
5	0.01N HCL	294.4	0.912

Preparation of Standard Stock Solutions

Standard stock solution was prepared by dissolving 10 mg of Moxifloxacin in 100mL of 0.01N HCL to get a concentration of 100 μg/mL.

Preparation of calibration curve

To prepare calibration standards, 0.2 to 0.8 mL of working standard solutions were diluted to obtain final drug concentration of 2 to 8 μg/mL and linearity was studied. Linearity relationship was observed in the range 2 to 8 μg / mL (Fig. 1) against a reagent blank as reference at 294.4 nm (Table 2).

Table 2: Linearity table of Moxifloxacin in Working Standard

Conc. (µg/mL)	Absorbance
2	0.375
3	0.539
4	0.728
5	0.912
6	1.081
7	1.265
8	1.449

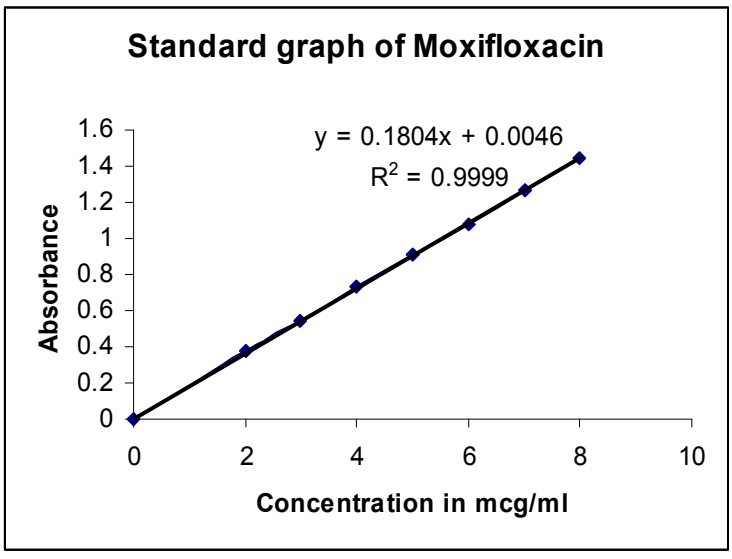

Fig 1 Linearity graph of Moxifloxacin

Estimation of Moxifloxacin in tablets.

For analysis of commercial formulations, twenty tablets were taken and powdered. Tablet powder equivalent to 400 mg of Formulation were transferred into 400 ml volumetric flask and dissolved in 0.01N HCI. Then the solution was sonicated for 30 minutes and filtered. 10ml from the filtrate were taken and further diluted with 0.01N HCI to form 100µg/mL. Then 0.2mL, 0.3mL, 0.4mL, 0.5mL, 0.6mL, 0.7mL, 0.8mL from the above 100µg/mL conc. were taken and further diluted with 0.01N HCI to 10mL.

The absorbance of the prepared solutions were measured at 294.4nm for Moxifloxacin solutions against 0.01N HCI as blank and the drug content was estimated. The results were shown in table 3.

Table 3 Analysis of tablets in commercial formulations

Formulation	Labeled Amount (mg.)	Amount obtained (mg)	%Drug present	%RSD
Moxif	400	397.38±0.3394	99.34	0.341
Staxom	400	397.88±0.7352	99.47	0.739

[*Each value is average of three determinations ± standard deviation]

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 4

Table 4 Precision Readings:

Conc. of Moxifloxacin (μg/mL)	Absorbance	Statistical analysis
6	1.081	Mean: 1.081 S.D: 0.0007 %R.S.D:0. 0647
6	1.082	
6	1.080	
6	1.081	
6	1.082	
6	1.083	
6	1.082	
6	1.082	

Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, 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. The readings were shown in Table 5.

Table 5 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 %	4.8	6	1.081	99.65	Mean	99.417
S_2 : 80 %	4.8	6	1.079	99.47	SD	0.240
S_3 : 80 %	4.8	6	1.079	99.31	% RSD	0.241
S_4 : 100%	6	6	1.085	99.90	Mean	99.76
S_5 : 100%	6	6	1.084	99.76	SD	0.183
S_6 : 100%	6	6	1.083	99.64	% RSD	0.1834
S_7 : 120%	7.2	6	1.083	99.72	Mean	99.84
S_8 : 120%	7.2	6	1.083	99.69	SD	0.3111
S_9 : 120%	7.2	6	1.087	100.13	% RSD	0.3114

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 -6. From the data % RSD was determined.

Table 6 Results for Repeatability studies

Amount taken (µg/mL)	Inter-day		Intra-day	
	Amount found (µg/mL)	%RSD	Amount found (µg/mL)	%RSD
4	3.98	0.176	3.95	0.540
4	3.93		3.90	
4	3.97		3.92	
6	5.85	0.240	5.97	1.07
6	5.90		5.92	
6	5.87		5.88	
8	7.87	0.356	7.96	0.448
8	7.98		7.89	
8	7.91		7.91	

Ruggedness

Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot in different laboratories using similar operational and environmental condition. The readings were shown in Table7.

Table 7 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
6 6 6	5.88 5.92 5.87	0.118	5.97 5.94 5.99	0.587

Results and discussion

The present study was carried out to develop a simple, accurate and sensitive UV spectrophotometric method for the determination of Moxifloxacin in tablets. In the present investigation 0.01N HCL was found to be a better solvent. From the optical characteristics of these proposed methods, it was found that the drugs obey linearity within the concentration range of 2-8µg/mL in uv region. % RSD is less than 2%; which indicates that these proposed method have good reproducibility (Table 4). Percentage recovery values of pure drug from the analyzed formulation were in between 99.31-100.13 % (Table 5). The assay values for the marketed formulation were found to be within limit as listed in table 3. All validation parameters are incorporated in table 8. 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.

Table 8 Validation Parameters

Parameters	Results
Beer's law limit (µg/ml)	2-8
Sand ell's sensitivity (µg/cm^2/0.001)	0.0055
Absorptivity ($1mole^{-1}$,cms^{-1})	0.181×10^4
% Relative standard deviation	0.0647
% Range of error	
0.05 confidence limits	0.048
0.01 confidence limits	0.064
Limit of detection	0.011
Limit of quantitation	0.038
Correlation coefficient	0.9999
Regression equations (Y^*)	
Slope (a)	0.1804
Intercept (b)	0.0046

$Y^* = aX + b$, X = concentration

Conclusion

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.

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