Open Access Contents
Int. J. Drug Dev. & Res.
| January - March 2014 | Vol. 6 | Issue 1 | ISSN 0975-9344 |
www.ijddr.in

Full Length Original Research Paper

Development and validation of HPLC method for the simultaneous estimation of Gatifloxacin and Loteprednol in bulk and dosage form

***Raval Kashyap[1]**

U. Srinivasa[2]

[1] Research scholar, Department of Pharmacy, Pacific University, Udaipur.
[2]Head of Department, Srinivas college of Pharmacy, Mangalore.

Corresponding Authors:
Raval Kashyap
Email:
raval.kashyap99@yahoo.com

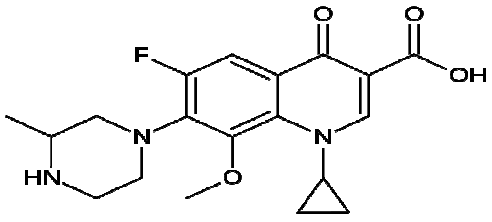
Abstract:
A simple, accurate and precise High Performance Liquid Chromatographic (HPLC) method has been developed for simultaneous determination of Gatifloxacin and Loteprednol in bulk and dosage form. The method has been validated as per the guidelines of ICH. The separation is achieved on 250*4.6mm C18, 5micron (Hypersil BDS) column with flow rate 1.0 mL per minute in isocratic mode using Buffer pH3.5: Acetonitrile (45:55) as mobile phase. Column oven temperature is maintained at 25°C and observations are recorded at 275 nm. The linearity range was found to be in the range of 15-45 µg/ml for Gatifloxacin and 25-75 µg/ml for Loteprednol. Correlation co-efficient for calibration curve of Gatifloxacin and Loteprednol was found to be 0.9977 and 0.9983 respectively. The method is simple, accurate, reproducible and short and can be used for simultaneous analysis of Loteprednol and Gatifloxacin.

Keywords: Gatifloxacin , Loteprednol, Buffer pH 3.5 And Acetonitrile.

Introduction:

A study of the interaction of light (or other electromagnetic radiation) with matter is an important and versatile tool for the chemist. Indeed, much of our knowledge of chemical substances comes from their specific absorption or emission of light. In this experiment, we are interested in analytical procedures based on the amount of light absorbed (or transmitted) as it passes through a sample.[1] Loteprednol and Gatifloxacin Ophthalmic Suspension is a combination eye drops, which contains antibiotic Gatifloxacin & an anti-inflammatory agent Loteprednol. ZYLOPRED name which contain these two drugs. Gatifloxacin is an antibiotic of the fourth-generation fluoroquinolone family that like other members of that family inhibits the bacterial enzymes DNA gyrase and topoisomerase IV. Bristol-Myers Squibb introduced Gatifloxacin in 1999 under the proprietary name Tequin® for the treatment of respiratory tract infections, having licensed the medication from Kyorin Pharmaceutical Company of Japan. Its Chemical name is (±) - 1- cyclopropyl-6-fluoro-1, 4-dihydro- 8- methoxy- 7- (3 –methyl - 1- piperazinyl)- 4- oxo - 3 quinolinecarboxylic acid sesquihydrate. Its Molecular formula and Molecular weight are $C_{19}H_{22}FN_3O_4$ and 402.42 respectevely.

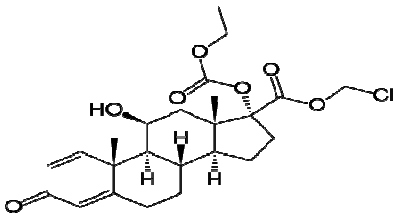
The structural formula is:



Its water solubility is 60mg/ml at pH 4. Gatifloxacin is a synthetic broad-spectrum 8-methoxyfluoroquinolone antibacterial agent for oral or intravenous administration. is bactericidal

and its mode of action depends on blocking of bacterial DNA replication by binding itself to an enzyme called DNA gyrase, which allows the untwisting required to replicate one DNA double helix into two. Notably the drug has 100 times higher affinity for bacterial DNA gyrase than for mammalian. Gatifloxacin is a broad-spectrum antibiotic that is active against Gram-positive and Gram-negative bacteria. Loteprednol is a white to off-white powder. Loteprednol as the ester Loteprednol etabonate. Its chemical name is chloromethyl 17α-((ethoxycarbonyl) oxy)-11β-hydroxy-3-oxoandrosta-1,4-diene-17β-carboxylate. Its Molecular formula and Molecular weight are $C_{21}H_{27}ClO_5$ and 394.889 respectively.

The structural formula is:



Its solubility in water in 4mg/ml. Loteprednol etabonate is structurally similar to other glucocorticoids. However, the number 20 position ketone group is absent. It is highly lipid soluble which enhances its penetration into cells. Loteprednol etabonate is synthesized through structural modifications of prednisolone- related compounds so that it will undergo a predictable transformation to an inactive metabolite.[2, 3, 4] there are spectroscopic and Chromatographic methods developed on single Loteprednol and Gatifloxacin [5-20]. But up to now there is no HPLC methods develop for simultaneous estimation Gatifloxacin and Loteprednol Analysis of the drug is important for development of drugs in their formulation and their use in therapies, for which we require standard analytical procedures. The USP has published specific guidelines for method validation for compound evaluation. USP defines eight steps for validation: Accuracy, Precision, Specificity, Limit of detection, Limit of quantitation , Linearity and range, Robustness[21-22] As quality control process is not static some form of validation/verification should continue till the validated procedure is in use. It should not be a concept that once the method is initially developed and validated it is forgotten.

MATERIALS AND METHODS:

Chromatographic methods offer an advantage in terms of sensitivity and selectivity. These methods can be used for routine analysis of dosage forms where two or more drugs are present together. HPLC method was developed for simultaneous estimation of Gatifloxacin and Loteprednol.

1. Reagents and Material

Loteprednol

Gatifloxacin

Acetonitrile for HPLC

Water for HPLC

Potassium Dihydrogen ortho phosphate AR grade

Sodium hydroxide AR Grade

2. Marketed formulation

The commercial formulation was purchased from Local pharmacy. Each ZYLOPRED Tablet contains 0.3% w/v Gatifloxacin and 0.5%w/v Loteprednol.

3. Selection of Mobile Phase

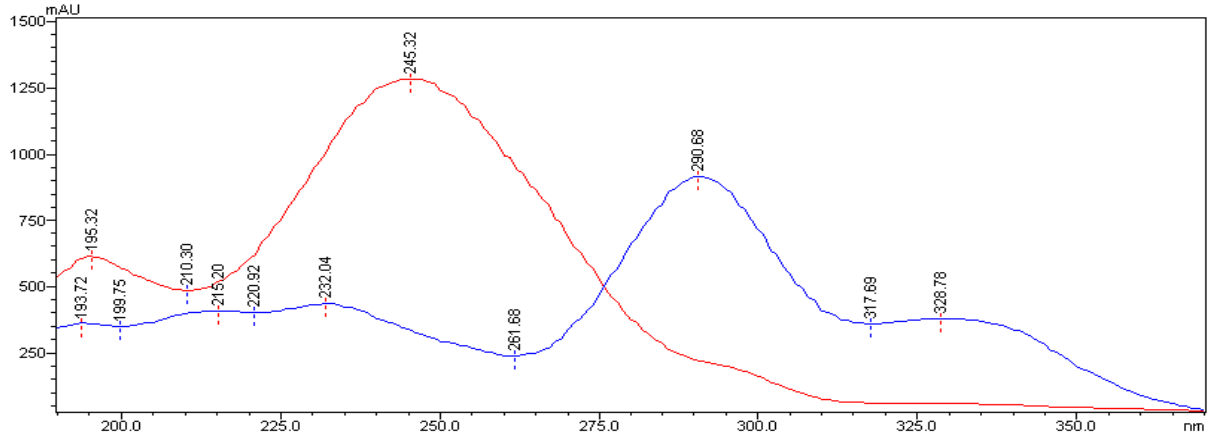
After assessing the solubility of drugs in different solvents as well on the basis of literature survey, the standard solution of Gatifloxacin and Loteprednol were injected into the HPLC system by using different solvent systems. Different mobile phases were tried in order to find the best conditions for the separation of both the drugs. It was found that Buffer pH 3.5: ACN give satisfactory results as compared to other mobile

phases. Finally, the optimal composition of the mobile phase was determined to be Buffer pH 3.5: Acetonitrile (45:55) which show in table no.1

4. Selection of Detection Wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study standard drug solutions of 15µg/ml Gatifloxacin and 25 µg/ml Gatifloxacin were, therefore, prepared in solvent mixtures of mixture of Buffer pH 3.5: Acetonitrile (45:55). This drug solution was than scanned in the UV region of 200-400 nm and the spectrum was recorded 275nm which is shown in figure no.1

Figure 1: Detection of wavelength 275nm



5. Optimized Chromatographic Conditions

To optimize the chromatographic conditions, the effect of chromatographic variables such as mobile phase pH, flow rate, and solvent ratio were studied. The resulting chromatograms were recorded and the chromatographic parameters such as capacity factor, asymmetric factor and column efficiency were calculated. The conditions that gave the best resolution, symmetry and capacity factor were selected for estimation show in table no.1 and figure 2-7.

1) Buffer preparation (0.1% triethylamine, pH3.5):
2) Mobile phase: Buffer: acetonitrile 45:55
3) Flow rate: 1.0ml/mint
4) Wavelength: 275nm
5) Column: 250*4.6mm C18, 5micron (Hypersil BDS)
6) Injection volume: 20 micro liter

6. Preparation of standard solutions

Preparation of buffer pH 3.5

1ml of triethylamine was taken into a 1000ml beaker; add 800ml water and mix. Adjust pH3.5 with 1% orthophosphoric acid. Make up volume with water up to 1000ml.

Preparation of mobile phase

450 ml of Buffer (pH 3.5) and 550 ml of Acetonitrile (HPLC grade) were mixed and filtered through 0.45µm filter, Sonicated for 10minutes to degas and used as mobile phase. Use mobile phase as a diluents.

Preparation of STD Stock solution of Gatifloxacin:

30mg of Gatifloxacin was taken as working standard into a 100ml volumetric flask. Add 60ml mobile phase and dissolve, make up volume with mobile phase (300 µg/ml)

Preparation of STD Stock solution of Lotepredlon:

50mg of Lotepredlon was taken as working standard into a 100ml volumetric flask. Add 60ml mobile phase and dissolve, make up volume with mobile phase (500 µg/ml)

7. Calibration curve for the 15-45 µg/ml Gatifloxacin and 25-75 µg/ml Loteprednol

Appropriate volume of aliquots from standard Gatifloxacin and Loteprednol stock solutions were transferred to same volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with mobile phase give a solution containing 15, 22.5, 30, 37.5 and 45 µg/ml Gatifloxacin and 25, 37.5, 50, 62.5, 75 µg/ml Loteprednol. Each of these mixed standard solutions was chromatographed for 10 minutes run time using mobile phase at 275nm at flow rate of 1 ml/min. The graphs were plotted for peak area vs. concentration for both the drugs. Data is recorded in table no. 2 and figure no. 8, 9 and 10.

8. Analysis of marketed formulation:

Solution-1: Took sample equivalent to 30mg of Gatifloxacin (10ml sample) into a 100ml volumetric flask. Add 60ml of mobile phase and shake for 15 minutes to dissolve. Make up with mobile phase. Filter this solution with 0.45micron membrane filter (This solution contains 300 µg/ml of Gatifloxacin and 500 µg/ml of Lotepredlon)

Solution-2: Take 1ml of solution-1 into a 10ml volumetric flask and make up with mobile phase. (This solution contains 30 µg/ml of Gatifloxacin and 50 µg/ml of Lotepredlon)

The prepared sample solution was chromatographed for 10 minutes run time using mobile phase at 275nm at flow rate of 1 ml/min. From the peak area obtained in the chromatogram, the amounts of both the drugs were calculated by fitting peak area responses into the equation of the straight line representing the calibration curves for Gatifloxacin and Loteprednol. And result shown in table no 3 and figure no. 11.

VALIDATION OF PROPOSED HPLC METHOD

9. System suitability

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated. System suitability test was carried out to verify that the analytical system is working properly to give accurate and precise results. Standard solution (15µg/ml Gatifloxacin and 25µg/ml Loteprednol) was injected six times and the chromatograms were recorded in table no. 4 and figure no. 12.

Acceptance Criteria: The % RSD for area response obtained from six replicate injections of Standard solution should be ≤ 2.0 %, Tailing factor should be ≤ 2.0, Theoretical plates should be ≥ 2000 and Resolution of drug peaks should be ≥ 2.0.

10. Solvent suitability:

Recorded in table no.5

11. Linearity

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity.

The linearity peak area response was determined by analyzing solutions having concentrations in the range of 15-45 µg/ml and 25-75 µg/ml for Gatifloxacin and Loteprednol respectively from same solution. Peak area of each solution was

measured using developed method. Calibration curve of peak area Vs concentration was plotted. The correlation coefficient and regression line equations for Gatifloxacin and Loteprednol were determined. Linearity is recorded in table no.6.

12. Precision

I. Repeatability

6 replicates of standard mixture solution having and Gatifloxacin (15 µg/ml) and Loteprednol (25µg/ml) were prepared and chromatograms were recorded and RSD was calculated and shown in table no. 7.

II. Intraday precision

Standard solutions containing 15, 30, 45 µg/ml Gatifloxacin and 25, 50, 75 µg/ml Loteprednol were analyzed 3 times on the same day. Chromatogram of each sample was recorded. SD and RSD were calculated and shown in table no. 8.

III. Interday precision

Standard solutions containing 15, 30, 45 µg/ml Gatifloxacin and 25, 50, 75 µg/ml Loteprednol were analyzed on three different days. Chromatogram of each sample was recorded. SD and RSD were calculated and shown in table no. 9.

13. Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. Recovery studies were carried out by addition of standard drug to the pre analysed sample at 3 different concentration levels (80, 100 and 120 %) taking into consideration percentage purity of added bulk drug samples. It was determined by calculating the recovery Gatifloxacin and Loteprednol by standard addition method.

✓ Preparation of sample solution for % recovery:

An accurately weighed powder equivalent to about 30 mg of Gatifloxacin and 50 mg of Loteprednol was transferred to 100 ml volumetric flask; dissolved and the volume was made up to the mark using mobile phase. The solution was sonicated for 20 minutes. The solution was filtered through whatman Filter Paper No.42. First few ml of filtrate were discarded. 1 ml of the solution from above filtrate was diluted to 10 ml with mobile phase. The prepared sample solution was chromatographed for 10 minutes using mobile phase at flow rate of 1 ml/min. concentration of Gatifloxacin and Loteprednol is calculated which is known as pre-analyzed sample.In pre-analyzed sample 80, 100 and 120% of Gatifloxacin and Loteprednol was spiked. Chromatogram of each spiked solutions was taken and total amount of drug was calculated and from which % recovery was calculated. This is shown in table no. 10 and 11.

14. Limit of Detection (LOD)

The LOD is estimated from the set of 6 calibration curves used to determine method linearity. The LOD may be calculated as;

LOD = 3.3 x (SD / Slope)

Where, SD = the standard deviation of Y-intercept of 6 calibration curves.

Slope = the mean slope of the 6 calibration curves.

This is shown in table no. 12.

15. Limit of Quantification (LOQ)

The LOQ is estimated from the set of 6 calibration curves used to determine method linearity. The LOQ may be calculated as;

LOQ = 10 x (SD / Slope)

Where, SD = the standard deviation of Y-intercept of 6 calibration curves.

Covered in Scopus & Embase, Elsevier
Int. J. Drug Dev. & Res., January - March 2014, 6 (1): 99-112

Slope = the mean slope of the 6 calibration curves. This is shown in table no. 12.

16. Robustness

The robustness of an analytical method was carried out to confirm that the method remained unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The standard solution was injected five times for each varied conditions of flow, column temperature, pH, and mobile phase ratio and chromatograms were recorded in table no. 13 & 14.

Change in Conditions for Robustness

Change in flow rate: ± 0.2 ml/min

Change in pH: ± 0.2

RESULT AND DISCUSSION:

High Performance Liquid Chromatographic (HPLC) method has been developed for simultaneous determination of Gatifloxacin and Loteprednol in bulk and dosage form. The linearity range was found to be in the range of 25-75 µg/ml for Loteprednol and 15-45 µg/ml for Gatifloxacin with using mobile phase Buffer pH 3.5: ACN (45:55). Correlation co-efficient for calibration curve Gatifloxacin and Loteprednol was found to be 0.9977 and 0.9983 respectively. The method is simple, accurate, reproducible and short and can be used for simultaneous analysis of Loteprednol and Gatifloxacin.

Optimization of Mobile phase

Conclusion Different mobile phases were tried in order to find the best conditions for the separation of both the drugs. It was found that Buffer pH 3.5: Acetonitrile (45:55) gives satisfactory results as compared to other mobile phases. This is shown in table no.1 and figure 2-7.

Table 1: Mobile Phase trial for Sample analysis

Trial No.	Mobile Phase	Ratio	Retention Time (min.)		Remark
			Gatifloxacin	Loteprednol	
Trial 1	Water: Methanol	50:50	-	10.900	Peak of Loteprednol was not found
Trial 2	Water: Methanol	30:70	-	7.710	Peak of Loteprednol was not found
Trial 3	Water: CAN	50:50	-	6.603	Peak of Loteprednol was not found
Trial 4	Buffer (water-pH3.5 with 1%H3po4): ACN	50:50	7.313	5.773	Peaks were resolved but tailing was observed
Trial 5	Buffer (water-0.1%TEA, pH3.5 with 1%H3po4): ACN	50:50	7.277	5.740	Resolution is good
Trial 6	Buffer (water-0.1%TEA, pH3.5 with 1%H3po4): ACN	45:55	4.950	3.977	Both peak separates with good resolution

Figure 2: Trial 1

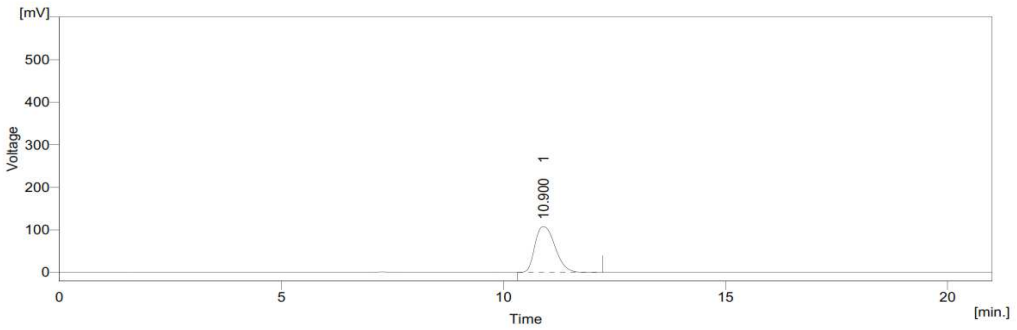


Figure 3: Trial 2

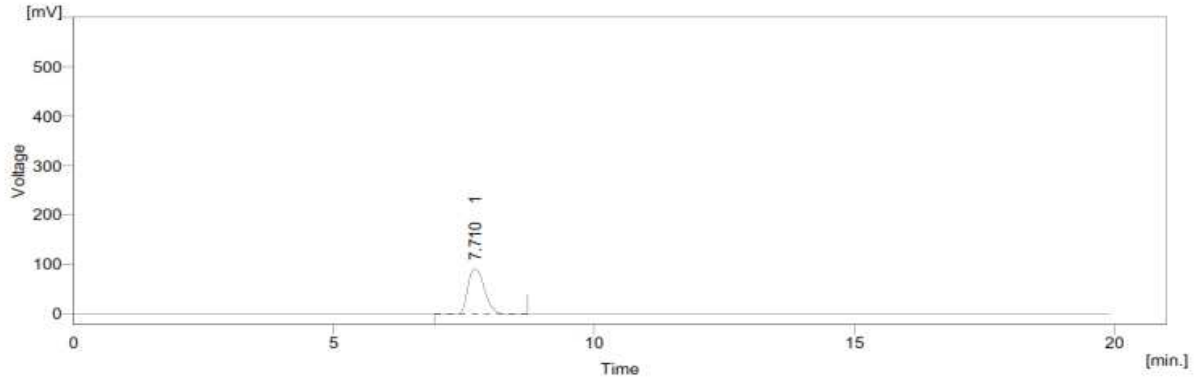


Figure 4: Trial 3

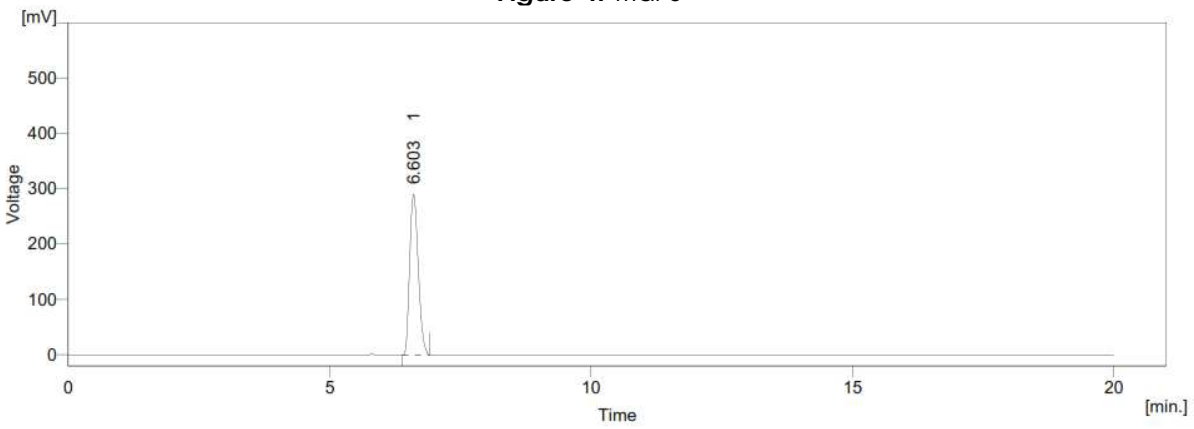


Figure 5: Trial 4

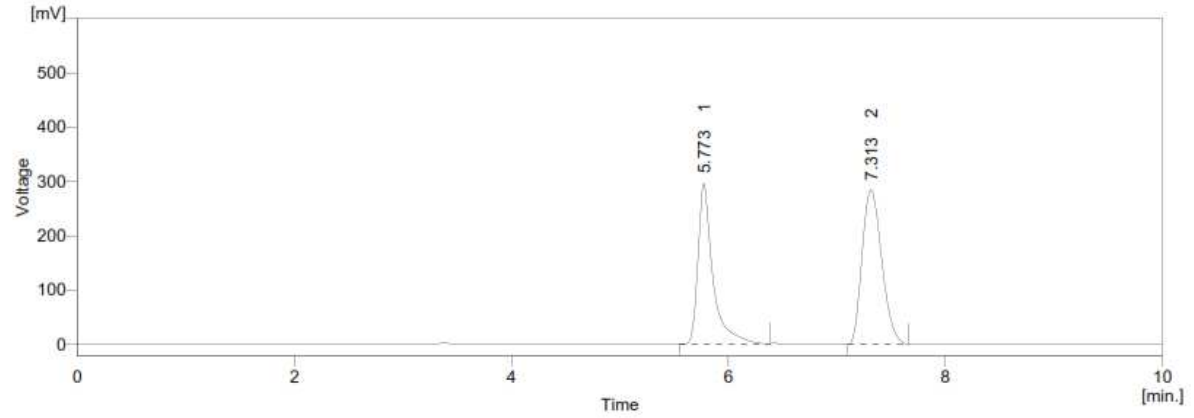


Figure 6: Trial 5

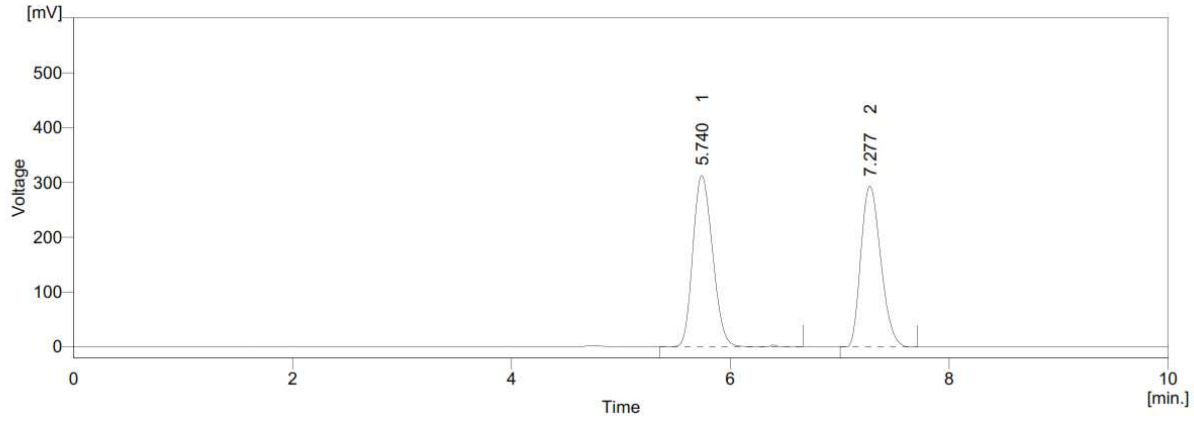
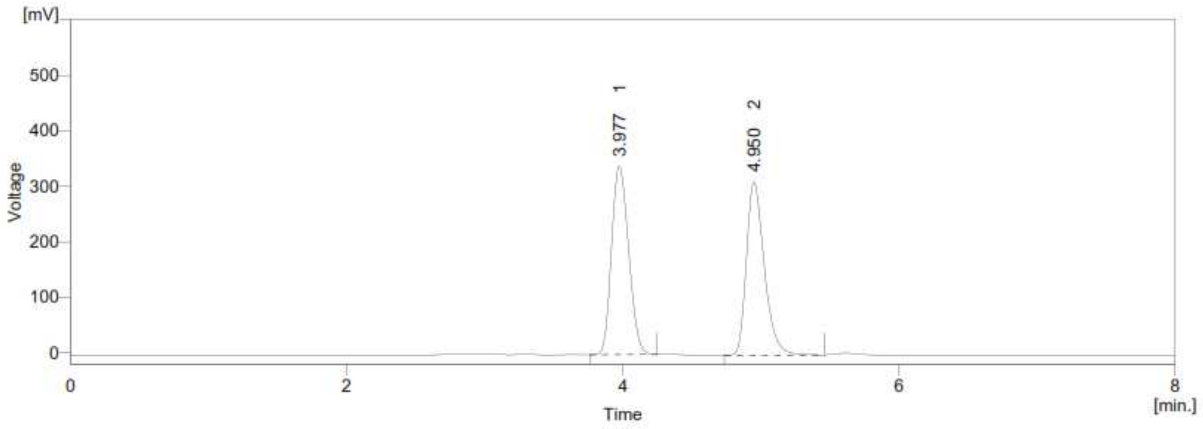


Figure 7: Trial 6



Calibration curve for the Gatifloxacin (15-45 µg/ml) and Loteprednol (25-75 µg/ml)

Conclusion:

The linearity range was found to be in the range of Gatifloxacin (15-45µg/ml). Loteprednol (25-75µg/ml). Correlation co-efficient for calibration curve Gatifloxacin and Loteprednol was found to be 0.9977 and 0.9983 respectively. Data is recorded in table no. 2 and figure no. 8,9,10.

Table 2: STD curve data for Gatifloxacin and Loteprednol

Gatifloxacin		Loteprednol	
Concentration (µg/ml)	Concentration (µg/ml)	Concentration (µg/ml)	Peak Area* (mAU*S)
15	1449.003	25	1387.602
22.5	2055.952	37.5	1949.108
30	2889.334	50	2766.875
37.5	3608.044	62.5	3455.199
45	4351.344	75	4167.018

Figure 8: STD curve linearity

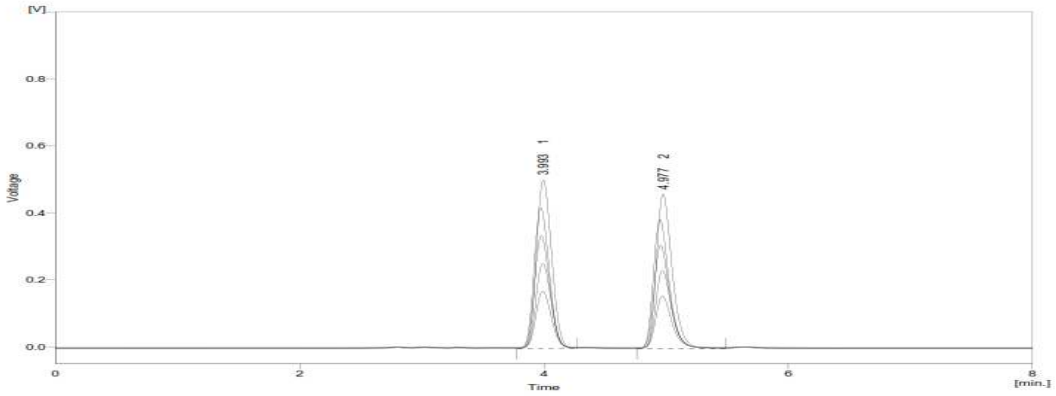
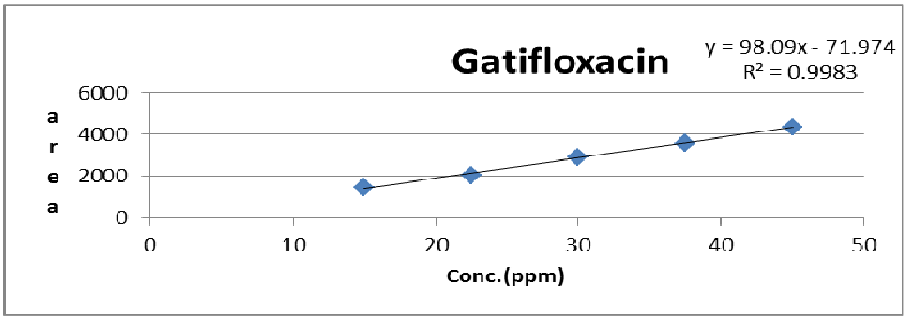
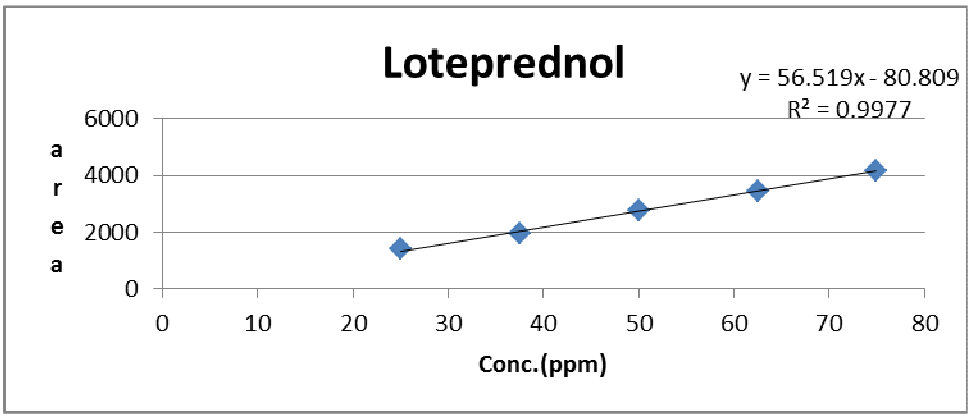


Figure 9: Calibration curve of Gatifloxacin



Covered in Scopus & Embase, Elsevier
Int. J. Drug Dev. & Res., January - March 2014, 6 (1): 99-112

Figure 10: Calibration curve of Loteprednol



Calibration curve Result

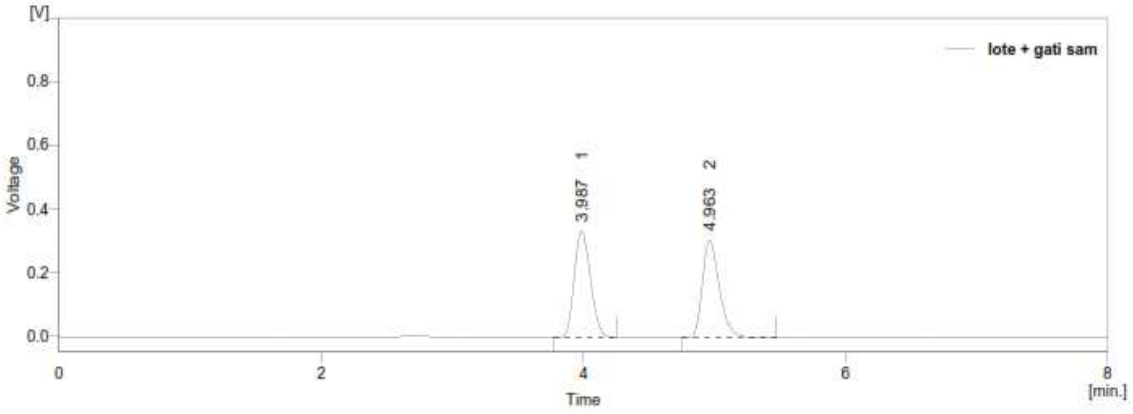
Parameter	Gatifloxacin	Loteprednol
Regression line Equation	y = 98.09x – 71.974	y= 56.519x – 80.809
Correlation coefficient	0.9983	0.9977

Analysis of marketed formulation:

Table 3: Analysis of marketed formulation

	Drugs	Label Claim (mg)	Amount Found(mg)	% Recovery
BRAND NAME: ZYLOPRED	Gatifloxacin	30mg	30.154	100.513
	Loteprednol	50mg	50.324	100.648

Figure 11: Chromatogram of marketed formulation



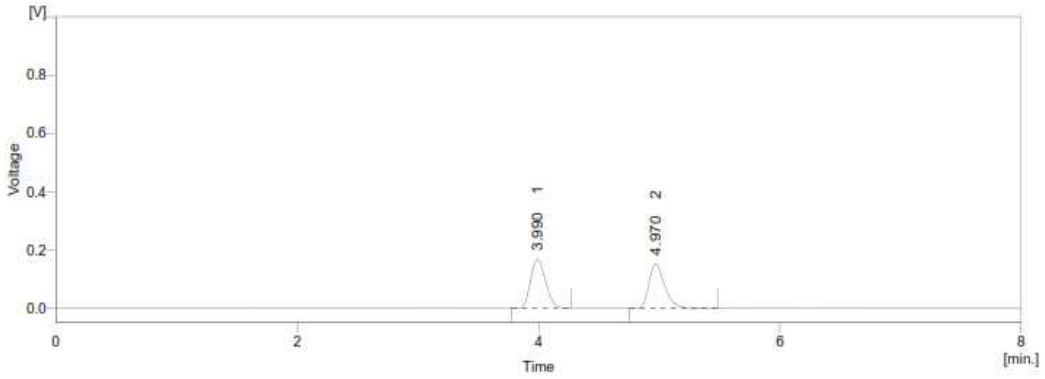
System suitability:

Conclusion: The column efficiency was more than 2000 theoretical plates and the tailing factor was less than 2.0 for Gatifloxacin and Loteprednol drugs. Resolution is 4.169. The study concludes the suitability of the HPLC system being use. And data was recorded in table no.4 and figure no.12.

Table 4: System suitability data for Gatifloxacin and Loteprednol

Sr No.	Standard Response (mAU*S)	
	Gatifloxacin (15 µg/ml)	Loteprednol (25µg/ml)
1	1449.003	1387.602
2	1452.425	1387.23
3	1447.588	1385.9
4	1447.455	1381.212
5	1448.784	1385.122
6	1446.325	1388.556
Average	1448.596	1385.937
SD	2.11	2.61
%RSD	0.14	0.18
Retention Time	3.990	4.970
Theoretical plates	4500	7327
Tailing Factor	1.313	1.581
Resolution	4.169	

Figure 12: Chromatogram of Standards for System Suitability



Solvent suitability:

Conclusion: The % RSD of response for both drugs was found to be less than 2. So, it was concluded that proposed mobile phase Buffer pH 3.5: ACN (45:55) is suitable for estimation of Gatifloxacin and Loteprednol in combined dosage form and shown in table no 5.

Table 5: Solvent suitability

Time	Standard Response (mAU*S)	
	Gatifloxacin (15 µg/ml)	Loteprednol (25µg/ml)
0 hrs	1450.733	1388.5
6 hrs	1449.2	1381.358
12 hrs	1448.5	1380.9
18 hrs	1447.762	1380.141
24 hrs	1446.8	1379.545
Average	1448.599	1382.088
SD	1.48	3.65
% RSD	0.10	0.26

VALIDATION

Linearity: 6 times chromatogram were taken for all five concentration and data were recorded table no.6

Table 6: Linearity data for Gatifloxacin and Loteprednol
*n=6

Gatifloxacin		Loteprednol	
Concentration (µg/ml)	Mean Peak Area* (mAU*S) ± SD	Concentration (µg/ml)	Mean Peak Area* (mAU*S) ± SD
15	1446.210±10.65	25	1388.395 ± 6.08
22.5	2045.766±12.23	37.5	1957.959 ±11.29
30	2887.099±8.12	50	2764.546 ±6.10
37.5	3613.587±9.63	62.5	3453.480 ±7.30
45	4350.842±9.63	75	4166.634± 6.14

Precision

I. Repeatability:

Discussion: The % RSD for Repeatability of both the drugs was found to be less than 2. So, it was concluded that proposed method for estimation of Gatifloxacin and Loteprednol is précised in nature and shown in table no.7.

Table 7: Repeatability data for Gatifloxacin and Loteprednol

Gatifloxacin		Loteprednol	
Concentration (µg/ml)	Peak Area (mAU*S)	Concentration (µg/ml)	Peak Area (mAU*S)
30	2889.334	50	2766.875
30	2896.784	50	2770.299
30	2870.746	50	2760.756
30	2894.892	50	2745.4
30	2865.148	50	2750.365
30	2895.656	50	2767.533
Mean	2885.426	Mean	2760.204
SD	13.89	SD	10.16
%RSD	0.48	%RSD	0.36

II. Intraday precision:

Discussion: The % RSD for Repeatability of both the drugs was found to be less than 2. So, it was concluded that proposed method for estimation of Gatifloxacin and Loteprednol is précised in nature and shown in table no.8.

Table 8: Intraday precision data for estimation of Gatifloxacin and Loteprednol

Gatifloxacin Concentration (µg/ml)	Peak Area(mAU*S)* ± S.D.	%RSD	Loteprednol Concentration (µg/ml)	Peak Area(mAU*S)* ± S.D.	%RSD
15	848.521±12.66	1.49	25	1127.820±11.29	1.00
30	1722.311±14.35	0.83	50	2250.723±15.85	0.70
45	2544.187±1.130	1.13	75	3361.771±41.22	1.22

III. Interday precision:

Discussion: The % RSD for Repeatability of both the drugs was found to be less than 2 so, it was concluded that proposed method for estimation of Gatifloxacin and Loteprednol is précised in nature and shown in table no.9.

Table 9: Interday precision data for estimation of Gatifloxacin and Loteprednol

Gatifloxacin Concentration (µg/ml)	Peak Area (mAU*S)* ± S.D.	%RSD	Loteprednol Concentration (µg/ml)	Peak Area (mAU*S)* ± S.D.	%RSD
15	862.313 ± 8.24	0.95	25	1130.692 ± 11.24	0.99
30	1710.632 ± 24.42	1.42	50	2243.816 ± 24.69	1.10
45	2556.962 ± 27.13	1.06	75	3378.680 ± 39.13	1.15

Accuracy:

Discussion: Result obtained reveals that % recovery of Gatifloxacin and Loteprednol was within acceptance criteria given in ICH guideline. And data recorded in table no. 10 and 11.

Table 10: Accuracy for Gatifloxacin

	Sample amount (mcg/ml)	amount added (mcg/ml)	amount recovered (mcg/ml)	% recovery	AVG	SD	%RSD
80%	30	24	23.99	99.99	99.99	0.5	0.54
80%	30	24	24.12	100.53			
80%	30	24	23.86	99.44			
100%	30	30	29.77	99.25	99.13	0.22	0.22
100%	30	30	29.78	99.27			
100%	30	30	29.66	98.86			
120%	30	36	36.31	100.86	99.56	1.15	1.15
120%	30	36	35.69	99.15			
120%	30	36	35.51	98.66			

Covered in Scopus & Embase, Elsevier
Int. J. Drug Dev. & Res., January - March 2014, 6 (1): 99-112

Table 11: Accuracy for Loteprednol

	Sample amount (mcg/ml)	amount added (mcg/ml)	amount recovered (mcg/ml)	% recovery	AVG	SD	%RSD
80%	50	40	40.00	100.00	99.99	0.54	0.54
80%	50	40	40.21	100.53			
80%	50	40	39.77	99.44			
100%	50	50	50.34	100.68	99.60	0.95	0.95
100%	50	50	49.64	99.28			
100%	50	50	49.43	98.86			
120%	50	60	60.51	100.85	99.95	0.85	0.85
120%	50	60	59.50	99.16			
120%	50	60	59.89	99.83			

LOD and LOQ:

Discussion: The proposed method can detect Gatifloxacin and Loteprednol at very low level .So, it was concluded that the proposed method is very sensitive in nature which shown in table no. 12.

Table 12: LOD & LOQ data for Gatifloxacin and Loteprednol

Parameters	Gatifloxacin	Loteprednol
Mean Slope (n=6)	98.3611	56.4158
SD (n=6)	14.50	12.45
LOD (µg/ml)	0.48	0.72
LOQ (µg/ml)	1.47	2.20

Robustness:

Discussion: The %RSD in both cases is less than 2.0. The study proves the reliability of test method for minor changes in chromatographic condition and which shown in table no. 13 and 14.

Table 13: Robustness for Gatifloxacin (30µg/ml)

Sr no.	Flow rate (+0.2ml/min)	Flow rate (0.2ml/min)	M.P. +0.2	M.P. -0.2	pH +0.2	pH -0.2
1	2744.854	3030.734	2883.53	2877.764	2872.003	2909.538
2	2720.264	3017.754	2854.695	2854.719	2843.162	2871.99
3	2776.93	3071.532	2917.291	2922.451	2892.969	2915.104
avg.area	2747.349	3040.007	2885.172	2884.978	2869.378	2898.877
SD	28.41	28.06	31.33	34.43	25.00	23.45
%RSD	1.03	0.92	1.085	1.19	0.87	0.80

Table 14: Robustness for Loteprednol (50µg/ml)

Sr no.	Flow rate (+0.2ml/min)	Flow rate (0.2ml/min)	M.P. +0.2	M.P. -0.2	pH +0.2	pH -0.2
1	2628.538	2902.319	2761.345	2755.872	2750.349	2786.292
2	2604.94	2889.851	2733.781	2733.796	2722.763	2750.282
3	2660.393	2942.758	2797.381	2808.538	2808.538	2791.659
avg.area	2631.29	2911.643	2764.169	2766.069	2760.55	2776.078
SD	27.82	27.65	31.89	38.40	43.78	22.50
%RSD	1.05	0.94	1.15	1.38	1.58	0.81

Covered in Scopus & Embase, Elsevier Int. J. Drug Dev. & Res., January - March 2014, 6 (1): 99-112

ACKNOWLEDGEMENT:

The author wishes to thanks mates who helped me lot for my work. And how can I forget U.Srinivas, my guide who suggests me in all way.

REFERENCE:

1) Chemistry 111 Lab: Intro to Spectrophotometry. Spectrophotometry 2005: E1-8
2) Loteprednol: http://www.drugbank.ca/drugs/DB00873
3) Gatifloxacin: http://www.drugbank.ca/drugs/DB01044
4) Gatifloxacin: http://www.rxlist.com/zymar-drug.htm
5) Sejal KP, Krishnakant PP. Spectrophotometric estimation of Loteprednol Etabonate and Moxifloxacin Hydrochloride in eye drops by derivative spectrophotometric method. Inventi Rapid: Pharm Analysis & Quality Assurance: Vol. 2013
6) Gaurang BB, Sandip KS, et al., Development and validation of first order derivative UV spectrophotometric method for simultaneous estimation of Moxifloxacin HCL and Loteprednol Etabonate in ophthalmic formulation. Inventi rapid: pharm analysis & quality assurance: vol. 2013.
7) Shin-ichi Yasueda, Masayo Higashiyama, Yoshihisa Shirasaki, et al. An HPLC method to evaluate purity of a steroidal drug, Loteprednol etabonate. Journal of Pharmaceutical and Biomedical Analysis 2004; 26(2):309-16.
8) Sejal KP, Krishnakant PP. Spectrophotometric estimation of Loteprednol etabonate and Moxifloxacin hydrochloride in eye drops by q-absorbance ratio method. International research journal of pharmacy 2013; 4(1):186-9.
9) Madhuri D, Chandraekhar KB. Spectrophothometric Determination of Gatifloxacin Through complexation with Surfactant. International Journal of Pharma Sciences and Research 2010;1 (2):84-89.
10) Bhanubhai NS ,Shailesh AS, Ishwarsinh SR, et al. De te rmination of Gatifloxacin and Ornidazole in Tablet Dosage Forms by High-Performance Thin-Layer Chromatography. The Japan Society for Analytical Chemistry, Analytical Sciences 2006; 22(5):743-5.
11) Sowmiya G, Gandhimathi M, Ravi TK, Sireesaa KR. HPTLC method for the determination of gatifloxacin in human plasma. Indian J Pharm Sci 2007; 69:301- 2.
12) Abida, Narasimhan B, Srinivas K,Mohd I. RP-HPLC Method Development and Validation for Gatifloxacin from Tablet Formulation. IJPI 2011; 2(7):1-8.
13) Salgado HRN, Oliveira CLCG. Development and validation of an UV spectrophotometric method for determination of gatifloxacin in tablets. Die Pharmazie- An International Journal of Pharmaceutical Sciences 2005; 60(1):263-4.
14) Venugopal K, Movva S, Saha RN. New, rapid, and sensitive spectrofluorimetric method for the estimation of Gatifloxacin in bulk and formulations. Indian J Pharm Sci 2006; 68(6):726-30.
15) Nagavallai D, Sankar A, Anandakumar K, Karunambigai K, Raju M. RP- HPLC method for simultaneous estimation of Gatifloxacin and Ornidazole in tablets. Indian J Pharm Sci 2007; 69 (2):333-5.
16) Hoang Anh Nguyen, Jean Grellet, et al. Simultaneous determination of Levofloxacin,Gatifloxacin and Moxifloxacin in serum by liquid chromatography with column switching. Journal of Chromatography B 2004; 810(1):77–83.
17) Shahed M, Nanda R, Dehghan MH, Nasreen H, Feroz S. Simultaneous determination of Gatifloxacin and Ambroxol hydrochloride from tablet dosage form using reversed-phase high performance liquid chromatography. Se Pu 2008; 26(3): 358-61.

18) Prabu SL, Thiagarajan S, et al. Simultaneous estimation of Gatifloxacin and Ambroxol hydrochloride by UV spectrophotometry. International Journal of Pharmaceutical Sciences review and research 2010; 3(2):123-6.

19) Basavaiah K and Anil Kumar. Sensitive Spectrophotometric Methods for Quantitative Determination of Gatifloxacin in Pharmaceutical Formulations using Bromate-Bromide, Thiocyanate and Tiron as Reagents. J. Mex. Chem. Soc. 2007; 51(2):106-112.

20) Mirza S, Rabindra N, et al. Simultaneous determination of Gatifloxacin and Ambroxol hydrochloride from tablet dosage form using reversed-phase high performance liquid chromatography. Chinese Journal of Chromatography 2008; 26(3):358–361

21) Ludwig H. Validation of Analytical Methods and Procedures; Labcompliance News, USA 2007

22) International Conference on Harmonisation. Guidance for industry in; Q2B Validation on Analytical Procedures: Methodology. Switcherland: IFPMA 1996: 1-8.

Article History: ----------

Date of Submission: 14-11-2013

Date of Acceptance: 14-12-2013

Conflict of Interest: NIL

Source of Support: NONE