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Stability Indicating HPLC Method for Determination of Gatifloxacin in Ophthalmic Dosage Form

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Abstract:

The objective of this study was to develop and validate a high performance liquid chromatographic method to identify, quantify and to establish the stability of gatifloxacin in marketed ophthalmic dosage form. Gatifloxacin (GAT) is a new fourth generation antibiotic and has the bactericidal action on the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV. The determination of gatifloxacin was achieved using Zorbax Eclipse plus C_{18}, (100 X 4.6 mm, 5µ) column with a mobile phase of acetonitrile-methanol (70:30): ammonium acetate buffer (20:80 v/v) at a flow rate of 1.0 ml/min with UV detection at 288 nm and the peak of gatifloxacin was eluted at the retention time of 9.812 minutes. The method validation results of the study were within the specified acceptance criteria as per ICH guidelines. The described method was linear over the range of 160-250 µg/ml with R^2 value of 0.9986. The average percentage recovery of the method was 99.8%. The method was applied in the assay of marketed eye drops of gatifloxacin and the mean % label claim was found to be 100.56. The results of the forced degradation study demonstrated that gatifloxacin was stable in acidic, basic and thermal degradation conditions and degraded in Oxidative and photolytic conditions. Hence it was proved that the suggested method has ability to identify, separate and quantify the gatifloxacin in presence of its degradation impurities. Hence the method can be applied for the analysis of gatifloxacin in bulk, marketed formulations and also in stability samples.

Keywords: Gatifloxacin, HPLC, Method validation, Ophthalmic dosage form, Stability indicating method.

INTRODUCTION

Gatifloxacin (GAT) (Fig. 1) is chemically 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-ihydroquinoline-3-carboxylic acid. The empirical formula of GAT is $C_{19}H_{22}FN_3O_4$ and the molecular weight is 384.40 g mol^{-1}. The bactericidal action of GAT results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, and recombination. It has been widely used in prophylaxis, treatment of ocular infections, respiratory tract infections and broad-spectrum anti-bacterial. [1-6]

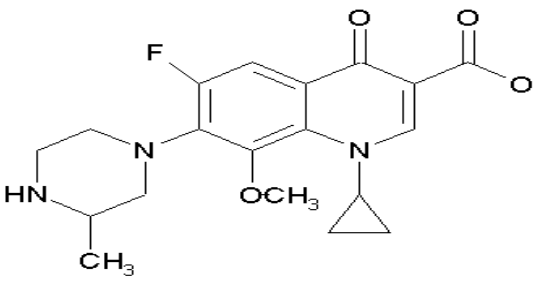

Figure 1: Structure of Gatifloxacin

Figure 1: Structure of Gatifloxacin

The literature survey revealed that limited numbers of analytical methods were reported for the estimation of gatifloxacin and its combination with other drugs which includes, determinations of GAT in pharmaceutical formulations by high-pressure liquid chromatography (HPLC),[7-12] UV spectrophotometry, [13-22] and capillary zone electrophoresis,[23] Spectrofluorometric,[24] HPTLC,[25,26] LC-MS,[27] the simultaneous determination of fluoroquinolones and corticosteroids in combined dosage form[28-31]. But in the present work an effort was made to ascertain detailed information about the stability of gatifloxacin in ophthalmic dosage form.

MATERIALS AND METHODS

Drugs and chemicals used

GAT pure drug was obtained as gift sample from Wockhardt R&D centre Aurangabad, GAT eye drops was purchased from Sun pharmaceuticals, Gujarat and all other analytical grade chemicals such as ammonia, ammonium acetate, and glacial acetic acid were purchased from Rankem Limited, New Delhi. HPLC grade methanol and acetonitrile were procured from E-Merck (India) Ltd, Mumbai.

Instruments used

Agilent-1100 series HPLC gradient system with the configurations of LC-10 ADVP solvent delivery system (pump), G 1315 B- photo diode array detector, SIL-10ADVP auto injector, 2695- Separations module. Ultra sonicator (3.5L100) PCI Analytics private ltd., Mumbai, pH meter LI613, ELICO Ltd, Hyderabad and MTT Sun Tester XLS+, Germany were used for this study.

Diluent

Water degassed in sonicator was used as diluents.

Preparation of acetate buffer

Accurately weighed amount of 3.85 g of ammonium Acetate was dissolved in 1000 ml of water and the pH was adjusted to 9.5 with ammonia solution (25% v/v) and then pH 6.5 with acetic acid.

Preparation of Mobile Phase

Acetonitrile - methanol (70:30) : ammonium acetate buffer were mixed in the ratio of 20:80% v/v and degassed in ultrasonicator for 5 minutes.

Preparation of standard solution

Accurately weighed and transferred about 200 mg of GAT standard into a 100 ml volumetric flask dissolved and diluted with diluent. From the above solution 10 ml was pipetted into a 100 ml volumetric flask and diluted with diluent to get the concentration of 200 µg/ml.

Preparation of sample solution (Eye drops)

From the Commercially available GAT eye drops (0.3% or 3 mg/ml), 6.6 mg of eye drops was weighed and transferred to 100 ml standard flask. It was dissolved in 50 ml of diluent by sonication for 15 min and made up to the volume with the diluent to get the concentration of GAT 200 µg/ml.

Optimized chromatographic conditions

The analysis was carriedout using Zorbax Eclipse plus C_{18}, (100 X 4.6 mm, 5µ) column; in isocratic elution mode with a mobile phase of acetonitrile-methanol (70:30): ammonium acetate buffer (20:80 v/v) at a flow rate of 1.0 ml/min with UV detection at 288 nm.

RESULTS

METHOD DEVELOPMENT

The several trials were done to elute the GAT by changing the mobile phase composition in different ratios such as acetonitrile and ammonium acetate buffer mixture (05:95 & 10:90) and the mixture of acetonitrile-methanol (70:30): ammonium acetate buffer (20:80) and also by altering the flow rate. In the initial trial conditions the peak was eluted at longer retention time, the peak shape was not good and more tailing was observed. The composition of solvents in the mobile phase was adjusted to achieve optimum analytical conditions to get satisfactory retention time and peak shape. Finally the analysis of GAT was accomplished using the method conditions of Zorbax Eclipse plus C_{18}, (100 X 4.6 mm, 5µ) column; in isocratic elution mode with a mobile phase of acetonitrile-methanol (70:30): ammonium acetate buffer (20:80 v/v) at a flow rate of 1.0 ml/min with UV detection at 288 nm. In the above chromatographic condition the peak was eluted at the retention time of 9.812 minutes with good peak shape.

METHOD VALIDATION [32, 33]

System suitability

The system suitability parameters were evaluated with the help of chromatogram obtained from the six replicate injections of GAT standard. The number of theoretical plates and asymmetry factor was found to be 6698 and 1.21 respectively.

Specificity

The specificity of the method was tested by comparing the chromatograms of blank, standard and sample solution. There was no interference of the excipients was detected at the retention time of GAT. The chromatograms of blank, GAT in standard and in sample were shown in **fig. 2, 3 & 4.**

Linearity

The linearity was established over the concentration level of 80% to 120% of the standard working concentration of GAT. The calibration curve obtained by plotting peak area versus concentration of GAT was found to be linear within the concentration range from 160 to 250 µg/ml. The linear regression data evaluation showed that the response of the analyte was found to be linear in the investigated concentration range and the correlation coefficient (R^2) value meets the acceptance criteria (Not less than 0.99). The linearity regression analysis data was given in **table 1**, linearity calibration curve was shown in **fig. 5.**

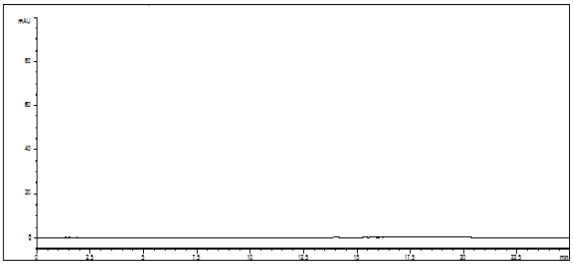

Figure 2: Specificity - Chromatogram of blank

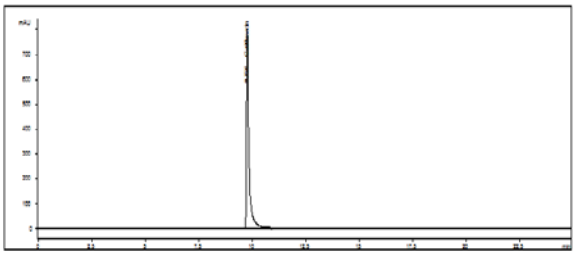

Figure 3: Specificity - Chromatogram of gatifloxacin standard

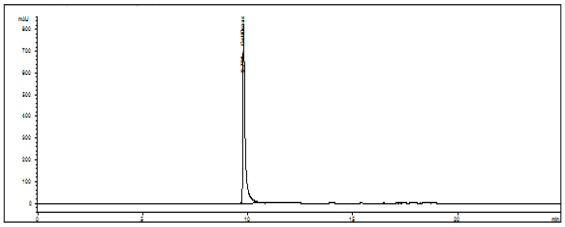

Figure 4: Specificity - Chromatogram of gatifloxacin sample

Table 1: Linearity data of gatifloxacin

Linearity Level	Concentration (µg/ml)	Peak Area	Statistical Analysis		
80%	160	5405.8	Slope	Intercept	Correlation Coefficient
90%	180	6096.6			
100%	200	6754.1	33.192	55.38	0.9986
110%	220	7443.8			
120%	250	8155.8			

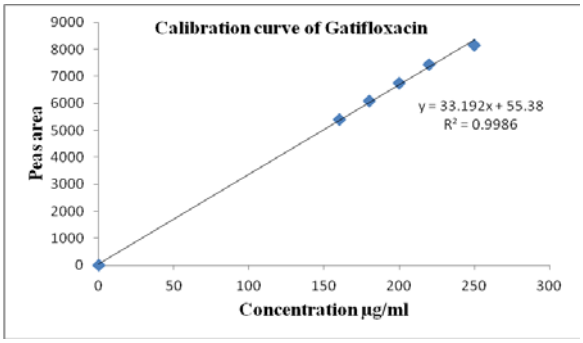

Figure 5: Calibration curve of gatifloxacin

Precision

The system precision of the method was evaluated by five replicate injections of standard solution and method precision was assessed by five replicate injections of sample solution. The intermediate precision was determined by carrying out the experiment on different day by different analyst. The %RSD of the system precision, method precision and the intermediate precision were found to be less than 2% which confirms that the developed method is precise. The data obtained from the precision study was given **table 2.**

Table 2: Results for precision study

Injection	System precision	Method precision		Intermediate precision (% Assay)	
	Peak area	Peak area	% Assay	Day-I, Analyst-I	Day-II, Analyst-II
1	7011.2	6731	100.61	100.61	100.11
2	6669.2	6748.4	100.87	100.87	100.37
3	6743.7	6739.7	100.74	100.74	101.86
4	6749.1	6749.1	100.88	100.88	101.97
5	6742.4	6688.2	99.97	99.97	100.77
6	6737.9	6727.6	100.56	100.56	99.13
Mean	6775.5	6730.6	100.6	100.6	100.7
SD	119.2239	22.5778	0.33	0.33	1.0849
%RSD	**1.75**	0.33	**0.33**	0.329	1.077
Overall %RSD				**0.70**	

Accuracy

The accuracy of GAT was determined by recovery at three concentration levels. The mean % recovery of the drug is within the acceptance limit 98-102% which indicates that the method is accurate. The spiked recovery result was presented in **table 3**.

Table 3: Results of Accuracy

Spiked concentration level (%)	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	Mean % Recovery	SD	% RSD
80	160	161.37	100.86	100.93	0.1594	0.15
80	160	161.79	101.12			
80	160	161.32	100.83			
100	200	201.94	100.97	101.17	0.1877	0.18
100	200	202.68	101.34			
100	200	202.42	101.21			
120	250	251.97	100.79	100.87	0.1026	0.10
120	250	252.12	100.85			
120	250	252.47	100.99			
Overall mean % recovery				**100.99**		

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ were calculated by using standard deviation of the y-intercept and slope of the calibration curve constructed at the concentration levels of the drug. The calculated LOD and LOQ were found to be 0.14 and 0.40 µg/ml respectively.

Robustness

The robustness of the method was demonstrated by making slight changes in the chromatographic conditions such as change in the ratio of acetonitrile in the mobile phase composition, flow rate and detection wavelength. The % RSD of % assay was calculated for the altered conditions which were found to be less than 2 %. This represents the ability of the method to produce accurate results during the slightly distorted method conditions. The data was presented in **table 4**.

Table 4: Results of robustness study

Altered conditions	Level	Peak area	Mean peak area	SD	% RSD
Ratio of acetonitrile in mobile phase	68	6698.0	6718.9	23.0816	0.34
		6715.2			
		6743.7			
	70	6749.1	6720.6	44.6531	0.66
		6669.2			
		6743.7			
	32	6669.2	6718.4	42.6422	0.63
		6743.7			
		6742.4			
Flow rate	0.8	6743.7	6745.0	3.5529	0.05
		6749.1			
		6742.4			
	1.0	6749.1	6720.6	44.6531	0.66
		6669.2			
		6743.7			
	1.2	6636.5	6644.1	7.6500	0.11
		6651.8			
		6644.2			
Detection wavelength	286	6792.9	6788.8	4.0501	0.05
		6784.8			
		6788.9			
	288	6749.1	6720.6	44.6531	0.66
		6669.2			
		6743.7			
	290	6899.3	6790.4	94.5304	0.39
		6742.4			
		6729.5			

Analysis of gatifloxacin in marketed ophthalmic dosage form

The amount of GAT present in marketed ophthalmic dosage form was determined as % label claim and the results were given in **table 5**. The % label claim was within the limit proves the applicability of the method in commercial dosage form.

Table 5: Analysis of gatifloxacin in marketed ophthalmic dosage form

Label claim (mg/unit)	Injection	Amount found (mg/unit)	% Label claim
15	1	15.065	100.45
	2	15.103	100.69
	3	15.094	100.63
	4	15.088	100.59
	5	15.093	100.62
	6	15.063	100.42
Mean % Label claim		15.08	**100.56**
SD		0.0164	0.1074
% RSD		0.01	0.10

Forced degradation studies

Acidic degradation

The GAT when exposed to acidic degradation condition showed no significant degradation and also no degraded peaks were observed. The percentage degradation was found to be 2.52%, which indicates the stability of gatifloxacin under acidic conditions. The chromatogram of gatifloxacin in acid degradation and peak purity plots were shown in **fig. 6 & 7.**

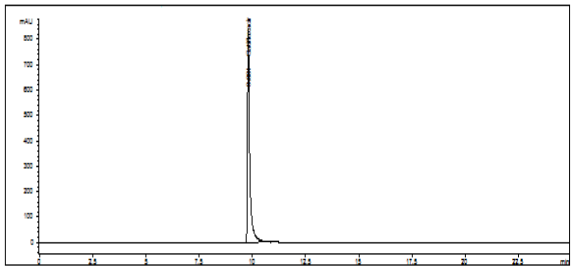

Figure 6: Chromatogram of gatifloxacin in acid degradation

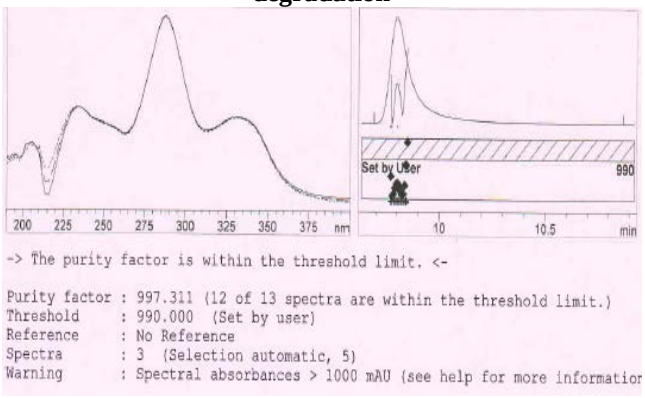

Figure 7: Peak purity of gatifloxacin in acid degradation

Alkaline degradation

The GAT when exposed to alkaline degradation condition showed no significant degradation and also no degraded peaks were observed. The percentage degradation was found to be 4.05%, which indicates the stability of gatifloxacin under alkaline conditions. The chromatogram of GAT in alkaline degradation and peak purity plots were shown in **fig. 8 & 9.**

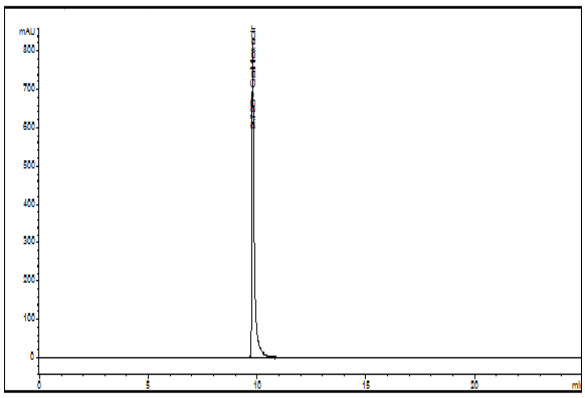

Figure 8: Chromatogram of gatifloxacin in alkaline degradation

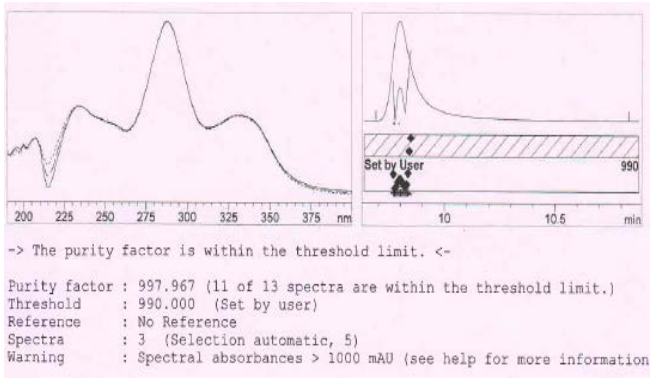

Figure 9: Peak purity of gatifloxacin in alkaline degradation

Oxidative degradation

The GAT when exposed to oxidative degradation condition showed the significant degradation and the additional peaks were observed at the RT of 1.312, 2.598, 8.969, 9.493, 10.838, 11.763, 12.209, 12.594, 13.823, 14.126 and 14.649 mins. The percentage degradation was found to be 34.5%, which indicates the gatifloxacin was degraded under Oxidative condition. The chromatogram of GAT in Oxidative degradation and peak purity plots were shown in **fig. 10 & 11.**

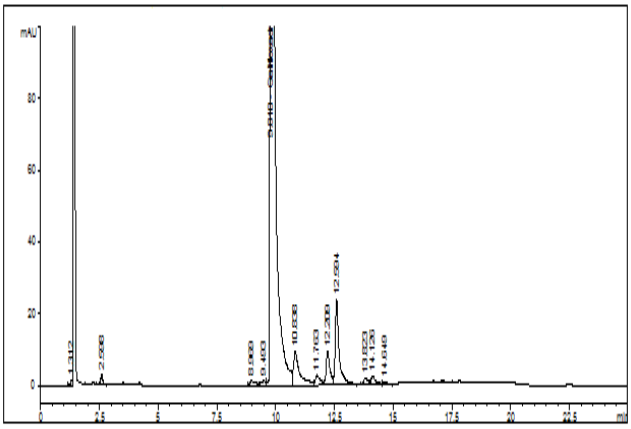

Figure 10: Chromatogram of gatifloxacin in oxidative degradation

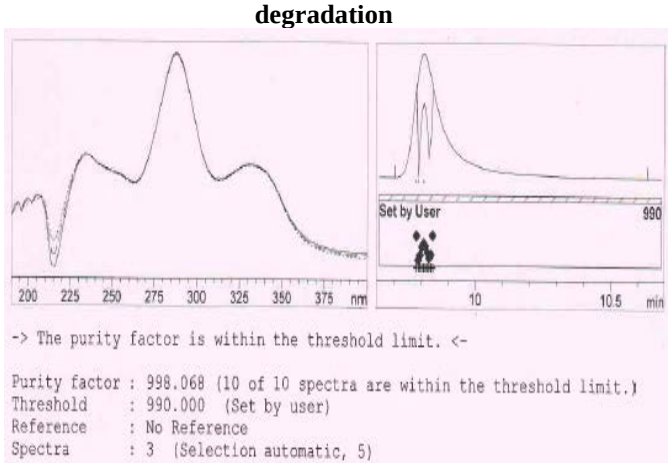

Figure 11: Peak purity of gatifloxacin in oxidative degradation

Thermal degradation

The GAT when exposed to thermal degradation condition showed no significant degradation. The percentage degradation was found to be 6.06%, which indicates the stability of gatifloxacin under thermal conditions. The chromatogram of GAT in thermal degradation and peak purity plots were shown in **fig. 12 & 13.**

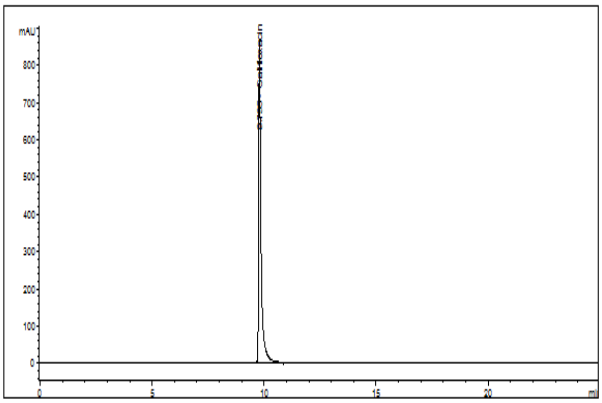

Figure 12: Chromatogram of gatifloxacin in thermal degradation

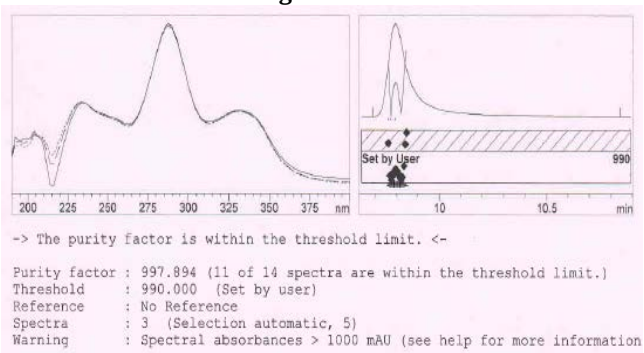

Figure 13: Peak purity of gatifloxacin in thermal degradation

Photolytic degradation

The GAT when exposed photolytic degradation condition, showed marginal degradation and also one additional peak was observed at the RT of 12.220. The percentage degradation was found to be 13.65%, which indicates the gatifloxacin was degraded to some extent under photolytic condition. The chromatogram of GAT in photolytic degradation and peak purity plots were shown in **fig. 14 & 15.**

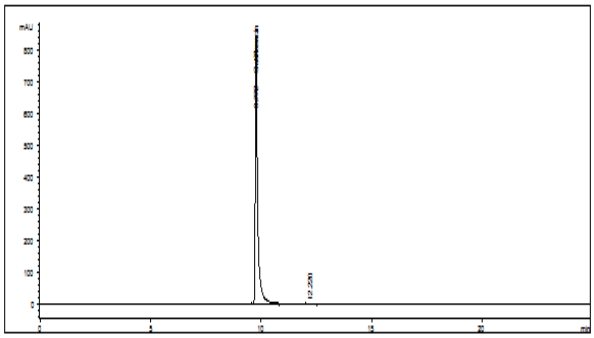

Figure 14: Chromatogram of gatifloxacin in photolytic degradation

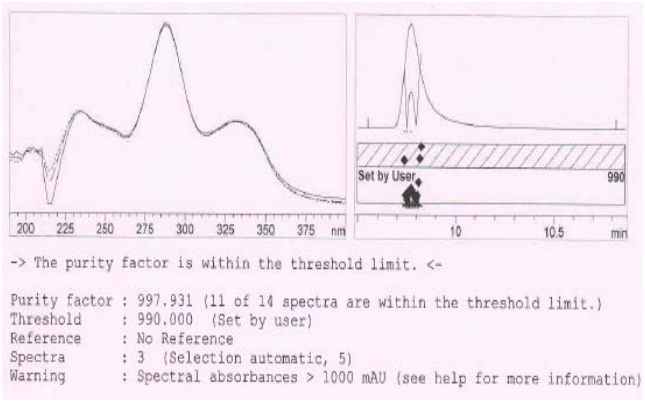

Figure 15: Peak purity of gatifloxacin in photolytic degradation

The percentage degradation was calculated by comparing the peak area of GAT under various stress conditions with the peak area of gatifloxacin control sample. The results of forced degradation studies of gatifloxacin were summarized in **table 6.**

Table 6: Results of forced degradation studies

Parameters	Stress conditions	Peak area* ± SD	% Degradation	% Assay	Peak purity**
Control sample	-	6745.2 ± 3.78	-	100.82	997.748
Acidic degradation	0.1M HCl for 4 hours	6576.5 ± 72.40	2.52	98.30	997.311
Alkaline degradation	0.1M NaOH for 4 hours	6474.5 ± 56.0	4.05	96.77	997.967
Oxidative degradation	0.3% H_2O_2 for 4 hours	4437.6 ± 372.55	34.5	66.32	998.068
Thermal degradation	At 90ºC for 24 hours	6340.2 ± 73.37	6.06	94.76	997.894
Photolytic degradation	1.2 x 106 lux hours of fluorescent light	5832.2 ± 39.74	13.65	87.17	997.931

*Mean of three determinations, **The peak purity values in the range of 990 – 1000 indicate a homogenous peak.

DISCUSSION

In this course of work a stability indicating analytical method by HPLC using UV detection was developed and validated to identify, determine and also to establish the stability of GAT in marketed ophthalmic dosage form. As an initial step, trials were done to elute the GAT by changing mobile phase composition in different ratio. The analysis of GAT was accomplished using the optimized method conditions and the peak of GAT was eluted at the satisfactory retention time of 9.812 min with good peak shape. The performance of the method was validated by all the parameters as per ICH guidelines. The Specificity of the method was ascertained by comparing the chromatograms of blank, standard and sample solution. There was no interference of the excipients was detected at the retention time of gatifloxacin. The system suitability was evaluated and the number of theoretical plates and asymmetry factor was found to be 6698 (not less than 2000) and 1.21 (not more than 2), the high counts of theoretical plates indicated the column efficiency and validity of the method. The

%RSD value of the precision and robustness were found to be in the range of 0.05-1.75, which indicated that the method was precise, rugged and robust enough to hold out the slight deviations in the method parameters. The mean % recovery was found to be 100.99, which was within the acceptance range, proved the accuracy of the method. The correlation coefficient calculated from the linearity study was 0.9986, indicated the linear response revealed by the method over the selected concentration range of 160-250 µg/ml. The lowest detection and quantification amount of GAT by the method were found to be 0.14 and 0.40. The very low LOD and LOQ signified the sensitivity of the method. The method was applied in the assay of marketed eye drops of GAT and the mean % label claim was found to be 100.56; the results demonstrated the applicability of the proposed method in commercial formulations.

To demonstrate the stability of GAT, the drug was exposed to forced degradation under various stressed conditions like acidic, alkaline, oxidative, thermal and photolytic degradation. The results of forced degradation study revealed that the percentage degradation was found to be 2.52, 4.05, 34.5, 6.06, and 13.65 in acidic, alkaline, oxidative, thermal and photolytic degradation correspondingly. Under the Oxidative degradation condition the drug was significantly degraded and the additional peaks were observed at the retention time of 1.312, 2.598, 8.969, 9.493, 10.838, 11.763, 12.209, 12.594, 13.823, 14.126 and 14.649 min. The chromatogram was subjected to peak purity spectral analysis by using diode array detector. The peak purity was 998.068 which in the acceptable range of 990-1000 revealed the ability of the method to separate the drug in presence of its degradation products. Further in the photolytic degradation condition drug was marginally degraded and one additional peak was observed at the retention time of 12.220 min. The peak purity was 997.931 which is in the acceptable range of 990-1000. The results of the forced degradation study demonstrated that GAT was stable in acidic, basic and thermal degradation conditions and degraded in Oxidative and photolytic conditions. Hence it was proved that the suggested method has ability to identify, separate and quantify the gatifloxacin in presence of its degradation impurities.

CONCLUSION

As per method development and validation report, the method was found to be better in the sense of simple preparation steps, isocratic elution, cost effectiveness, time of analysis, well resolved degraded peaks and also wide range of linearity, precision, robustness, accuracy and sensitivity when compared to reported methods. The developed method for Estimation of GAT ophthalmic dosage form was said to be rapid, simple, accurate, precise, sensitive, robust, and specific and stability indicating that can be successfully applied for the routine analysis of GAT in their marketed ophthalmic dosage form. Thus the developed method is stability indicating, it can be used to separate the degradants and to determine the stability of GAT samples and it will be helpful to maintain the safety and quality of drug products.

REFERENCES

1. Data base of Gatifloxacin, compilation prepared by drug bank, http://www.drugbank.ca/drugs/DB01044.
2. Long M., Jensen HG., Allergan Gatifloxacin Study Group, Ocular bacteria from conjunctivitis patients: susceptibility to gatifloxacin and older fluoroquinolones. *Invest Ophthalmol Visual Sci.* 2003; 44: U355.
3. Donnenfeld E., Perry HD., Chruscicki DA., Bitterman A., Cohn S., Solomon RA., Comparison of the fourth-generation fluoroquinolones gatifloxacin 0.3% and moxifloxacin 0.5% in terms of ocular tolerability. *Curr Med Res Opin*. 2004; 11: 1753–8.
4. Bucci Jr FA., Amico LM., Evans RE., Antimicrobial efficacy of prophylactic gatifloxacin 0.3% and moxifloxacin 0.5% in patients undergoing phacoemulsification surgery. *Eye Contact Lens.* 2008; 1: 39–42.
5. Callegan MC, Novosad BD, Ramadan RT, Wiskur B, Moyer AL. Rate of bacterial eradication by ophthalmic solutions of fourth-generation fluoroquinolones. *Adv Ther*. 2009; 4: 447–54.
6. Cervantes LJ., Mah FS.. Clinical use of gatifloxacin ophthalmic solution for treatment of bacterial conjunctivitis. *Clin Ophthalmol* 2011;5: 495–502.
7. Venugopal K., Snehalatha M., Bende G., Saha R., Development and validation of an ion-pairing RP-HPLC method for the estimation of gatifloxacin in bulk and formulations. *J Chromatogr Sci*. 2007; 4: 220–5.
8. Abida B., Srinivas NK., Mohd I., RP-HPLC method development and validation for gatifloxacin from tablet formulation. *J Anal Chem.* 2011; 2: 1–8.
9. Gopi P., Payal C., Samir S., Application of RP-HPLC method for simultaneous estimation of Gatifloxacin and Flurbiprofen Sodium in ophthalmic formulation. *Am J PharmTech Res.* 2014; 4(2): 658-68.
10. 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 J Pharm and Cli Res.* 2015; 8(1): 242-6.
11. 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. *Inter J Univ Pharm Bio Sci.* 2014; 3(3): 59-70.
12. Sireesha KR., Prakash K., Simultaneous determination of Gatifloxacin and Dexamethasone Sodium Phosphate in bulk and pharmaceutical formulations by HPLC. *African J Pharm Pharmacol.* 2011; 5(17): 1990-5.
13. 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. *Am J Anal Chem.* 2012; 3: 147-52.
14. Salgado HRN., Oliveira CLCG., Development and validation of an UV spectrophotometric method for determination of gatifloxacin in tablets. *Pharmazie* 2005; 4: 263–4.
15. Venugopal K., Saha RN., New, simple and validated UVspectrophotometric methods for the estimation of gatifloxacin in bulk and formulations. *Il Farmaco.* 2005; 11(12): 906–12.
16. Jane J., Subrahmanyam EVS., Sathyanarayana D., Spectrophotometric determination of gatifloxacin. *Asian J Chem.* 2006; 4: 3210– 2.
17. Amin AS., Gouda AEF., El-Sheikh R., Zahran F.. Spectrophotometric determination of gatifloxacin in pure form and in pharmaceutical formulation. *Spectrochim Acta Part A.* 2007; 5: 1306– 12.
18. Kanakapura B., Rangachar AKU., Sensitive spectrophotometric methods for quantitative determination of gatifloxacin in pharmaceutical formulations using bromate-bromide, thiocyanate and tiron as reagents. *J Mex Chem Soc.* 2007; 2: 106–12.
19. Darwish IA., Sultan MA., Al-Arfaj HA., Selective kinetic spectrophotometric method for determination of gatifloxacin based on formation of its N-vinyl chlorobenzoquinone derivative. *Spectrochim Acta Part A.* 2010; 1: 334–9.
20. Patel, Gopi, Chauhan, Payal, Shah, Samir, Simultaneous estimation of Gatifloxacin and Flurbiprofen Sodium in ophthalmic formulation by UV-Specrophotometric method. *J Chem Pharm Res.* 2014; 6(7): 96-101.
21. Upadhyay N., Maheshwari RK., Jain J., Patani M., Pandey R., New spectrophotometric analysis of Gatifloxacin tablets utilizing mixed solvency concept. *Der Pharmacia Letter*. 2012; 4 (1): 1-4.

22. 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 J Chem.* 2012; 28(2): 639-50.
23. 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. *Inter J Pharm Sci Rev Res*. 2014; 27(2): 96-9.
24. Sane RT., Menon S., Pathak AR., Deshpande AY., Mahale M., Capillary electrophoretic determination of gatifloxacin from pharmaceutical preparation. *Chromatographia*. 2005; 5(6): 303–6.
25. 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 Quality Assur*. 2014; ppaqa/1421/14.
26. Rote AR., Kumbhoje PA., Development and validation of HPTLC method for simultaneous estimation of Gatifloxacin and Ornidazole in human plasma. *J Chromato Separ Tech.* 2011; 2(115): 2157-64.
27. Shah SA., Rathod IS., Suhagia BN., Baldaniya M., A simple and sensitive HPTLC Method for estimation of Gatifloxacin in tablet dosage forms. *Ind J Pharm Sci.* 2004; 66(3): 306-8.
28. 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. *J Pharm Biomed Anal.* 2011; 54: 359-67.
29. Prakash K., Sireesha KR., Simultaneous determination of ciprofloxacin hydrochloride and dexamethasone sodium phosphate in eye drops by HPLC. *Eur J Chem.* 2012; 3: 1077–84.
30. Barot HN., Dave JB., Patel CN., Development and validation of spectrophotometric method for simultaneous determination of prednisolone acetate and ofloxacin in eyedrop. *Int J Pharm Sci Res.* 2012; 3: 1817–21.
31. Patel HB., Sejal PK., Spectrophotometric method for simultaneous estimation of gatifloxacin sesquihydrate and prednisolone acetate in combined pharmaceutical dosage form. *Am J Pharm Tech Res.* 2013; 3: 478–86.
32. International Conference on Harmonization (ICH) guidelines Q2(R1), Text on validation of analytical procedures, methodology. International Conference on Harmonization, Geneva. 2005.
33. 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.