Development and Validation of HPLC Method for the Simultaneous Estimation of Loteprednol and Gatifloxacin

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Abstract: ***A simple, rapid, precise and HPLC method for determination of Loteprednol and Gatifloxacin drugs in combined dosage form. The proposed HPLC method carried out on Hypersil BDS C18 (250 X 4.6mm, 5µm) as stationary phase by using mobile phase consisting of 0.02M Phosphate buffer (pH 4.0 with orthophosphoric acid) : acetonitrile (75 : 25). Mobile phase was pumped through chromatographic system at a flow rate of 1.0 ml/min. The UV detector was operated at 272nm. The retention time was found to be 3 min for Loteprednol And 7 min for Gatifloxacin . This optimize method was validated as recommended by ICH guidelines. The specificity studies shows that the analytic peaks were well resolved from the intermediates. The correlation coefficient were found 0.999 for Loteprednol and Gatifloxacin. The precision study showed that the percentage relative standard deviation was within the range of acceptable limits respectively. The limit of detection for Gatifloxacin is 0.248 and limit of quantification is 0.752 and for Lotprednol limit of detection is 0.486 and limit of quantification is 1.474.***

Keywords: Loteprednol , Gatifloxacin, High performance liquid chromatography (HPLC) .

1. Introduction

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 , The corticosteroid (glucocorticoid) binds to the glucocorticoid receptoron the surface of the cell . After binding, the resultant complex travels to the cell nucleus, where it binds with glucocorticoid response elements (GREs) on target genes. Clinically susceptible infections affecting the anterior chamber (front portion) of the eye such as the Allergic conjunctivitis, Acne rosacea , Cyclitis , as well as chronic form of Keratitis, Keratoconjuctivitis, Pingueculitis, Episoleritis. Corticosteroid. It is also used in optometry of eye due to allergic condition. The drug has little or no effect on intraocular pressure. Gatifloxacin is a fourth generation fluroquinolones antibiotic. Chemically, It is 1- cyclopropy l-6-fluoro -8- methoxy -7 - (3- methylpiperazin-1-yl)- 4-oxo-quinoline-3- carboxylic acid . The drug inhibits replication of the bacatrial DNA by interfering with the action of DNA gyrase [topoisomeraseII and topoisomerase IV]. After binding the quinolone to both the enzyme and DNA forms a ternary complex. It is highly active against pencillin and resistant stains of streptococcus pneumonaie. It is indicated in the treatment of acute sinusitis and acute bacterial exerberation of bronchitis, effective against Gram-positive and Gram-negative a typical aerobes.

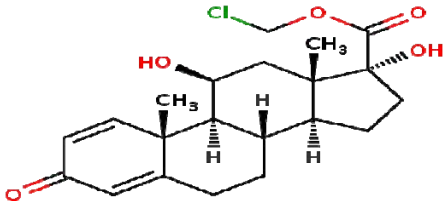

Figure 1 Loteprednol Chemical Structure[15]

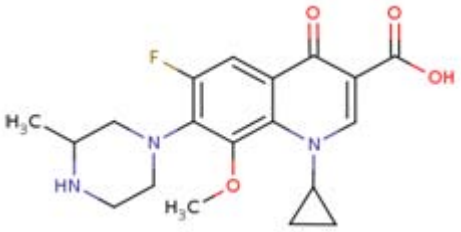

Figure 2 Gatifloxacin Chemical Structure[15]

2. Experimental

2.1 Apparatus and software

The Shimadzu HPLC system was consisting of gradient pump (LC-20AT Prominence pump), Rheodyne injector, SPD-M 20A PDA detector or SPD -20A UV detector . The separations were achieved on a Gemini, C18 (250×4.6mm, 5µm size) column with UV detection at 272nm. The injection volume was 20µL.Analytical weighing balance (Shimadzu AX-200) was used for weighing, Ultra-Sonicator (Toshcon by Toshniwal) , Millipore filtration kit for

solvents and sample filtration were used throughout the experiment. The Spinchrom CFR software was used for acquisition, evaluation and storage of chromatographic data.

2.2 Reagent and Pharmaceutical preparations

Standard bulk drug sample Loteprednol and Gatifloxacin were kindly donated by Molecule Laboratory., Ahmedabad . Acetonitrile , Methanol (HPLC grade Rankem) , Potassium dyhydrogen phosphate , Sodium dyhydrogen phosphate , Orthophosphoric acid (AR grade Rankem), Triethyl amine(AR grade Finar) .Commercial pharmaceutical preparation Zylopred Allergan India) is claimed to contain 0.5% W/V of Loteprednol and 0. 3% W/V of Gatifloxacin.

2.3 Selection of wavelength

Loteprdlone and Gatifloxacin Sodium, 272 nm was selected as detection wavelength. At the selected wavelength Loteprdlone and Gatifloxacin Sodium showed good absorbance

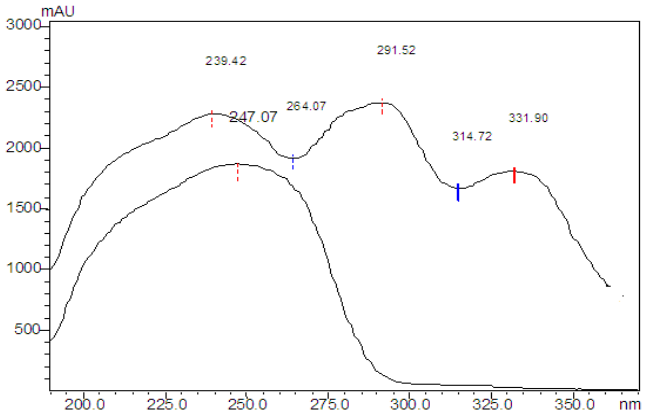

Figure 3: UV spectrum of Loteprednol and Gatifloxacin

2.4 Chromatographic Conditions

The mobile phase containing acetonitrile Buffer Potassium dihydrogen phosphate(0.5% , pH 4.0) (75:25) was found suitable to resolve Loteprednol etabonate and Gatifloxacin , orthophospheric acid was used for pH adjustment of buffer ,and the mobile phase was filtered on a 0.45µm embrane filter and then ultrasonicator was used . The flow rate was set to 1.0 ml/min. Both drugs shows good absorbance at 272nm, which was selected as wave length for further analysis.

2.5 Working standard preparation

Take 1ml of Loteprednol stock solution and 1ml of gatifloxacin stock solution and dilute with mobile phase up to 10ml. (Loteprdlone-100mcg/ml & Gatifloxacin 60mcg/ml) .

2.6 Sample preparation: (Label claim: 0.5%w/v Loteprdnol & 0.3%w/v Gatifloxacin)

Take 1ml sample and dilute with mobile phase into 10ml volumetric flask (500mcg/ml of Loteprdnol and 300mcg/ml of Gatifloxacin) Take 2ml of above solution and dilute with mobile phase up to 10 ml (100mcg/ml of Loteprdnol and 60mcg/ml of Gatifloxacin .

2.7 Calibration curves

Calibration curves were prepared by taking appropriate aliquots of standard Loteprednol and Gatifloxacin. Stock solution dilute with mobile phase up to 10ml volumetric flask (lotepredlone-100mcg/ml & gatifloxacin 60mcg/ml) to obtain final concentration of both drugs. Standard solutions were injected through 20µL loop system and chromatograms were obtained using 1.0µL/ml flow rate .The effluent was monitored at 272nm. A representative chromatograms' showing the retention time for both the drugs in laboratory prepared mixture and individually drug. Calibration curve was constructed by plotting average peak area against concentration.

3. Method Validation

3.1 Calibration curves (linearity)

Calibration curve were constructed by plotting peak area vs concentration of Loteprednol and Gatifloxacin. After that the regression equations were calculated. The calibration curves were plotted over the concentration range 10-30mcg/ml for Loteprednol and 6-18mcg/ml for Gatifloxacin. The accurately measured standard working solution of Loteprednol (0.2,0.5,1.0,1.5,2.0,2.5,3.0ml) and Gatifloxacin (0.2,0.5,1.0,1.5,2.0,2.5,3.0ml) from 100mcg/ml of stock solution were transferred to a series of 10ml of volumetric flasks and diluted to mark with methanol. 20µL of each solution were injected under the operating chromatography condition.

3.2 Accuracy (%recovery)

The accuracy of the method was determined by calculating recover of Loteprednol and Gatifloxacin by standard addition method. The known amounts of standard solutions of Loteprednol were added to prequantified sample solution of Gatifloxacin. Then known amounts of standard solution of Gatifloxacin were added to pre quantified sample solution of loteprednol. The amounts of loteprednol and gatifloxacin were estimated by applying obtained values to the regression equation of calibration curve.

3.3 Method precision (%Repeatability)

The precision of Method was checked by repeatedly injecting six sample solution of loteprednol (20mcg/ml)and gatifloxacin (12mcg/ml)under the same chromatographic condition and measurement of peak area, retention time and tailing factor, percentage relative standard deviation(RSD)or % coefficient of variation should not more than 2.0.

LOD calculated as:

Based on the standard deviation of the response (σ) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula:

$LOD = 3.3\ \sigma / s$

The standard deviation of the response can be determined based on the standard deviation of the blank, on the residual standard deviation of the regression line, or the standard deviation of y-intercepts of regression lines.

LOQ calculated as:

Based on the standard deviation of the response (σ) and the slope of the calibration curve (S) at levels approximating the LOQ according to the formula:

LOQ = 10 σ/s

The standard deviation of the response can be determined based on the standard deviation of the blank, on the residual standard deviation of the regression line, or the standard deviation of y-intercepts of regression lines.

4. Analysis of Loteprednol and Gatifloxacin synthetic mixture

The response of the sample solution was measured at 272 nm under the chromatographic condition mentioned above for the quantification of Loteprednol and Gatifloxacin. The amounts of Loteprednol and Gatifloxacin present in sample solution were determined by applying values of the peak area to the regression equations of the calibration curve.

4.1. Results and Discussion

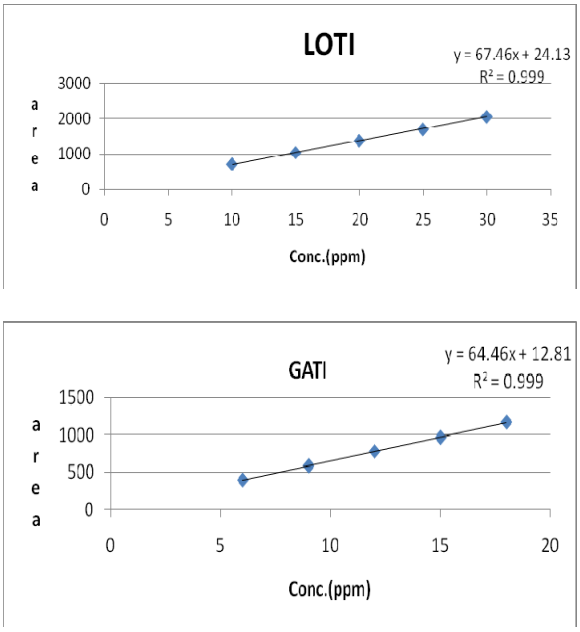

Figure 4 Chromatogram showing of Linearity

The calibration curve plotted over the concentration range 10-30 µg /ml of Loteprednol and 6-18µg/ml for Gatifloxacin.

4.1.1Method Precision (% Repeatability)

The RSD values for Loteprednol and Gatifloxacin were found to be 1.10 and 1.13 %, respectively. The RSD values were found to be <2 %, which indicates that the proposed method is repeatable.

4.1.2. LOD and LOQ

LOD values for Loteprednol and Gatifloxacin were found to be 0.48 µg/mland 0.24 µg/ml, respectively and LOQ values for Loteprednol and Gatifloxacin were found to be 1.47µg /ml and 0.75µg/ml, respectively. These data show that the proposed method is sensitive for the determination of Loteprednol and Gatifloxacin.

Table 4.1.3: Regression Analysis Data and Summary of Validation Parameter for the proposed Method

Parameters	*HPLC method*	
	Loteprednol	*Gatifloxacin*
Detection wavelength(nm)	272	272
Concentration range (µg/ml)	10-30	6-18
Regression equation Y= mX + c	Y= 67.46X + 24.13	Y=64.46X +12.81349
Correlation coefficient	0.999	0.999
LOD(µg/ml)	0.48	0.24
LOQ(µg/ml)	1.47	0.75
RT	3.78	7.39
Slope	67.46	64.46

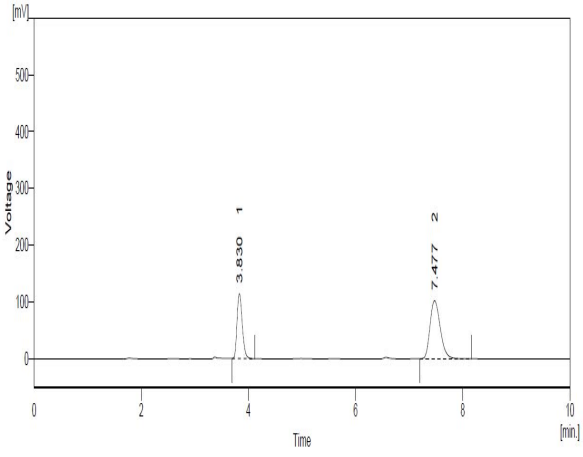

Figure 5: Chromatogram of standard solution of Loteprednol and Gatifloxacin.

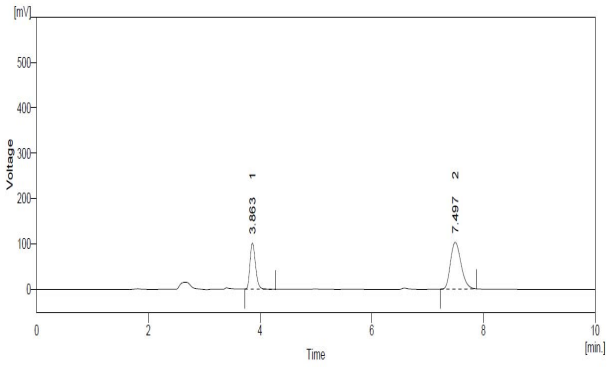

Figure 6: Chromatogram of sample solution of Loteprednol and Gatifloxacin.

Table 4.1.4: Assay of Gatifloxacin and Loteprednol

Stock solution of Gatifloxacin	12 mg	100ml	120ppm
Stock solution of Loteprednol	20mg	100ml	200ppm

Gatifloxacin: Area of standard-704.829

S.no	*Area of sample*	*%assy*
1	692.996	98.321
2	699.2	99.201
3	683.95	97.037

Average of assay-98.1867
Sd - 1.08806
%RSD of assay-1.10816

Loteprednol

Area of standard-1354.435

S.no	*Area of sample*	*%assay*
1	1353.372	99.921
2	1379.601	101.8580
3	1352.415	99.8508

Average of assay-100.5434
Sd-1.138999
%RSD - 1.132825

4. Conclusion

A sensitive and specific isocratic HPLC was developed for quantitative estimation of Loteprednol and Gatifloxacin in formulation. The developed method consisting the mobile phase 0.02M phosphate buffer and acetonitrile with isocratic programming, C18 Hypersil BDS column (250 X 4.6mm C18) as stationary phase. The specificity studies shows that the analytic peaks were well resolved from the intermediates. The correlation coefficient was found to be 0.999 for Loteprednol and Gatifloxacin. The developed method was validated for the various parameter as per ICH guideline like specificity, accuracy, precision, Linearity, and robustness. Gatifloxacin limit of detection is 0.248 and limit of quantification is 0.752 and Lotprednol limit of detection is 0.486 and limit of quantification is 1.474.

5. Acknowledgement

The authors are thankful to Molecule laboratory Pharmaceutical Ltd., Ahmedabad, India for providing gift sample of Loteprednol and Gatifloxacin for carry out the research work.

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