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RESEARCH ARTICLE

Simultaneous Spectrophotometric Estimation of Loteprednol Etabonate and Gatifloxacin in Bulk and Ophthalmic Formulation

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ABSTRACT

Two simple, sensitive, accurate, precise, rapid and economical methods were developed and validated for the quantitative determination of simultaneous estimation of Loteprednol etabonate (LOTE) and Gatifloxacin (GAT) in bulk and ophthalmic formulations. Method I is based on simultaneous equations and Method II on Q-absorbance ratio. The absorption maxima were found to be at 243nm and 291nm for LOTE and GAT and Isoabsorptive point at 267nm. Beer's law is obeyed in the concentration range of 3-18 µg/ml and 2-12 µg/ml for LOTE and GAT respectively. The label claim of LOTE and GAT was found to be 101.30% and 103.05% for method I and 102.50% and 98.66% for method II. The methods was successfully applied to pharmaceutical dosage form because no interference from the synthetic mixture excipients was found. The suitability of this method for the quantitative determination of LOTE and GAT was proved by validation. The proposed method was found to be simple and sensitive for the routine quality control application of LOTE and GAT in pharmaceutical dosage form. The results of analysis have been validated statistically and by recovery studies.

KEYWORDS

Loteprednol etabonate, Gatifloxacin, Simultaneous Equation Method, Q-absorption Method, Isoabsorptive Point, Validation

INTRODUCTION

Gatifloxacin sesquihydrate (GAT) [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[1]. From the literature available, it is noted that HPLC, HPTLC and UV spectrophotometric methods are described for GAT with other drugs in combination[2].

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Loteprednol Etabonate is a topical corticosteroid anti-inflammatory. Chemically, it is Chloromethyl 17- ethoxycarbonyloxy-11-hydroxy-10, 13-dimethyl-3-oxo-7, 8, 9, 11, 12, 14, 15, 16-octahydro-6H-cyclopenta-phenanthrene-17-carboxylate[3]. It is not an official compound in any pharmacopoeia. Literature survey reveals HPLC methods for the determination of LOTE in bile, blood and urine[4]. The use of HPLC to identify Loteprednol etabonate in ophthalmic formulation[5]. However, there is no Spectrophotometric method for simultaneous estimation of LOTE and GAT in combined dosage forms. Hence, the present work describes the development of a simple, precise, accurate and cost effective

spectrophotometric method for the simultaneous estimation of LOTE and GAT from the pharmaceutical formulation. The methods reported are based on Q-Analysis and simultaneous equation methods. The structure of GAT and LOTE was shown in figure 1 and figure 2.

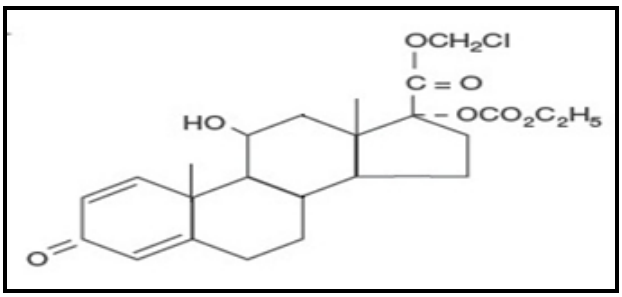

Figure 1: Structure of Loteprednol Etabonate

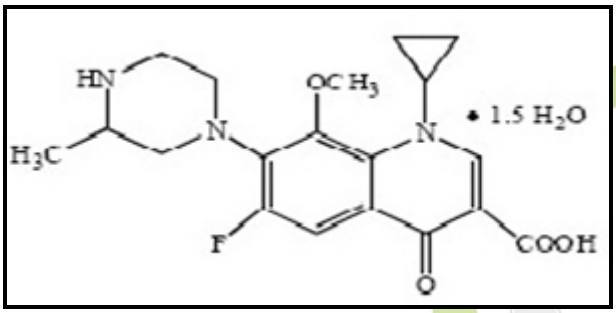

Figure 2: Structure of Gatifloxacin Sesquihydrate

EXPERIMENTAL

Materials

The standard samples of Loteprednol etabonate and Gatifloxacin were collected from Micro Labs Limited, Bangalore, India. Chemicals used for this experiment were methanol, hydrochloric acid. These chemicals were purchased from MERCK. The Ophthalmic suspension contains Loteprednol etabonate 0.5% and Gatifloxacin 0.3% were procured from local market under brand name Zylopred manufactured by Allergan Pharmaceuticals ltd.

Instrumentation

A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with matched quartz cells corresponding to 1cm path length and spectral bandwidth of 2nm was used in the study.

Method I: Simultaneous Equation Method[6]

The sample containing two absorbing drugs, each of which absorbs at the λ_{max} of the other were determined by simultaneous equation method. The two wavelengths selected were 243nm and 291nm, which represents the λ_{max} of LOTE and GAT respectively.

The Concentrations of both the drugs in mixture can be calculated by using the following equations.

$Cx = A_2ay_1\text{-}A_1ay_2/ax_2ay_1\text{-}ax_1ay_2$ -------- (1)

$Cy = A_1ax_2\text{-}A_2ax_1/ax_2ay_1\text{-}ax_1ay_2$---------- (2)

Where, A_1 and A_2 are absorbances of mixture at 243nm and 291nm respectively

Ax1 and ax_2 are the absorptivities of LOTE at 243nm and 291nm respectively

Ay1 and ay_2 are the absorptivities of GAT at 243nm and 291nm respectively.

Method II: Absorption Ratio Method[7]

In the quantitative assay of two components in admixture by absorption ratio method, absorbance were measured at two wavelengths one being the λ_{max} of one of the components and the other being a wavelength of equal absorptivity of the two components (Isoabsorptive point). From the overlain spectra LOTE and GAT two wavelengths were selected one at 243nm (λ_{max} of LOTE) and the other at 267nm (Isoabsorptive point). From the following set of equations, the concentration of each component in sample solution was calculated.

$Cx = (Qm\text{-}Qy) / (Qx\text{-}Qy) \times A_1/ax_1$---------- (3)

$Cy = (Qm\text{-}Qx) / (Qy\text{-}Qx) \times A_1/ay_1$---------- (4)

Where, Cx and Cy are the concentration of LOTE and GAT respectively.

A_1 = Absorbance of sample at 267nm

Qm= (Absorbance of sample at 243 nm) / (Absorbance of sample at 267nm)

Qx = (Absorptivity of LOTE at 243nm) / (Absorptivity of LOTE at 267nm)

Qy = (Absorptivity of GAT at 243nm) / (Absorptivity of GAT at 267nm)

Ax1 and ay_2 are the absorptivity of LOTE and GAT at 267nm.

Preparation of Standard Solutions

Primary stock solution was prepared by weighing 10mg of LOTE and 10mg of GAT separately into a clean, dry 100ml volumetric flask and dissolved with sufficient volume of alcohol. The volume was made up to 100ml with methanol to get concentration of 100µg/ml. Finally, the stock solution was further diluted in a 10 ml volumetric flask with methanol to get a concentration 10µg/ml and 6µg/ml of LOTE and GAT. Working standard solutions of both the drugs were scanned in the UV range of 200 to 400nm, using methanol as blank.

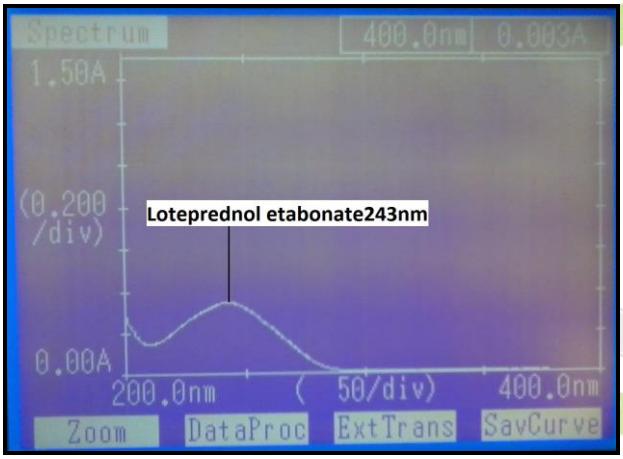

Figure 3: Spectra of Loteprednol etabonate at 243nm

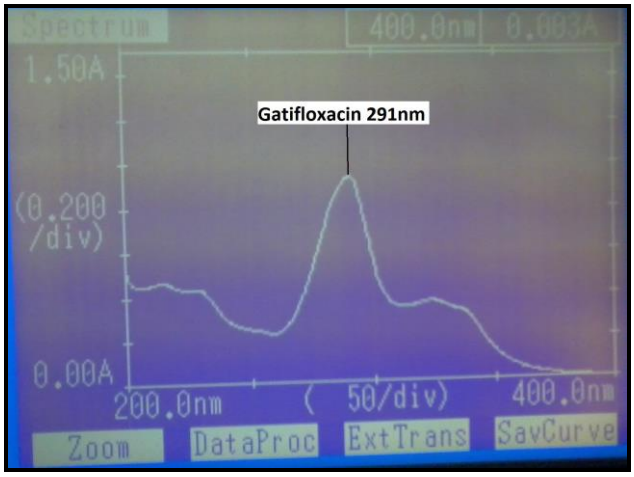

Figure 4: Spectra of Gatifloxacin at 291nm

The peaks obtained were noted and the wavelength having highest absorbance was taken as wavelength maxima and the wavelength at which these spectra cross each other corresponds to the isobestic point. The λ_{max} of LOTE and GAT were found to be 243nm and 291nm, respectively whereas 267nm is the isobestic wavelength. The Spectra's were shown in Figure 3-5.

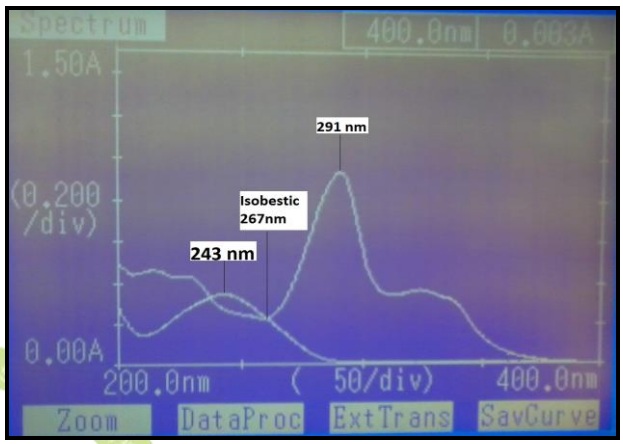

Figure 5: Overlain spectra of Loteprednol and Gatifloxacin

Preparation of Sample

From the Ophthalmic formulation of Zylopred (0.5%w/v LOTE and 0.3% w/v GAT). 1ml was taken in 50ml volumetric flask and the volume was adjusted up to the mark with methanol. From this solution 1ml was taken into 10 ml volumetric flask and the volume was made up with methanol to get the final concentration of LOTE (10µg/ml) and GAT (6µg/ml). Absorbance of working sample solutions of LOTE and GAT were taken at 243nm, 267nm and 291nm. Concentrations of LOTE and GAT in the solution were calculated and shown in table 1.

Method Validation[8]

The proposed UV methods were validated as per ICH guidelines were linearity, accuracy, precision, LOD and LOQ were studied which were well in accordance to ICH guidelines.

Linearity

The linearity was evaluated by analyzing different concentration of the standard solution

of LOTE and GAT. The linearity range for LOTE and GAT was found to be 3-18 µg/ml and 2-12 µg/ml, respectively for method I and method II. The regression equation is given in figure 6-8, table 2 & 3.

Accuracy

To check the degree of accuracy of the proposed methods, recovery studies were performed by standard addition method at three different levels (80%, 100% and 120%). Known amounts of standard LOTE and GAT were added to pre-analyzed samples and were subjected to the proposed HPLC method. Results of recovery studies were shown in table 4.

Precision

System precision

Mixed standards solutions were measured for six times. Observe the standard deviation and % RSD which is to be NMT 2.0 and shown in table 5.

Method precision

Six different test samples were prepared. The absorbance values were taken and % RSD was calculated and shown in table 6.

Inter-day Precision

The absorbance of six replicate solutions of working sample solution was recorded at wavelength of 243nm, 267nm and 291nm on different days. Concentration of LOTE and GAT in each replicate was calculated. The standard deviation and % RSD was calculated and shown in table 7.

Intra-day Precision

The absorbance of six replicate solutions of working sample solution were recorded on the same day at 243nm, 291nm and 267nm at different time intervals. The standard deviation and % RSD was calculated and shown in table 8.

Limit of detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ was derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

RESULTS AND DISCUSSION

The methods discussed in the present work provide a convenient and accurate way for simultaneous analysis of LOTE and GAT. In method I, the absorption maxima of LOTE and GAT were found to be at 243nm and 291nm respectively. In method II, the isoabsorptive point at 267nm for GAT and 243nm for LOTE were selected. In both the methods linearity was observed in the concentration range of 3-18µg/ml and 2-12µg/ml for LOTE and GAT respectively. The label claim for LOTE and GAT was found to be 101.30% and 103.05% respectively for method I and 102.50% and 98.66% respectively for method II. Accuracy of the proposed method was ascertained by recovery studies and the results were expressed as percentage recovery and found in the range of 98.0-102.0%. Standard deviation and %RSD for inter day and intraday precision studies was

Table 1: Analysis of LOTE and GAT in Eye Suspension

Eye Suspension	Label Claim (mg)		Amount found (mg)		*%Label Claim ± S.D.	
	LOTE	GAT	LOTE	GAT	LOTE	GAT
Method I	5	3	5.065	3.09	101.30± 0.6641	103.05± 0.7120
Method II	5	3	5.125	2.96	102.50± 0.7614	98.66± 0.4217

*Average of three determinations

Found to be less than 2%. It indicates that the precision of proposed method is within the ICH limit LOD and LOQ was found to be 0.51µg/ml and 1.56µg/ml respectively for LOTE, 0.22µg/ml and 0.76µg/ml respectively for GAT.

These data showed that the method is accurate for the determination of LOTE and GAT. The proposed methods were validated as per the ICH guidelines. The summary of validation parameters for the proposed method were summarised in Table.9.

Table 2: (Method I) Linearity Range Data of LOTE and GAT

Volume of stock solution (ml)	Total Volume (ml)	Conc. (µg/ml)	Absorbance at 243nm	Volume of stock solution (ml)	Total Volume (ml)	Conc. (µg/ml)	Absorbance at 291nm
0.3	10	3	0.105*	0.2	10	2	0.194*
0.6	10	6	0.202*	0.4	10	4	0.385*
0.9	10	9	0.303*	0.6	10	6	0.563*
1.2	10	12	0.401*	0.8	10	8	0.755*
1.5	10	15	0.491*	1.0	10	10	0.934*
1.8	10	18	0.589*	1.2	10	12	1.112*

*Average of six determinations

Table 3: (Method II) Linearity Range Data of LOTE and GAT

Volume of stock solution (ml)	Volume Adjusted (ml)	Conc. (µg/ml)	Absorbance at 243nm	Volume of stock solution (ml)	Volume Adjusted (ml)	Conc (µg/ml)	Absorbance at 267nm
0.3	10	3	0.105*	0.2	10	2	0.068*
0.6	10	6	0.202*	0.4	10	4	0.125*
0.9	10	9	0.303*	0.6	10	6	0.190*
1.2	10	12	0.401*	0.8	10	8	0.244*
1.5	10	15	0.491*	1.0	10	10	0.302*
1.8	10	18	0.589*	1.2	10	12	0.358*

*Average of six determinations

Table 4: Accuracy Recovery Data of Standard Mixture LOTE and GAT

--	Level	Conc. of formulation (µg/ml)		Conc. of std drug spiked (µg/ml)		*%Recovery ± RSD	
		LOTE	GAT	LOTE	GAT	LOTE	GAT
Method I	80	5	3	3	1.8	100.46± 0.4474	102.16± 0.3509
	100	5	3	5	3	103.02± 0.4915	102.50± 0.3368
	120	5	3	7	4.2	101.02± 0.2698	103.81± 0.4641
Method II	80	5	3	3	1.8	101.20± 0.1932	99.85± 0.1023
	100	5	3	5	3	100.96± 0.5315	99.33± 0.3094
	120	5	3	7	4.2	101.30± 0.1540	98.33± 0.4779

*Average of three determinations.

Table 5: System Precision

Replicate	LOTE (243nm)	GAT (291nm)	Isoabsorptive (267nm)
1	0.634	0.612	0.374
2	0.632	0.611	0.378
3	0.635	0.612	0.379
4	0.631	0.612	0.374
5	0.635	0.612	0.375
6	0.634	0.613	0.379
Mean	**0.6335**	**0.6115**	**0.3765**
Standard deviation	**0.0016**	**0.0007**	**0.0022**
%RSD	**0.2593**	**0.1249**	**0.5889**

Table 6: Method Precision

Replicate	Method I		Method II	
	LOTE (243nm)	GAT (291nm)	LOTE (243nm)	GAT (267nm)
1	9.63	5.6	11.614	5.54
2	9.58	5.65	11.569	5.65
3	9.62	5.59	11.585	5.85
4	9.68	5.64	11.625	5.64
5	9.54	5.75	11.422	5.39
6	9.65	5.63	11.424	5.26
Mean	**9.62**	**5.64**	**11.53**	**5.64**
Standard deviation	**0.0457**	**0.0512**	**0.0846**	**0.0521**
%RSD	**0.4752**	**0.9024**	**0.7331**	**0.9052**

Table 7: Inter-day Precision

Replicate	Date Interval	Method I		Method II	
		LOTE 243nm	GAT 291nm	LOTE 243nm	GAT 267nm
1	25/06/13; 10am	0.597	0.762	0.595	0.416
2	25/06/13; 4pm	0.595	0.764	0.595	0.413
3	26/06/13; 10am	0.599	0.763	0.599	0.412
4	26/06/13; 4pm	0.597	0.764	0.597	0.413
5	27/06/13; 10 am	0.596	0.768	0.596	0.411
6	27/06/013; 4pm	0.594	0.765	0.594	0.415
Mean		**0.5963**	**0.7643**	**0.5960**	**0.4133**
Standard Deviation		**0.0015**	**0.0018**	**0.0016**	**0.0017**
% RSD		**0.2680**	**0.2467**	**0.2739**	**0.4112**

Table 8: Intra-day Precision

Replicate	Time Interval	Method I		Method II	
		LOTE 243nm	GAT 291nm	LOTE 243nm	GAT 267nm
1	11 am	0.384	0.58	0.56	0.374
2	12 Noon	0.385	0.58	0.565	0.376
3	1 pm	0.386	0.579	0.568	0.374
4	2 pm	0.387	0.582	0.561	0.372
5	3 pm	0.385	0.579	0.562	0.377
6	4 pm	0.387	0.581	0.568	0.372
Mean		**0.3856**	**0.5801**	**0.5640**	**0.3741**
Standard deviation		**0.0012**	**0.0010**	**0.0032**	**0.0018**
%RSD		**0.3140**	**0.1839**	**0.5699**	**0.4980**

Table 9: Summary of Validation parameters for the proposed method

Parameters	Method I		Method II	
	LOTE	GAT	LOTE	GAT
Wavelength (nm)	243nm	291nm	243nm	267nm
Linearity Range (µg/ml)	3-18	2-12	3-18	2-12
Correlation coefficient(R^2)	0.999	0.999	0.999	0.999
Regression equation (y= mx + c)	y = 0.032x+0.005	y = 0.092x+0.007	y = 0.032x+0.005	y = 0.029x+0.005
Slope (m)	0.032	0.092	0.032	0.29
Intercept (c)	0.005	0.007	0.005	0.005
Molar Extinction coefficient ($1mol^{-1}cm^{-1}$)	335.32	945.86	335.32	313.74
Accuracy	102.94± 0.6564	101.32± 0.8493	101.15± 0.1414	99.65± 0.0229
System precision (% RSD, n=6)	0.2024	0.1834	0.2024	0.1249
Method Precision (%RSD, n=6)	0.4752	0.9052	0.7331	0.9245
Inter day precision (% RSD, n=6)	0.2680	0.2467	0.2739	0.4112
Intraday precision (% RSD, n=6)	0.3140	0.1839	0.5699	0.4980
LOD	0.51	0.22	0.51	0.50
LOQ	1.56	0.76	1.56	1.72

CONCLUSION

Both the developed methods were found to be simple, accurate, precise and inexpensive for the routine analysis of LOTE and GAT using environmentally friendly solvents. Moreover the assay results obtained showed that the ophthalmic formulation met the ICH acceptance criteria of 95 to 105% of the label claim.

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