ESTIMATION OF GATIFLOXACIN IN PHARMACEUTICAL DOSAGE FORMS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT
A simple, rapid, sensitive and precise High Performance Liquid Chromatographic (HPLC) method has been developed for the estimation of gatifloxacin in its pharmaceutical dosage forms. In this method RP-C₁₈ column (150mmx4.6mm I.D., 5µm particle size) with mobile phase consisting of acetonitrile and 0.05M phosphate buffer in the ratio of 25:75 v/v in isocratic mode was used. The detection wavelength is 293nm and the flow rate is 0.8ml/min. Ciprofloxacin is used as internal standard. The linearity was found to be in the range of 0.1 to 10 µg/ml and shows a correlation coefficient of 0.9999. The intra- and inter-day variation was found to be less than 1.2% showing high precision of the assay method. Hence, this method is simple, fast, specific, accurate, precise and less time consuming for the estimation of gatifloxacin in pharmaceutical dosage forms.

Keywords: HPLC, Gatifloxacin, Estimation, Pharmaceutical dosage forms.

INTRODUCTION
Gatifloxacin(GTF)¹ is a broad-spectrum antimicrobial fluoroquinolone that is active against both gram-positive and gram-negative bacteria, is used in the treatment of wide range of infections. Chemically GTF² is 1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quino lonecarboxylicacid. GTF acts by inhibiting bacterial DNA synthesis by inhibiting DNA gyrase³. Literature survey reveals that a few methods based on Spectrophotometric⁴, Spectrofluorimetric⁵, HPTLC⁶ and HPLC⁷⁻¹⁵ have been reported for the determination of GTF. In this present study a simple, rapid, accurate, sensitive and precise HPLC method was developed for the estimation of GTF in pharmaceutical dosage forms.

EXPERIMENTAL
GTF was a gift sample from M/s Panacea Biotec. Ltd., New Delhi and Ciprofloxacin was a gift sample from M/s Dr. Reddy's Lab Ltd., Hyderabad. Acetonitrile of HPLC grade (E- Merck) and Triple distilled water was used. All other reagents used in the study were of AR quality (E- Merck).
mark. Daily working standard solutions of GTF and ciprofloxacin were prepared by suitable dilutions of the stock solution with the mobile phase.

**Calibration solutions**

Nine sets of the GTF solutions were prepared in mobile phase at concentrations of 0.1, 0.2, 0.4, 0.6, 1, 2, 4, 6 and 10 µg/ml along with a fixed concentration (4 µg/ml) of ciprofloxacin as internal standard.

**Procedure**

The standard solutions prepared as above were filtered through 0.45 µm membrane filter and the filtrate was injected (20 µl) six times into the column at a flow rate of 0.8 ml/min. The ratio of GTF peak area to that of internal standard for each of the drug concentrations was calculated. The regression of the GTF concentration over the ratio of GTF peak area to that of internal standard was obtained. This regression was used to estimate the amount of GTF in pharmaceutical dosage forms.

GTF solutions containing 2 µg/ml, 4 µg/ml and 6 µg/ml were subjected to the proposed HPLC analysis for finding out the intra- and inter-day variations. The recovery studies were carried out by adding known amount of GTF to the preanalysed samples and subjecting them to the proposed HPLC method.

**Estimation of GTF in Pharmaceutical dosage forms**

**Tablets:** Twenty tablets were weighed and powdered. An accurately weighed portion of the powder, equivalent to 100 mg of GTF was transferred to a 100 ml volumetric flask containing 50 ml of 0.1M HCl. The contents of the flask were sonicated to dissolve GTF, made up to volume to get a solution of 1 mg/ml and the resulting solution was filtered through 0.45 µm membrane filter.

**Injections:** An aliquot of injection equivalent to 100 mg of GTF was transferred into a 100 ml volumetric flask and volume was made up to 100 ml with a 0.1M HCl to get a solution of 1 mg/ml. Further dilutions were made with mobile phase to get a working standard solution of 100 µg/ml.

Aliquots of this solution was transferred to volumetric flasks and made up to sufficient volume with mobile phase to give an expected concentration along with a fixed concentration (4 µg/ml) of internal standard. This solution (20 µl) was injected six times into the column. The mean values of peak area ratio of GTF to internal standard of six such determinations were calculated and the GTF content in the tablet and injection dosage forms were quantified using the regression equation obtained below. The same procedure was used to estimate the concentration of the GTF in two more commercial brands.

**RESULTS AND DISCUSSION**

The present study was carried out to develop a specific, sensitive, precise and accurate HPLC method for the analysis of GTF in pharmaceutical dosage forms. A typical chromatogram is shown in Fig. 1. The retention times for GTF and internal standard (ciprofloxacin) were 3.21 and 2.48 min respectively. Each of the samples were injected five times and the same retention times were observed in all cases. The ratio of peak area of GTF to peak area of internal standard for different concentrations as above were calculated and the average values for six such determinations are shown in Table 1. The peak areas of both the GTF and internal standard were reproducible as indicated by low coefficient of variation (0.42 %). A good linear relationship (r = 0.9999) was observed between the concentration of GTF and the respective ratio of peak areas. The regression of GTF concentration over its peak area ratio was found to be $Y = 0.0061 + 0.6083X$ (Where $Y = \text{ratio of peak area of GTF to that of internal standard}$; $X = \text{concentration of GTF}$) in the range of 0.1-10 µg/ml.

When GTF solution containing 2, 4 and 6 µg/ml were analysed by the proposed HPLC method for finding out intra- and inter-day variation, a low coefficient of variation was observed (Table 2). This shows that the present HPLC method is highly precise. The amounts of GTF from the preanalysed samples containing known amounts of the GTF are shown in Table 3. About 100.5 % of GTF could be recovered from the preanalysed samples indicating the high accuracy of the proposed HPLC method.

The GTF content in the pharmaceutical dosage forms tablets and injections were quantified using the proposed analytical method. The mean content of GTF in four brands of dosage forms are shown in the Table 4. The absence of additional
peaks indicates no interference of the excipients used in the dosage forms. The dosage forms were found to contain 103.37 to 104.25 % of the labelled amount. The low % CV indicates the reproducibility of the assay of GTF in pharmaceutical dosage forms.

Table 1: Calibration of the HPLC method for the estimation of GTF

<table>
<thead>
<tr>
<th>Concentration of GTF (µg/ml)</th>
<th>Mean peak area ratio (n = 6)</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.0682</td>
<td>0.95</td>
</tr>
<tr>
<td>0.2</td>
<td>0.1265</td>
<td>0.42</td>
</tr>
<tr>
<td>0.4</td>
<td>0.2504</td>
<td>0.47</td>
</tr>
<tr>
<td>0.6</td>
<td>0.3772</td>
<td>0.54</td>
</tr>
<tr>
<td>1</td>
<td>0.6020</td>
<td>0.36</td>
</tr>
<tr>
<td>2</td>
<td>1.2242</td>
<td>0.97</td>
</tr>
<tr>
<td>4</td>
<td>2.4662</td>
<td>1.21</td>
</tr>
<tr>
<td>6</td>
<td>3.6255</td>
<td>0.44</td>
</tr>
<tr>
<td>10</td>
<td>6.0984</td>
<td>1.53</td>
</tr>
</tbody>
</table>

Regression equation (from 0.1 to 10 µg/ml), \( Y = 0.0061 + 0.6083X \) (r = 0.99997)

Fig. 1: Typical Chromatogram of Gatifloxacin
### Table 2: Precision of the proposed HPLC method

<table>
<thead>
<tr>
<th>GTF concentration (µg/ml)</th>
<th>Concentration of GTF (µg/ml) found on</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intra-day Mean (n = 6) (%) CV</td>
<td>Inter-day Mean (n = 6) (%) CV</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.15 0.93</td>
<td>2.05 1.20</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4.07 1.75</td>
<td>3.98 0.97</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6.12 1.21</td>
<td>6.03 0.75</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3: Recovery of GTF

<table>
<thead>
<tr>
<th>Amount of drug added (µg)</th>
<th>Mean amount found (n = 6)</th>
<th>Mean % of recovery (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1.99 ± 0.02</td>
<td>99.5 ± 1.0</td>
</tr>
<tr>
<td>4</td>
<td>4.02 ± 0.08</td>
<td>100.5 ± 2.06</td>
</tr>
<tr>
<td>6</td>
<td>5.98 ± 0.10</td>
<td>99.6 ± 1.6</td>
</tr>
</tbody>
</table>

### Table 4: Assay of GTF in different dosage forms

<table>
<thead>
<tr>
<th>Dosage form</th>
<th>Labell ed amount (mg)</th>
<th>Mean amount found (mg) by the proposed method (n=6)</th>
<th>Mean % labelled amount (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tab-I</td>
<td>400</td>
<td>413.5 ± 0.10</td>
<td>103.37 ± 0.03</td>
</tr>
<tr>
<td>Tab-II</td>
<td>200</td>
<td>207.25 ± 0.15</td>
<td>103.625 ± 0.12</td>
</tr>
<tr>
<td>Inj-I</td>
<td>400</td>
<td>417 ± 0.07</td>
<td>104.25 ± 0.01</td>
</tr>
<tr>
<td>Inj-II</td>
<td>400</td>
<td>415.3 ± 0.29</td>
<td>103.82 ± 0.01</td>
</tr>
</tbody>
</table>

### CONCLUSION

The proposed HPLC method enables quantitative determination of third generation fluoroquinolones such as gatifloxacin in pharmaceutical dosage forms. Preparation of samples is easy and efficient. UV detection at 293nm was found to be suitable without any interference from tablets or injections excipients or solvents. All calibration curves were found to be linear with correlation coefficient of 0.9999. Analytical results of samples were in accordance with those of standard solution in the same concentrations. The proposed HPLC method is simple, specific, sensitive, accurate and precise and can be used in routine analysis in quality control laboratories.

### ACKNOWLEDGEMENTS

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REFERENCES


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