New, Rapid, and Sensitive Spectrofluorimetric Method for the Estimation of Gatifloxacin in Bulk and Formulations

K. VENUGOPAL, SNEHALATHA MOVVA, R. N. SAHA*
Pharmacy Group, Birla Institute of Technology and Science (BITS), Pilani-333 031, India.

A new rapid, sensitive, simple, and cost-effective spectrofluorimetric method was developed for the estimation of gatifloxacin in bulk and pharmaceutical formulations. The relative fluorescence intensity of gatifloxacin in 10 mM hydrochloric acid (pH 2.0) was measured at excitation wavelength (λ_{exc}) of 292 nm and emission wavelength (λ_{em}) of 482 nm. Linearity range was found to be 20-160 ng/ml with regression equation, relative fluorescence intensity = 36.05 × concentration in ng/ml + 12.60 with regression coefficient (r^2) = 0.9998. The method was tested and validated for various parameters according to ICH guidelines and USP. The detection and quantitation limits were found to be 5.48 and 16.61 ng/ml, respectively. The results demonstrated that the procedure is accurate, precise, and reproducible (relative standard deviation <2%), while being simple and less time consuming. The method is applicable for the estimation of gatifloxacin in different dosage forms and the results are in good agreement with label claims.

Gatifloxacin is a fourth-generation 8-methoxy fluoroquinolone derivative (1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid) with a broad spectrum of activity encompassing gram-positive and gram-negative pathogens, including *S. epidermidis, S. aureus, S. pneumoniae, S. pyogenes, H. influenzae, E. coli, B. cereus, N. gonorrhoeae*, and *P. mirabilis*. Additionally, gatifloxacin is highly active against atypical pathogens, such as *Mycoplasma, Legionella*, and *Chlamydia* species, as well as the anaerobic organism *P. acnes*.[1-2] More favorable pharmacokinetic properties of gatifloxacin are due to modification in substitutents of the original fluoroquinolone moiety. Due to clinical advantages of gatifloxacin, there was increase in number of gatifloxacin formulations in market in recent past, which necessitated a simple analytical method for routine analysis. A survey of literature has not revealed any simple spectrofluorimetric method for estimation of gatifloxacin in bulk, formulations and for dissolution studies of oral and ophthalmic formulations. Recently, a UV-spectrophotometric method has been reported for estimation of gatifloxacin in tablet formulations with linearity range of 4-14 µg/ml.[3] High performance liquid chromatography (HPLC) and microbiological assay methods were reported for the estimation of gatifloxacin in biological fluids such as plasma, serum, and urine.[4-7] Reported HPLC methods used different detectors like electron-spray tandem mass spectrometry, ultraviolet and fluorescence. Overholser *et al.*, have developed and validated a HPLC method for the estimation of gatifloxacin in human serum and urine samples.[8] But, chromatographic techniques demand a lot of time and expertise in their operation with higher cost. Thus there is a need to develop a simple, rapid, and cost effective method with low estimation range for routine analysis.

The objective of the present study was to develop simple, sensitive, precise, accurate, and economic analytical method with better detection range for the estimation of gatifloxacin in bulk, and in pharmaceutical formulations, and for *in vitro* dissolution studies of oral and ophthalmic formulations. Analytical method was developed in 10 mM hydrochloric acid (pH 2.0). In the selected medium the relative fluorescence intensity was measured at λ_{exc} of 292 nm and λ_{em} of 482 nm. No extraction step was involved in the proposed method, thereby decreasing time and error in quantitation. The developed method was validated as per ICH guidelines

***For correspondence**
E-mail: rnsaha@bits-pilani.ac.in

and USP requirements.[9,10] Suitable statistical tests were performed on validation data.[11]

MATERIALS AND METHODS

Gatifloxacin was obtained as a gift sample from Venkar Chemicals Pvt. Ltd., India. Formulations containing gatifloxacin: Gaity-200 tablets, labeled to contain 200 mg of gatifloxacin per tablet (Dr. Reddy's Laboratories Ltd., India), Gaity-400 tablets, labeled to contain 400 mg of gatifloxacin per tablet (Dr. Reddy's Laboratories Ltd., India) and Gatilox concentrated injection, labeled to contain gatifloxacin 10 mg/ml (Solares, Sun Pharma, India) were collected. Gatifloxacin ophthalmic solution of 0.3 %w/v strength was prepared in the laboratory using phosphate buffer saline (pH 7.4) as vehicle, under aseptic conditions. Apart from common excipients Gaity-200 and Gaity-400 tablets contain excipients like iron oxide yellow and titanium oxide. Gatilox concentrated injection contains excipients like dextrose and water for injection. Gatifloxacin ophthalmic solution contains 0.01 %w/v of benzalkonium chloride (preservative) in aqueous vehicle. All other chemicals and reagents used were of analytical grade. All fluorescence measurements were done on a spectrofluorimeter (Jasco FP777, Japan) loaded with in-built software and equipped with a 150 W Xenon lamp, using 10 mm quartz cells. Measurement parameters were: excitation band width - 3 nm, emission band width - 5 nm, photo-multiplier tube response – high, slit width - 0.5 nm, λ_{exc} = 292 ± 1 nm and λ_{em} = 482 ± 1 nm.

Method development:

Different pH media alone and in combination with different organic solvents, in various proportions, were tried. For selection of media the criteria employed was sensitivity of the method, ease of sample preparation, solubility of the drug, cost and applicability of the method for various purposes. Primary stock solution of 200 µg/ml of gatifloxacin was prepared in 10 mM hydrochloric acid (pH 2.0). Using aliquot of primary stock solution, secondary stock solution of 1 µg/ml was prepared in 10 mM hydrochloric acid. For preparation of different concentrations, aliquots of secondary stock solution were transferred into series of 10 ml standard flasks and volume was made with 10 mM hydrochloric acid. Five different concentrations (20, 60, 100, 140, and 160 ng/ml) of gatifloxacin were prepared for calibration curve. Relative fluorescence intensity was measured at λ_{exc} of 292 nm and λ_{em} of 482 nm against blank (Table 1). To establish linearity of the proposed method, six separate series of solutions of the drug in selected medium were prepared from the stock solution and analysed. Least square regression analysis was done for the obtained data. ANOVA test (one-way) was performed based on the relative fluorescence intensity values observed for each pure drug concentration during the replicate measurement of the standard solutions.

TABLE 1: CALIBRATION DATA OF GATIFLOXACIN

Con. (ng/ml)	Mean relative fluorescence intensity (± SD[a])	% RSD[b]	Predicted con. (ng/ml)
20	731 ± 17	2.38	19.93
60	2180 ± 17	0.76	60.12
100	3624 ± 35	0.96	100.19
140	5051 ± 35	0.69	139.76
160	5781 ± 19	0.33	160.03

[a]Standard deviation, [b]Percentage relative standard deviation. Each value is result of six separate determinations

Analytical validation:

Gatifloxacin solutions (100 ng/ml) were prepared in the selected medium with and without common excipients (lactose, starch, methyl cellulose, hydroxypropylmethylcellulose, microcrystalline-cellulose, dextrose, iron oxide yellow, titanium oxide, magnesium stearate, talc and benzalkonium chloride) separately. All the solutions were scanned (400 nm/min) for emission spectrum by fixing the λ_{exc} at 292 nm and checked for the change in emission spectrum. In a separate study drug concentration of 150 ng/ml was prepared independently from pure drug stock and formulation sample stock in selected medium and analysed (n = 5). Paired t-test at 95% level of significance was performed to compare the means of relative fluorescence intensity.

As a part of determining accuracy of the proposed method, different levels of drug concentrations (LQC – 25 ng/ml, MQC – 75 ng/ml, and HQC – 150 ng/ml) were prepared independently from stock solution and analysed (n = 6). Accuracy was assessed as the percentage relative error and mean percentage recovery (Table 2). To give additional support to accuracy of the developed assay method, standard addition method was done. In this study, different concentrations of pure drug (20, 40, 60, 80, and 100 ng/ml) were added to a known pre-analysed formulation sample (drug concentration of 50.3 ng/ml) and the total concentration was determined using the proposed method (n = 3). The percent recovery of the added pure drug was calculated as, % recovery = [(Cv-Cu)/Ca] × 100, where Cv is the total drug concentration measured after standard addition; Cu, drug concentration in the formulation; Ca, drug concentration added to formulation.

TABLE 2: ACCURACY AND PRECISION DATA FOR THE DEVELOPED METHOD

Level	Predicted con. (µg/ml)[a]			Mean % recovery (±SD)	Accuracy (%)[b]
	Range	Mean (±SD)	% RSD		
LQC	24.44-25.50	25.02 ± 0.286	1.142	100.06 ± 1.142	0.060
MQC	72.24-75.10	74.83 ± 0.651	0.870	99.78 ± 0.868	-0.221
HQC	149.59-150.28	149.99 ± 0.230	0.153	99.99 ± 0.153	-0.009

[a]Predicted concentration of gatifloxacin was calculated by linear regression equation, [b]Accuracy is given in relative error % (= 100 × [(predicted concentration – nominal concentration)/nominal concentration)]. Each value is result of six separate determinations

Repeatability was determined by using different levels of drug concentrations (as mentioned in accuracy), prepared from independent stock solution and analysed (n = 6) (Table 3). Inter- and intra-day variation and analyst variation was studied to determine intermediate precision of the proposed method. Different levels of drug concentrations in triplicates were prepared two different times in a day and studied for intra-day variation. Same protocol was followed for three different days to study inter-day variation (n = 18). Different analysts prepared different solutions on different days. The relative standard deviation (in %) of the predicted concentrations from the regression equation was taken as precision (Table 3).

The detection limit (DL) and quantitation limit (QL) of gatifloxacin by the proposed method was determined using calibration standards. DL and QL were calculated as 3.3 σ/*S* and 10 σ/*S* respectively, where *S* is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation.[9] Robustness of the proposed method was determined by (a) changing pH of the media by ±0.1 units and (b) stability of drug in the selected medium at room temperature for 8 h. Three different concentrations (LQC, MQC and HQC) were prepared in different pH media and mean percentage recovery was determined.

Estimation from formulations:

Twenty tablets were weighed and pulverized. Amount of the powder equivalent to 5 mg of gatifloxacin was taken and extracted with selected medium for 30 min. The solution was diluted suitably to prepare a 200 µg/ml concentration. This primary stock solution was filtered through Whatman filter paper number 40 and the filtrate was further diluted suitably to prepare a secondary stock solution of 1 µg/ml concentration. Aliquot of the secondary stock solution was diluted to a concentration of 50 ng/ml and the samples were analyzed using proposed method. Same procedure was followed for the tablets of strength 200 and 400 mg.

Aliquot of gatifloxacin injection (10 mg/ml strength) equivalent to 5 mg of drug was taken and diluted in the selected medium to get a 200 µg/ml concentration primary stock. Rest of the sample preparation was same as used for tablets and samples were analyzed.

Aliquot of gatifloxacin ophthalmic solution (0.3% w/v strength) equivalent to 300 µg of drug was taken and diluted in the selected medium to get a 3 µg/ml concentration primary stock. Aliquot of the primary stock solution was diluted to a concentration of 150 ng/ml and the samples were analysed. Five replicates were prepared in all the cases.

TABLE 3: RESULT OF INTERMEDIATE PRECISION STUDY

Level	Intra-day repeatability- % RSD[a] (*n* = 3)			Inter-day repeatability % RSD[a] (*n*=18)
	Day 1	Day 2	Day 3	
LQC	0.911	1.346	1.316	0.518
	0.655	1.426	0.384	
MQC	0.037	0.033	0.014	0.372
	2.151	0.094	0.018	
HQC	0.039	0.028	0.013	0.177
	0.012	0.063	0.016	

[a]percentage relative standard deviation

RESULTS AND DISCUSSION

For media optimization various aqueous media like 10 mM hydrochloric acid, acetate buffers (pH 3.6-5.8), phosphate buffers (pH 5.8-8.0) and 100 mM sodium hydroxide were investigated. Addition of acetonitrile/methanol in various proportions with various aqueous media did not improve the sensitivity of the method. Sensitivity was found to be maximum in 10 mM hydrochloric acid medium. The final decision of using 10 mM hydrochloric acid (pH 2.0) as a medium was based on certain criteria like; sensitivity of the method, cost, ease of preparation and applicability of the method to different purposes. The excitation and emission spectra of gatifloxacin in 10 mM hydrochloric acid are shown in fig. 1.

Different concentrations and their relative fluorescence intensities were shown in the Table 1. At all the concentration levels the standard deviation was low and

the relative standard deviation (RSD) did not exceed 2.5%. The predicted concentrations were nearly matching with the nominal concentration. In selected medium the linearity range was found to be 20-160 ng/ml. According to linear regression analysis, the slope (± standard error) and intercept (± standard error) were found to be 36.05 ($\pm 8.38 \times 10^{-2}$) and 12.60 (± 8.641), respectively. These mean values were found to be within the 95% confidence limits (confidence limits of slope: 35.88-36.22; confidence limits of intercept: from -4.451 to 29.64). Goodness of fit of regression equation was supported by high regression coefficient value (0.9998), low standard error of estimate (0.591) and low calculated *F*-value (calculated *F*(5,42)-value - 0.244 and critical *F*-value - 2.437 at $P = 0.05$ level of significance). Lower values of parameters like standard error of slope, intercept, and estimate indicated high precision of the proposed method.

The emission spectrum of gatifloxacin was not changed in the presence of common excipients in selected medium. When the relative fluorescence intensity of same concentration of pure drug sample and formulation sample were compared by paired t-test (at 8 df), the calculated t-value (0.244) was found to be less than that of the critical t-value (2.306), indicating that statistically there was no significant difference between mean relative fluorescence intensities. The interference of excipients was insignificant in the estimation of drug. Therefore proposed method was specific and selective for the drug.

All the three concentration levels showed accuracy ranged from -0.221 to 0.060% (Table 2). The high (nearly 100%) mean % recovery values and their low standard deviation values (SD < 1.5) represented accuracy of the method. In standard addition method, the mean percentage analytical recoveries (±SD) for 20, 40, 60, 80, and 100 ng/ml concentrations were found to be 100.54 (±1.649), 98.70 (±0.701), 99.22 (±1.957), 101.47 (±0.951), and 100.38 (±0.568), respectively. This result revealed the validity and reliability of the proposed method.

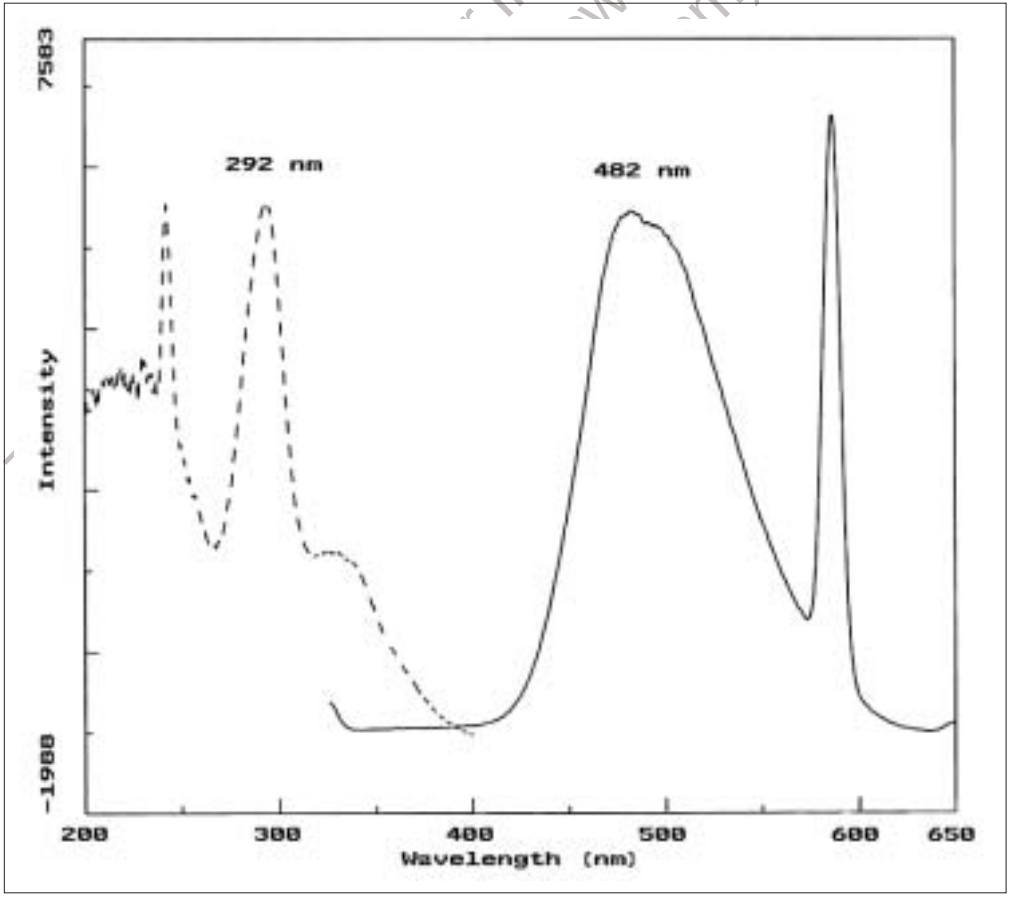

Fig. 1: Excitation and emission spectra of gatifloxacin
Excitation spectra indicated by (---) and emission spectra indicated by (—). Concentration of gatifloxacin was 160 ng/ml in selected medium. Gatifloxacin was estimated at λ_{exc} of 292 nm and λ_{em} of 482 nm in selected medium

TABLE 4: RESULTS OF ASSAY OF GATIFLOXACIN IN PHARMACEUTICAL FORMULATIONS

Commercial products	Amount found	% Assay
Gaity-200 tablets (200 mg) Mean ± SD (mg)	203.17 ± 0.81	101.59 ± 0.41
Gaity-400 tablet (400 mg) Mean ± SD (mg)	405.42 ± 4.67	101.36 ± 1.17
Gatilox concentrated injection (10 mg/ml) Mean ± SD (mg/ml)	9.98 ± 0.07	99.83 ± 0.67
Ophthalmic solution (0.3% w/v = 3 mg/ml)[a] Mean ± SD (mg/ml)	3.00 ± 0.02	99.89 ± 0.67

[a]Formulation was prepared in laboratory. Each value is average of five separate determinations

In repeatability study, the RSD was ranged from 0.153 to 1.142% (Table 2). At all three concentration levels, precision showed satisfactory levels. Intermediate precision expresses within-laboratory variations in different days and by different analysts. Results of intermediate precision study, RSD values for each set (all three levels) were given in Table 3. In all the cases the RSD values were not more than 2.151%. RSD values within the acceptable range indicating that these methods have excellent repeatability and intermediate precision.

DL and QL were found to be 5.48 and 16.61 ng/ml, respectively. Robustness was found to be very high as variation of pH of the selected media by ±0.1 did not have any significant effect on relative fluorescence intensity. The mean percentage of recovery (±SD) was found to be 99.94 (±0.826). The gatifloxacin solution in selected medium exhibited no spectrofluorimetric changes for 8 h when kept at room temperature.

The proposed method was evaluated by estimation of gatifloxacin in pharmaceutical formulations. The assay values of gatifloxacin for different formulations ranged from 99.83 to 101.59% with standard deviation not more than 1.17. Assay values of formulations were very close to the label claim. This indicated that the interference of excipient matrix is insignificant in estimation of gatifloxacin by the proposed method (Table 4).

In summary, the proposed method was sensitive, simple, rapid, accurate, precise, and inexpensive and can be used for routine analysis of gatifloxacin in bulk, pharmaceutical formulations and dissolution studies of oral and ophthalmic formulations. Detection limit of the proposed method is lower than the reported UV-Spectrophotometric method[3] and HPLC method.[8] The sample recoveries in all formulations were in good agreement with their respective label claims, indicating non-interference of excipients in the estimation. The usage of any organic solvent for extraction of gatifloxacin from the formulations is not required.

ACKNOWLEDGMENTS

The authors are grateful to University Grant Commission (UGC), New Delhi for their financial support. The authors are thankful to Venkar Chemicals Pvt. Ltd., Hyderabad for providing gatifloxacin as a gift sample.

REFERENCES

1. Bauernfeind, A., **J. Antimicrob. Chemother.**, 1997, 40, 639.
2. Ednie, L.M., Jacobs, M.R. and Appelbaum, P.C., **Antimicrob. Agents Chemother.**, 1998, 42, 2459.
3. Salgado, H.R. and Oliveira, C.L., **Pharmazie.**, 2005, 60, 263.
4. Wallace, A.W., Victory, J.M. and Amsden, G.W., **J. Clin. Pharmacol.**, 2003, 43, 92.
5. Vishwanathan, K., Bartlett, M.G. and Stewart, J.T., **Rapid Commun. Mass Spectrum.**, 2001, 15, 915.
6. Trampuz, A., Laifer, G., Wenk, M., Rajacic, Z. and Zimmerli, W., **Antimicrob. Agents Chemother.**, 2002, 46, 3630.
7. Liang, H., Kays, M.B. and Sowinski, K.M., **J. Chromatogr. B**, 2002, 772, 53.
8. Overholser, B.R., Kays, M.B. and Sowinski, K.M., **J. Chromatogr. B**, 2003, 798, 167.
9. The European Agency for the Evaluation of Medical Products. ICH Topic Q2B Note for Guidance on Validation of Analytical Procedures: Methodology GPMP/ICH/281/95, 1996.
10. United States Pharmacopoeia: Validation of compendial methods, Pharmacopoeial Convention, Inc., Rockville, MD, 2003, 2439.
11. Bolton, S., Eds., In; Pharmaceutical Statistics: Practical and Clinical Applications, 3rd Edn., Marcel Dekker, New York, 1997, 216.

Accepted 20 November 2006
Revised 7 March 2006
Received 20 June 2005
Indian J. Pharm. Sci., 2006, 68 (6): 726-730