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Development and validation of a stability indicating UPLC method for determination of moxifloxacin hydrochloride in pharmaceutical formulations

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ABSTRACT

Simple, rapid, sensitive, accurate, robust & rugged stability indicating analytical method for determination of voriconazole in pharmaceutical formulations is developed and validated by using UPLC & applied the developed and validated method for determining the assay of Moxifloxacin HCl in tablets (Avelox®), as there is no official monograph & no analytical method by UPLC. Chromatography was performed with mobile phase containing potassium dihydrogen ortho phosphate (adjusted to pH 1.8 with orthophosphoric acid), Methanol & acetonitrile in the ratio of 60:20:20, with a flow rate of 0.3mL/min, C-18 column & UV detection at 296nm.The method was validated for linearity, accuracy, ruggedness, robustness, precision & bench top stability of sample & standard solution. Moxifloxacin tablets were subjected to different stress conditions like acid, alkali, peroxide, thermal, water & UV studies and checked for its specificity, degradation & stability. The developed method was very rapid with a run time of 3 min, accurate, robust, rugged and stable.

KEYWORDS: *Moxifloxacin, Assay method, UPLC, Stability indicating method.*

INTRODUCTION

Ultra performance liquid chromatography TM (UPLC) takes advantage of technological strides made in particle chemistry performance, system optimization, detector design, and data processing and control. Using sub-2 mm particles and mobile phases at high linear velocities, and instrumentation that operates at higher pressures than those used in HPLC, dramatic increases in resolution, sensitivity, and speed of analysis can be obtained. This new category of analytical separation science retains the practicality and principles of HPLC while creating a step function improvement in chromatographic performance.[1]

According to an FDA guidance document, a stability-indicating method is "a validated quantitative analytical procedure that can detect the changes with time in the pertinent properties of the drug substance and drug product. A stability-indicating method accurately measures the active ingredients, without interference from degradation products, process impurities, excipients, or other potential impurities."[2]

Moxifloxacin is slightly yellow crystalline mono-hydrochloride salt.[3] Moxifloxacin Hydrochloride is designated chemically as ((1'S,6'S)-1-Cyclopropyl-7-(2,8-diazabicyclo[4.3.0]non-8-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride with an empirical formula of $C_{21}H_{24}FN_3O_4 \cdot HCl$ and a molecular weight of 437.90.(**Fig.1**)[4].

Moxifloxacin can be used to treat respiratory infections, including acute sinusitis, acute exacerbations of chronic bronchitis, and community-acquired pneumonia, as well as skin and skin structure infections. Moxifloxacin is also used for the treatment of complicated intra-abdominal infections. [5]. Moxifloxacin inhibits bacterial topoisomerase II (DNA gyrase) and topoisomerase IV. Topoisomerases are essential enzymes which play a crucial role in the replication

and repair of bacterial DNA. This mechanism is lethal to susceptible bacteria. Moxifloxacin is often referred to as a chemotherapeutic drug because its mode of action has so far not been noted in any naturally occurring or semi-synthetic antibiotic.A few methods for the determination of Moxifloxacin Hydrochloride in pharmaceutical formulations by HPLC,
HPTLC and UV appear in literature. So far no systematic UPLC method has been reported for determination of Moxifloxacin Hydrochloride in pharmaceutical formulations. This paper reports a rapid and sensitive UPLC method with UV detection, useful for routine quality control of Moxifloxacin Hydrochloride in pharmaceutical formulations. The method was validated by parameters such as linearity, accuracy, precision, robustness, ruggedness, sample and standard solution stability and forced degradation studies.

EXPERIMENTAL

Reagents

HPLC grade Acetonitrile (HPLC Grade, Merck), Potassium dihydrogen orthophosphate (AR, Rankem), Hydrochloric Acid (AR, Rankem) Sodium hydroxide (AR, Rankem), Hydrogen peroxide (AR, Rankem), Ortho phosphoric acid (AR,Rankem),Water (Milli Q water), Acetonitrile (HPLC Grade, Merck). Moxifloxacin pure drug substance was kindly supplied by MSN Laboratories Limited, India. Ingredients used for placebo were Microcrystalline cellulose, croscarmellose sodium, PVPK-30, Ethanol, Magnesium stearate.

Instrumentation

A liquid chromatograph (Waters Acquity) system equipped with an injection valve (Rheodyne), & PDA detector. The UPLC system was well equipped with Empower 2 software for data processing. Other instruments like Sartorius Analytical Balance, Metrohm pH Meter and Biotechnics sonicator were used in sample and standard preparations and for forced degradation studies.

METHODOLOGY

Chromatographic conditions:

The analytical column used was Waters HSS, C-18, 100X2.1; 1.8µm .The mobile phase was potassium dihydrogen ortho phosphate, adjusted to pH 1.8 with ortho phosphoric acid, methanol & acetonitrile in the ratio of 60:20:20. It has a flow rate of 0.3mL/min, injection volume of 1µL with ambient column oven temperature and sample tray temperature with isocratic elution & UV detection at 296nm & a run time of 3 min.

Standard, sample, mobile phase and diluent preparation:

Diluent: Mobile phase is used as diluent.

Preparation of mobile phase:

Dissolved 3.4g of potassium dihydrogen ortho phosphate in one litre water and adjusted the pH to 1.8 with ortho phosphoric acid.Filtered through 0.22µ membrane filter.Mixed the buffer,acetonitrile and methanol in the ratio of 60:20:20 and sonicated to degas.

Preparation of standard solution:

Accurately weighed and transferred 44mg of Moxifloxacin HCl in to a 100mL volumetric flask and added 70mL of diluent.Sonicated for 5 min and made up to the mark with diluent.Transferred 5mL of above solution to 20mL volumetric flask and made up to volume with diluent.Filtered with 0.45µm PFTE filter.

Preparation of Test solution:

Weighed 20 tablets and determined the average weight.Weighed 2 tablets and transferred in to a 200mL volumetric flask and added 150mL of diluent.Sonicated in cold water for 20minutes with intermittent shaking.Allowed it to cool to room temperature and diluted to volume with diluent.Filtered atleast 12mL of the above solution with 0.45µm PTFE filter and transfered 5mL of filtered solution to 200mL volumetric flask and made up to volume with diluent.

RESULTS & DISCUSSION

Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. [6]
Specificity was demonstrated by injecting a blank, placebo and standard solution. No interference was seen at the retention time of analyte. The

specificity was also demonstrated by induced degradation of Moxifloxacin formulation and placebo samples to acid degradation, alkali degradation, peroxide degradation, thermal degradation, water degradation, U.V. degradation. Purity angle is less than purity threshold for all the stress conditions. The results are tabulated in **Table 1.Figures 2-15** represents different stress conditions.

Table-1

Moxifloxacin Forced Degradation		
Stress Condition	**Purity Angle**	**Purity Threshold**
Acid Stress	**0.120**	**0.271**
Alkali Stress	**0.124**	**0.274**
Peroxide Stress	**0.138**	**0.297**
Water Stress	**0.140**	**0.277**
Heat Stress	**0.118**	**0.272**
U.V. Stress	**0.170**	**0.278**
Acceptance Criteria	**Peak Purity shall pass**	

Fig. No.1: Moxifloxacin Hydrochloride

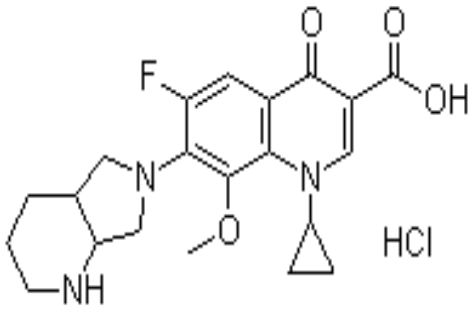


Fig. No.2: Blank-Diluent

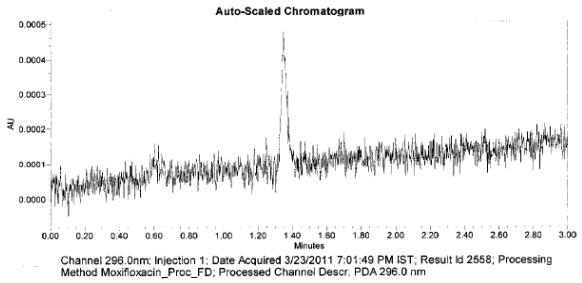


Fig.No.3: Standard

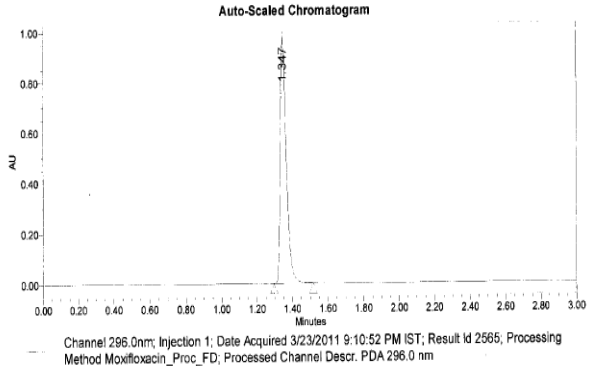


Fig. No.4: Acid Stressed Placebo Solution

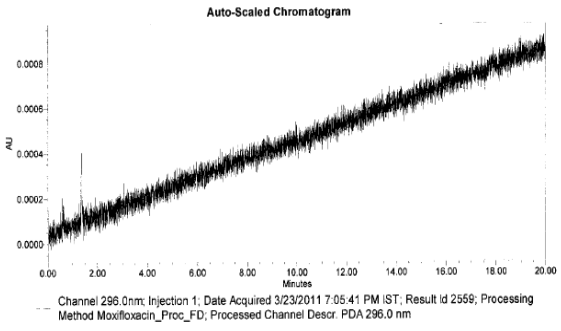


Fig.No.5: Alkali Stressed Placebo

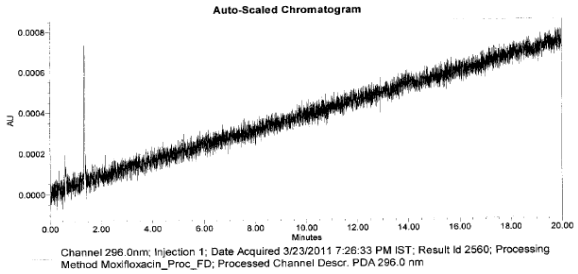


Fig.No.6: Peroxide Stressed Placebo

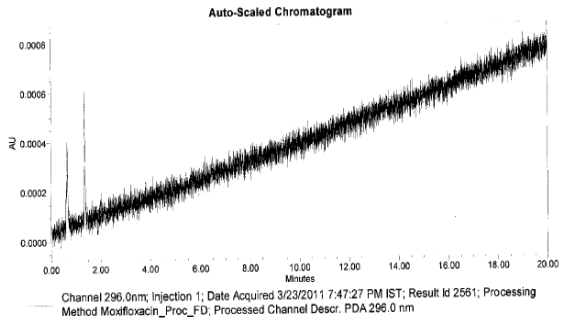


Fig.No.7: Water Stressed Placebo

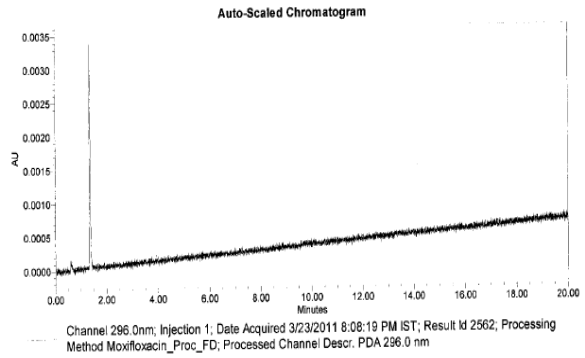


Fig.No.8: Heat Stressed Placebo

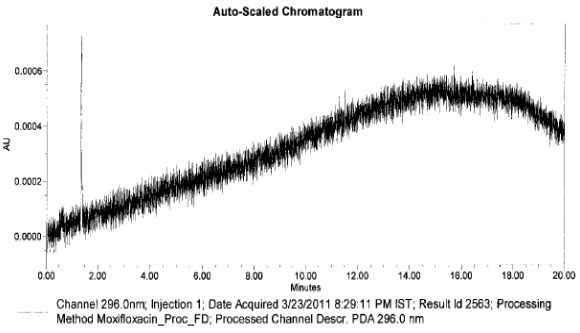


Fig.No.9: UV Stressed Placebo

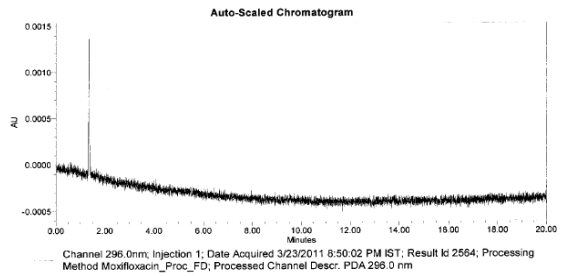


Fig.No.10: Acid Stressed Sample

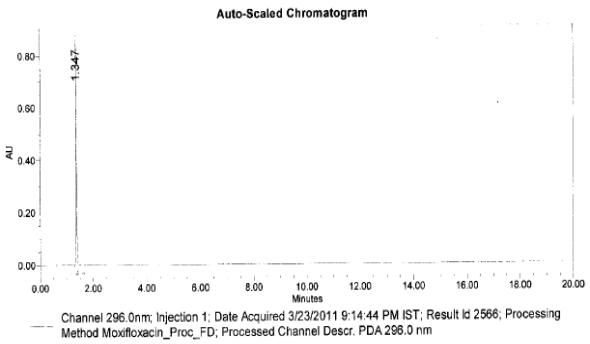


Fig.No.11: Alkali Stressed Sample

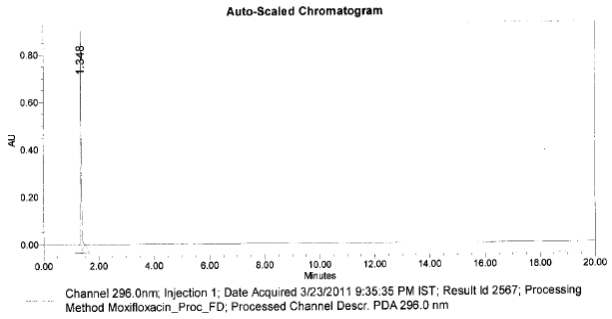


Fig.No.12: Peroxide Stressed Sample

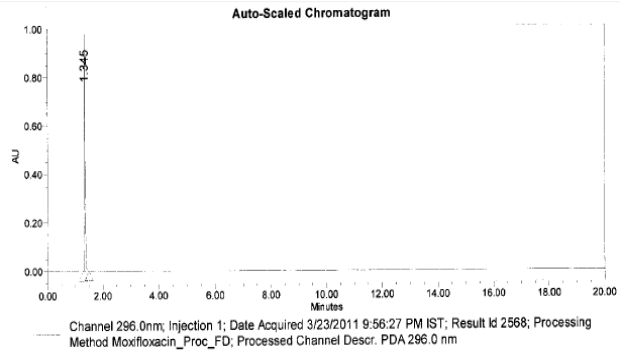


Fig.No.13: Water Stressed Sample

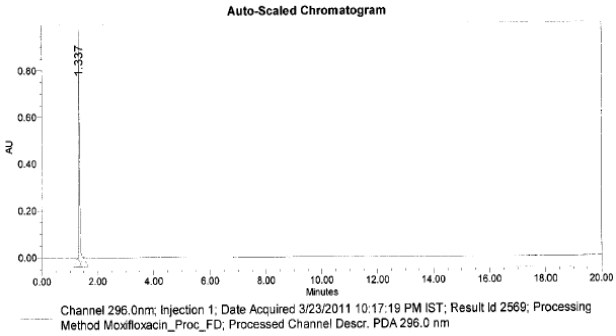


Fig.No.14: Heat Stressed Sample

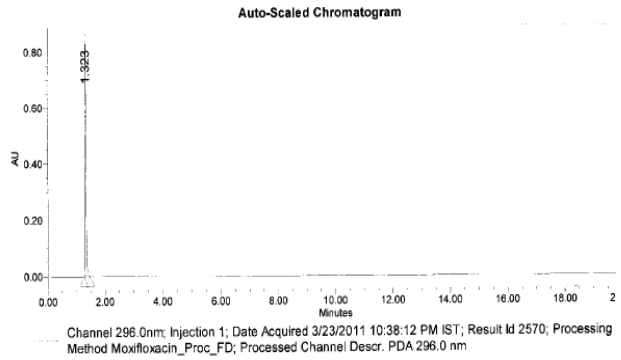


Fig.No.15: UV Stressed Sample

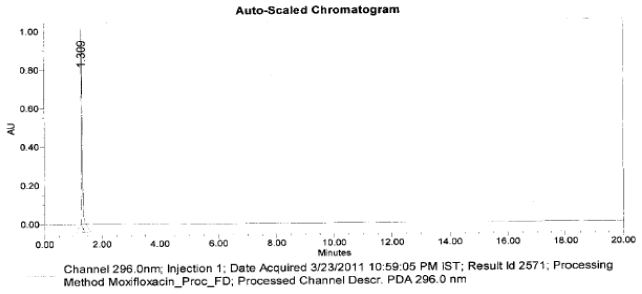
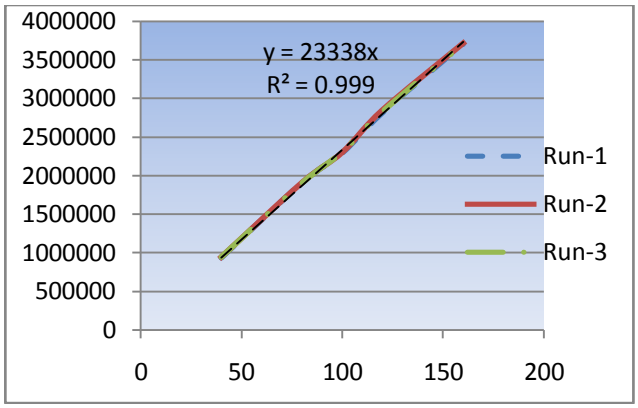


Fig.No.16: Peroxide Stressed Sample



System suitability Testing:

System suitability testing is used to verify that the reproducibility of the system is adequate for the analysis to be performed. System suitability is done by preparing and injecting the standard solution 5 times and calculating its RSD. Other parameters like tailing and theoretical plates should also be taken in to consideration. Results are tabulated in **Table No.:2**

Table-2

Moxifloxacin System Suitability

Injection No.:	1	2	3	4	5	Mean	STDEV	RSD	***Limits***
Standard Area:	2305687	2302824	2311478	2300543	2283295	2300765	10589	0.5	**RSD NMT 2.0%**
Theoretical Plates	7818	7835	7825	7826	7829	7827	6.19	0.1	**NLT 2000**
USP tailing	1.54	1.54	1.54	1.54	1.53	1.54	0.00	0.3	***NMT 2.0***
RT	1.259	1.260	1.263	1.265	1.267	1.263	0.00	0.3	

Linearity:

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample [6]. The linearity of the test method was performed by plotting a graph between concentration of the test solution on X-axis and response of the corresponding solutions on Y-axis from 40% to 160% of test concentration and calculated the correlation coefficient, it was found to be 0.999.The results are tabulated in **Table No.3** and the graphs are represented as **Fig No.16**.

Moxifloxacin-Linearity

Run	% Conc.	Conc. Of Moxifloxacin	Area of Moxifloxacin	Slope	Y-intercept	R^2
1	40%	40.02	937722	23059.4	27156.504	0.999
	80%	80.04	1908256			
	100%	100.05	2295800			
	120%	120.05	2819056			
	160%	160.07	3709937			
2	40%	40.02	942173	23184.9	25399.4381	0.999
	80%	80.04	1908189			
	100%	100.05	2301865			
	120%	120.05	2852614			
	160%	160.07	3719921			
3	40%	40.02	943469	23070.2	31121.98583	0.999
	80%	80.04	1902911			
	100%	100.05	2306901			
	120%	120.05	2831549			
	160%	160.07	3711182			
Average				23104.80869	27892.64264	0.999
Standard Deviation				69.54	2931.44	0.00
Acceptance criteria: Coefficient of correlation shall be NLT 0.999						

Limit of detection (LOD) and limit of quantification (LOQ):

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy [6]. Calculated the LOD & LOQ, with the calculations obtained from evaluation of the calibration curve of the linearity. LOD and LOQ values are less than the minimum linearity concentration.

The calculations and results are tabulated in **Table. No.4**

Bench top stability of standard & test preparation:

Performed the assay of Moxifloxacin as per the test method in duplicate and kept the standard and test solutions on the bench top for 48 Hrs. Injected at initial, 24 Hrs and 48 Hrs. Calculated the difference between initial and bench top stability samples for % assay of Moxifloxacin for test solutions and similarity factor for standard solutions were found to be within limits. The results are tabulated in **Table No.5**

Table-4

Moxifloxacin- Limit of detection (LOD) & Limit of Quantification (LOQ)				
S.No.	Injection No.	Slope	Y-Intercept	R^2
1	Inj-1	23059.4	27156.504	0.999
2	Inj-2	23184.9	25399.4381	0.999
3	Inj-3	23070.2	31121.98583	0.998
Average		23104.8333	27892.6426	0.9987
STDEV		69.550	2931.435	0.001
LOD=3.3 x σ/S				
LOD	0.4	Ppm		
LOQ=10 x σ/S				
σ = Standard deviation of y-intercepts of regression line				
S= slope of the linearity curve				
LOQ	1.3	Ppm		
Acceptance Criteria: LOD & LOQ values shall be less than the minimum linearity concentration				

Table-5

Moxifloxacin Bench Top Stability of Standard Solution						
Time(Hrs)	Day	Std. Wt.	Response	Fresh Std Wt.	Response of fresh std.	Similarity Factor
Initial	Initial	44.02	2300765			
24 Hrs	Day-1	44.02	2311082	44.13	2316978	1
48 Hrs	Day-2	44.02	229288	43.89	2268919	0.99
Acceptance Limits: Similarity Factor should be NMT 2.0						
Moxifloxacin Bench Top Stability of Test Solution-1						
Time(Hrs)	Day	Weight(mg)	Response of sample	% Assay	Difference from Initial	Difference in Assay results of Initial,24 & 48 Hrs shall be NMT 2.0
Initial	Initial	1353.34	2337254	101.29	NA	
24 Hrs	Day-1	1353.34	2331881	100.6	0.7	
48 Hrs	Day-2	1353.34	2305445	101.01	0.3	
Moxifloxacin Bench Top Stability of Test Solution-2						
Time(Hrs)	Day	Weight(mg)	Response of sample	% Assay	Difference from Initial	Difference in Assay results of Initial,24 & 48 Hrs shall be NMT 2.0
Initial	Initial	1351.89	2321427	100.6	NA	
24 Hrs	Day-1	1351.89	2320794	100.12	0.5	
48 Hrs	Day-2	1351.89	2327728	101.99	1.4	

Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found[6]. Performed the accuracy of test method using Moxifloxacin placebo at 50%, 70%, 100%, 125%, 150% spike levels. The % assay at each spike level was found to be between 98.0-102.0% of the labeled amount. The results are tabulated in **Table No.6**

Table No.6:

Moxifloxacin-Accuracy

Spike level	Wt. of sample taken in mg	Sample area	mg/mL added	mg/mL found	% Recovery	Average
50%_01	674.46	1159290	0.04996	0.05454	100.1	100.1
50%_02	672.90	1155954	0.04984	0.05438	100.1	
50%_03	673.11	1158198	0.04986	0.05449	100.2	
70%_01	1018.65	1753515	0.07545	0.08249	100.3	100.0
70%_02	1018.42	1746671	0.07544	0.08217	99.9	
70%_03	1016.46	1744562	0.07529	0.08207	100.0	
100%_01	1349.09	2292178	0.09993	0.10783	98.9	98.6
100%_02	1348.20	2281190	0.09987	0.10732	98.5	
100%_03	1347.63	2272375	0.09982	0.10690	98.2	
125%_01	1686.17	2867979	0.1249	0.13492	99.1	98.9
125%_02	1685.31	2856118	0.12484	0.13436	98.7	
125%_03	1685.91	2866778	0.12488	0.13487	99.0	
150%_01	2015.68	3400552	0.14931	0.15998	98.3	98.2
150%_02	2023.69	3406155	0.1499	0.16024	98.0	
150%_03	2021.14	3411601	0.14971	0.16050	98.3	
Acceptance criteria:% Average recovery shall be between 98.0% -102.0%						

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. [6]

Method precision:

Determined the precision of the test method by preparing & injecting 6 test solutions of Moxifloxacin formulations in to the chromatograph and recorded the results. The average % assay was found to be 100.4 with % RSD of 0.62. The results are tabulated in **Table No.7**

Intermediate precision:

Performed the assay of Moxifloxacin by following the same procedure as that of Method precision but on a different day and by a different analyst. The average % assay was found to be 99.4% with % RSD of 0.39.Overall RSD when compared with Method precision is 0.73. The results are tabulated in **Table No.8&9**

Table-7

Moxifloxacin Analytical Method Validation-Assay								
Method Parameter			Method Precision					
Std. wt. & Dilution	44.02	5	Tablet Wt.	Spl. wt. & Dilution	Wt. of sample taken	5	Label claim (mg)	400
	100	20	675.01		200	200	Potency (%)	98.8
Molecular factor for Moxifloxacin				0.917				
Std. No.	Standards	USP Tailing	Weight of sample taken	Area of sample	Assay %	Average (%)	STDEV	% RSD
1	2310915	1.54	1353.34	2337254	101.04	100.4	0.61837	0.62
2	2290693	1.54	1351.89	2321427	100.46			
3	2300684	1.54	1358.15	2317128	99.81			
4	2300777	1.54	1353.97	2341249	101.16			
5	2300755	1.54	1355.02	2324067	100.34			
			1356.39	2310208	99.64			
Average	2300765	1.54	1354.79	2325222	100.41			
STDEV	7149.73	0.00	Limits	% RSD of 6 replicate injections is not more than 2				
%RSD	0.31	0.0						

Table-8

Moxifloxacin Analytical Method Validation-Assay								
Method Parameter			Intermediate Precision					
Std. wt. & Dilution	44.13	5	Tablet Wt.	Sample wt. & Dilution	Wt. of sample taken	5	Label claim (mg)	400
	100	20	675.01		200	200	Potency(%)	98.8
Molecular factor for Moxifloxacin				0.917				
Std. No.	Standards	USP Tailing	Wt. of sample taken	Area of sample	Assay %	Average (%)	STDEV	% RSD
1	2315498	1.52	1351.91	2303175	99.22	99.4	0.388	0.39
2	2302693	1.52	1360.40	2318575	99.26			
3	2314434	1.52	1355.75	2314650	99.43			
4	2321577	1.52	1353.39	2305262	99.20			
5	2330688	1.52	1352.51	2325271	100.13			
6			1356.55	2306776	99.03			
Average	2316978	2	1355	2312285	99.38			
STDEV	10269.35	0.00	Limits	% RSD of 6 replicate injections is not more than 2				
%RSD	0.4	0.0						

Table-9

Moxifloxacin Analytical Method Validation-Assay							
Method Parameter		Method & Intermediate Precision combined					
Method Precision		**Intermediate Precision**					
S.No.	**% Drug content**	**S. No.**	**% Drug content**	**Difference**	**Average of both Method & Intermediate precision**	**STDEV of both Method & Intermediate precision**	%RSD of both Method & Intermediate precision
1	**101.04**	**1**	**99.2**	**1.8**	**99.9**	**0.730**	0.73
2	**100.46**	**2**	**99.3**	**1.2**			
3	**99.81**	**3**	**99.4**	**0.4**			
4	**101.16**	**4**	**99.2**	**2.0**			
5	**100.34**	**5**	**100.1**	**0.2**			
6	**99.64**	**6**	**99.0**	**0.6**			
Limits: Overall RSD when compared with Method precision should be not more than 2%.							

Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage [6]. Robustness was performed by injecting the Moxifloxacin standard solution in to the UPLC by altering the Flow rate, Column oven temperature and also by changing the pH of the buffer & composition of the organic solvent from the normal chromatographic conditions. The results are tabulated in **Table No.10**

Table-10

Moxifloxacin Analytical Method Validation-Assay					
Method Parameter			**Robustness**		
Change in Flow Rate(0.25mL/min)			**Change in Flow Rate(0.35mL/min)**		
Std. No.	**Standards**	**USP Tailing**	**Std. No.**	**Standards**	**USP Tailing**
1	2743760	1.55	1	1973875	1.49
2	2774673	1.55	2	1943344	1.49
3	2740829	1.55	3	1960245	1.49
4	2732432	1.55	4	1952056	1.49
5	2734277	1.55	5	1958542	1.49
Average	2745194	1.55	**Average**	1957612	1.49
STDEV	17118.49	0.00	**STDEV**	11255.31	0.00
%RSD	0.62	0.0	**%RSD**	0.57	0.0
Change in pH of Mobile Phase(1.6)			**Change in pH of Mobile Phase(2.0)**		
Std. No.	**Standards**	**USP Tailing**	**Std. No.**	**Standards**	**USP Tailing**
1	2271424	1.49	1	2263481	1.53

2	2252217	1.49	2	2258739	1.53
3	2249439	1.49	3	2276006	1.53
4	2244184	1.49	4	2272593	1.53
5	2241573	1.48	5	2276184	1.53
Average	2251767	1.49	**Average**	2269401	1.53
STDEV	11762.64	0.00	**STDEV**	7882.71	0.00
%RSD	0.52	0.3	**%RSD**	0.35	0.0
Change in Org Phase Composition (90%)			**Change in Org Phase Composition (110%)**		
Std. No.	**Standards**	**USP Tailing**	**Std. No.**	**Standards**	**USP Tailing**
1	2311223	1.43	1	2265737	1.53
2	2313683	1.43	2	2269570	1.53
3	2305552	1.43	3	2290266	1.53
4	2315524	1.43	4	2291368	1.53
5	2306395	1.43	5	2290691	1.53
Average	2310475	1.43	**Average**	2281526	1.53
STDEV	4393.90	0.00	**STDEV**	12742.53	0.00
%RSD	0.19	0.0	**%RSD**	0.56	0.00

Calculation:

%Assay:

$$\frac{At}{As} \times \frac{Ws}{100} \times \frac{5}{20} \times \frac{200}{Wt} \times \frac{200}{5} \times \frac{P}{100} \times \frac{100}{L} \times 100 \times MF =$$

Where

At=Area of test solution
As=Area of standard solution
Ws=Weight of standard taken
Wt=Weight of two tablets
P=Potency of Moxifloxacin HCl Working Std.on as is basis
Avg. Wt. =Avg. Wt. of 20 tablets
LC=Label claim of the tablet as Moxifloxacin
MF=Molecular Factor for Moxifloxacin (0.917)

CONCLUSION

The reported UPLC method was proved to be simple, rapid with a runtime of 3 min & reproducible. The validation data indicates good specificity, precision, accuracy & reliability of the method. The developed method has many advantages like isocratic mode of elution, easy sample preparation, short run time and can be used for routine quality control analysis of Moxifloxacin formulations.

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