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A Green Approach For The Simultaneous Estimation Of Gatifloxacin And Flurbiprofen by a Very Sensitive Spectrofluorimetric Method

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Abstract: *Gatifloxacin (GAT), an antibiotic and Flurbiprofen (FLU), a Non-steroidal anti-inflammatory drug are co-formulated as an ophthalmic solution for the reduction of post operative ocular inflammation & ocular infections. Quality control of the ophthalmic solution of GAT and FLU is very essential and hence this work aims at the development of an economic and ecofriendly method for simultaneous estimation of GAT and FLU in an ophthalmic solution. Spectrofluorimetric method is selected due to the known fact of its inherent sensitivity. 10mM SLS was used as the solvent and micellar solubilisation resulted in an enhanced fluorescence for GAT and FLU. The excitation wavelengths selected for GAT and FLU were 294 nm and 259 nm respectively. The emission wavelengths for GAT and FLU were 470 nm and 315 nm respectively. A linear relationship was found in the concentration range of 0.005-2.5 µg/mL and 0.005-0.35 µg/mL for GAT and FLU, respectively. The percentage recovery was 98.2-100.1% for GAT and 98.6-102% for FLU.*

Keywords: *Flurbiprofen, Gatifloxacin, Sodium lauryl sulphate, Validation*

I. Introduction

Gatifloxacin (GAT) is chemically 1-Cyclopropyl-6-fluoro- 8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-quinoline-3-carboxylic acid (chemical structure in fig 1), an antibiotic of the fourth generation fluoroquinolone group, which inhibits the bacterial enzymes, DNA gyrase and topoisomerase IV. It is used in the treatment of bacterial infections and is official in Indian Pharmacopoeia (IP) [1] and British Pharmacopoeia (BP) [2]. Chemically, Flurbiprofen (FLU) is Sodium (±)-2-(2-fluoro-4-biphenylyl) propionate dehydrate (chemical structure in fig 2), a non-steroidal anti-inflammatory drug (NSAIDs) with antipyretic and analgesic activity. FLU is a non-selective COX inhibitor and inhibits the activity of both COX-1 and 2. It is used in the treatment of arthritis, bursitis, tendinitis, ankylosing spondylitis, soft tissue injuries and dysmenorrhea. It is official in IP [3], BP [4], USP [5]. GAT and FLU are co-formulated as anophthalmic solution for the reduction of post operative ocular inflammation & ocular infections.

Literature survey revealed analytical methods for GAT by ultraviolet spectrophotometry[6-8], spectrofluorimetry [9], high performance liquid chromatography [10-15], high-performance thin layer chromatography [16,17] and for FLU by UV spectrophotometry and HPLC [18]. For the simultaneous estimation of GAT and FLU, very less analytical methods such as UV spectrophotometry[19-21] and RP-HPLC [22,23] methods were available.

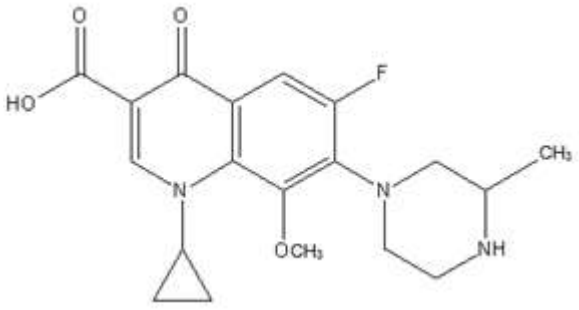

Fig 1: Structure of GAT

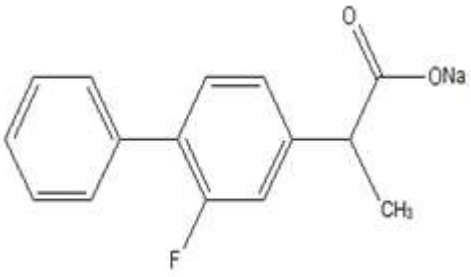

Fig 2: Structure of FLU

However, there is no spectrofluorimetric method for the simultaneous estimation of GAT and FLU in their combined dosage form. Spectrofluorimetric method is a preferred choice due to its inherent sensitivity, economical and less time consumption, on comparing with HPLC or other hyphenated techniques. For GAT and

FLU, there is wide variation in the dose of the two drugs (0.3% of GAT and 0.03% of FLU in 5 mL bottle) and hence, a sensitive method is mandatory. It is more economical, as an organic solvent free method is adopted by selecting 10mM SLS as the solvent. It also has the advantage of using an ecofriendly solvent and hence the method is titled as the green approach.

The developed method was validated for different parameters like linearity, accuracy, precision, robustness, ruggedness, limit of detection (LOD) and limit of quantification (LOQ) as per International conference of Harmonization Guidelines.[24]

II. Experimental

2.1 Instrument

RF-5301 PC Spectrofluorophotometer, Shimadzu, Japan, equipped with 150W Xenon arc lamp with a 1 cm non-florescence quartz cell was used for sample analysis. RFPC software was used for data assimilation.

2.2 Materials

Cirex Pharmaceutical Ltd. supplied the gratis samples of Standard GAT and FLU. Sodium lauryl sulphate (SLS) was purchased from SD Fine Chemicals Ltd, Mumbai, India.

2.3 Preparation of standard stock solutions and calibration curve

Standard stock solutions of pure drug containing 1000 µg/mL of GAT and FLU were prepared separately in SLS. It was further diluted with SLS to get working standard solutions of analytes in the concentration range of 0.005-2.5 µg/mL and 0.005-0.35 µg/mL for GAT and FLU, respectively. Fluorescence intensity was measured at λ_{EX} of 294 nm and λ_{EM} of 470 nm for GAT and at λ_{EX} of 259 nm and λ_{EM} of 315 nm for FLU.

2.4 Preparation of sample solution and formulation analysis

The commercial eye drops consist of 0.3% of GAT and 0.03% of FLU in 5 mL bottle. Twenty bottles were taken and the contents were quantitatively transferred into a beaker. Pipetted out 5 mL of sample to a 10 mL volumetric flask and the final volume was made up with 10mM SDS. (I.e.1500 µg/mL of GAT and 150 µg/mL of FLU). From the above stock solution, pipetted out 2 mL into a 10 mL volumetric flask and the final volume was made up to 10 mL with the same diluent. The fluorescence intensity at the appropriate wavelength (λ_{EX} 294 nm, λ_{EM} 470 nm for GAT and λ_{EX} 259 nm, λ_{EM} 315 nm for FLU) were noted. The emission intensities were substituted into the corresponding equation of the straight line representing the calibration curves of GAT and FLU respectively.

2.5 Method Validation

The method was validated according to the international conference on harmonization (ICH) Q2B guidelines for the following validation parameters such as linearity, accuracy, precision, specificity, limit of detection(LOD), limit of quantification (LOQ) and robustness.

2.5.1 Linearity and range

Linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of drug in the sample. Standard calibration curves were prepared with six calibrators over a concentration range of 0.005 µg/mL to 2.5 µg/mL for GAT and 0.005 µg/mL to 0.35 µg/mL for FLU. The data of emission intensity versus concentrations were plotted to get the corresponding regression equations of each drug.

2.5.2 Accuracy

The method of standard additions was used for the recovery studies of GAT and FLU. Ophthalmic solution equivalent to 10 mg of GAT was spiked separately with 80%, 100% and 120% of GAT standard solution. Similar procedure was adopted for FLU as well. The fluorescence intensity was noted at their appropriate wavelengths (λ_{EX} 294 nm and λ_{EM} 470 nm for GAT and λ_{EX} 259 nm and λ_{EM} 315 nm for FLU). The accuracy determination was verified in triplicate preparations at each specified concentration level.

2.5.3 Precision

The intra-day precision of the proposed spectrofluorimetric method was performed by estimating the corresponding response three times on the same day for three different concentrations of GAT (5, 500, 1000 ng/mL) and FLU (5, 50, 100 ng/mL). Similarly, the inter-day precision of the proposed spectrofluorimetric method was performed using the same concentrations of GAT and FLU and the corresponding responses were

recorded three times on 3 different days. The results of both intra-day and inter-day precision were reported in terms of relative standard deviation (% RSD).

2.5.4 LOD and LOQ

The limit of detection (LOD) and the limit of quantification (LOQ) of GAT and FLU were determined by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations as per International Conference on Harmonization (ICH) guidelines.

$$\mathbf{LOD = 3.3\ \sigma/S} \qquad \mathbf{LOQ = 10\ \sigma/S}$$

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

III. Results and Discussion

3.1 Optimization of solvent

Various solvents like water, urea and SDS were used. Fluorescence intensity was highest in SDS and second highest in urea, on comparing with other solvents. There was a tremendous increase in the fluorescence intensity of GAT when SLS was used as the solvent and is attributed to the micellar enhanced solubilisation. For FLU also, there was slightly higher fluorescence intensity compared to other solvents. Hence, for preparation of the stock solution as well as for the further dilutions, 10mM SLS.was used. A comparative study of the solvent influence on the fluorescence intensity of GAT and FLU is given in Fig 3.

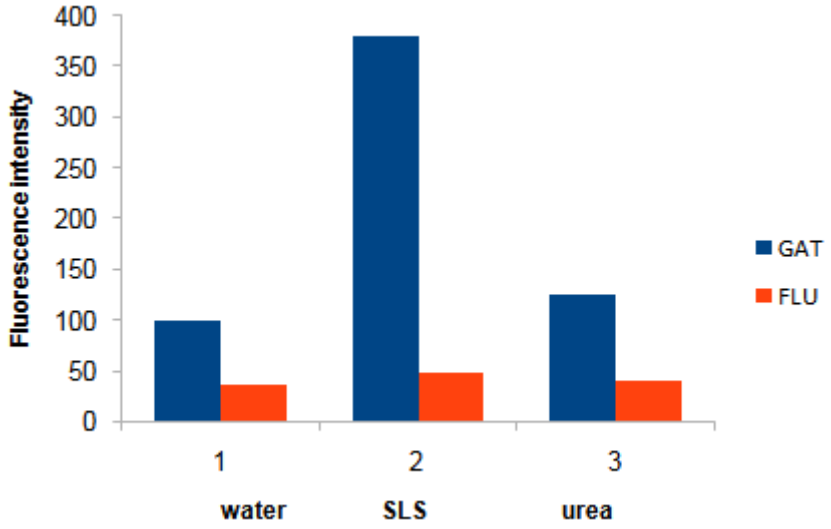

Fig 3: Effect of solvents on the fluorescence intensities of GAT and FLU

3.2 Method validation

3.2.1 Linearity

GAT and FLU were showing a linear relationship between concentration (µg/mL) and fluorescence intensity. GAT and FLU were linear in the concentration range of 0.005-2.5 µg/mL and 0.005-0.35 µg/mL respectively. The overlaid emission spectra shown in Fig 4, indicated the progressive increase in fluorescence intensity of both the drugs at their respective emission wavelengths with respect to concentration. From the linear regression analysis, correlation coefficient value (R^2) for GAT and FLU was 0.9998 and 0.999 respectively, which indicated the fit of the graph between the X and Y coordinates. The fluorescence intensity was measured at an emission wavelength of 470 nm for GAT and for FLU, it was 315 nm. The linearity data for GAT and FLU is shown in the Table 1 and 2. The calibration curve of GAT and FLU with regression equation and R^2 value, is shown in Fig 5 and 6, respectively.

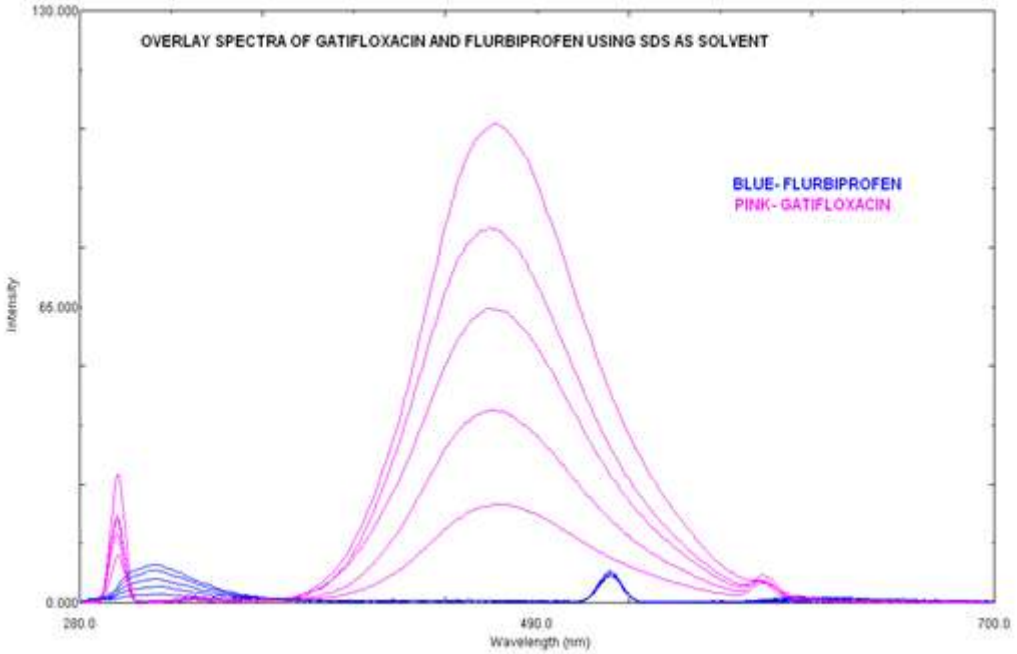

Fig 4: Overlaid emission spectra of GAT and FLU

Table 1: Linearity data of GAT

S.No	Concentration (µg/mL)	Fluorescence intensity (AM±SD) (n=6)
1	0.005	1.301 ± 0.01126
2	0.01	1.702 ± 0.0195
3	0.05	3.537 ± 0.150
4	0.5	21.358 ± 0.46
5	1	42.116 ± 0.49
6	1.5	63.935± 0.585
7	2	83.393±0.38
8	2.5	106.048±0.52

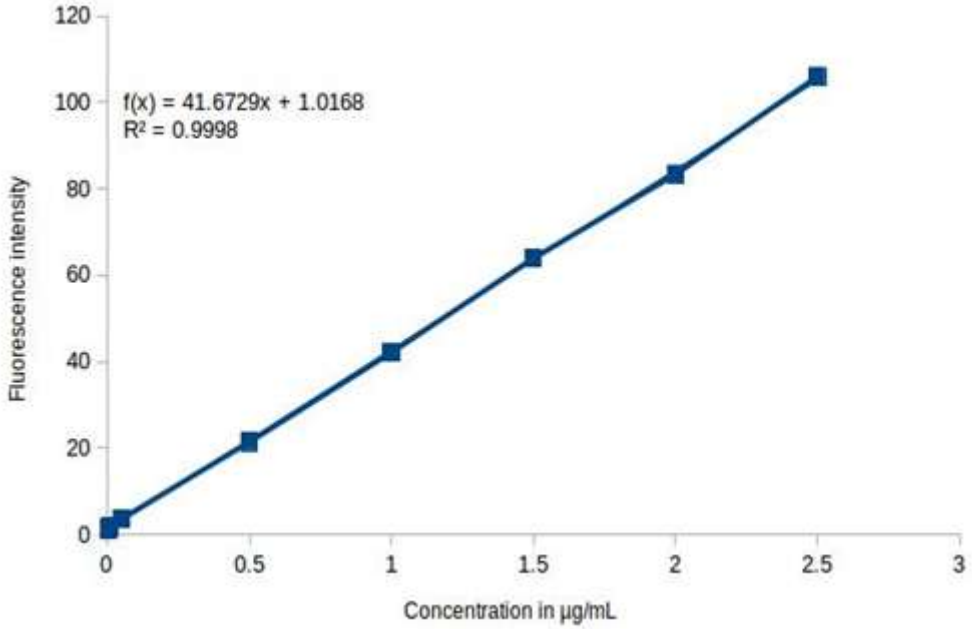

Fig 5: Standard plot of GAT at an emission wavelength of 470 nm

Table 2: Linearity data of FLU

S.No	Concentration (µg/mL)	Fluorescence intensity (AM±SD) (n=6)
1	0.005	0.608±0.024
2	0.05	1.998±0.130
3	0.1	3.354±0.171
4	0.15	5.448±0.10
5	0.2	7.204±0.126
6	0.25	8.864±0.209
7	0.3	10.596±0.024
8	0.35	12.514±0.0146

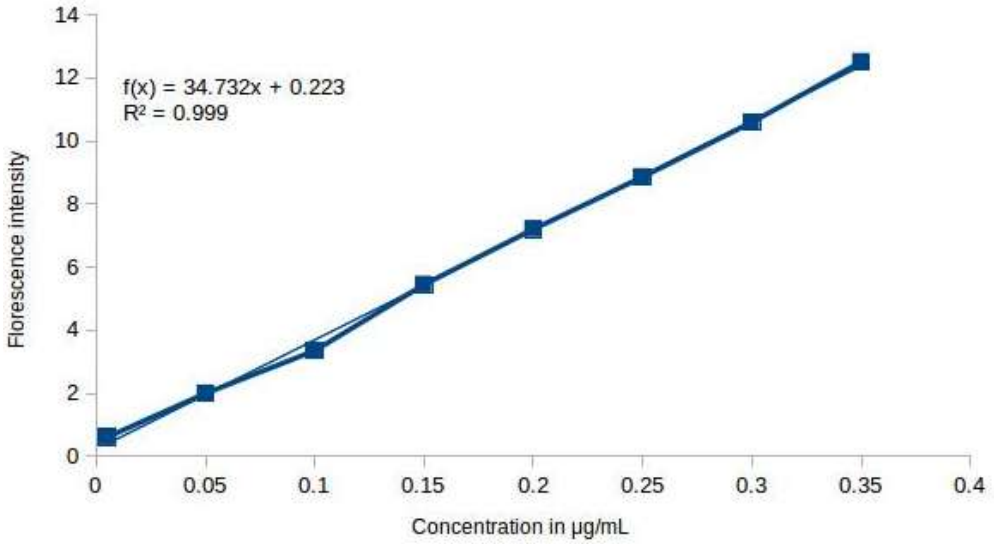

Fig 6: Standard plot of FLU at an emission wavelength of 315nm

3.2.2 Recovery study

Standard addition method was used for recovery studies. Three different levels (80%, 100% and 120%) of standards were spiked to commercial tablets in triplicate. The mean of percentage recoveries and % RSD values were calculated and reported in Table 3. The % recovery of GAT and FLU was found to be in the range 98.2-100.1% and 98.6-102%, which is satisfactory.

Table 3: Accuracy data of GAT and FLU (recovery studies)

Brand	Spiking level (%)	Drug	Theoretical content (mg)	Amount found (mg)±SD	% Recovery	% RSD
Flubigat	80	GAT	5.4	5.406 ± 0.007	100.1	0.137
		FLU	0.54	0.537 ± 0.003	99.4	0.559
	100	GAT	6	5.894 ± 0.004	98.2	0.068
		FLU	0.6	0.612 ± 0.004	102	0.637
	120	GAT	6.6	6.594 ± 0.004	99.9	0.061
		FLU	0.66	0.651 ± 0.003	98.6	0.399

Acceptance Criteria: % RSD should not be more than 2

3.2.3 Precision

The repeatability (intra-day precision) of the method was determined by intra-day (n = 3) analysis of three standard solutions of GAT and FLU at the concentration of 5, 500 & 1000ng/mL and 5, 50 & 100ng/mL respectively. The % RSD of repeatability was <2.0 for both the drugs. Intermediate precision was determined by the inter-day (n = 3) analysis of three standard solutions of GAT and FLU at the concentration of 5, 500 & 1000 ng/mL and 5, 50 & 100 ng/mL respectively and reported in Table 4. The % RSD for inter-day analysis was <2.0 for both the drugs. These statistical data were indicative of good precision.

Table 4: Precision data of GAT and FLU

Drug	Theoretical amount (ng/mL)	Intra-day		Inter-day	
		Amount found ±SD(ng/mL)	% RSD	Amount found ±SD(ng/mL)	% RSD

	5	4.8 ± 0.012	0.25	4.2 ± 0.02	0.476
	500	523 ± 0.03	0.006	487.2 ± 0.042	0.008
GAT	1000	980 ± 0.042	0.004	1040 ± 0.034	0.003
	5	4.9 ± 0.062	1.265	5.1 ± 0.08	1.568
	50	52 ± 0.032	0.061	51.4 ± 0.002	0.005
FLU	100	108 ± 0.0248	0.023	970 ± 0.01	0.001

Acceptance Criteria: % RSD should not be more than 2

3.2.4 Analysis of commercial tablets (assay)

The assay of commercially available ophthalmic formulation (FLUBIGAT) containing 0.3% of GAT and 0.03% of FLU in 5 mL bottle, was performed by the optimized method. The results obtained for GAT and FLU were compared with the corresponding labeled amounts and reported in Table 5.The amount of GAT and FLU were 0.298 % and 0.029 % respectively. These amounts were within the limits. The % RSD for the assay result was less than 2, which indicated the accuracy of the proposed method.

Table 5: Analysis of commercial ophthalmic formulation (assay of GAT and FLU)

Formulation with label claim		**Amount found in % (AM)± SD, % RSD**	
Flubigat		GAT	FLU
GAT= 0.3%	FLU= 0.03%	0.298 ± 0.02, 0.0067	0.029 ± 0.013, 0.044

3.2.5 Specificity

The formulation was assayed in presence of the excipients by the proposed methods. There is no interference of the excipients, which justified the specificity of the method for the drugs.

3.2.6 LOD and LOQ

LOD and LOQ of GAT was 1.5 ng/mL and 4.79 ng/mL respectively. For FLU, LOD and LOQ were 1.23 ng/mL and 03.74 ng/mL, respectively.

Table 6: System suitability parameters of GAT and FLU

Parameter	**Values**	
	GAT	**FLU**
Excitation wavelength (nm)	294	259
Emission wavelength(nm)	470	315
Beer's Law Limit (µg/mL)	0.005-2.5	0.005-0.35
Correlation coefficient	0.9998	0.999
Regression equation	y = 41.67x+1.017	y= 34.73+0.223
LOD (ng/mL)	1.5	1.23
LOQ (ng/mL)	4.79	3.74

IV. Conclusion

The developed method is a simple, sensitive, economical and ecofriendly one, that can be used for the simultaneous estimation of GAT and FLU in ophthalmic dosage form. The percentage RSD for all validated parameters was less than 2, which indicated the validity of the method. Hence, the method can be used for the routine QC analysis of the aforementioned drugs in their ophthalmic dosage formulation.

References

[1]. Indian Pharmacopoeia. Government of India, Ghaziabad: The Indian Pharmacopoeia Commission; 2007.Vol.2. p.540.
[2]. British Pharmacopoeia. London, the stationary office, 2003; vol-I, p.104-05.
[3]. Indian Pharmacopoeia. Vol.2. Government of India, Ghaziabad: The Indian Pharmacopoeia Commission; 2010.p.1378.
[4]. British Pharmacopoeia 2008. Vol.1. London: Stationery office; 2007. Flurbiprofen; p.953.
[5]. The United state Pharmacopoeia. USP 34-NF 29. Rockville, MD: US Pharmacopoeial convention Inc.; 2010; 31(4):1069.
[6]. A.V. Mali, R.P. Dhavale, V.L. Mohite, A.R. Mahindrakar, Y.V. Pore and B.S. Kuchekar, Spectrophotometric estimation of gatifloxacin in tablets, Indian Journal of Pharmaceutical Sciences, 68 (3), 2006, 386-387.
[7]. A.S. Amin, A.A.F. Gouda, R. El-Sheikh, F. Faten-Zahran, Spectrophotometric determination of gatifloxacin in pure form and in pharmaceutical formulation, Spectrochimica Acta Part A, 67, 2007, 1306–1312.
[8]. A.A.F. Gouda, R. El-Sheikh, A.S. Amin, Utility of some π-acceptors for spectrophotometric determination of gatifloxacin in pure form and in pharmaceutical preparations, Chemical and Pharmaceutical Bulletin, 56, 2008, 34–40.
[9]. K. Venugopal, S. Movva, R.N. Saha, New, rapid and sensitive spectrofluorimetric method for the estimation of gatifloxacin in bulk and formulations, Indian Journal of Pharmaceutical Sciences, 68, 2006, 726-730.

[10]. A. Lakshmana Rao, B.N.V. Ravi Kumar, G. Girija Sankar, Estimation of gatifloxacin in pharmaceutical dosage forms by high performance liquid chromatography. Asian Journal of Pharmaceutical Research and Health Care,3(3), 2011, 72-76.
[11]. Ibrahim A. Aljuffali, Mohd Abul Kalam, Yasmin Sultana, Ahamad Imran, Aws Alshamsan, Development and validation of stability indicating high performance liquid chromatography method to analyze gatifloxacin in bulk drug and pharmaceutical preparations,Saudi Pharmaceutical Journal, 23(1), 2014.
[12]. T.N.V.S.S. Satyadev, C.H. Bhargavi, Tata Santosh, B. Syama Sundar, Development and validation of RP-HPLC method for determination of gatifloxacin in human plasma, American Journal of Pharmatech Research, 4(2), 2015.
[13]. S. Mirza, N. Rabindra, D.M. Hassan, N. Huda, F. Shaikh, Simultaneous determination of gatifloxacin and ambroxol hydrochloride from tablet dosage form using reversed phase high performance liquid chromatography, Chinese Journal of Chromatography, 26, 2008, 358–361.
[14]. H.A. Nguyen, J. Grellet, B.B. Ba, C. Quentin, M. Saux, Simultaneous determination of levofloxacin, gatifloxacin and moxifloxacin in serum by liquid chromatography with column switching, Journal of Chromatography B, 810, 2004, 77–83.
[15]. L. Tasso, T.D. Costa, High performance liquid chromatography for quantification of gatifloxacin in rat plasma following automated on-line solid phase extraction, Journal of Pharmaceutical and Biomedical Analysis, 44, 2007, 205–210.
[16]. B.N. Suhagia, S.A. Shah, I.S. Rathod, H.M. Patel, D.R. Shah and B.P. Marolia, Determination of gatifloxacin and ornidazole in tablet dosage forms by HPTLC, Analytical Sciences, 22, 2006, 743-747.
[17]. S.K. Motwani, R.K. Khar, F.J. Ahmad, S. Chopra, K. Kohli, S. Talegaonkar, Z. Iqbal , Stability indicating high-performance thin-layer chromatographic determination of gatifloxacin as bulk drug and from polymeric nanoparticles, Analytica Chimica Acta, 576, 2006, 253–260.
[18]. C. Sajeev, Pravin R. Jadhav, D. Ravi Shankar, R.N. Saha, Determination of flurbiprofen in pharmaceutical formulations by UV spectrophotometry and liquid chromatography, Analytica Chimica Acta, 2002, 207–17.
[19]. K.M. Patel, B.D. Patel, H.J. Vekaria, A.R. Parmar, Development and validation of uv-visible spectroscopy for simultaneous estimation of flurbiprofen sodium and gatifloxacin in eye drops, Inventi: ppaqa/1442/14.2014.
[20]. Mehal M. Patel, H.U. Patel, Chaganbhai N. Patel, Development and validation of first derivative spectroscopic method for simultaneous estimation of gatifloxacin and flurbiprofen in bulk and eye drops,Inventi: ppaqa/1506/14.2014.
[21]. Gopi Patel, Payal Chauhan, Samir Shah, Simultaneous estimation of gatifloxacin and flurbiprofen sodium in ophthalmic formulation by UV-Spectrophotometric method, Journal of Chemical and Pharmaceutical Research, 6(7), 2014, 96-101.
[22]. Sridhar Siddiraju, Rayala Kavitha, Muvvala Sudhakar, Reverse phase high performance liquid chromatography method development and validation for the simultaneous estimation of gatifloxacin and flurbiprofen in pharmaceutical dosage form, Asian Journal of Pharmaceutical and Clinical Research, 8 (1), 2015, 242-46.
[23]. Islam Ullah Khan, Syed Naeem Razzaq, Irfana Mariamc, Muhammad Ashfaq, Syed Saleem Razzaq, Stability- Indicating RP-HPLC method for simultaneous determination of gatifloxacin and flurbiprofen in binary combination, Quimica Nova, 37(2), 2014, 349-54.
[24]. International Conference on Harmonization, Harmonized Tripartite Guideline, Validation of Analytical Procedures, Text and Methodology, Q2 (R1); 2005.