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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF LOTEPREDNOL ETABONATE AND MOXIFLOXACIN HYDROCHLORIDE IN EYE DROPS

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ABSTRACT

A simple, rapid, economical, precise and accurate Stability indicating RP-HPLC method for simultaneous estimation of Moxifloxacin Hydrochloride and Loteprednol Etabonate in Their Combined Dosage Form has been developed.

A reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Moxifloxacin Hydrochloride and Loteprednol Etabonate in Their Combined Dosage Form. The separation was achieved by LC- 20 AT C18 (250mm x 4.6 mm x 2.6 µm) column and Buffer (Potassium Phosphate, pH 3.0): Acetonitrile (60:40) as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 229 nm. Retention time of Loteprednol Etabonate and Moxifloxacin Hydrochloride were found to be 3.550 min and 5.307 min respectively. The method has been validated for linearity, accuracy and precision. Linearity observed for Moxifloxacin Hydrochloride 5-15 µg/ml and for Loteprednol Etabonate 5-15 µg/ml. Developed method was found to be accurate, precise and rapid for simultaneous estimation of Moxifloxacin Hydrochloride and Loteprednol Etabonate in their Combined Dosage Form. The drug was subjected to stress condition of Hydrolysis, Oxidation, Photolysis and Thermal degradation. The proposed method was successfully applied for the simultaneous estimation of both the drugs in commercial Combined dosage form.

Keywords: Moxifloxacin Hydrochloride, Loteprednol Etabonate, Stability indicating RP-HPLC Method, Validation

INTRODUCTION:

Moxifloxacin Hydrochloride - 7-[(4aS,7aS)-octahydro-1H-pyrrolo[3,4-b] pyridin-6-yl]-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid is a synthetic fluoroquinolone antibiotic agent **[1-2]** and Loteprednol Etabonate- Chloromethyl, 17 ethoxycarbonyloxy11hydroxy 10,13 dimethyl3 oxo7,8,9,11,12,14,15,16 octahydro 6Hcyclopenta [a] phenanthrene 17carboxylate is a topical corticoid anti-inflammatory **[3-4]**. Structure of MOX and LOT is shown in **Figure 1, 2**. They are used in treatment of eye infection with inflammation. It is used in ophthalmic solution for the treatment of steroid responsive inflammatory conditions of the eye such as allergic conjunctivitis, uveitis, acne, rosacea. The combination gives Additive effect so, formulation is used for the treatment of uveitis and reduction of post operative inflammatory conditions of the eye **[5-6]**. Literature review reveals that numbers of individual analytical methods available for estimation of Moxifloxacin Hydrochloride and Loteprednol Etabonate in their individual and Combined dosage forms. But no method has been reported for stability indicating simultaneous estimation of Moxifloxacin Hydrochloride and Loteprednol Etabonate in combined pharmaceutical dosage form by RP-HPLC **[7-11]**. With the advent of International Conference on Harmonization (ICH) guidelines, the requirement of establishment of stability -indicating assay method (SIAM) has become more clearly mandated. The guidelines explicitly require conduct of forced decomposition studies under a variety of conditions, like pH, light, oxidation, etc. and separation of drug from degradation products **[12]**. Thus, the objectives of this work are to develop a new sensitive stability indicating RP-HPLC method for simultaneous determination of Moxifloxacin Hydrochloride and Loteprednol Etabonate in mixture **[13-19]**. Also, it is validated for market product containing Moxifloxacin Hydrochloride and Loteprednol Etabonate in ophthalmic dosage form (eye drops).

MATERIALS AND METHODS

Standard Moxifloxacin Hydrochloride and Loteprednol Etabonate were obtained as gift sample from OREX PHARMA and RIVAN LABORATORY respectively. Shimadzu HPLC was used. Methanol - HPLC grade, Water - HPLC grade and Potassium Dihydrogen Phosphate and Ammonium Acetate of AR grade from Merck India Ltd., Mumbai, was used. A commercial dosage form MOXINIX-LP was purchased from local market.

Selection of Detection wavelength:

10 mg Moxifloxacin Hydrochloride was added into 100ml Volumetric flask and volume was made up to the mark with Methanol. (10 µg/ml in methanol) Further 1ml of above solution was taken and transferred into 10ml volumetric flask and volume was made up to the mark with Methanol.

10 mg Loteprednol Etabonate was added into 100ml Volumetric flask and volume made up to the mark with Methanol. (10 µg/ml in methanol) Further 1ml of above solution was taken and transferred into 10ml volumetric flask and volume was made up to the mark with Methanol.

Both solutions were scanned between 200 - 400 nm.

Wavelength was selected from the overlay spectra of above solutions.

Selection of Mobile phase

Various mobile phases were tried. Trial contains various mobile phases which consisted of Methanol, Water, Buffer (potassium dihydrogen Phosphate) and Acetonitrile in different proportions with various pH and different volumes at flow rate 1 ml/min were tried. Chromatogram in optimized mobile phase is shown in Figure.

Preparation of standard and stock solution

(A) Moxifloxacin HCl standard stock solution: (100 µg/ml)

A 10 mg of Moxifloxacin HCl was weighed and transferred to a 100 ml volumetric flask. volume was made up to the mark with mobile phase.

(B) Loteprednol Etabonate standard stock solution: (100 µg/ml)

A 10 mg of Loteprednol Etabonate was weighed and transferred to a 100 ml volumetric flask. volume was made up to the mark with mobile phase

(C) Preparation of standard solution of binary mixtures of Moxifloxacin HCl (10 µg/mL) and Loteprednol Etabonate (10 µg/ml)

Take 1 mL from the Moxifloxacin HCl stock solution and 1ml from Loteprednol Etabonate stock solution and transferred to 10 ml volumetric flask and volume made up to the mark by mobile phase which was used in particular trials.

Optimized Chromatographic Conditions

Parameters	Chromatographic Condition
Mode of elution	Isocratic
Mobile Phase	Buffer (Potassium Phosphate, pH 3.0): Acetonitrile (60:40)
Column	C18 (25cm x 0.46 cm) Hypersil BDS
Flow rate	1ml/min
Runtime	8 min
Injection volume	20 µl
Detection wavelength	229 nm

Calibration of standards

Calibration curve of MOX and LOT were prepared for concentration range of 5-15 µg/ml was prepared by pipette out different volumes from each stock solution and dilute up to the marks with mobile phase.

METHOD VALIDATION

Linearity and Range

The linearity for Moxifloxacin Hydrochloride and Loteprednol Etabonate were assessed by analysis of combined standard solution in range of 5-15 µg/ml and 5-15 µg/ml respectively,

5,7.5,10,12.5,15 ml solutions were pipette out from the Stock solution of Moxifloxacin Hydrochloride (100 µg/ml) and Loteprednol Etabonate (100 µg/ml) and transferred to 100 ml volumetric flask and volume was made up with mobile phase to obtain 5,7.5,10,12.5 and 15 µg/ml, and 5,7.5,10,12.5 and 15 µg/ml for Moxifloxacin Hydrochloride and Loteprednol Etabonate respectively. Linearity plots were shown in Figure. Results for linearity are shown in table.

Repeatability

The data for repeatability of peak area measurement for Moxifloxacin Hydrochloride (10 µg/ml) and Loteprednol Etabonate (10 µg/ml) based on six measurements of same solution. The % RSD for Moxifloxacin Hydrochloride and Loteprednol Etabonate are shown in table.

Intra-day Precision

Standard solution containing (5,10,15 µg/ml) of Moxifloxacin Hydrochloride and (5,10,15 µg/ml) of Loteprednol Etabonate were analysed three times on the same day and % R.S.D was calculated.

Inter-day Precision

Standard solution containing (5,10,15 µg/ml) of Moxifloxacin Hydrochloride and (5,10,15 µg/ml) of Loteprednol Etabonate were analysed three times on the different days and % R.S.D was calculated.

Accuracy

For Moxifloxacin Hydrochloride

5µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 229 nm. The amount of Moxifloxacin Hydrochloride was calculated at each level and % recoveries were computed.

For Loteprednol Etabonate

5µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 229 nm. The amount of Moxifloxacin Hydrochloride was calculated at each level and % recoveries were computed.

LOD and LOQ

Calibration curve was repeated for Three times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD = 3.3 * SD/slope of calibration curve

LOQ = 10 * SD/slope of calibration curve

Where, SD = Standard deviation of intercepts. The results were shown in table.

Robustness

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (± 0.2 ml/min) 0.8 ml/min and 1.2 ml/min.
2. pH of Mobile phase was changed (± 0.2) 3.2 and 2.8.
3. Ratio of Mobile phase was changed (±2) Buffer: Acetonitrile (62:38) and Buffer: Acetonitrile (58:42). The results were shown in table.

Assay of marketed formulation

Sample Stock Solution (Loteprednol Etabonate 100 µg/mL, and Moxifloxacin Hydrochloride 100 µg/mL):

Take Eye drops equivalent to 10 mg of Loteprednol Etabonate, and 10 mg of Moxifloxacin Hydrochloride was transferred to a 100 ml volumetric flask, Add 60 ml Mobile phase and Shake for 15 min and Volume made up to the mark with Mobile phase. The solution was filtered through Whatman filter paper no. 42.

Working Sample Preparation (Loteprednol Etabonate 10 µg/mL, and Moxifloxacin Hydrochloride 10 µg/mL):

1 ml was taken from standard stock solution and transferred to 10 ml volumetric flask and made-up volume up to the mark with the mobile phase

Inject above Solution 20 µl for Assay Analysis. Results are shown in table.

Forced degradation

Acid degradation

Acid decomposition studies were performed by taking one ml of stock solution and transferred in to a 10 ml of volumetric flask. Two ml of 0.1 N Hydrochloride solutions was added and mixed well and put for 4 hours. After time period, the volume was adjusted with diluent to get 10 µg/ml for Moxifloxacin Hydrochloride and 10 µg/ml for Loteprednol Etabonate.

Base degradation

Base decomposition studies were performed by taking one ml of stock solution and transferred in to a 10 ml of volumetric flask. Two ml of 0.1 N NaOH solutions was added, mixed well and put for 2.5 hours. After time period, the volume was adjusted with diluent to get 10 µg/ml for Moxifloxacin Hydrochloride and 10 µg/ml for Loteprednol Etabonate.

Oxidative Degradation

Oxidative decomposition study was performed by taking one ml of stock solution and transferred in to a 10 ml of volumetric flask. Two ml of 3% H2O2 solutions was added, mixed well and put for 4 hours. After time period, the volume was adjusted with diluent to get 10 µg/ml for Moxifloxacin Hydrochloride and 10 µg/ml for Loteprednol Etabonate.

Thermal degradation

Thermal Degradation studies were performed One ml of stock solution was transferred in to 10 ml of volumetric flask. The volumetric flask was stored in oven at 110°C for 3 hours. Then the volume was adjusted with diluent to get 10 µg/ml for Moxifloxacin Hydrochloride and 10 µg/ml for Loteprednol Etabonate.

Photolytic degradation

Photo Degradation studies were performed One ml of stock solution was transferred in to 10 ml of volumetric flask. The volumetric flask was kept in presence of Sunlight for 3.5 hours. Then the volume was adjusted with diluent to get 10 µg/ml for Moxifloxacin Hydrochloride and 10 µg/ml for Loteprednol Etabonate.

RESULT AND DISCUSSION

The present work aimed development and validation of stability indicating RP-HPLC method for simultaneous estimation of MOX and LOT. The melting point of MOX (236-242^{o}C) and LOT (220-222^{o}C) was found in the range. Method was developed in mobile phase containing Buffer (Potassium dihydrogen Phosphate) pH 3: Acetonitrile (60:40). Detection was carried out at 229 nm. Method was validated as per ICH guidelines. Linearity and regression data were shown in table and Figure. % recovery was within the range (98% - 102%). Results were shown in table. Hence it is found that the developed method is accurate. %RSD values were <2 for repeatability, intra-day and inter-day precision. Results were shown in table. So, the developed method was found to be precise. LOD and LOQ values were shown in table. LOD & LOQ confirms the method to be sensitive. Small changes were carried out in mobile phase and flow rate for robustness study, in that % RSD of area was found to be <2. Results were shown in table. So, the developed method was found to be robust. Various forced degradation conditions were performed in proposed method and it can efficiently separate all the degradation products from the drugs. % degradation values are 5% to 20% degradation of the drug substance, have been considered as reasonable and acceptable for validation of chromatographic assays. Results were shown in table. So, the developed method is stability indicating.

Table 1: Interpretation of IR Spectra of sample and standard of Moxifloxacin Hydrochloride and Loteprednol etabonate

Sr No.	Functional Group	Moxifloxacin Hydrochloride Frequency cm^{-1}	Loteprednol etabonate frequency cm^{-1}
1.	Secondary N-H Stretching	3529 cm-1	-
2.	OH Stretching	2929 cm-1	3300 cm-1
3.	C=O Stretching of keto group	1708 cm-1	1741 cm-1
4.	OH, Bending of COOH	1469 cm-1	-
5.	Aromatic C=H Stretching	-	2945 cm-1
6.	C-Cl Stretching	-	887 cm-1
7.	C-O Stretching of carbonyl group	-	1112.85 cm-1

Table 2: List of Mobile Phase trials

Sr No	Mobile Phase	Remark
1	Water: Methanol (60:40)	No peak observed
2	Water: Methanol (40:60)	No Peak Observed
3	Water: Methanol (20:80)	One Peak Observed
4	Water: Methanol (20:80)	Peak of Loteprednol Etabonate Confirmed
5	Water: Methanol (20:80)	Observed peak was not of Moxifloxacin Hydrochloride
6	Water: Acetonitrile (50:50)	No other peak observed but peak shape became good
7	Water: Acetonitrile (30:70)	Retention time reduced
8	Water: Acetonitrile (10:90)	Still second peak did not observe
9	Buffer (pH 4.0): Acetonitrile (40:60)	Second peak observed by using the buffer
10	Buffer (pH 4.0): Acetonitrile (50:50)	Resolution increased but first peak observed at solvent peak time
11	Buffer (pH 3.0): Acetonitrile (60:40)	Both peak follow SST Parameters
12	Buffer (pH 3.0): Acetonitrile (70:30)	Run time increased

Table 3: System Suitability Parameter

Parameters	Loteprednol Etabonate	Moxifloxacin Hydrochloride
Retention Time	3.550	5.307
Theoretical Plates	6982	7594
Asymmetry	1.208	1.294
Resolution	8.496	

Table 4: Linearity data for Moxifloxacin Hydrochloride

Sr. No	Concentration (µg/ml)	Area ± S.D (n=3)
1	5	1015.093
2	7.5	1527.068
3	10	2044.278
4	12.5	2449.249
5	15	3066.567

Table 5: Linearity data for Loteprednol Etabonate

Sr. No	Concentration (µg/ml)	Area ± S.D (n=3)
1	5	1328.029
2	7.5	1998.002
3	10	2675.351
4	12.5	3234.582
5	15	4012.424

Table 6: Repeatability data for Moxifloxacin Hydrochloride

Moxifloxacin Hydrochloride				
Sr No.	Conc (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
1.	10	2042.23	2043.99 ±8.55	0.42
		2046.27		
		2050.43		
		2044.22		
		2028.38		
		2052.46		

Table 7: Repeatability data for Loteprednol Etabonate

Loteprednol Etabonate				
Sr No.	**Conc (µg/ml)**	**Area**	**Mean ± S.D (n=6)**	**% R.S.D**
1.	**10**	**2672.67**	**2661.57±39.83**	**1.50**
		2678.04		
		2676.38		
		2675.36		
		2580.82		
		2686.19		

Table 8: Intraday precision data for estimation of Moxifloxacin Hydrochloride

	Moxifloxacin Hydrochloride		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	**5**	**1015.67 ± 11.74**	**1.16**
2	**10**	**2021.98 ± 17.78**	**0.88**
3	**15**	**3038.17 ± 18.38**	**0.60**

Table 9: Intraday precision data for estimation of Loteprednol Etabonate

	Loteprednol Etabonate		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	**5**	**1328.44 ± 18.72**	**1.41**
2	**10**	**2640.84 ± 27.46**	**1.04**
3	**15**	**3965.84± 32.75**	**0.83**

Table 10: Interday precision data for estimation of Moxifloxacin Hydrochloride

	Moxifloxacin Hydrochloride		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	**5**	**1016.67 ± 11.72**	**1.15**
2	**10**	**2027.37± 12.26**	**0.60**
3	**15**	**3045.58 ± 13.26**	**0.44**

Table 11: Interday precision data for estimation of Loteprednol Etabonate.

	Loteprednol Etabonate		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	**5**	**1326.36 ± 13.01**	**0.98**
2	**10**	**2642.31± 26.23**	**0.99**
3	**15**	**3974.03± 29.37**	**0.74**

Table 12: Recovery data for Moxifloxacin Hydrochloride For Loteprednol Etabonate

SR. NO.	**Conc. Level (%)**	**Sample Amount**	**Amount Added**	**Amount recovered (µg/ml)**	**% Recovery**	**% Mean Recovery ± S.D**
	80 %	**5**	**4**	**3.95**	**98.68**	**100.40 ± 1.52**
2		**5**	**4**	**4.04**	**101.00**	
3		**5**	**4**	**4.06**	**101.54**	
4	**100 %**	**5**	**5**	**4.95**	**98.98**	**99.71 ± 0.70**
5		**5**	**5**	**5.02**	**100.38**	
6		**5**	**5**	**4.99**	**99.77**	
7	**120 %**	**5**	**6**	**6.01**	**100.11**	**99.71 ± 0.46**
8		**5**	**6**	**5.95**	**99.21**	
9		**5**	**6**	**5.99**	**99.82**	

Table 13: Recovery data for Loteprednol Etabonate

SR. NO.	**Conc. Level (%)**	**Sample amount (µg/ml)**	**Amount Added (µg/ml)**	**Amount recovered (µg/ml)**	**% Recovery**	**% Mean Recovery ± S. D**
1	**80 %**	**5**	**4**	**3.94**	**98.39**	**99.77 ± 1.20**
2		**5**	**4**	**4.02**	**100.40**	

3		5	4	4.02	100.52	
4	100 %	5	5	4.96	99.16	99.70 ± 0.76
5		5	5	5.03	100.56	
6		5	5	4.97	99.37	
7	120 %	5	6	6.02	100.27	99.63 ± 0.76
8		5	6	5.93	98.79	
9		5	6	5.99	99.82	

Table 14: Limit of Detection data for Loteprednol Etabonate and Moxifloxacin Hydrochloride

Loteprednol Etabonate	Moxifloxacin Hydrochloride
LOD = 3.3 x (SD / Slope) = 3.3 x (52.26/264.2) = 0.65 µg/ml	LOD = 3.3 x (SD / Slope) = 3.3 x (50.90/201) = 0.84 µg/ml

Table 15: Limit of Quantitation data for Loteprednol Etabonate and Moxifloxacin Hydrochloride

Loteprednol Etabonate	Moxifloxacin Hydrochloride
LOQ = 10 x (SD / Slope) = 10 x (52.26/264.2) = 1.99 µg/ml	LOQ = 10 x (SD / Slope) = 10 x (4.921/284.5) = 2.53 µg/ml

Table 16: Robustness data for Moxifloxacin Hydrochloride

SR NO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (- 0.2)	Area at pH (+ 0.2)	Area at Mobile phase (-2)	Area at Mobile phase (+2)
1	2113.91	1990.96	2089.28	1946.06	2091.38	1968.97
2	2126.37	2003.22	2101.62	1958.26	2103.73	2001.21
3	2168.70	2025.50	2113.91	1990.46	2136.03	2013.49
% R.S.D	1.34	0.87	0.59	1.17	1.09	1.15

Table 17: Robustness data for Loteprednol Etabonate

SR NO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (-0.2)	Area at pH (+0.2)	Area at Mobile phase (-2)	Area at Mobile phase (+2)
1	2766.59	2605.71	2734.37	2546.90	2737.03	2603.09
2	2782.81	2621.76	2750.41	2562.87	2763.17	2649.13
3	2759.89	2678.74	2659.96	2527.52	2757.86	2601.21
% R.S.D	0.43	1.46	1.78	0.70	0.50	1.04

Table 18: Analysis on marketed formulation

Eye drop	Moxinix-LP	
Label claim	Loteprednol Etabonate (0.5 % w/v)	Moxifloxacin Hydrochloride (0.5 % w/v)
Assay (% of label claim*) Mean ± S. D.	99.80±0.59	99.45±1.15

Table 19: Analysis on marketed formulation at 0 hours

Eye drop	Moxinix-LP	
Label claim	Loteprednol Etabonate (0.5 % w/v)	Moxifloxacin Hydrochloride (0.5 % w/v)
Assay (% of label claim*) Mean ± S. D.	98.65±0.61	98.61±0.43

Table 20: Analysis on marketed formulation at 24 hours

Eye drop	Moxinix-LP	
Label claim	Loteprednol Etabonate (0.5 % w/v)	Moxifloxacin Hydrochloride (0.5 % w/v)
Assay (% of label claim*) Mean ± S. D.	96.98±0.59	96.65±0.65

Table 21: Analysis on marketed formulation at 48 hours

Eye drop	Moxinix-LP	
Label claim	Loteprednol Etabonate (0.5 % w/v)	Moxifloxacin Hydrochloride (0.5 % w/v)
Assay (% of label claim*) Mean ± S. D.	93.39±0.62	92.83±0.45

Table 22: Summarization of validation results

Parameters		Result	
		Moxifloxacin Hydrochloride	Loteprednol Etabonate
Linearity		0.996	0.998
Range		5-15 µg/ml	5-15 µg/ml
Accuracy	80%	100.40 1.52	99.77 ± 1.20
	100%	99.71 ± 0.70	99.70 ± 0.76
	120%	99.71 ± 0.46	99.63 ± 0.76
Precision Repeatability Inter-day Intra-day		 %RSD= 0.42 %RSD= 1.16-0.60 %RSD= 1.15-0.44	 %RSD= 1.50 %RSD= 1.41-0.83 %RSD= 0.99-0.74
LOD		0.65 µg/ml	0.65 µg/ml
LOQ		2.53 µg/ml	1.99 µg/ml
Assay		99.45±1.15	99.80±0.59
Robustness Variation in flow rate Variation in Mobile phase Variation in pH		 %RSD= 1.34-0.87 %RSD= 0.59-1.17 %RSD= 1.09-1.15	 %RSD= 0.43-1.46 %RSD= 1.78-0.70 %RSD= 0.50-1.04

Table 23: Moxifloxacin Hydrochloride and Loteprednol Etabonate Acid Degradation Sample at 4 hours

Loteprednol Etabonate			Moxifloxacin Hydrochloride		
Area of std = 2737.83		% Degradation	Area of std = 2077.56		% Degradation
4 hrs	1610.942	37.70 %	4 hrs	1347.139	32.76 %

Table 24: Moxifloxacin Hydrochloride and Loteprednol Etabonate Acid Degradation Sample at 4 hours

Parameter	Sample for Loteprednol Etabonate		Sample of Moxifloxacin Hydrochloride	
	Area	%Degradation	Area	%Degradation
Acid	2252.85	17.71	1647.76	20.69

Table 25: Moxifloxacin Hydrochloride and Loteprednol Etabonate Base Degradation Standards at 2.5 hours

Loteprednol Etabonate			Moxifloxacin Hydrochloride		
Area of std = 2737.83		% Degradation	Area of std = 2077.56		% Degradation
2.5 hrs	2238.97	18.22 %	2.5 hrs	1566.55	24.60 %

Table 26: Moxifloxacin Hydrochloride and Loteprednol Etabonate Base Degradation Sample at 2.5 hours

Parameter	Sample for Loteprednol Etabonate		Sample of Moxifloxacin Hydrochloride	
	Area	% Degradation	Area	% Degradation
Base	2178.25	20.44	1674.37	19.41

Table 27: Moxifloxacin Hydrochloride and Loteprednol Etabonate Oxidation Degradation Standards at 4 hours

Loteprednol Etabonate			Moxifloxacin Hydrochloride		
Area of std = 2737.83		% Degradation	Area of std = 2077.56		% Degradation
4 hrs	2012.51	26.49	4 hrs	1771.07	14.75

Table 28: Moxifloxacin Hydrochloride and Loteprednol Etabonate Oxidation Degradation sample at 4 hours

Parameter	Sample for Loteprednol Etabonate		Sample of Moxifloxacin Hydrochloride	
	Area	% Degradation	Area	% Degradation
Oxidation	2040.59	25.47	1725.22	16.96

Table 29: Moxifloxacin Hydrochloride and Loteprednol Etabonate Photo Degradation Standards at 3.5 hours

Loteprednol Etabonate			Moxifloxacin Hydrochloride		
Area of std = 2737.83		% Degradation	Area of std = 2077.56		% Degradation
3.5 hrs	1907.25	30.34	4 hrs	1937.08	29.25

Table 30: Moxifloxacin Hydrochloride and Loteprednol Etabonate Photo Degradation sample at 3.5 hours

Parameter	Sample for Loteprednol Etabonate		Sample of Moxifloxacin Hydrochloride	
	Area	%Degradation	Area	%Degradation
Photo	1937.08	29.25	1615.35	22.25

Table 31: Moxifloxacin Hydrochloride and Loteprednol Etabonate Thermal Degradation Standards at 3 hours

Loteprednol Etabonate			Moxifloxacin Hydrochloride		
Area of std = 2737.83		% Degradation	Area of std = 2077.56		% Degradation
3 hrs	2265.3	17.26	3 hrs	1793.96	13.65

Table 32: Moxifloxacin Hydrochloride and Loteprednol Etabonate Thermal Degradation sample at 3 hours

Parameter	Sample for Loteprednol Etabonate		Sample of Moxifloxacin Hydrochloride	
	Area	%Degradation	Area	%Degradation
Thermal	2293.58	16.23%	1805.69	13.09%

Table 33: Moxifloxacin Hydrochloride % Degradation

Moxifloxacin Hydrochloride				
Parameter	Standard		Sample	
	Area	% Degradation	Area	% Degradation
Acid	1609.58	22.53	1647.76	20.69
Base	1566.55	24.60	1674.37	19.41
Thermal	1793.96	13.65	1805.69	13.09
Oxidation	1771.07	14.75	1725.22	16.96
Photo	1643.16	20.91	1615.35	22.25

Table 34: Loteprednol Etabonate % Degradation

Loteprednol Etabonate				
Parameter	Standard		Sample	
	Area	%Degradation	Area	%Degradation
Acid	2198.68	19.69	2252.85	17.71
Base	2238.97	18.22	2178.25	20.44
Thermal	2265.37	17.26	2293.58	16.23
Oxidation	2012.51	26.49	2040.59	25.47
Photo	1907.25	30.34	1937.08	29.25

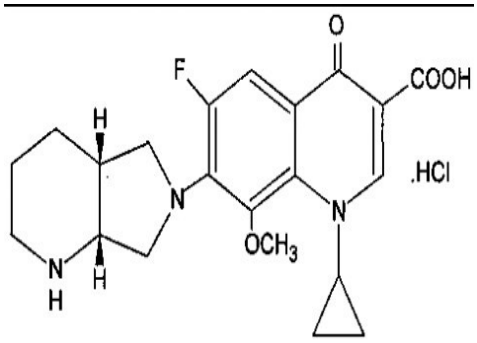

Figure 1: Structure of Moxifloxacin Hydrochloride

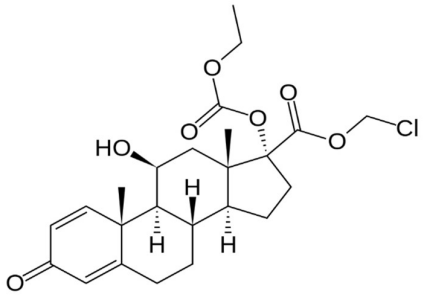

Figure 2: Structure of Loteprednol Etabonate

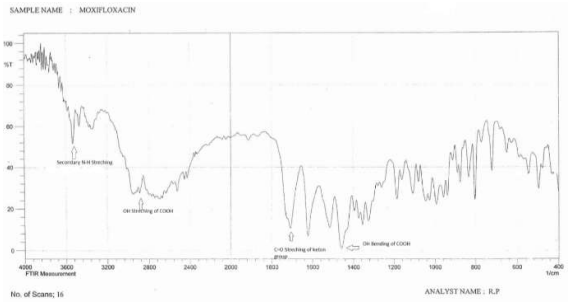

Figure 3: IR spectra of Sample moxifloxacin

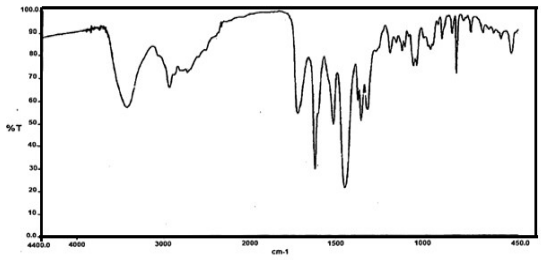

Figure 4: IR spectra of standard moxifloxacin

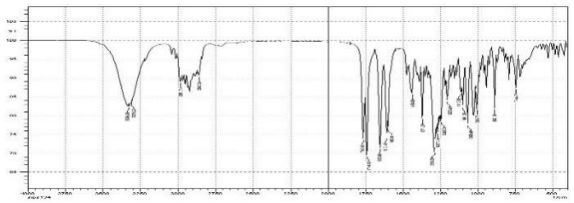

Figure 5: IR spectra of sample loteprednol

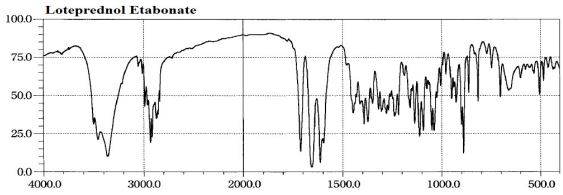

Figure 6: IR sample of standard loteprednol

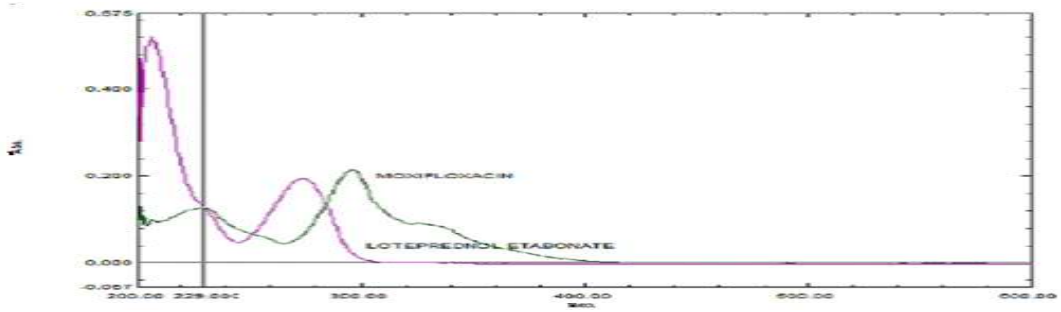

Figure 7: Overlain Spectra of Moxifloxacin Hydrochloride (10 ppm) and Loteprednol Etabonate (10 ppm) in Methanol (Isoabsorptive point 229 nm)

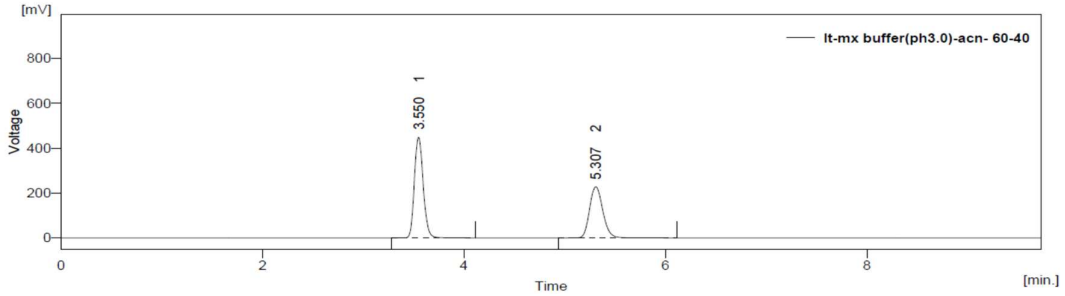

Figure 8: HPLC Chromatogram of Moxifloxacin Hydrochloride 10 µg/ml and Loteprednol Etabonate 10 µg/ml in Buffer (pH 3.0): Acetonitrile (60:40)

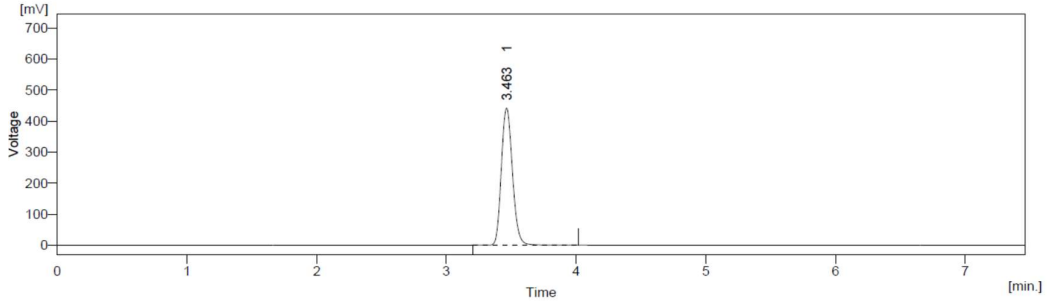

Figure 9: Chromatogram of Loteprednol Etabonate (10 µg/ml) Standard

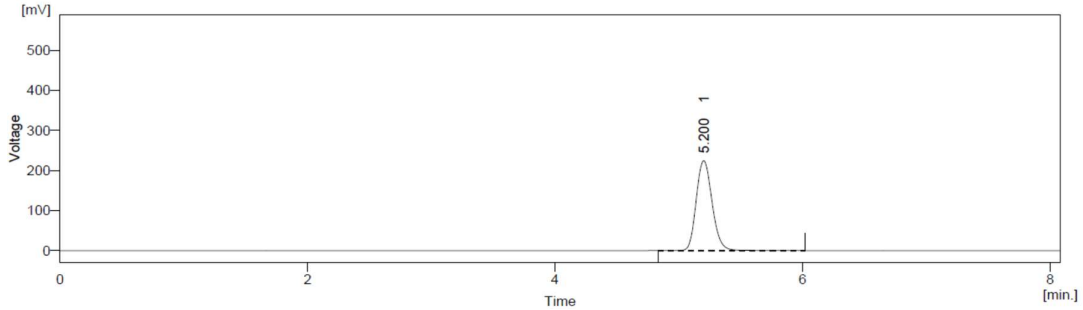

Figure 10: Chromatogram of Moxifloxacin Hydrochloride (10 µg/ml) Standard

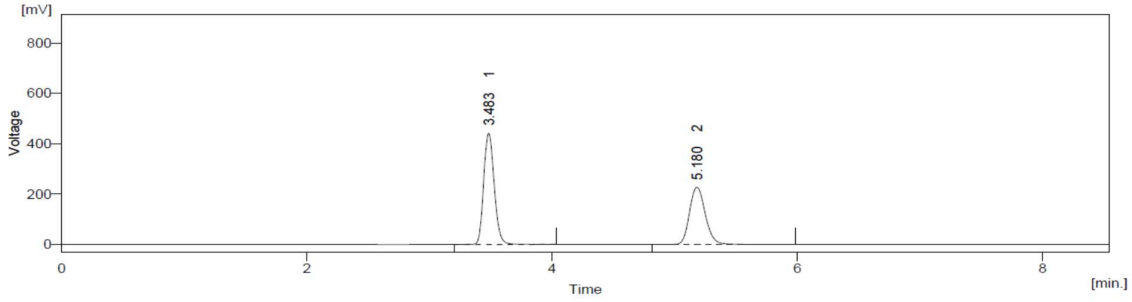

Figure 11: Chromatogram of Loteprednol Etabonate (10 µg/ml) and Moxifloxacin Hydrochloride (10 µg/ml) Standard

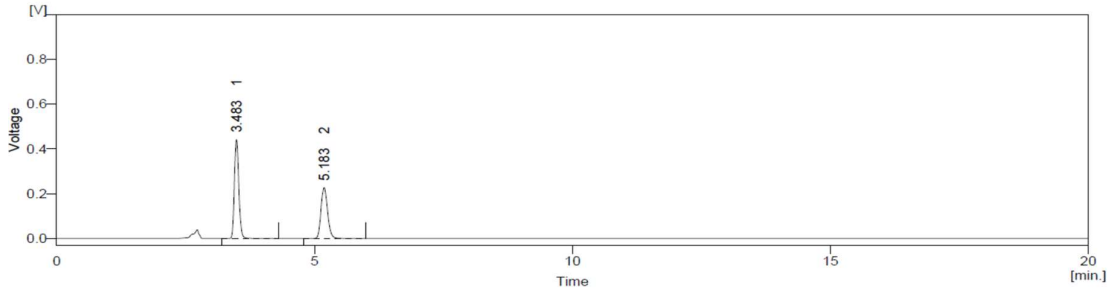

Figure 12: Chromatogram of Loteprednol Etabonate (10 µg/ml) and Moxifloxacin Hydrochloride (10 µg/ml) Sample

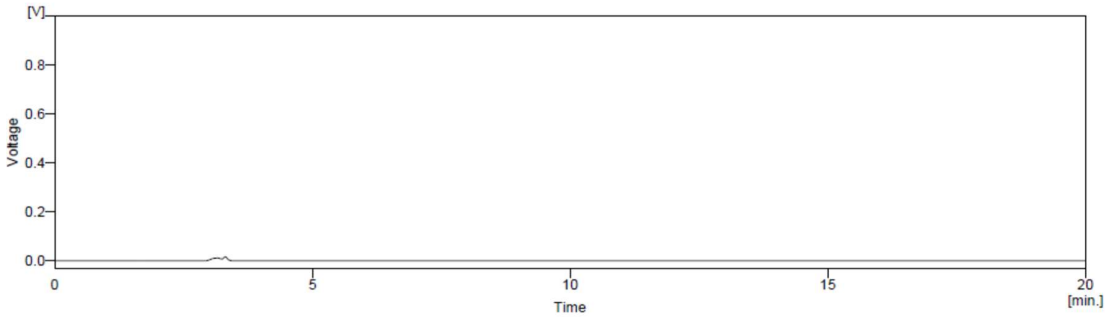

Figure 13: Chromatogram of Loteprednol Etabonate and Moxifloxacin HCl Blank

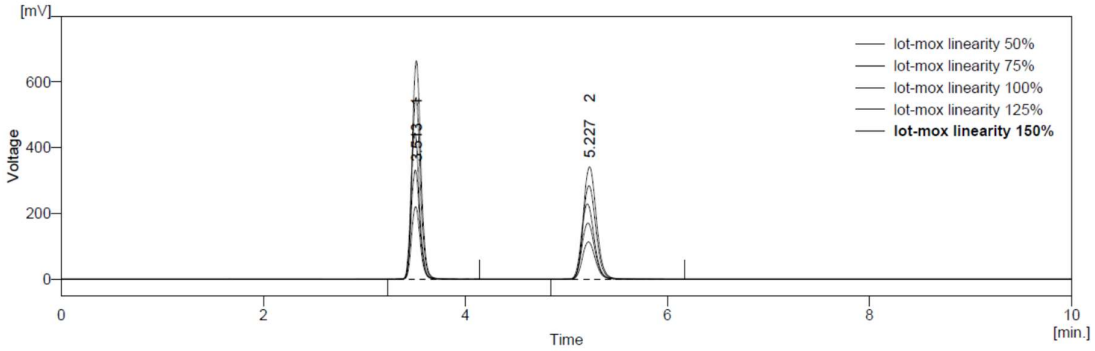

Figure 14: Overlain chromatogram of Moxifloxacin Hydrochloride and Loteprednol Etabonate

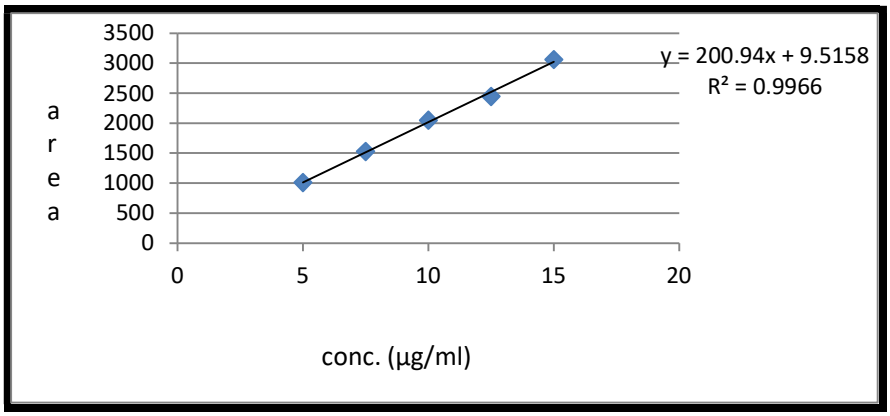

Figure 15: Calibration Curve of Moxifloxacin Hydrochloride (5-15 µg/ml)

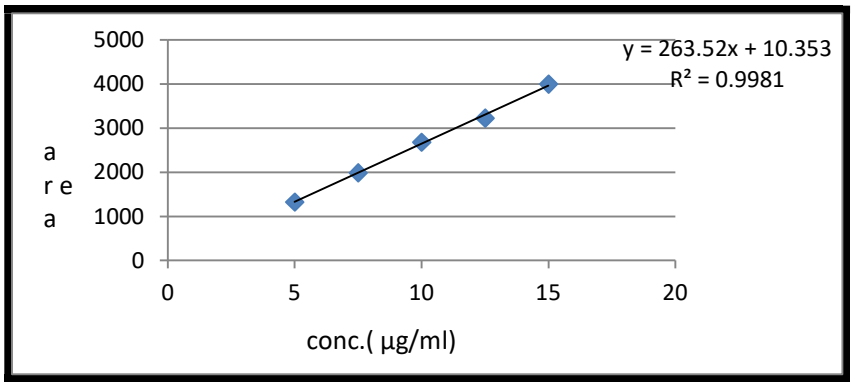

Figure 16: Calibration Curve of Loteprednol Etabonate (5-15 µg/ml)

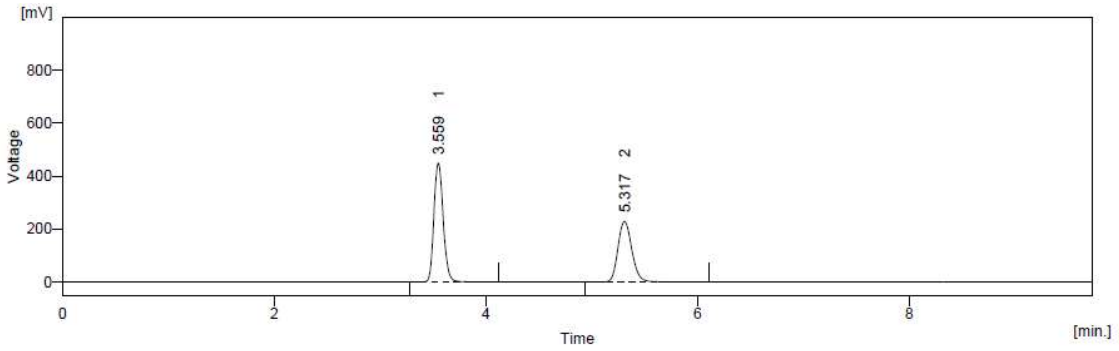

Figure 17: Moxifloxacin Hydrochloride and Loteprednol Etabonate Standard for stability

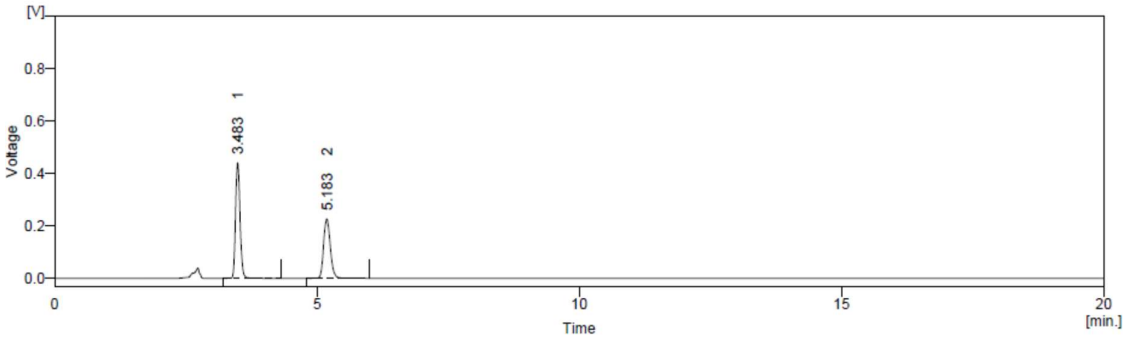

Figure 18: Moxifloxacin Hydrochloride and Loteprednol Etabonate sample

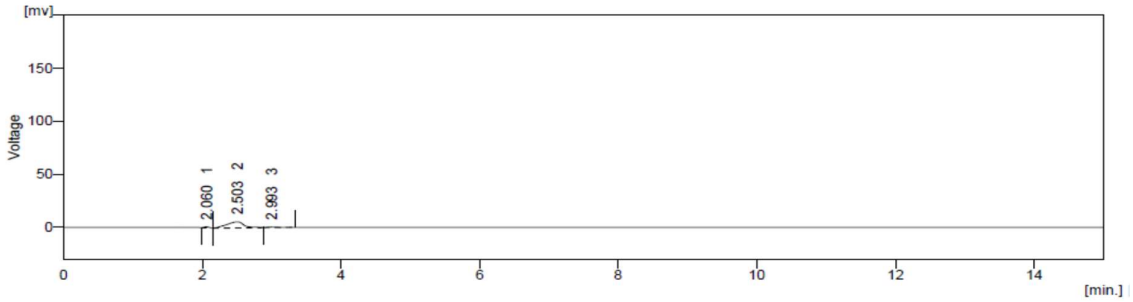

Figure 19: Moxifloxacin Hydrochloride and Loteprednol Etabonate Acid Degradation Blank

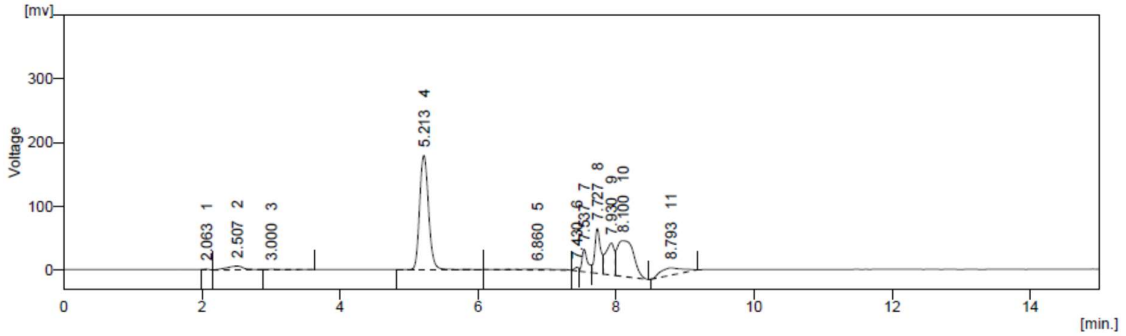

Figure 20: Moxifloxacin Hydrochloride Acid Degradation Standard at 4 hours

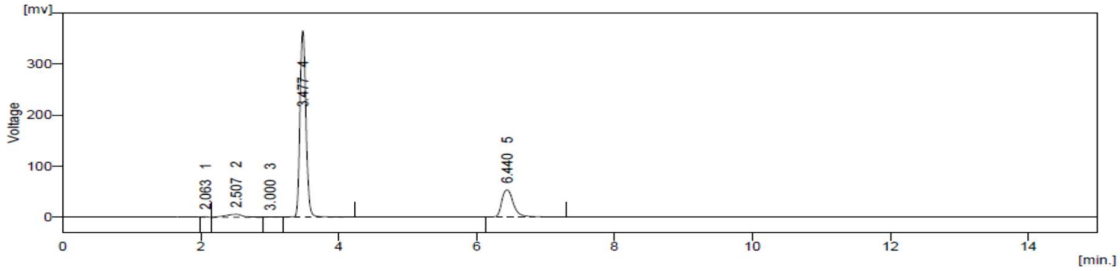

Figure 21: Loteprednol Etabonate Acid Degradation Standard at 4 hours

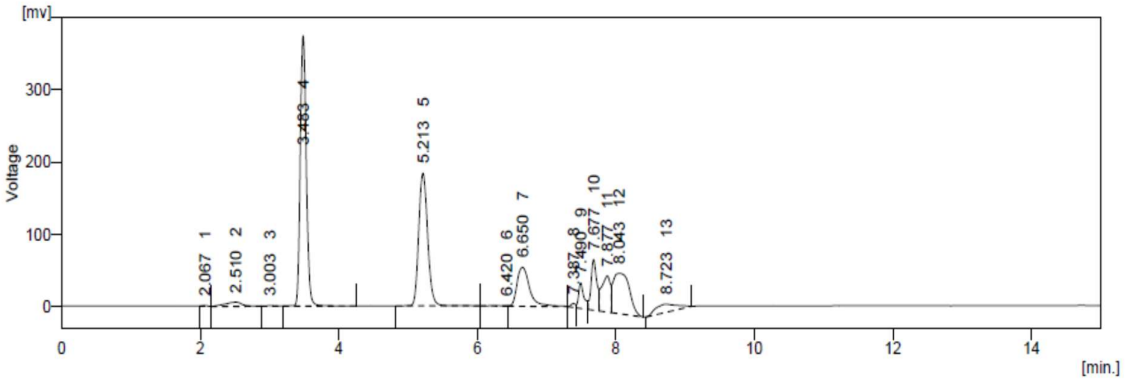

Figure 22: Moxifloxacin Hydrochloride and Loteprednol Etabonate Acid Degradation Sample at 4 hours

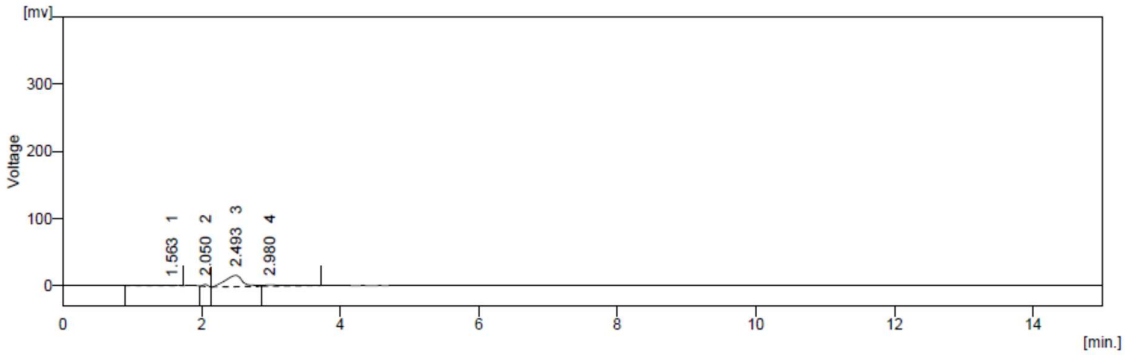

Figure 23: Moxifloxacin Hydrochloride and Loteprednol Etabonate Base Degradation blank

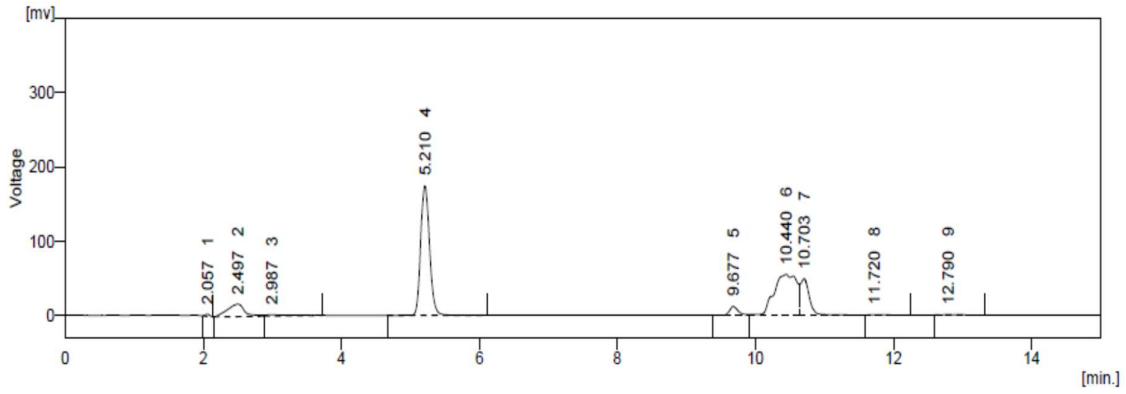

Figure 24: Moxifloxacin Hydrochloride Base Degradation at 2.5 hours

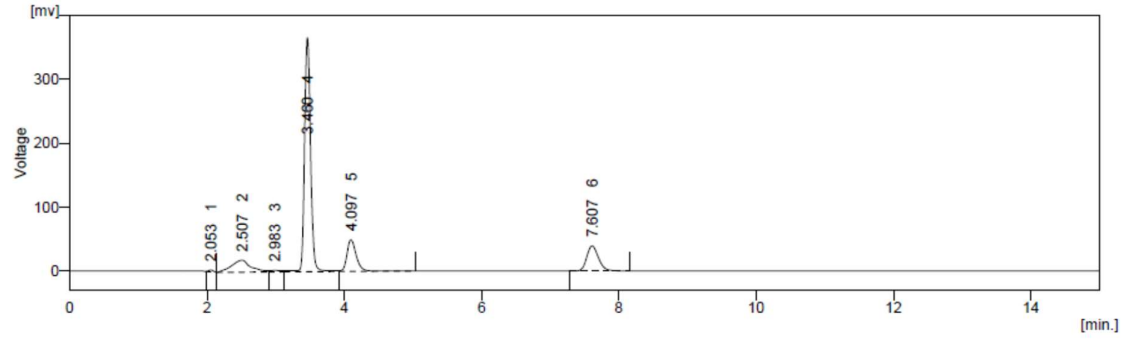

Figure 25: Loteprednol Etabonate Base Degradation at 2.5 hours

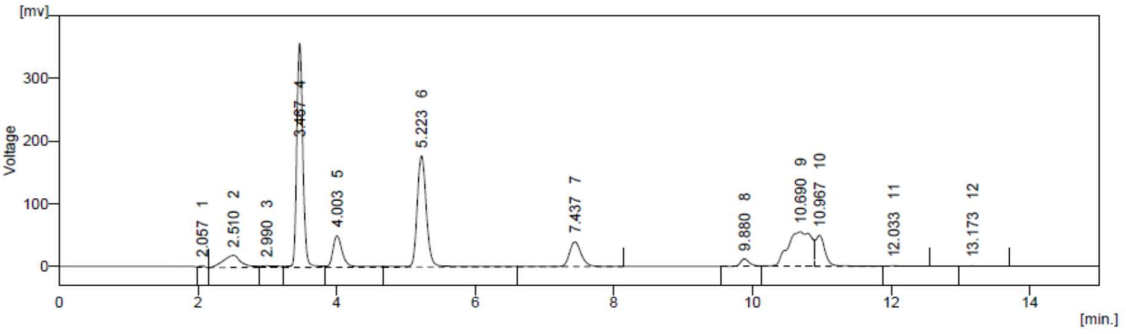

Figure 26: Moxifloxacin Hydrochloride and Loteprednol Etabonate Base Degradation Sample at 2.5 hours

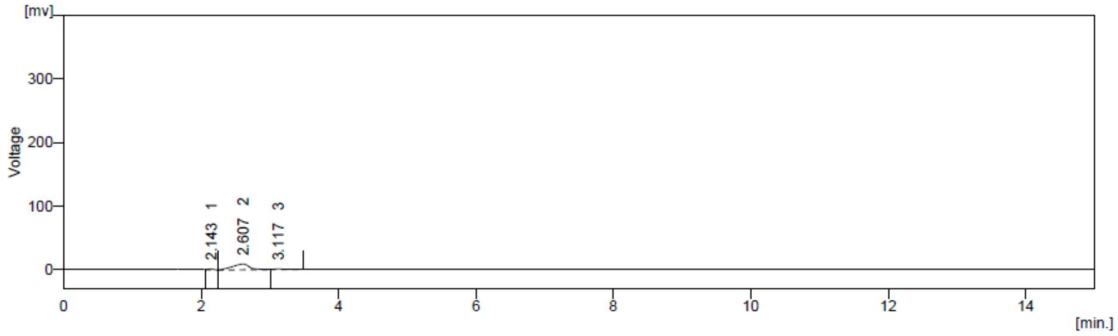

Figure 27: Moxifloxacin Hydrochloride and Loteprednol Etabonate Oxidation Degradation Blank

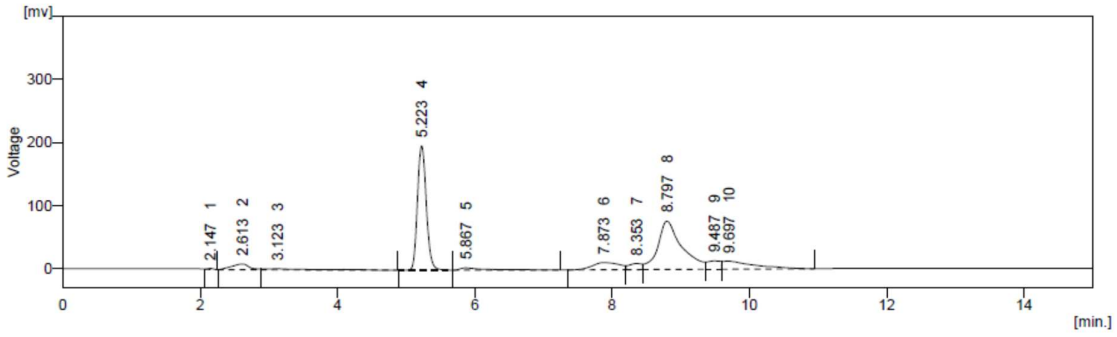

Figure 28: Moxifloxacin Hydrochloride Oxidation Degradation at 4 hours

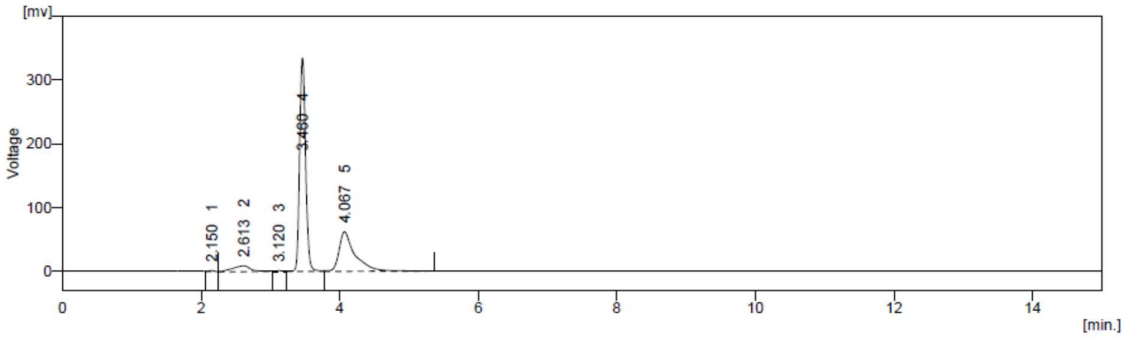

Figure 29: Loteprednol Etabonate Oxidation Degradation at 4 hours

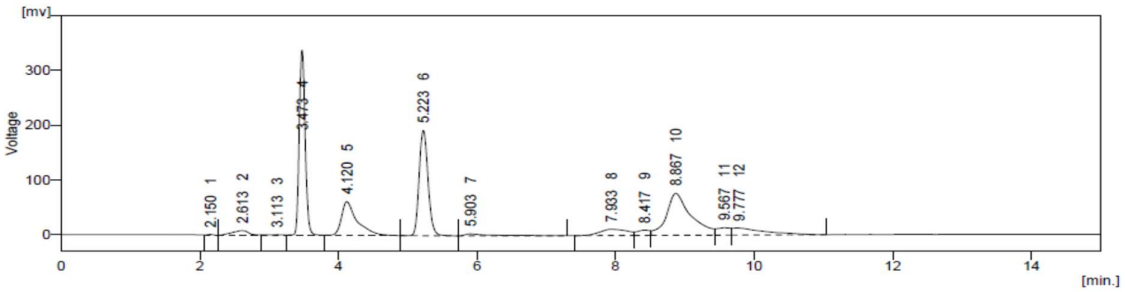

Figure 30: Moxifloxacin Hydrochloride and Loteprednol Etabonate Oxidation Degradation sample at 4 hours

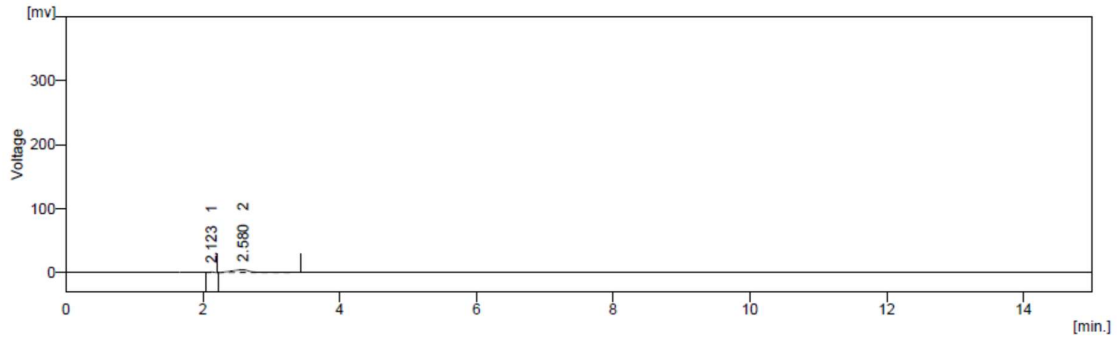

Figure 31: Moxifloxacin Hydrochloride and Loteprednol Etabonate Photo Degradation Blank

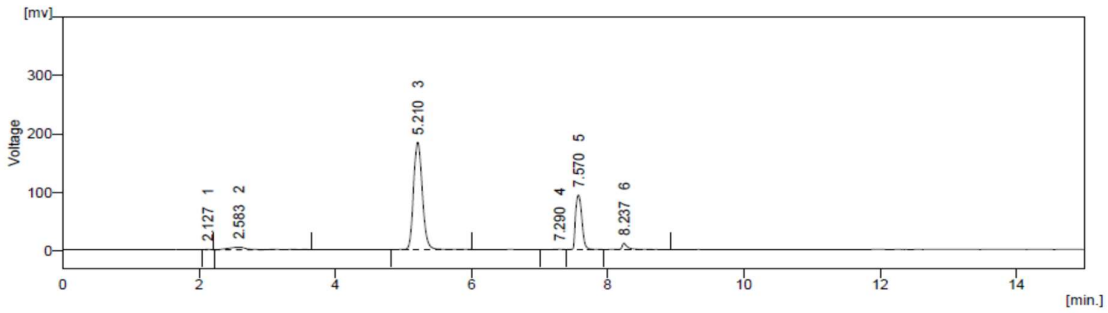

Figure 32: Moxifloxacin Hydrochloride Photo Degradation at 3.5 hours

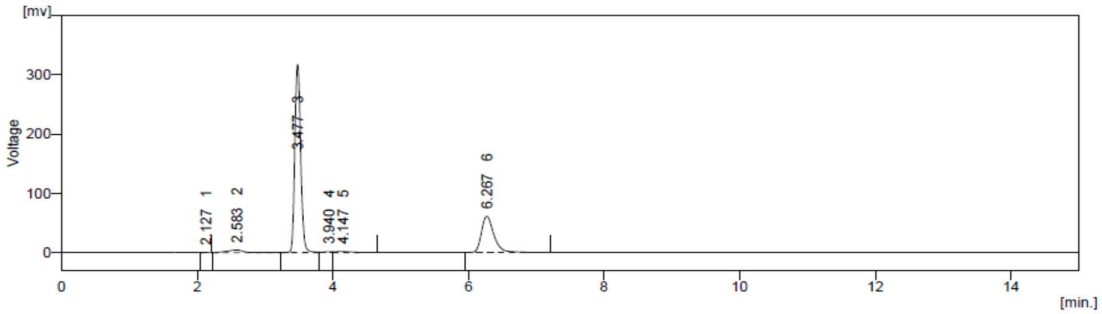

Figure 33: Loteprednol Etabonate Photo Degradation at 3.5 hours

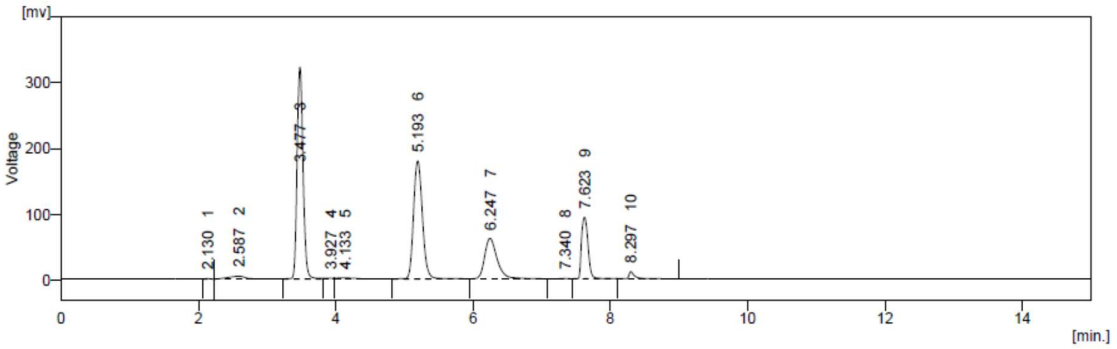

Figure 34: Moxifloxacin Hydrochloride and Loteprednol Etabonate Photo Degradation sample at 3.5 hours

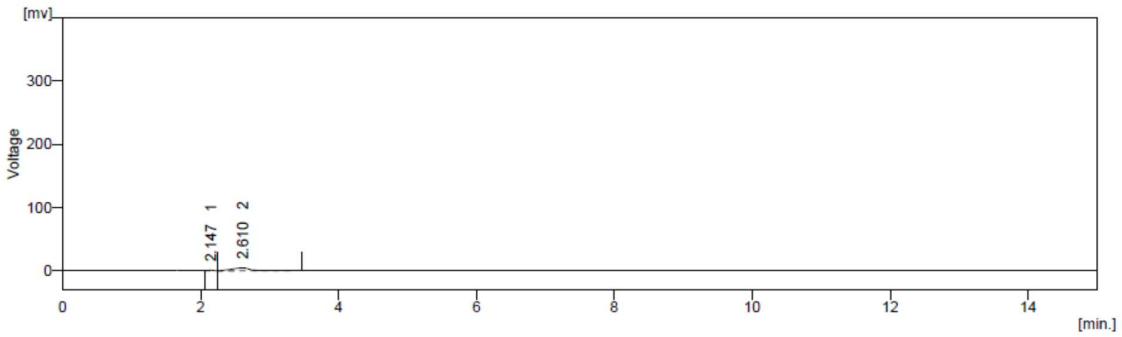

Figure 35: Moxifloxacin Hydrochloride and Loteprednol Etabonate Thermal Degradation Blank

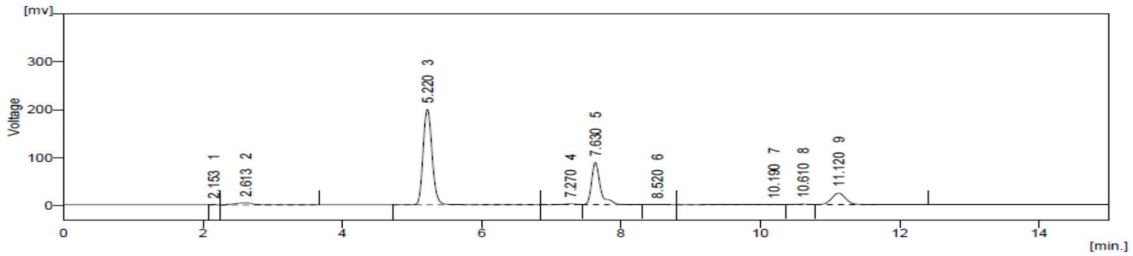

Figure 36: Moxifloxacin Hydrochloride Thermal Degradation at 3 hours

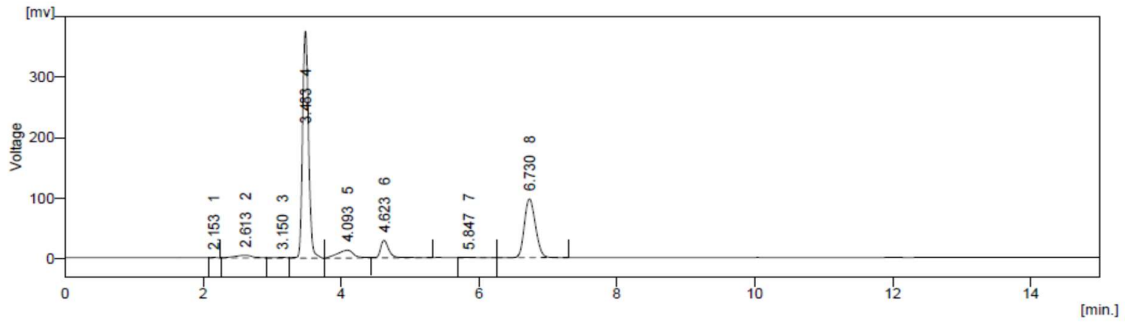

Figure 37: Loteprednol Etabonate Thermal Degradation at 3 hours

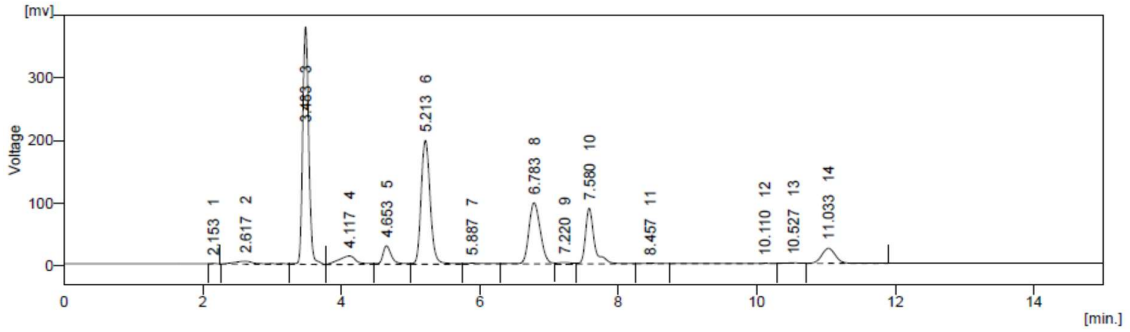

Figure 38: Moxifloxacin Hydrochloride and Loteprednol Etabonate Thermal Degradation sample at 3 hours

CONCLUSION

Moxifloxacin Hydrochloride is a fluorinated quinolone antibacterial. The bactericidal action of Moxifloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and

topoisomerase IV. DNA gyrase is an essential enzyme that is involved in the replication, transcription and repair of bacterial DNA. Topoisomerase IV is an enzyme known to play a key role in the partitioning of the chromosomal DNA during bacterial cell division. Loteprednol Etabonate is a topical corticoid anti-inflammatory. It is used in ophthalmic solution for the treatment of steroid responsive inflammatory conditions of the eye such as allergic conjunctivitis, uveitis, acne rosacea

RP-HPLC method was developed for simultaneous estimation Moxifloxacin HCl and Loteprednol Etabonate. In RP-HPLC method, good resolution and separation of two drugs was achieved. 0.05 M Sodium dihydrogen phosphate (pH 3): Acetonitrile (60:40 v/v) was used as mobile phase. Retention time of Loteprednol Etabonate and Moxifloxacin HCl were found to be 3.550 and 5.307 min respectively with a flow rate of 1 ml/min. The proposed method was accurate and precise. Therefore, proposed method can be used for routine analysis of Moxifloxacin HCl and Loteprednol Etabonate in tablets.

Forced degradation study of Moxifloxacin HCl and Loteprednol Etabonate was performed by RP-HPLC method which includes Acid, Base, Oxidative, Photo and Thermal degradation. Results of degradation were found within limit.

REFERENCES

[1] Drug profile for Moxifloxacin Hydrochloride, Sept-2015, http://www.drugbank.ca/drugs/DB00218

[2] Drug profile for Moxifloxacin Hydrochloride, Sept-2015, http://en.wikipedia.org/wiki/Moxifloxacin

[3] Drug profile for Loteprednol Etabonate, Sept-2015, http://www.drugbank.ca/drugs/DB00873

[4] Drug profile for Loteprednol Etabonate, Sept-2015, http://en.wikipedia.org/wiki/Loteprednol

[5] " Introduction to Uveitis", sept-2015, https://en.wikipedia.org/wiki/Uveitis

[6] Mitchel RM, Cortan RS. Basic pathology; 7th Edn; 2001, pp. 841-846.

[7] Singh RN, Sahoo S, Mishra U and Garnaik B, "Stability Indicating RP-HPLC Method Development And Validation Of Moxifloxacin." *Int. J. of Res. in Pharm. and Chem..,* **2014,** 4(1), 131-140.

[8] Reddy NK, Prasad SR, Maiti NJ, Nayak D and Maharana PK, "Development and Validation of a Stability Indicating UPLC Method for Determination of Moxifloxacin Hydrochloride in Pharmaceutical Formulations." *Pharm. Anal. Acta.,* **2011,** 2(9), 1-10.

[9] Sanapala AK, Anusham MK and Priyadarshani JV, " A Validated RP-HPLC Method For The Analysis Of Moxifloxacin Hydrochloride In Pharmaceutical Dosage Forms." *Int.J. adv. in Pharm.Sci.,* **2010,** 1(2), 347-352.

[10] Dhilon V and Choudhry AK, "A validated HPTLC method for estimation of moxifloxacin hydrochloride in tablets." *Pharm. Methods.,* **2010,** 1(1), 54-56.

[11] Tarkse KN, Admane SS and Sonkhede NG, "Development and Validation of UV-Spectrophotometric Methods for Determination of Moxifloxacin HCL in Bulk and Pharmaceutical Formulations." *Der Pharma Chemical.,* **2012,** 4(3), 1180-1185.

[12] ICH, Validation of Analytical Procedures; Methodology, Q2 (R1), International Conference on Harmonization, IFPMA, Geneva 1996.

[13] Dong WM. Modern HPLC for Practicing Scientists; A Wiley- Inter science publication, USA, 2006, pp 1-9.

[14] Kazakevich Y and LoBrutto R. HPLC for pharmaceutical Scientists; A John Wiley and sons, 2007, pp 1-6.

[15] Snyder LR., Kirkland JJ and Glajch LJ. Introduction to Modern Liquid Chromatography; 2nd Edn; A Wiley-Inter science publication, NY, USA, 1997, pp 5-42.

[16] Snyder LR., Kirkland JJ and Glajch LJ. Practical HPLC Method Development; 2nd Edn; A Wiley-Inter science publication, NY, USA, 1997, pp 3-35.

[17] Skoog DA., Holler FJ and Nieman TA. Introduction to UV Spectroscopy, Principle of instrumental analysis; 5th Edn; Thomson Brooks/Cole publication, 2005, pp 301.

[18] Beckett AH and Stenlake JB. UV-visible Spectrophotometry: Practical Pharmaceutical Chemistry; 4th Edn; Part-II, C.B.S. Publishers, Delhi, 2001, pp. 275-299.

[19] FDA, "Guidance for Industry; Analytical Procedures and Methods Validation (Draft guidance), Food & Drug Administration," Rockville, US Department of Health and Human Services, 2000.