Ashdin Publishing
Journal of Drug and Alcohol Research
Vol. 11 (2022), Article ID 236193, 06 pages
DOI:10.4303/jdar/236193

Research Article

Robust, Sensitive and Validated RP-HPLC Modus Operandi for the Quantitation of Fixed Dose Combination of Gatifloxacin and Flurbiprofen

K. Shankar[1*], M. Ramayyappa[2] and G. Raveendra Babu[3]

[1]*Pharmacy Department, CDSCO, India*
[2]*Department of Pharmaceutical Analysis, Oxbridge College of Pharmacy, India*
[3]*Department of Pharmaceutical Analysis, QIS College of Pharmacy, India*

[*]*Address Correspondence to K. Shankar, upendragudimitla@gmail.com*

Received: 03 August 2022; Manuscript No: jdar-22-73051; **Editor assigned:** 05 August 2022; PreQC No: jdar-22-73051 (PQ); **Reviewed:** 19 August 2022; QC No: jdar-22-73051; **Revised:** 24 August 2022; Manuscript No: jdar-22-73051 (R); **Published:** 31 August 2022; **DOI:** 10.4303/jdar/236193

Abstract

In this study, we aim to create a technique for the simultaneous determination of stability indicators of Gatifloxacin (GTF) and Flurbiprofen sodium (FLU) using reversed phase high performance liquid chromatography in ophthalmic dosage form that is easy to perform and replicate with high reliability. For the RP-HPLC analysis, a thermosil C18 column (4.6 x 250 mm, 5 µm) is used with a mobile phase comprising of 0.02 M phosphate buffer: acetonitrile (70:30) at a velocity of 1.0 ml/min. The 236 nm wavelength was used for the detection. GTF and FLU were shown to have retention durations of 2.152 min and 7.881 min, respectively. It was determined that the linearity ranges for GTF and FLU were 20-60 µg/ml and 2-6 µg/ml, respectively. For marketed formulation, both GTF and FLU had recoveries of 98.73 and 99.21 percent, respectively. Results showed that the medications had correlation coefficients greater than 0.99. Acceptance was also achieved with respect to other aspects such as ruggedness, robustness, etc. The RP-HPLC methods for showing stability were found to be reliable, quick, exact, and easy to use. Using this simple technique, the selected medicaments in bulk and ophthalmic dose forms can be estimated simultaneously.

Keywords: Gatifloxacin; Flurbiprofen sodium; Validation; RP-HPLC

Introduction

1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid is the chemical name for gatifloxacin (Figure 1). It is a fluoroquinolone antibiotic that blocks the replication of bacterial DNA by obstructing the mediator's topoisomerase IV and DNA gyrase. Infections of the respiratory system are its primary target [1].

Figure 2 shows the chemical structure of FLU, also known as 2-(3-fluoro-4-phenylphenyl) propanoic acid, which is an NSAID with antipyretic and analgesic properties. Symptomatic therapy of anklylosing spondylitis, osteoarthritis and rheumatoid arthritis with oral flurbiprofen is possible [2].

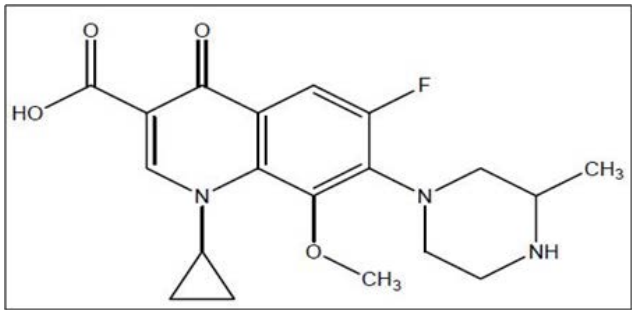

Figure 1: Chemical Structure of Gatifloxacin

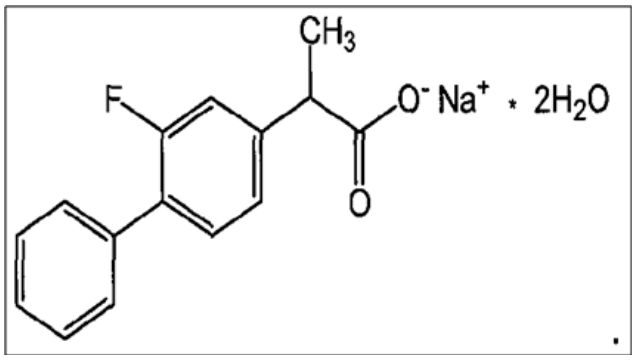

Figure 2: Chemical Structure of Flurbiprofen sodium

In depth research into the published literature revealed that UV-Spectrophotometric [3-6], Spectrofluorometric [7], HPTLC [8,9], LC-MS [10,11], and HPLC [12-15] methods are available for the assessment of these medications sin-

gly or in combination. We aimed to create straightforward spectrophotometric and RP-HPLC procedures for the combined assessment of these medicines. The procedures proposed were verified as valid in accordance with ICH regulations [16]. Under stress, pharmacological compounds were degraded forcibly using the RP-HPLC technique in order to establish the stability indicating [17] nature of the technique (different conditions for forced degradation studies). The proposed procedures were fine-tuned and verified in accordance with ICH standards.

Materials and Methods

Chemicals and reagents

Yarrow Chemicals Ltd., Mumbai was contacted to acquire working standards of flurbiprofen sodium and gatifloxacin. The FLUBIGAT eye drops were acquired from a local pharmacy and are available for commercial use. Merck specialties Pvt. Ltd., Mumbai supplied the HPLC grade acetonitrile and methanol. Milli-Q System double-distilled water was utilised in all studies (Millipore). Universal Laboratories Pvt. Ltd., Mumbai supplied the 30% AR grade hydrogen peroxide, while Merck Specialties Pvt. Ltd., Mumbai supplied the concentrated hydrochloric acid and purified sodium hydroxide pellets.

Instrumentation and analytical conditions

The absorbance of solutions was determined using a Double beam LABINDIA UV-Visible spectrophotometer, 3092, with a spectral bandwidth of 2 nm and wavelength accuracy of 0.5 nm, and a set of matching quartz cells of 1 cm in diameter. The RP-HPLC technique was carried out utilising a binary gradient pump HPLC system (Shimadzu) and a UV detector (LC-AD20) for analysis. Lab solutions software was used to collect chromatographic data. We used a Thermosil C18 column (4.6 mm i.d., 250 mm) as our stationary phase to accomplish this separation. To isocratically elute GTF and FLU, a mobile phase of 0.02 M phosphate buffer: acetonitrile (70:30 v/v) was used at a flow rate of 1.0 mL/min. Adjusting the UV detector's setting to 236 nm (Figures 3 and 4). The mobile phase was filtered using a 0.45 m membrane filter after being sonicated before each use (Millipore). In, we saw a rundown of the main criteria for judging a system's fitness for use (Table 1).

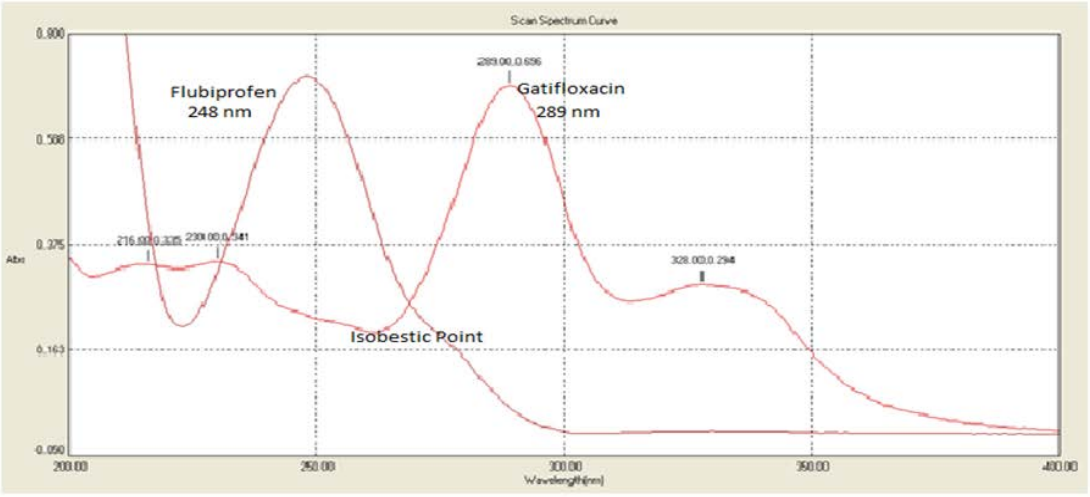

Figure 3: Overlain spectrum of Gatifloxacin and Flurbiprofen sodium

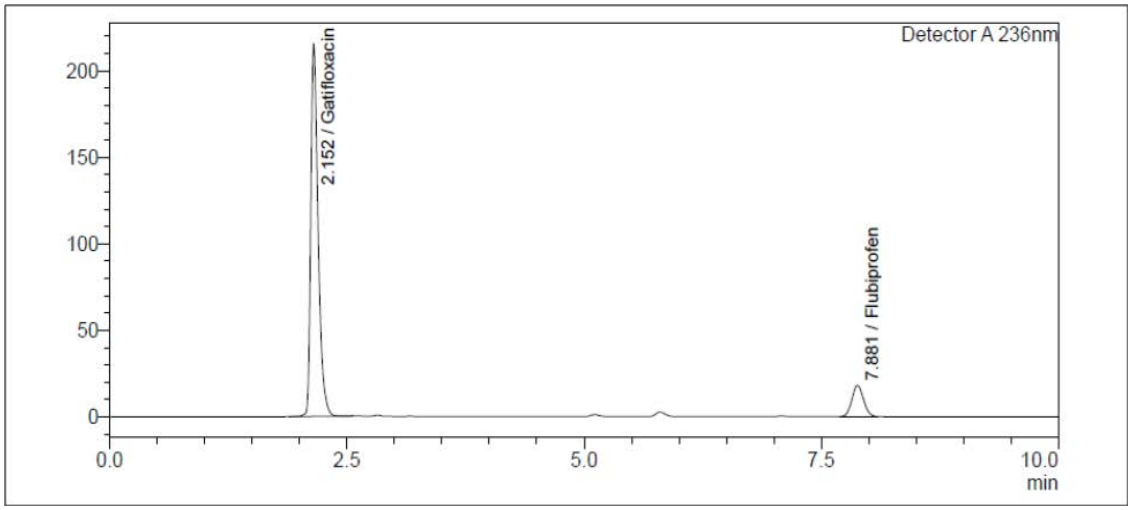

Figure 4: Chromatogram showing well resolved peaks of Gatifloxacin and Flurbiprofen

Table 1: RP-HPLC System suitability parameters

Parameter	Observation*	
	GTF	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 Tailing Factor		

Preparation of standard solutions

A standard solution of GTF (100 µg/ml) and FLU (100 µg/ml) was prepared by adding accurately weighed quantities of GTF (10 mg) and FLU (10 mg) to separate 100 mL volumetric flasks, dissolving them in a solvent comprising acetonitrile: water in the ratio of 70:30 v/v, and diluting them to the mark. A 10 ml volumetric flask was used to create standard stock solutions of 40 µg/ml GTF and 4 µg/ml FLU by diluting the corresponding stock solutions with the mobile phase.

Preparation of the sample solutions

Sample (FLUBIGAT Eye drops) included 0.3% (w/v) Gatifloxacin and 0.03% (w/v) Flurbiprofen, which is in compliance with the labelling. By adding 1 ml of the formulation to a 50 ml volumetric flask and diluting it to the necessary amount using a solvent consisting of acetonitrile: water in the ratio of 30:70 v/v, we were able to reach concentrations of 60 µg/ml GTF and 6 µg/ml FLU. After transferring 5 ml of the solution to a 10 ml volumetric flask, the volume was diluted to 10 ml to achieve a final concentration of 30 µg/ml of GTF and 3 µg/ml of FLU.

Procedure for forced degradation study

For the degradation tests, solutions with 30 µg/ml of GTF and 3 µg/ml of FLU were used.

Stress degradation by hydrolysis under acidic conditions

The final drug solution was refluxed for 1 hour at 60° C after 1 mL of 2M HCl was added to it. This solution was then injected after 1 hour at room temperature and under ideal chromatographic conditions.

Stress degradation by hydrolysis under alkaline conditions

After adding 1 mL of 2M NaOH to the final drug solution and refluxing it for 1 hour at 60° C, the medication was degraded by the alkali. This solution was then injected after 1 hour at room temperature and under ideal chromatographic conditions.

Oxidative degradation

The finished drug solution was refluxed for 1 hour at 60° C after 1 mL of 10% v/v H2O2 was added. After waiting 1 hour, optimum chromatographic conditions allowed for the injection of this solution.

Photo hydrolysis

The finished medication solution underwent photolytic testing by being stored at room temperature and subjected to UV light at 200 watt hours/m2 for 7 days. Under ideal chromatographic conditions, this solution was injected after 7 days.

Thermal hydrolysis

The final drug solution was kept at 60 degrees Celsius for six hours for thermal studies. Under ideal chromatographic conditions, this solution was injected after 6 hours.

Neutral hydrolysis

A one hour period of refluxing at 60 degrees Celsius is used to achieve the desired drug concentration in Neutral Hydrolysis. This solution was then injected after 1 hour at room temperature and under ideal chromatographic conditions.

Method validation

International Conference on Harmonization criteria for validation of analytical processes were used to verify the accuracy of the established methodologies.

Linearity

GTF and FLU RP-HPLC calibration curves at 2-6 µg/ml and 20-60 µg/ml, respectively. There were three copies of each solution made. Regression analysis, with the least squares regression approach used to determine linearity, was performed.

Precision

Analyses of the samples (at 50%, 100%, and 150% concentrations) were performed at three separate times during a single day (intraday precision) and on separate days (interday precision).

Accuracy

Recovery studies were conducted in triplicate using the standard addition method at the 80%, 100%, and 120% levels to evaluate the precision of the RP-HPLC method.

Robustness

We tested the RP-HPLC method's robustness by analysing materials under varying chromatographic settings. After starting with a mobile phase flow rate of 1 ml/min, we subsequently adjusted it to 0.9 ml/min and then 1.1 ml/min. There was a 5% variation in the organic phase ratios, therefore 25% and 35% acetonitrile were used instead. There was an investigation into how this affected the retention time and peak parameter.

Limit of detection and limit of quantitation

The limits of detection and quantification (LOD, LOQ) for the RP-HPLC technique were determined by utilising the slope and intercept values of the calibration curves for both drugs.

Result and Discussion

The mobile phase was determined by experimenting with various mixtures of acetonitrile and water. Finally, the mobile phase was settled upon as a 70:30 (v/v) mixture of 0.02 M phosphate buffer and acetonitrile. The standard solution of GTF and FLU was analysed using the suggested ap-

proach, and the resulting chromatogram is shown in Figure 4. The elution sequence at a flow rate of 1.0 ml/min was GTF (Rt=2.152 min) followed by FLU (Rt=7.881). At 236 nm, the chromatogram was captured.

We produced calibration curves for GTF and FLU over the concentration range of 2-6 g/ml and 20-60 g/ml, respectively, and found that the coefficient of regression for both medications was closer to 1 than to 0.99 (Table 2).

Table 2: Linearity values of Gatifloxacin and Flurbiprofen sodium

Method	Parameter	GTF	FLU
HPLC	Regression equation	Y=55546x	Y=94224x-1529
	Linearity (µg/ml)	20-60	2-6
	Correlation coefficient	0.999	0.999

It was found that the proposed method was accurate (Table 3), meaning that the calculated value was in line with the target value.

Table 3: Recovery values of Gatifloxacin and Flurbiprofen sodium

Drug	Recovery			% RSD		
	80 %	100 %	120 %	80 %	100 %	120 %
GTF	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

The accuracy of both medications was measured by comparing the daily and hourly shifts in their concentrations. Estimation of GTF and FLU during intraday and interday changes was shown to have relative standard deviations of less than 2 (Table 4). Slope and intercept values from the calibration curves were used to determine the LOD and LOQ for each drug (Table 5), and it was observed that the percent relative standard deviations for robustness studies in all purposefully altered settings were less than 2% (Table 6). The results of the experiments performed on the ophthalmic formulation to determine GTF and FLU were within the specified ranges (Table 7).

Table 4: Precision values of Gatifloxacin and Flurbiprofen sodium

Drug	Concentration (µg/ml)	Intraday (% RSD)	Interday (% RSD)	System Precison(% RSD)
Flurbiprofen	20	0.22	0.92	1.01
	40	0.30	0.57	
	60	0.37	0.42	
Flurbiprofen	2	0.21	0.21	0.34
	4	0.66	0.28	
	6	0.16	1.4	

Table 5: LOD and LOQ of Gatifloxacin and Flurbiprofen sodium

Drug	LOD (µg/ml)	LOQ (µg/ml)
GTF	0.09	2.70
FLU	0.06	0.20

Table 6: Robustness parameters of GAT and FLU

S. No.	Parameter	% Target conc.	GTF	FLU
			Rt (min.)	Rt (min.)
1	Initial Sample	50 %	2.174	7.769
		100 %	2.177	7.751
		150 %	2.169	7.741
2	Flow 0.9 ml/min	50 %	2.365	8.235
		100 %	2.370	8.242
		150 %	2.370	8.237
3	Flow 1.1 ml/min	50 %	1.948	7.315
		100 %	1.958	7.326
		150 %	1.964	7.398
4	Organic phase, 10 % more (35%)	50 %	2.055	6.594
		100 %	2.056	6.612
		150 %	2.080	6.629
5	Organic phase, 10 % less (25 %)	50 %	2.241	8.168
		100 %	2.248	8.204
		150 %	2.259	8.215

Table 7: Assay data of marketed formulation

Drug	Amount labeled	Amount found	% Label claim	% RSD
GTF	3 mg/ml	3.012	100.40	0.38
FLU	0.3 mg/ml	0.299	99.66	0.50

In an acidic environment, hydrolysis of GTF and FLU led to the degradation findings displayed in Figure 5.

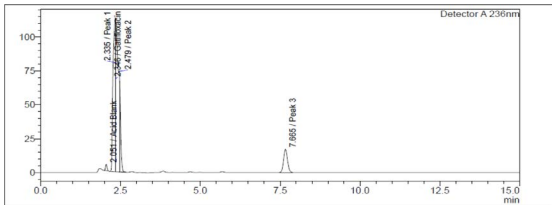

Figure 5: Chromatogram of GAT and FLU in 2M HCL

Both medicines were shown to be destroyed by alkaline hydrolysis, as demonstrated in Figure 6. Gatifloxacin degraded in the presence of hydrogen peroxide (3%), whereas Flurbiprofen remained stable (Figure 7). Poor Stability Under Photolytic Conditions; Neither drugs was significantly degraded by light exposure displayed in Figure 8. Hydrolysis at high temperatures: Gatifloxacin Degrades, Flurbiprofen Stays Stable (Figure 9). Both medicines demonstrated negligible photodegradation when exposed to neutral hydrolysis and is manifested in Figure 10.

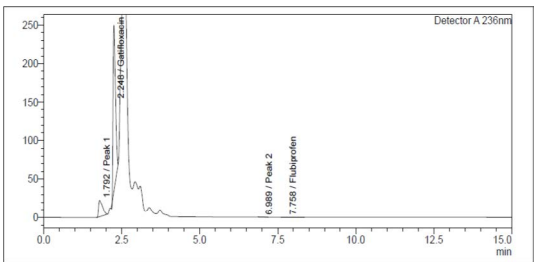

Figure 6: Chromatogram of GTF and FLU in 2M NAOH

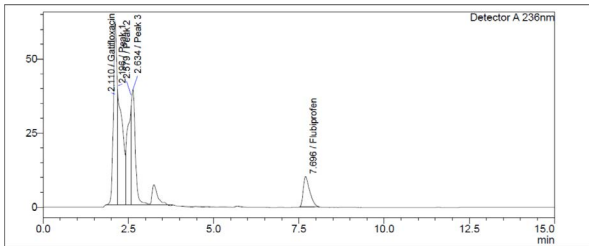

Figure 7: Chromatogram of GTF and FLU in 10% H_20_2

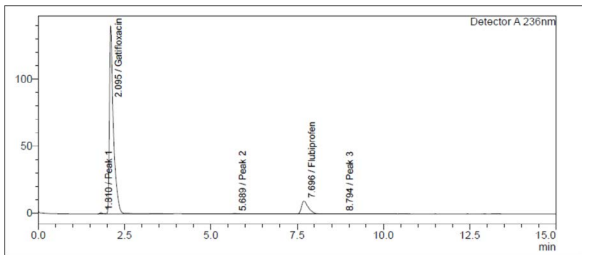

Figure 8: Chromatogram of GTF and FLU in UV photolytic condition

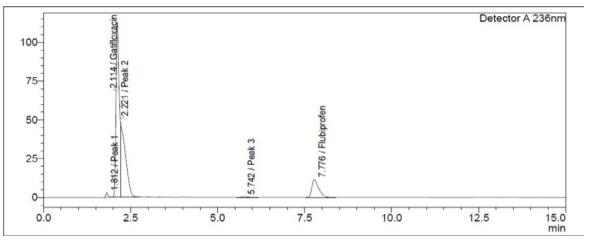

Figure 9: Chromatogram of GTF and FLU in thermal stress condition

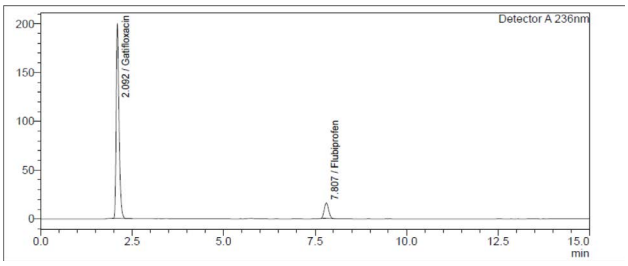

Figure 10: Chromatogram of GTF and FLU in neutral stress condition

Degradation studies reveal the percentage of medication lost to degradation, and the Rt of degradation products are listed here Table 8.

Table 8: Degradation data of stress studies

Drug	Stress Condition (% degradation)					
	Acid	Base	Perox-ide	UV	Ther-mal	Neutral
GTF	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 RP-HPLC method was designed to be quick, sensitive, and easy to use, and it was verified against ICH standards. When compared to other approaches, the proposed ones have a low standard deviation and % RSD, indicating a high degree of precision. It has been shown through extensive recovery study findings that the proposed approaches are highly accurate. In spite of the presence of its degradation products, the RP-HPLC method was able to selectively quantify GTF and FLU, allowing it to be used as a stability indicating technique. Results from the experiments show that the spectrophotometric and stability indicating chromatographic methods devised are reliable, precise, and selective, and may be used for the determination of GTF and FLU in ocular dose form.

Conflict of Interest

The authors have no conflicts of interest regarding this investigation.

Acknowledgment

None

Source of Funding

None

References

1. Data base of gatifloxacin, compilation prepared by drug bank.
2. Data base of Flurbiprofen, compilation prepared by drug bank.
3. G. Patel, P. Chauhan, S. Shah, Simultaneous estimation of gatifloxacin and flurbiprofen sodium in ophthalmic formulation by uv-specrophotometric method, J Chem Pharm Res, 6(2014), 96-101.
4. N. Upadhyay, R.K. Maheshwari, J. Jain, M. Patani, R. Pandey, New spectrophotometric analysis of gatifloxacin tablets utilizing mixed solvency concept, Der Pharmacia Lettre, 4(2012), 1-4.
5. A.A. Shalaby, R.A. Sayed, W.S. Hassan, M.Y. El-mammli, A new extractive spectrophotometric method for the determination of gatifloxacin and cefotaxime sodium in pure and pharmaceutical dosage forms, Orien J Chem, 28(2012), 639-650.
6. P.K. Pradhan, P.N. Rajput, N. Kumar, B. Joshi, U.M. Upadhyay, Simultaneous estimation of flurbiprofen and gatifloxacin by dual wavelength uv spectroscopy method in an eye drops, Int J Pharm Sci RevRes, 27(2014), 96-99.
7. M.D. Jinal, M.P. Vishnu, P. Rajesh, S. Dushyant, Development and validation of spectrofluorometric method for analysis of gatifloxacin and flurbiprofen sodium in ophthalmic dosage form, Inv Rap Pharm Anal Qty Ass, 2(2014), 160-168.
8. A.R. Rote, P.A. Kumbhoje, Development and validation of hptlc method for simultaneous estimation of gatifloxacin and ornidazole in human plasma, J Chrom sep tech, 2(2011), 2157-7064.
9. S.A. Shah, I.S. Rathod, B.N. Suhagia, M. Baldaniya, A simple and sensitive hptlc method for estimation of gatifloxacin in tablet dosage forms, Ind J Pharm Sci, 66(2004), 306-308.
10. A. Giancarlo, C. Marina, O. Marica, M.F. Roberto, L.W. Gary, Metabolic profile of no-flurbiprofen in rat brain and plasma: A lc-ms study, Life Sci, 71(2002), 1487-1500.

11. J. Deglon, A. thomas, Y. daali, E. lauer, C. samer, et al. Automated system for on-line desorption of dried blood spots applied to LC/MS/MS pharmacokinetic study of flurbiprofen and its metabolite, J Pharm Biomed Anal, 54(2011), 359-367.

12. S. Sridhar, R. Kavitha, M. Sudhakar. Reverse phase high performance liquid chromatography method development and validation for the simultaneous estimation of gatifloxacin and flurbiprofen in pharmaceutical dosage form, Asian J Pharm Clin Res, 8(2015), 242-246.

13. R.R. Sandip, M.P. Laxman, A.K. Joshi, K.L. Mahammadali, RP-HPLC method for the simultaneous estimation of gatifloxacin and flurbiprofen sodium in their ophthalmic dosage form, Int J Univ Pharm Bio Sci, 3(2014), 59-70.

14. K.R. Sireesha, K. Prakash, Simultaneous determination of gatifloxacin and dexamethasone sodium phosphate in bulk and pharmaceutical formulations by hplc, Afr J Pharm Pharmac, 5(2011), 1990-1995.

15. N. Sultana, M. Arayne, R. Siddiqui, S. Naveed, RP-HPLC method for the simultaneous determination of lisinopril and nsaids in api, pharmaceutical formulations and human serum, Ame J Ana Chem, 3(2012), 147-152.

16. ICH guidelines Q2 (R1), text on validation of analytical procedures, methodology international conference on harmonization, Geneva.

17. International Conference on Harmonization (ICH) Harmonized Tripartite Guideline on, Topic Q1A, Notes for Guidance on stability testing: Stability testing of new drug substances and new drug products.