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Simultaneous Estimation of Loteprednol Etabonate and Gatifloxacin in Pharmaceutical Dosage Form by UHPLC Method

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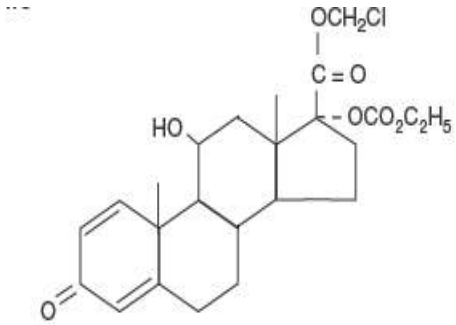
Abstract: *A reverse phase ultra-high performance liquid chromatography (RP-UHPLC) method was developed and validated for the simultaneous estimation of Loteprednoletabonate (LOTE) and Gatifloxacin (GAT) in pharmaceuticaldosage form. The separation was carried on zorbax eclipse plus column C_{18} (4.6 × 50 mm, 1.8 µ particle size) column with mobile phase (pH 6.0 withtriethylamine) containing Acetonitrile, Methanol and 0.02M potassiumdihydrogen phosphate in the ratio of 40:30:30 v/v/v with a flow rate of 0.7 mL/min and UV detection at 272 nm. The linearity was found to be in range of 15.34–46.03 µg/mL (R^2 = 0.999) and 24.94–74.81 µg/mL (R^2 = 0.999) for GAT and LOTE respectively. The method has shown good, consistent recoveries for GAT 98.88-101.24% and LOTE 98.42 - 100.94% respectively. The method was found to be accurate, precise, specific,robust and linear for the determination of GAT and LOTE in pharmaceutical dosage form.*

Keywords: *Gatifloxacin, Loteprednol etabonate, RP-UPLC, ICH guidelines, Method validation.*

I. Introduction



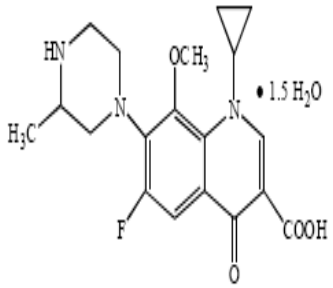


Figure 1: Structure of Loteprednol etabonate **Figure 2:** Structure of Gatifloxacin Sesquihydrate

Loteprednol etabonate is a topical corticosteroid anti-inflammatory. Chemically, it is chloromethyl 17-ethoxycarbonyloxy-11-hydroxy-10,13-dimethyl-3oxo-7,8,9,11,12,14,15,16-octahydro-6H-cyclopenta phenanthrene-17-carboxylate. It is not an official compound in any pharmacopoeia. Literature survey reveals HPLC methods for the determination of LOTE in bile, blood and urine. To the best of our knowledge there is no UHPLC method reported for the simultaneous estimation of GAT and LOTE in ophthalmic dosage forms. Therefore, attempts were made in this study to develop a rapid, sensitive and selective RP-UHPLC method for the simultaneous determination of GAT and LOTE in combined dosage form as per ICH guidelines[17]. The chemical structure of LOTE was shown in figure 1.

Gatifloxacin sesquihydrate[1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid], it is a synthetic broad spectrum antimicrobial fluoroquinolone that is active against both gram-negative and gram positive. From the literature available, it is noted that HPLC, HPTLC and UV spectrophotometric methods are described for GAT with other drugs in combination. The chemical structure of GAT was shown in figure 2.

II. Experimental Details

Chemicals and reagents

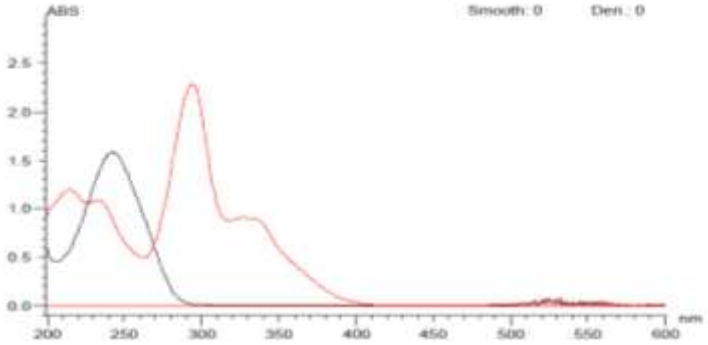
Loteprednol etabonate and Gatifloxacin were collected as a gift sample from Micro Labs Limited, Bangalore, India. All other chemicals required for the experiment were of HPLC grade and were purchased from MERCK chemicals. Chemicals used for this experiment were methanol, acetonitrile, water, triethylamine and potassium dihydrogen phosphate.

Instrument and chromatographic conditions

Chromatography was performed on UPLC Agilent technologies. The chromatographic system equipped with 1220 infinity LC, auto sampler, binary solvent and UV detector. The chromatographicseparation was performed using zorbax eclipse plus column C18 (4.6x50mmx1.8 µ particle size).

Separation was achieved using a mobile phase consisting of acetonitrile, methanol and 0.02M potassium dihydrogen phosphate buffer (40:30:30v/v/v) adjusted to pH 6.0 with triethylamine, pumped at a flow rate of 0.7ml/min. The eluent was monitored using UV detector at a wavelength of 272nm. The mobile phase was vacuum filtered through 0.22µm nylon membrane filter followed by degassing in an ultrasonic bath prior to use. Data acquisition and integration was performed using open lab CDS chemstation software.

Fig.3 Overlay spectra of LOTE and GAT



Preparation of Mobile phase/Diluent

A mixture of 30 volumes of 0.02M potassium dihydrogen orthophosphate previously adjusted to pH 6.0 with triethylamine,30 volumes of methanol and 40 volumes of acetonitrile.

Preparation of standard solution

Weighed accurately about 15mg of Gatifloxacin working standard and 25mg Loteprednol working standard in 50ml volumetric flask. Dissolved with diluent, and diluted with mobile phase up to the mark. Pipetted out 5ml of this solution into 50ml volumetric flask, made up the volume with diluent to get 50µg/ml and 30µg/ml.

Sample preparation for calibration curve of GAT and LOTE

Aliquots were taken from working standard solutionflask and diluted to 10ml with mobile phase to give final concentration of 15.34µg/ml to 46.03µg/ml for GAT and 24.94µg/ml to 74.81µg/ml for LOTE. Separatelyinjected the linearity solution in increasing concentration levels into the chromatograph and recorded the peak response.Calibration graph was constructed by plotting peak area versus concentration.

Robustness

Toevaluate LC method robustness, afew parameters were deliberately varied. The parameters included variation of flow rate, percentage of organic medium in the mobile phase and wavelength. One factor at a time was changed to estimate the effect. Thus 3 injections of standard solution was performed under small changes of three chromatographic parameters.

Linearity

The calibration curves constructed for GAT and LOTE were checked for linearity over the concentration range of 15.34µg/ml to 46.03µg/ml for GAT and 24.94µg/ml to 74.81µg/ml for LOTE. Calculated the correlationcoefficient.

Accuracy

The known quantity of the drug substance corresponding to 50,100,150% of standard GAT and LOTE and the mixture were analyzed by the proposed method. At each level of the amounts six determinations were performed. This was done to check the accuracy of the drug at different levels in the formulations.

Precision

Six injections of three different concentrations were given on the same day and the values of the %RSD were calculated to determine intra-day precision. Threeinjectionsof three different concentration were given on three different days to determine inter-day precision.

Limit of quantitation (LOQ) and limit of detection (LOD)

The LOQ and LOD were determined based on a signal-to-noise ratios and were based on analytical responses of 10 and 3 times the background noise respectively. LOD and LOQ were experimentally verified by diluting known concentration of GAT and LOTE until the average responses were approximately 3 or 10 times the standard deviation of the responses for six replicate determinations.

Stability of solution

Three different concentration of GAT and LOTE were prepared from sample solution and stored at room temperature for 6 hours and 24 hours at room temperature. They were then injected into HPLC system.

Analysis of marketed and developed formulation of GAT and LOTE

To determine the content of GAT and LOTE in formulations, 1ml of the sample was transferred into 10ml volumetric flask, dissolved with diluent. Filtered the solution through 0.45µ membrane filter. Then pipetted out 1ml of the above solution into 10ml volumetric flask, made up the volume with the diluent. The concentration of GAT and LOTE in sample stock solution was 30µg/ml and 50µg/ml. The analysis was repeated in triplicate.

III. Results And Discussion

Validation of developed UPLC method was carried out as per ICH guidelines Q2 (R1). To develop an effective method for the analysis of the drugs, preliminary tests were performed in order to select adequate and optimum conditions. Parameters such as detection wavelength, ideal mobile phase and its combination, optimum pH and concentration of the standard solutions were studied. The mobile phase consisted of Acetonitrile: methanol: 0.02M potassium dihydrogen phosphate buffer (40:30:30 v/v/v) and adjusted the mobile phase to pH 6.0 with triethylamine with a flow rate of 0.7ml/min was selected for analysis after preliminary tests. The retention time of GAT and LOTE were found to be 1.34 and 3.31 min, respectively.

The System suitability tests were carried out on freshly prepared standard solutions and the % RSD values were within the limit (<2.0). The linearity was found in the concentration range of 15.34-46.03µg/ml for GAT and 24.94-74.81µg/ml for LOTE. The correlation coefficient was found to be 0.999 and 0.999 for both the drugs respectively.The results are presented in table 3 and figure 4 & 5. The % accuracy was found between the range of 99.96 - 100.20 for GAT and 99.37-100.14 for LOTE and represented in table 4. By performing system precision and method precision studies, the % RSD values were within the limits (<2.0) and the method was found to be highly precise and represented in table 5. Robustness of the method was studied by changing the chromatographic conditions slightly. Specificity was evaluated by injecting the blank, placebo and sample. There was no other interfering peak around the retention time of GAT and LOTE. So the proposed method was found to be simple, accurate and specific, hence it could be used for routine analysis of GAT and LOTE in combined dosage form.

Table 1 optimized parameters of UPLC method

Column	Zorbax Eclipse Plus, C18 (50 mm x 4.6 mm, 1.8µm)
Mobile phase	Acetonitrile:Methanol:0.02M dihydrogen orthophosphate adjusted the pH to 6.0 with triethylamine (40:30:30v/v/v).
Column tempt.	30°C and sampler cooler 10°C
Flow rate	0.7ml/min
Flow	Isocratic
Detection wavelength	272nm

Table 2 Robustness evaluation

Parameter	Proposed	Variation	GAT	LOTE
			%RSD	%RSD
Wavelength	272nm	270nm	0.61	0.91
		274nm	0.45	0.44
Flow Rate	0.7ml/min	0.65ml/min	0.85	0.39
		0.75ml/min	0.21	0.81
Mobile Phase	30:30:40v/v/v	25:30:45	0.24	0.41
		35:30:35	0.44	0.97

Table 3 Linearity of GAT and LOTE

Concentration (µg/ml)		Peak Area	
GAT	**LOTE**	**GAT**	**LOTE**
15.34	24.94	117.47	27.43
21.48	34.91	165.06	38.91
24.55	39.90	190.92	43.94
27.62	44.88	212.07	49.27
30.69	49.87	238.00	55.04
33.76	54.86	261.49	60.37
36.83	59.84	285.14	65.59
39.90	64.83	310.37	70.99
46.03	74.81	357.73	82.00
Correlation coefficient		**0.999**	**0.999**

Figure 4 Calibration curve of Gatifloxacin

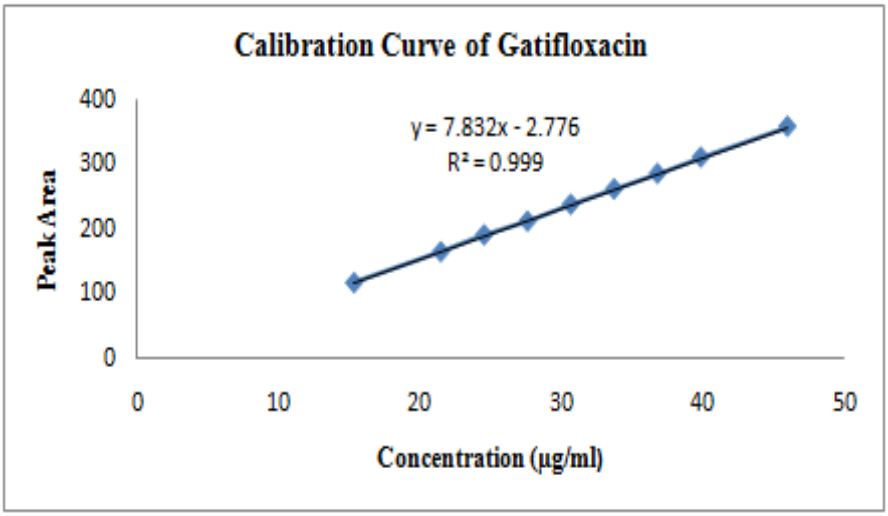


Figure 5 Calibration curve of Loteprednol etabonate

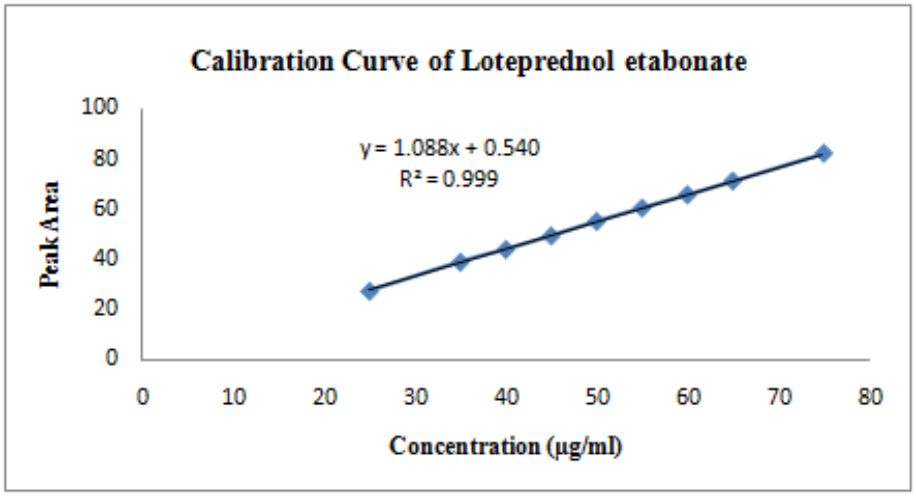


Table 4 Accuracy

S.No	Conc. of Std spiked		Amount added (mg/100ml)		Amount Recovered (mg/100ml)		*%Recovery ± SD	
	GAT	**LOTE**	**GAT**	**LOTE**	**GAT**	**LOTE**	**GAT**	**LOTE**
1	50	50	30.12	50.09	30.108	50.095	99.96± 0.16	100.01± 0.23
2	100	100	60.24	100.18	60.359	99.548	100.20± 0.35	99.37± 0.25
3	150	150	90.36	150.27	90.443	150.479	100.09± 0.35	100.14± 0.56

Table 5 Precision

S.No	Sample	GAT		LOTE	
		Area	Assay %	Area	Assay %
1	Low-1	149.37	99.53	34.37	99.96
2	Low-2	149.79	99.81	34.49	100.31
3	Low-3	148.87	99.20	34.09	99.15
4	Mid-1	245.68	98.22	57.12	99.68
5	Mid-5	246.60	98.59	57.11	99.66
6	Mid-6	249.01	99.55	56.93	99.35
7	High-1	355.93	101.64	79.40	98.97
8	High-2	350.21	100.01	80.27	100.05
9	High-3	350.14	99.99	80.19	99.95
Average :			**99.54**		**99.67**
S.D. :			**0.48**		**0.18**
% R.S.D.:			**0.76**		**0.48**

Limit of quantitation (LOQ) and limit of detection (LOD)

The LOQ was found to be 12 µg/mlfor GAT and 20 µg/ml for LOTE. The LOD was found to be 2 µg/mlfor GAT and 5 µg/ml for LOTE.

Stability in sample solutions

No additional peak was found in chromatogram indicating the stability of LOTE and GAT in the sample solution.

Analysis of marketed and developed formulations of LOTE and GAT

The validated UPLC method was successfully applied for the assay of GAT and LOTE in marketed formulations. Assay results are represented in Table 6.

Table 6 Assay of GAT and LOTE

Drug	Label Claim (%w/v)		Amount found (%w/v)		*%Label Claim ±S.D.	
	GAT	LOTE	GAT	LOTE	GAT	LOTE
Zylopred	**0.3**	**0.5**	**0.3011**	**0.4982**	**100.36± 0.4806**	**99.36± 0.1738**

System suitability

This was performed by injecting six consecutive injections of solutions having concentration of 30.69 µg/ml and 49.87 µg/mlduring the start of method validation and start of each day. Differentpeak parameters were observed like retention time, tailing factor, theoretical plates and %RSD of area. These are summarised in Table 7.

Table 7 Summary of Validation Parameters

Parameter		Gatifloxacin	Loteprednol etabonate
System suitability	Tailing factor	1.40	1.03
	Theoretical plates/meter	1796	14336
	% RSD of 6 injections	0.633	0.967
System Precision		0.633	0.967
Method Precision		0.76	0.48
Ruggedness		0.31	0.47
Accuracy (Mean)		99.40	99.53
Linearity (r2)		0.999	0.999
Robustness		Within Acceptance criteria	Within Acceptance criteria
Specificity		No interference due to blank and placebo	No interference due to blank and placebo

IV. Conclusion

UPLC method has been developed and validated for the determination of Gatifloxacin and Loteprednol etabonate in combined pharmaceutical dosage forms. The developed method was validated as per ICH guidelines and was found to be accurate, precise, robust, specific and less time consuming.No interference from any components of pharmaceutical dosage form observed, and the method has been successfully used to perform rapid and accurate analysis of Gatifloxacin and Loteprednol etabonatein their combined pharmaceutical dosage form.

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