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VALIDATED CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS ESTIMATION OF GATIFLOXACIN AND PREDNISOLONE IN BULK AND MARKETED FORMULATION

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ABSTRACT

The New HPLC method was developed to estimate and validate the Gatifloxacin and Prednisolone in bulk and marketed formulation. Method development was carried out by HPLC Shimadzu 2030C 3D Plus with stationary phase as Hypersil ODS Column(150X4mm) using Mobile phase of Methanol and 0.1% TEA in water was pumped into the system at a flow rate of 0.6ml/min with injection volume 20 µL and Photo diode array detector at 266 nm. Validation was done according to ICH Q2 (R1) guidelines. Linearity for Gatifloxacin 10-50 µg/mL and prednisolone is 50-125 µg/mL, the Retention time of Gat and Pred was found to be 1.960,6.812 Respectively. Average correlation coefficient for linearity was found to be R^2=0.999. With % RSD values ≤2.0, the recovery studies was found to be 100.12 for Gatifloxacin and 99.85 for Prednisolone. The developed method is accurate for simultaneous estimation of two drugs. The LOD for Gatifloxacin is 1µg/mL and 3µg/mL for Prednisolone. The LOQ of Gatifloxacin is 3 µg/mL of gatifloxacin and 15 µg/mL of Prednisolone. The run time is 10 min. All the parameters like theoretical plates, resolution, tailing factor and %RSD was within acceptance limits. The developed method was useful for routine analysis of Gatifloxacin and Prednisolone.

Keywords: Gatifloxacin, Prednisolone, Gatiquin p, HPLC, Assay

INTRODUCTION

Gatifloxacin: Gatifloxacin (GFC) is chemically known as 1-cyclopropyl 6-fluoro 1,4dihydro 8-methoxy 7 (3- methyl 1-piperazinyl) 4 -oxo 3 -quinoline carboxylic acid. It has a broader antibacterial activity than older fluoroquinolones and is superior to Gram + ve and Gram-ve microorganisms **[1]**. It acts intravenously by inhibiting topoisomerase II (DNA gyrase) or topoisomerase IV **[2]**.

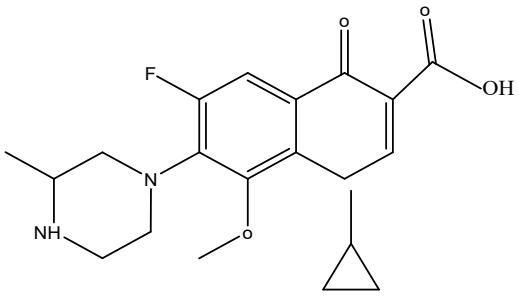

Figure 1: Structure of gatifloxacin [3]

Prednisolone: Prednisolone(PRE) is chemically known as (8S,9S,10R,11S,13S,14S,17R)-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl7,8,9,11,12,14,15,16-octahydro-6Hcyclopenta[a]phenanthren-3-one.Prednisolone is used as immunosuppressive agent and anti-inflammatory **[4]**.

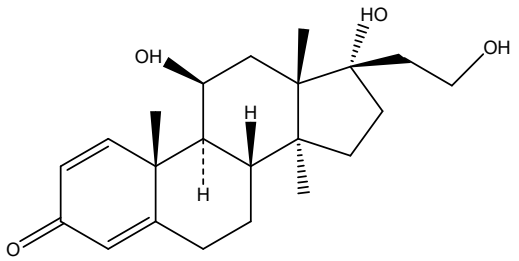

Figure 2: Structure of Prednisolone [5]

METHODOLOGY:

Materials:

Reagents and solvents such as Methanol, HPLC water of analytical grade procured from thermo scientific pvt. Ltd. was used. Gatiquin p eye drops containing was procured from local market.

Instrumentation:

The chromatographic separation was carried out on HPLC Shimadzu 2030C 3d Plus with photodiode array detector, thermoscientific Hypersil C_{18} column with ambient temperature. The mobile phase consisting of methanol and 0.1% TEA in water. The solvents are sonicated before use. The flow rate was 0.6ml/min. The injection volume was set to be 20 µL with detection at 266 nm.

Preparation of Gatifloxacin, prednisolone standard solution:

Accurately weigh about 10 mg of gatifloxacin and prednisolone and transfer to two different10 ml volumetric flask and make up to volume with diluents Acetonitrile: water in the ratio of (1:1) v/v (1000ug/mL).

Preparation of Gatifloxacin, prednisolone working solution:

From above stock solution pipette out 1 ml solution transfer into two different volumetric flasks,and make up to volume with diluents (100µg/mL)

Preparation of Gatifloxacin and Prednisolone sample solution:

5 ml of solution was measured and transferred to 10 ml volumetric flask and made up to 10 ml with ACN: Water in the ratio of 1:1

Method Validation:

The proposed method was validated according to the ICH guidelines which include system suitability, specificity, linearity, accuracy, precision, and robustness. Under the validation study, the following parameters were studied.

System suitability:

HPLC system was optimized as per the chromatographic conditions, 20 µL of standard solution of drugs were injected into chromatographic system. To ascertain system suitability for the proposed method, the parameters such as retention time, number of theoretical plates, resolution, tailing factor and % RSD were calculated and compared with standard specification of system.

Specificity:

The specificity of method was determined, by comparing the chromatogram of blank, standard and sample

Linearity:

Linearity of the method was analysed by preparing calibration curves using different concentrations of the standard solutions. Linearity of method was analysed by preparing calibration curves using different concentrations of the standard solutions. The calibration curve was plotted using peak area and concentration of standard solutions. Linearity was established from 10 to 50µg/mL for gatifloxacin and 50 to 150µg/mL for prednisolone.

Accuracy:

It is analyzed by conducting three different concentrations of the working standards with the % of 50%,100% and 150% inject each concentrations three times into HPLC and calculate the average percentage recovery.

Precision:

Six replicate injections of known concentrations of Gatifloxacin (0.3µg/mL) and prednisolone (1.0µg/mL) are studied and evaluated.

Limit of Detection and limit of Quantification:

LOD and LOQ are performed according to ICH guidelines. The LOD is the lowest amount of analyte that can be detected. LOQ is the lowest amount of analyte quantitatively determined.LOD is analyzed by taking 1µg/mL of gatifloxacin and 5 µg/mL of Prednisolone and LOQ is determined by taking 3 µg/mL of gatifloxacin and 15 µg/mL of Prednisolone.

Robustness:

Robustness should be considered during development phase and also depends on the type of procedure under study. The robustness of method is ability to remain

unaffected by small changes in parameters such as pH of mobile phase, temperature, % organic solvent strength, and buffer concentrations etc., to determine the robustness of the method experimental conditions were purposely altered, and chromatographic characters were evaluated. In this work we altered wavelength i.e., ± 5 nm, ratios of mobile phase ± 5 respectively.

RESULTS AND DISCUSSIONS

System suitability:

Table 1: System suitability parameters for Gatifloxacin and Prednisolone

Parameters	Gatifloxacin	Prednisolone
Retention time	1.960	6.812
Number of theoretical plates	4530	3480
Tailing factor	1.525	1.476
%RSD	0.84	1.08

Specificity:

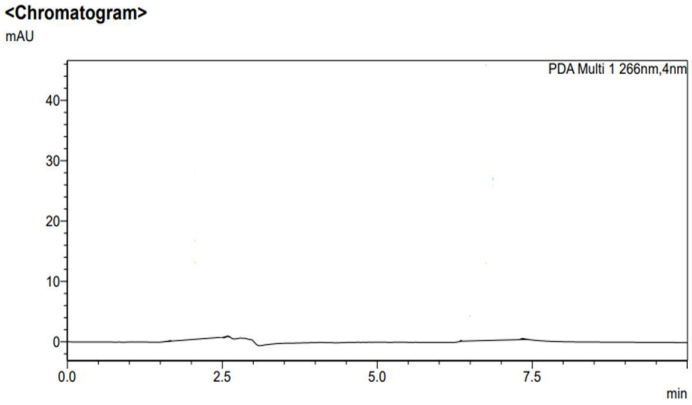

Figure 3: Blank chromatogram

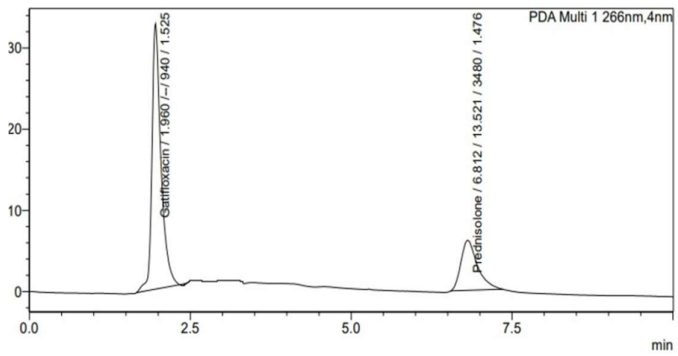

Figure 4: Optimized chromatogram

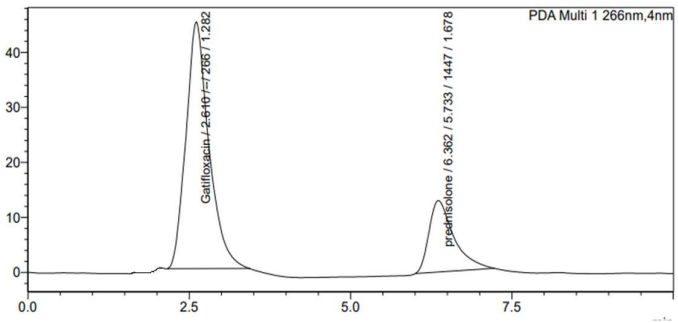

Figure 5: Marketed formulation

Linearity

Table 2: Linearity for Gatifloxacin and Prednisolone

S.no	Gatifloxacin		Prednisolone	
	concentration	Peak area	Concentration	Peak area
1	10	406991	50	177301
2	20	803771	75	252111
3	30	1284958	100	338772
4	40	1654689	125	435879
5	50	2068467	150	513853
6	Correlation coefficient	$R^2 = 0.999$	Correlation coefficient	$R^2 = 0.999$

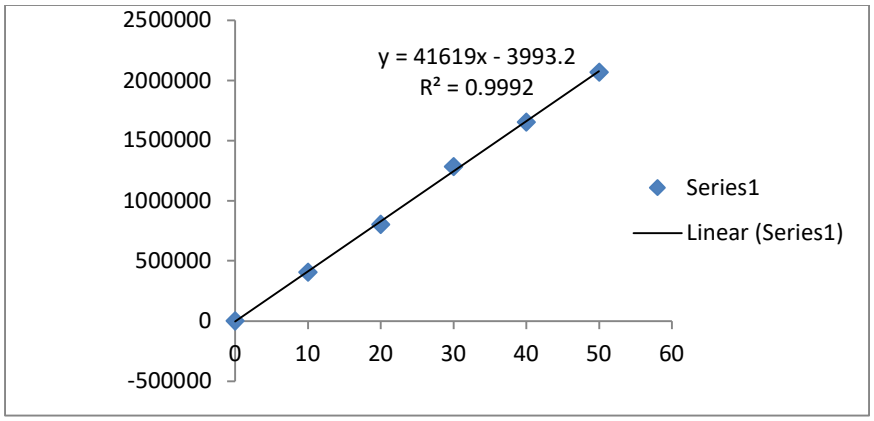

Figure 6: Calibration curve for Gatifloxacin

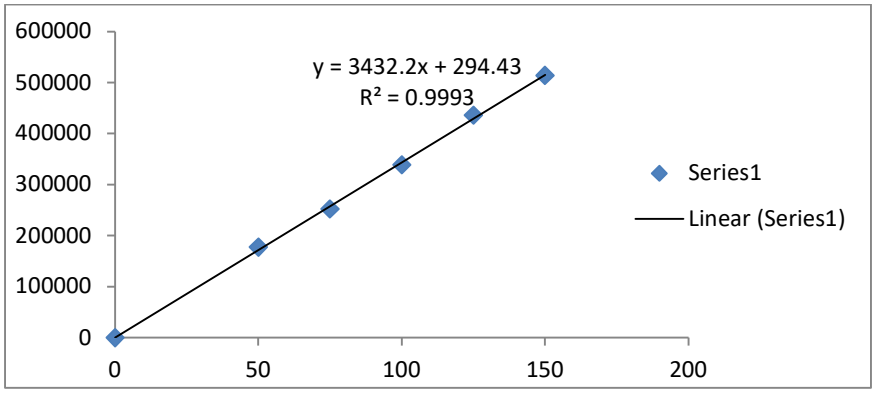

Figure 7: Calibration curve for Prednisolone

Table 3: Precision for gatifloxacin and Prednisolone

S.no	System precision		Method precision	
	Peak area		Peak area	
	Gatifloxacin	Prednisolone	Gatifloxacin	Prednisolone
1.	1161681	324017	1161686	324049
2.	1177589	322862	1177591	322882
3.	1168573	326799	116589	325799
4	1177636	331501	1177646	331505
5	1162895	330276	1162896	330277
6	1186903	329673	1186911	329679
AVG	1172546	327521.3	1172553	327365.2
SD	9843.151	3537.432	9844.134	3592.198
%RSD	0.84	1.08	0.84	1.10

Table 4: Accuracy for Gatifloxacin and Prednisolone

% Level	% Recovery		Average % Recovery	
	Gatifloxacin	Prednisolone	Gatifloxacin	Prednisolone
50%	99.80	100.85	100.12	99.85
	100.05	100.73		
	99.88	100.77		
100%	100.65	99.62		
	100.54	99.64		
	100.47	99.69		
150%	99.85	99.02		
	99.92	99.22		
	99.94	99.43		

Robustness:

The method was robust enough with %RSD values NMT 2 for various robustness parameters such as change in flow rate, ratio of mobile phase, wavelength and pH of mobile phase.

Assay

The % assay of Gatifloxacin and Prednisolone was represented.

Table 5: Assay of Gatifloxacin and Prednisolone

Drug	%Assay
Gatifloxacin	99.80
Prednisolone	99.68

CONCLUSION:

The simple, reliable, novel, economical method was developed for simultaneous estimation of Gatifloxacin and Prednisolone. The 0.1% TEA in water and methanol are used as mobile phase, By RP-HPLC the isocratic mode was used for separation of two drugs, and the peaks were eluted at 1.960, 6.812 min for Gatifloxacin and Prednisolone respectively. The theoretical plates, peak area, retention time, were found to be satisfactory. Analytical method was validated according to ICH guidelines. The correlation coefficient, % recovery, %RSD was found to be within limits. There is no Interference of excipients in sample analysis. So, developed method was found to be accurate. This method can be used for quality control in pharmaceutical industries and for academic purposes.

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