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Research Article

## Method Development and Validation of Gemifloxacin in Tablet Dosage Form by RP-HPLC

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### ABSTRACT

A simple, precise and accurate RP-HPLC method was developed and validated for the estimation of gemifloxacin in the tablet dosage form. The separation was achieved on a reversed-phase C-18 column (250 x 4.6 mm i.d., 5 μm) using a mobile phase consisting of acetonitrile/acetate buffer of pH 4.5 (70:30 v/v) at a flow rate of 1.0 ml/min and a detection wavelength of 244 nm. The separation was carried out on an isocratic mode at room temperature. The method was validated as per ICH guidelines for linearity, accuracy, precision, robustness, LOD, LOQ and specificity. The developed method showed good linearity over the concentration range of 50-150 μg/ml ( $r^2=0.995$ ). The average percentage recovery was 99.77%. The LOD and LOQ were 12.678 μg/ml and 14.261 μg/ml, respectively. Based upon validation studies, the developed method can be successfully applied for the routine analysis of gemifloxacin in bulk drugs as well as pharmaceutical dosage forms.

**Keywords:** Gemifloxacin, Tablet dosage form, RP-HPLC, Validation, ICH guidelines

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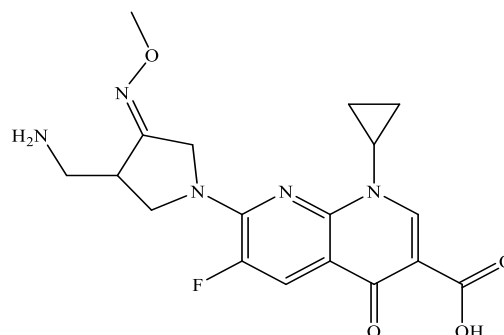
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### INTRODUCTION

Gemifloxacin is an oral broad-spectrum quinolone antibacterial agent used in the treatment of chronic bronchitis and mild-to-moderate pneumonia. Chemically, gemifloxacin is 7-[(4Z)-3-(aminomethyl)-4-(methoxyimino)pyrrolidin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (Figure 1)<sup>1,2</sup>. It is an important antibacterial compound fall under the class of fluoroquinolone antibiotics and is available as the mesylate salt in the sesquihydrate form. The bactericidal action of gemifloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, and recombination<sup>3,4</sup>. It occurs as white to off-white crystalline powder usually soluble in methanol, dimethyl sulfoxide, and water<sup>5</sup>. In this paper, a simple and specific RP-HPLC method was developed and validated (as per ICH guidelines) for the estimation gemifloxacin in the tablet dosage form.



**Figure 1:** Chemical structure of gemifloxacin

### MATERIALS AND METHODS

#### Chemicals

Acetonitrile, methanol and water (Merck, Rankem) used in the study were of HPLC grade. All other chemicals were of analytical grade. The gemifloxacin bulk drug was obtained as gift sample from Chandra Laboratories, Hyderabad. The tablet dosage form of gemifloxacin was procured from the local medical store.

## Apparatus and Instruments

UV-Visible spectrophotometer (Analytical Technologies Ltd.), HPLC (Waters), ultra sonicator (Citizen, Digital Ultrasonic Cleaner), pH meter (Elico) and electronic weighing balance (Shimadzu) were used for analytical studies.

## Chromatographic Parameters

Chromatographic analysis was performed on a RP-HPLC equipped with a pump, manual sampler and a UV detector. The chromatographic column was a Develosil Rp Aqueous-AR-5 C18 column (250 mm × 4.6 mm i.d., 5 μm). The column temperature was maintained at 25 °C. The mobile phase consisted of acetonitrile/acetate buffer (pH 4.5)/ (70:30% v/v). The separation was achieved on an isocratic mode at ambient temperature. The flow rate was 1.0 ml/min and the injection volume was 20 μl. The run time was 5.0 min. The wavelength of UV detection was set at 244 nm.

## Preparation of Solutions<sup>6,7</sup>

About 100 mg of gemifloxacin weighed into a 100 ml volumetric flask, added to this 25 ml of mobile phase, sonicated for 20 minutes and the volume was made up to the mark with the mobile phase to obtain a concentration of 1000 μg/ml. Further dilutions were made with the same

solvent to prepare a series of standard solutions in the concentration range of 50-150 μg/ml of gemifloxacin.

Twenty tablets were accurately weighed and finely powdered. Powder equivalent to 100 mg of gemifloxacin was accurately weighed, transferred into a 100 ml volumetric flask and dissolved in mobile phase to obtain a sample stock solution of 1000 μg/ml. The solution was sonicated for 20 minutes and filtered through Whatman filter paper. Using the stock solution, final sample solutions were prepared in the concentration range of 50-150 μg/ml using the same solvent.

## RESULTS AND DISCUSSION

Preliminary studies involved several trial runs using C<sub>18</sub> reversed-phase columns, various mobile phase compositions and different flow rates for the separation of gemifloxacin with optimum chromatographic parameters (resolution, symmetry, tailing factor etc.). A C<sub>18</sub> column (250 mm × 4.6 mm i.d., 5 μm.) stationary phase with a mobile phase consisting of acetonitrile/ acetate buffer (pH 4.5) (70:30 v/v) at a flow rate of 1.0 ml/min and a detection wavelength of 244 nm afforded the best separation with well-resolved and sharp peak. The summary of optimum chromatographic conditions is depicted in Table 1.

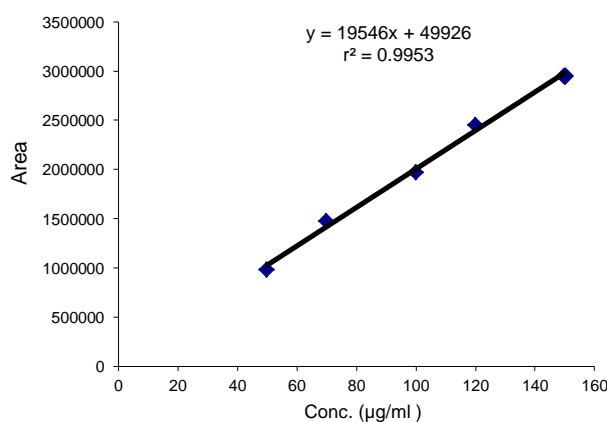
**Table 1:** Optimum chromatographic conditions

| Chromatographic parameter | Condition   |
|---------------------------|---|
| Mobile phase              | Acetonitrile/ammonium acetate buffer (pH 4.5) (70:30) |
| Column                    | Develosil Rp Aqueous-AR-5 (250 x 4.6 mm, 5 μm)        |
| Flow rate                 | 1 ml/min  |
| Column temperature        | Room temperature (20-25 °C)                           |
| Sample temperature        | Room temperature (20-25 °C)                           |
| Wavelength                | 244 nm  |
| Injection volume          | 20 μl   |
| Run time                  | 5 min.  |
| Retention time            | 3.084 min.  |

After method development, validation of the developed method was performed in terms of the following parameters: linearity, accuracy, precision, robustness, limit of detection (LOD), limit of quantitation (LOQ) and specificity<sup>8-12</sup>.

### Linearity

The linearity of the method was evaluated by analyzing six (n=6) calibration standards of gemifloxacin for a concentration range of 50-150 μg/ml at 244 nm. The plot of peak area versus concentration was found linear in the range of in the range of 50-150 μg/ml (Figure 2). The regression equation was obtained as follows:  $y = 19546x + 49926$  ( $r^2=0.9953$ ), where  $y$  = peak area,  $x$  = concentration of solution,  $r$  = the square of determined correlation coefficient. From results it is clear that the developed method is linear over the specified range.



**Figure 2:** Calibration curve of gemifloxacin

### Accuracy

The accuracy of the method was determined by percentage recovery assay. A known amount of each standard powder (with the same proportion as in the drug formulation) was added to blank sample composed of all the excipients equivalent to the ratio of the tablet formulation, which was then mixed, extracted and subsequently diluted to obtain three different levels of concentration (50, 100 and 150 µg/ml). The study was performed three times (n=3) at 50%, 100% and 150% concentration. The method was found to be accurate with % recovery of 99.77%. The RSD values of peak areas were found to be less than 1% at each level.

### Precision

The system precision and method precision were determined by analysing sample solution of gemifloxacin in six replications (n=6). The % RSD values for system and method precision studies were found to be within the acceptance criteria of less than 2.0%. Results indicate good precision of the developed method.

### Robustness

The robustness of the methods was investigated by analyzing six replicates (n=6) of sample solution by

deliberate variations in the UV spectrometric measurements such as temperature and chromatographic conditions such as flow rate. Results of robustness studies indicate that the developed method is practically robust. The % RSD values of the method determined under robustness conditions were less than 2.0%.

### LOD and LOQ

The LOD and LOQ for the developed RP-HPLC method were found to be 12.678 µg/ml and 14.261 µg/ml, respectively. Results of LOD and LOQ imply that the developed method is sensitive for the precise determination of gemifloxacin in the marketed formulation.

### Specificity

The standard and sample solutions (100 µg/ml) of gemifloxacin were injected and the chromatograms were recorded. No additional peaks due to impurities or other related substances were observed adjacent to the peak of interest in the chromatogram of gemifloxacin. The analyte peak for gemifloxacin was obtained with the retention time of 3.084 min. Result of specificity studies demonstrates that the RP-HPLC method is highly specific. Chromatograms also indicate that the method is free from interferences due to excipients and/or related components (Figure 3).

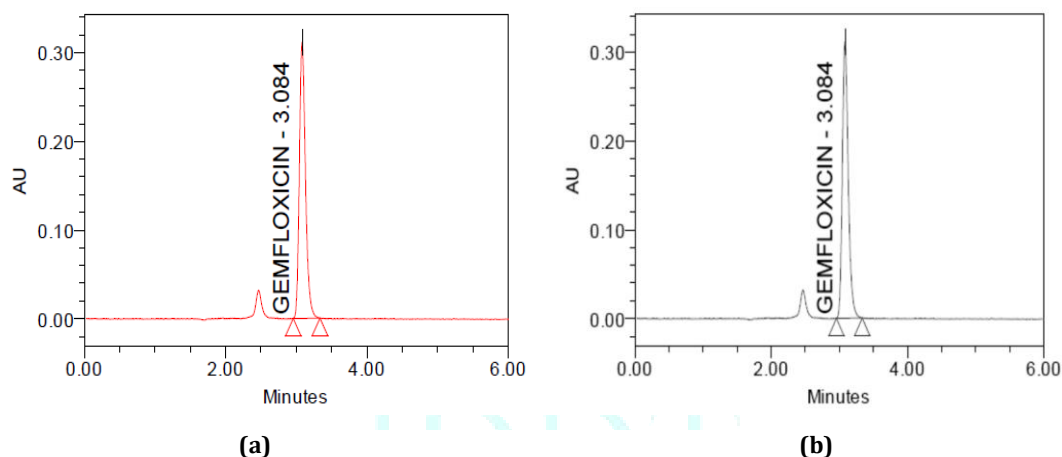


Figure 3: Chromatogram of standard (a) and sample (b)

### Assay

Weighed a quantity of powder equivalent to 100 mg of gemifloxacin in 100 ml volumetric flask and the volume was made up to the mark with mobile phase. From this stock solution, a sample solution of 100 µg/ml was prepared and used for the estimation of gemifloxacin. A chromatogram was recorded (Figure 4). The amount of gemifloxacin in the tablet dosage form was calculated from the peak area of recorded chromatogram. The amount estimated was in good agreement with the label claimed. The assay of gemifloxacin (% gemifloxacin) in the tablet dosage form was found to be 99.90%. The percentage purity of gemifloxacin was found to be within the standard limit i.e., 98-102 %.

### System suitability

System suitability was determined by injecting six replicate injections of the standard solution (100 µg/ml) of gemifloxacin<sup>13-15</sup>. Results of system suitability parameters depicted in Table 2 are within acceptable limit.

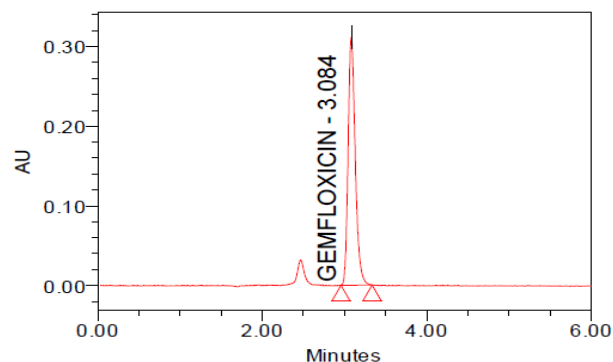


Figure 4: Assay chromatogram of gemifloxacin

**Table 2:** Results of validation parameters

| Parameter   | Result                     |                   |
|---|----------------------------|-------------------|
| Wave length of detection ( $\lambda_{\max}$ , nm) | 244                        |                   |
| Linearity   | Range ( $\mu\text{g/ml}$ ) | 50-150            |
|   | Slope                      | 19546             |
|   | Intercept                  | 49926             |
|   | Coefficient of correlation | 0.9953            |
| Accuracy  | % Recovery*                | %RSD (<2.0)       |
| 50%   | 99.13                      | Average:<br>99.77 |
| 100%  | 100.02                     |                   |
| 150%  | 100.18                     |                   |
| Precision   | Peak area**                | %RSD (<2.0)       |
| System precision                                  | 1964334.83                 | 0.13              |
| Method precision                                  | 1963997.00                 | 0.12              |
| Robustness**                                      | Peak area**                | %RSD (<2.0)       |
| Wavelength (nm)                                   | 0.13                       |                   |
| 242   | 2101531                    | 0.13              |
| 246   | 2101435                    |                   |
| Flow rate (ml/min)                                | 0.12                       |                   |
| 0.8   | 218891                     | 0.12              |
| 1.2   | 2182155                    |                   |
| LOD ( $\mu\text{g/ml}$ )                          | 12.678                     | 0.12              |
| LOQ ( $\mu\text{g/ml}$ )                          | 14.261                     | 0.12              |
| System suitability                                | Retention time (min.)      | 3.084             |
|   | Peak area                  | 1962155           |
|   | Theoretical plates         | 6193              |
|   | Tailing factor             | 1.266             |
| Assay*  | Amount (mg)                | % Estimated       |
| Label claim                                       | 320.0                      | 99.90             |
| Amount found                                      | 319.8                      |                   |

\*mean of three determinations (n=3)

\*\*mean of six replicate observations (n=6)

## CONCLUSION

In this paper, a RP-HPLC method was developed and validated for the estimation of gemifloxacin in the marketed tablet dosage form. The developed method was successful for the estimation of gemifloxacin in the tablet formulation. The proposed method is claimed to be simple, accurate and precise. The developed method is also reported to be highly valid, specific and reliable. Based upon validation studies, the proposed method can be applied for the routine analysis of gemifloxacin in bulk drugs as well as pharmaceutical dosage forms.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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