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DEVELOPMENT AND VALIDATION OF A REVERSED–PHASE HPLC METHOD FOR THE ANALYSIS OF GATIFLOXACIN IN BULK AND ITS PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A rapid and sensitive high–performance liquid chromatographic method was developed for the estimation of gatifloxacin in bulk and its pharmaceutical formulations. Gatifloxacin was chromatrographed on a reverse phase C–18 column using ciprofloxacin as internal standard in a mobile phase consisting of acetonitrile and buffer pH 3.0 in the ratio of 25 : 75 v/v. The mobile phase was pumped at a flow rate of 1.0 mL / min, and the eluents were monitored at 293 nm. The calibration curve was linear in the range of 0.1–50 µg/mL. The intra– and inter–day variation was found to be less than 1% showing high precision of the assay method. The method was found to be simple, precise sensitive, less time consuming and accurate for the estimation of gatifloxacin in bulk and its pharmaceutical formulations.

Key words: Gatifolxacin, Ciprofloxacin, Pharmaceutical formulations, HPLC, Estimation.

INTRODUCTION

Gatifloxacin[1] (GTL) is a synthetic broad – spectrum 8–methoxyfluoroquinolone antibacterial agent for oral or intravenous administration. It offers enhanced gram–positive activity and anaerobic coverage compared to other fluoroquinolones. Chemically GTL is (±) –1 –cyclopropyl – 6–fluoro–1, 4–dihydro–8–methoxy –7– (3–methyl–1–piperzinyl)–4–oxo–3–quinolinecarboxylic acid sesquihydrate. The literature survey reveals that few HPLC[2–9], and spectrophotometric[10] methods were reported for its analytical monitoring in either biological fluids or formulations. This paper reports a sensitive and precise HPLC method for the determination of GTL in bulk samples and pharmaceutical formulations by using a C 18 column [Bondapak C–18 (250 x 4.6 mm, packed with 5 micron) mobile phase combination acetonitrile: buffer pH 3.0 – 25 : 75 (1 liter water + 1.6 mL orthophosphoric acid and pH adjusted with

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triethylamine to 3.0)] and internal standard [ciprofloxacin]. The results were found to be accurate, reproducible and free from interference and better than the earlier reported methods.

EXPERIMENTAL

Chemicals: Gatifloxacin was obtained as a gift samples from Torrent Pharmaceuticals, Ahmedabad, India. HPLC grade acetonitrile and water was from Qualigens. Commercially available tablets of gatifloxacin were selected from the local market on a random basis. These tablets normally contain common additives like diluents (lactose, aerosil, etc) glidants and lubricants (magnesium sterate, etc).

Equipment: For LC estimation, an isocratic HPLC waters with water 510 HPLC pump, equipped with a 20 µL sample loop, and water 486 tunable absorbance detector. The output signal has monitored and integrated using Millenium NT workstation software was used.

Chromatographic conditions: The chromatographic column used was a reverse phase 4.6 x 250 mm Bondapak C 18 HPLC column (waters) with 5 µm particles. The column and the HPLC system was kept in ambient conditions. The mobile phase was acetonitrile : buffer pH 3.0–25 : 75 delivered at a flow rate of 1.0 mL/min. The injection volume was 20 µL. The elute was analyzed at a wavelength of 293 nm.

Method development: To develop a rugged and suitable LC method for the analysis of gatifloxacin in formulations, different solvents systems were used. The criteria employed for assessing the suitability of a particular solvent system for the drug was cost, time required for analysis, sensitivity of the assay, solvent noise, preparatory steps involved and use of the same solvent system for extraction of the drug from the formulation excipients matrix for estimation of the drug content.

Preparation of standard curve : A stock solution (1000 µg/mL) of pure drug was prepared by dissolving 100 mg of gatifloxacin and 100 mg of ciprofloxacin (internal standard) separately in 100 mL volumetric flasks containing 70 mL of triple distilled water, sonicated for at least 15 min and then made upto volume with triple distilled water. Daily working standard solutions of gatifloxacin and internal standard were prepared by suitable dilution of the stock solution with appropriate mobile phase.

Composition and flow rate of the mobile phase was programmed from mother pump and the mobile phase acetonitrile : buffer pH 3.0 (25 : 75) was passed through the 0.45 µm membrane filter using Millipore HPLC solvent filtration assembly, was delivered at 1.0 mL/min for column stabilization. During this period, the baseline was continuously monitored. The wavelength of detection was selected at 293 nm. The prepared dilutions containing (0.1 µg/mL, 1.0 µg/mL, 2.0 µg/mL, 4.0 µg/mL, 6.0 µg/mL, 8.0 µg/mL. 10 µg/mL, 20 µg/mL, 40 µg/mL) gatifloxacin and fixed concentration (4µg/mL) of internal standard were injected serially. The peak area ratios to the internal standard were calculated. The stability of the solution of

gatifloxacin during analysis was determined by repeated analysis of samples during the course of the experiment of the same day and also on different days after storing at laboratory bench conditions and in the refrigeration. The results are listed in Table 1. Chromatogram parameters, retention time and asymmetry factor, were standardized.

Table 1. Calibration curve points of the proposed method for estimation gatifloxacin

Concentration of the solution (µg/mL)	Peak area ratio*	CV (%)
0.1	0.429	0.88
1.0	0.4394	0.41
2.0	0.8189	0.54
4.0	1.642	0.42
6.0	2.452	0.85
8.0	3.278	0.37
10.0	4.079	1.21
20.0	8.1569	0.68
40.0	16.349	0.74

* Mean of six determinations
Regression equation: Y= 0.01867 + 0.40652 X, (r = 0.9999)

Method validation

Accuracy and precision: Five separate solutions of gatifloxacin (5 µg/mL) standard and test solution were prepared in duplicated from freshly prepared stock solution and analyzed as per the procedure.

Linearity: Five separate series of solutions of the drug 0.1–40 µg/mL were prepared form the stock solution and analyzed.

Specificity: Series of five solutions of the drug in 5 µg/mL were prepared from the stock solution meant for the method validation and analyzed.

Limit of detection (LOD) and quantitation (LOQ): LOQ and LOD were calculated on the basis signal to noise ratio. Experiments are performed to analyze the actual concentration that can be accurately quantified or detected by the method.

Ruggedness: It was determined for the method by varying the analyst, instrument and different columns of it, for the proposed method.

Robustness: For the robustness of method, the % of acetonitrile was varied (25, 30, 45%) and the effect on retention time and peak parameters was studied.

Estimation of gatifloxacin from the commercial tablets by the proposed method: Commercially available tablets of gatifloxacin were taken randomly form the Indian market for estimation of total drug content per capsule by the proposed method. 20 tablets were weighed, contents were thoroughly mixed and accurately weighted. Aliquot amount (equivalent to 100 mg gatifloxacin) was dissolved in 20 mL of water. The weighed amount 100 mg of active ingredient was extracted with water and made to get a stock solution of 1 mg/mL. This solution was filtered through a 0.45 µm membrane filter. This solution was further diluted stepwise with mobile phase as under preparation of standard solution to get different concentrations required. The area under the curve, the drug content per tablet (on an average weight basis) was calculated. The results are tabulated in Table 3.

RESULTS AND DISCUSSION

For the determination of gatifloxacin different mobile phases were employed. Initially a mobile phase consisting of acetonitrile : buffer in the ratio of 85 : 15 was tried. Symmetry RP – C18 column 250 mm was used. Early elution with tailing of peaks were observed in the above condition. Then the composition of mobile phase was changed to 70 : 30. Under these conditions, broad peaks shape and pronounce tailing was observed. For the same mobile phase, if the ratio was changed to 25 : 75, gatifloxacin was eluted at around 3.34 min with symmetric peak shape.

A typical chromatogram for gatifloxacin using C18 RP HPLC column with mobile phase, composed of acetonitrile : buffer (25 : 75) at 1.0 mL/min flow rate is shown in Fig. 1. The λ_{max} of detection was fixed at 293 nm so that there was less interference from mobile phase with

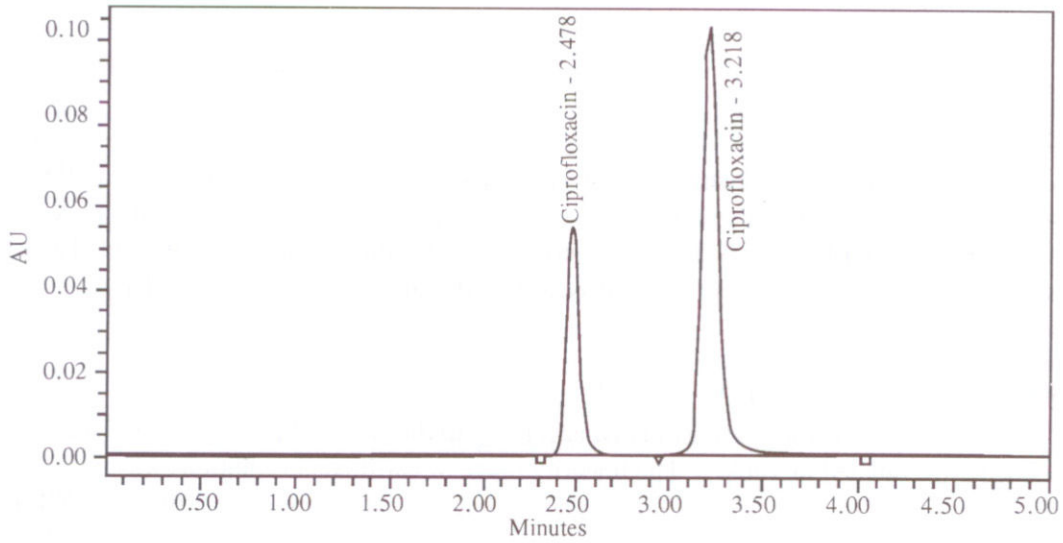

Fig. 1 : A typical chromatograph of Gatifloxacin

highest sensitivity according to UV analysis. The statistical analysis[11] of data obtained for the estimation of gatifloxacin in pure solution indicated a high level of precision of the proposed method.

The peak area ratio to the internal standard verses concentration (µg/mL) was found to be linear. Values obtained for the calibration curve points and their standard deviation, coefficient of variance and standard error are presented in Table 2. The drug was stable during analysis and for a period of 48 h stored at room temperature and under refrigerated conditions in acetonitrile : buffer (25 : 75) mixture.

Table 2. Optical and regression characteristics, precision and accuracy of the proposed hplc method for GTL

Parameter	Method
Detection wavelength (nm)	293
Linearity range (µg/mL)	0.1 – 40
Detection limits (µg/mL)	0.07837
Regression equation (Y = a + bX)	
Slope (b)	0.40652
Standard deviation of slope (S_b)	0.00216
Intercept (a)	0.01867
Standard deviation of intercept (S_a)	0.01062
Standard error of estimation (S_e)	0.01236
Correlation coefficient	0.9999
% error in bulk sample**	0.1112

** Average of three determinations.

The linear regression equation obtained for the proposed method was $Y = 0.01867 + 0.40652 \times X$, ($r = 0.9999$) where Y is the peak area ratio to the internal standard and X is the concentration of gatifloxacin. The correlation coefficient value was highly significant (Table 2). The retention time and asymmetry factor were found to be 3.34 ± 0.04 min and 1.18 ± 0.041, respectively.

Validation of the developed method

The developed method was validated according to the standard procedures and the results obtained are tabulated in Table 3. The linearity range of gatifloxacin solutions was found to be 0.1 – 40 µg/mL. The limit of detection (LOD) (50 ng/mL) and limit of quantitation (LOQ) 75 ng/mL) are given in Table 3. The proposed method has found to be rugged when analyst or equipment of column was varied.

Table 3. Validation report for the determination of gatifloxacin in standard solutions by HPLC method

Analytical parameter	Results
Accuracy (%)	99.83 ± 0.15
Precision (%)	99.08
	99.12
	99.79
	99.67
	99.88
	RSD* = 0.38
Linearity	0.1–40 (µg/mL)
Specificity	A 4µg/mL solution of gatifloxacin will give an area of 304462 ± 0.42 at 293 nm using RP–C 18 column in, acetonitrile buffer (pH 3.07 (25 : 75) mobile phase at a flow rate of 1.0 mL/min.
Limit of detection	50 ng / mL
Limit of quantitation	75 ng/ mL
Ruggedness (%)	98.93 ± 0.52

** Relative standard deviation

Recovery studies

The method was evaluated by estimation of gatifloxacin in pharmaceutical formulations by the proposed method and analysis of pure drug solution as reference. The results are presented in Table 4. The estimated drug content with low values of standard deviation established the precision of the proposed method. The accuracy of results of estimation was further tested by recovery experiments by adding known amount of pure drug to pre–analyzed samples of the formulations. The average accuracy was found to be 99.65%. Common formulation excipients in the concentration normally used did not effect. Recovery experiments using the developed assay procedures further indicated the absence of interference from commonly encountered pharmaceutical excipients used in the selected formulations.

Table 4. Estimation of GTL in tablets

Pharmaceutical formulation	Labeled amount (mg)	Amount obtained by proposed method	% Recovery of proposed method
Tablets	200	200.60	100.31
Tablets	400	398.12	99.94

CONCLUSIONS

The proposed method was found to be simple, precise, accurate and rapid for determination of gatifloxacin in pure form and its pharmaceutical formulations. As the LOQ of the proposed method is very low (75 ng/mL), the method can be adopted for sensitive quality testing and dissolution studies. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non–interference of formulation excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of gatifloxacin in pure form and its dosage forms and can also be used for dissolution or similar studies.

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