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Development and Validation of HPTLC Method for the Simultaneous Estimation of Gatifloxacin and Loteprednol Etabonate in Pharmaceutical Dosage Form.

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ABSTRACT

A simple, accurate, precise high-performance thin-layer chromatographic method for simultaneous estimation of Gatifloxacin (GAT) and Loteprednol etabonate (LOTE) in ophthalmic formulation has been developed and validated. The method employed TLC aluminium plates precoated with silica gel $60F_{254}$ as stationary phase. The solvent system consisted of methanol: ethylacetate: triethylamine (7:3:0.2v/v/v). Densitometric analysis was carried out at 272nm for LOTE and GAT. The system was found to give compact spots for LOTE and GAT at R_f value of0.70 and 0.29respectively. The linear regression analysis data showed a good linear relationship in the concentration range of 1-5µg/spot for LOTE and GAT. The% recovery was found to be 99-101% for GAT and 99-102% for LOTE. The correlation coefficient was found to be 0.999 for LOTE and GAT. The % RSD values indicated that the proposed method was precise. The specificity of the method was ascertained by peak purity profiling studies and the developed method was specific. The method has been successfully applied in the analysis of combined dosage form.

Keywords: Gatifloxacin, Loteprednol etabonate, HPTLC and Method Validation.

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INTRODUCTION

Gatifloxacin sesquihydrate[1] (GAT) is a well known standard Antimicrobial drug, Official compound in Indian Pharmacopoeia (IP) in which the estimation is done by HPLC. From the literature available for the estimation of GAT in single dosage form. It is also noted that HPLC, HPTLC and UV spectrophotometry methods are described for GAT with other drugs in combination. Loteprednol etabonate[2] (LOTE) is a commonly used steroidal anti-inflammatory drug which is not an official compound in any pharmacopoeia. HPLC methods for the determination of LOTE in bile, blood and urine is found in the existing literature and also in Ophthalmic formulation. The combination of drug is not official in any pharmacopoeia, hence no official method is available for simultaneous estimation of these two drugs However, the above two drugs is pharmacopoeia; there are no Spectrophotometric method for simultaneous estimation of LOTE and GAT in combined dosage forms. In the present investigation, an attempt has been made to develop simple, sensitive, specific, economic HPTLC method for the simultaneous determination of GAT and LOTE in combined tablet dosage forms. The structure of GAT and LOTE was shown in Figure.1 and Figure.2.

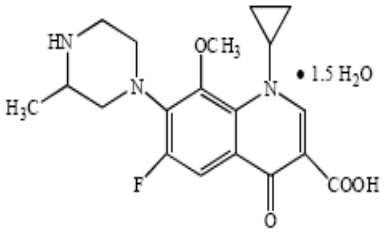

Figure 1: Structure of GAT

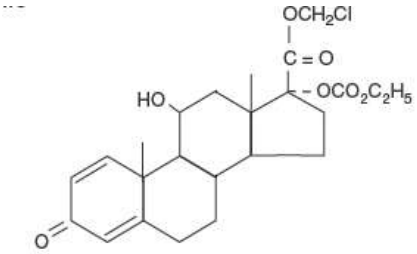

Figure 2: Structure of LOTE

MATERIALS AND METHODS

Materials

Methanol, ethylacetate, triethylamine and chloroform were procured from merck chemicals. Loteprednol etabonate and Gatifloxacin were collected as a gift sample from Micro Labs Limited, Bangalore, India. Zylopred eye suspension (Label claim: Loteprednol etabonate 0.5% and Gatifloxacin 0.3%) manufactured by Allergan Pharmaceuticals Ltd were procured from local market.

Instrumentation

A Camag HPTLC system (Switzerland) comprising of Camag Linomat V sample applicator, Camag TLC Scanner 3, Camag Plate heater, Camag twin-trough glass chamber, Precoated plates (20cm X 20cm) with 200µm layer thickness, UV lamp(190-400nm), Camag winCATS software, Hamilton syringe100µl and Sartorius Analytical balance were used for the study.

Chromatographic conditions

The experiment was performed on silica gel 60F254 aluminum sheets (20 x 20 cm) as stationary phase, using mobile phase comprised methanol: ethylacetate: triethylamine in the ratio of 7:3:0.2v/v/v. The solutions were applied on TLC plate in the form of bands of 6 mm width under a stream of nitrogen gas using a Camag Linomat V automatic sample applicator. A constant application rate of 0.1 ml/s was employed and space between two bands was fixed at 5 mm. Ascending development to 80 mm was performed in 10 cm x 10 cm Camag twin trough glass chamber saturated with the mobile phase for 30 min at room temperature. The developed TLC plate was air dried and then scanned between 200 to 400 nm using Camag TLC scanner 3 using WinCATS software. Both components show reasonably good response at 272 nm.

Preparation of standard solution

LOTE (100mg) and GAT (100mg) were weighed accurately, transferred to 100ml volumetric flask, dissolved and diluted with methanol to get 1000µg/ml (1µg/µl). Different volumes of mixed standard solution (1µl, 2µl, 3µl, 4µl and 5µl) were spotted on the TLC plate to obtain the concentrations of 1µg, 2µg, 3µg, 4µg and 5µg/spot for LOTE and GAT respectively.

Preparation of sample solution

From the Ophthalmic formulation of Zylopred (0.5%w/v LOTE and 0.3% w/v GAT). 3.3ml was taken in 10ml volumetric flask and the volume was adjusted upto the mark with methanol. 1µl of the sample solution was spotted on the TLC plate to obtain the concentrations of 1µg/spot for GAT and 1.66µg/spot for LOTE.

Validation of the proposed method[13]

The Proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.

Linearity (Calibration curve)

Calibration curves were plotted over the concentration range of 1-5µg/spot and 1-5µg/spot for GAT and LOTE, respectively. Accurately measured mixed standard solutions of GAT and LOTE were applied to the TLC plate. The TLC plate was developed and photometrically analyzed as described under chromatographic separation. The calibration curve was prepared by plotting peak area versus concentration (µg/spot) corresponding to each spot. Each reading was an average of five determinations.

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of GAT and LOTE by the standard addition method. Known amounts of standard solutions of GAT and LOTE was added at

50, 100 and 150 % level to pre quantified sample solution of GAT and LOTE respectively. The amount of GAT and LOTE was estimated by applying obtained values to the respective regression line equations.

Method Precision (% Repeatability)

The precision of the instrument was checked by repeatedly injecting (n = 6) solutions of GAT and LOTE without changing the parameters of the proposed method. The results were reported in terms of relative standard deviation (% RSD).

Intermediate Precision (Reproducibility)

The intraday and interday precision of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for different concentration of standard solution of GAT and LOTE for the proposed method. The results were reported in terms of relative standard deviation (% RSD).

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

LOD and the LOQ of the drug were calculated using the following equations as per international Conference on Harmonization (ICH) guidelines.

LOD = 3.3 X σ/S

LOQ = 10 X σ/S

Where = Standard deviation of the response

S = Slope of calibration curve

Specificity

The specificity of the method was ascertained by analyzing standard drugs and the sample. The spots for GAT and LOTE in the samples were confirmed by comparing the Rf and spectra of the spots with that of the standards.

Analysis of the marketed formulations

Five microlitres of sample solution from formulation was applied separately on TLC plate, developed and scanned as described in chromatographic separation. The amount of GAT and LOTE present in the sample solution was determined by fitting area values of peak corresponding to GAT and LOTE into the respective calibration curve.

RESULTS AND DISCUSSIONS

The TLC procedure was optimized with a view to develop an assay method for the simultaneous estimation of GAT and LOTE. The standard solutions of both the drugs were spotted on the TLC plates and run in different solvent systems. The mobile phase consisting of methanol: ethylacetate:

triethylamine (7:3:0.2, v/v/v) gave sharp and symmetrical peaks with the Rf values of 0.29 ± 0.002 and0.70 ± 0.006 for GAT and LOTE, respectively. Well defined spots were obtained when the chamber was saturated with mobile phase for 30 min at room temperature (27 ±30ºC). A combined densitogram of mixed standards and3-D chromatogram showing peaks of GAT and LOTE indifferent concentrations at 272nm are depicted in Figure3 and Figure 4, respectively. The proposed HPTLC method was validated in terms of linearity, precision, accuracy, LOD, LOQ and specificity. The calibration plot was found to be linear over the concentration range 1-5µg/spot for GAT and LOTE, respectively with a correlation coefficient of 0.999 and 0.999 for GAT and LOTE, respectively. LOD for GAT and LOTE were found to be0.0112µg/spot and 0.0634 µg/spot, respectively. LOQ for GAT and LOTE were found to be 0.0341µg/spot and 0.1921µg/spot, respectively indicate the sensitivity of the method. The low % RSD values of intraday (0.874 for GAT and 0.760 for LOTE) and interday (0.794 for GAT and 0.749 for LOTE) precision reveals that the proposed method is precise. To study the accuracy of the method, recovery studies were performed. The percent average recoveries obtained were 99-101% and 99-102% for GAT and LOTE, respectively indicating that the proposed HPTLC method is highly accurate (Table 1).The proposed validated method was successfully applied to determine GAT and LOTE in tablet dosage forms. The percent average assay was found to be 99.76 ± 0.99 and be 99.98 ± 1.14 for GAT and LOTE, respectively (Table2). The low values of standard deviation indicate the suitability of this method for routine analysis of GAT and LOTE in pharmaceutical dosage forms. To confirm the specificity of the proposed method, the solution of formulation was spotted on TLC plate, developed and scanned. It was observed that the excipients present in the formulation did not interfere with the sample peak. The regression analysis data and validation parameters are presented in Table 5.

Table 1: Recovery data of proposed method

S.No	Conc. of Standard added (µg/spot)		Amount Recovered		*%Recovery ± SD	
	GAT	LOTE	GAT	LOTE	GAT	LOTE
1	2	2	2.9964	3.6863	99.6411± 0.1699	101.5865 ±0.6326
2	3	3	4.0032	4.6531	100.3209±0.3534	99.5897 ± 0.1975
3	4	4	5.0063	5.6635	100.6302±0.3534	100.2130±0.1975

S. D. is Standard deviation and n is number of determinations

Table 2: Assay of GAT and LOTE

Drug	Label Claim (mg)		Amount found (mg)		*%Label Claim ±S.D.	
Zylopred	GAT	LOTE	GAT	LOTE	GAT	LOTE
	3	5	2.993	4.999	99.76± 0.4806	99.98± 0.1738

Table 3: Intraday Precision of GAT and LOTE

Replicate	Time Interval	Concentration (µg/spot)	GAT	LOTE
1	11 am	3	9345.0	6459.4
2	12 Noon	3	9367.7	6408.3
3	1 pm	3	9356.0	6334.8
4	2 pm	3	9345.0	6458.2
5	3 pm	3	9546.7	6429.8
6	4 pm	3	9457.4	6459.8
Mean			9402.96	6425.05
Standard deviation			82.2096	48.8561
%RSD			0.8742	0.7604

Table 4: Interday Precision of GAT and LOTE

Replicate	Date Interval	Concentration (µg/spot)	GAT	LOTE
1	16/12/13; 10am	3	9367.9	6489.5
2	16/12/13; 4pm	3	9467.9	6497.5
3	17/12/13; 10am	3	9369.0	6432.9
4	17/12/13; 4pm	3	9364.0	6498.7
5	18/12/13; 10 am	3	9547.8	6375.0
6	18/12/013; 4pm	3	9458.5	6457.0
Mean			9429.18	6458.43
Standard deviation			74.9064	48.4311
%RSD			0.7944	0.7498

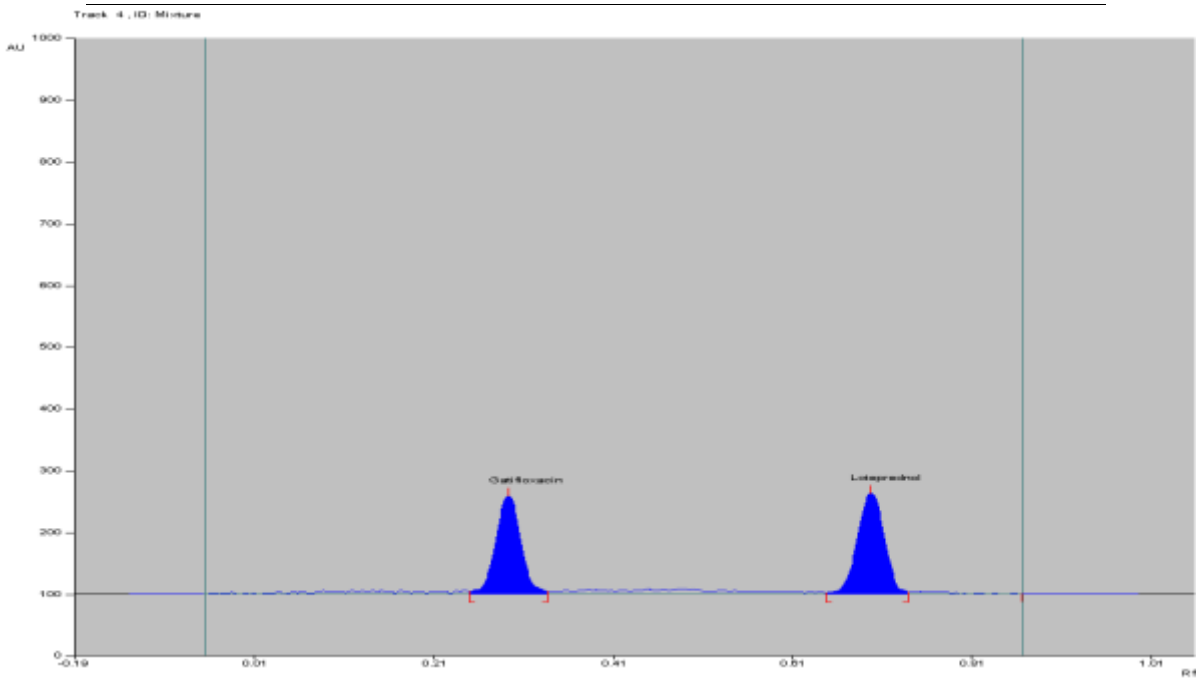

Figure 3: Chromatogram of LOTE (50 ng/spot) and GAT (100 ng/spot) with corresponding Rf values at 272 nm. Stationary phase: 10 X 10 cm HPTLC silica gel 60F254 aluminium plates, Mobile phase: methanol: ethylacetate: triethylamine (7:3:0.2, v/v/v), Detection: UV at 272 nm.

Table 5: Validation Parameters of Gatifloxacin and Loteprednol etabonate

Validation Parameters	Gatifloxacin	Loteprednol etabonate
Linearity	1-5µg/spot	1-5µg/spot
Correlation Coefficient	0.999	0.999
Accuracy	99-101%	99-102%
Precision(%RSD)	Less than 2%	Less than 2%
a. Intraday Precision	0.8742	0.7604
b. Interday Precision	0.7944	0.7498
LOD	0.0112	0.0634
LOQ	0.0341	0.1921
Specificity	Specific	Specific

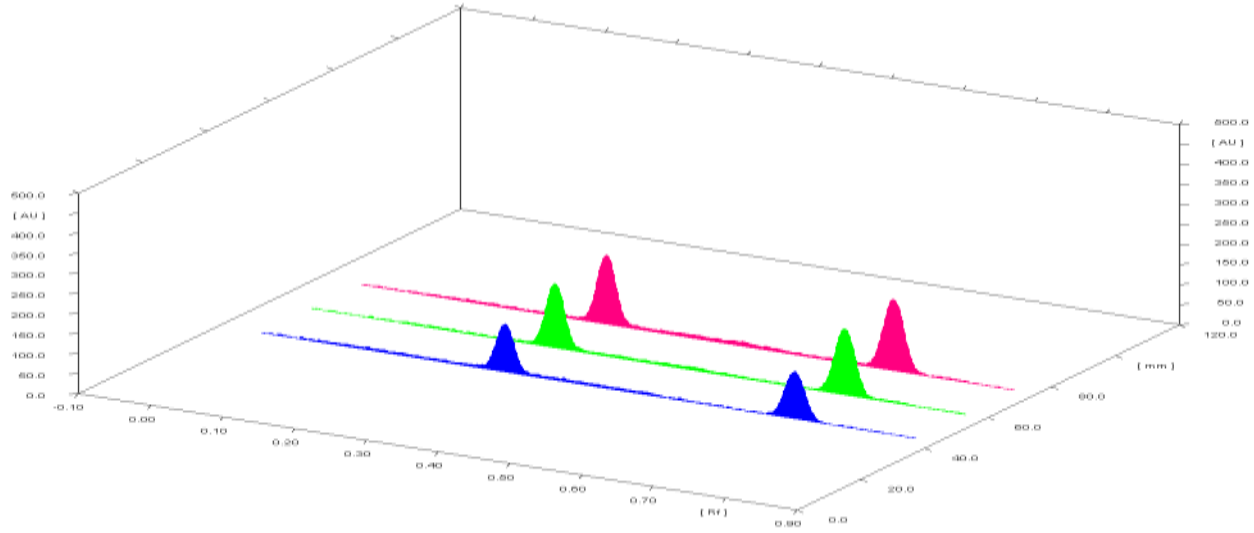

Figure 4: 3-D Chromatogram showing peaks of GAT and LOTE indifferent concentrations at 272 nm reproducible, reliable and are in good agreement with the labelled claim of the drug.

CONCLUSION

The proposed HPTLC method for simultaneous estimation of LOTE and GAT in combined dosage forms was validated and found to be applicable for routine quantitative analysis of LOTE and GAT. The result of linearity, precision, accuracy and specificity were proved to be within the limits. The method provides selective quantitative of LOTE and GAT. Therefore this method can be employed for the routine analysis for simultaneous estimation of LOTE and GAT in quality control of formulation.

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