

REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF GATIFLOXACIN AND FLURBIPROFEN IN PHARMACEUTICAL DOSAGE FORM

SRIDHAR SIDDIRAJU¹, RAYALA KAVITHA^{2*}, MUVVALA SUDHAKAR²

¹Department of Pharmaceutical Chemistry, Malla Reddy College of Pharmacy, Hyderabad, Telangana, India. ²Department of Pharmaceutical Analysis and Quality Assurance, Malla Reddy College of Pharmacy, Hyderabad, Telangana, India.
Email: kavitha.rayala31@gmail.com

Received: 23 October 2014, Revised and Accepted: 10 November 2014

ABSTRACT

Objective: The aim of this work was focused on to develop and validate an accurate, simple reverse phase high-performance liquid chromatography (HPLC) method for simultaneous estimation of gatifloxacin and flurbiprofen in pharmaceutical dosage form.

Methods: The chromatographic separation was performed on a kromosil C18 column (150 mm × 4.6 mm × 5 μ), with a mobile phase comprising of a mixture of potassium dihydrogen orthophosphate and acetonitrile (48:52) and pH is adjusted to 7.5 by adding 1 ml triethylamine, at a flow rate of 0.8 ml/minutes, with detection at 245 nm.

Results: Retention times of gatifloxacin and flurbiprofen were found to be 2.3 minutes and 5.6 minutes respectively. The developed method was validated according to ICH guidelines. Linearity of gatifloxacin was found to be in the range of 75-450 μg/ml and that for flurbiprofen was found to be in the range of 7.5-45 μg/ml. The percentage recoveries for both drugs were found in the range of 99-101%. The limit of detection and limit of quantification values for gatifloxacin were found to be 0.38 and 1.16 μg/ml and that for flurbiprofen were found to be 0.05 and 0.14 μg/ml, respectively.

Conclusion: The proposed HPLC method is simple, rapid, isocratic, specific, accurate and precise. Hence, this method can be recommended for the estimation of gatifloxacin and flurbiprofen in pharmaceutical dosage form.

Keywords: Gatifloxacin, Flurbiprofen, Reverse phase high performance liquid chromatography method, Validation.

INTRODUCTION

Gatifloxacin is an antibiotic of the fourth-generation fluoroquinolone family. IUPAC name of 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (Fig. 1). Gatifloxacin acts by inhibiting bacterial enzymes DNA gyrase and topoisomerase IV. Gatifloxacin is a broad spectrum antibiotic indicated for the treatment of bacterial conjunctivitis, keratitis as well as for pre- and post-operative infections of the eye and is effective against many Gram-positive and Gram-negative bacteria and it is administered orally in the treatment of chronic bronchitis, acute sinusitis, community acquired pneumonia, gonorrhoea, pyelonephritis, urinary tract infections. Flurbiprofen is a propionic acid derivative, and it is a non-steroidal anti-inflammatory agent with antipyretic and analgesic activity. IUPAC name of flurbiprofen is 2-(3-fluoro-4-phenylphenyl) propanoic acid (Fig. 2). Flurbiprofen acts by inhibiting prostaglandin synthesis by interfering with cyclooxygenase needed for biosynthesis. Flurbiprofen is administered orally in the treatment of arthritis, bursitis, tendinitis, ankylosing spondylitis, soft tissue injuries and dysmenorrhoea. Flurbiprofen may also be used topically prior to ocular surgery to prevent or reduce intraoperative miosis. A single enantiomer of flurbiprofen tarenflurbil (R-flurbiprofen) is currently in clinical trials for the treatment of metastatic prostate cancer [1-5].

Literature reveals that some methods are available for estimation of gatifloxacin by ultraviolet (UV) spectrophotometry [6], high-performance liquid chromatography (HPLC) [1,3,6,7], europium-sensitized fluorescence spectrophotometry [8], spectrofluorimetry [9], potentiometry [10], high-performance thin layer chromatography [11], capillary zone electrophoresis [12,13]. Methods available for the estimation of flurbiprofen are UV

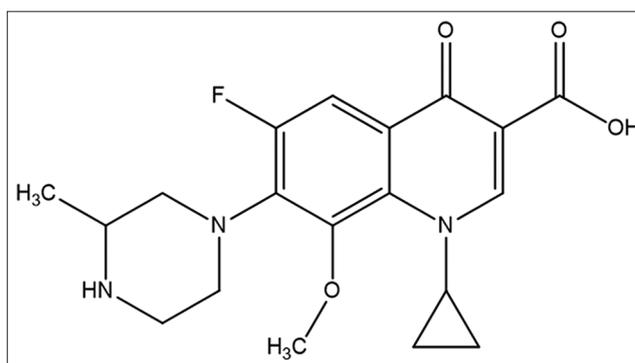


Fig. 1: Structure of gatifloxacin

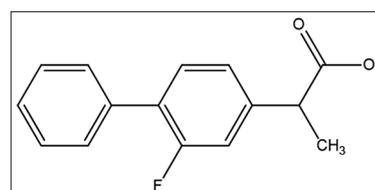


Fig. 2: Structure of flurbiprofen

spectrophotometry [14,15], HPLC [2,3,16,17], high-performance thin layer chromatography [18], spectro fluorimetry [9], liquid chromatography-mass spectrometry (LC-MS) [19-21], capillary zone electrophoresis [22], gas chromatography-MS [23], chiral phase LC [24].

The literature survey was carried out for the simultaneous estimation of gatifloxacin and flurbiprofen, there was only single method was available in combination of both the drugs. Individual methods are also available for both the drugs. Therefore, attempts were made to develop and validate a simple, accurate, precise, selective reverse phase HPLC (RP-HPLC) method for simultaneous determination of both the drugs.

METHODS

Active pharmaceutical ingredients gatifloxacin and flurbiprofen were kindly gifted by spectra Pharma Research Solutions, Hyderabad. Pharmaceutical dosage form was purchased from the local pharmacy. Solvents used are of HPLC grade and obtained from Merck Specialties Private Limited, Mumbai.

Instrumentation

Analysis was performed on an HPLC system consist of waters 2695 with 2996 module-photo diode array detector equipped with a quaternary solvent delivery pump, automatic sample injector and column thermostat. The data acquisition and analysis was performed using Empower software.

Chromatographic conditions

The chromatographic separation was performed using kromosil C18 column (150 mm × 4.6 mm × 5 μ). Flow rate was kept at 0.8 ml/minutes. The column temperature was maintained at 30°C. The mobile phase was made up of potassium dihydrogen orthophosphate buffer and acetonitrile in 48:52 ratio. The method was optimized at 245 nm. Run time was taken as 10 minutes, and all the determinations are carried out at an ambient temperature.

Standard solution preparation

Accurately weighed and transferred 30 mg and 3 mg of gatifloxacin and flurbiprofen into 10 ml volumetric flasks separately. Add 3/4th amount of diluent and sonicated for 30 minutes and make up the final volume with water and acetonitrile in 50:50 ratio, then the solutions consist of 3000 μg/ml and 300 μg/ml of gatifloxacin and flurbiprofen, respectively. From the above stock, solutions take 1 ml into 10 ml volumetric flask and makeup the final volume with diluent. The final standard solution consists of 300 μg/ml of gatifloxacin and 30 μg/ml of flurbiprofen.

Sample preparation

The commercial eye drops consist of 0.3% of gatifloxacin and 0.03% of flurbiprofen in 5 ml bottle. 20 bottles were taken and transferred into a beaker. From the above solution pipette out 5 ml of sample and transferred into clean and dry 10 ml volumetric flask and the final volume was made up to 10 ml with water and acetonitrile in 50:50 ratio. The final sample solution consists of 1500 μg/ml of gatifloxacin and 150 μg/ml of flurbiprofen, respectively. From the above stock solution, pipette out 2 ml into 10 ml volumetric flask and the final volume was made up to 10 ml with diluent.

Buffer (0.02 N KH₂PO₄) preparation

Accurately weighed 2.72 g of potassium dihydrogen orthophosphate is taken in a 1000 ml of volumetric flask, add about 900 ml of milli-Q water and degas to sonicate and finally make up the volume with water.

Preparation of linearity solutions

Pipette out 0.25, 0.5, 0.75, 1, 1.25, 1.5 ml from stock solution into clean and dry 10 ml volumetric flasks and made the volume up to 10 ml using acetonitrile and water (50:50) as diluent to prepare concentration range of 75-450 μg/ml of gatifloxacin and 7.5-45 μg/ml of flurbiprofen.

Method validation

The developed method was validated according to ICH guidelines. Validation parameters included are linearity, accuracy, limit of detection (LOD) and limit of quantification (LOQ), precision, and robustness.

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of gatifloxacin and flurbiprofen and the solutions were injected 5 times and the parameters like peak tailing, resolution and US Pharmacopeia (USP) plate count were determined.

Linearity

The linearity of the method was determined by preparing six different concentrations of both gatifloxacin and flurbiprofen in the concentration range of 75-450 μg/ml and 7.5-45 μg/ml. Each solution was prepared in triplicate. The calibration curves were obtained by plotting peak area versus concentration.

Precision

Method precision (repeatability)

The method precision/repeatability was determined by injecting six working standard solutions and six sample injections. The areas of all the injections were taken and standard deviation, % relative standard deviation (RSD), % assay were calculated.

Intermediate precision

The intermediate precision was determined by injecting six working standard solutions and six sample injections on different days by different operators or by different instruments. The areas of all the injections were taken and standard deviation, % RSD, % assay were calculated. The results obtained were within the acceptance criteria.

Accuracy

The accuracy of the method was determined at three different concentration levels 50%, 100% and 150% by spiking known quantities of the drug analytes and % recoveries were calculated.

LOD and LOQ

LOD and LOQ of gatifloxacin and flurbiprofen were determined by calibration curve method. Prepare the solutions of gatifloxacin and flurbiprofen in the linearity range and inject the solutions in triplicate. Average peak areas were calculated and plot a graph between average peak areas versus concentration. The LOD and LOQ are calculated as: LOD=3.3 σ/S and LOQ=10 σ/S; where σ is the standard deviation of the lowest standard concentration and S is the slope of the standard curve.

Robustness

The robustness can be determined by varying the following parameters:

1. Flow rate: It was determined by varying the flow rate from 0.8 ml/minutes to 1 ml/minutes. The standard solution of gatifloxacin and flurbiprofen were prepared and was injected by varying the flow rate from 0.8 ml/minutes to 1 ml/minutes along with the optimized method
2. Column temperature: A study was conducted to determine the effect of variation in column temperature. Standard solution of gatifloxacin and flurbiprofen was prepared and was injected into the HPLC system by keeping the column temperature at 25°C and 35°C
3. Mobile phase: It was determined by changing the organic mobile phase composition by ±10%. The standard solution of gatifloxacin and flurbiprofen was prepared and was injected by changing organic mobile phase composition by ±10% along with the optimized method.

RESULTS AND DISCUSSIONS

The aim of this work was focused on to develop and validate an accurate, simple, precise RP-HPLC method for simultaneous estimation of gatifloxacin and flurbiprofen in pharmaceutical dosage form. Solubility of active pharmaceutical ingredients was checked in different solvents like methanol, acetonitrile and water in different ratios. Finally, the standards were soluble in water and acetonitrile in 50:50 ratio. So water and acetonitrile in 50:50 ratio was used as diluent. The different

mobile phases such as acetonitrile and sodium dihydrogen phosphate and acetonitrile and potassium dihydrogen orthophosphate were used in different compositions with different flow rates. Hence, peak resolution, tailing factor, retention time were not satisfactory. Hence, finally acetonitrile and potassium dihydrogen orthophosphate was selected as a buffer in the composition ratio of 52:48 with a flow rate of 0.8 ml/minutes. The chromatographic separation was done by using different columns like Altima, XDB (150 mm × 4.6 mm × 5 μ) and base deactivated silica (250 mm × 4.6 mm × 5 μ). But resolution, retention time, and tailing were not within the specified limits. So, finally the actual chromatographic separation was done by using kromosil column (150 mm × 4.6 mm × 5 μ) with a flow rate of 0.8 ml/minutes with a run time of 10min and with the optimized wavelength of 245 nm (Fig. 3).

System suitability parameters

The system suitability tests were conducted before performing the validation and the parameters were within the acceptance criteria like retention times were 2.3 and 5.6 minutes for gatifloxacin and flurbiprofen, plate count was >2000, peak tailing was <2 and the % RSD of peak areas of six injections were ≤2% (Table 1). Hence, the proposed method was successfully applied to routine analysis without any problems.

Linearity range

The linearity range was in the interval of 75-450 μg/ml gatifloxacin and 7.5-45 μg/ml of flurbiprofen, respectively. These were represented by a linear regression equation as follows:

y (gatifloxacin) = 10431x + 2002 ($r^2 = 0.999$) (Fig. 4),
 y (flurbiprofen) = 65063x + 7555 ($r^2 = 0.999$) (Fig. 5) and regression line was established by least squares method. Hence, the curves established were linear (Table 2).

Precision

Six replicates injections in the same concentration were analyzed on the same day and 2 different days for verifying the variation in the precision and the % RSD for gatifloxacin and flurbiprofen were within an acceptable limit of ≤2. Hence, the method is reproducible on different days with different analyst and column. This indicates that the method is precise (Table 3).

Accuracy

The percentage recoveries for gatifloxacin and flurbiprofen were found to be 99.93% and 100.09%, respectively. The results of the recovery studies undoubtedly demonstrate accuracy of the proposed method (Table 4).

LOD and LOQ

The determined values of LOD and LOQ were calculated by using slope and Y-intercept. The LOD and LOQ values for gatifloxacin were found

Table 2: Determination of linearity

S. No.	Gatifloxacin		Flurbiprofen	
	Concentration	Peak area	Concentration	Peak area
1	75	746516	7.5	506287
2	150	1608339	15	1003491
3	225	2383069	22.5	1471913
4	300	3106397	30	1919532
5	375	3895122	37.5	2437405
6	450	4703181	450	2961625
Slope	10431		65063	
Y-intercept	2002		7555	
Correlation coefficient	0.999		0.999	

Table 1: System suitability of gatifloxacin and flurbiprofen

S. No	Gatifloxacin				Flurbiprofen			
	Rt	Area	USP plate count	USP tailing	Rt	Area	USP plate count	USP tailing
1	2.297	2942639	3958	1.32	5.658	1928452	7907	1.19
2	2.299	2904792	3888	1.28	5.663	1910940	8053	1.18
3	2.300	2895786	3848	1.29	5.664	1920293	8027	1.17
4	2.304	2934212	4036	1.29	5.667	1926049	7971	1.18
5	2.312	2985983	4151	1.35	5.676	1922498	7989	1.17
Mean		2932682				1921646		
SD		35642.9				6761.6		
% RSD		1.2				0.4		

SD: Standard deviation, RSD: Relative standard deviation, Rt: Retention time, USP: US Pharmacopeia

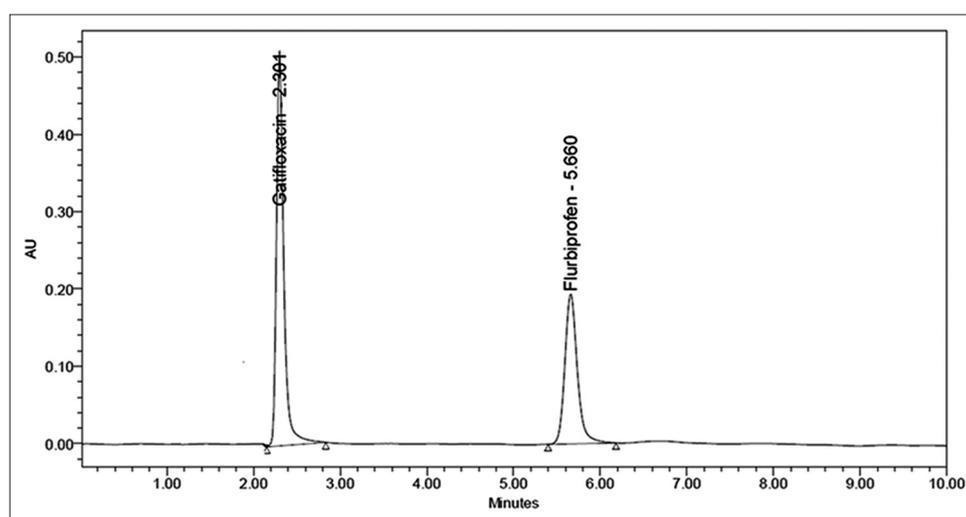


Fig. 3: High performance liquid chromatography of standard solution (i.e. gatifloxacin and flurbiprofen)

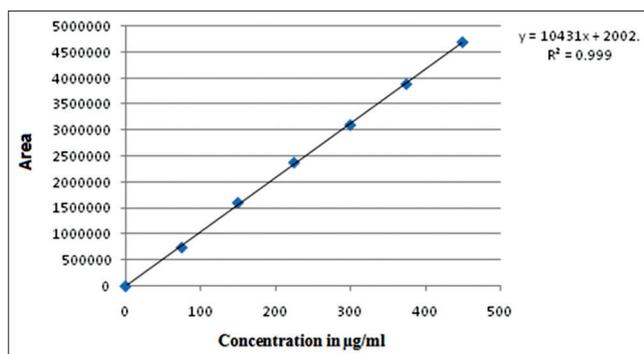


Fig. 4: Linearity graph of gatifloxacin

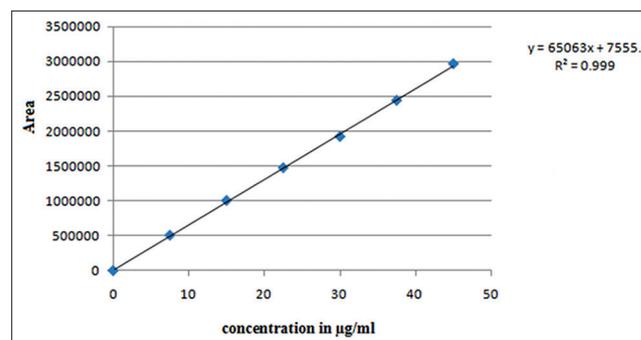


Fig. 5: Linearity graph of flurbiprofen

Table 3: Determination of repeatability and intermediate precision for gatifloxacin and flurbiprofen

Drug	Repeatability			Intermediate precision		
	Peak area	SD	% RSD	Peak area	SD	% RSD
Gatifloxacin	2943526	20135.1	0.68	2924704	8675.8	0.3
Flurbiprofen	1927824	10296.3	0.53	1918754	11616.3	0.6

RSD: Relative standard deviation, SD: Standard deviation

Table 4: Determination of accuracy of gatifloxacin and flurbiprofen

Gatifloxacin				Flurbiprofen			
Standard ppm	Added ppm	Found ppm	% Recovery	Standard ppm	Added ppm	Found ppm	% Recovery
300	150	150.48	100.33	30	15	14.96	99.73
300	150	148.72	99.15	30	15	15.07	100.49
300	150	150.34	100.23	30	15	15.04	100.29
300	300	300.47	100.16	30	30	29.87	99.57
300	300	298.05	99.35	30	30	30.20	100.70
300	300	299.00	99.67	30	30	30.10	100.35
300	450	448.82	99.74	30	45	45.19	100.42
300	450	450.77	100.17	30	45	44.69	99.33
300	450	452.64	100.59	30	45	44.98	99.96
Mean			99.93				100.09
SD			0.48				0.47
RSD			0.48				0.47

SD: Standard deviation, RSD: Relative standard deviation

Table 5: LOD and LOQ for gatifloxacin and flurbiprofen

S.No	Gatifloxacin		Flurbiprofen	
	Slope	Y-intercept	Slope	Y-intercept
1	10458	1860	65155	6792
2	10401	3272	64884	8608
3	10433	873.7	65148	7265
Mean	10431	2002	65062.33	7555
SD		1205.4		942.1
LOD		0.38		0.05
LOQ		1.16		0.14

SD: Standard deviation, LOD: Limit of detection, LOQ: Limit of quantification

to be 0.38 and 1.16 µg/ml and flurbiprofen were found to be 0.05 and 0.14 µg/ml, respectively (Table 5).

Robustness

Robustness of the proposed method demonstrated a non-significant alteration through analysis of the sample and standard gatifloxacin and flurbiprofen solution. After this, the results obtained were compared with that of optimized method. It was confirmed that by the deliberate changes in the parameters there was no any significant changes in standard deviation, RSD, theoretical plates, retention time and USP tailing factor (Table 6).

Assay

The content of gatifloxacin and flurbiprofen in the pharmaceutical dosage form was found by using the developed method. The percentage purity of gatifloxacin and flurbiprofen were found to be 99.97% and 99.92% and % RSD values for both gatifloxacin and flurbiprofen were within the limit of ≤ 2 (Table 7).

CONCLUSION

The proposed HPLC method was simple, rapid, isocratic, specific, accurate and precise than existing method. The newly developed method differs from already existing method in the aspects of parameters like mobile phase composition, column, retention times of the drugs. In newly developed method the column used was kromasil C18 column and mobile phase was buffer (potassium dihydrogen orthophosphate pH 7.5) and acetonitrile in 48:52 (v/v) ratio, retention times of gatifloxacin and flurbiprofen were 2.3 and 5.6 minutes, respectively. Method validation was performed by testing its linearity, accuracy, precision, LOD, LOQ, robustness. Hence, this method can be recommended for the estimation of gatifloxacin and flurbiprofen in pharmaceutical dosage form.

ACKNOWLEDGMENTS

The authors were thankful for Spectra Pharma Research Solutions,

Table 6: Robustness data

Parameter	Gatifloxacin			Flurbiprofen		
	Mean area	SD	% RSD	Mean area	SD	% RSD
Flow minus (0.8 ml/minutes)	2862982	3797.9	0.1	1905337	17188	0.9
Flow plus (1 ml/minutes)	2621903	27357.8	1	1717843	7584.4	0.4
Temperature minus (25°C)	2830328	28693.2	1.1	1965513	1332.3	0.1
Temperature plus (35°C)	2974557	10584.2	0.4	1969590	4061.6	0.2
Organic solvent ratio (-10%)	2912727	8355	0.3	1909563	4441.5	0.1
Organic solvent ratio (+10%)	2892868	4551.4	0.2	1908925	15979.2	0.8

SD: Standard deviation, RSD: Relative standard deviation

Table 7: Assay of gatifloxacin and flurbiprofen

Brand name	Drugs	Label claim (%)	% Assay	Amount recovered (%)	Amount found
Flurgat	Gatifloxacin	0.3	99.97	0.299	0.29±0.68
	Flurbiprofen	0.03	99.92	0.029	0.029±0.53

Hyderabad for providing gatifloxacin and flurbiprofen reference standards as a gift sample to carry out the research work.

REFERENCES

- Prathap B, Rajendran SS, Dinakar A, Srinivasa G, Ashok Kumar J, Roosewelt C. Simultaneous determination of gatifloxacin and ambroxol hydrochloride from tablet dosage form using RP-HPLC. *Int J Res Pharm Sci* 2010;1(3):325-7.
- Najma S, Saeed Arayne M, Rubina S, Safila N. RP-HPLC Method for the simultaneous determination of lisinopril and NSAIDS in API, pharmaceutical formulations and human serum. *Am J Anal Chem* 2012;3:147-52.
- Khan IU, Naeem SR, Irfana M, Muhammad A, Syed SR. Stability-indicating RP-HPLC method for simultaneous determination of gatifloxacin and flurbiprofen in binary combination. *Quim Nova* 2012;35(4):651-61.
- Razaq SN, Mariam I, Khan IU, Ashfaq M. Development and validation of liquid chromatographic method for gatifloxacin and ketorolac tromethamine in combined dosage form. *J Liq Chromatogr* 2012;35(4):651-61.
- Prathap B, Nagarajan G, Dinakar A, Srinivasa G, Ranjit Singh BR, Shahul H. Spectrophotometric method for simultaneous estimation of gatifloxacin and ambroxol hydrochloride in tablet dosage form. *Der Pharm Lett* 2011;3(3):62-8.
- Srinivas N, Narasu L, Shankar BP, Mullangi R. Development and validation of a HPLC method for simultaneous quantitation of gatifloxacin, sparfloxacin and moxifloxacin using levofloxacin as internal standard in human plasma: Application to a clinical pharmacokinetic study. *Biomed Chromatogr* 2008;22(11):1288-95.
- Mahmoud MS, El-Shanawany AA, El-Adl SM, Abdel-Aziz LM, Hisham AH. Rapid RP-HPLC method for simultaneous estimation of sparfloxacin, gatifloxacin, metronidazole and tinidazole. *Asian J Pharm Res* 2011;1(4):119-25.
- Guo C, Dong P, Chu Z, Wang L, Jiang W. Rapid determination of gatifloxacin in biological samples and pharmaceutical products using europium-sensitized fluorescence spectrophotometry. *Luminescence* 2008;23(1):7-13.
- 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 Anal Qual Assur* 2014;ppaqa/1421/14.
- Hasna M, Amir AS, Bassam N. Potentiometric determination of gatifloxacin, ciprofloxacin in pharmaceutical formulations. *Int J Pharm Pharm Sci* 2012;4(4):537-42.
- Suhagia BN, Shah SA, Rathod IS, Patel HM, Shah DR, Marolia BP, et al. Determination of gatifloxacin and ornidazole in tablet dosage forms by high-performance thin-layer chromatography. *Anal Sci* 2006;22(5):743-5.
- Faria AF, de Souza MV, de Oliveira MA. Validation of a capillary zone electrophoresis method for the determination of ciprofloxacin, gatifloxacin, moxifloxacin and ofloxacin in pharmaceutical formulations. *J Braz Chem Soc* 2008;19(3):389-96.
- Sun H, Wei L, Zuo Y, Wu Y. Effective separation and simultaneous detection of gatifloxacin, amino methyl benzoic acid, cefazolin and cefminox in human urine by capillary zone electrophoresis. *J Iran Chem Soc* 2011;8(4):1043-51.
- Sajeev C, Pravin RJ, Ravi Shankar D, Saha RN. Determination of flurbiprofen in pharmaceutical formulations by UV spectrophotometry and liquid chromatography. *Anal Chim Acta* 2002;463:207-17.
- Emrah A, Bilal Y. Determination of flurbiprofen in pharmaceutical preparations by first-order derivative spectrophotometry method. *Indian J Pharmacol* 2014;1(2):130-7.
- Hirai T, Matsumoto S, Kishi I. Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high-performance liquid chromatography with normal solid-phase extraction. *J Chromatogr B Biomed Sci Appl* 1997;692(2):375-88.
- Park KM, Gao ZG, Kim CK. Assay of flurbiprofen in rat plasma using HPLC with fluorescence detection. *J Liq Chromatogr* 1997;20(12):1849-55.
- Dhavs VV, Parmar DV, Devarajan PV. High-performance thin-layer chromatographic determination of flurbiprofen in plasma. *J Chromatogr B Biomed Sci Appl* 1997;694(2):449-53.
- Vinci F, Fabbrocino S, Fiori M, Serpe L, Gallo P. Determination of fourteen non-steroidal anti-inflammatory drugs in animal serum and plasma by liquid chromatography/mass spectrometry. *Rapid Commun Mass Spectrom* 2006;20(22):3412-20.
- 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 Sci* 2002;71(13):1487-500.
- Déglon J, Thomas A, Daali Y, Lauer E, Samer C, Desmeules J, 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* 2011;54:359-67.
- Hamoudová R, Pospíšilová M. Determination of ibuprofen and flurbiprofen in pharmaceuticals by capillary zone electrophoresis. *J Pharm Biomed Anal* 2006;41:1463-7.
- Chao SH, Ho HT, Chen FA, Lin PY, Yu YC, Wu AB, et al. Identification of flurbiprofen and its photoproducts in methanol by gas chromatography-mass spectrometry. *Biomed Chromatogr* 2007;21(5):527-33.
- Patel BK, Valentova J, Hutt AJ. Stereospecific analysis of flurbiprofen and its major metabolites in plasma and urine by chiral-phase liquid chromatography. *Chromatographia* 2003;57(1):7-18.