Available online on www.ijddt.com
International Journal of Drug Delivery Technology 2019; 9(1); 83-88
doi: 10.25258/ijddt.9.1.13

ISSN: 0975 4415

Research Article

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Gatifloxacin and flurbiprofen Sodium in Eye Drops

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Received: 24th Jan, 19; Revised and Accepted: 14th Feb, 19; Available Online: 25th Mar, 2019

ABSTRACT

A simple, selective and rapid reversed phase High Performance Liquid Chromatographic (RP-HPLC) method has been developed and validated for the simultaneous analysis Gatifloxacin and flurbiprofen sodium in eye drops. The separation was carried out using a mobile phase consisting ACN: Buffer (pH 3.5) in the ratio of 55:45 v/v. The column used was Phenomenex luna ODS C18 (250mm X 4.6 mm i.d., 5 µm particle size) with flow rate of 1 ml / min using UV detection at 268 nm. The described method was linear over a concentration range of 2-12 µg/ml for both of Gatifloxacin and flurbiprofen sodium. The retention times of Gatifloxacin and flurbiprofen sodium were found to be 3.710 min. and 6.797 min respectively. Method was validated statistically and recovery studies were carried out. The proposed method has been applied successfully to the analysis of cited drugs either in pure form or in pharmaceutical formulations with good accuracy and precision. The method here in described can be employed for quality control and routine analysis of drugs in pharmaceutical formulations.

Keywords: Gatifloxacin, flurbiprofen sodium, RP-HPLC, Validation.

INTRODUCTION

Chemically, Gatifloxacin is 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1- yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid[1]. Gatifloxacin is a fourth generation fluoroquinolones family antibiotic inhibits the enzyme topoisomerase (DNA gyrase) which are require for bacterial DNA replication, transcription, repair and recombination. It is available for oral and parenteral administration It is official in IP[1] that describes HPLC method for its estimation. Flurbiprofen sodium is chemically, RS)-2(2-fluorobiphenyl-4-yl) propionate dehydrate[1]. It is official in IP[3], BP[4] and USP[5] which describes chromatographic methodfor its estimation.The chemical structure of Gatifloxacin and flurbiprofen sodium is shown in the (Fig 1 and 2) respectively.

A detailed literature survey revealed spectrophotometric, HPLC and HPTLC, and stability indicating RP-HPLC method for both Gatifloxacin and flurbiprofen sodium as individual and with other drug combination[6-26]. The combination of these two drugs is not official in any pharmacopoeia; hence, no official method is available for the simultaneous estimation Gatifloxacin and flurbiprofen sodium in their combined dosage forms. Literature survey does not reveal any simple spectrophotometric or chromatographic method for simultaneous estimation of Gatifloxacin and flurbiprofen sodium in combined dosage forms.

MATERIALS AND METHODS

Gatifloxacin and flurbiprofen sodium API were obtained as a gift sample from Biomatrix health care, Ahmedabad and Balaji drugs, Surat, respectively. Methanol, Water, Acetonitrile and distilled water was used in the study. The commercial fixed dose combination product containing 0.1% w/v betamethasone sodium phosphate and 0.3% w/v ofloxacin was procured from the local market.

Preparation of standard stock solutions

A stock solution of GATI and FLUR (100 µg/ml) was prepared, by taking 10 mg of each drug, accurately weighed, in separate 100-ml volumetric flasks and dissolving in methanol and diluted to 100 ml with same solvent upto the mark.

Preparation of sample solution

5 ml equivalent to 3 mg Gatifloxacin and 0.3 mg Flurbiprofen sodium was transferred into 10 ml volumetric flask, initially 2.5 ml of mobile phase was added and sonicated for 5 min, and then diluted to volume with mobile phase & filtered through 0.45 µm membrane filter paper. The resulted solution was 30 µg/ml Gatifloxacin and 3 µg/ml Flurbiprofen sodium. Above solution was diluted with mobile phase to give working standard solution containing 3 µg/ml Gatifloxacin and 0.3 µg/ml Flurbiprofen sodium. After the preparation of sample solution this solution was analysed by RP-HPLC and the content of Gatifloxacin and Flurbiprofen sodium in eye drop was calculated by calibration curve.

Validation of the proposed method

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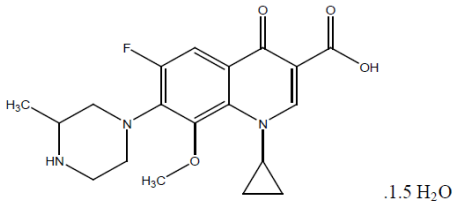

Figure 1: Chemical structure of Gatifloxacin.

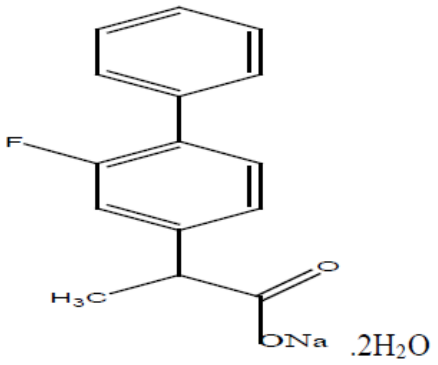

Figure 2: Chemical structure of flurbiprofen sodium.

Table 1: Repeatability and intermediate precision data of gatifloxacin and flurbiprofen sodium (n=6).

Parameter	Gatifloxacin	Flurbiprofen sodium
Repeatability (%RSD)	0.39	1.06
Interday (%RSD)	0.56-0.65	0.51-1.00
Intraday (%RSD)	0.29-0.50	0.56-0.65

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[27].

Calibration curve (Linearity)

Calibration curves were constructed by plotting Peak areas vs. Concentrations of BMS and OFLO, and the regression equations were calculated. The calibration curves were plotted over the concentration range 15-45 µg/ml for GATI and 1.5-4.5 µg/ml for FLUR. Aliquots (20 µl) of each solution were injected under the operating chromatographic conditions described above. The graphs were plotted for peak area vs. concentration for both the drugs and expressed in terms of slope, intercept and correlation coefficient values

Precision (Repeatability)

6 replicates of standard mixture solution having Gatifloxacin (30 µg/ml) and Flurbiprofen sodium (3 µg/ml) were prepared and chromatograms were recorded and % RSD was calculated..

Intermediate Precision

Intraday Precision

Standard solutions containing 15, 30 and 45 µg/ml Gatifloxacin and 1.5, 3.0 and 4.5 µg/ml Flurbiprofen sodium was analyzed 3 times on the same day as per the procedure. Chromatogram of each sample was taken. SD and % RSD were calculated.

Interday Precision

Standard solutions containing 15, 30 and 45 µg/ml Gatifloxacin and 1.5, 3.0 and 4.5 µg/ml Flurbiprofen sodium were analyzed on three different days as per the procedure. Chromatogram of each sample was taken. SD and % RSD were calculated.

Robustness

To determine the robustness of the method, experimental conditions such as the composition of the mobile phase, pH of the mobile phase, and flow rate of the mobile phase were altered and the chromatographic characteristics were evaluated. No significant change was observed.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were 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.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

To develop suitable RP-HPLC method for simultaneous estimation of gatifloxacin and flurbiprofen sodium, different chromatographic conditions were applied and optimized chromatographic conditions were developed [Figure 3].

Optimized chromatographic conditions are as follows:

Instrument: HPLC Shimadzu separation module LC-20AD Prominence liquid chromatograph,

Mobile phase: ACN: Buffer (pH 3.5) (55:45)

Column: Phenomenex LunaC18 (250mm X 4.6mm i.d., 5µm particle size)

Injection volume: 20 µL,

Flow rate: 1.0 mL/min,

Detection wavelength: 268 nm

Run time: 10 min,

Temperature: Ambient (25°C).

Validation

Linearity

The chromatographic method showed good linearity for gatifloxacin and flurbiprofen sodium in the range of 15-45 µg/ml and 1.5-4.5 µg/ml, respectively [Figure 4, 5and 6]. Correlation of coefficient value was found to be 0.999 and 0.999 for gatifloxacin and flurbiprofen sodium respectively.

Precision
Repeatability

The RSD values for gatifloxacin and flurbiprofen sodium were found to be 0.39% and 1.06% respectively (Table 1). Relative standard deviation was less than 2%, which

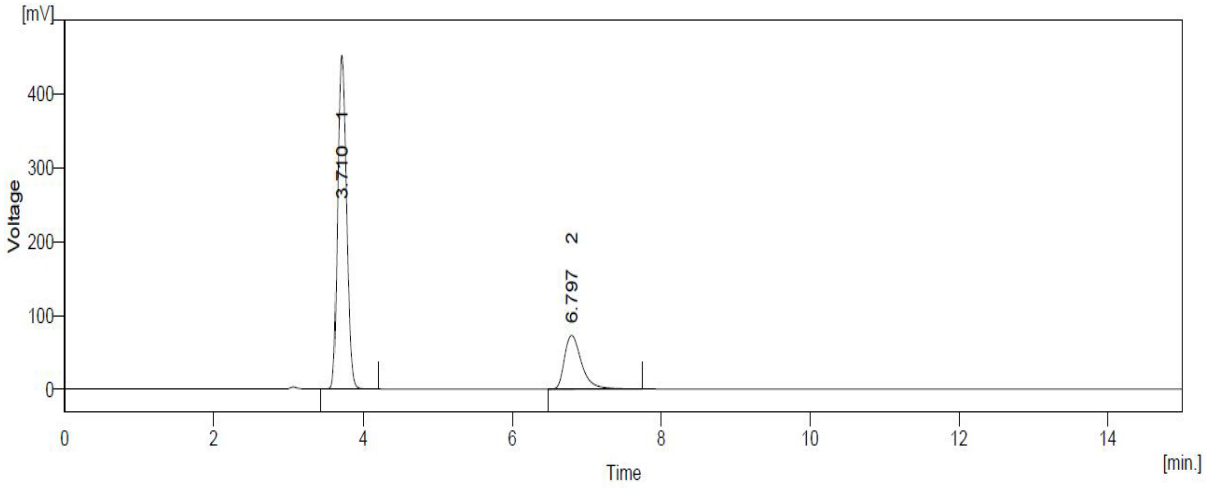

Figure 3: Optimized chromatogram of gatifloxacin and flurbiprofen sodium

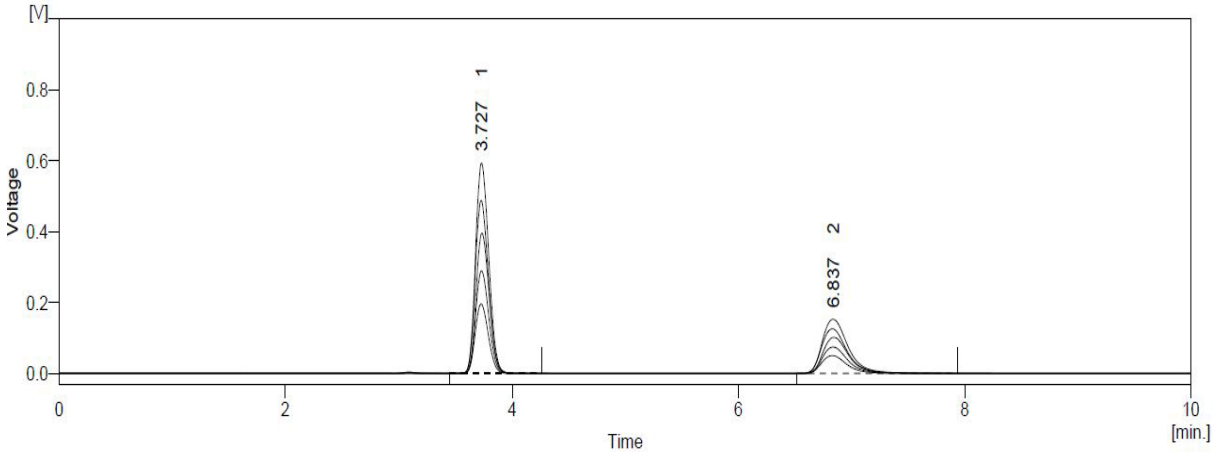

Figure 4: 3D overlay chromatogram of Gatifloxacin and Flurbiprofen sodium in combination for Linearity

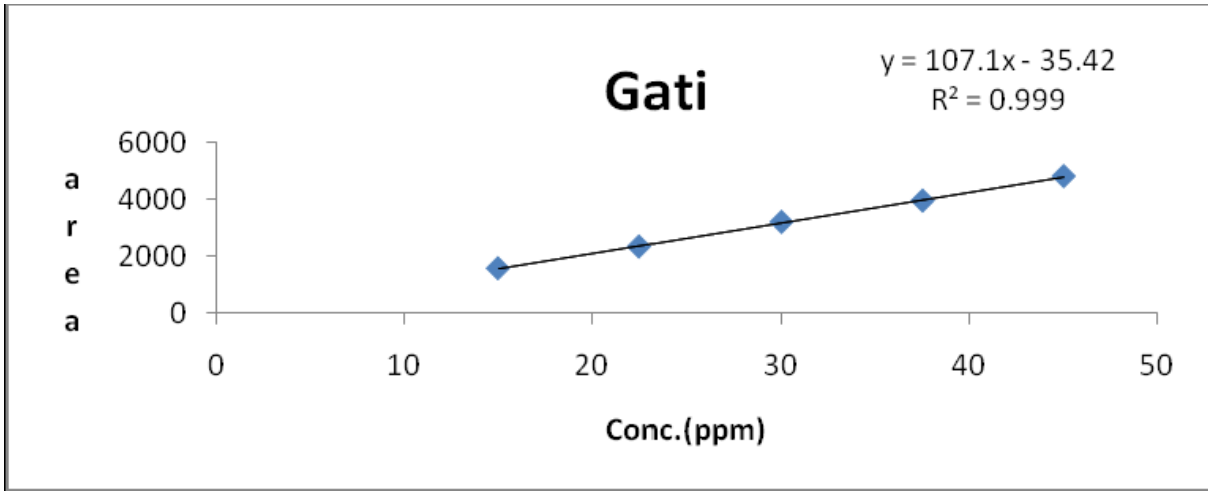

Figure 5: Linearity of Gatifloxacin (15-45 µg/ml)

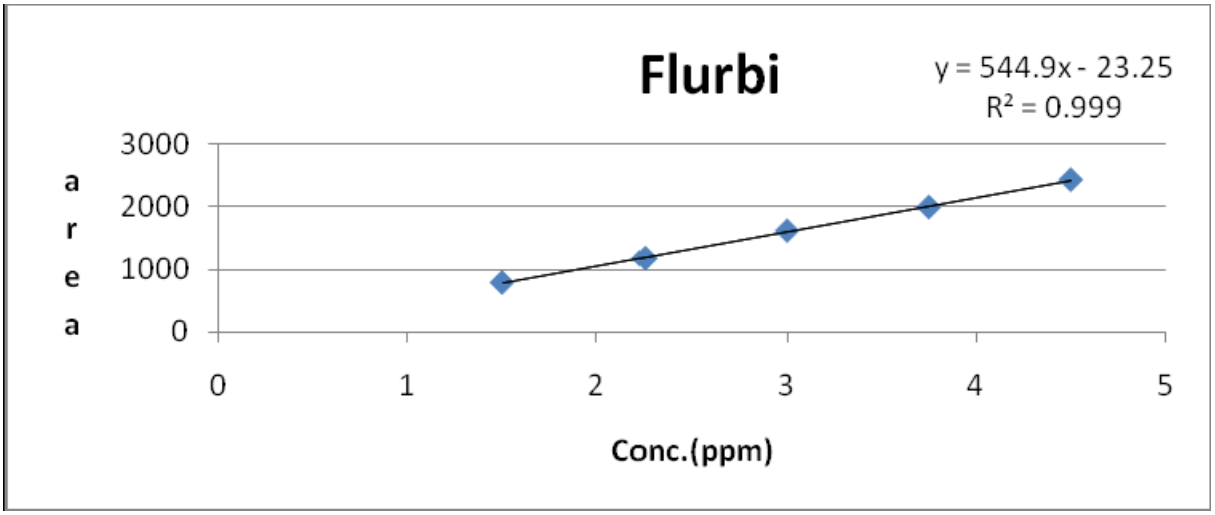

Table 2: Result of recovery study (n=6).

Drugs	Level	Amount present (µg/ml)	Amount added (µg/ml)	Total amount of drug (µg/ml)	% Recovery (n=3)	% RSD
Gatifloxacin	80%		12	27	99.38	0.21
	100%	15	15	30	98.88	0.50
	120%		18	33	99.19	0.33
Flurbiprofen	80%		1.2	2.7	99.16	0.91
sodium	100%	1.5	1.5	3.0	99.99	1.33
	120%		1.8	3.3	99.62	0.84

Table 3: Specificity Data.

Drug	Concentration	Area before addition of Excipients	Area after addition of Excipients	% Interference
Gatifloxacin	15	1582.60	1570.12	0.78
	22.5	2340.66	2322.16	0.74
	30	3208.28	3190.11	0.56
	37.5	3948.12	3913.24	0.88
	45	4605.16	4588.08	0.37
Flurbiprofen	1.5	802.61	796.14	0.80
Sodium	2.25	1187.70	1176.4	0.95
	3.0	1625.97	1613.23	0.79
	3.75	2003.02	1996.87	0.30
	4.5	2438.46	2427.56	0.44

Table 4: Robustness data for Gatifloxacin and Flurbiprofen sodium.

Sr. No	Parameters	Variations	% Assay ± SD	
			Gatifloxacin	Flurbiprofen sodium
1	Flow rate	0.9 ml/min	99.26±0.02	99.15±0.05
	(1± 0.1 ml/min)	1.0 ml/min	99.16±0.31	100.05 ±0.21
		1.1 ml/min	99.53±0.02	99.71±0.05
2	Mobile Phase (57:43 ± 2 v/v)	59:41(v/v)	99.65±0.05	99.71±0.05
		57:43(v/v)	99.16 ±0.31	100.05±0.21
		55:45(v/v)	99.50±0.08	99.63±0.07

indicates that the proposed method is repeatable.

Intermediate precision

The low RSD values of intraday and interday for gatifloxacin and flurbiprofen sodium, reveals that the proposed method is precise (Table 1).

Accuracy

Accuracy was performed at 80%, 100% and 120% levels by spiking method. Each concentration was analyzed three times and average recoveries were measured (Table 2). Result obtained reveals that % recovery of gatifloxacin and flurbiprofen sodium was within acceptance criteria given in ICH i.e. 98-102%.

Specificity

Robustness

The robustness of the method was determined by making slight changes in the chromatographic conditions. The

Table 5: LOD and LOQ for Gatifloxacin and Flurbiprofen sodium.

Drug	LOD	LOQ
Gatifloxacin	0.1743	0.5281
Flurbiprofen sodium	0.1168	0.3527

Table 6: System suitability parameters.

Parameters	Data obtained	
	Gatifloxacin	Flurbiprofen sodium
Retention time (min)	3.7	6.7
Theoretical plates per column	4512	4443
Symmetry factor/ tailing factor	1.300	1.654
Resolution	9.8	

Table 7: Assay of combined dosage form.

Drug	Conc. Dosage Form (mg)	Conc. taken for assay (µg/ml)	Conc. Found	%Assay
Gatifloxacin	3	3	3.064	102
Flurbiprofen sodium	0.3	0.3	0.272	91.49

robustness of the proposed HPLC method was assessed for peak resolution and peak resolution and symmetric factor. The parameters investigated are: (1) Flow rate (±0.1 ml/min) (2) Mobile phase composition (±2). The robustness of the method shows that there were no marked changes in the chromatographic parameters, which demonstrates that the method developed is robust (Table 4).

Limit of Detection and Limit of Quantitation

The proposed method can detect and quantify small amount of drugs with precisely. So, it was concluded that the proposed method is very sensitive in nature.

System suitability testing

The resolution, number of theoretical plates, and peak asymmetry were calculated for the standard solutions. The stock solution containing 10 µg/mL was injected and repeated five times and the chromatograms were recorded. The resolution, number of theoretical plates, and peak asymmetry were calculated to determine whether the result complies with the recommended limit. (Table 6)

Analysis of Gatifloxacin and Flurbiprofen sodium in marketed formulation (Eye Drops)

% Assay of Gatifloxacin and Flurbiprofen sodium was found in an acceptance limit so this method could be used for analysis of this combination.

DISCUSSION

Gatifloxacin and Flurbiprofen sodium were given linear response from 15-45 µg/ml and 1.5-4.5 µg/ml in RP-HPLC, respectively. Correlation of coefficient value was found to be 0.999 for both Gatifloxacin and Flurbiprofen sodium. %RSD was less than 2, which indicates that the proposed method is repeatable and precise. Result obtained from accuracy study reveals that % recovery of Gatifloxacin and Flurbiprofen sodium was within acceptance criteria given in ICH i.e. 98-102%. The robustness study suggested that all the parameters have no significant influence on the determination. Results indicate that the selected factors remained unaffected by small variation of these parameters and %RSD was less than 2, which demonstrates that the proposed method was robust.

CONCLUSION

The proposed RP-HPLC method with UV detection was used for the simultaneous estimation of Gatifloxacin and Flurbiprofen was found to be sensitive, accurate, precise, simple, and rapid. Hence the present RP-HPLC method may be used for routine analysis of the raw materials, in vitro dissolution study of combinational dosage formulations containing Gatifloxacin and Flurbiprofen.

CONFLICT OF INTERESTS

The authors do not have a direct financial relation with the commercial identity mentioned in paper that might lead to a conflict of interests for any of the authors.

ACKNOWLEDGEMENT

The authors are highly thankful to Parul Institute of Pharmacy and Research, Vadodara, Gujarat, India for providing all the facilities to carry out the work.

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