



## RP-HPLC METHOD FOR THE DETERMINATION OF GEMIFLOXACIN MESYLATE IN BULK AND PHARMACEUTICAL FORMULATION

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### ABSTRACT

A reverse phase high performance liquid chromatography (RP-HPLC) has been developed for the estimation of gemifloxacin mesylate in bulk drug and pharmaceutical dosage form. The quantification was carried out using C18 column and mobile phase consisting of acetonitrile: 20mM phosphate buffer at pH 3 (25:75v/v), at flow rate of 1 ml/min. The separation was performed at ambient temperature. Eluents were monitored by UV detector set at 275 nm. The method was statistically validated for the linearity, precision, accuracy, LOD and LOQ. The linearity was found to be in the range of 1-6 µg/ml. The proposed method was found to be simple, precise, accurate, rapid and reproducible for the estimation of Gemifloxacin mesylate in bulk drug and tablets.

**Keywords:** Gemifloxacin mesylate, RP-HPLC method, Gemistar, validation.

### INTRODUCTION

Gemifloxacin mesylate (Gemi) is a third group of fluoroquinolone active against *s.pneumonia*. It is useful in treatment of chronic bronchitis and mild to moderate pneumonia. It is chemically, (R, S)-7-[4z-3-(amino methyl)-4-(methoxy imino)-1-pyrrolidiny]-1-cyclopropyl-6-fluoro-1, 4 dihydro-4-oxo-1, 8- naphthyridine-3-carboxylic acid<sup>1</sup>. A through survey of literature revealed only LCMS was reported for the determination of Gemifloxacin mesylate in human plasma<sup>2</sup>. The objective of this study was to develop a rapid and sensitive RP-HPLC method for the analysis of Gemifloxacin mesylate in bulk drug and in its tablet formulation using the most commonly employed RP-C<sub>18</sub> column with UV-detection.

### MATERIALS AND METHODS

The pure drug sample of Gemi was obtained as gift sample from Orchid Pharmaceuticals, Chennai. The formulation (Gemistar) used was purchased from local pharmacy. Qualigens fine chemicals, Mumbai, supplied HPLC grade acetonitrile, methanol and water, sodium dihydrogen orthophosphate AR grade and Phosphoric acid AR grade. An isocratic high pressure liquid chromatograph (Schimadzu HPLC class VP series) with LC-10 ATVP pump, variable wavelength programmable UV/Vis detector SPD-10 AVP system and operating software winchrome was used. The chromatography column used was a reverse phase phenomenax C<sub>18</sub> column (250mm × 4.6 mm i.d, particle size 5µ). A mixture of acetonitrile and 20mM phosphate buffer (adjusted to pH 3 using ortho phosphoric acid) in the ratio of 25:75 v/v was used as mobile phase and was filtered before use through 0.45µ membrane filter. The flow rate of mobile phase was maintained at 1 ml/ min. Detection was carried out at 273nm at ambient temperature.

### Preparation of standard stock solution:

About 25 mg of Gemi was weighed accurately and dissolved in a minimum quantity of mobile phase and the total volume was brought to 25 ml with more mobile phase to get the concentration of 1000 µg/ml.

### Assay procedure:

From the standard solution, 1 ml was transferred into a 100ml standard flask and made up to the mark to produce 10 µg/ml. To the series of five 10ml standard flask, added (1 - 6ml) of above solution and made up to the mark to obtain the concentration range from 1 to 6µg/ml and the calibration curve was plotted between concentration and peak area.

### Quantification of gemifloxacin mesylate in formulation:

Twenty tablets containing 320mg of Gemi were accurately weighed and finely powdered. An accurately weighed sample of powdered tablets equivalent to 100mg of Gemi was placed in a 100ml volumetric flask, added 25ml mobile phase and shaken vigorously for few minutes and repeated the extraction consequently by three times (3×25) to produce 100 ml with mobile phase and filtered through a 0.45 µ membrane filter paper. Further dilutions were made to get a concentration of 4 µg/ml of drug solution. An aliquot of 20 µl of test solution was injected and the amount of Gemi was calculated from the calibration curve.

### RESULTS AND DISCUSSION

The development of an analytical method for the determination of drug by RP-HPLC has received considerable attention in recent years because of their importance in quality control of drugs and drug products.

The mobile phase containing acetonitrile and phosphate buffer pH 3, in the proportion 25:75v/v was selected



because it was found to give a peak for Gemi with minimal tailing. With the above mentioned composition of mobile phase, sharp peak was achieved with reasonable short run time of 10 min. The criteria employed for assessing the suitability of above said solvent system were cost. Time required for analysis, solvent noise, preparatory steps involved in the use of same solvent system for the extraction of the drug from formulation excipient matrix for the estimation of drug content. UV detection was carried out at 273 nm as Gemi showed good absorbance at this wavelength. The retention time for Gemi was found to be 5.54 min. A typical chromatogram of test solution is shown in Fig.1.

The peak shape was symmetrical and asymmetry factor was less than 2. When the concentrations of Gemi and its respective peak areas were subjected to regression analysis by least squares method, a good linear relationship ( $r=0.9996$ ) was observed between the

concentration of Gemi and the respective peak areas in the range of 1-6 mcg/ ml. The regression of Gemi was found to be  $Y = -124569x + 1909476.2$ , where Y is the peak area and X is the concentration of Gemi. The regression equation was used to estimate the amount of Gemi either in tablet formulations or in validation study. The RP-HPLC method developed in the present study has been used to quantify Gemi in tablet dosage forms. Gemi tablets were analyzed as per procedure described above and the average drug content was found to be 99.59% of the labeled amount (Table 1). The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on the standard deviation of response of the calibration curve. The standard deviation of y-intercepts of regression lines and slope of the calibration curves were used to calculate LOD and LOQ. The LOD and LOQ values are 0.56917 and 1.7247  $\mu\text{g/ml}$  respectively.

**Table 1:** Quantification of formulation (Gemistar)

Labeled amount (mg/tab)	Amount found (mg)	Percentage obtained	S.D	%RSD
320	319.40	99.50	0.35276	0.35421
320	318.07	99.30		
320	318.40	99.50		
320	317.60	99.20		
320	320.00	100.00		
320	320.70	100.02		

**Table 2:** Recovery studies of formulation (Gemistar)

Amount present ( $\mu\text{g/ml}$ )	Amount added ( $\mu\text{g/ml}$ )	Total amount Present ( $\mu\text{g/ml}$ )	Amount recovered ( $\mu\text{g/ml}$ )	% recovery
1.97	0.5	2.49	0.52	99.59%
1.97	1.0	2.99	1.02	
1.97	1.5	3.50	1.53	
1.98	2.0	3.86	1.94	
2.00	2.5	4.50	2.50	

The proposed method was validated as per the standard analytical procedures<sup>3</sup>. Each sample was injected six times and the retention times were same. Accuracy of the method was calculated by recovery studies ( $n=3$ ) at five levels. Standard drug solution containing drugs in the range 25%, 50%, 75%, 100% and 125% of nominal concentration was added to previously analyzed test solution. Amount of drug recovered at each level was calculated. The sample recovery in the formulation (Table 2) was in good agreement with the label claim.

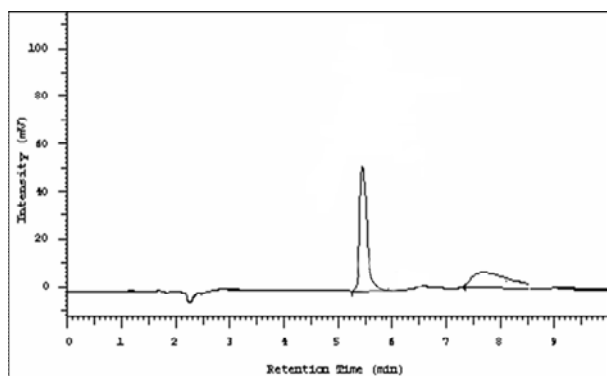
High percentage recovery showed that the method was free from interference of the excipients used in the formulation. System suitability parameters of the proposed method for Gemi are given in Table 3. No interfering peaks were found in the chromatogram

indicating that excipients used in the tablet formulation did not interfere with the estimation of the drug by the proposed RP-HPLC method.

**Table 3:** System suitability parameters

Parameter	Gemifloxacin mesylate
Retention Time	4.97
Tailing factor	1.62
Capacity factor	3.83
Asymmetric factor	1.81



**Figure 1:** Typical chromatogram of the sample solution

The results of the study indicate that the proposed HPLC method is simple, precise, accurate and less time consuming. Therefore, this method can be applied for the routine quality control analysis of Gemifloxacin mesylate in its tablets dosage forms.

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