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Research Article

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A validated RP- HPLC method for the analysis of Moxifloxacin Hydrochloride in pharmaceutical dosage forms and stability studies

M. Vishnu Priya*[1], P. Madhavan[2] and Pramod Kumar[3]

[1]Jawaharlal Nehru Technological University, Kukatpally, Hyderabad, India
[2]CPS, Dr. Reddys Laboratories LTD, Miyapur, Hyderabad, India
[3]CCST, IST, JNTU, Hyderabad, India

ABSTRACT

A stability indicating High performance Liquid chromatography method was developed for the Moxifloxicin in pharmaceutical dosage forms. The chromatographic separation was achieved on C18, 150mm X 4.6mm,5 µm particle size column. The mobile phase contains a mixture of 0.1% TEA in water and methanol in isocratic elution. The retention time of Moxifloxicin was found to be 2.8min. The total run time was 10min. The proposed method is found to be having linearity in the concentration range of 20-60µg mL-1 with correlation coefficient of 0.999. The developed method has been statistically validated and found simple and accurate. The mean recoveries obtained for Moxifloxacin HCL are in the range 99.3-102%. Due to its simplicity, rapidness, high precision and accuracy of the proposed method it may be used for determining Moxifloxacin HCL in bulk and dosage forms.

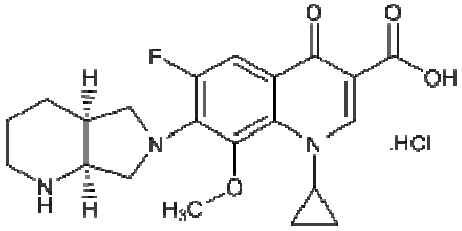
Key words: Method development, validation, Moxifloxicin and stability indicating.

INTRODUCTION

Moxifloxacin is a fourth-generation fluoroquinolone antibiotic that exerts its effects by trapping a DNA drug enzyme complex and specifically inhibiting ATP-dependent enzymes topoisomerase II (DNA gyrase) and topoisomerase IV. Currently, moxifloxacin is being extensively used in the treatment of respiratory system diseases such as community-acquired pneumonia (CAP), chronic bronchitis (CB) and chronic obstructive pulmonary disease (COPD) for the broad spectrum of antimicrobial activity against respiratory tract pathogens, including Gram-positive and Gram negative organisms, anaerobic bacteria, and atypical respiratory tract pathogens [1–4]. The favorable pharmacokinetics of moxifloxacin, including a high mean apparent volume of distribution and a long terminal half life, supports a once-daily dosing regimen in the treatment of infectious disease [5]. It is revealed that moxifloxacin is primarily eliminated in the liver [6].

In recent years, a variety of methods on high-performance liquid chromatography (HPLC) for measuring moxifloxacin concentration in plasma have been reported. Fluorescence detector was applied in several methods for its advantage of sensitivity [7–12]. However, some complex techniques such as gradient elution and on-column focusing [7], precolumn derivatisation [8], or special column [9], were employed. In addition, these expensive specific instruments would increase the experiment cost and not brief enough for clinical application. Although a few methods applied HPLC with UV detector to determine moxifloxacin in plasma [13–16], automated extraction

methods with a polymeric cartridge [13], poor extraction recovery [14], or complicated flow phase [15] were involved. LC/ESI-MS/MS methods have also been reported [17,18], but these advanced techniques are not suitable for clinical routine.



Chemical Formula: $C_{21}H_{25}ClFN_4O_4$
Exact Mass: 437.15
Moxifloxacin, HCl

Figure 1: Chemical structure of Moxifloxicin

EXPERIMENTAL SECTION

Chemicals and reagents:
Moxifloxacin HCL was obtained as a gift sample from Msn labs. Triethylamine and Orthophosphoric acid (AR grade) was used for preparing buffer and acetonitrile HPLC grade was purchased from Merck.

Instrumentation and Chromatographic condition
Analysis was performed using High Performance Liquid Chromatography System (HPLC) Shimadzu Item CBM-20A, control of LC-20A/10Avp/10A-Series solvent Delivery Module (Pump), SPD-M20A Photodiode Array Detector. Inersil ODS C18 50x4.6mm,5µm Particles was used as a stationary phase. 0.1% TEA in water as a solvent A and Methanol was used as solvent B. The mobile phase was pumped as isocratic elution mode with 1.0ml min-1. The elution was monitored at 293nm. The injection volume of sample and standard were 2 µL. Diluent used as a diluent.

Preparation of solutions:
A standard solution containing 100µg/ml of Moxifloxicin was prepared by dissolving appropriate amount. All solutions were covered with aluminium foil to prevent photolytic reaction until the time of analysis.

Sample Preparation:
Ten tablets, each containing 100 mg of Moxifloxicin was dissolved in 500ml diluent get 5000 µg/m of Moxifloxicin. 2 mlof above solution was diluted to 100ml to get 100 µg/ml .The solution was filtered through 0.45 micron PVDF filter. Then 10 µL of these solutions were injected into the column and chromatogram was recorded. The retention time of Moxifloxicin was found to be 3.6min.

System suitability solution criteria:
The system suitability was assessed by five replicate analyses of the drugs at concentration of 100 µg/ml of Moxifloxicin. The acceptance criteria was not more than 2.0% for the RSD for the peak area and not more than 2.0 for tailing factor for the peaks of the drug.

Method validation:
Method validation was performed as per ICH guidance (28-29) for determination of Moxifloxicin in the formulation. The following validation characteristics were addressed linearity, detection limit, quantification limit, precision, Accuracy and Specificity.

System suitability Criteria:
The system suitability test solution was injected and the chromatographic parameters like relative standard deviation for replicate injections of Moxifloxicin and the tailing factor for Moxifloxicin peaks were evaluated for proving the system suitability.

Specificity- Forced degradation studies:
Forced degradation studies were performed on Moxifloxicin eye drops to prove the stability indicating property of the method. The stress conditions employed for degradation study of Moxifloxicin include light exposure (29), heat (1050C), water hydrolysis at 500C and oxidation (3% H2O2 at 300C). for light studies, the monitoring period was 10 days were as for heat, acid, base and water hydrolysis it was 48hrs.Oxidation was carried out for 24 hrs. Peak purity of the principle peak in the chromatogram of stressed samples of Moxifloxicin eye drops was checked using photodiode array detector.

Linearity of response:
Linearity solutions were prepared from stock solution at five concentration levels from 20 to 60 µg/ml Moxifloxicin. The slope, Y-Intercept and correlation coefficient were calculated.

Precision:
Repeatability (Intraday):
The precision of the assay method was evaluated by carrying out six independent assays of Moxifloxicin (20 µg/ml) test samples against qualified reference standard. The percentage of RSD of six assay values was calculated.

Intermediated Precision (Interday):
Different analyst from the same laboratory evaluated the intermediate precision of the method. This was performed by assaying the six samples of Moxifloxicin eye drops against qualified reference standard. The percentage of RSD of six assay values was calculated.

Accuracy (Recovery study):
Recovery of the assay method for Moxifloxicin was established by three determinations of test sample using eye drops at 50%,100% and 150% of analyte concentrations (20 to 60 µg/ml). Each solution was injected tries (N=3) into HPLC system and the average peak area of Moxifloxicin peaks was calculated.

Limit of detection and Limit of Quantification:
The LOD and LOQ for Moxifloxicin were estimated at a signal to Noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations.

Robustness :
To determine the robustness of the method the experimental conditions were deliberately changed and the resolution of Moxifloxicin, tailing factor and % RSD for five replicate injections was evaluated. The mobile phase flow rate was 10.ml/min; to study the effect of flow rate on resolution it was changed to 0.9 and 1.1 ml/min. The effect of Column temperature was studied at 25 and 350C (instead of 300C). In all these experiments the mobile phase components were not changed.

Solution stability and Mobile phase stability:
The stability of Moxifloxicin in solution was determined by leaving test solutions of the sample and reference standard in tightly capped volumetric flasks at room temperature for 48hrs during which they were assayed at 24hrs intervals. Stability in the Mobile phase was determined by analysis of Freshly prepared sample solutions at 24hrs intervals for 48hr and comparing the results those obtained from freshly prepared reference standard solutions. The Mobile phase prepared at the beginning of the study period and not changed during the experiment. The RSD(%) of the results was calculated for both the Mobile phase and solution-stability experiments.

Method development and optimization of stability indicating assay method:
The method was optimized to separate major degradation products formed under various stress conditions from Moxifloxicin. The main target of the chromatographic method is to get the separation for closely eluting degradation products, mainly for the degradation products at rt, which is eluting vary closely to the Moxifloxicin. The degradation samples were run using different stationary phases like C18, C8 and Mobile phases containing buffers like Phosphate acetate different pH (2 to7) and using organic modifiers like acetonitrile and methanol in the mobile phase. But the separations were satisfactory in the adopted chromatographic conditions only. It indicated that the gradient elution with 0.2% OPA in water as solvent A and Acetonitrile and water in the ratio 90:10v/v; was as solvent B for Mobile phase was successful in separating drugs and all chromatographic degradation products. The detailed experimentation is reported in the table 1.

RESULTS AND DISCUSSION

Method validation: Validation of an analytical procedure is the process by which it is established, by laboratories, studies, that the performance Characteristics of the procedure meets the requirements for the intended applications (28).

System suitability:
The system suitability test solution was injected and the chromatographic parameters like relative standard deviation for replicate injections of I and DC and the tailing factor for Moxifloxicin peaks are evaluated. The relative standard deviation for replicate injections of Moxifloxicin was 0.5% and 0.3% respectively. The tailing factor for Moxifloxicin peak was 1.2%. This indicates the suitability of the system.

Table 1. Results from different method development trails

Trail No	HPLC condition		Remarks
1	Column	Inertsil ODS 3V,150,4.6,5µm	Peak shape was not good
	Mobile phase	Solvent A :0.2% OPA in water pH (2.2) Solvent B:Methanol and water in the ratio (90:10)	
	Flow rate	1.0 ml/min.	
2	Column	Inertsil ODS 3V,150,4.6,5µm	Peak shape was not good and tailing factor high.
	Mobile phase	Solvent A :0.2% OPA in water pH (2.2) Solvent B:Acetonitrile and water in the ratio (50:50)	
	Flow rate	1.0 ml/min.	
3	Column	Hypersil BDS, C18, 250,4.6, 5µm	Un knows impurity was not separated in degradation conditions.
	Mobile phase	Solvent A :0.1% TEA in water pH Solvent B:Acetonitrile and water in the ratio (70:30)	
	Flow rate	1.0 ml/min.	
4	Column	(Inertsil ODS 3v 150,4.6,5µm	Un knows impurity was separated in degradation conditions. Peak shape was good.
	Mobile phase	Solvent A :0.1% TEA in water Solvent B: Methanol	
	Flow rate	1.0 ml/min.	

Table 2b: Residual summary of Linearity results of Moxifloxicin

Concentration in µg/ml	Mean area response achieved	Response calculated thru trend line	Residual	Residual square
12.5	294426	288932	-5493.2	988990
25	430896	436335	5439.2	1133160
50	580852	583737	2885.6	207613417
75	731256	731140	-116.0	200366856
100	881258	878542	-275.6	308163981
Residual sum Squares	75474769.6			
Correlation coefficient	0.999			
Tried line equation	Y=8620x-5872			

Table 3.Precision results

S.no	Parameter	%RSD for Assay of Moxifloxicin
1	Repeatability	0.7
2	Intermediated precision	0.6

Table 4: Recovery study.

S.No	Concentration	Mean recovery	%RSD
1	50	99.8	0.31
2	100	99.3	0.20
3	150	100.3	0.25

Table 5: Solution and Mobile phase stability

S.No	Interval	% Assay solution stability	% Assay Mobile phase stability
1	0h	99.4	98.2
2	24h	99.0	98.1
3	48h	98.2	98.3
% RSD		0.6	0.1

Table 6: Forced degradation results

Stress condition	Time	% Degradation
Acid Hydrolysis	24h	0.5
Base hydrolysis	24h	0.4%
Oxidation	24h	11.5%
Water hydrolysis	24h	0.5%
Thermal	24h	0.2%
Light	10days	0.3%

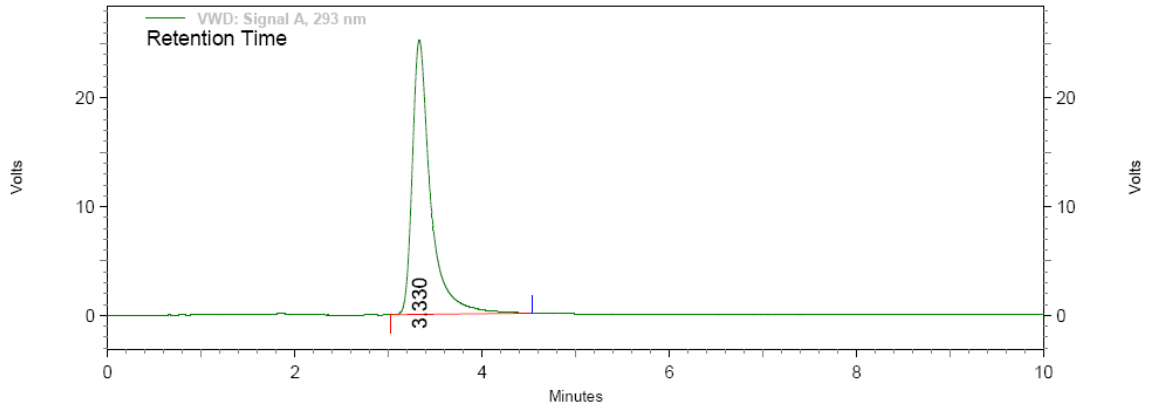


Figure 2: A typical HPLC chromatogram of Moxifloxicin

Linearity of the response:
Calibration curve obtained by least square regression analysis between average peak area and the concentration showed (Table 2a) linear relationship with a regression coefficient of 0.999. The best fit linear equation obtained was Y= 8620Con- 5872 for Moxifloxicin. Analysis of residuals indicated that the residuals were normally distributed around the mean with uniform variance across all concentrations suggesting the homosccedastic nature of data. Selected linear model with univarieant regression showed minimum % bias indicating goodness off it which was further supported by the low standard error of estimate and mean sum of residual squares.

Precision:
The precision of an analytical method gives conformation on the random error. It express of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. The % RSD values for the precision study was 0.7% (Interday Precision).And 0.6% (intraday precision) for Moxifloxicin. This is confirming good precision of the method (Table 3).

Accuracy- Recovery test:
The percentage recovery of Moxifloxicin was ranged from 99.8 to 101.6. Excellent recoveries was made each added concentration (Table 4).

Limit of detection and Limit of Quantification:
The limit of detection of Moxifloxicin was 1.8 µg/ml for 10µL injection volume. The limit of quantification of Moxifloxicin was 5.6µg/ml.

Robustness:
When the mobile phase was flow rate and column temperature were deliberately varied resolution was greater than 3.0, tailing factor and % RSD for five replicate injections of Moxifloxicin was less than 1.5,illustrating the robustness of the method.(Table 5).

Stability in solution and in the mobile phase:
Degradation was not observed in Moxifloxicin stressed samples that were subjected to light, acid, base and water hydrolysis. However the degradation was observed under oxidation. The peak purity results derived from PDA

conformed that the Moxifloxicin peaks were pure and homogeneous in all the analyzed stress conditions. This indicates that the method is specific and stability indicating (Figure 2 and Table 6).

CONCLUSION

A simple Specific stability indicating liquid chromatographic method is developed for the quantification of Moxifloxicin Pharmaceutical dosage forms. This method is validated and it is found to be Specific, precise, accurate, Robust and linear for the detection and quantification of Moxifloxicin. The method is stability-indicating and can be used for routine analysis of production sample and to check the stability samples of Moxifloxicin in Pharmaceutical dosage forms.

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