DEVELOPMENT AND VALIDATION FOR HPLC METHOD OF ESTIMATION OF MOXIFLOXACIN IN EYE DROP FORMULATION

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Abstract:
Moxifloxacin shows most effectiveness towards antibacterial antibiotic activity, as in eye drops. It is marketed worldwide under the brand name 4-Quin. We have developed a HPLC method to estimate Moxifloxacin from an eye drop formulation. For effectiveness Moxifloxacin 98% Purity was used. Sodium acetate buffer and Methanol were taken as a mobile phase. For identifying and determining Moxifloxacin, the Younglin HPLC system was used. The assay of Moxifloxacin 98.24% was at the standard limit of 90.0 % to 110.0 % by Trial and test Sample Observation. Y=22.563x+13.923 and R2= 0.9994 Linearity was observed in 4 trials of Standard Moxifloxacin. The SD of the peak area and the slop of the calibration curve of Moxifloxacin was found to be 23.37µg and its lineality was found to be y=-29.25x+124.8 and R 2 = 0.645. The repeatability on Interday and interraday precision was found to be above 99%.There was not any deviation under subjected to stress with 5N HCl, 5N NaOH, 3% H2O2; 105°C, UV and Thermal degradation at 600C in presence of 80% RH. Validate 100% Recovery with Moxifloxacin assay. The Project's objectives were successful when compared with the marketed product.
Key words: Moxifloxacin, Eye Drop, Development, Validation, Formulation, Antibacterial

Introduction:
Modern pharmaceutical formulations are complex mixtures including one or more medicinally active ingredients, a number of inert materials such as diluents, disintegrates, colors and flavours. In order to ensure quality and stability of the final product, the pharmaceutical analyst must be able to separate these mixtures into individual components prior to quantitative analysis.
Pharmaceutical products formulated with more than one drug, typically referred to as combination products, are intended to meet previously unmet patients 'needs by combining the therapeutic effect of two or more drugs in one product.
Surveys revealed that, day by day, new drugs and their combinations with other drugs are being introduced as these are more effective than a single drug. So, analytical chemists are facing the challenges of developing a simple, accurate and reproducible method for estimation of drug combination.

Method: Analytical method validation according to USP is performed to ensure that an analytical methodology is accurate, specific, reproducible, precise and rugged over the specified range that an analyte will be analyzed. Validation of method is the process by which a method is tested by a developer or user for reliability, accuracy and preciseness of its intended purpose.
The international conference on harmonization (ICH) of technical requirements for the registration of pharmaceuticals for human use has developed a suitable text on the validation of analytical procedures. The document includes definitions for eight validation parameters. The United State Food and Drug Administration (US-FDA) has proposed guidelines on submitting samples and analytical data for method validation. The United State Pharmacopoeia (USP) has published specific guidelines for method validation for compound evaluation.
Materials and Instruments: All reagents and chemical used were of AR grade and HPLC grade

Sr.No.	Name Of Instrument	Model
1	UV-VIS	(Shimaduz) UV-1700 Double beam with software UV probe 2.33
2	HPLC System	Younglin-HPLC system, Column-C18 (Cosmosil) (4.6 ×250mm, 5µm)
3	Analytical Balance	(ESSAE) DS-852J Series~) ~(``Micro Analytical Balance)
4	pH Meter	(Digisun electronics) PH System 7007`
5	Ultrasonicator	(Servewell Instrument) RC-System MU-1700

Sr.No.	Name of chemic	Grade	Manufacturer
1	Methanol	HPLC& AR Grade	Merk Ltd., India
2	Water	HPLC Grade	Merk Ltd., India
3	Ortho-phosphoric acid	AR Grade	Merk Ltd., India
4	Triethyl amine	AR Grade	Merk Ltd., India

Materials: ICH Guideline number: Q2A & Q2B of CPMP / ICH / 281 / 95.

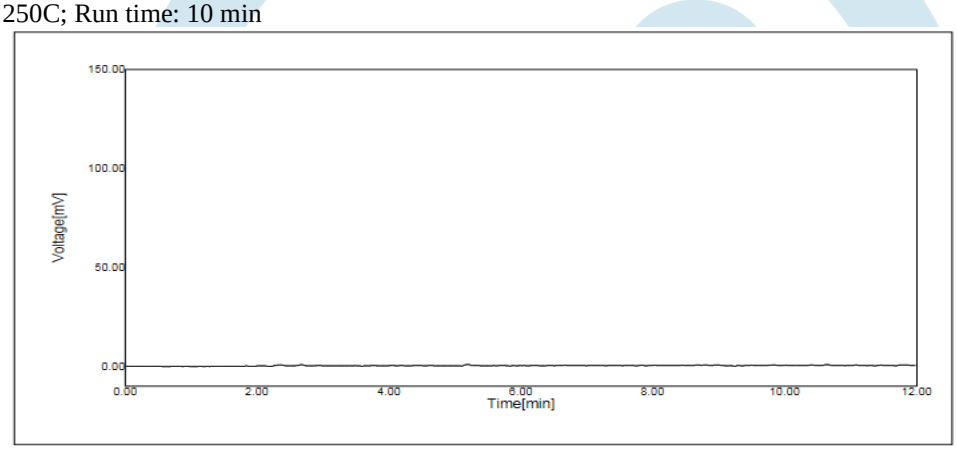
Sr.No.	Name	W.S. number	Purity on dried basis
1	**Moxifloxacin**	**63-2**	**98.0%**

Sr. No.	Mobile phase	Wavelength (nm)	Retention Time	Flow Rate (ml/min)	Remark
1	Methanol: Water (80:20)	290	-	1.2ml/min	Peak splitting observed

2	Acetonitrilie:Water (50:50)	290	5.18	1.2ml/min	Peak broad, TP not observed
3	Methanol: Buffer (60:40)	290	5.18	1.2ml/min	Peak splitting observed
4	Methanol: Buffer (50:50)	290	5.15	0.9ml/min	TP,TF is observed
5	Methanol: Buffer c(60:40)	290	5.17	0.9ml/min	TP,TF is observed

Trials of various mobile phases

Each mobile phase was filtered through Whatman filter paper No 41 and degassed by sonication for 20 min prior to use:
METHOD DEVELOPMENT: By Younglin HPLC system High Performance Liquid Chromatography Method Development for The Eye Drop Preparation Different trials taken were as follows Trial 1: Principle: High Performance liquid chromatography Preparation of mobile phase: water: Methanol (20:80)
Preparation of diluents: water
Preparation of standard solution (common for all samples): transfer an accurately weighed quantity of about 20 mlof Moxifloxacin WS into a 100 ml volumetric flask, add 50ml of water, sonicate for 2 min to dissolve and make up to the mark with water. Filter the solution through a membrane filter. (The final concentration of the solution is 0.2mg/ml)
Chromatographic System: (Common for all Trials)
HPLC: HPLC - UV detector Analytical Column: Hypersil BDS C18, 250mm x 4.6mm x 5µ (BDS)
Flow rate: 1.2 ml/minute; Detector Wavelength: 290nm; Injection volume: 20 µl
Temperature: 250C; Run time: 10 min



Chromatographic Peak Observation (Sample 1)

Sr. No.	Name	RT (min)	Area(Mv*s)	TP	TF
1	Moxifloxacin	Blank sample	0.0000	Blank sample	Blank sample

TRIAL: 2: Principle: High Performance liquid chromatography
Preparation of mobile phase: **Water: ACN (50:50)**

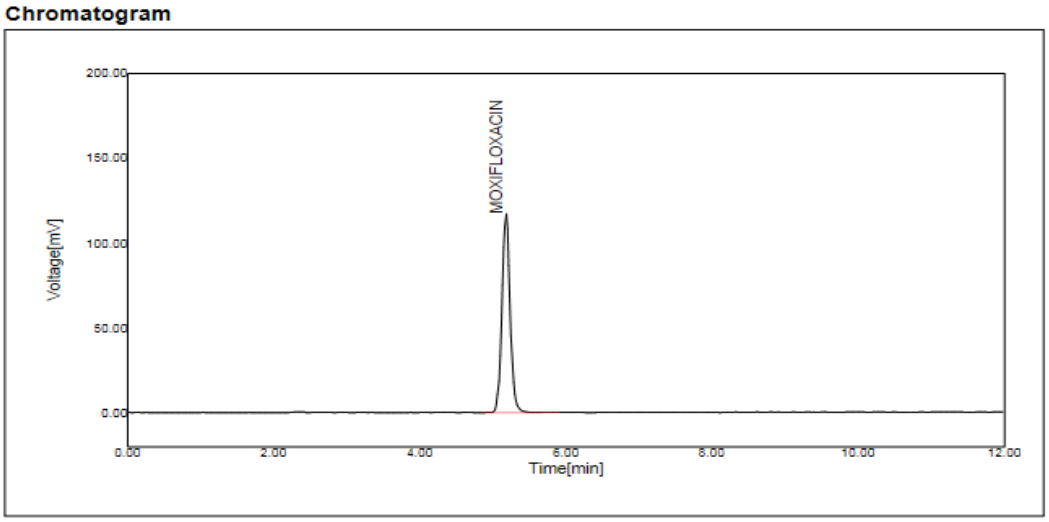


Fig-Trial 2 (Sample)

Table 7.2: Trial 2 Chromatographic Peak Observation

` Sr. No	Name	RT (min)	Area(Mv*s)	TP	TF
1	Moxifloxacin	5.18	911.6141	11915	1.04

Trial 3: Principle: High Performance liquid chromatography

Preparation of mobile phase: **Methanol: Buffer (60:40)**

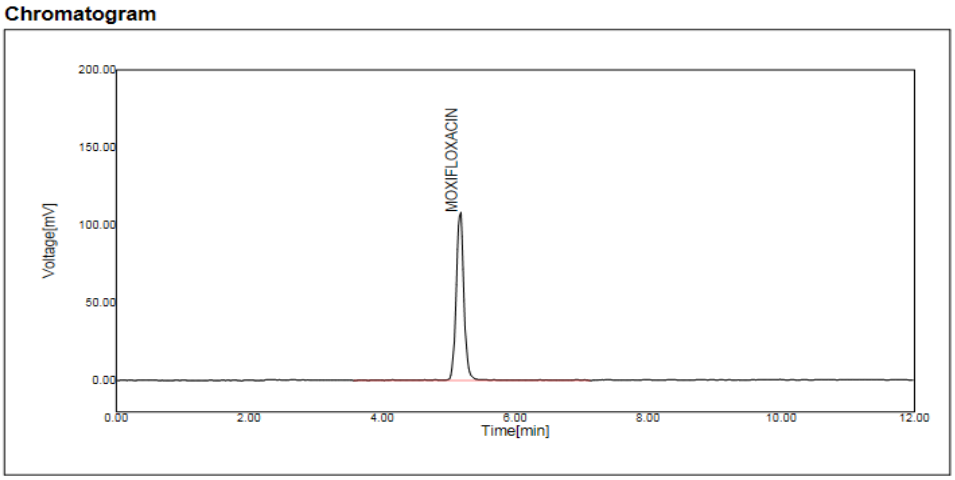


Fig-Trial 3 (Sample)

Table 7.3: Trial 3 Chromatographic Peak Observation

Sr. No	Name	RT (min)	Area(Mv*s)	TP	TF
1	Moxifloxacin	5.18	912.1934	13174	0.97

Trial 4: **Principle:** High Performance liquid chromatography

Preparation of mobile phase: **Methanol : Buffer(50:50)**

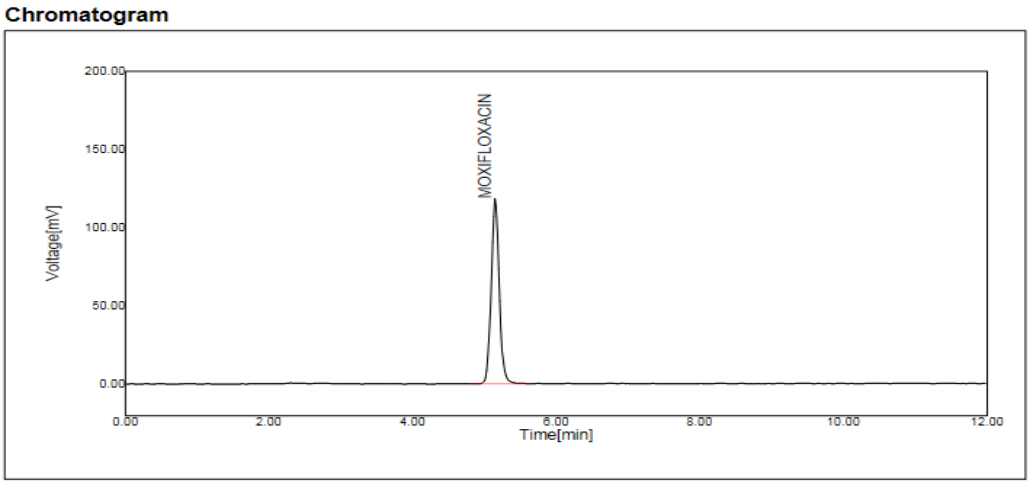


Fig-Trial 4 (Sample)

Table 7.4: Trial 4 Chromatographic Peak Observation

Sr. No	Name	RT (min)	Area(Mv*s)	TP	TF
1	Moxifloxacin	5.15	922.5414	12985	1.09

Trial 5: **Principle:** High Performance liquid chromatography

Preparation of mobile phase: **Methanol: Buffer (60:40)**

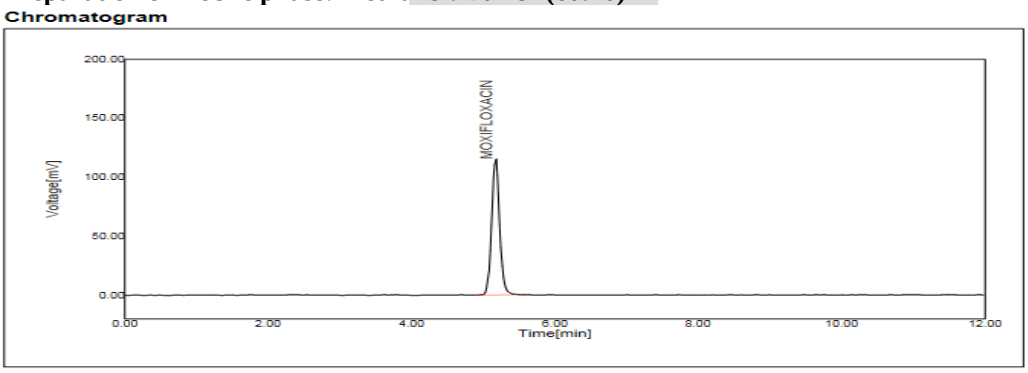


Fig-Trial 5 (Test)

Table 7.5: Trial 5 Chromatographic Peak Observation

Sr. No	Name	RT (min)	Area(mV*s)
1	Moxifloxacin	5.18	894.4362

Table 7.6 Peak Area and there Average

	Area			
Solution	1	2	3	Average
Blank	-	-	-	0.0000
Standard	911.6141	912.1934	922.5414	915.4496
Test	913.2239	894.4362		903.8300

1) Average Area of Standard = 915.446
2) Average Area of Sample (Test) = 903.8300
3) Potency of Working Standard Moxifloxacin l = 99.26% (On as is basis)

Calculation:

%Assay of 'Moxifloxacin solution

$$= \frac{903.8300}{915.446} \times \frac{20.1}{50} \times \frac{2}{20} \times \frac{50}{20.2} \times \frac{20}{2} \times 100$$

$$= \times \frac{At}{As} \times \frac{Wt.\ std}{Wt.\ test} \times 100$$

Moxifloxacin per mg = 98.24mg

Assay of Moxifloxacin 98.24% was in standard limit 90.0 % to 110.0 %

METHOD VALIDATION

Linearity and range

The linearity & range of the method is established by injecting duplicates of Standard solutions of 5 different test concentration. A plot of Concentration in ppm vs. Peak Response of Moxifloxacin WS drawn and expressed in terms of the correlation coefficient, slope & intercept

Sr.No.	Area of Moxifloxacin	Tailing factor
1	463.4545	**5.18**
2	701.9647	
3	905.6029	
4	1137.3634	
5	1373.9296	
Mean	**916.4630**	
Standard deviation±	**200.5662**	
(%) Relative standard	**0.21**	

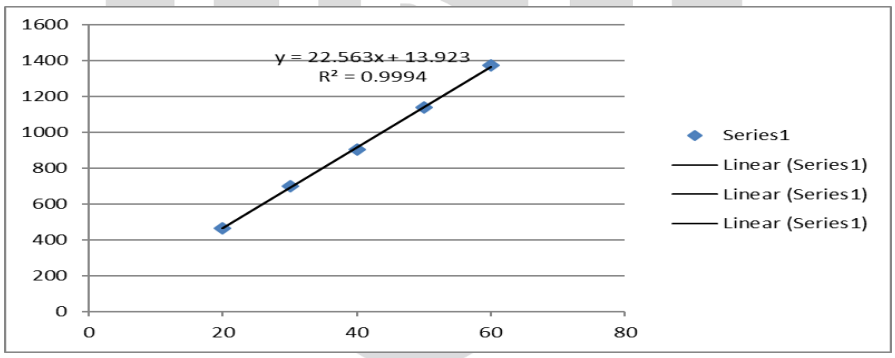


Table 7.13: Linearity concentration for Moxifloxacin

Linearity level	Sample concentration (in %)	Sample concentration (in ppm) '	Average area (n = 3)
1	50	20	463.4545
1.5	75	30	701.9647
2	100	40	905.6029
2.5	125	50	1137.3634
3	150	60	1373.9296
Slop	**22.563**		
Intersect	**13923**		
Regression Cofficient	**0.9997**		

Standard Deviation	200.5662

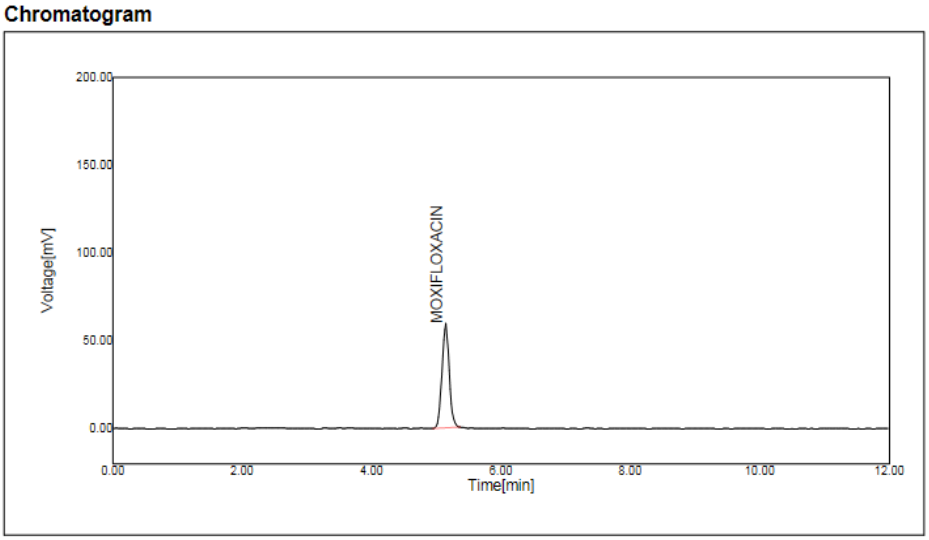


Fig:- Linearity of Level 1

Observation:

Sr.No.	Name	RT	Area
1	Moxifloxacin	5.15	463.4545

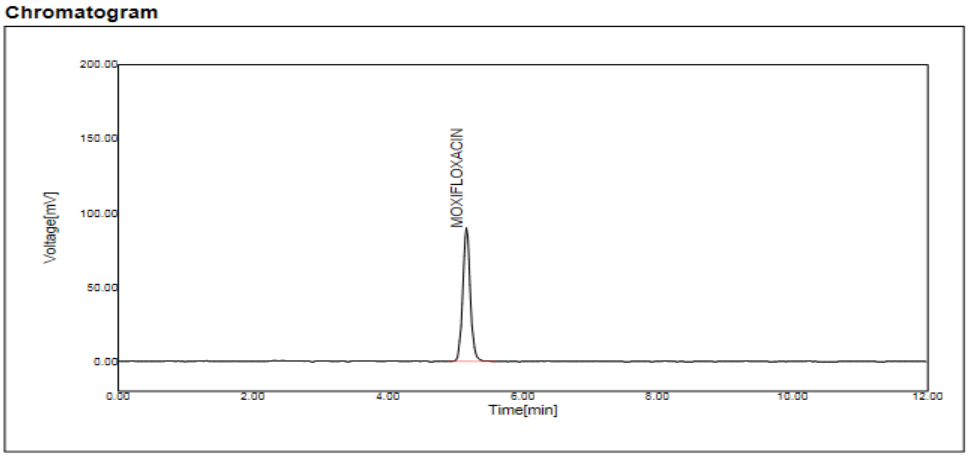


Fig:- Linearity of Level 2

Observation:

Sr.No.	Name	RT	Area
1	Moxifloxacin	5.17	701.9647

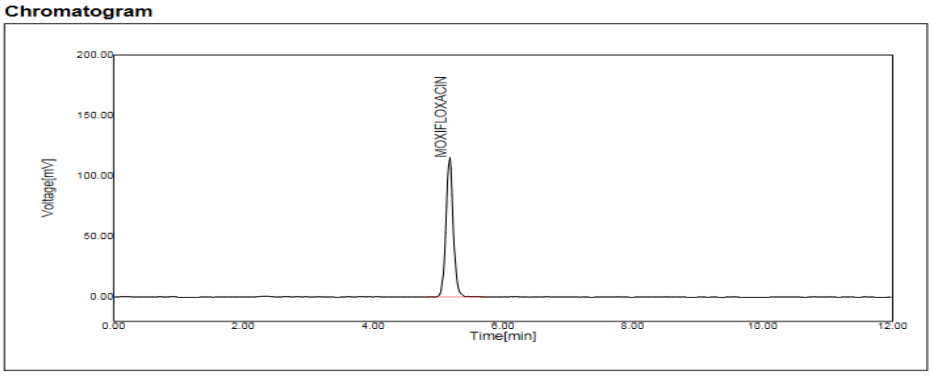


Fig:- Linearity of Level 3

Observation:

Sr.No.	Name	RT	Area

1	Moxifloxacin	5.18	905.6029

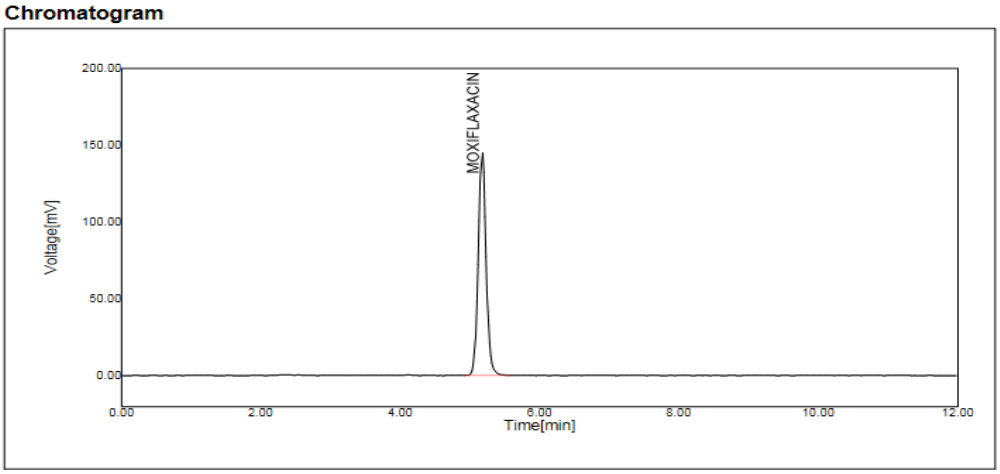


Fig:- Linearity of Level 4

Observation:

Sr.No.	Name	RT	Area
1	Moxifloxacin	5.18	1137.3634

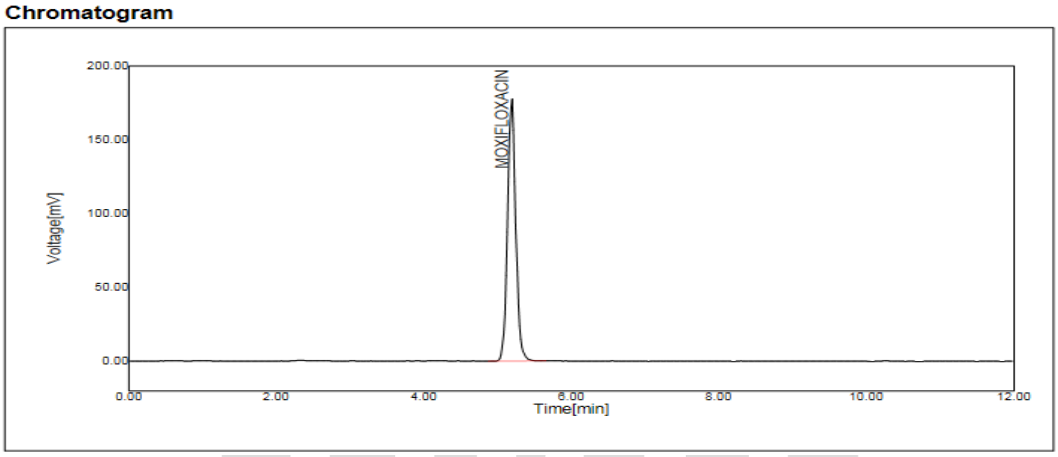


Fig:- Linearity of Level 5

Observation:

Sr.No.	Name	RT	Area
1	Moxifloxacin	5.20	1373.9296

The correlation or regression coefficient of Concentration vs. peak response of Moxifloxacin in Standard preparations, Recovery preparations and Sample preparations are 0.99999, 0.9997 and 0.9998 respectively. The relationship between the concentration and the response (peak area) of Moxifloxacin is linear in the range examined as all the points lie in a straight line and the correlation coefficient is well within limits

Analysis: The relationship between the concentration of Moxifloxacin taken and the response (peak area) measured for Moxifloxacin is linear in the range examined and the regression coefficient is more than 0.99 in all the three cases. Hence, the method is linear in the range of 20% to 60% of Moxifloxacin test concentration (i.e. 0.20mg/ml of Moxifloxacin).

ACCURACY (RECOVERY)

The accuracy of the method is determined by recovery experiments. The recovery is performed by adding Moxifloxacin WS in the range of 80%-120%of test concentration i.e. 0.2mg/ml of Moxifloxacin WS. (80%, 100%, and 120%) and expressed as % RSD.

Table 7.14: System suitability: Linearity study of Moxifloxacin

Sr.No.	Area of Moxifloxacin	Tailing factor
1	463.4545	**5.18**
2	701.9647	
3	905.6029	
4	1137.3634	
5	1373.9296	
Mean	**916.4630**	
Standard deviation±	**200.5662**	

(%) Relative standard	**0.21**	

Recovery of Moxifloxacin (80%-120% of Test concentration

S. No	Test Conc.	Std Added (mg)	Replicate (Injection) No.	Peak Response	Moxifloxacin Recovered (mg)	Percentage Recovery	Mean % Recovery
1	80	32	1	1648.7076	32.0392	100.12	99.76
			2	1636.8592	31.5215	98.50	
			3	1652.6576	32.2118	100.66	
2	100	40	1	1819.8060	39.5153	98.69	99.63
			2	1827.5689	39.8545	99.64	
			3	1835.2225	40.1845	100.46	
3	120	48	1	2024.0778	48.4408	100.92	100.74
			2	2032.9078	48.8266	101.72	
			3	2009.5058	47.8041	99.59	
Mean Percentage Recovery						**99.63**	**99.63**
Standard Deviation						**0.83**	**0.83**
%RSD						**0.84**	**0.84**

Chromatogram

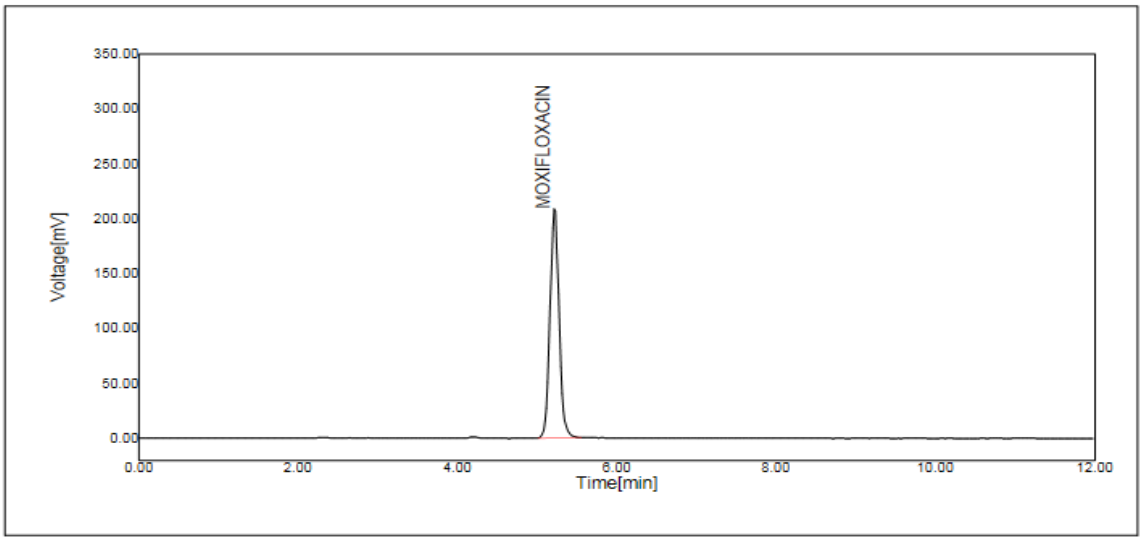


Fig:- 80% recovery

Observation:

Sr.No.	Name	RT (min)	Area(mV*s)
1	Moxifloxacin	5.22	1648.7076

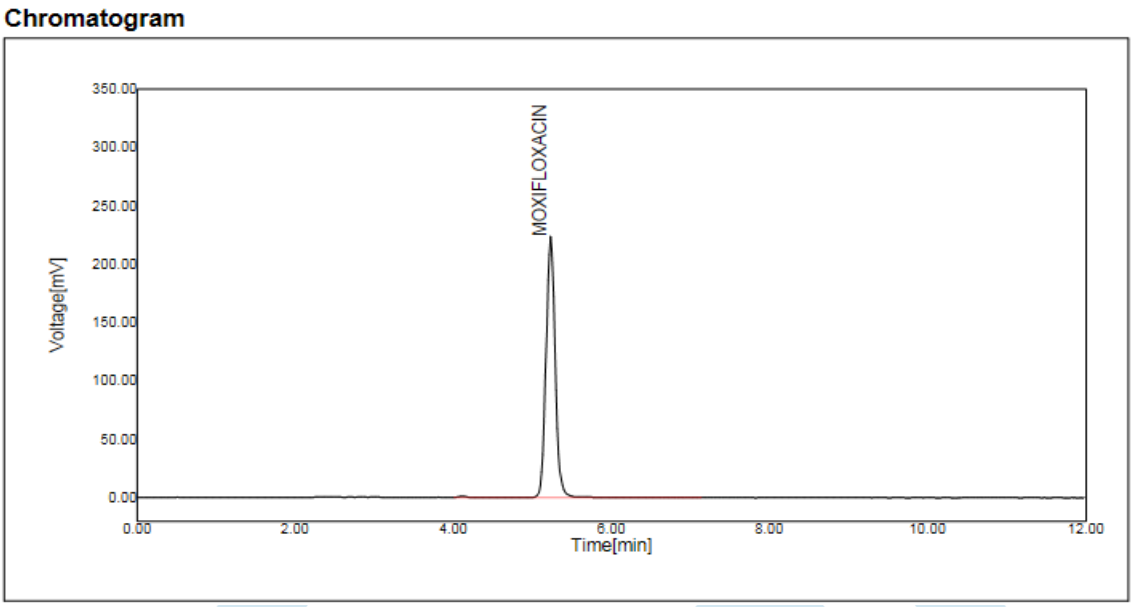


Fig:- 100% recovery

Observation:

Sr.No.	Name	RT (min)	Area(mV*s)
1	Moxifloxacin	5.23	1819.8060

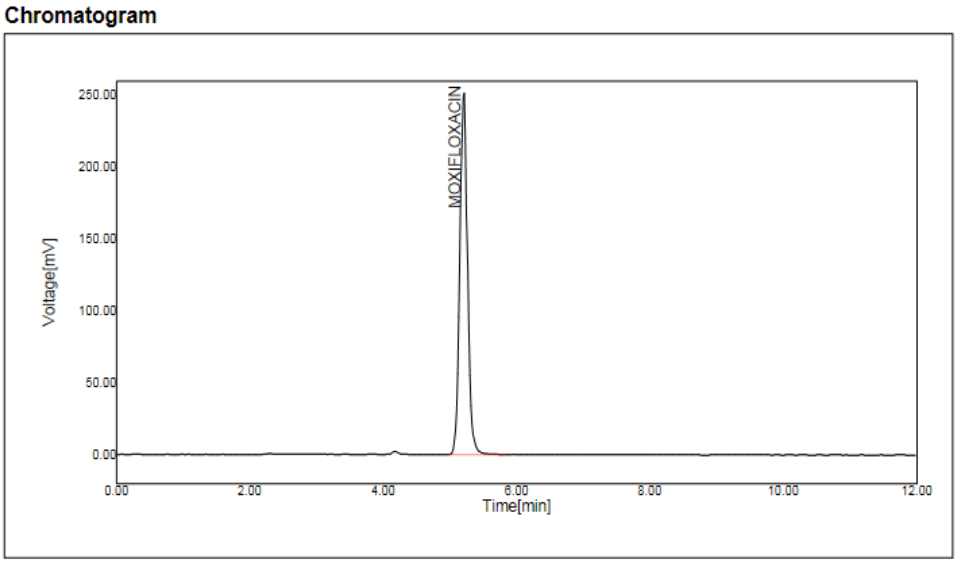


Fig:- 120% recovery

Observation:

Sr.No.	Name	RT (min)	Area(mV*s)
1	Moxifloxacin	5.22	2024.0776

Analysis: The recovery obtained as a measure of accuracy, expressed in percent of mean recovery is 99.96%. The acceptance criterion is 97 % to 103 %. Hence, the method is accurate.

- Also, the accuracy of the method is determined by measuring the 21 assay determinations (7 concentrations / 3 replicates) over seven different test concentrations i.e. 80%, 100%and 120% of test concentration. The average assay obtained is 20ml
- The accuracy of the method is measured and reported as %RSD of the 21 assay determinations. The RSD of 3 assay determinations is 0.94%, which is well within acceptable limit of 2.0%.
- Hence, the method is accurate within the range of 80%-120%of Test concentration and the difference between the mean assay of 3 determinations over 9 concentrations and the assay value obtained from precision is less than 2% of the absolute assay value.

PRECISION

Intraday `Precision

The repeatability of the method is established by estimating the assay for different sample preparations of same batch. The average assay for Moxifloxacin determinations. The RSD of assays is found to be 0.66%, which is well within the acceptance criteria of 2.0%.

Name	Preparation	% Assay
Set-1	Prep-1	99.26
	Prep-2	98.19
Set-2	Prep-1	99.28
	Prep-2	99.89
Mean		**99.16**
SD		**0.7067**
%RSD		**0.71**

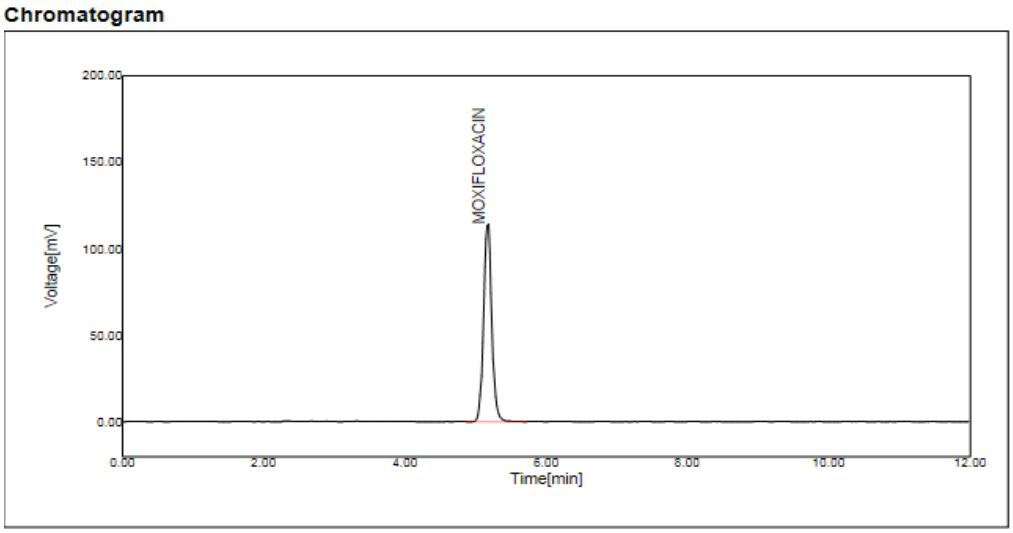


Fig:- Intraday Precision of Std Solution

Observation:

Sr.No.	Name	RT (min)	Area(mV*s)	TP	TF
1	Moxifloxacin	5.18	924.5242	12665	0.98

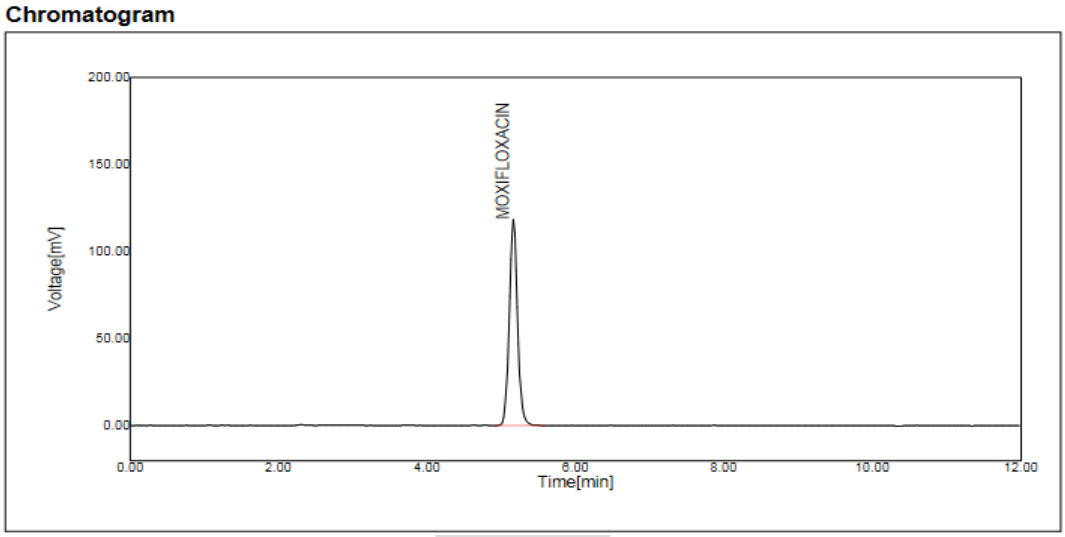


Fig:- Intraday Precision of Test Solution

Observation:

Sr.No.	Name	RT (min)	Area(mV*s)
1	Moxifloxacin	5.17	913.7983

Interday Precision / Ruggedness

The Intermediate Precision of the method is established by estimating the assay of different sample preparations of the same batch by different Chemist on a different system using different column and on a different day. The average assay form Moxifloxacin assay determination.The RSD of assays is found to be 1.10%, which is well within the acceptance criteria of 2.0%

Table 7.2: Assay of sample preparations of same batch

Name	Preparation	% Assay
Day-1	Prep-1	99.26

	Prep-2	98.19
Day-2	Prep-1	100.84
	Prep-2	99.65
Mean		**99.49**
SD		**1.0941**
%RSD		**1.10**

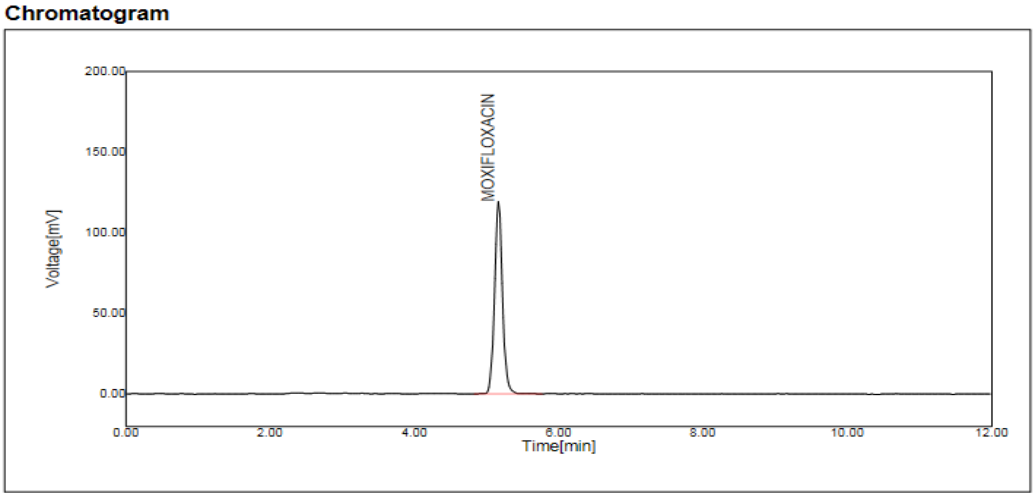


Fig:- Interday Precision of Std Solution

Observation:

Sr.No.	Name	RT (min)	Area(mV*s)	TP	TF
1	Moxifloxacin	5.17	912.2319	12032	1.06

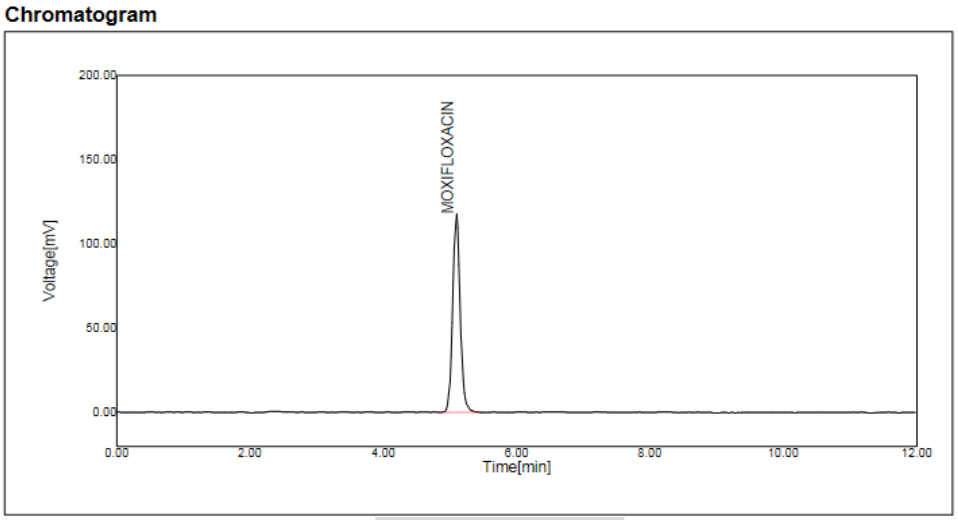


Fig:- Interday Precision of Test Solution

Observation:

Sr.No.	Name	RT (min)	Area(mV*s)
1	Moxifloxacin	5.10	923.9946

Analysis: Two different analysts carried out assay of the same batch of Moxifloxcin on two different instruments and two different columns. The assay results of two analysts are shown in the Table respectively. The difference in the assay of Moxifloxacin between two analysts is less than 2.0% of absolute assay value.

Table 7.23: Observation of Precision of Moxifloxcin

Sr.No.	Analyst-1	Analyst-2
Number of sample	1	1
Mean(assay)	99.16	0.71
%RSD	99.49	1.10

Analysis:

The assay results of two analysts carried out on the same batch of Moxifloxacin on different instruments, different columns and on different day respectively. The difference in the assay of Moxifloxacin between two analysts is less than 2.0% of absolute assay value. Hence, the method is reproducible and very precise with any make of HPLC instruments and with any qualified analyst for the estimation of Assay of Moxifloxacin

Observation of Robustness method of determining assay of Moxifloxacin

Sr.No.	Condition	Std.Wt	%Assay	Average Assay
1	Flow rate (0.9ml/min)	20	99.04	99.18
	Flow rate (1.10ml/min)	20.1	99.32	
2	MeOH:Buffer (55:45)	20	98.65	99.6
	MeOH:Buffer(65:35)	20.1	100.55	
3	λ- 288	20	99.32	98.71
	λ- 292	20.1	98.11	
			Mean	99.16
			SD	0.45
			%RSD	0.45

Chromatogram

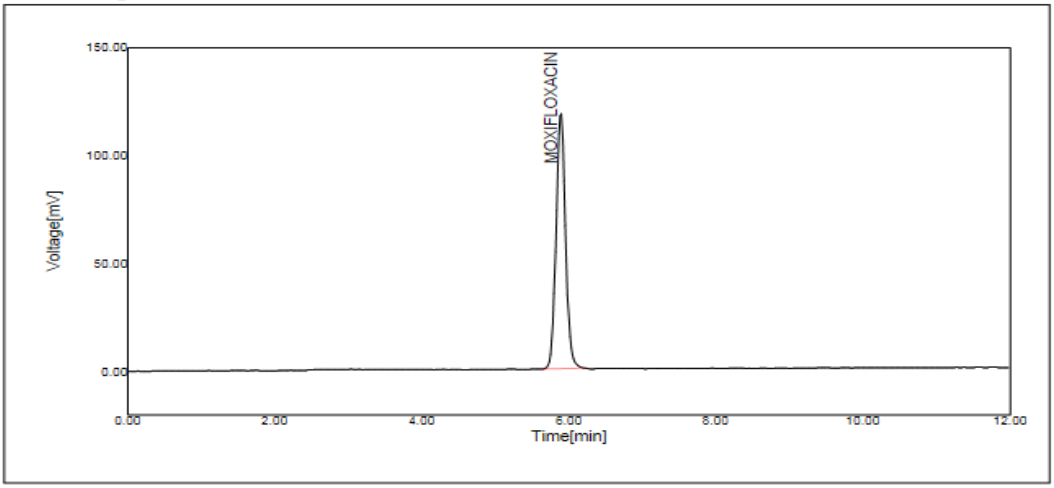


Fig :- Flow Rate 0.9ml/min (Standard)

Observation:

Sr.No.	Name	RT (min)	Area(mV*s)	TP	TF
1	Moxifloxacin	5.90	1056.7548	11663	1

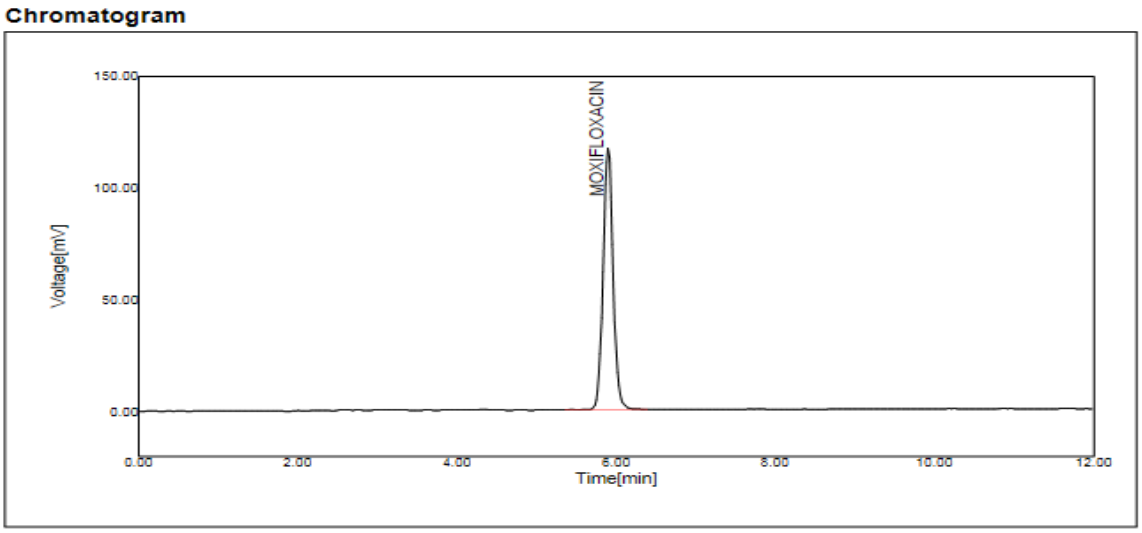


Fig :- Flow Rate 0.9ml/min (Test)

Observation:

Sr.No.	Name	RT (min)	Area(mV*s)
1	Moxifloxacin	5.90	1025.9115

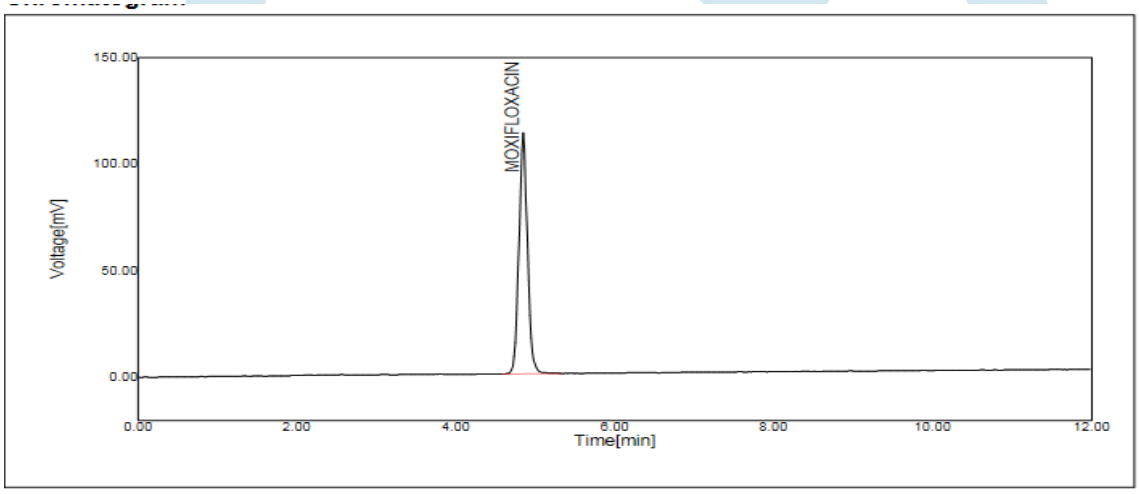


Fig :- Flow Rate 1.10 ml/min (Standard)

Observation:

Sr.No.	Name	RT (min)	Area(mV*s)	TP	TF
1	Moxifloxacin	4.85	849.8355	12639	1.05

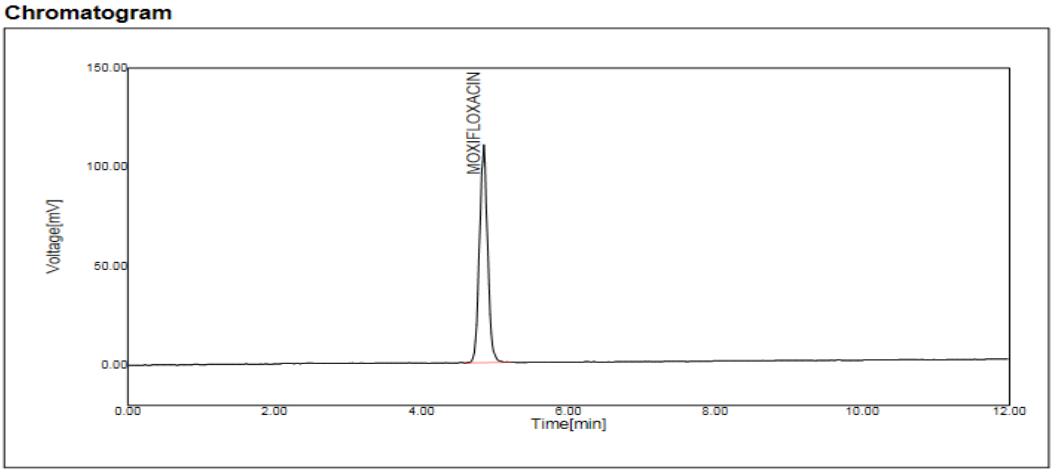


Fig :- Flow Rate 1.10 ml/min (Test)

Observation:

Sr.No.	Name	RT (min)	Area(mV*s)

1	Moxifloxacin	4.85	851.0593

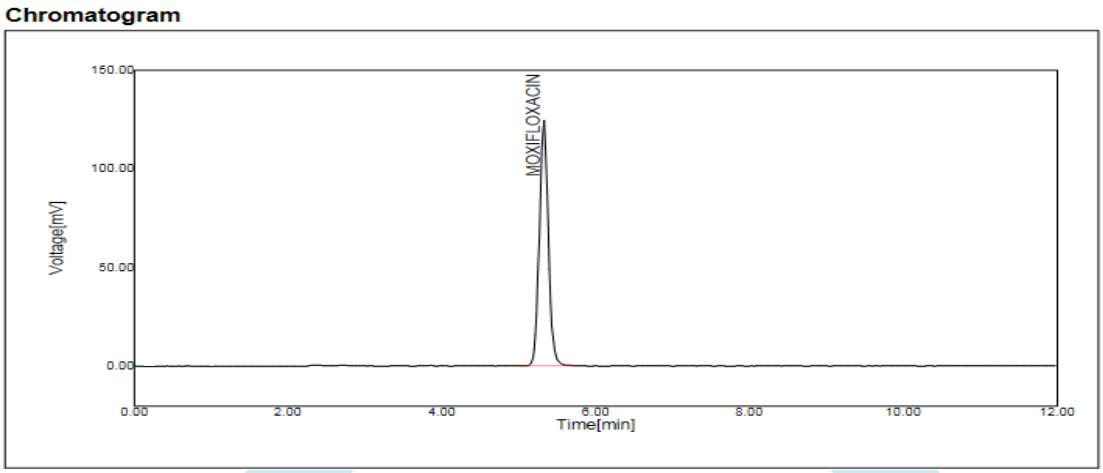


Fig :- Wavelength(λ) 288nm (Standard)

Observation:

Sr.No.	Name	RT (min)	Area(mV*s)	TP	TF
1	Moxifloxacin	5.33	1016.9319	9217	1.01

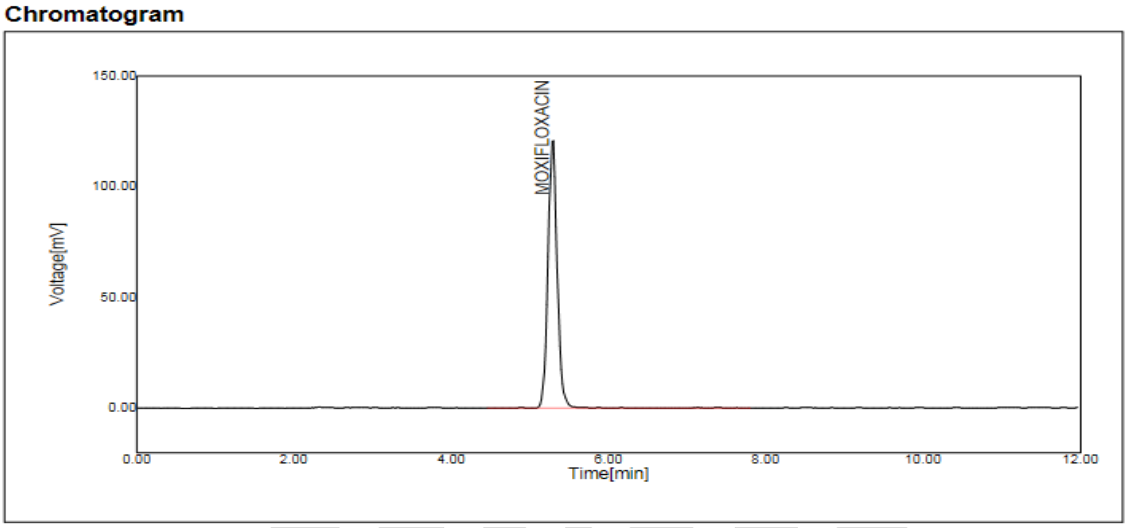


Fig :- Wavelength(λ) 288nm (Test)

Observation:

Sr.No.	Name	RT (min)	Area(mV*s)
1	Moxifloxacin	5.30	1004.7930

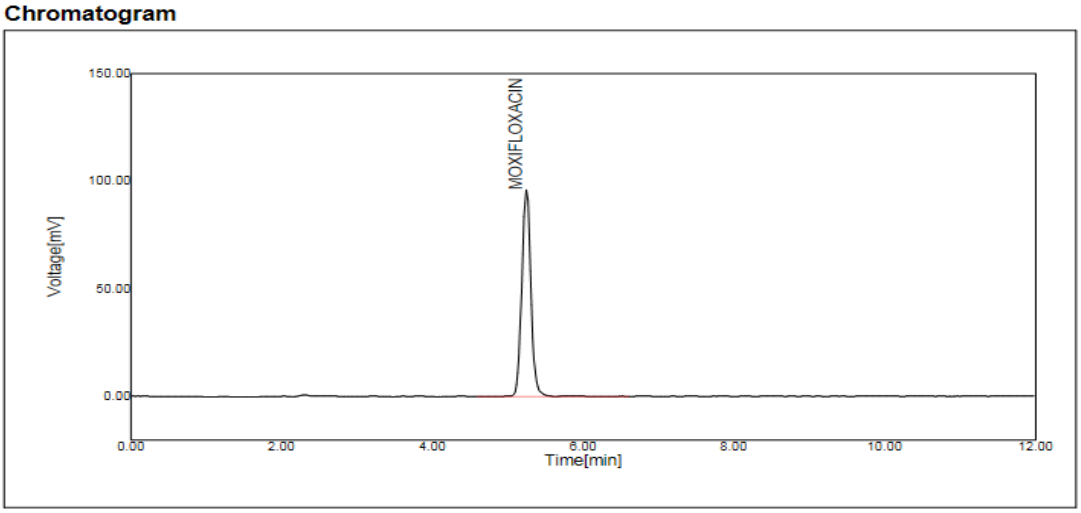


Fig :- Wavelength(λ) 292nm (Standard)

Observation:

Sr.No.	Name	RT (min)	Area(mV*s)	TP	TF
1	Moxifloxacin	5.25	814.0369	8858	1.04

Chromatogram

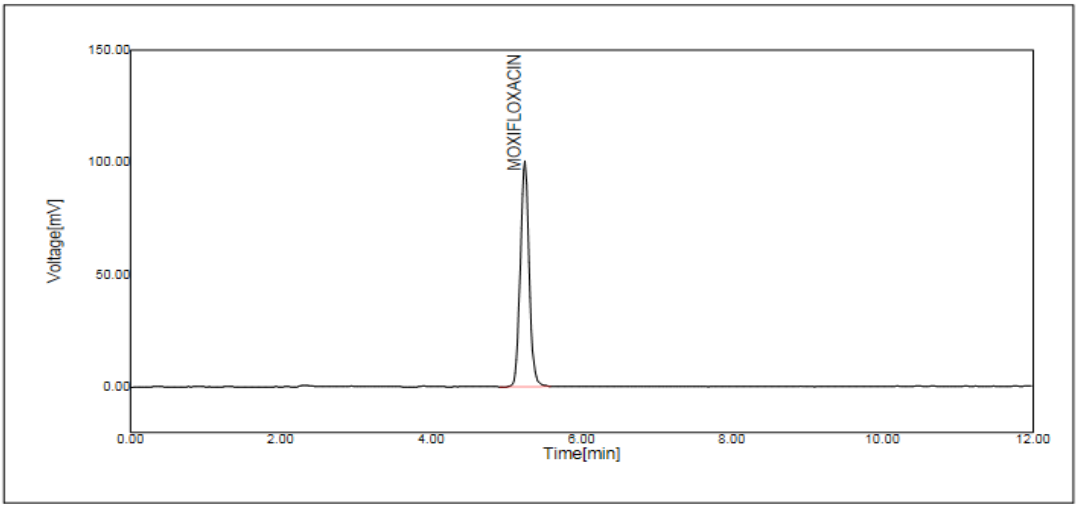


Fig :- Wavelength(λ) 292nm (Test)

Observation:

Sr.No.	Name	RT (min)	Area(mV*s)
1	Moxifloxacin	5.25	811.9684

Chromatogram

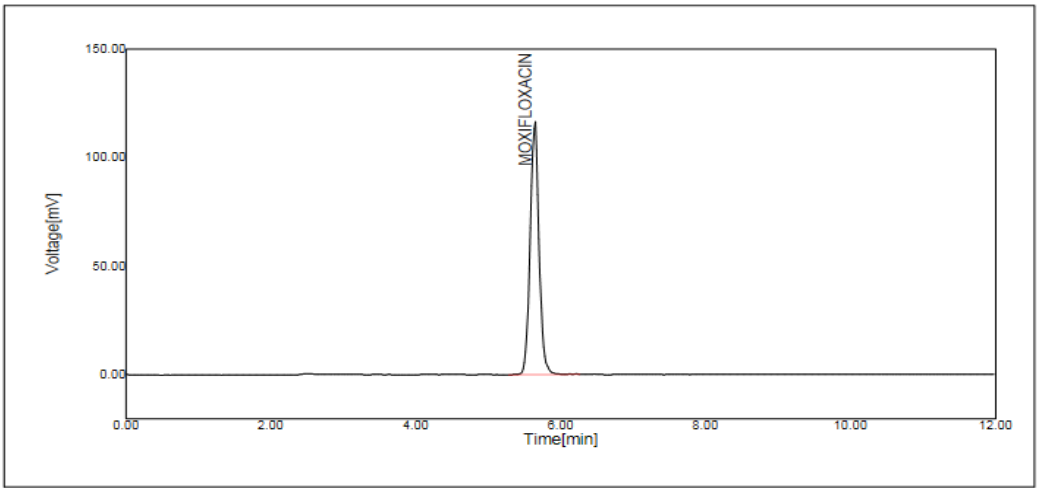


Fig :- Methanol: Buffer (55:45) (Standard)

Observation:

Sr.No.	Name	RT (min)	Area(mV*s)	TP	TF
1	Moxifloxacin	5.65	987.9197	11584	1

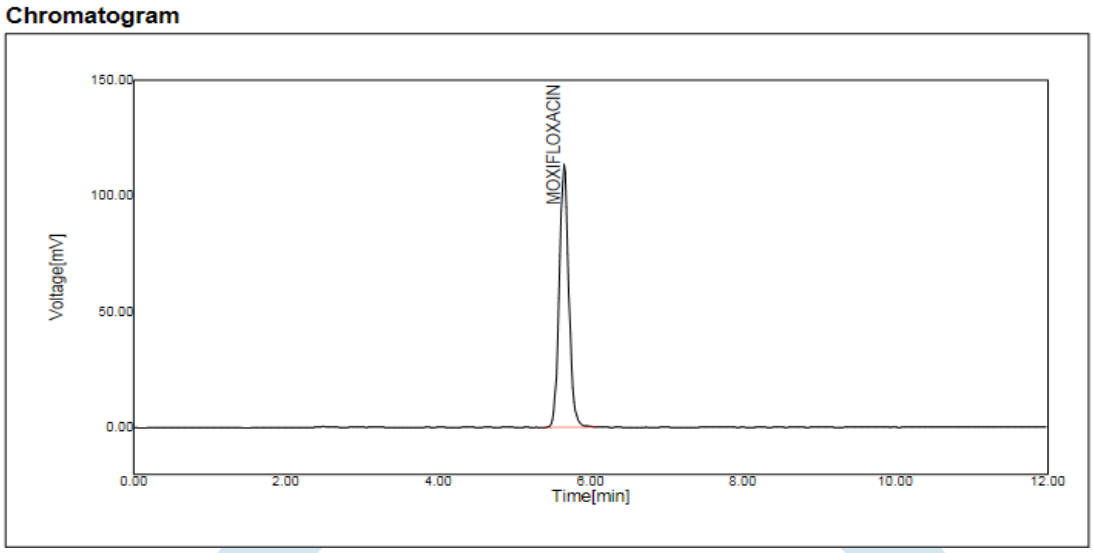


Fig: - Methanol: Buffer (55:45) (Test)

Observation:

Sr.No.	Name	RT (min)	Area(mV*s)
1	Moxifloxacin	5.65	983.2440

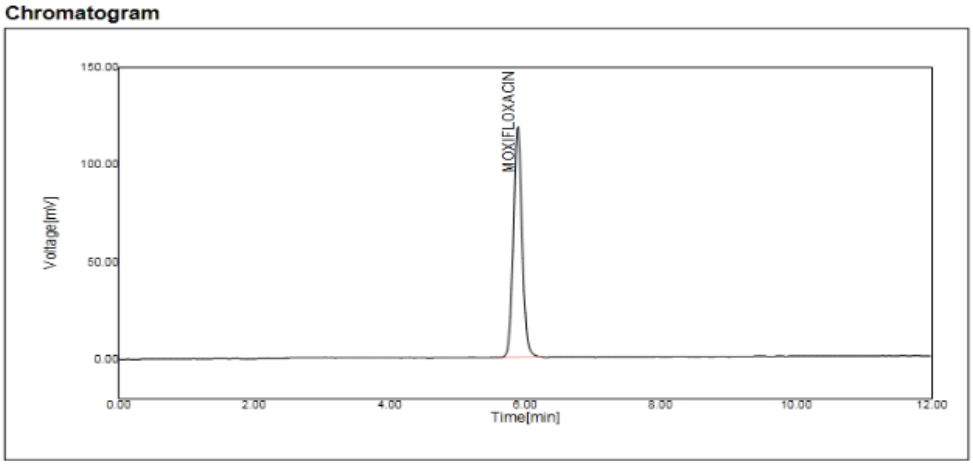


Fig :- Methanol: Buffer (65:35) (Standard)

Observation:

Sr.No.	Name	RT (min)	Area(mV*s)	TP	TF
1	Moxifloxacin	4.98	987.5648	11586	1.06

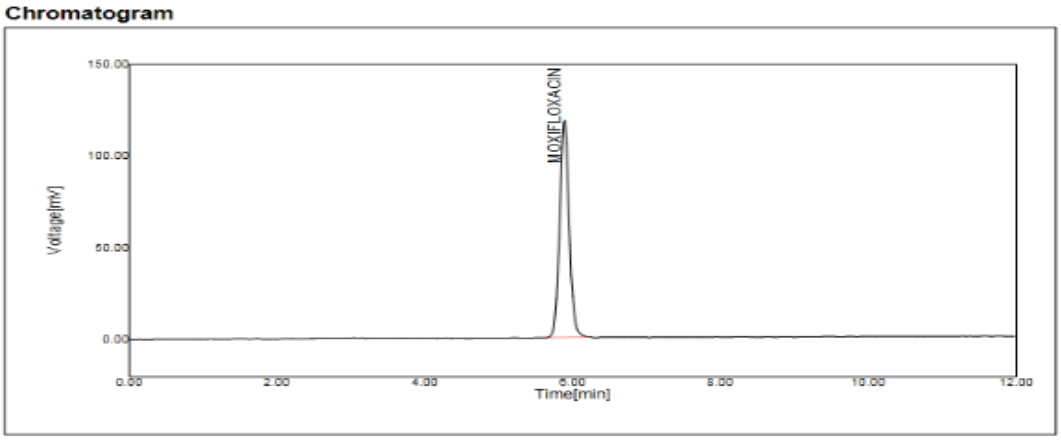


Fig :- Methanol: Buffer (55:45) (Test)

Observation:

Sr.No.	Name	RT (min)	Area(mV*s)	TP	TF
1	Moxifloxacin	4.97	981.5671	11127	1.04

Observation: The average assay obtained for Moxifloxacin under deliberately modified chromatographic condition ml per sample.The RSD for the assay values of Moxifloxacin obtained under deliberately modified chromatographic conditions is 0.45%. The difference between the assay under deliberately modified chromatographic conditions and the assay obtained under Precision is less than 2.0% of absolute value.

Analysis: The RSD of assay of Moxifloxacin under deliberately modified chromatographic conditions is less than 2.0% and the difference between the assays under deliberately modified chromatographic conditions and the assay obtained under Precision is less than 2.0% of absolute value.

7.2.7 FORCE DEGRADATION

The forced degradation studies are performed to establish the stability indicating nature of the assay method and to observe any degraded compounds. Moxifloxacin WS and Sample are subjected to stress with 5N HCl, 5N NaOH, 3% H2O2; 105°C, UV and Thermal degradation at 60^0C in presence of 80%RH. All the above solutions are chromatographed and recorded the chromatograms.

Table 6.26 The following stress conditions are followed for degradation

Sample stress condition	Description of stress condition
Acid degradation	5N HCl heated at about 60^0C for 10 min on a water bath.
Alkali degradation	5N NaOH heated at about 60^0C for 10 min on a water bath.
Oxidation degradation	3%v/v H2O2 heated at about 60^0C for 10 min on a water bath.
UV degradation	Exposure to UV light for 7 days
Thermal degradation in presence of humidity	60°C @ 80%RH for 7 days
Thermal degradation	105°C for 12 hours

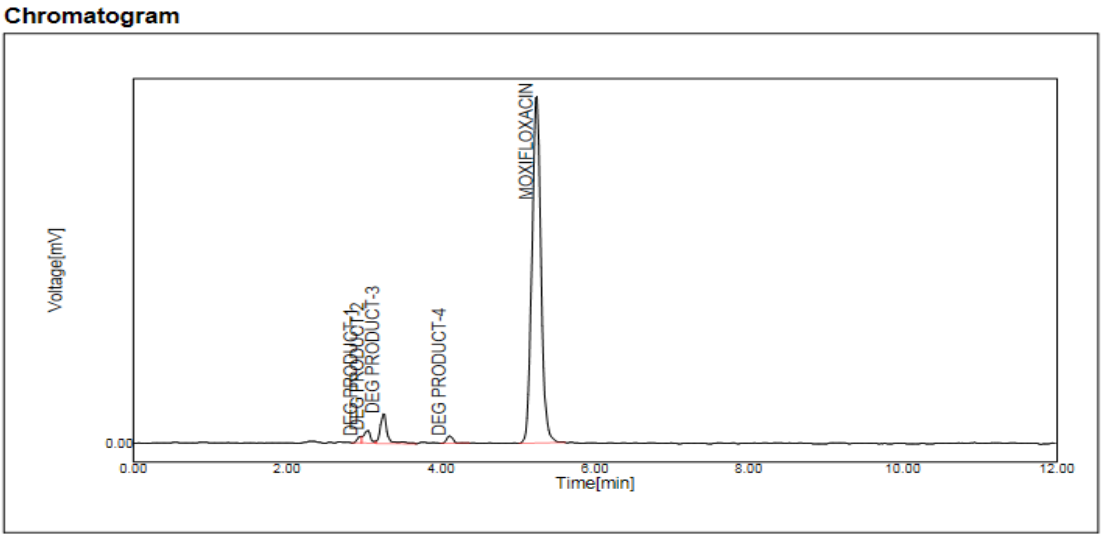


Fig:- Force degradation of Acid Hydrolysis

Observation:

Sr.No.	Name	RT	Area	%Area	Resolution
1	DEG Product-1	2.97	6.6357	0.75	0.00
2	DEG Product-2	3.05	21.7058	2.44	0.56 1
3	DEG Product-3	3.25	52.2246	5.87	1.76
4	DEG Product-4	4.12	11.9612	1.34	8.24

5	Moxifloxacin	5.25	797.3650	89.60	6.98
sum			889.8922		

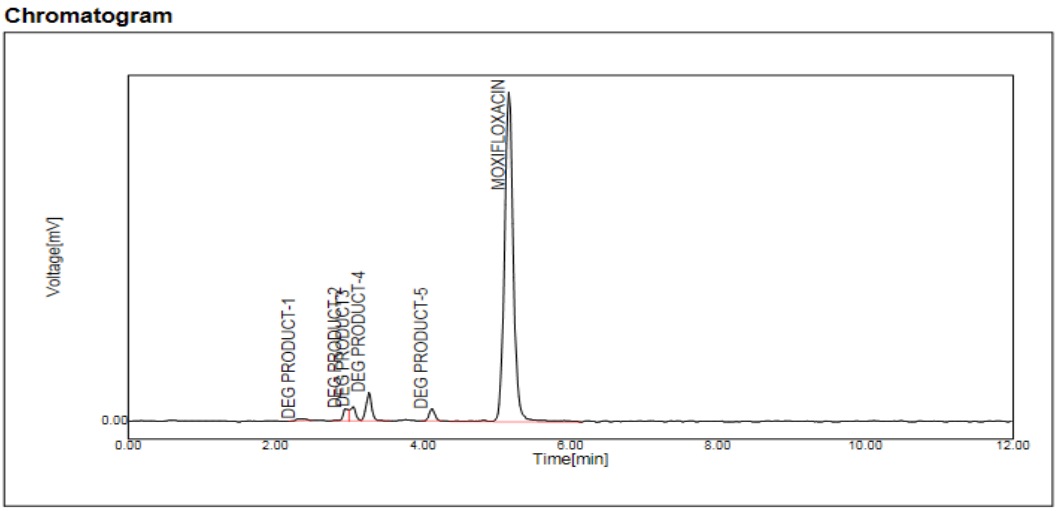


Fig:- Force degradation of Base Hydrolysis

Observation:

Sr.No.	Name	RT	Area	%Area	Resolution
1	DEG Product-1	2.32	5.8197	0.61	0.00
2	DEG Product-2	2.95	18.5355	1.95	3.17
3	DEG Product-3	3.05	22.6949	2.39	0.68
4	DEG Product-4	3.27	46.0941	4.86	1.49
5	DEG Product-4	4.12	20.1817	2.13	7.37
5	Moxifloxacin	5.17	835.6838	88.06	6.34
sum			949.0098		

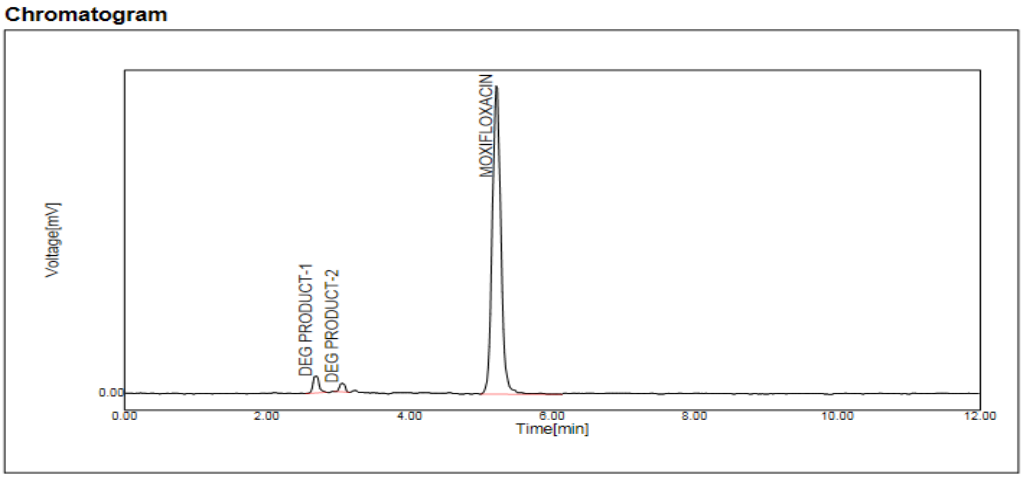


Fig:- Force degradation of Perhydroxide

Observation:

Sr.No.	Name	RT	Area	%Area	Resolution
1	DEG Product-1	2.68	33.7144	3.64	0.00

2	DEG Product-2	3.05	15.4856	1.67	2.96
3	Moxifloxacin	5.22	877.3475	94.69	12.72
sum			926.5475		

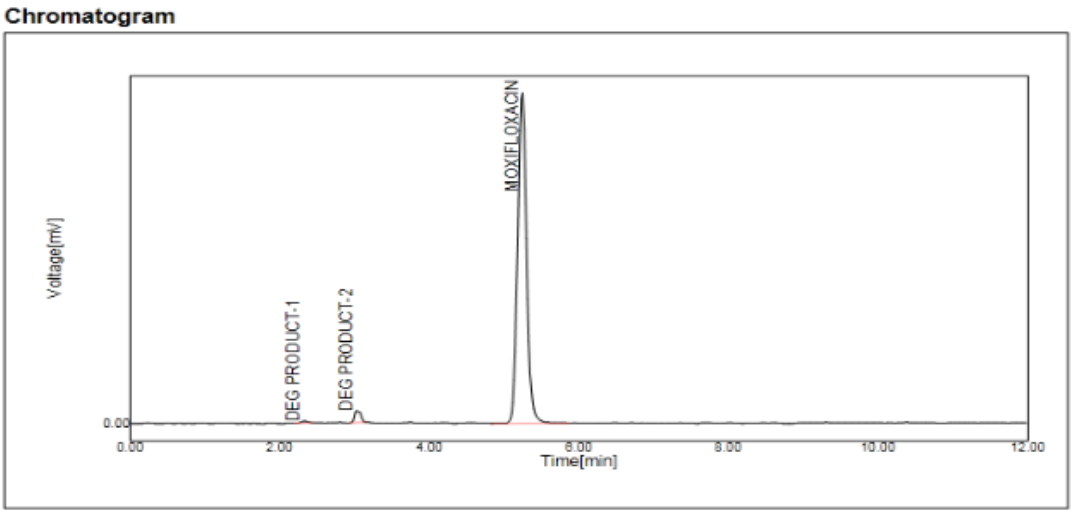


Fig:- Force degradation of Photolytic

Observation:

Sr.No.	Name	RT	Area	%Area	Resolution
1	DEG Product-1	2.32	3.4050	0.37	0.00
2	DEG Product-2	3.03	20.7002	2.24	5.26
3	Moxifloxacin		898.5419	97.22	12.49
sum			922.6471		

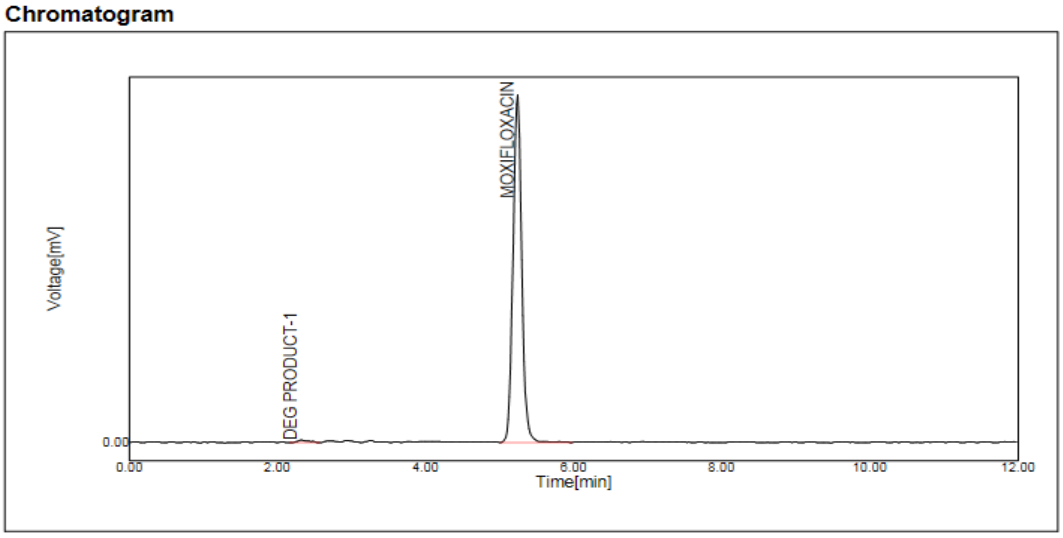


Fig:- Force degradation of Thermal

Observation:

Sr.No.	Name	RT	Area	%Area	Resolution
1	DEG Product-1	2.32	8.1383	0.91	0.00
2	Moxifloxacin	5.25	887.3117	99.09	10.18

Sum			895.4500		

Analysis: There is no interference between the peaks obtained for the chromatograms of degradation preparations. The degradation peaks under forced degradation are well separated from each other and the resolution between peaks to peak is more than 1.0. The peak purity for Moxifloxacin peak is passing. Hence, the method is very precise, selective and specific to the estimation of Assay of Moxifloxacin HPLC and the same method is stability indicating, as the degraded products are well separated from Moxifloxacin and as well from each adjacent peaks.

7.2.8 LOD & LOQ

Determination of LOD & LOQ is based on the comparison of the SD of the peak area and the slop of calibration curve of Moxifloxacin was found to be 23.37µg, respectively where as Moxifloxacin w as found tobe 0.44µg/ml and 1.31µg/ml

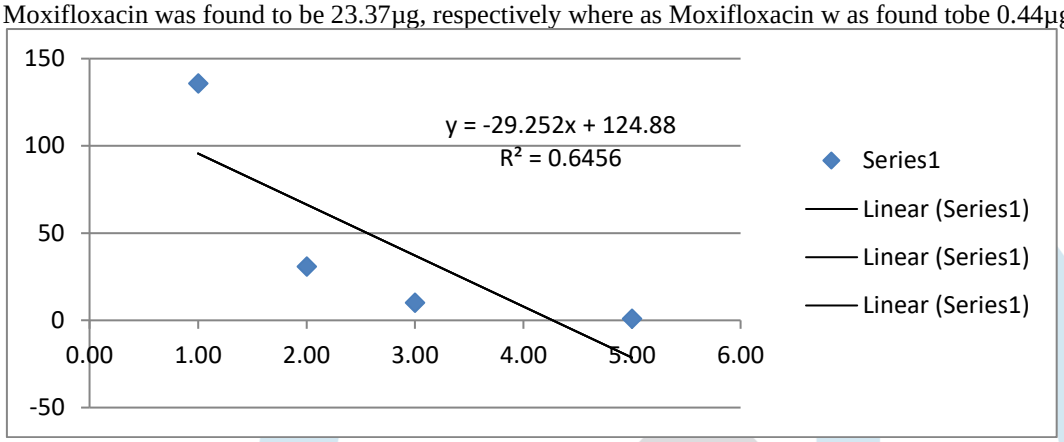


Table 7.27 the following LOD & LOD are followed

LOD LOQ	**Con.(ppm or µg/ml)**	**Area**
1	**40**	**935.2259**
2	**20**	**467.2849**
3	**10**	**230.1173**
4	**6**	**135.8012**
5	**1**	**30.7886**
6	**0**	**10.1381**
Correlation		**0.99997**
STEYX		**3.0721**
SLOPE		**23.3786**

Analysis: The assay result of two analysts carried out of LOD and LOQ of Moxifloxacin was found to be 0.99µg/ml. This result is passed in acceptance criteria.

Marketed Preparation:

The marketed preparation of analytical solutions is established by injecting the standard and sample preparations. The RSD of peak responses for Standard and Sample preparation is found to be 0.87 respectively, which is well within the acceptance criteria of 2.0%.

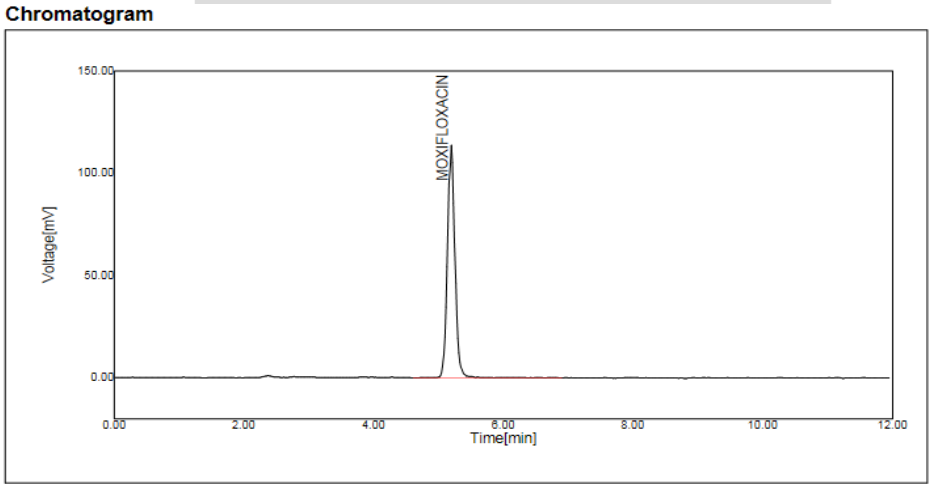


Fig : Marketed Product of Standard

No.	Name	RT(min)	Area(mV*s)	TP	TF
1	MOXIFLOXACIN	5.20	939.1890	11164	1.06

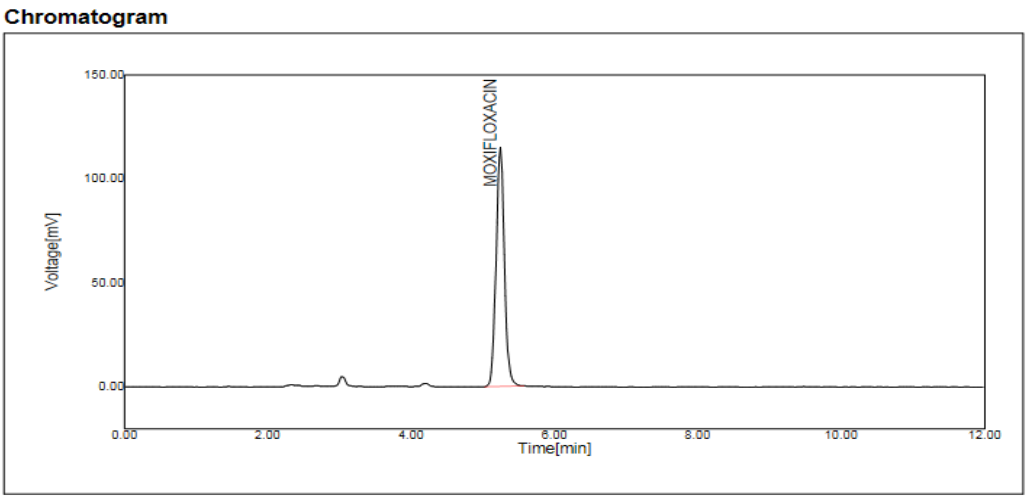


Fig : Marketed Product of Test

No.	Name	RT(min)	Area(mV*s)
1	MOXIFLOXACIN	5.25	921.1875

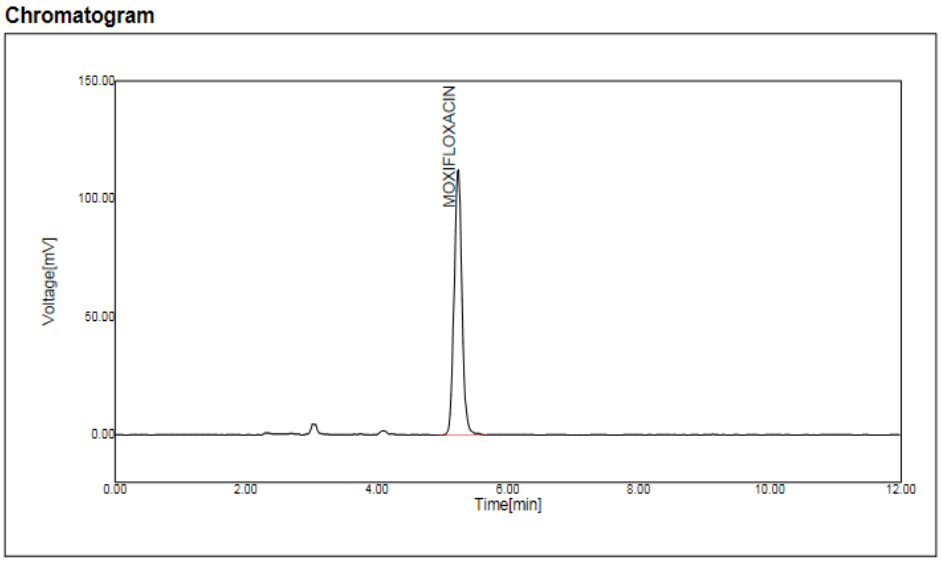


Fig : Marketed Product of Test

No.	Name	RT(min)	Area(mV*s)
1	MOXIFLOXACIN	5.25	932.1566

Results:

The average assay obtained for Moxifloxacin under deliberately modified chromatographic condition is ml per sample. The RSD for the assay values of Moxifloxacin obtained under deliberately modified chromatographic conditions is 0.87%. The difference between the assay under deliberately modified chromatographic conditions and the assay obtained under precision is less than 2.0% of the absolute value.

Conclusion:

The RSD of an assay of Moxifloxacin under deliberately modified chromatographic conditions is less than 2.0% and the difference between the assays under deliberately modified chromatographic conditions and the assay obtained under precision is less than 2.0% of the absolute value.

The relationship between the concentration of Moxifloxacin taken and the response (peak area) measured for Moxifloxacin is linear in the range examined and the regression coefficient is more than 0.99 in all the three cases. Hence, the method is linear in the range of 20% to 60% of Moxifloxacin test concentration (i.e. 0.20mg/ml of Moxifloxacin).

The assay result of two analyses carried out of LOD and LOQ of Moxifloxacin was found to be 0.99µg/ml. This result is passed through the acceptance criteria. In this way, all the objectives of the project are fulfilled here.

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