Research Article

Development and Validation of A Stability-Indicating Reversed Phase HPLC Method for Determination of Moxifloxacin in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

The present study describes a novel stability indicating Reversed Phase High-Performance LC method for the determination of Moxifloxacin (MOXI) in bulk and pharmaceutical formulation. The chromatographic separation was carried out on Agilent technologies model SPD 20A prominence UV detector, utilizing Kromasil model C_{18} Column (based on 99.999 % ultra high purity silica) 150 mm × 4.6 mm, 3.5 µm particle size, utilizing methanol: phosphate buffer pH 2.5, (60:40 % v/v) as mobile phase at flow rate of 1.2 ml/minute with an injection volume of 20 µl was selected for this study. The separation was carried out at a room temperature and the eluents were observed by photo diode array detector set at 292 nm. The retention time of MOXI obtained was at 5.495 minutes. The calibration curve for MOXI was linear (r^2 = 0.9999) over the concentration range of 6-18 µg/ml with LOD and LOQ of 0.001 µg/ml and 0.003 µg/ml respectively. A recovery of MOXI in tablet formulation was observed in the range of 99.68-99.90 %. Percentage assay of MOXI tablets (Avelox) and bulk drug was found to be 99.95 ± 2.92 and 99.97 ± 3.05 respectively. The stability of the method was demonstrated by forced degradation studies under conditions of acidic, alkaline, oxidation, photolytic, thermal and UV stress conditions as per ICH Q1A (R2) guidelines. Thus the proposed method for MOXI was found to be feasible for the estimation of MOXI in bulk as well as pharmaceutical dosage form.

Keywords: Moxifloxacin, RP-HPLC, Validation, Forced degradation.

INTRODUCTION

Moxifloxacin (1-cyclopropyl-6-fluoro-1, 4-dihydro-8-methoxy-7-[(4aS, 7aS)-octahydro-6H-pyrrolo [3, 4b]pyridin-6-yl]-4-oxo-3-quinoline carboxylic acid). Moxifloxacin is used to treat a variety of bacterial infections such as acute sinusitis, bronchitis, pneumonia, lung disorder and skin/skin structure infections. It works on pathogens such as H. influenza, S. pneumonia, M. catarrhalis, S. aureus, S. pyogens. Moxifloxacin will not work on virus infections like flu and common cold. Advanced generation this drug synthesized and developed to cover respiratory tract pathogens by excellent Gram -ve coverage plus enhancing Gram positive and a typical anaerobe activity, favorable safety profile. A 400 mg dose once a day for 7 days is recommended for optimal patient compliance and convenience. This fourth generation antibiotic treats only bacterial infections which act by targeting of both DNA gyrase and topoisomerase IV has been achieved. The very important results are achieved via the methoxy at C_8 which significantly delays the selection of resistance and the bicyclic amine at C_7 minimizes drug efflux.

Literature Survey shows that the MOXI has been determined by UV spectrophotometric method [1-2], HPLC[3-4], RP-HPLC [5-6], HPTLC [7] and LC-MS/MS [8] in biological fluids like human and rat plasma. However no stability indicating High Performance LC method has been reported for the estimation of MOXI in bulk and pharmaceutical dosage forms hitherto. Hence the major objective of the present research is to develop and validate a simple, precise, sensitive liquid chromatography method for MOXI in its bulk and tablet dosage form and stress degradation studies of MOXI as per International Conference on Harmonization (ICH) Q1A (R2) guidelines. Figure 1 shows the chemical structure of Moxifloxacin.

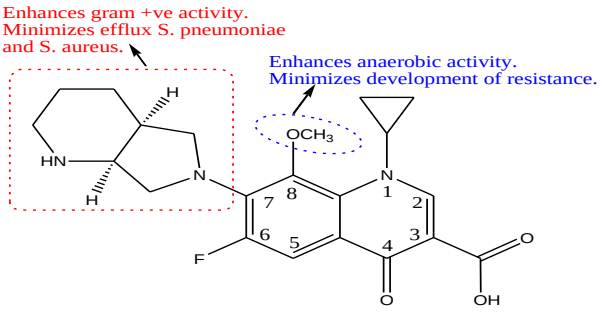

Figure 1: Chemical structure of Moxifloxacin

MATERIALS AND METHODS

Chemicals and Reagents

MOXI pure drug was supplied as gift sample by Hetero Drugs Ltd., Hyderabad, Telangana, India. The marketed formulation Avelox tablets containing 400 mg of MOXI were obtained from local market. Methanol was obtained from E. Merck specialties private Ltd., Mumbai, India.

Instrument

The HPLC system utilized was Agilent technologies HPLC with a gradient pump connected to UV detector set at 292 nm. Empower software was utilized for data acquisition. A digital balance (Essae vibra AJ (0.001 g)) and

a sonicator (Model no-91250 mode) were utilized in this study.

Method development

Selection of chromatographic technique: MOXI is a fairly non-polar molecule which is very soluble in methanol. So, RP-HPLC method was tried first for the analysis of MOXI and it showed decorous elution during the preliminary trials.

Selection of detection wavelength: The UV spectrum of the MOXI was recorded by scanning ranging from 200-400 nm using methanol/water (50:50) as medium. From this absorption spectrum λ_{max} at 292 nm was selected for the decorous study. Figure 2 shows the absorption spectra of MOXI.

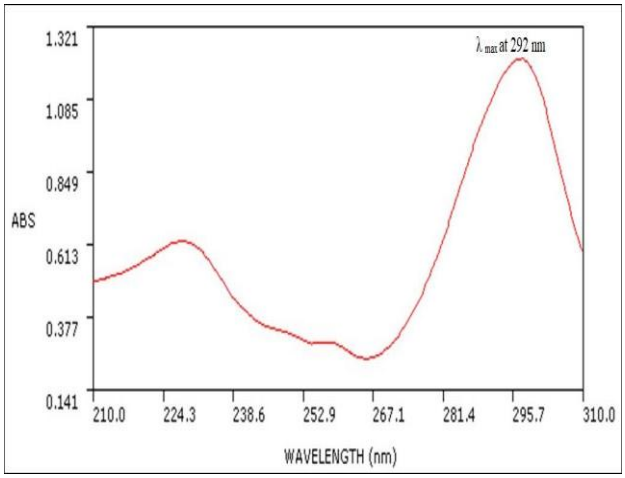

Figure 2: Absorbance spectrum of Moxifloxacin (λ_{max} 292 nm)

Chromatographic conditions

Chromatographic separation was attained on Kromasil C_{18} (150 mm × 4.6 mm, 3.5 µm) column utilizing methanol and phosphate buffer (pH 2.5) as mobile phase. The mobile phase was filtered and degassed through 0.22 mm filter paper. The Flow rate was kept at 1.2 ml/min. Column temperature is set to ambient (30 °C), for a good peak shape with required peak area was obtained with injection volume of 20 µl with a flow rate of 1.2 ml/min and the detector wavelength of 292 nm were set for the chromatographic study. The retention time obtained of MOXI was at 5.495 min. Diluent used was mobile phase, filtered through 0.45 micron filter and degassed before use. (See table 1).

Preparation of phosphate buffer pH 2.5

Dissolve 2.76 g sodium dihydrogen phosphate (NaH_2PO_4) in 1000 ml of HPLC grade water and add 1 ml of triethyl amine and adjust the pH to 2.5 ± 0.05 with diluted ortho phosphoric acid solution. Filter through 0.45 µm membrane filter.

Table 1: Optimum chromatographic conditions and system suitability results

Parameter	Chromatographic conditions
Instrument	Agilent Technologies
Column	Kromasil C_{18} Column (150 mm X 4.6 mm, 3.5 µm particle size)
Detector	photo diode array detector
Flow rate	1.2 ml/min
Detection wavelength	292 nm
Run time	10 minutes
Temperature	Ambient temperature (30 °C)
Volume of injection loop	20 µl
Retention time (Rt)	5.495 minutes
System suitability results	
Theoretical plates (acceptance criteria NLT 2000)	8348
USP tailing factor (Acceptance criteria NMT 1.5)	1.1
% RSD for MOXI peak area from 6 replicate injections of standard solution (Acceptance criteria NMT 2.0)	0.1185

Preparation of mobile phase

The above stated prepared phosphate buffer (pH 2.5) 630 ml is mixed with 370 ml volume of methanol and degassed by sonication. The prepared solution is used as mobile phase.

Preparation of standard stock solution and standard solution

For preparation of standard stock solution, 30 mg of MOXI was precisely weighed and transferred to 100 ml of volumetric flask which contains 50 ml mobile phase and sonicate to dissolve and dilute to volume with mobile phase and mix well. For preparing standard solution pipette out 4 ml of the above said stock solution is taken into a 100 ml of volumetric flask and dilute to volume with mobile phase and mix well. Filter the above prepared solution with 0.45 µm millipore filter paper.

Test preparation for 400 mg strength tablets and assay procedure of marketed formulation

Twenty Avelox® (MOXI) tablets were weighed, average weight was calculated, and was made to fine powder with mortar and pestle. MOXI tablet powder equivalent to 250 mg is taken into a 250 ml volumetric flask to which 200 ml of Mobile phase was added. The flask is then ultra-sonicated for 20 minutes and volume is made up with the mobile phase and mix well. From the above solution 3 ml is pipetted out and transferred into 250 ml volumetric

flask and filled up with mobile phase and mix well. The tablet MOXI solution is then filtered through 0.45 micron filter. Inject mobile phase as blank, standard preparation should be injected 5 injections and test preparation (in duplicate) record the chromatogram and measure the peak response.

Analytical method validation

The developed method was validated for different parameters like linearity, precision, accuracy, specificity, ruggedness, robustness, LOD and LOQ as per Q1A (R2) guidelines.

System suitability

The system suitability test was carried out on freshly prepared MOXI standard solution was used for the evaluation (as per test method) of the system suitability parameters such as theoretical plate count, tailing factor, % RSD and results were observed. The system suitability data and the optimum chromatographic conditions are reported.

System precision

The system precision was evaluated by injecting the standard solution 6 times and the results were noted. Infact the result was within the limit.

Linearity

Linearity of detector response was established by plotting a graph between concentrations versus area. A series of dilutions of MOXI standard were prepared in the concentration range of 6 µg/ml to 18 µg/ml from MOXI standard stock solution which is 12 µg/ml and analyzed as per test method. A graph was plotted with concentration in µg/ml on X- axis versus peak area on Y-axis and the correlation coefficient was determined.

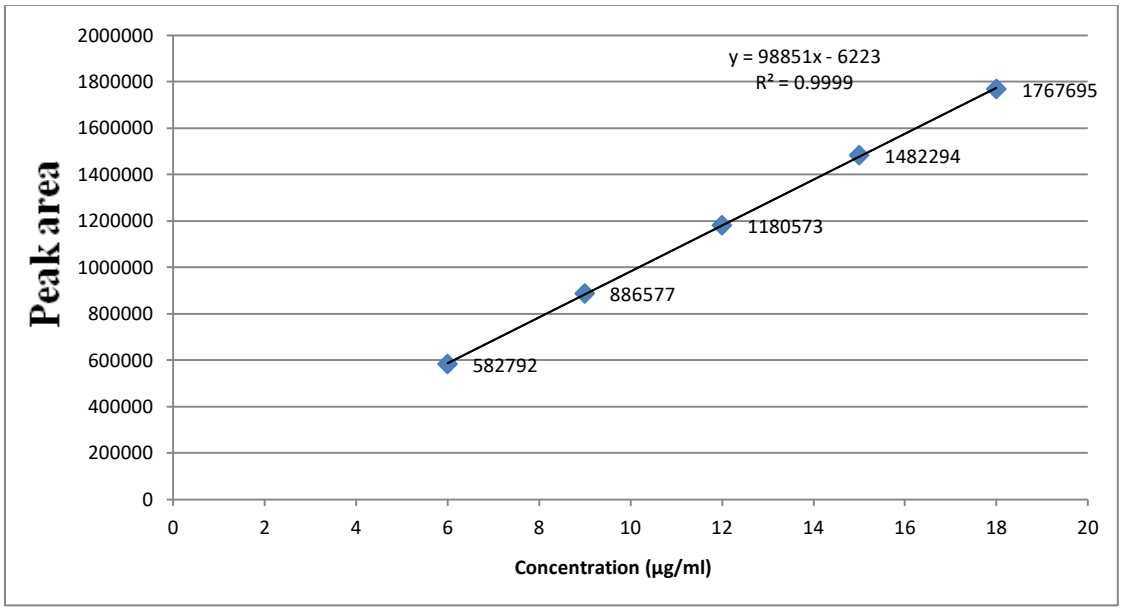

Figure 3a: Calibration graph of Moxifloxacin

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.999949296
R Square	0.999898594
Adjusted R Square	0.999864792
Standard Error	5452.481939
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	8.7943E+11	8.794E+11	29581.087	4.3341E-07
Residual	3	89188677.9	29729559		
Total	4	8.7952E+11			

	Coefficients	*Standard Erro*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-6223	7315.27216	-0.850686	0.4574527	-29503.461	17057.4608	-29503.46	17057.4608
X Variable 1	98850.76667	574.742061	171.99153	4.334E-07	97021.6809	100679.852	97021.681	100679.852

Figure 3b: Summary output of Moxifloxacin

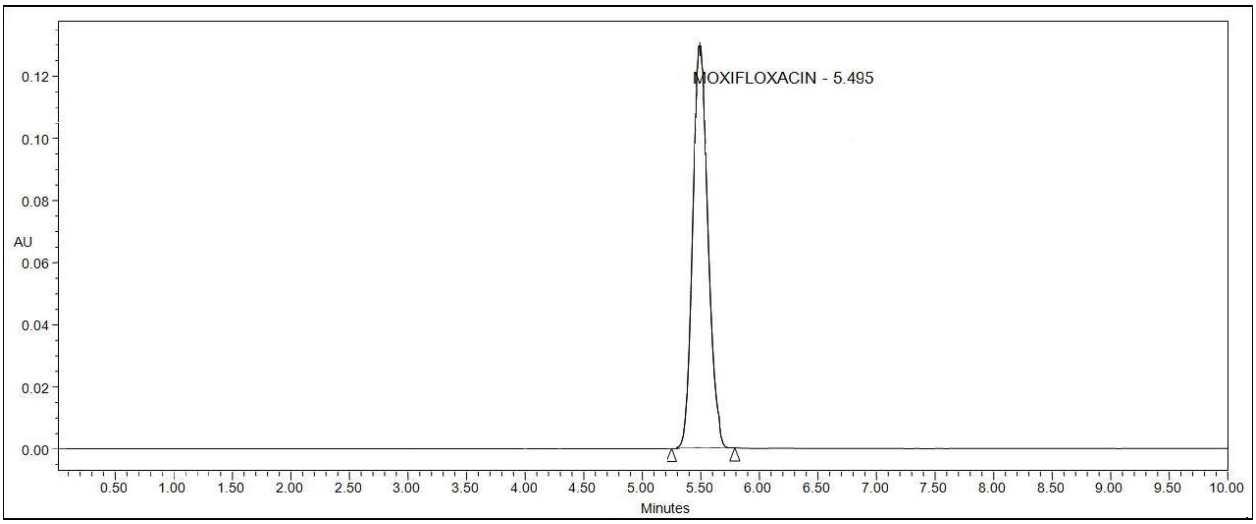

Figure 4a: Standard chromatogram of Moxifloxacin

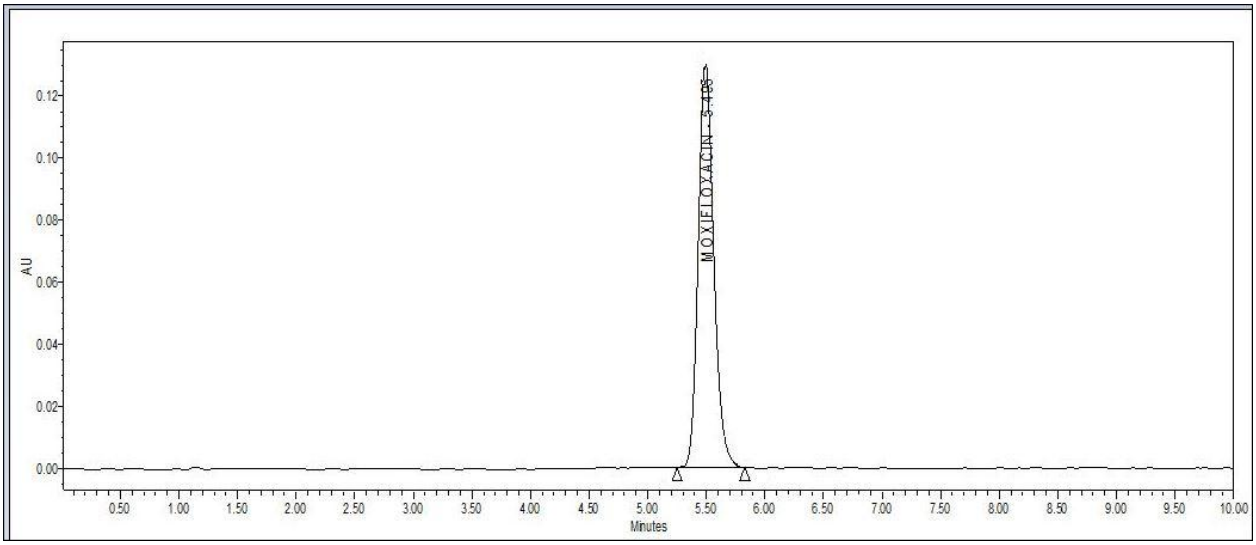

Figure 4b: Sample chromatogram of Moxifloxacin

Precision

Precision of the test method was determined by repeatability, intra-day and inter-day precision. Repeatability was confirmed by injecting same concentration in six replicates and corresponding areas were calculated. Intra-day and Inter-day variation MOXI was analyzed by selecting three concentrations which were 9, 12 and 15 µg/ml from linearity range. Intra-day analysis assay was performing same day whereas inter-day analysis assay was carried on three different days in replicates of three as per test method. The respective peak areas for different concentrations were reported. The % relative standard deviation for % assay results is found to be within the limit. Indeed no significant variation in % assay.

Table 2a: Results of system precision

Injection Number	Peak area in AU
1	1183573
2	1180569
3	1180366
4	1180574
5	1180689
6	1180672
Mean	11881120.833
SD	1399.8
% RSD (acceptance criteria: The % RSD for peak area of MOXI form 6 replicate injections of standard solution should not be more than 2.0)	0.1185

Table 2b: Intra-day precision studies of Moxifloxacin

Amount of Standard taken (µg/ml)	Area (mAU)	Retention Time (Rt) (minutes)	Tailing factor	Theoretical plates	% Relative Standard Deviation (n =3)
Day-1 (Morning)					
9	886577	5.493	1.2	9348	0.23
12	1180573	5.492	1.4	9359	0.12
15	1482294	5.493	1.3	9327	0.14
Day-1 (Afternoon)					
9	886573	5.493	1.0	9342	0.22
12	1180571	5.493	1.4	9355	0.11
15	1482291	5.493	1.1	9329	0.12
Day-1 (Evening)					
9	886571	5.493	1.2	9347	0.11
12	1180570	5.492	1.3	9357	0.13
15	1482293	5.493	1.0	9324	0.22

Table 2c: Inter-day precision studies of Moxifloxacin

Amount of Standard taken (µg/ml)	Area (mAU)	Retention Time (Rt) (minutes)	Asymmetry factor	Theoretical plates	% Relative Standard Deviation (n =3)
Day-1					
9	886576	5.494	1.1	9346	0.12
12	1180571	5.493	1.2	9357	0.14
15	1482292	5.493	1.3	9329	0.13
Day-2					
9	886570	5.493	1.0	9348	0.13
12	1180573	5.492	1.2	9359	0.14
15	1482294	5.493	1.2	9322	0.24
Day-3					
9	886572	5.492	1.4	9342	0.23
12	1180570	5.493	1.1	9351	0.11
15	1482291	5.493	1.0	9327	0.12

Accuracy

Accuracy study was determined by recovery studies. This accuracy study was conducted for MOXI intact tablet from about 50 %, 100 % and 150 % of the initial assay concentration. Sample solutions were prepared in triplicate for each level and analyzed as stated by test method. The individual % recovery, mean % recovery and % relative standard deviation for recovery at each level were calculated and analyzed. Indeed the individual recovery should be between 95.0 and 105.0. The average recovery of each level should be between 97.0 and 102 %. As a matter of fact the results are found to be within limit.

Specificity

The purpose of this study is to evaluate by determining the effect of excipients, additives present in the formulations interfered with the analysis or not. MOXI standard was injected with known quantities of impurities. All the impurities were resolved and are not interfering with the retention time of MOXI.

Placebo interference

The purpose of this study is to evaluate the interference from placebo. Samples were prepared in duplicate by taking placebo equivalent to the amount present in the test preparation and analyzed as indicated by the test method. Chromatograms of placebo preparations are not showing any peak at the retention time of analyte peak i.e., MOXI.

Robustness

Robustness of the method was determined by varying the method parameters such as change in flow rate (± 0.2 ml/min), wavelength (± 1 nm) and mobile phase composition.

Table 3: Robustness studies of Moxifloxacin

S. No	Parameter	Optimized	Used	Retention time (Rt), min	Plate count	Tailing factor	% RSD
1	Flow rate (± 0.2 ml/min)	1.2 ml/min	1.0 ml/min	5.595	9442	1.1	0.11
			1.2 ml/min	5.495	9348	1.06	0.12
			1.4 ml/min	5.392	9341	1.1	0.14
2	Detection wavelength (± 1 nm)	292 nm	291 nm	5.495	9344	1.12	0.13
			292 nm	5.495	9348	1.06	0.12
			293 nm	5.495	9392	1.06	0.22
3	Mobile phase composition	Methanol : Phosphate buffer (pH 2.5)	55:45 %	5.493	9345	1.1	0.11
			60:40 %	5.495	9348	1.06	0.12
			65:50 %	5.491	9320	1.1	0.25

Acceptance criteria: Plate count NLT 2000, tailing factor NMT 1.5, % RSD NMT 2.0.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

A limit of detection (LOD) and a limit of quantification (LOQ) were calculated according to the formulae:

LOD = 3.3* σ/s

LOQ = 10* σ/s

Where, 'σ' is the standard deviation of 'y' intercept of regression line and 's' is the slope of the calibration curve.

Forced degradation studies

To conduct the forced degradation study, 10 mg MOXI was subjected to acidic, alkaline, oxidation, thermal, UV light, humidity and photolytic conditions. For acidic degradation, 10 mg MOXI was dissolved in 5 ml of mobile phase to which 5 ml of 0.1 N HCl was added and heated under reflux at 40 °C for 3 days. The mixture was neutralized by the addition of 0.1 N NaOH. For alkaline degradation, 10 mg drug was dissolved in 5 ml of mobile phase to which 5 ml of 0.1 N NaOH was added and heated under reflux at 40 °C for 3 days before the mixture was neutralized by the addition of 0.1 N HCl.

For degradation under oxidising conditions the drug was heated under reflux with 30 % H_2O_2 (v/v) at 40 °C for 3 days. For thermal degradation the powdered drug was exposed at 105 °C for 72 hours. Regarding UV light degradation, powdered MOXI was exposed to UV light for 3 days. Pharmaceutical MOXI dosage forms were also subjected to the same stress conditions to determine whether any peaks arose from the degraded excipients. After completing the treatments, the MOXI solutions were left to return to room temperature diluted with solvent mixture to obtain 10 µg/ml solutions. Stressed samples were analyzed as per test method with PDA detector. Chromatograms of stressed samples were determined for peak purity. For all forced degradation samples the purity angle is less than purity threshold with no purity flag for MOXI which indicates that there is no interference from degradants in estimation of MOXI.

Table 4: Stability studies of Moxifloxacin

S. No.	Stress Conditions	Observation	Moxifloxacin			Purity Flag
			Type	Purity Angle	Purity Threshold	
1	Treated with 0.1 N HCl (5 ml) solution for 3 days (sonicated at 40°C)	No degradation of drug is seen	Standard	0.180	0.380	No
			Sample	0.160	0.365	
2	Treated with 0.1 N NaOH (5 ml) solution for 3 days (sonicated at 40°C)	No degradation of drug is seen	Standard	0.187	0.370	No
			Sample	0.150	0.360	
3	Treated with 30 % H_2O_2 (5 ml) solution for 3 days	No degradation of drug is seen	Standard	0.175	0.310	No
			Sample	0.148	0.420	
4	Kept in oven at 105 °C for 72 hours	No degradation of drug is seen	Standard	0.168	0.290	No
			Sample	0.170	0.420	
5	Kept in humidity chamber for five days	No degradation of drug is seen	Standard	0.165	0.360	No
			Sample	0.155	0.420	
6	Kept in photolytic chamber for 3 days	No degradation of drug is seen	Standard	0.160	0.260	No
			Sample	0.158	0.420	

Assay of marketed formulation

Twenty Avelox® (MOXI) tablets were weighed, average weight was calculated, and was made to fine powder. MOXI powder proportionate to 10 mg was taken in a 10 ml volumetric flask to which small amount of mobile phase was added. The flask is then ultra-sonicated for 15 minutes and volume is made up with the mobile phase. The tablet MOXI solution is then filtered through whatmann filter paper (No. 42) to get rid of insoluble materials. From the above solution 10 ml is added to 100 ml with diluent so as to attain concentration of 100 µg/ml for the assay. It was further diluted according to the need and then analyzed following the proposed procedure. The content of the Avelox was calculated either from the previously plotted calibration graph or utilizing regression equation.

Determination of Avelox in Bulk drug

For the analysis of bulk drug accurately weighed 10 mg MOXI was taken in a 100 ml volumetric flask and the volume was filled upto the mark with mobile phase to get 100 µg/ml concentration. From this 1 ml was taken and transferred to a 10 ml volumetric flask and the volume was made up to the mark with mobile phase to get 10 µg/ml concentration. The concentration of the bulk drug was calculated from the linear regression equation.

RESULTS AND DISCUSSION

Several mobile phases of different compositions were tested so as to develop an optimization of chromatographic conditions such as tailing factor, decorous peak shape, and theoretical plates. For the selection of the mobile phase primarily methanol, acetonitrile, CH_3OH : water, ACN : water has been tried in different compositions. Eventually Methanol and phosphate buffer (pH 2.5) used at a flow rate of 1.2 ml/min was found to be satisfactorily and decorous system suitability parameters. The average retention time (Rt) got for MOXI was at 5.495 min. The tailing factor and theoretical plates for MOXI were found to be 1.1 and 8348 respectively. Accuracy of MOXI was determined by calculating the % recovery. The method was found to be accurate with % recovery between 99.68-99.90 %. Intra and inter-day precision was calculated. Infact the method was precise with percentage RSD < 2%. Intra and inter-day precision are shown in tables 2b and 2c respectively. The % RSD value of robustness which is less than 2% for Moxifloxacin reveals that the proposed method is robust (table 3) (Change in flow rate, wavelength and mobile phase composition). The % RSD values of ruggedness for Moxifloxacin reveal that the proposed method is quite rugged. The LOD and LOQ of Moxifloxacin were found to be 0.001 µg/ml and 0.003 µg/ml respectively. The % assay of the bulk was found to be 99.97 ± 3.05. The average content of MOXI was 99.95 ± 2.92, which was in good agreement with labelled claim. The method was specific and has no interference observed when the MOXI were estimated in presence of excipients.

Degradation behaviour of MOXI under various stress conditions are shown in table 4. The assay method of MOXI in pharmaceutical formulation was successfully developed and validated for its intended purpose. Infact there was no particular precaution necessary during manufacturing and storage of MOXI formulation because there was no degradation studied at room temperature.

CONCLUSION

The current research deals with the development of a stability indicating RP-HPLC method for determination of MOXI in bulk as well as pharmaceutical dosage form. The values of accuracy, precision, robustness, ruggedness, LOD and LOQ were within the limits. Infact stressed samples were evaluated as per test method with PDA detector. When the chromatograms of stressed samples were analysed for peak purity of peak utilizing Agilent empower software. For all forced degradation samples the purity angle was less than purity threshold with no purity flag for MOXI peak which indicates that there is no interference from degradants in estimation of MOXI in MOXI sample. As a matter of fact the results of the study shows that the developed method was found to be simple, accurate, sensitive, rapid and reproducible and have short run time and only requires low cost technology which makes this method economically good for all most all clinical laboratories hence this study of novel RP-HPLC method for the determination of MOXI in a bulk and tablet formulation can be successfully utilized for its intended purpose.

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