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METHOD DEVELOPMENT AND VALIDATION OF MOXIFLOXACIN BY USING RP- HPLC AND UV-SPECTROSCOPY

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ARTICLE INFO

Key Words

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ABSTRACT

A rapid, sensitive and specific RP-HPLC and UV-Spectroscopy method was developed and validated for determination and quantification of Moxifloxacin HCl in tablet dosage form. Chromatography was carried out on a pre-packed luna C-18, 5µ (250 x 4.6) mm phenomex column using filtered and degassed mixture of phosphate buffer: Methanol (65:35)pH(4) as mobile phase at a flow rate of 1.0 ml/min and effluent was monitored at 296 nm. The pH of the mobile phase was adjusted with glacial acetic acid. The retention time of the drug is 3.4mins . The method does require only 10 min as run time for analysis which proves the adoptability of the method for the routine quality control of the drug. For UV method also same buffer is used. The method was validated in terms of linearity, precision, accuracy, and specificity, limit of quantification and limit of detection. The assay was linear over the concentration range of 2-6ug/ml for RP-HPLC method and 2-7.5ug/ml for uv method. Accuracy of the method was determined and % recovery was found to be 99.3 %-100.7 %for RP-HPLC and 99.1%-100.1% for UV-method. The % RSD is less than 2% for all parameters for RP-HPLC method and UV-method.The method is developed and validated as per ICH guidelines.

INTRODUCTION

Fluoroquinolones (FQs) are among the most important antibacterial agents used in human medicine. They are active against both Gram-positive and Gram negative bacteria through inhibition of their DNA gyrase and also possess some activity against mycobacteria, mycoplasmas and rickettsias. Moxifloxacin hydrochloride (MOXI) chemically is 1-Cyclopropyl-6-fluoro-8-methoxy-7-[(4a*S*,7a*S*) octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo 1,4 dihydroquinoline-3-carboxylic acid(fig-1). It is used to treat a number of infections ,including: respiratory tract infections, cellulitis, anthrax, meningitis and tuberculosis.

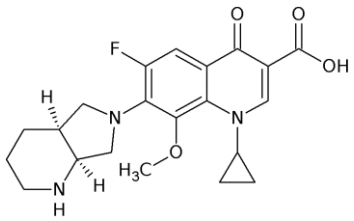

Fig-1Structure of Moxifloxacin

MATERIALS AND METHODS

Working standard of Moxifloxacin HCl was obtained from MSN laboratories.

Materials for RP-HPLC: HPLC grade Methanol, HPLC grade glacial acetic acid, HPLC grade water, AR grade Disodiumhydrogen phosphate and potassiumdihydrogen phosphate were procured from market.Luna C_{18} 5 u (250x4.5)mm column and the mobilephase was filtered and degassed prior to use.

Materials for UV-Spectroscopy: AR grade methanol, double distill water, AR grade Disodiumhydrogen phosphate and potassium dihydrogen phosphate were procured from market

Instrumentation and Conditions: A High Performance Liquid Chromatographic system, with Spinchrom data handling system (Shimadzu-LC 20AD) with Analytical Column-Phenomenex ODS C18 (250 X 4.5 mm, 5 micron particle size) equipped with binary gradient pump, 20A PDA detector was used for the analysis. Calibrated electronic single pan balance (Contech). pH-meter (Elico-210),UV-Visible Spectro photometer(Elico210).Ultrasonicator were also used during the analysis of drug.

A reverse phase C-18 column was equilibrated with the mobile phase dual phosphate buffer : methanol (65:35) and pH adjusted 4.0 with glacial acetic acid. Mobile phase flow rate was maintained at 1ml/min and eluents was monitored at 296nm for Moxifloxacin. The sample was injected using a 20sµl fixed loop. The determination was performed at ambient temperature for a run time of 10min.

METHOD DEVELOPMENT

Buffer Preparation

Dissolve 5.04gms of Disodiumhydrogen phosphate and 3.01 gms of potassium dihydrogen phosphate in 1000ml water and adjust the pH of the buffer with glacial acetic acid to pH -4

Mobile phase: Buffer and methanol were mixed in the ratio of 65:35 and sonicated to degas.

Standard preparation: Accurately Weighed and transferred Moxifloxacin HCl, equivalent to 5 mg of Moxifloxacin. Working Standard into a 5 ml clean dry volumetric flask, and 3 ml of mobile phase was added, sonicated for 5 minutes, and volume was made upto5ml(1000ug/ml).further dilute the stock solution to 100ug/ml.

Calibration curve of moxifloxacin for RP-HPLC: Appropriate aliquots of standard stock solution of the drug was taken in 5 ml volumetric flask and diluted up to the mark with mobile phase in such a way that final concentration of drug was in the range of 2-6 µg/ml Moxifloxacin respectively. The solution was injected using a 20 µl fixed loop system and chromatogram was recorded. Calibration curve was plotted by taking peak area on y-axis and respective concentration of drug on x-axis the values were shown in Table 1

Preparation of sample solution

Accurately weigh and powder 10 tablets. Weigh and transfer a quantity of tablet powder equivalent to 5mg of moxifloxacin into 5ml volumetric flask. Add 3ml diluent and sonicate for 30 minutes with occasional shaking, dilute to volume with diluents. Filter through 0.45µ filter.(1000ug/ml). Pipette 0.5ml of standard stock solution into a 5ml volumetric flask and dilute to volume with diluent. (100ug/ml) Pipette 0.5ml of primary standard solution into a 5ml volumetric flask and dilute to volume with diluent. (10ug/ml). 2.5ml of secondary standard solution were pipette into 5ml volumetric flask and diluted up to the mark with mobile phase. (5ug/ml)

Calibration curve of moxifloxacin for UV-Visible Spectroscopy: Standard stock solution was suitably diluted with mobile phase to obtain concentrations ranging from 2-7.5µg/ml. Absorbance of these solutions was measured at 290nm. Calibration curve was obtained by plotting graph between concentration and absorbance shown in table -2.

Preparation of sample stock solution

Weigh accurately equivalent to 10mg of moxifloxacin and transfer into 10ml volumetric flask. Add 6ml of diluents and sonicate for 2mins and make up the volume.(1000ug/ml). Pipette 1ml from stock solution and transfer into 10ml flask and add 6ml of diluents and sonicate for 2mins and make up the volume with diluent. (100ug/ml). Pipette 1ml from primary std solution and transfer into into 10ml volumetric flask. Add 6ml of diluent and sonicate for 2mins and make up the volume with diluents.(10ug/ml)

Preparation of 6ug/ml sample solution of moxifloxacin: 3.0ml of secondary standard solution were pipette into 5ml volumetric flask and diluted up to the mark with mobile phase

METHOD VALIDATION

1. Linearity

The linearity range for the detection of moxifloxacin was 2-6ug/ml with ($y = 195961x + 83871$ $R^2 = 0.999$).the prepared diluention wasinjected into the column to obtain chromatogram

2. Accuracy

"The closeness of agreement between the conventional true value or an accepted reference value and the value found**".** The accuracy of the method was determined by calculating recoveries of drug by method of standard addition. Known amounts of standard drug corresponding to 80%, 100%, and 120% of the label claim was added to pre quantified sample solution, and the amounts of drug were estimated by measuring the peak areas.

3. Precision: The precision of an analytical procedure "expresses the closeness of agreement between a series of measurement obtained from multiple sampling from the same homogenous sample under the prescribed conditions".

Repeatability: Repeatability is established by injecting six injections of sample prepared

Inter day precision: The same sample prepared above is injected six times on the next day.

4. Specificity

"Specificity as the ability to assess unequivocally the analyte in presence of components which may be expected to be present"
The specificity of the proposed RP-HPLC method was determined by complete separation of two peaks with parameters like retention time (Rt), resolution (Rs) and tailingfactor (T).

5. Robustness

Robustness of the method was studied by deliberate variations of the analytical parameters such as flow rate (1.0+0.1 ml/min), concentration of acetonitrile (30+2%).

6. Sensitivity: The sensitivity of the method was determined with respect to LOD and LOQ. Calibration curves were plotted by using concentration in the expected detection limit range (0.1-5µg/ml) for each drug. The standard deviation of y-intercept of regression line wasdetermined and substituted in the following equation for the determination of detection limit and quantification limit.

Detection limit = 3.3σ/s;

Quantification limit = 10 σ/s

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

7. System suitability: The system suitability test is used to verify that the chromatographic system is suitable for the intended analysis or not"

RESULTS AND DISCUSSION

1. LINEARITY: Typical chromatograms for linearity were shown in fig.1.the caliberation curve for moxifloxacin for RP-HPLC and UV were shown in fig.2 and fig.3 respectively. The results of linearty for RP-HPLC and UV were shown in table-1&2**.**

2. Accuracy: The chromatograms for 80%, 100%&120% were shown in fig 5-7.The results of accuracy were shown in table 3&4.

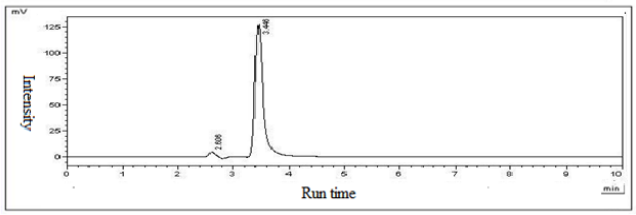

Figure 2: Typical chromatograms for linearity

Table :1 Linearity results for moxifloxacin of RP-HPLC

s.no	Concentration	Area
1	2ug/ml	458416
2	4ug/ml	674856
3	6ug/ml	874928
4	8ug/ml	1069453
5	10ug/ml	1240923

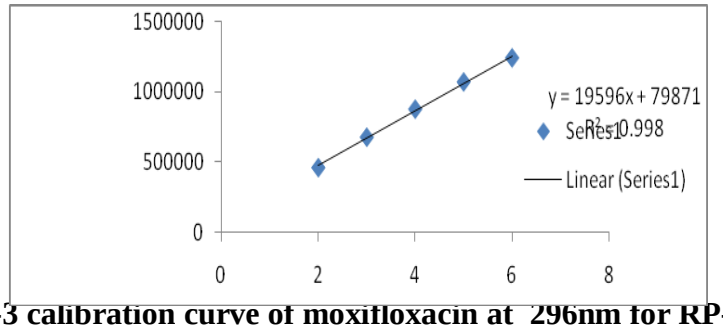

Fig-3 calibration curve of moxifloxacin at 296nm for RP-HPLC

Table 2: Linearity results for moxifloxacin of UV

S.No	Conc	absorbance
1.	2 ug/ml	0.2382
2.	3ug/ml	0.3656
3.	4.5ug/ml	0.5524
4.	6ug/ml	0.7766
5.	7.5ug/ml	0.9792

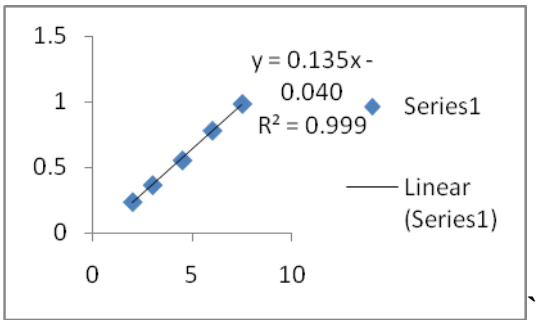

Fig-4 Calibration curve of moxifloxacin at 296nm for UV

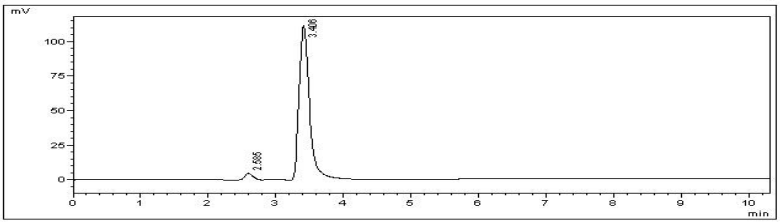

Fig-5 Chromatogram of 80% spike

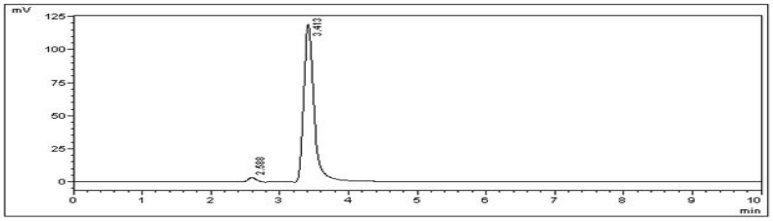

Fig-6 Chromatogram of 100% spike

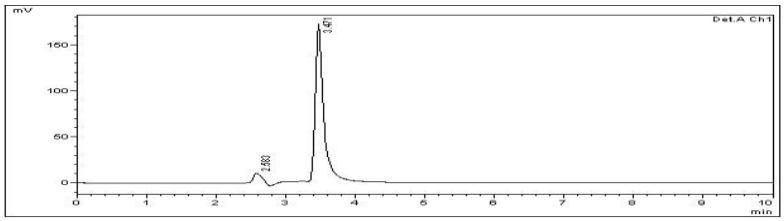

Fig-7 Chromatogram of 120% spike

Table-3 Results of accuracy for RP-HPLC

Sample ID	Concentration(ug/ml)			Area	% recovery	Avg % recovery	S.D	R.S.D
	Amount of pure drug	Amount of sample	Total conc					
80%	2ug/ml	2.5ug/ml		961848	99.9%			
80%	2ug/ml	2.5ug/ml	4.5ug/ml	964643	100.2%	99.9%	2925.53	0.304%
80%	2ug/ml	2.5ug/ml		959531	99.6%			
100%	2.5ug/ml	2.5ug/ml		1062268	99.3%			
100%	2.5ug/ml	2.5ug/ml	5ug/ml	1068529	99.9%	99.9%	6273.53	0.59%
100%	2.5ug/ml	2.5ug/ml		1077559	100.7%			
120%	3ug/ml	2.5ug/ml		1179632	100.2%			
120%	3ug/ml	2.5ug/ml	5.5ug/ml	1172225	99.6%	99.86%	3053.5	0.26%
120%	3ug/ml	2.5ug/ml		1175028	99.9%			

Table-4 Results of accuracy for UV

Sample ID	CONCENTRATION(ug/ml)			Absorbance	% recovery	Avg % recovery	S.D	R.S.D
	Amount of pure drug	Amount of sample	Total Conc					
80%	2.4ug/ml	3ug/ml		0.6966	99.6%			
80%	2.4ug/ml	3ug/ml	5.4ug/ml	0.6943	99.3%	99.4%	0.001159	0.17%
80%	2.4ug/ml	3ug/ml		0.6957	99.5%			
100%	3ug/ml	3ug/ml		0.7711	99.1%			
100%	3ug/ml	3ug/ml	6ug/ml	0.7693	99.0%	99.1%	0.00074	0.12%
100%	3ug/ml	3ug/ml		0.7699	99.1%			
120%	3.6ug/ml	3ug/ml		0.8492	99.4%			
120%	3.6ug/ml	3ug/ml	6.6ug/ml	0.8488	99.3%	99.3 %	0.0006658	0.08%
120%	3.6ug/ml	3ug/ml		0.8479	99.2%			

PRECISION

A. Repetability: The chromatogram of repeatability is shown in fig-8.The results of repetability were shown in table 5&6 for RP-HPLC and UV

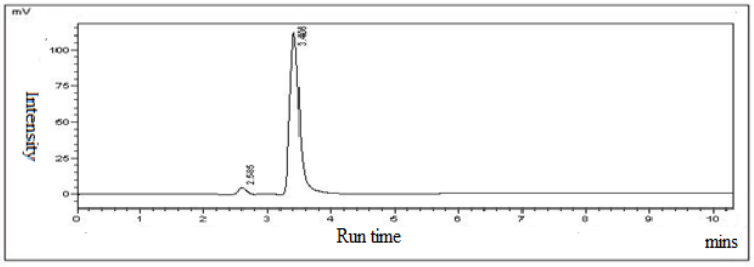

Fig-8 Chromatogram of repeatability

Table-5 Results of repeatability

Conc	Area	%Assay
5ug/ml	1042976	99.8%
5ug/ml	1054154	101.04%
5ug/ml	1044961	100.08%
5ug/ml	1047695	100.36%
5ug/ml	1046150	100.21%
5ug/ml	1039386	99.5%
Mean	**1045887**	**100.16**
S.D	**4965.39**	**0.527**
R.S.D	**0.47%**	**0.53%**

Table -6 Results of repeatability for UV

Conc	Area
6ug/ml	0.7457
6ug/ml	0.7416
6ug/ml	0.7699
6ug/ml	0.7566
6ug/ml	0.7521
6ug/ml	0.7632
Mean	**0.7548**
S.D	**0.00899**
R.S.D	**1.19%**

B. Interday precision:

Chromatogram for interday precision is shown in fig-7.the results of interday precision were shown in table-7&8 for RP-HPLC and UV.

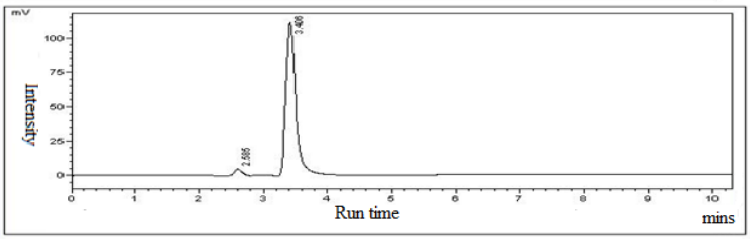

Fig -9 Chromatogram of interday precision

Table-7 results of interday precision for RP-HPLC

S.NO	Conc	Area	% Assay
1	5ug/ml	1057620	101.4%
2	5ug/ml	1054154	101.04%
3	5ug/ml	1054962	101.04%
4	5ug/ml	1057343	101.3%
5	5ug/ml	1057279	101.3%
6	5ug/ml	1057588	101.4%
	Mean	**1056491**	**101.24**
	S.D	**1391.89**	**0.166**
	R.S.D	**0.13%**	**0.16%**

Table-8 results of interday precision for RP-HPLC

Conc	Area
6ug/ml	0.7457
6ug/ml	0.7416
6ug/ml	0.7699
6ug/ml	0.7566
6ug/ml	0.7521
6ug/ml	0.7632
Mean	**0.7548**
S.D	**0.00899**
R.S.D	**1.19%**

System suitability: The chromatogram of system suitability is shown in fig-10 the results are shown in table-9

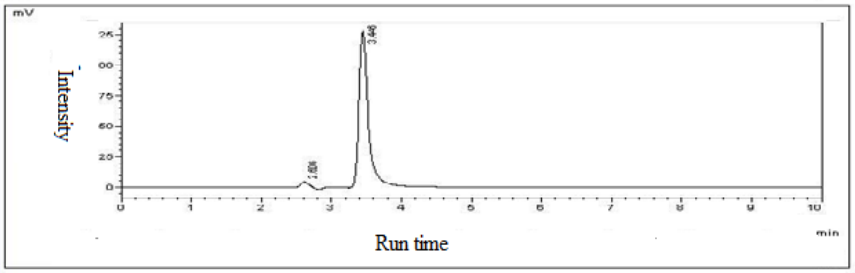

Fig 10 Chromatogram of system suitability

Table- 9 Results of system suitability for moxifloxacin

s.no	Conc	R.T	Area	Theorectical plate	Tailing factor
1.	5ug/ml	3.44	1069543	3604.04	1.37
2.	5ug/ml	3.44	1069126	3617.68	1.41
3.	5ug/ml	3.44	1067798	3604.04	1.38
4.	5ug/ml	3.44	1068475	2780.11	1.26
5.	5ug/ml	3.45	1065600	2850.07	1.25
6.	5ug/ml	3.44	1074479	3605.09	1.39
	Mean		**1068161.33**		
	S.D		**1271.51**		
	R.S.D		**0.12%**		

Specificity :

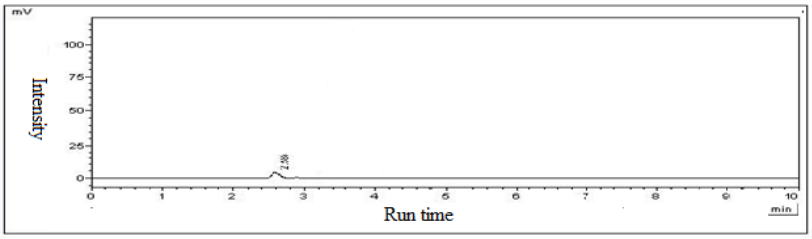

Fig -11Chromatogram of blank

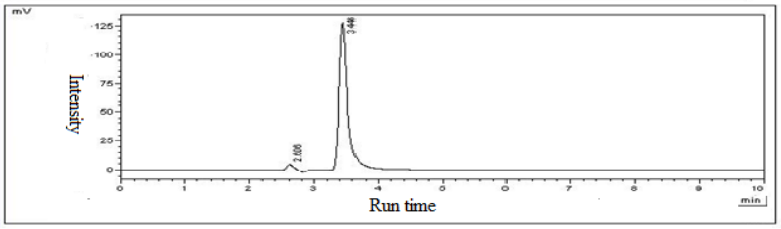

Fig -12 Chromatogram of standard 5 ug/ml conc

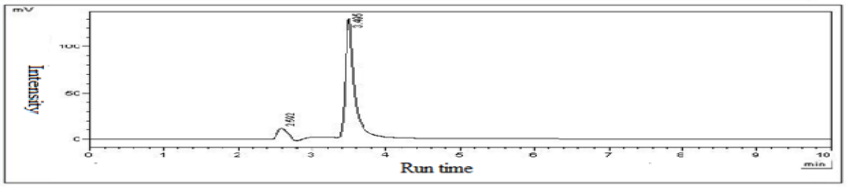

Fig -13 Chromatogram of MOXI sample

Robustness: ***Change in flow rate (± 0.1 ml/min):*** A study was conducted to determine the effect of variation in flow rate. The system suitability parameters were evaluated at the flow rate of 1.1 ml/min. and 0.9 ml/min. Chromatograms were recorded and listed below

Flow rate -1.1ml/min:

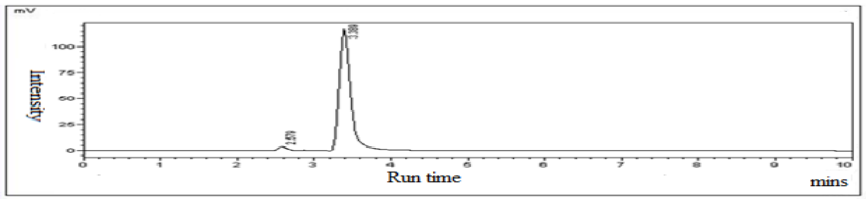

Fig- 14 Chromatogram of flow rate-1.1ml/min

Flow rate -0.9ml/min :

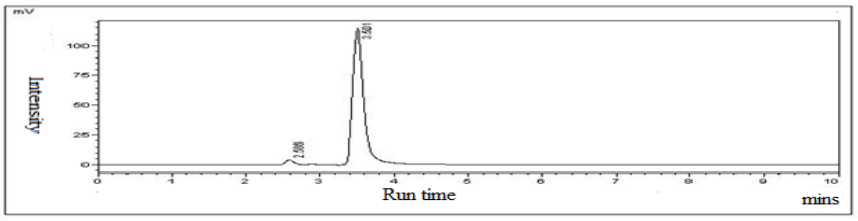

Fig -15 Chromatogram of flow rate -0.9ml/min

Table– 10 Results for change in flow rate

Parameter	**Flow rate -0.9ml/min**				**Flow rate-1.1ml/min**			
s.no	Conc	RT	Area	%Assay	**Conc**	**RT**	**Area**	**%Assay**
1	5ug/ml	3.51	1045043	100.6%	5ug/ml	3.39	1049403	101%
2	5ug/ml	3.56	1030563	98.9%	5ug/ml	3.38	1046787	100.6%
3	5ug/ml	3.56	1026365	98.6%	5ug/ml	3.37	1041089	100.1%
Mean		3.543	1033990.33	99.3%		**3.38**	**1045759.67**	**100.5%**
S.D		**0.0288**	**8001.12**	**1.078**		**0.01**	**3471.04**	**0.45**
%R.S.D		**0.81%**	**0.77%**	**1.09%**		**0.30%**	**0.33%**	**0.45%**

Change in Mobile phase ratio (± 2%)

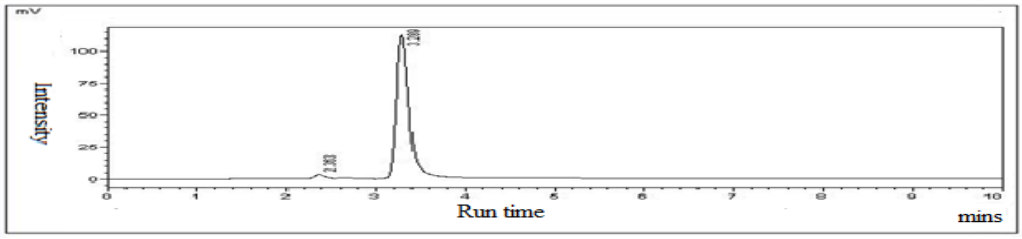

Fig -16 Chromatogram of mobilephase ratio(33:67)

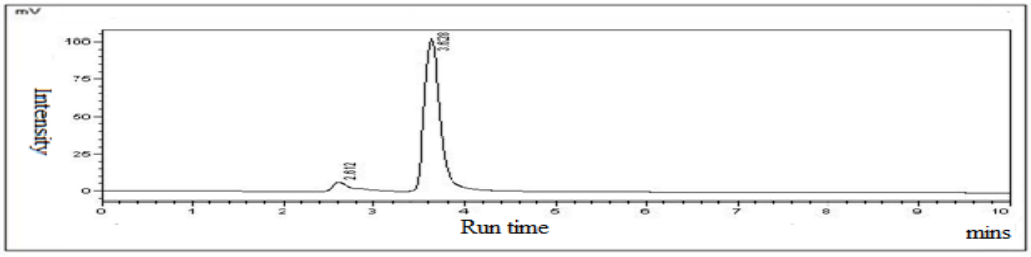

Fig -17 Chromatogram of mobile phase ratio (37:63)

Table -11 Results of change in ratio of mobile phase

Parameters	**Ratio -buffer : Methanol(67:33)**				**Ratio –buffer: Methanol(63:37)**			
S.No	Conc	RT	Area	%Assay	**Conc**	**RT**	**Area**	**%Assay**
1	5ug/ml	3.28	1047430	100.77%	5ug/ml	3.62	1047586	100.77%
2	5ug/ml	3.28	1041560	100.14%	5ug/ml	3.62	1050153	101.04%
3	5ug/ml	3.28	1049442	100.96%	5ug/ml	3.63	1044665	100.47%
Mean		3.543	1046144	100.62%		**3.38**	**1047468**	**100.5%**
S.D		**0.028**	**3343.84**	**1.078**		**0.01**	**2242.02**	**0.45**
%R.S.D		**0.81%**	**0.32%**	**1.09%**		**0.30**	**0.21%**	**0.45%**

Change in pH (± 0.2)

pH of Buffer: methanol– 3.8

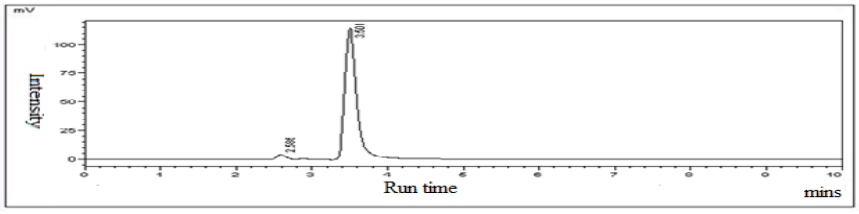

Fig -18 Chromatogram of pH -3.8

pH of Buffer: methanol – 4.2

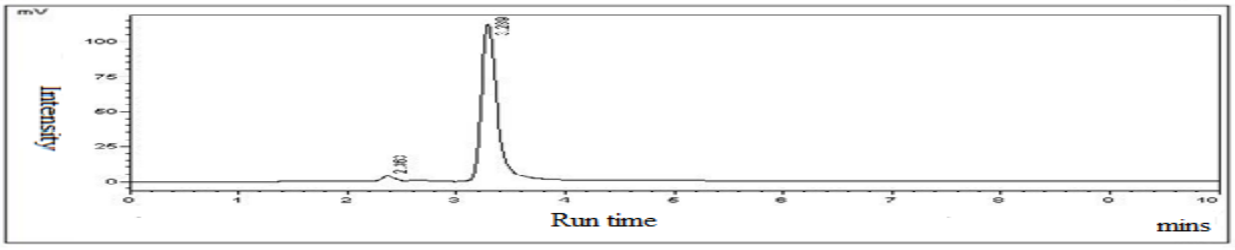

Fig -19 Chromatogram of pH-4.2

Table- 12 Results of change in pH

Parameter	pH -3.8				pH-4.2			
	Conc	**RT**	**Area**	**%Assay**	**Conc**	**RT**	**Area**	**%Assay**
1	5ug/ml	3.51	1052381	101.2%	5ug/ml	3.28	1046492	100.66%
2	5ug/ml	3.52	1043548	100.3%	5ug/ml	3.3	1042976	100.19%
3	5ug/ml	3.50	1040142	99.99%	5ug/ml	3.29	1040530	100.03%
Mean			**1045357**	**100.46%**		**3.47**	**1043332.67**	**100.2%**
S.D			**5157.68**	**0.513**		**0.023**	**2447.01**	**0.29**
%R.S.D			**0.49%**	**0.51%**		**0.66%**	**0.23%**	**0.079%**

Limit of Detection and Limit of Quantification for RP-HPLC:

LOD and LOQ of drug were calculated using the following equations designated by international Conference on Harmonization (ICH)guidelines.

LOD =3.3 x σ/S

LOQ= 10 x σ/S

Where σ is the standard deviation of response

S is slope of the calibration curve

Table –13 Results of LOD and LOQ

Sample	Intercept	Slope	LOD	LOQ
MOXIFLOXACIN	83871	195961	3.3 x σ/S= 3.3x3823.18/196613 =0.064ug/ml	10 x σ/S = 10x3823.18/196613 =0.19ug/ml
	77129	197258		
	77376	196620		
Mean	**79458.6**	**196613**		
S.D	**3823.18**			

Table-14 Results of LOD and LOQ OF UV

Sample	Intercept	Slope	LOD	LOQ
MOXIFLOXACIN	0.052	0.137	3.3 x σ/S= 3.3x0.00917/0.136 =0.22ug/ml	10 x σ/S = 10x0.00917/0.136 =0.67ug/ml
	0.034	0.134		
	0.046	0.137		
Mean	**0.044**	**0.136**		
S.D	**0.00917**			

CONCLUSION

Development of methods to achieve the final goal of ensuring the quantity of drug substances and drug products is not easy task . Hence they can be regarded as simple, specific and sensitive methods for the estimation of Moxifloxacin HCl in tablet dosage forms. The proposed & validated RP-HPLC method is highly sensitive, reproducible, reliable, rapid, robust and specific. The developed method is validated as per the International Conference on Harmonisation **ICH** (Q2B) Guidelines, and were found to be applicable for routine Quantitative analysis of Moxifloxacin HCl by RP-HPLC using UV detector in tablet dosage forms. The developed and validated RP-HPLC method was found to be better than previous methods because of short analytical run time of 10.0 minutes leading to an environmental friendly chromatographic procedure that allows the analysis of a large number of samples in a

short period of time and this method has been found to be better than previously reported methods, due to its high lack of extraction procedures. Hence above method can be used in quality control for routine analysis of tablets of Moxifloxacin HCl without any interference

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