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Application of RP-HPLC Method for Simultaneous Estimation of Gatifloxacin and Flurbiprofen Sodium In Ophthalmic Formulation

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ABSTRACT

A simple, accurate, precise and sensitive RP- HPLC method has been developed for the determination of Gatifloxacin and Flurbiprofen Sodium in their pharmaceutical formulation. Chromatographic separation was carried out on InertsilODS – 3 column (250 mm ×4.6 mm, 5µm) as stationary phase by using mobile phase consisting of 0.02 M Phosphate buffer (pH 3.5 adjusted with orthophosphoric acid) : Methanol (80 : 20 v/v). The flow rate was 1.5 ml/min with UV-detection at 245 nm. The retention time was found to be 2.59 min for Gatifloxacin and 5.41min for Flurbiprofen Sodium. The method was validated for various parameters according to ICH guideline. The linear regression analysis data for the calibration plots showed good linear relationship in the concentration range of 30 – 90 µg/ml and 3 – 9 µg/ml and correlation coefficient was found to be 0.9988 and 0.9992 for Gatifloxacin and Flurbiprofen Sodium respectively. The Limit of Detection for Gatifloxacin and Flurbiprofen Sodium were 1.45 and 0.028 respectively. The Limit of Quantification for Gatifloxacin and Flurbiprofen Sodium were 4.39 and 0.28 respectively.

Keywords : Gatifloxacin, Flurbiprofen Sodium, RP- HPLC, Validation, Ophthalmic Formulation.

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INTRODUCTION

Gatifloxacin(GFC) is chemically 1-Cyclopropyl-6-fluoro- 8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-quinoline-3-carboxylic acid(Figure 1)[1,2].GFC is a fluoroquinolones family of synthetic broad-spectrum antibiotics, which eradicate bacteria by interfering with DNA replication. However, the fluoroquinolones are relatively ineffective against intracellular pathogens[3]. Flurbiprofen Sodium (FS) is chemically Sodium(±)-2-(2-fluoro-4-biphenylyl) propionate dehydrates (Figure 2)[4,5]. FS is a propionic acid derivative and Non - Steroidal Anti – Inflammatory Drugs (NSAIDs) with antipyretic and analgesic activity[6]. Gatifloxacin is combination with Flurbiprofen sodium is used for reduction of post – operative inflammatory condition of eye, when bacterial infection exists.

In the literature, methods were described for the individual estimation of Gatifloxacin by Titrimetric[7], UV - Visible Spectrophotometry[8,9], RP- HPLC[10] and HPTLC[11]and for Flurbiprofen Sodium by Titrimetric[12], HPTLC[13]and RP- HPLC[14]methods. The methods were also given for simultaneous estimation of Gatifloxacin and Flurbiprofen Sodium combine with other drugs by UV- Visible Spectrophotometry[15, 16], RP- HPLC [17-22]and HPTLC [23, 24] methods. Literature survey dose not reveal simultaneous determination of these drugs in their combined pharmaceutical formulation and has not been reported in official pharmacopoeia. Therefore, it was thought of interest to develop and validate the RP- HPLC method for Gatifloxacin and Flurbiprofen Sodium in their ophthalmic formulation.

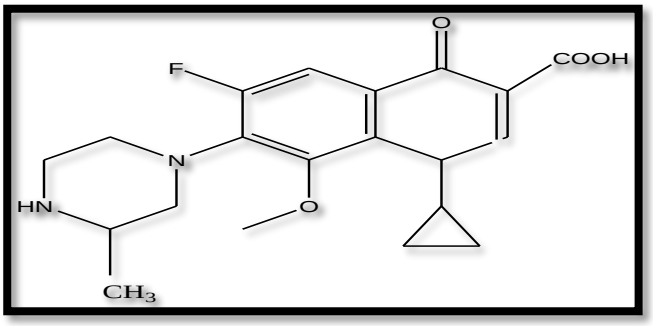

Figure 1: Chemical Structure of Gatifloxacin

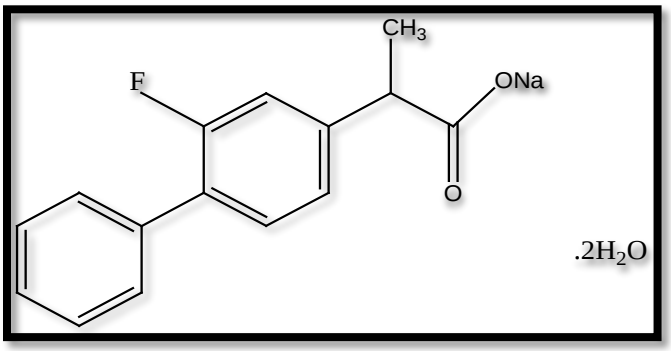

Figure 2: Chemical Structure of Flurbiprofen Sodium

MATERIALS AND METHODS

Instruments:

RP- HPLC system (Agilent Technologies, 1260 infinity, EZ Chrom Elite Software), UV – Visible Spectrophotometer (Agilent 8453),Sonicator – Ultrasonic (PCI Analytics), Sartorous Analytical weighing balance (PA 225 D) and pH meter (Eutech Instrument)

Chemicals:

GFC and FS bulk powder were procured as a gift samples from Yash Laboratories, Chikhali, Gujarat. HPLC grade Water(Merck Ltd.), HPLC grade Methanol and Acetonitrile(S. D. Fine Chem Limited, Mumbai), Potassium Dihydrogen Phosphateand Orthophosphoric acid (Merck Ltd.) Commercial pharmaceutical preparation FLUBIGAT Eye Drops, Mfg. by, Entod Pharma is claimed to contain 0.3 % w/v of Gatifloxacin and 0.03 % w/v of Flurbiprofen Sodium.

METHOD DEVELOPMENT

Selection of wavelength:

Stock solution of GFC (9µg/ml) and FS (0.9µg/ml) were prepared in methanol for the selection of wavelength. The spectrum scans in range of 200 - 400 nm. The wavelength 245nm was selected for estimation of Gatifloxacin and Flurbiprofen Sodium. At the selected wavelength both drugs showed good absorbance.

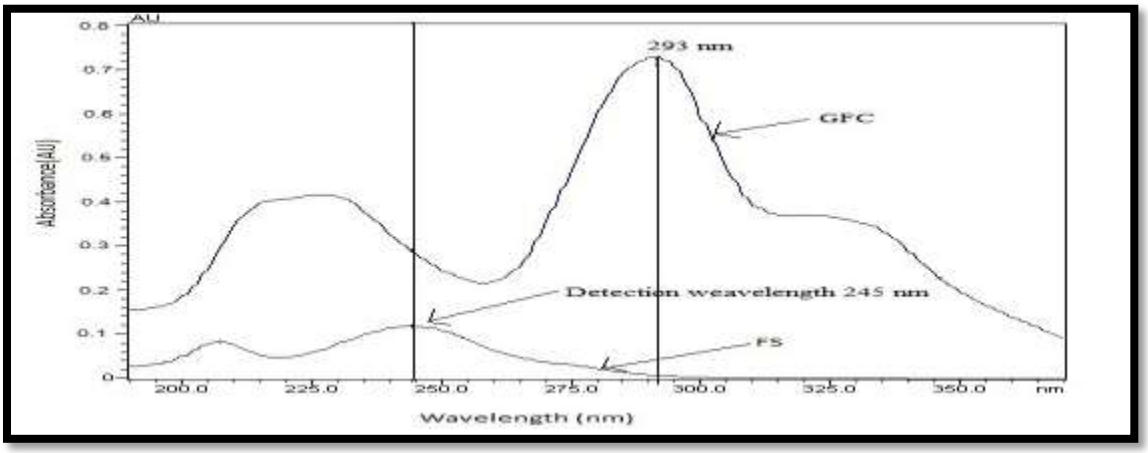

Figure 3 : UV overlain spectra of Gatifloxacin and Flurbiprofen Sodium

Chromatographic condition:

The Mobile phase was prepared by 0.02 M Phosphate buffer (pH 3.5 with orthophosphoric acid): Methanol (80: 20 v/v). The mobile phase was filter through 0.45 µm membrane filter and sonicated for 5 mins. The flow rate was 1.5 ml/ min. The run time was set at 10 mins. The volume of injection was 20 µl prior to injection of the drug solution the column was equilibrated with mobile phase following through system.

Preparation of Standard stock solution from bulk drugs:

An accurately weighed 300 mg of standard GFC powder and 30 mg of standard FS powder transfer in to 100 ml volumetric flask and dissolve with methanol to get concentration 3000 µg/ml solution for GFC and 300 µg/ml solution for FS. Take 5ml of that solution diluted with 50 ml of methanol and get final concentration 300µg/ml for GFC and 30 µg/ml for FS. Standard solutions were prepared by dilution of stock solution with mobile phase to give the final concentration range of 30 – 90 µg/ml and 3 - 9 µg/ml for GFC and FS respectively. The solutions were sonicated for 5mins.

Sample preparation:

(Label claim: 0.3 % w/v Gatifloxacin and 0.03 % w/v Flurbiprofen Sodium). Take 2 ml sample and dilute with mobile phase in to 10 ml volumetric flask (60µg/ml of Gatifloxacin and 6µg/ml of Flurbiprofen Sodium). The solution was sonicated for 5mins.

METHOD VALIDATION [25]:

Linearity :

Five working standard solutions of each analyte in the concentration range 30 - 90 µg/ml for GFC and 3 - 9 µg/ml for FS were prepared and injected (n=5).Calibration curve was constructed by plotting the peak area against concentration using linear regression analysis.R^2 value was found 0.9988 for GFC and 0.9992 for FS. (Table 1)

Limit of Detection and Limit of Quantification:

Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated based on standard deviation of the response and slope of the calibration curve. The LOD for GFC and FS were 1.45 and 0.028 respectively. The LOQ for GFC and FS were 4.39 and 0.286 respectively. (Table 1)

LOD and LOQ are calculated by formula:

LOD = 3.3 x σ / s and LOQ = 10 x σ / s

Where, σ= standard deviation of the y- intercepts of the regression line., s = slope of the calibration curve.

Precision :

Method precision: The precision of the method was evaluated by inter-day and intra-day precision. For intra-day precision, three different concentrations of GFC (30, 60 and 90 µg/ml) and FS (3, 6 and 9 µg/ml) were prepared in triplicate and analyzed during same day. For Inter-day precision the same concentrations were analyzed different day. The % RSD values were calculated. (Table 2)

System precision: The Precision of system was evaluated by repeatability. This was analyzed by repeated injection of six sample solution of GFC (60 µg/ml) and FS (6 µg/ml)under same chromatographic condition. The % RSD was calculated for both analyte. The % RSD values were calculated. (Table 3)

Accuracy:

The accuracy of the method was determined by recovery studies. These studies were carried out by addition of known amount of GFC and FS to a sample solution of known concentration. The percentage of recovery was calculated from the amount of drug found in the solution. (Table 4)

Specificity:

The specificity of the method was analyzed by standard drug, pharmaceutical product and placebo and comparing the retention time of the standard with that of the sample to determine whether the pharmaceutical product and placebo led to interfere.

Robustness:

By introducing small but deliberated changes in method parameters like change in the mobile phase pH (± 0.1), mobile phase composition (± 2.0 %), and flow rate (± 10.0 %). Effect of these changed parameters was studied by injecting the sample in to the system.

System suitability parameters:

System suitability was established to determine the adequate resolution and reproducibility of the proposed method. Parameters including Retention time, Tailing factor, Resolution and No. of theoretical plates were calculated. (Table 5)

Assay of the marketed formulation:

The develop method was applied to the simultaneous estimation of GFC and FS in ophthalmic formulation. (Table 6)

RESULTS AND DISCUSSION

Mobile phase optimization:

The composition of mobile phase for the development of chromatographic method was optimized by testing different solvent mixtures in various ratios. After various trials, finally a mobile phase consisting of a mixture of 0.02 M Phosphate buffer (pH 3.5 adjusted with orthophosphoric acid): Methanol (80:20 v/v) was adopted, which produces good resolution, reasonable retention time and acceptable peak shape for the both drugs. Chromatogram for Mobile phase (Blank chromatogram) is shown in Figure4 and show no interference with the drug peaks. The retention time for GFC was around 2.6 min and 5.4 min for FS in standard and sample solution (Figure 5 and 6

respectively).The optimum wavelength for the detection was set at 244 nm, which show good response and better resolution.

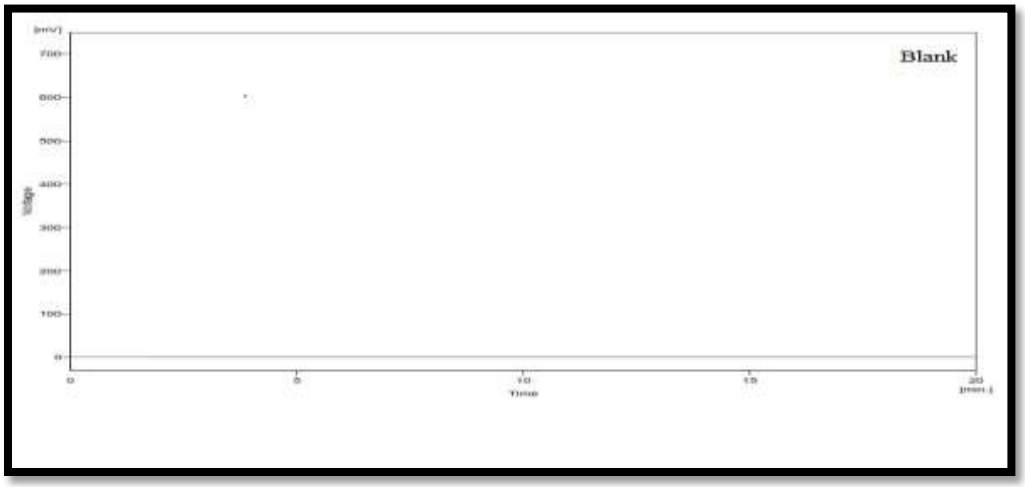

Figure 4: Chromatogram of Blank

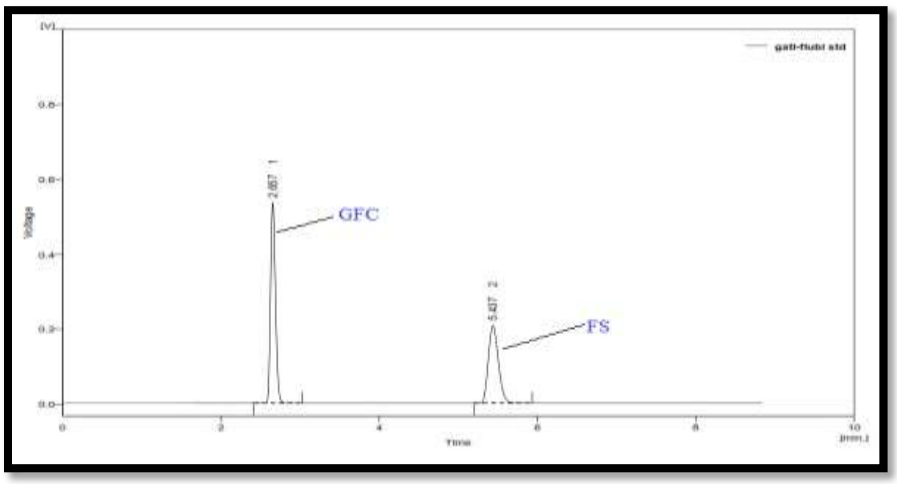

Figure 5: Chromatogram of Standard solution of GFC (60 µg/ml) and FS (6 µg/ml)

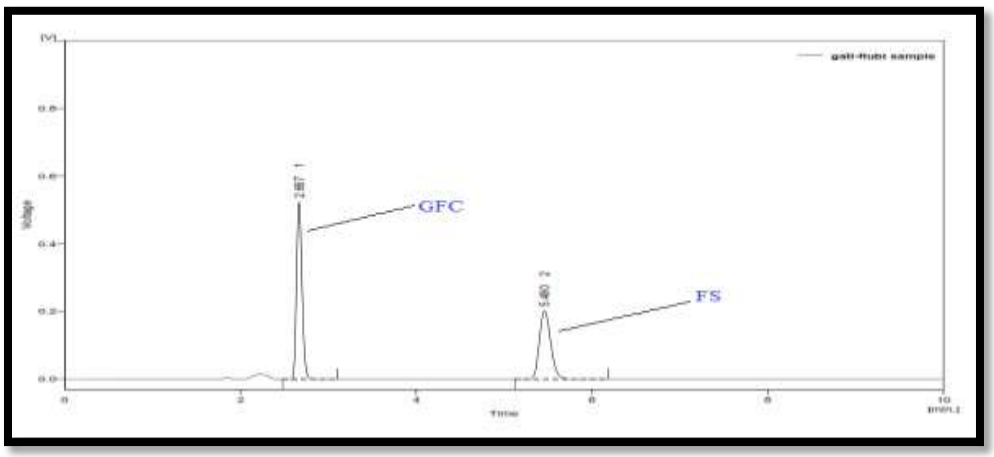

Figure 6: Chromatogram of Sample solution of GFC (60 µg/ml) and FS (6 µg/ml)

Validation:

Linearity:

Calibration graphs were constructed by plotting the peak area versus their corresponding concentrations. Good linearity was obtained in the range30 - 90 µg/ml for GFC and 3 - 9µg/ml for FS. The results are shown in Table 1. LOD and LOQ were calculated from slope and standard deviation y- intercepts of the regression line of the calibration curve shown in figure 7 and 8.

Table 1: Linearity Data by Regression Analysis (n = 5)

Parameters	GFC	FS
Concentration range	30 – 90 µg/ml	3 – 9 µg/ml
Regression equation	y = 39.383x - 72.803	y = 310.75x - 38.467
R^2 Value	0.9988	0.9992
LOD	1.45	0.028
LOQ	4.39	0.286

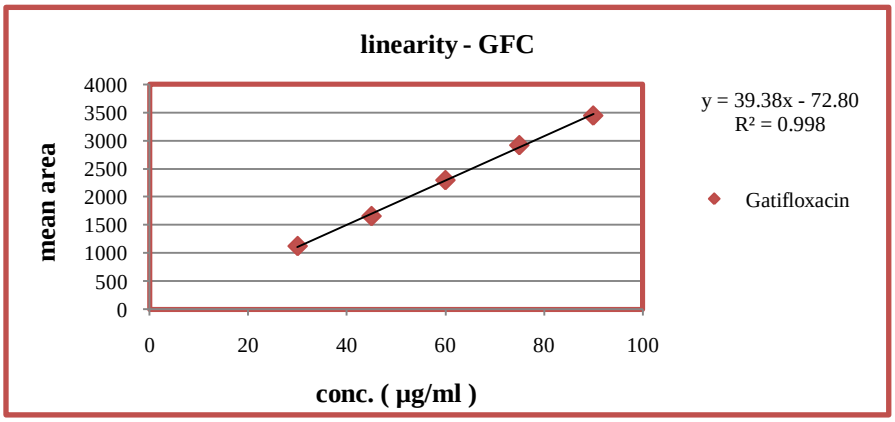

Figure 7: Calibration curve of Gatifloxacin

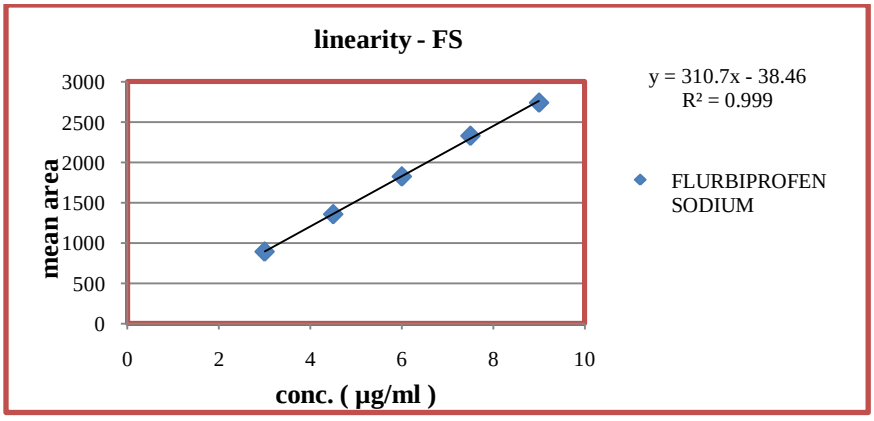

Figure 8: Calibration curve for Flurbiprofen Sodium

Precision:

The precision of method and system were evaluated and % RSD values were calculated. The precision of the method was satisfactory. The results are shown in Table 2 and 3.

Table 2: Intra-day and Inter-day precision Data (n=3)

Conc.(µg/ml)	Intra – day Precision	Inter – day Precision
	% RSD	% RSD
GFC		
30	0.15	0.23
60	0.14	0.26
90	0.15	0.22
FS		
3	0.20	0.36
6	0.12	0.30
9	0.21	0.25

Table 3: Repeatability Data (n=6)

Conc. (µg/ml)	% RSD
GFC	
60	0.21
FS	
6	0.26

Accuracy :

The accuracy of the method was determined by recovery studied. These studies carried out at 80 %, 100 % and 120 % level. The results are shown in Table 4.

Table 4: Recovery Studies Data

Drugs	Amount taken µg/ml)	% Added	Mean % Recovery ± SD (n=3)
GFC	30	80	100.62 ± 0.43
		100	99.86 ± 0.26
		120	99.34 ± 0.26
FS	3	80	99.59 ± 0.96
		100	99.42 ± 0.32
		120	99.99 ± 0.25

System Suitability Parameters:

System suitability tests were carried out on standard stock solution of GFC (60 µg/ml) and FS (6 µg/ml) and these solution were injected under optimized chromatographic condition. The following parameters were studied. The parameters are shown in Table 5.

Table 5: System suitability parameters (n=3)

Parameters	GFC	FS
Retention Time (min) ± SD	2.64 ± 0.0035	5.40 ± 0.0090
Tailing factor ± SD	1.308 ± 0.0701	1.327 ± 0.0415
No. of theoretical plate ± SD	7665 ± 389.26	8262 ± 27.03
Resolution	15.46	

Table 6: Analysis Data of Marketed formulation(n=5)

Label claim		Amount taken(µg/ml)		Mean Amount found (µg/ml)		Mean % Assay	
GFC	FS	GFC	FS	GFS	FS	GFC	FS
0.3 % w/v	0.03 % w/v	60	6	59.30	5.88	98.51	99.02

CONCLUSION:

The developed RP- HPLC method was suitable for the simultaneous estimation of Gatifloxacin and Flurbiprofen Sodium as bulk and marketed formulation without any interfering from the excipients. The developed method was validated for the various parameters as ICH guideline.

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