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A NOVEL STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF MOXIFLOXACIN AND PREDNISOLONE IN BULK AND THEIR COMBINED DOSAGE FORM

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Keywords:

Moxifloxacin (MFX), Prednisolone (PDS), RP-HPLC, Stability, and validation

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ABSTRACT: A simple, specific, and precise stability indicating reverse phase high performance liquid chromatography method was developed and validated as per the ICH guidelines for the simultaneous determination of Moxifloxacin and Prednisolone in bulk and combined dosage forms. The quantification was carried out by using Zodiac C_{18} (250mm*4.6mm, 5µ) column at 25^0c with Acetate Buffer pH 4.5: Methanol: Acetonitrile in ratio of 50:20:30 % V/V as mobile phase. The flow rate is 1 mL/min and the estimation was carried out by using PDA detector at 271 nm. The retention time of MFX and PDS were 2.317 and 4.310 minutes respectively. The linearity was observed from 30-70µg/mL with correlation coefficient 0.9999 for Moxifloxacin and 60-140 µg/mL with correlation coefficient 0.9998 for Prednisolone. The LOD and LOQ of Moxifloxacin and Prednisolone were found to be 4.85 & 14.69µg/mL and 9.07 and 27.47µg/mL respectively and the Statistics data for the MFX and PDS were concluded that the method was found to be simple, reliable, selective, reproducible and accurate. The method was successfully used for quality control analysis of Moxifloxacin and Prednisolone.

INTRODUCTION: Moxifloxacin 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-quinoline carboxylic acid [1] is an orally active fourth generation fluoroquinolone antibiotic. MFX acts by binding and inhibiting Topoisomerase- II (DNA-gyrase) and Topoisomerase-IV enzymes, which are responsible for the coiling and uncoiling of DNA, which are needed for bacterial cell repair and replication [2] and it is active against both Gram-positive and Gram-negative bacteria [3]. Moxifloxacin differs from other quinolones in that it has a methoxy function at C-8 position and a diazabicyclonyl moiety with S, S-configuration at the C-7 position [4] and its molecular formula and molecular weight is $C_{21}H_{24}FN_3O_4$ and 401.43 g/mol. It is mainly used for the treatment of bacterial infections of community-acquired pneumonia, chronic bronchitis, soft tissue infections [5], conjunctivitis (pink eyes), and lung infection [6].

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Prednisolone is 11β, 17α, 21-trihydroxypregna-1, 4-diene-3, 20-dione [7, 8] and Prednisone is a prodrug which is bio transformed to Prednisolone in the

liver [9]. It is mainly used for the treatment of a wide range of inflammatory and auto-immune diseases [10] such as asthma [11], multiple sclerosis [12], rheumatoid arthritis [13], autoimmune hepatitis [14]. Moxifloxacin and Prednisolone is a one of the newer combination of dosage form which is beneficial for the treatment of various bacterial infections. Chemical structures of Moxifloxacin and Prednisolone are shown in **Figure 1 & 2** respectively.

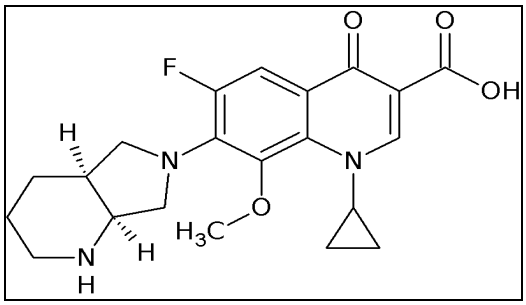


FIG.1: MOXIFLOXACIN

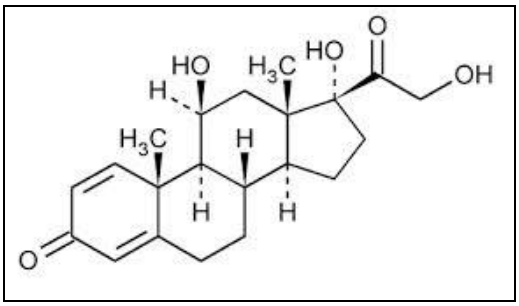


FIG. 2: PREDNISOLONE

MATERIALS AND METHODS:

Materials:

Moxifloxacin and Prednisolone pure drugs were obtained as a gift sample from Cipla pharmaceuticals Ltd, Mumbai, India. HPLC grade Acetonitrile, Methanol and water [filtered through 0.2µ filters] were purchased from Merck, Mumbai, India.

Preparation of Solutions:

Stock and Standard solution:

The stock solution prepared from pure drugs of 0.05g of Moxifloxacin and 0.1g of Prednisolone were taken in 100 mL volumetric flask and dissolved in 25mL of HPLC grade methanol, and diluted up to the mark with mobile phase.

The standard solution prepared from 1mL of stock solution was taken in 100 mL volumetric flask and diluted up to the mark with mobile phase to get a concentration of 50 µg/mL of Moxifloxacin and 100µg/mL of Prednisolone.

Acetate Buffer pH 4.5:

Dissolve 77.1 g of ammonium acetate in water add 70 ml of glacial acetic acid, and dilute up to 1000 mL with HPLC grade water (filtered through 0.2µ filters) and degassed. Adjust the pH to 4.5 by 0.1M ortho phosphoric acid.

Sample solution:

1mL of eye drops solution was taken and transferred to the 100 mL volumetric flask, then made up to the volume with mobile phase. This solution was placed in an ultrasonicator for 20 minutes and filtered through a 25 mm, 0.45 µm nylon syringe filter.

HPLC Instrumentation and Conditions:

Instrumentation:

Prominence SHIMADZU HPLC system consisting of an inbuilt SIL-10A auto sampler, CTO-10A column oven, LC 20 AT pump, SPD 20A detector, and DGU-14A degasser was employed throughout the analysis. The method development and forced degradation studies were performed on Zodiac C_{18} column and a sonerex sonicator was used for sonication. The data were acquired by using the CLASS-VP^{TM} software.

Optimized chromatographic conditions:

Chromatography was performed on a Zodiac C_{18} column using mobile phase containing mixture of Acetate Buffer (pH 4.5): Methanol: ACN in ratio of 50:20:30% V/V. The mobile phase was filtered through membrane filter (0.45 µm), and vacuum degassed by sonication prior to use. The pump pressure and run time was maintained at 1500-2500 psi and 6 minutes respectively. Chromatography was performed at 25^0C with flow rate at 1 mL/min and detection was carried out at 271 nm. Instrumentation and optimized chromatographic conditions for proposed method details are shown in **Table 1**.

RESULTS AND DISCUSSION:

Validation study of Moxifloxacin and Prednisolone:

The Method validation was performed as per ICH guidelines for the simultaneous estimation of

Moxifloxacin and Prednisolone in bulk and combined dosage form. The method was validated with respect to parameters including accuracy, precision, linearity, robustness, specificity, system suitability, LOD and LOQ [15].

Assay of Moxifloxacin and Prednisolone:

The developed method was applied to the assay of Moxifloxacin and Prednisolone in combined dosage forms. The drug content was estimated with an average of six determinations, and results were given in **Table 2**. The results were similar to the labeled claim of market formulations. The standard and sample chromatograms of Moxifloxacin and Prednisolone were shown in **Figure 3** and **4** respectively.

Specificity:

The specificity of the proposed method was established by injecting the placebo and mobile phase solution in triplicate and the chromatograms were recorded. Comparison of chromatograms revealed that there were no interactions between the placebo and sample peaks. Finally, it was indicated that the method was specific.

Accuracy:

The accuracy was determined by calculating the recovery of Moxifloxacin and Prednisolone at 100%, 120%, and 140% and they were added to pre quantified sample solution. The recovery studies were carried out in the dosage form in triplicate each in the presence of placebo.

The mean percentage recovery of MFX and PDS at each level was not less than 99%, and not more than 102%. The percentage recovery of Moxifloxacin and Prednisolone was found to be in the range of 100 to 101%. The results are shown in the **Table 3** and **4**.

TABLE 1: INSTRUMENTATION AND OPTIMIZED CHROMATOGRAPHIC CONDITIONS FOR PROPOSED METHOD

S. No	Instrumentation	Optimized Chromatographic Conditions
1	HPLC	Prominence SHIMADZU-SPD-20A
2	Column	Zodiac C_{18} (250mm*4.6mm,5µ)
3	Column temperature	25^0C
4	Flow rate	1 mL/min
5	Injection volume	20µL
6	Wavelength	271 nm
7	Run time	6 minutes
8	Mobile phase composition	Acetate Buffer (pH 4.5): Methanol: ACN in 50:20:30 % V/V

TABLE NO 2: ASSAY RESULTS OF MOXIFLOXACIN AND PREDNISOLONE FORMULATIONS

S. No	Formulations		Labeled Amount (mg/mL)	Amount Found (mg/mL)±S.D	%Assay ±RSD
1	Moftrex-P	Moxifloxacin	5	4.97	99.4±0.74
2		Prednisolone	10	10.01	100.1±0.18
3	Occumox-P	Moxifloxacin	5	4.92	98.4±0.73
4		Prednisolone	10	9.97	99.7±0.12

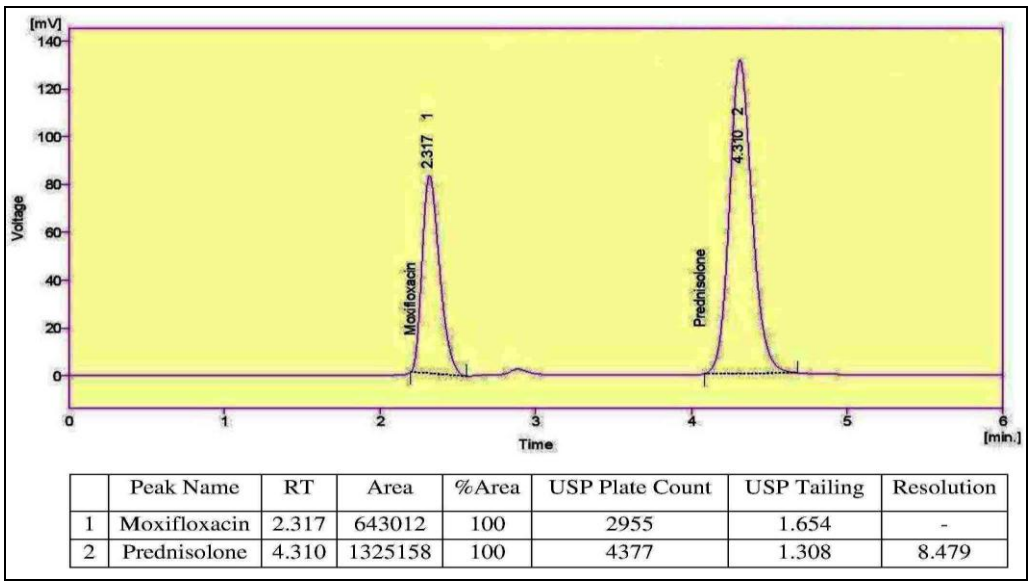
TABLE 3: RECOVERY DATA FOR THE PROPOSED RP-HPLC METHOD FOR MFX

S. No	Concentration level	Amount added (µg/mL)	Amount found (µg/mL)	Area obtained	Mean %Recovery ± S.D*	%RSD*
1	100	50	50.11	659853	100.493±0.316	0.314
			50.21	655692		
			50.42	678663		
2	120	60	59.91	763289	100.00±0.178	0.178
			60.12	778442		
			59.98	752608		
3	140	70	69.98	847488	100.05±0.295	0.295
			70.27	859993		
			69.87	848284		

TABLE 4: RECOVERY DATA FOR THE PROPOSED RP-HPLC METHOD FOR PDS

S. No	Concentration level	Amount added (µg/mL)	Amount found (µg/mL)	Area obtained	Mean %Recovery ± S.D*	%RSD*
1	100	100	99.87	1348275	99.92±0.180	0.1804
			99.77	1341057		
			100.12	1383789		
2	120	120	120.02	1583524	100.04±0.083	0.0834
			119.98	1604410		
			120.17	1553698		
3	140	140	140.17	1766886	100.09±0.100	0.0999
			139.97	1789564		
			140.24	1767220		

*S.D & %RSD is Standard Deviation and percentage of Relative Standard Deviation



	Peak Name	RT	Area	%Area	USP Plate Count	USP Tailing	Resolution
1	Moxifloxacin	2.317	643012	100	2955	1.654	-
2	Prednisolone	4.310	1325158	100	4377	1.308	8.479

FIG. 3: RP-HPLC CHROMATOGRAM OF MOXIFLOXACIN AND PREDNISOLONE

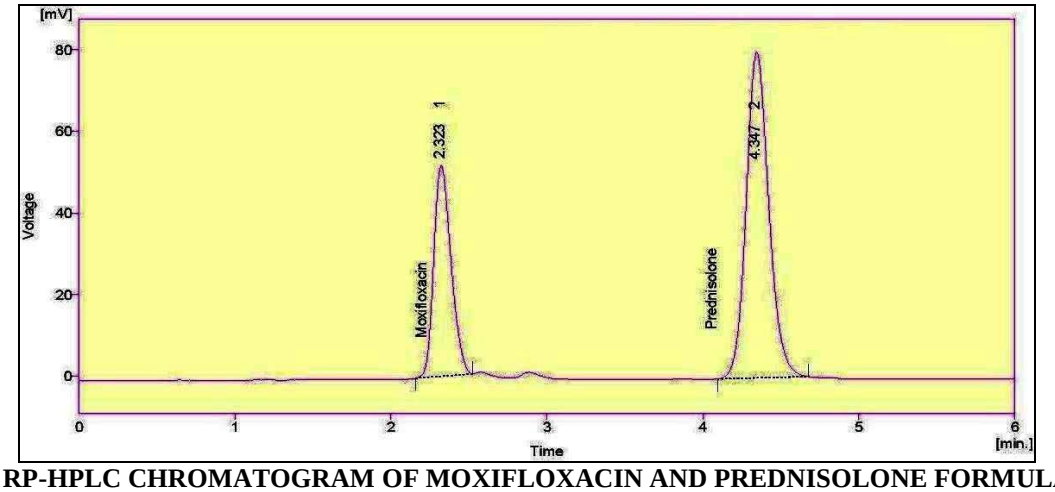


FIG. 4: RP-HPLC CHROMATOGRAM OF MOXIFLOXACIN AND PREDNISOLONE FORMULATION

Precision:

Precision should be investigated by using authentic, and homogeneous samples. The precision of this method was expressed as S.D and %RSD of series of repeated measurements. Precision of MFX and PDS determination by proposed method were ascertained by repeated analysis of homogeneous samples of moxifloxacin and prednisolone standard solutions in the intraday under the similar conditions. The method precision results were shown in **Table 5**.

TABLE 5: METHOD PRECISION RESULTS OF THE PROPOSED RP-HPLC METHOD

S. No	Injections	MOXIFLOXACIN		PREDNISOLONE	
		Retention Time	Peak Area	Retention Time	Peak Area
1	1	2.360	671698	4.337	1361388
2	2	2.343	666466	4.307	1346031
3	3	2.333	666242	4.287	1351976
4	4	2.323	658514	4.267	1347300
5	5	2.313	660578	4.253	1332036
6	6	2.317	658668	4.310	1330331
7	MEAN	2.3315	663694.3	4.2935	1344844
8	SD	0.017729	5292.312	0.030762	11888.5
9	%RSD	0.760391	0.797402	0.716478	0.884006

Linearity:

Linearity of the proposed method was established by using series of standard solutions of Moxifloxacin and Prednisolone and these studies are repeated in triplicate with different stock solutions. The curve obtained by concentration on x-axis and peak area on y-axis against showed linearity in the concentration range of 30 to 70 µg/mL for Moxifloxacin and 60-140 µg/mL Prednisolone and linearity graph is shown in **Graph 1 and 2**. The regression equation and correlation coefficient of Moxifloxacin and Prednisolone were found to be Y=12880x+3060 and 0.9999 and Y=13312x+11292 and 0.9998 respectively. The Linearity and statistical analysis of data are shown in **Table 5** and **6.**

TABLE 5: LINEARITY AND STATISTICAL ANALYSIS DATA FOR MOXIFLOXACIN

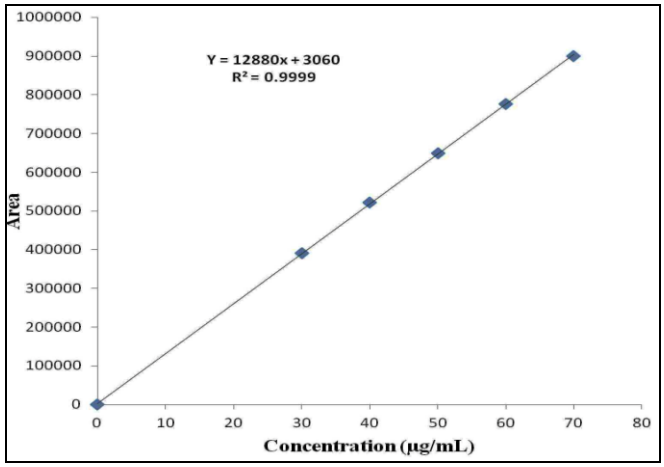
S. No	Concentration (µg/mL)	Area	Statistical Analysis		
			Slope	Y-Intercept	Correlation Coefficient
1	30	391031			
2	40	511577			
3	50	649419	12880	3060	0.9999
4	60	776386			
5	70	899984			

TABLE 6: LINEARITY AND STATISTICAL ANALYSIS DATA FOR PREDNISOLONE

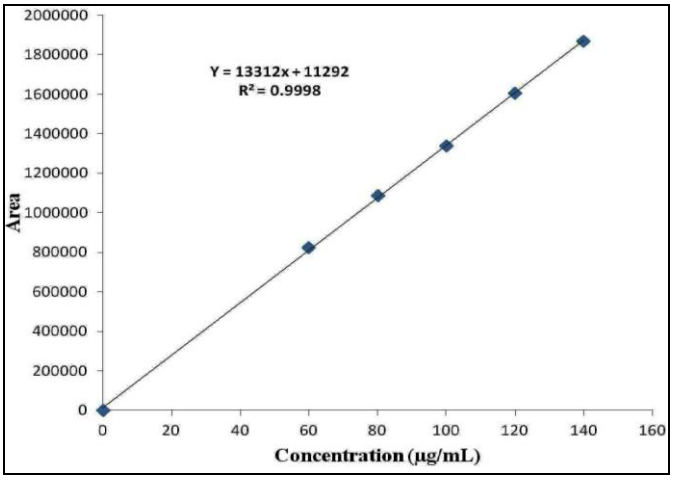
S. No	Concentration (µg/mL)	Area	Statistical Analysis		
			Slope	Y-Intercept	Correlation Coefficient
1	60	825151			
2	80	1085876			
3	100	1339850	13312	11292	0.9998
4	120	1603698			
5	140	1869220			

Robustness:

The robustness was evaluated by the analysis of Moxifloxacin and Prednisolone under different experimental conditions such as slight changes in chromatographic conditions like change of temperature (±5^{0}C), flow rate (±0.2 ml/min), and wavelength (±2%). It was distinguished that there were no changes in the chromatograms, and the parameters were within the limits, which indicates that the method was robust and suitable for routine use. The complete results are shown in **Table 7** and **8**, and the method is having good system suitability.



GRAPH 1:LINEARITY GRAPH OF MOXIFLOXACIN



GRAPH 2:LINEARITY GRAPH OF PREDNISOLONE

Limit of Detection:

The limit of detection (LOD) has established the minimum concentration at which the analyte can be reliably detected. LOD is determined by the signal to noise ratio and generally acceptable detection limit ratio is 3:1. It was found to be 0.485µg/mL for Moxifloxacin and 0.571µg/mL for Prednisolone respectively.

Limit of Quantification:

The limit of quantification (LOQ) has established the minimum concentration at which the analyte can be reliably quantified. LOQ is determined by the signal to noise ratio and a typical signal to noise ratio is 10:1 is acceptable for estimating the quantification limit. It was found to be 1.425µg/mL for Moxifloxacin and 1.677µg/mL for Prednisolone respectively.

System suitability:

This test was conducted on freshly prepared Moxifloxacin and Prednisolone standard solution was used for the evaluation of the system suitability parameters such as retention time, area, USP tailing and theoretical plates, limit of detection and limit of quantification. Five replicate injections for a system suitability test were injected into the chromatographic system. Finally the proposed method is having good system suitability and its parameters are shown in **Table 9**.

TABLE 7: ROBUSTNESS RESULTS OF MOXIFLOXACIN

S. No	Parameters			Peak Area	RT	USP	
	Optimized		Used			Plate Count	Tailing Factor
1	Flow rate (±0.2)	1 mL/min	0.8	103486	3.647	1842	1.643
			1.2	487985	1.727	1652	1.435
2	Temperature (±5^{0}c)	25^{0}c	20	544028	1.892	1498	1.891
			30	767158	2.991	1989	1.252
3	Wave length (± 2)	271 nm	269	888446	2.330	1780	1.552
			273	474233	2.323	1682	1.500

TABLE 8: ROBUSTNESS RESULTS OF PREDNISOLONE

S. No	Parameters			Peak Area	RT	USP		Resolution
	Optimized		Used			Plate Count	Tailing Factor	
1	Flow rate (±0.2)	**1 mL/min**	0.8	1034886	6.783	1842	1.643	8.025
			1.2	989232	3.230	3251	1.435	7.582
2	Temperature (±5^{0}c)	**25^{0}c**	20	1292954	5.985	2645	1.271	8.540
			30	1865465	4.981	3477	1.647	7.787
3	Wave length (± 2)	**271 nm**	269	1340164	4.277	3127	1.552	7.390
			273	1280135	5.421	3206	1.213	7.314

TABLE 9: SYSTEM SUITABILITY PARAMETERS OF PROPOSED RP-HPLC METHOD

S. No	Parameters	MOXIFLOXACIN	PREDNISOLONE
1	Linearity range(µg/mL)	30-70	60-140
2	Regression equation	Y=12880x+3060	Y=13312x+11292
3	Correlation coefficient(r^2)	0.9999	0.9998
4	Retention time (minutes)	2.317	4.310
5	Theoretical plates	2955	4377
6	Tailing factor	1.654	1.308
7	Wavelength- Isosbestic point	271	

8	Limit of Detection (µg/mL)	0.485	0.571
9	Limit of Quantification (µg/mL)	1.425	1.677
10	Capacity factor (k)	0.127	0.174

Forced Degradation Study:

Forced degradation studies were conducted to evaluate the stability and specificity of the method. The acceptable limit for consideration in the present study is between 5 to 20% for chromatographic assays [16, 17]. The specificity of the developed method was evaluated by using different ICH prescribed stress conditions like acidic, basic, oxidative, thermal and photolytic.

Acidic Degradation:

These studies can be performed by taking 10 mL stock solution of Moxifloxacin and Prednisolone, each in separate 50 mL volumetric flask. 10 mL of 5N HCL was added to the stock solution and these solutions were kept at reflux for 4 hours. Finally this solution was neutralized with 5 N NaOH.

Alkali Degradation:

These studies can be performed by taking 10 mL stock solution of Moxifloxacin and Prednisolone, each in separate 50 mL volumetric flask. 10 mL of 5 N NaOH was added to the stock solution and these solutions were kept at reflux for 4 hours. Finally this solution was neutralized with 5N HCL.

Oxidative Degradation:

These studies can be performed by taking 10 mL stock solution of Moxifloxacin and Prednisolone, each in separate 50 mL volumetric flask. 10 mL of 3% hydrogen peroxide added to each flask. These mixtures were kept for up to 3 days in the dark.

Thermal Degradation:

These studies can be performed by taking 10 mL stock solution of Moxifloxacin and Prednisolone, each in separate 50 mL volumetric flask, then sample solution were heated to 80^0c for 15-60 minutes.

Photolytic degradation:

These studies can be performed by taking 10 mL stock solution of Moxifloxacin and Prednisolone, each in separate 50 mL volumetric flask, then sample solution were directly exposed to sunlight for 15-60 minutes.

Finally forced degradation studies of Moxifloxacin and Prednisolone concluded that purity of angle less than purity of threshold and forced degradation chromatogram were shown in Figure No 5 to 8. All the Degradation summary results were shown in **Table 10**

TABLE 10: FORCED DEGRADATION RESULTS OF PROPOSED RP-HPLC METHOD

S. No	Degradation condition	Moxifloxacin		Prednisolone		Observation
		Purity of				
		Angle	Threshold	Angle	Threshold	
1	Control sample	--	--	--	--	Not applicable
2	Acidic Degradation	0.14	0.22	0.24	0.49	No significant degradation
3	Alkali Degradation	0.15	0.21	0.33	0.51	Substantial
4	Oxidative Degradation	0.14	0.31	0.24	0.49	Substantial
5	Thermal Degradation	0.17	0.34	0.26	0.53	No significant degradation
6	Photolytic degradation	0.11	0.28	0.24	0.56	No significant degradation

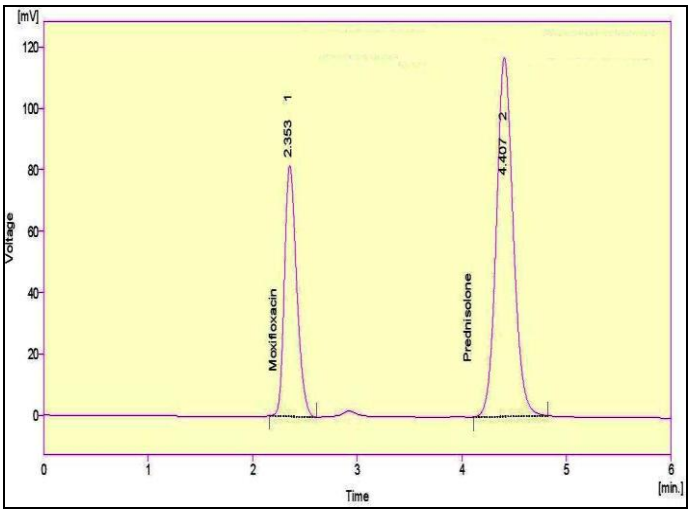


FIG. 5: CHROMATOGRAM OF MOXIFLOXACIN AND PREDNISOLONE FOR ACIDIC DEGRADATION

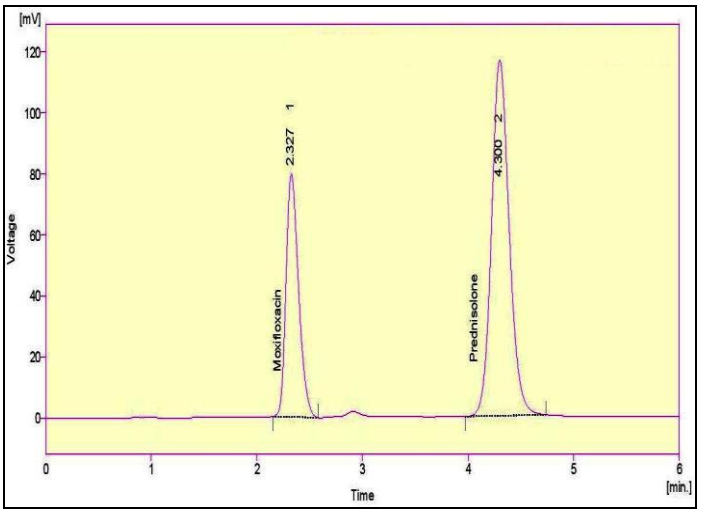


FIG. 6: CHROMATOGRAM OF MOXIFLOXACIN AND PREDNISOLONE FOR ALKALI DEGRADATION

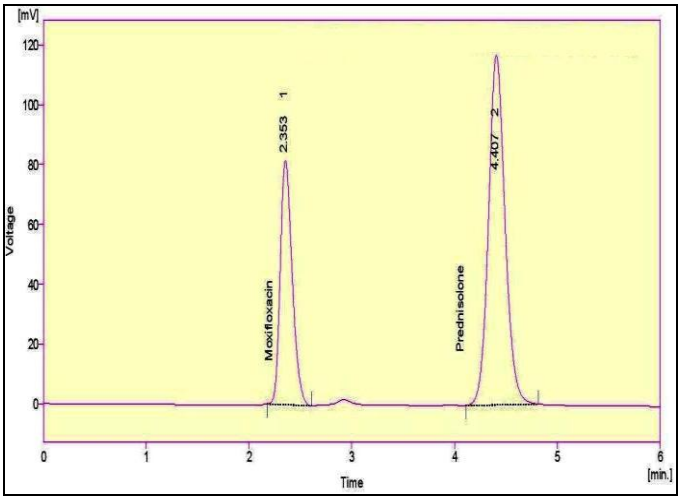


FIG. 7: CHROMATOGRAM OF MOXIFLOXACIN AND PREDNISOLONE FOR OXIDATIVE DEGRADATION

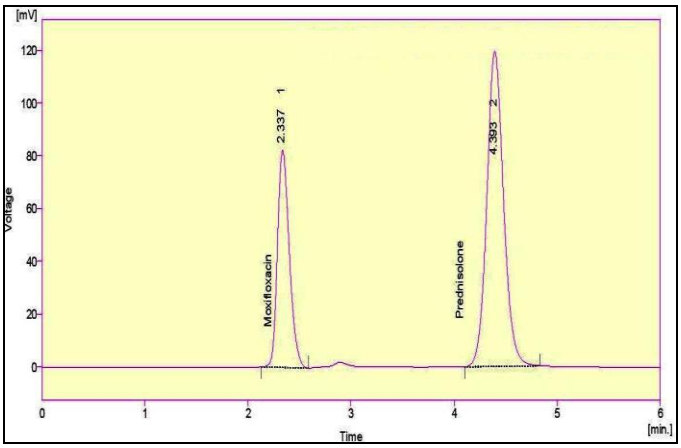


FIG. 8: CHROMATOGRAM OF MOXIFLOXACIN AND PREDNISOLONE FOR THERMAL DEGRADATION

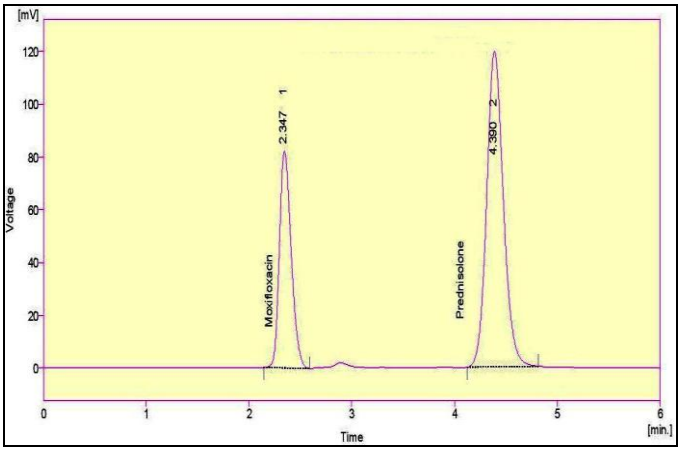


FIG. 9: CHROMATOGRAM OF MOXIFLOXACIN AND PREDNISOLONE FOR PHOTOLYTIC DEGRADATION

CONCLUSION: A stability indicating RP-HPLC method for simultaneous estimation of Moxifloxacin and Prednisolone in bulk and pharmaceutical dosage forms is established. The method is simple, accurate, linear, sensitive and reproducible as well as economical for the effective quantitative analysis of Moxifloxacin and Prednisolone in bulk and dosage forms. The method was validated, and all the method validation parameters were tested and shown to produce satisfactory results. The method is free from interactions of the other ingredients and excipients used in the formulations. Finally concluded that the method is suitable for use in the routine quality control analysis of Moxifloxacin and Prednisolone in API and in pharmaceutical dosage forms

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