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Research Article

STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF MOXIFLOXACIN

Raghabendra Narayan Singh*, Shisir Sahoo, Umashankar Mishra, Bamakanta Garnaik, Sudhir Kumar Sahoo and Deepak Hati

Laboratories of Royal College of Pharmacy and Health Sciences, Berhampur-760 002, Orissa, India.

ABSTRACT

A stability indicating high performance liquid chromatographic method has been developed for the determination of moxifloxacin. Optimum separation was achieved in less than 10 minutes using Phenomenex ODS C_{18} (250x 4.6mm packed with 5µ) column. The analyte was resolved by using a mobile phase 20 mmol L^{-1} ammonium formate and acetonitrile (70:30) pH adjusted to 4.0 with formic acid at flow rate 1 ml/min on a isocratic high performance liquid chromatographic system at a wavelength of 295 nm. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision, ruggedness and robustness and can be successfully applied for determination of this drug in commercial tablets. For stress studies the drug was subjected to acid, alkali and neutral hydrolysis, oxidation, dry heat and photolytic degradation. The degradation studies indicated the drug to be susceptible to acid, alkali hydrolysis and oxidative degradation. The analytical conditions and solvent developed provided good resolution within a short analysis time and economic advantages. The proposed method not required highly sophisticated and expensive instrumentation.

Keywords: Moxifloxacin, RP-HPLC, Validation, Stability, Degradation.

INTRODUCTION

Moxifloxacin is the second generation fluoroquinolone antibacterial drug. It was available in market for the treatment of urinary tract infection for many years. Chemically, it is 1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b] pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid[1]. The bactericidal action of Moxifloxacin results from inhibition of DNA gyrase and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair and recombination. The mechanism of action of fluoroquinolones, including moxifloxacin, is different from that of penicillins, cephalosporins, aminoglycosides, macrolides, and tetracyclines; therefore, microorganisms resistant to these classes of drugs may be susceptible to moxifloxacin and other fluoroquinolones. There is no known cross-resistance between moxifloxacin and other classes of antimicrobials. In vitro studies with cytochrome (CYP) P450 enzyme indicate that moxifloxacin does not inhibit 80 CYP3A4, CYP2D6, CYP2C9, CYP2C19, or CYP1A2, suggesting that moxifloxacin is unlikely to alter the pharmacokinetics of drugs metabolized by this enzymes[2-3]. The focus of present study was to develop and validate a rapid, stable and economic RP-HPLC method for the estimation of moxifloxacin in pure.

Several analytical methods for moxifloxacin have been described in scientific literature such as UV spectrophotometry, liquid chromatography etc, amongst others[4-9]. The high performance Liquid Chromatography (HPLC) has become an important tool for the routine determination of anti microbial drugs, with specific emphasis on fluoroquinolones, in various animal products, biological fluida, pharmaceutical products, with emphasis on fluoroquinolones[10-13].

In the literature there are some references about the determination of moxifloxacin using

HPLC methodology. De Smet described a high-performance liquid chromatographic (HPLC) method with fluorescence detection was developed and validated for the simultaneous quantification of moxifloxacin in human plasma[14]. Laban-Djurdjevic et al described simple and rapid RP-HPLC method for the direct determination of moxifloxacin in human plasma is described[15]. Xu et al. described a specific, sensitive and widely applicable high performance liquid chromatography with ultraviolet detection (HPLC-UV) method for the determination of moxifloxacin in human plasma[16]. Hemanth Kumar et al. developed a high performance liquid chromatographic method for determination of moxifloxacin in human plasma[17]. Most of the methods were involve human plasma.

Our investigation involved the optimization of the method described above using a reliable stability indicating and one new development, as wel as validating a simple, sensitive accurate and reproducible HPLC method for the determination of moxifloxacin pharmaceutical dosage form.

MATERIALS AND METHODS

Chemicals & Reagents

Analytically pure Moxifloxacin was obtained as a gift sample from Aurobindo Pharma, Hyderabad, India. Commercial tablet formulations were purchased from the local market. All chemicals and reagents used were of AR/HPLC grade, obtained from Merck, Qualigens and Loba Chemie.

INSTRUMENT

A High Performance Liquid Chromatographic system, with Spinchrom data handling system (Shimadzu-LC 2010) with Analytical Column-Phenomenex ODS C18 (250 X 4.6 mm, 5 micron particle size), equipped with quaternary isocratic pump, 2010C UV-VIS detector in isocratic mode was used for the analysis. Calibrated electronic single pan balance (Sigma 200/A Super), pH Meter (Thermo Fisher scientific), RK 102 CH liter 3,0 Ultrasonicator were also used during the analysis.

Chromatographic conditions

A reverse phase C-18 column was equilibrated with the mobile phase Ammonium formate: Acetonitrile (70:30) and pH adjusted 4.0 with formic acid. Mobile phase flow rate was maintained at 1ml/min and eluents was monitored at 295nm for Moxifloxacin. The sample was injected using a 20 µl fixed loop. The determination was performed at 30^0C for a run time of 10min.

Preparation of mobile phase and Standard Stock Solution

Mobile phase was prepared by mixing 700 ml of 20mM ammonium formate solution with 300 ml of HPLC grade acetonitrile to get the proportion of 70:30 v/v and finally the pH was adjusted to 4.0 with formic acid. The mobile phase was sonicated for 10 minutes and filtered through 0.45µ membrane filter. The standard stock solution of Moxifloxacin was prepared by dissolving 50mg moxifloxacin in 50ml of mobile phase to get a concentration of 1000µg/ml volume was made up to the mark.

Calibration curve for Moxifloxacin

Appropriate aliquots of standard stock solution of the drug was taken in 10 ml volumetric flask and diluted up to the mark with mobile phase in such a way that final concentration of drug was the range of 10-150 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.

Method Validation[18-20]

1. Linearity

Various working standard solutions were prepared and the linearity range was calculated from the observation.

2. Accuracy

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 and by fitting these values to the straight line equation of calibration curve.

3. Precision

The intraday and interday precision study of the drug was carried out by estimating the corresponding responses on the same day and consecutive three days respectively. The results were reported in terms of standard deviation and %RSD.

4. Specificity

The specificity of the proposed RP-HPLC method was determined by complete separation of two peaks with parameters like retention time (R_t), resolution (R_s) and tailing factor (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. Ruggedness
Ruggedness is the degree of reproducibility of the results obtained under a verity of conditions, expressed as %RSD. These conditions include different laboratory conditions and different analysts.

7. 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 (0.1-5 µg/ml) for each drug. The standard deviation of y-intercept of regression line was determined and substituted in the following equation for the determination of detection limit and quantification limit. Detection limit = 3.3 σ/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.

Forced Degradation Studies[18-20]
The specificity of the method can be demonstrated through forced degradation studies conducted on the sample using acid, alkaline, oxidative, thermal, photolytic, and UV degradations. The sample was exposed to these conditions and the main peak was studied for the peak purity, thus indicating that the method effectively separated the degradation products from the pure active ingredient.

1. Degradation in Neutral Condition
About 10mg of pure drug was accurately weighed and taken in a 10ml volumetric flask and dissolved in minimum volume of acetonitrile. Then the volume was made up to the mark with water and kept at 70^{0}C. At different time interval solutions were prepared and 20 µl of the sample solution was injected into the HPLC system.

2. Degradation in Acidic Condition
About 10mg of pure drug was accurately weighed and taken in a 10ml volumetric flask and dissolved in minimum volume of acetonitrile. Then the volume was made up to the mark with 1N HCl and kept at 70^{0}C. At different time interval solutions were prepared and 20 µl of the sample solution was injected into the HPLC system.

3. Degradation in Basic Condition
About 10mg of pure drug was accurately weighed and taken in a 10ml volumetric flask and dissolved in minimum volume of acetonitrile. Then the volume was made up to the mark with 1N NaOH and kept at 70^{0}C. At different time interval solutions were prepared and 20 µl of the sample solution was injected into the HPLC system.

4. Oxidative Degradation
About 10mg of pure drug was accurately weighed and taken in a 10ml volumetric flask and dissolved in minimum volume of acetonitrile. Then the volume was made up to the mark with 6% w/v H2O2 and kept at 70^{0}C. At different time interval solutions were prepared and 20 µl of the sample solution was injected into the HPLC system.

5. Photolytic Degradation
About 100 mg of pure drug was taken in a clean petridish and
exposed to day light. Sampling was done at an interval of 10h, 1week and 2weeks. From this sample, different solutions were prepared and 20 µl of the sample solution was injected into the HPLC system.

6. UV- Degradation
About 100 mg of pure drug was taken in a clean petridish and subjected to UV illumination of 1.2x10^6 lux hours. Sampling was done at an interval of 12h, 24h, and 48h and from this sample, different solutions were prepared and 20µl of the sample solution was injected into the HPLC system.

7. Thermal Degradation
About 100 mg of pure drug was taken in three separate clean
petridishes and subjected to dry heat at 70^{0}C. Sampling was done at intervals of 10 days, 20 days and 30 days. Solutions of the drugs were prepared and 20 µl of the sample solution was injected into the HPLC system.

RESULTS AND DISCUSSION

Calibration Curve
The peak areas for the different concentrations (10-150 µg/ml) were recorded at 295 nm. The calibration curve (Figure 2) and the HPLC chromatogram (Figure 3) is shown in Table 1.

Accuracy
The percentage recovery was found to be in the range of 99.97% to 100.06% as shown in Table 2.

Precision
From Table 3, the %RSD for precision was found to be 0.86% and 0.90%.

Sensitivity
The LOD was found to be 0.35μg/ml and the LOQ was found to be 1.16μg/ml at 295 nm respectively.

Intraday and Interday Assay
The %RSD for Intraday and Interday Assay were found to be 0.33% to 0.53% and 0.60% to 0.93% respectively. Low values of %RSD indicate that the proposed method is accurate. The data is shown in Table 4 and 5.

Ruggedness
To evaluate ruggedness of the developed method, deliberate variations were made in the method parameters such as analysts and temperature of the system. The results are found to be %RSD of 0.52% to 0.52% as presented in Table 6.

Robustness
To evaluate robustness of the developed method, deliberate variations were made in the method parameters such as the flow rate of the mobile phase and ratio of mobile phase. The %RSD for different pH was 0.48% to 0.55% and flow rate was 1.01% to 1.03% are presented in Table 7 and 8.

Stability Results
The results obtained in acidic degradation, alkaline degradation, neutral degradation, thermal degradation, oxidative degradation, photolytic degradation and UV degradation are depicted as chromatograms and given in figure 4, 5, 6, 7, 8 and 9 respectively and represented in Table 9.

CONCLUSION
From the results of method development it is found that the developed method is simple, reliable, sensitive and accurate. The developed RP-HPLC method was found suitable for the analysis of selected drug in its pure and dosage form in presence of their respective degradants since the resolution between the drugs with their corresponding degradants is better. The optimized chromatographic condition for the selected drug was a reverse phase C-18 column, mobile phase Ammonium Formate solution: Acetonitrile (70:30) pH adjusted to 4.0 with formic acid, flow rate was maintained at 1ml/min and eluents was monitored at 295nm for moxifloxacin. The sample was injected using a 20 µl fixed loop. The determination was performed at 30^0C for a run time of 10 min.
The method was found to be fast, simple, reliable, sensitive, economical, accurate and precise. The method was found to be linear within the range of 10mcg/ml to 150mcg/ml with regression coefficient of 0.999. The method was found to be accurate with %recovery within 99.97 to 100.06 for moxifloxacin with the standard deviation and percentage standard deviation was less than 1. The method was found to be precise according to the repeatability data, intraday precision data and interday precision data with the standard deviation and % relative standard deviation less than 2. The method was rugged and robust with the standard deviation and % relative standard deviation less than 2.
Stability studies for moxifloxacin were performed which showed 15.7%, 18.9% and 22.32% degradation in neutral, acidic and basic conditions respectively. Moxifloxacin in presence of hydrogen peroxide showed 32.81% degradation after 11days. Moxifloxacin was found to degrade up to 7.4% after 11days of exposure to day light. Degradation carried out in presence of UV light showed 8.09% degradation after 11days. The thermal degradation study showed a degradation of 12.35% after 11days.

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Table 1: Calibration Table of moxifloxacin for RP-HPLC Method

Conc. (µg/ml)	PA1	PA2	PA3	PA4	PA5	PA6	Mean	Stdev	%Rsd
10	672.967	669.822	671.113	670.024	671.111	671.988	671.171	1.19	0.18
20	1394.23	1395.73	1398.34	1396.66	1394.13	1396.90	1396.00	1.64	0.12
30	2183.45	2185.09	2186.17	2185.03	2184.00	2183.19	2184.49	1.14	0.05
40	2971.4	2971.86	2970.94	2971.9	2972.38	2970.88	2971.56	0.59	0.02
50	3736.95	3735.34	3734.99	3736.01	3737.23	3736.01	3736.09	0.87	0.02
60	4473.97	4475.67	4474.08	4474.17	4473.98	4473.99	4474.31	0.67	0.02
70	5351.47	5351.56	5352.1	5351.9	5352.2	5351.9	5351.85	0.29	0.01
80	6002.54	5999.62	6003.32	6001.99	6003.47	6002.13	6002.18	1.39	0.02
90	6675.96	6677.81	6676.39	6675	6675.99	6675.91	6676.18	0.92	0.01
100	7506.21	7501.09	7503.13	7502.74	7501.01	7502.39	7502.76	1.90	0.03
150	11044.8	11041.3	11046.05	11045.71	11045.32	11044.81	11044.62	1.68	0.02
								1.12	0.04

Table 2: Accuracy Data of the RP-HPLC Method for Moxifloxacin

No. of preparations	Concentration (µg/ml)		% Recovery	Statistical Analysis
	Formulation	Pure Drug		
S1 : 80 %	30	24	99.938	Mean=100.06 SD=0.18 %Rsd=0.18
S2 : 80 %	30	24	100.274	
S3 : 80 %	30	24	99.979	
S4 : 100 %	30	30	100.025	Mean=99.97 SD=0.067 %Rsd=0.07
S5 : 100 %	30	30	99.896	
S6 : 100 %	30	30	99.993	
S7 : 120 %	30	36	99.997	Mean=100.01 SD=0.037 %Rsd=0.037
S8 : 120 %	30	36	100.056	
S9 : 120 %	30	36	99.988	

Table 3: Precision Data Showing Repeatability of the RP-HPLC Method for Moxifloxacin

S. No	Conc.(µg/ml)	Peak Area	Calc. Amt.	Statistical Analysis
1	30	2218.98	29.9912	Mean=29.91 SD.=0.26 %Rsd=0.86
2	30	2228.46	30.1183	
3	30	2191.44	29.6218	
4	40	2959.26	39.9184	Mean=40.34 SD.=0.36 %Rsd=0.90
5	40	3007.47	40.5649	
6	40	3004.97	40.5314	

Table 4: Intraday Precision Data of the RP-HPLC Method for Moxifloxacin

S. No	Conc. (µg/ml)	Peak Area1	Peak Area2	Peak Area3	Statistical Analysis
1	30	2221.55	2279.53	2218.11	Mean=30.16 SD=0.16 %Rsd=0.53
2	30	2235.13	2231.34	2223.2	
3	30	2226.83	2224.12	2225.55	
	Mean	2227.83	2244.99	2222.29	
	Calc. Amt.	30.1099	30.34	30.0355	
1	40	2991.58	2979.53	2978.15	Mean=40.11 SD=0.13 %Rsd=0.33
2	40	2985.13	2951.34	2951.2	
3	40	2976.83	2964.12	2985.57	
	Mean	2984.51	2964.99	2971.64	
	Calc. Amt.	40.2571	39.9954	40.0845	

Table 5: Interday Precision Data of the RP-HPLC Method for Moxifloxacin

S. No	Conc.(µg/ml)	Day1	Day2	Day3	Statistical Analysis
1	30	2181.55	2289.53	2188.11	Mean=29.75 SD=0.28 %Rsd=0.93
2	30	2175.13	2191.34	2191.2	
3	30	2226.87	2192.12	2175.56	
	Mean	2194.51	2224.33	2184.96	
	Calc. Amt.	29.663	30.0629	29.5349	
1	40	2977.49	2982.28	2938.02	Mean=39.90 SD=0.24 %Rsd=0.60
2	40	2982.72	2943.93	2951.62	
3	40	2973.81	2931.68	2942.12	
	Mean	2978.01	2952.63	2943.92	
	Calc. Amt.	40.1699	39.8296	39.7127	

Table 6: Ruggedness Data of the RP-HPLC Method by Different Analysts for Moxifloxacin

Analyst-1				Analyst-2			
Conc. (µg/ml)	Peak Area	Calc. Amt.	Statistical Analysis	Conc. (µg/ml)	Peak Area	Calc. Amt.	Statistical Analysis
30	2271.3	30.69	Mean=30.46	30	2218.01	29.98	Mean=30.09
30	2258.6	30.52	SD.=0.27	30	2239.77	30.27	SD.=0.16
30	2231.99	30.17	%Rsd=0.88	30	2221.78	30.03	%Rsd=0.52
40	2959.26	39.92	Mean=39.80	40	2969.13	40.05	Mean=39.95
40	2947.47	39.76	SD.=0.10	40	2943.55	39.71	SD.=0.21
40	2944.97	39.73	%Rsd=0.26	40	2971.04	40.08	%Rsd=0.52

Table 7: Robustness Data of the RP-HPLC Method at Different pH for Moxifloxacin

pH-3.9				pH-4.1			
Conc. (µg/ml)	Peak Area	Calc. Amt.	Statistical Analysis	Conc. (µg/ml)	Peak Area	Calc. Amt.	Statistical Analysis
30	2198.01	29.71	Mean=30.00	30	2185.42	29.54	Mean=29.69
30	2268.75	30.66	SD.=0.57	30	2194.12	29.66	SD.=0.16
30	2191.94	29.63	%Rsd=1.91	30	2209.66	29.87	%Rsd=0.55
40	2959.19	39.92	Mean=39.95	40	2948.03	39.77	Mean=39.99
40	2967.23	40.03	SD.=0.07	40	2973.1	40.10	SD.=0.19
40	2958.12	39.90	%Rsd=0.17	40	2972.99	40.10	%Rsd=0.48

Table 8: Robustness Data of the RP-HPLC Method at Different Flow Rate for Moxifloxacin

Flow Rate 0.9ml/min				Flow Rate 1.1ml/min			
Conc. (µg/ml)	Peak Area	Calc. Amt.	Statistical Analysis	Conc. (µg/ml)	Peak Area	Calc. Amt.	Statistical Analysis
30	2228.06	30.11	Mean=30.04	30	2193.98	29.66	Mean=29.95
30	2221.91	30.03	SD.=0.07	30	2239.64	30.27	SD.=0.31
30	2216.99	29.96	%Rsd=0.25	30	2213.07	29.91	%Rsd=1.03
40	2949.32	39.79	Mean=39.89	40	2961.12	39.94	Mean=40.37
40	2957.41	39.89	SD.=0.10	40	2996	40.41	SD.=0.41
40	2964.64	39.99	%Rsd=0.26	40	3021.92	40.76	%Rsd=1.01

Table 9: Stability Study Results of Moxifloxacin

Conditions	Conc. (µg/ml)	Time	% Degraded
Acidic Degradation	100	11days	18.9
Alkaline Degradation	100	11days	22.32
Neutral Degradation	100	1 week	15.7
Thermolytic Degradation	100	11days	12.35
Oxidative Degradation	100	11days	32.81
Photolytic Degradation	100	11days	7.4
UV Degradation	100	11days	8.09

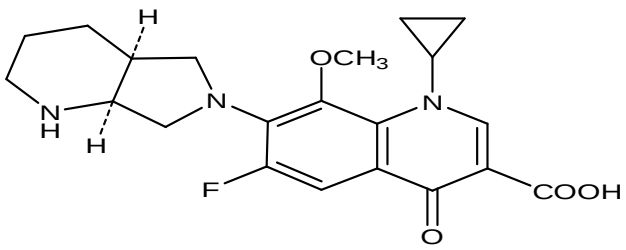

Fig. 1: Chemical Structure of Moxifloxacin

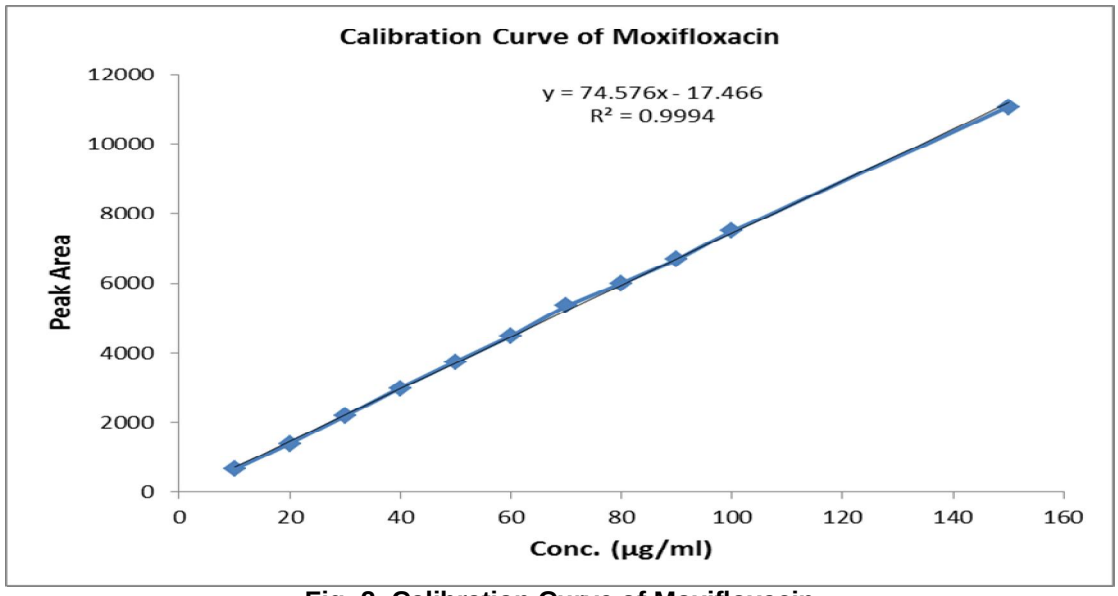

Fig. 2: Calibration Curve of Moxifloxacin

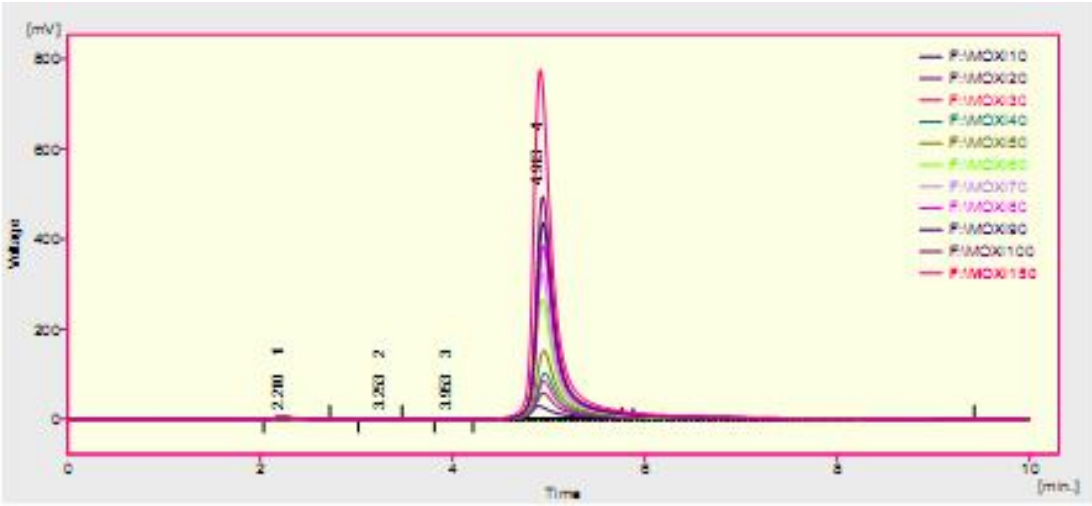

Fig. 3: Typical HPLC Overlay Chromatogram of Moxifloxacin

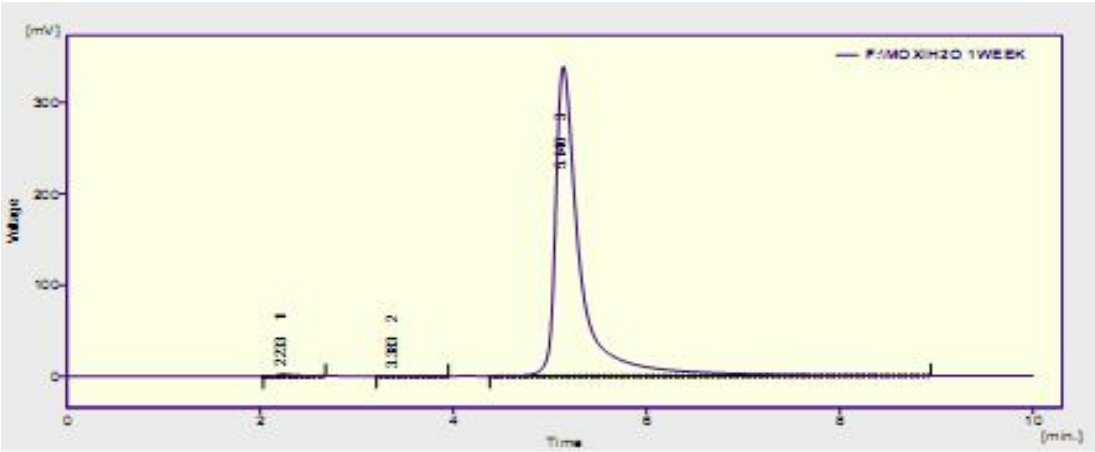

Fig. 4: Representative Chromatogram of Hydrolytic Degradation of Moxifloxacin

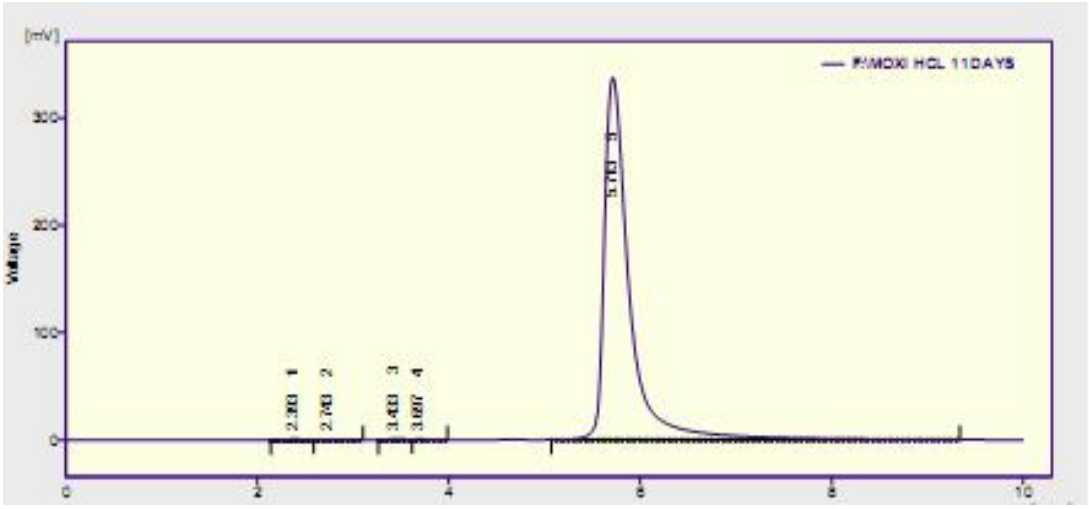

Fig. 5: Representative Chromatogram of Acidic Degradation of Moxifloxacin

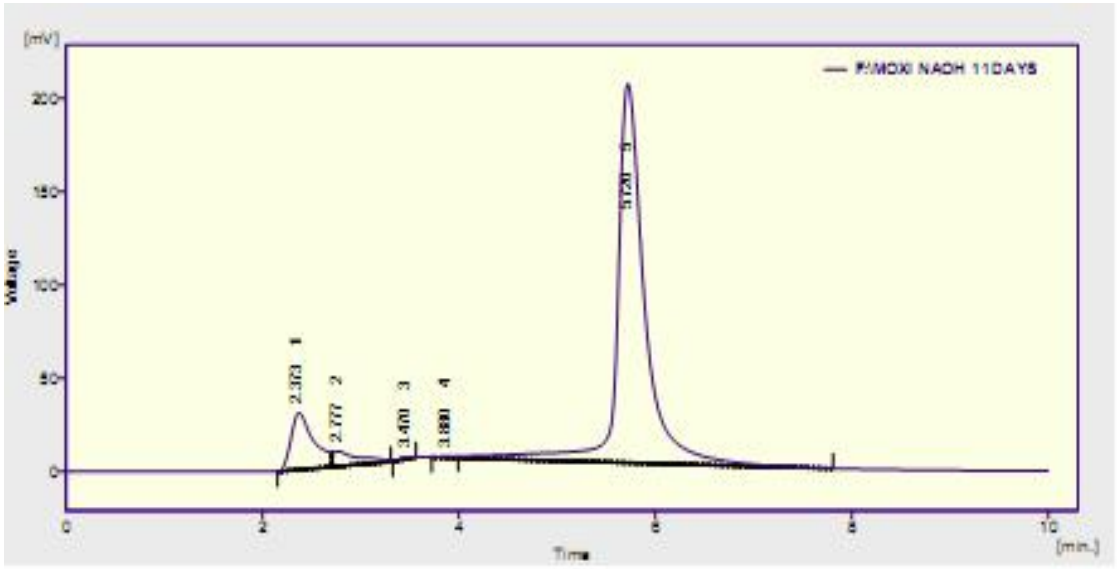

Fig. 6: Representative Chromatogram of Basic Degradation of Moxifloxacin

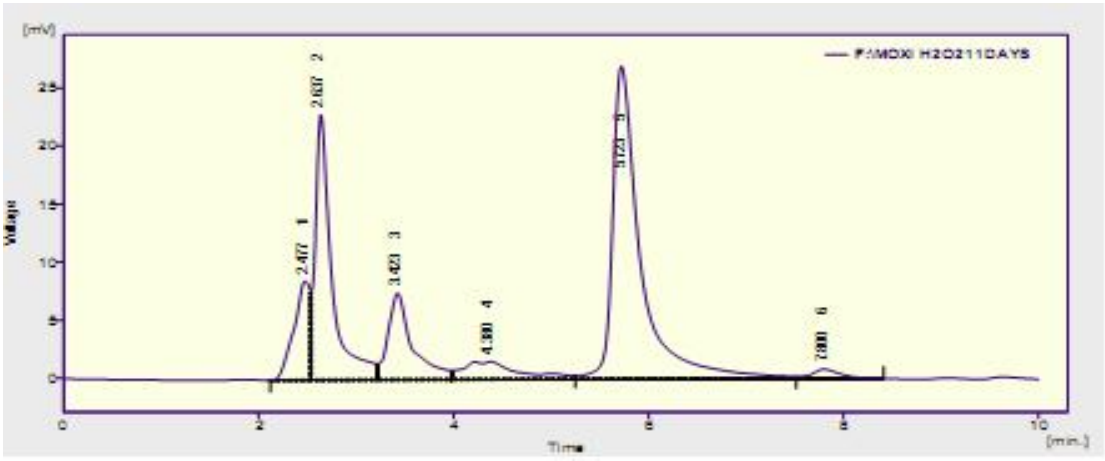

Fig. 7: Representative Chromatogram of Oxidative Degradation of Moxifloxacin

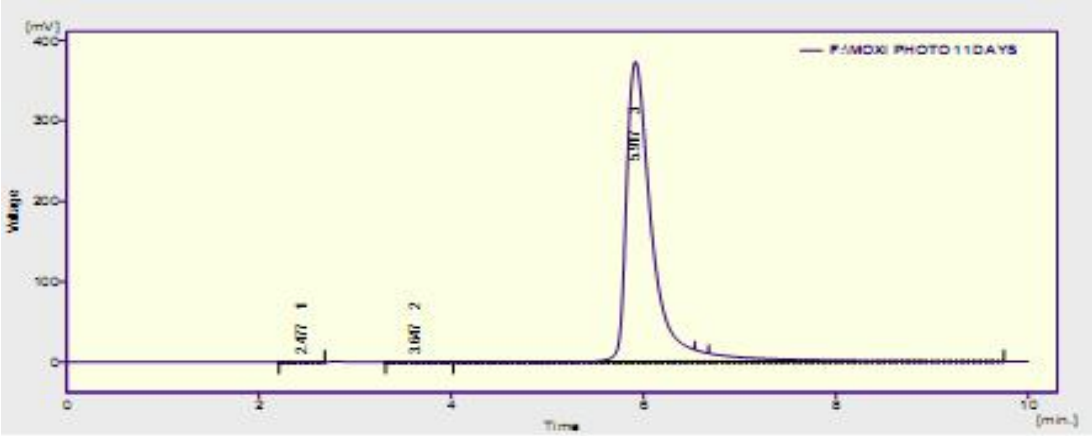

Fig. 8: Representative Chromatogram of Photolytic Degradation of Moxifloxacin

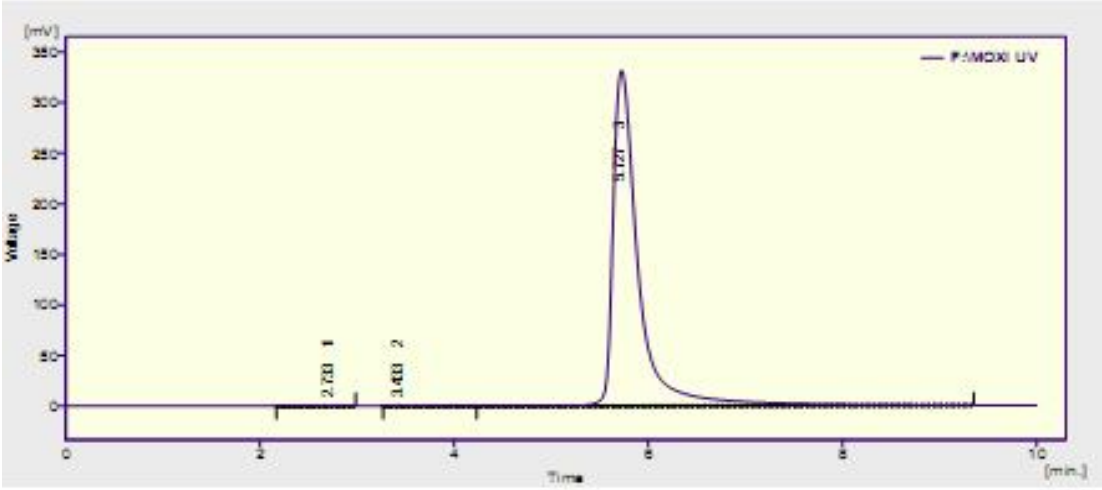

Fig. 9: Representative Chromatogram of UV- Degradation of Moxifloxacin

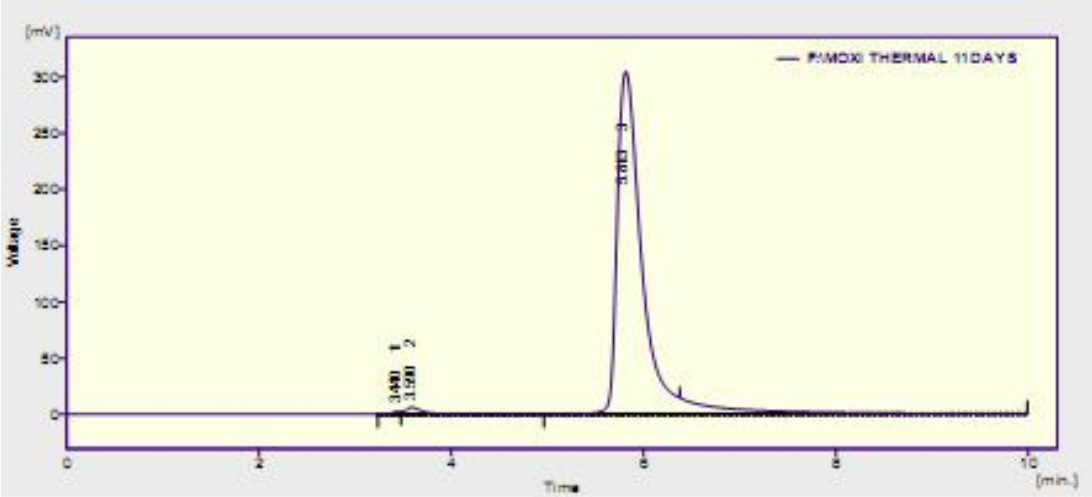

Fig. 10: Representative Chromatogram of Thermal Degradation of Moxifloxacin

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