Development and Validation of Spectrophotometric and Stability Indicating RP-HPLC Method for the Simultaneous Estimation of Gatifloxacin and Flubiprofen Sodium in Ophthalmic Dosage Form

Mohan Gandhi B[1], Lakshmana Rao A[2,*], Venkateswara Rao J[3]

[1]Assistant Professor, Sri Vasavi Institute of Pharmaceutical Sciences, Tadepalligudem, A.P., India.
[2]Professor and Principal, V. V. Institute of Pharmaceutical Sciences, Gudlavalleru, A.P., India.
[3]Professor, Bharat School of Pharmacy, Mangalpalli, Telangana, India.

*Corresponding Author:
E-mail: dralrao@gmail.com

ABSTRACT

Objective: *The main objective of this work is to develop simple, precise, accurate and reproducible UV-Spectrophotometric and Stability indicating RP-HPLC methods for simultaneous estimation of Gatifloxacin (GAT) and Flubiprofen sodium (FLU) in ophthalmic dosage form.*

Methods: *Dual wavelength spectrophotometric method which involves solving of simultaneous equations based on the measurement of absorbances at 289 nm and 248 nm, which are the absorption maxima (λmax) of GAT and FLU respectively. The RP-HPLC analysis is carried out on Shiseido C18 column (250 mm × 4.6 mm I.D.), using 1% orthophosphoric acid in water and acetonitrile in the ratio of (40:60 % v/v) as the mobile phase with a flow rate of 0.9 ml/min. The detection was carried out at a wavelength of 236 nm.*

Results: *The retention times were found to be 2.152±0.2 min and 7.881±0.2 min for GAT and FLU respectively. The linearity range was found to be 10-20 μg/ml and 1-2 μg/ml for Gatifloxacin and Flubiprofen sodium respectively by UV method and 10-30 μg/ml and 1-3 μg/ml for Gatifloxacin and Flubiprofen sodium respectively by HPLC method. The percentage recoveries of both the drugs GAT and FLU from the ophthalmic form were 99.51% and 99.58% respectively by UV method and 99.08% and 99.43% respectively by HPLC method. The correlation coefficients of both the drugs were found to be more than 0.99 by two methods. Other parameters like ruggedness, robustness etc. was well within the acceptance criteria.*

Conclusion: *Both UV-spectrophotometric and stability indicating RP-HPLC methods were found to be accurate, rapid, precise and simple. These simple methods can be used for the simultaneous estimation of GAT and FLU in bulk and in ophthalmic dosage forms.*

Keywords: *Gatifloxacin, Flubiprofen sodium, Simultaneous equation, Validation, RP-HPLC.*

INTRODUCTION

Gatifloxacin (Fig. 1) is chemically 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid. It is an antibiotic of the fourth-generation fluoroquinolone family which inhibits the bacterial enzymes DNA gyrase and topoisomerase IV. It is mainly used to treat respiratory tract infections[1].

Flurbiprofen (Fig. 2) is chemically 2-(3-fluoro-4-phenylphenyl) propanoic acid, is a non-steroidal anti-inflammatory agent (NSAIDs) with antipyretic and analgesic activity. Oral formulations of flurbiprofen may be used for the symptomatic treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis[2].

Detailed literature survey revealed analytical methods like UV- Spectrophotometric[3-6], Spectrofluoro-metric[7], HPTLC[8,9], LC-MS[10,11] and HPLC[12-16] methods are available for the estimation of these drugs individually or in combination. We tried to develop simple spectrophotometric and RP-HPLC methods for the simultaneous estimation of these drugs. The developed methods were validated as per the guidelines of ICH[17]. To establish stability indicating nature of the RP-HPLC method, forced degradation of drug substances were performed under stress conditions (peroxide, acid, base, thermal, UV and neutral hydrolysis)[18]. The proposed methods were optimized and validated as per ICH guidelines.

MATERIALS AND METHODS

Chemicals and Reagents: Gatifloxacin and Flurbiprofen sodium working standards were procured from Yarrow Chemicals Ltd., Mumbai. Commercially available FLUBIGAT eye drops were purchased from the local pharmacy. HPLC grade acetonitrile and methanol were purchased from Merck Specialities Pvt. Ltd., Mumbai. Double distilled water used in all experiments was obtained from Milli-Q System (Millipore). Concentrated hydrochloric acid AR grade, sodium hydroxide pellets purified were procured from Merck specialties Pvt. Ltd., Mumbai and hydrogen peroxide 30% AR grade was obtained from Universal Laboratories Pvt. Ltd., Mumbai.

Instrumentation and analytical conditions: The UV method was performed on a Double-beam LABINDIA UV-Visible spectrophotometer, 3092,

with spectral bandwidth of 2 nm, wavelength accuracy ±0.5 nm and a pair of 1 cm matched quartz cells was used to measure absorbance of solution. The method is based upon determination of Gatifloxacin at 289 nm and Flubiprofen sodium at 248 nm. RP-HPLC method was performed on HPLC system (Shimadzu) consisting of binary gradient pump, and UV detector (LC-AD20) was employed for analysis. Chromatographic data was acquired using Lab solutions software. Shiseido C18 column (250 mm × 4.6 mm I.D.) was used as stationary phase. GAT and FLU were eluted isocratically with a flow rate of 0.9 ml/min using a mobile phase consisting of 1 % orthophosphoric acid in water and acetonitrile in a proportion of 40:60 v/v respectively. The wavelength of UV detector was set at 236 nm. The mobile phase was prepared daily, filtered through 0.45 μm membrane filter (Millipore) and sonicated before use. The summary of system suitability parameters were shown in Table 1.

Preparation of standard solutions

For UV method: Standard stock solution of GAT and FLU were prepared by transferring accurately weighed GAT (10 mg) and FLU (10 mg) to a 100 ml volumetric flask separately, dissolved and diluted to a mark with the solvent consisting of acetinitrile:water in the ratio of 50:50 v/v, to obtain a standard solution of GAT (100 μg/ml) and FLU (100 μg/ml). From these solutions, standard stock solutions were prepared in 10 ml volumetric flask and made up the volume with the same solvent, to get the concentration of 15 μg/ml of GAT and 1.5 μg/ml of FLU.

For HPLC method: Standard stock solution of GAT and FLU were prepared by transferring accurately weighed GAT (10 mg) and FLU (10 mg) to a 100 ml volumetric flask separately, dissolved and diluted to a mark with the solvent consisting of acetinitrile: water in the ratio of 50:50 v/v, to obtain a standard solution of GAT (100 μg/ml) and FLU (100 μg/ml). From these solutions, standard stock solutions were prepared in 10 ml volumetric flask and made up the volume with the mobile phase to get the concentration of 30 μg/ml of GAT and 3 μg/ml of FLU.

Preparation of the sample solutions

For UV method: 1 ml of the test sample (FLUBIGAT Eye drops) contains 0.3 w/v of Gatifloxacin and 0.03 % w/v of Flubiprofen as per the labelled claim. One ml of the formulation was taken into 50 ml volumetric flask and diluted up to the mark with the solvent consisting of acetonitrile: water in the ratio of 50:50 v/v, to obtain a concentration of 60 μg/ml of GAT and 6 μg/ml of FLU. 2.5 ml of the above solution was taken in 10 ml volumetric flask and diluted to 10 ml with the same

solvent to obtain a final concentration of 15 μg/ml of GAT and 1.5 μg/ml of FLU.

For HPLC method: 1 ml of the test sample (FLUBIGAT Eye drops) contains 0.3 w/v of Gatifloxacin and 0.03 % w/v of Flurbiprofen as per the labelled claim. One ml of the formulation was taken into 50 ml volumetric flask and diluted upto the mark with the solvent consisting of acetinitrile: water in the ratio of 50:50 v/v, to obtain a concentration of 60 μg/ml of GAT and 6 μg/ml of FLU. Five ml of the above solution was taken in 10 ml volumetric flask and diluted to 10 ml to obtain a final concentration of 30 μg/ml of GAT and 3 μg/ml of FLU.

Procedure for forced degradation study

Degradation studies were performed in sample solutions containing 30 μg/ml of GAT and 3 μg/ml of FLU.

Stress degradation by hydrolysis under acidic conditions: For acid degradation, 1 ml of 2M HCl was added to final drug solution, and it was refluxed for 1 hr at 60°C. After 1 hr, this solution was injected under optimized chromatographic conditions.

Stress degradation by hydrolysis under alkaline conditions: For alkali degradation, 1 ml of 2M NaOH was added to final drug solution, and it was refluxed for 1 hr at 60°C. After 1 hr, this solution was injected under optimized chromatographic conditions.

Oxidative degradation: For oxidation, 1 ml of 10 % v/v H_2O_2 was added to final drug solution, and it was refluxed for 1 hr at 60°C. After 1 hr, this solution was injected under optimized chromatographic conditions.

Photo hydrolysis: For photolytic studies, the final drug solution was kept at a room temperature and exposed to UV light of 200 watt hours/m² for 7 days. After 7 days, this solution was injected under optimized chromatographic conditions.

Thermal hydrolysis: For thermal studies, the final drug solution was kept at a temperature of 60°C for 6 hrs. After 6 hrs this solution was injected under optimized chromatographic conditions.

Neutral hydrolysis: For neutral hydrolysis, the final drug concentration is refluxed for 1 hr at 60°C. After 1 hr this solution was injected under optimized chromatographic conditions.

METHOD VALIDATION

The developed methods were validated according to International Conference on Harmonization guidelines for validation of analytical procedures.

Linearity: The calibration curves for UV method were obtained with concentrations of the standard solutions 10-20 μg/ml and 1-2 μg/ml of GAT and FLU respectively and for RP-HPLC method 10-30 μg/ml and 1-3 μg/ml of GAT and FLU respectively. The solutions were prepared in triplicate. Linearity was evaluated by regression analysis, which was

calculated by the least square regression method.

Precision: Precision of UV and RP-HPLC method were checked by analyzing the samples (50%, 100% and 150%) at three different time intervals of the same day (intra-day precision) as well as on different days (inter-day precision).

Accuracy: To check the degree of accuracy of UV and RP-HPLC method, recovery studies were performed in triplicate by standard addition method at 80%, 100% and 120% levels.

Robustness: Robustness for RP-HPLC method was determined by analysis of samples under deliberately changed chromatographic conditions. The flow rate of the mobile phase was changed from 0.8 ml/min to 0.9 ml/min and 1.0 ml/min. The ratio of the organic phase was changed by ± 5%, i.e., 55%, 60%, 65% of acetonitrile. The effect on retention time and peak parameter were studied.

Limit of detection and limit of quantitation: LOD, LOQ of UV and RP-HPLC method were calculated by using the values of slopes and intercepts of the calibration curves for both the drugs.

RESULT AND DISCUSSION

UV method: The proposed UV methods, allows a rapid and accurate quantitation of GAT and FLU in ophthalmic preparation without any time consuming sample preparation. Moreover, the spectrophotometric methods involve simple instrumentation compared with other instrumental techniques. The absorption spectra of GAT and FLU in ACN: H_2O (50:50 v/v) are shown in Figure 3. Wavelengths selected for analysis are 289 nm (λ_{max} of GAT) and 248 nm (λ_{max} of FLU). Calibration curves were constructed in the concentration range of 10-20 µg/ml and 1-2 µg/ml for GAT and FLU respectively. Beer's law was obeyed over this concentration range, and the coefficient of regression for both the drugs was found to be nearer to 1 (Table 2). The accuracy of proposed method were determined (Table 3), indicating an agreement between the true value and found value. Precision was calculated as inter-day and intra-day variations for both the drugs. Percent relative standard deviations for estimation of GAT and FLU under intra-day and inter-day variations were found to be less than 2 (Table 4). The LOD and LOQ were calculated using the values of slopes and intercepts of the calibration curves for both the drugs (Table 5). The assay values obtained for the determination of GAT and FLU in ophthalmic formulation was within the claimed limits (Table 7).

HPLC method: Different proportions of acetonitrile and othophosphoric acid were tried for selection of mobile phase. Ultimately, 1% OPA in water and acetonitrile in a proportion of 40:60 v/v respectively

was finalized as the mobile phase. Figure 4 shows typical chromatogram obtained from the analysis of standard solution of GAT and FLU using the proposed method. The elution order was GAT (Rt = 2.094 min) and FLU (Rt = 7.777 min), at a flow rate of 0.9 ml/min. The chromatogram was recorded at 236 nm. The calibration curves for GAT and FLU were constructed in the concentration range of 10-30 µg/ml and 1-3 µg/ml of GAT and FLU respectively and the coefficient of regression for both the drugs was found to be nearer to 1 (Table 2). The accuracy of proposed method was determined (Table 3), indicating an agreement between the true value and found value. Precision was calculated as inter-day and intra-day variations for both the drugs. Percent relative standard deviations for estimation of GAT and FLU under intra-day and inter-day variations were found to be less than 2 (Table 4). The LOD and LOQ were calculated using the values of slopes and intercepts of the calibration curves for both the drugs (Table 5) and for robustness studies in all deliberately varied conditions, percent relative standard deviations were found to be less than 2 % (Table 6). The assay values obtained for the determination of GAT and FLU in ophthalmic formulation was within the claimed limits (Table 7).

The following degradation results were found when GAT and FLU were subjected to,

Acid hydrolysis: Both the drugs were degraded in acidic condition shown in Fig. 5.

Alkaline hydrolysis: Both the drugs were degraded in alkaline condition shown in Fig. 6.

Oxidative degradation: Gatifloxacin showed degradation in hydrogen peroxide (10%) conditions whereas Flubiprofen showed stablility shown in Fig. 7.

Photolytic degradation: Both the drugs showed good stability under photolytic conditions with very less degradation shown in Fig. 8.

Thermal hydrolysis: Gatifloxacin showed degradation under thermal conditions whereas Flubiprofen showed stablility shown in Fig. 9.

Neutral hydrolysis: Both the drugs showed good stability under photolytic conditions with very less degradation shown in Fig. 10.

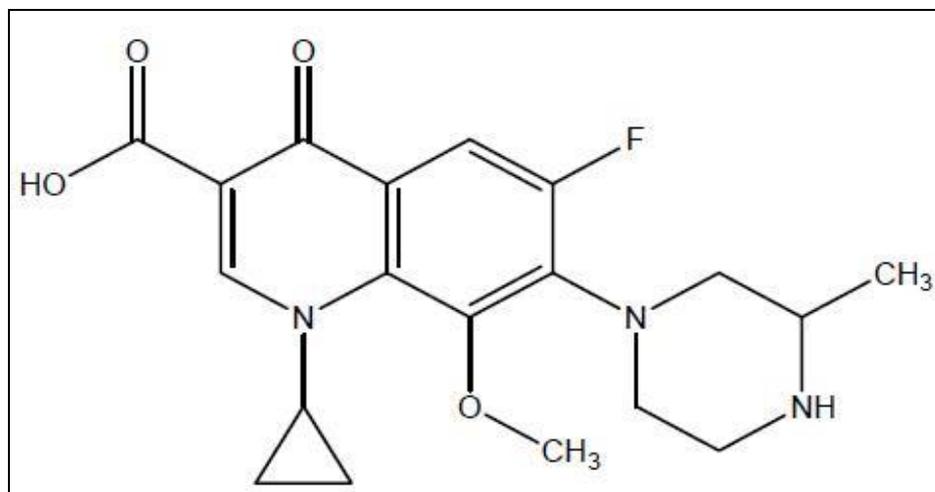The percent amount of drug degraded after degradation studies were given in Table 8.

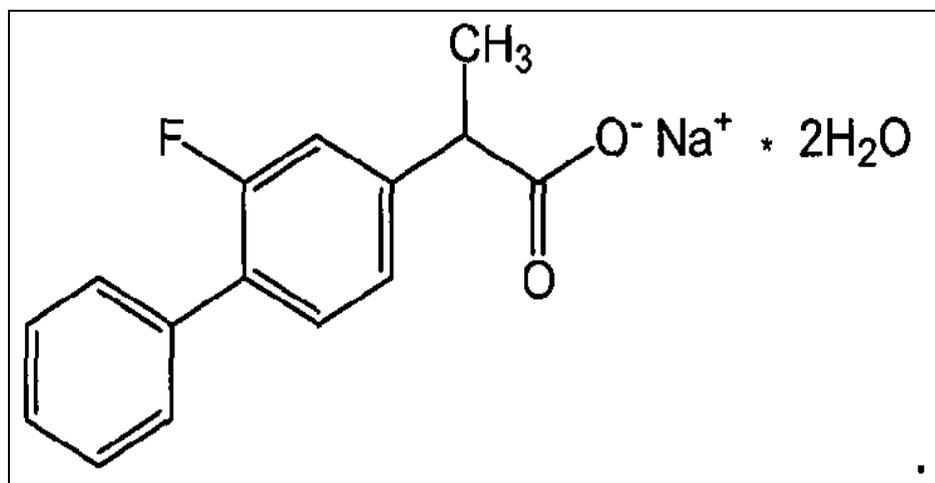**Fig. 1: Chemical structure of Gatifloxacin**

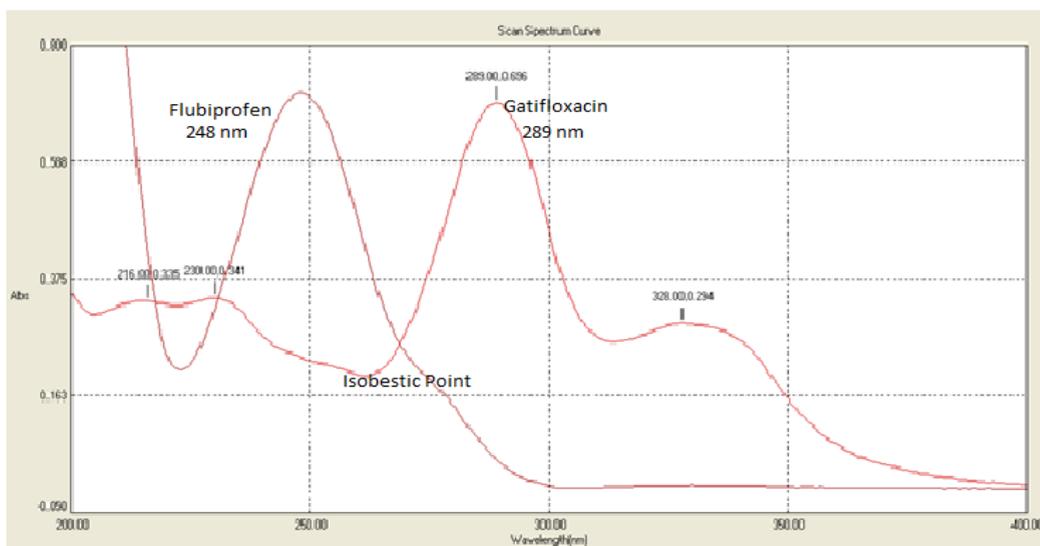**Fig. 2: Chemical structure of Flurbiprofen Sodium**

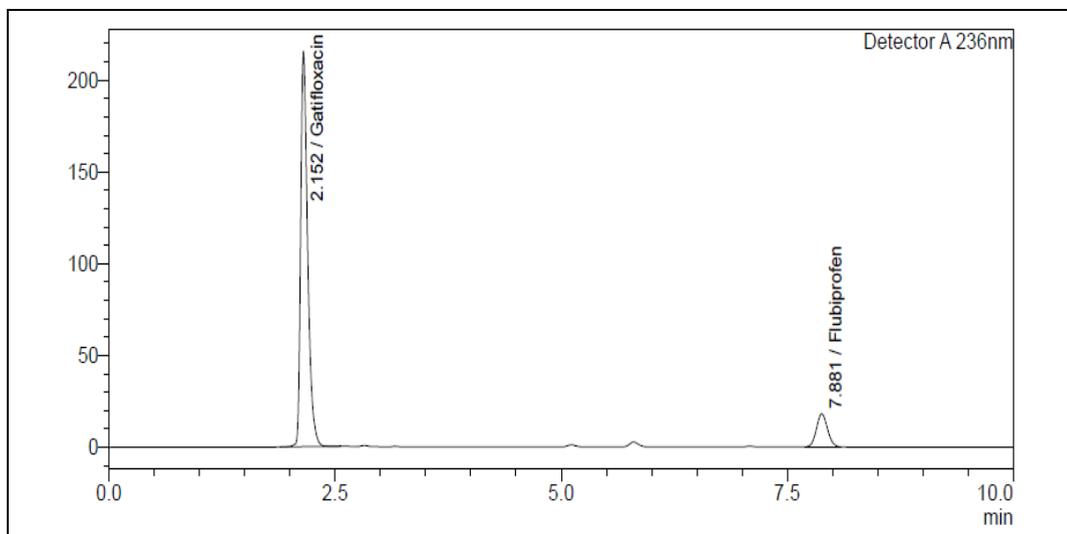**Fig. 3: Overlain spectrum of Gatifloxacin and Flurbiprofen Sodium**

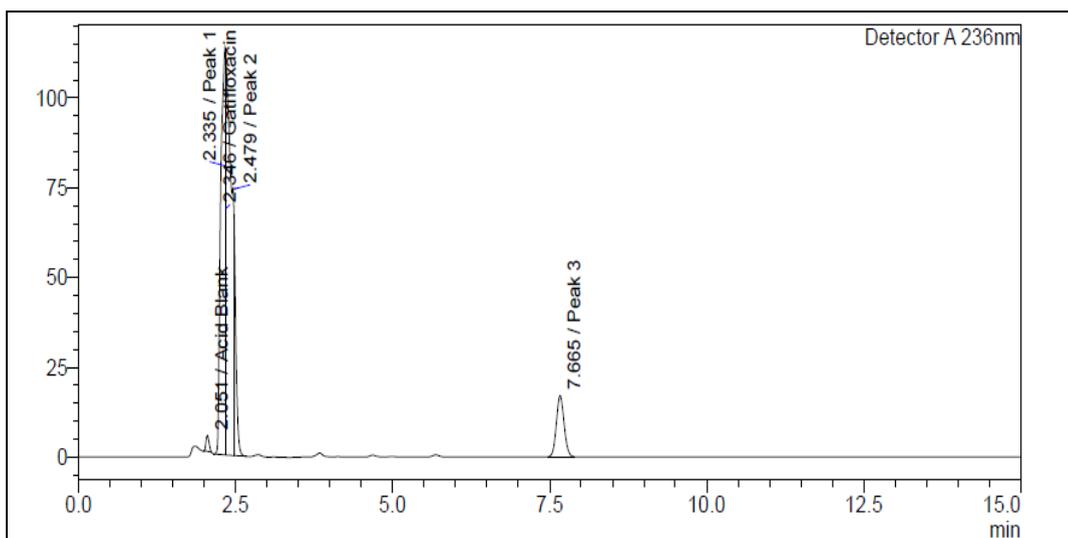**Fig. 4: Chromatogram showing well resolved peaks of Gatifloxacin and Flurbiprofen**

Fig. 5: Chromatogram of GAT and FLU in 2M HCl

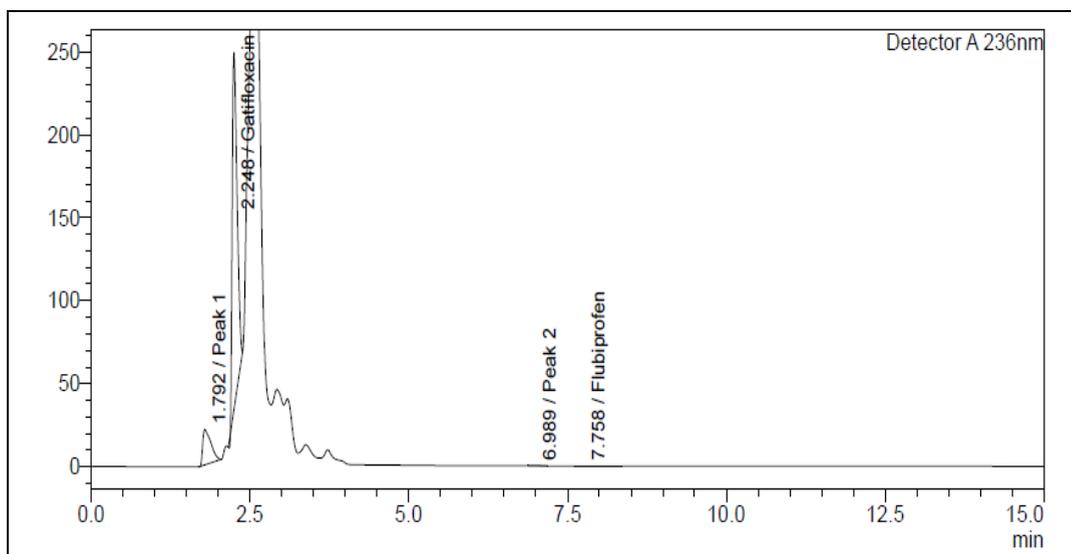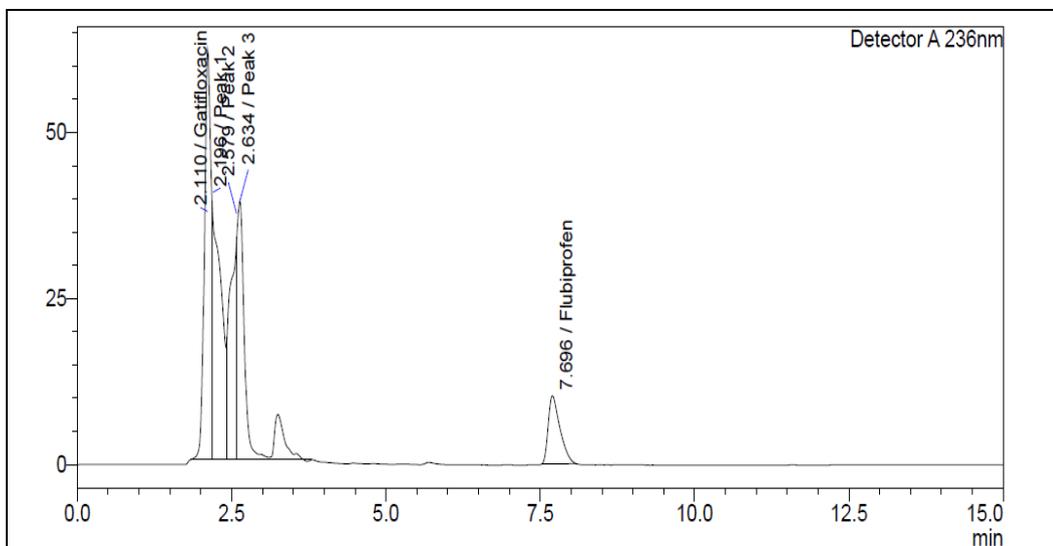**Fig. 6: Chromatogram of GAT and FLU in 2M NaOH**

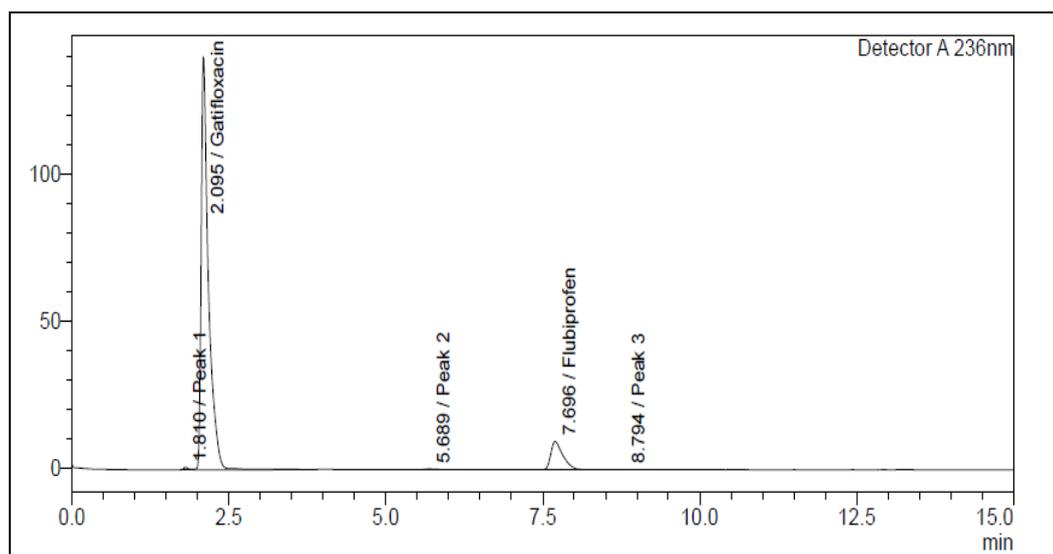**Fig. 7: Chromatogram of GAT and FLU in 10% H_2O_2**

Fig. 8: Chromatogram of GAT and FLU in UV photolytic condition
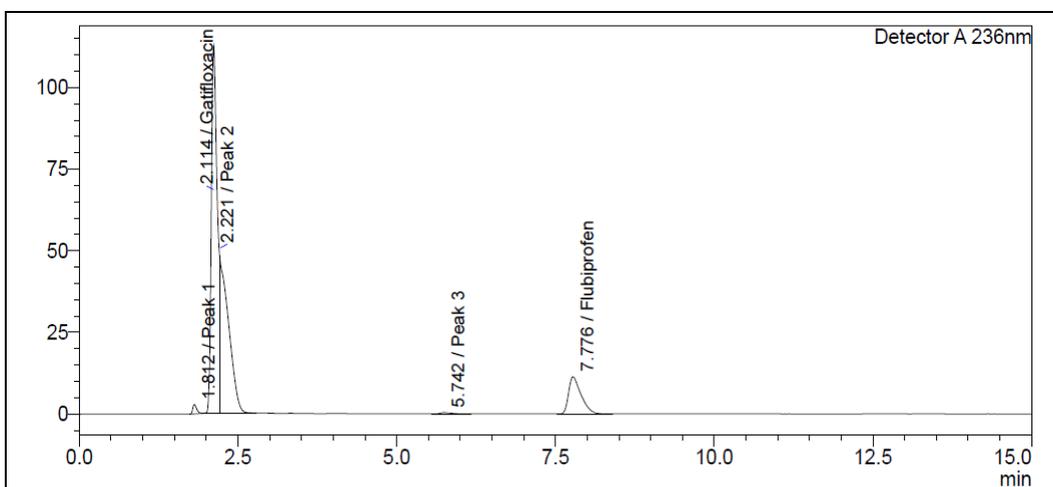

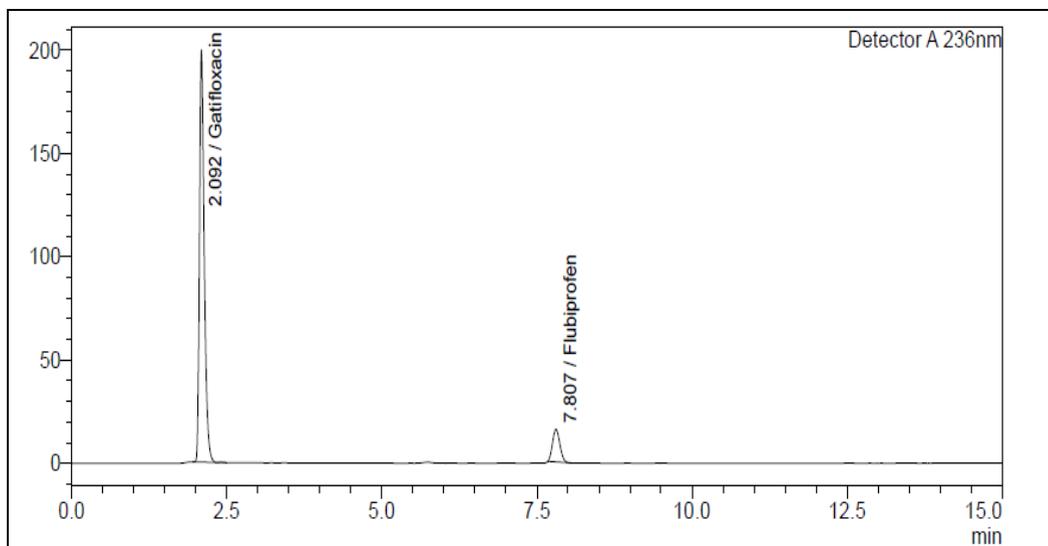**Fig. 9: Chromatogram of GAT and FLU in thermal stress condition**

Fig. 10: Chromatogram of GAT and FLU in neutral stress condition

Table 1: RP-HPLC System suitability parameters

Parameter	Observation*	
	GAT	**FLU**
Retention time	2.152 min.	7.881 min.
No. of Theoretical plates	6534	5985
Tailing Factor	1.181	1.064

Average of six readings

Table 2: Linearity values of Gatifloxacin and Flurbiprofen Sodium

Method	Parameter	GAT	FLU
UV	Regression equation	Y= 0.071X	Y= 0.167X - 0.100
	Linearity (µg/ml)	10-20	1.0-2.0
	Correlation coefficient	0.996	0.995
HPLC	Regression equation	Y= 55546X	Y= 94224X - 1529
	Linearity (µg/ml)	10-30	1.0-3.0
	Correlation coefficient	0.999	0.999

Table 3: Recovery values of Gatifloxacin and Flurbiprofen Sodium

UV method						
Drug	**Recovery**			**% RSD**		
	80%	**100%**	**120%**	**80%**	**100%**	**120%**
GAT	99.21	100.21	99.12	0.51	0.29	0.19
FLU	98.64	98.95	101.15	0.28	0.56	0.52
HPLC method						
Drug	**Recovery**			**% RSD**		
	80%	**100%**	**120%**	**80%**	**100%**	**120%**
GAT	98.52	99.60	99.14	0.08	0.12	0.94
FLU	98.24	99.04	101.03	0.04	0.88	0.59

Table 4: Precision values of Gatifloxacin and Flurbiprofen Sodium

Method	Drug	Concentration (µg/ml)	Intra-day (% RSD)	Inter-day (% RSD)	System Precison (% RSD)
UV	GAT	10	0.5	0.53	0.58
		15	0.47	0	
		20	0.31	0.48	
	FLU	10	0.40	0.15	0.47
		15	0.15	0.13	
		20	0	0.52	
HPLC	GAT	10	0.22	0.92	1.01
		20	0.30	0.57	
		30	0.37	0.42	
	FLU	10	0.21	0.21	0.34
		20	0.66	0.28	
		30	0.16	1.4	

Table 5: LOD and LOQ of Gatifloxacin and Flurbiprofen Sodium

Method	Drug	LOD (µg/ml)	LOQ (µg/ml)
UV	GAT	1.86	5.63
	FLU	0.10	0.31
HPLC	GAT	0.09	2.70
	FLU	0.06	0.20

Table 6: Robustness parameters of Gatifloxacin and Flurbiprofen Sodium

Parameter	% Target Conc.	GAT Rt (min.)	FLU Rt (min.)
Initial Sample	50%	2.111	7.769
	100%	2.104	7.751
	150%	2.093	7.741
Flow 0.8 ml/min	50%	2.365	9.225
	100%	2.370	9.235
	150%	2.370	9.227
Flow 1.0 ml/min	50%	1.884	7.277
	100%	1.883	7.267
	150%	1.892	7.267
Organic phase, 10% more (65%)	50%	2.055	6.392
	100%	2.056	6.521
	150%	2.080	6.725
Organic phase, 10% less (55%)	50%	2.148	10.03
	100%	2.158	10.12
	150%	2.146	10.01

Table 7: Assay of marketed formulation of Gatifloxacin and Flurbiprofen Sodium

Method	Drug	Amount labeled	Amount found	% Label claim	% RSD
UV	GAT	3 mg/ml	2.980	99.33	0.19
	FLU	0.3 mg/ml	0.297	99.00	0.52
HPLC	GAT	3 mg/ml	3.012	100.40	0.38
	FLU	0.3 mg/ml	0.299	99.66	0.50

Table 8: Degradation data of Gatifloxacin and Flurbiprofen Sodium

Drug	Stress Condition (% degradation)					
	Acid	Base	Peroxide	UV	Thermal	Neutral
GAT	67.70	82.90	40.87	2.37	48.69	0.36
FLU	6.87	87.85	7.06	2.06	0.15	0.88

CONCLUSION

The two proposed methods based on the spectrophotometry and RP-HPLC were developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods are low, indicating high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy for the proposed methods. The RP-HPLC method could selectively quantitate GAT and FLU in presence of its degradation products hence; it can be employed as a stability indicating method. From the found experimental data it can be concluded that the developed spectrophotometric and stability indicating chromatographic methods are accurate, precise and selective and can be employed successfully for the estimation of GAT and FLU in ophthalmic dosage form.

REFERENCES:

1. Data base of Gatifloxacin, compilation prepared by drug bank, http://www.drugbank.ca/drugs/DB01044.
2. Data base of Flurbiprofen, compilation prepared by drug bank, http://www.drugbank.ca/drugs/DB00712.
3. Patel, Gopi, Chauhan, Payal, Shah, Samir. Simultaneous estimation of Gatifloxacin and Flurbiprofen Sodium in ophthalmic formulation by UV-Specrophotometric method. Journal of Chemical and Pharmaceutical Research. 2014; 6(7): 96-101.
4. Upadhyay N, Maheshwari RK, Jain J, Patani M, Pandey R. New spectrophotometric analysis of Gatifloxacin tablets utilizing mixed solvency concept. Der Pharmacia Lettre. 2012; 4 (1): 1-4.
5. Shalaby AA, Sayed RA, Hassan WS, El-mammli MY. A new extractive spectrophotometric method for the determination of Gatifloxacin and Cefotaxime Sodium in pure and pharmaceutical dosage forms. Oriental Journal of Chemistry 2012; 28(2): 639-650.
6. Pradhan PK, Rajput PN, Kumar N, Joshi B, Upadhyay UM. Simultaneous estimation of Flurbiprofen and Gatifloxacin by dual wavelength UV spectroscopy method in an eye drops. International Journal of Pharmaceutical Sciences Review and Research 2014; 27(2): 96-99.
7. Jinal MD, Vishnu MP, Rajesh P, Dushyant S. Development and validation of spectrofluorometric method for analysis of Gatifloxacin and Flurbiprofen Sodium in ophthalmic dosage form. Inventi Rapid-Pharm Analysis & Quality Assurance. 2014; ppaqa/1421/14.
8. Rote AR, Kumbhoje PA. Development and validation of HPTLC method for simultaneous estimation of Gatifloxacin and Ornidazole in human plasma. Journal of Chromatography and Separation Techniques. 2011; 2(115): 2157-7064.
9. Shah SA, Rathod IS, Suhagia BN, Baldaniya M. A simple and sensitive HPTLC Method for estimation of Gatifloxacin in tablet dosage forms. Indian Journal of Pharmaceutical Sciences. 2004; 66(3): 306-308.
10. Giancarlo A, Marina C, Marica O, Roberto MF, Gary LW. Metabolic profile of NO-Flurbiprofen in rat brain and plasma: A LC-MS study. Life Sciences. 2002; 71(13): 1487-500.
11. Deglon J, Thomas A, Daali Y, Lauer E, Samer C, Desmeules J. Automated system for on-line desorption of dried blood spots applied to LC/MS/MS pharmacokinetic study of Flurbiprofen and its metabolite. Journal of Pharmaceutical and Biomedical Analysis. 2011; 54: 359-67.
12. Gopi P, Payal C, Samir S. Application of RP-HPLC method for simultaneous estimation of Gatifloxacin and Flurbiprofen Sodium in ophthalmic formulation. American Journal of PharmTech Research 2014; 4(2): 658-668.
13. Sridhar S, Kavitha R, Sudhakar M. RP-HPLC method development and validation for the simultaneous estimation of Gatifloxacin and Flurbiprofen in pharmaceutical dosage form. Asian Journal of Pharmaceutical and Clinical Research. 2015; 8(1): 242-246.
14. Sandip RR, Laxman MP, Joshi AK, Mahammadali KL. RP-HPLC Method for the simultaneous estimation of Gatifloxacin and Flurbiprofen Sodium in their ophthalmic dosage form. International Journal of Universal Pharmacy and Bio Sciences. 2014; 3(3): 59-70.
15. Sireesha KR, Prakash K. Simultaneous determination of Gatifloxacin and Dexamethasone Sodium Phosphate in bulk and pharmaceutical formulations by HPLC. African Journal of Pharmacy and Pharmacology. 2011; 5(17): 1990-1995.
16. Sultana N, Arayne M, Siddiqui R, Naveed S. RP-HPLC method for the simultaneous determination of Lisinopril and NSAIDs in API, pharmaceutical formulations and human serum. American Journal of Analytical Chemistry. 2012; 3: 147-152.
17. International Conference on Harmonization (ICH) guidelines Q2(R1), Text on validation of analytical procedures, methodology. International Conference on Harmonization, Geneva. 2005.
18. International Conference on Harmonization (ICH) guidelines Q1A, Stability testing: stability testing of new drug substances and new drug products. International Conference on Harmonization, Geneva. 2003.