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A Simple, Rapid and Validated Reverse Phase High Performance Liquid Chromatographic Method for the Estimation of Gemifloxacin in Pharmaceutical Dosage Form

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Abstract: A simple, specific and precise reverse phase HPLC method was developed and validated for the determination of gemifloxacin present in pharmaceutical dosage form. Separation of the drug was achieved on a reverse phase X terra C_{18} 5 µm column having 4.6 mm × 15 mm id in isocratic mode with mobile phase containing acetonitrile and phosphate buffer in the ratio of 40:60 (v/v). The pH of the final mobile phase consisting buffer was adjusted to 3.5 with dilute phosphoric acid. The compound was eluted at a flow rate of 0.5 ml/min and effluent was monitored at wavelength 272 nm. The retention time of gemifloxacin was reported to be 5.164 min. Calibration curve for gemifloxacin was linear over the concentration range of 0.01– 0.50 µg/ml. The limit of detection and limit of quantification were found to be 0.06 and 0.19 µg/ml, respectively. The mean recovery of gemifloxacin was 99.9% for pharmaceutical preparation. The results of the proposed method have been validated statistically. The proposed reverse phase high performance liquid chromatography method was found to be simple, reproducible, accurate and economical. The developed and validated RP-HPLC method can be used successfully for the routine quality control analysis of commercial gemifloxacin to quantify the drug.
Keywords: RP-HPLC, Gemifloxacin, Estimation, Validation, Tablet dosage form.

Introduction

Gemifloxacin is a synthetic broad-spectrum fourth generation fluoroquinolone antibacterial compound having affinity towards bacterial topoisomerase IV and DNA gyrase[1]. This quinolone antibacterial agent is available as the mesylate salt in the sesquihydrate form and is used in the treatment of chronic bronchitis and community-acquired pneumonia[1-4]. It is also used for the treatment of the respiratory and urinary tract infections [5-7]. Gemifloxacin has been shown to be active against both Gram-positive and Gram-negative microorganisms [8-11]. The bactericidal action of gemifloxacin results by preventing DNA synthesis through the inhibition of both DNA gyrase and topoisomerase IV enzymes, which are required for bacterial growth[12]. Gemifloxacin belongs to the naphthyridine carboxylic acids and derivatives. These are compounds containing a naphthyridine moiety, where one of the ring atoms bears a carboxylic acid group[13]. Chemically, gemifloxacin is (R,S)-7[(4Z)-3-(aminomethyl)-4-(methoxyimino)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo1,8-naphthyridine-3-carboxylic acid[13,14] (Figure 1).

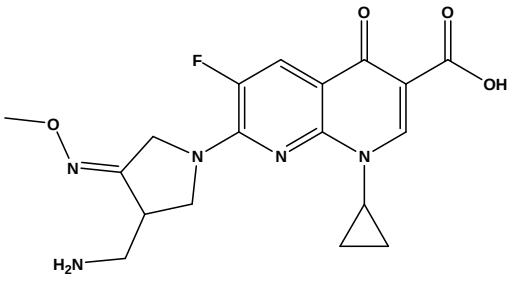

Figure 1. Chemical structure of gemifloxacin

A review of the literature revealed that a number of analytical methods have been reported for the determination of Gemifloxacin in pharmaceutical dosage forms and biological samples which include potentiometry[15], spectrophotometry[16-18], spectrofluorimetry[19], capillary electrophoresis[20], high performance liquid chromatography (HPLC)[21], HPTLC[22], liquid chromatography coupled with mass spectrometry[23] and reverse phase liquid chromatography[24,25] methods. The objective of the present study is to develop a rapid, accurate, time consuming and economical reverse-phase high performance liquid chromatographic method for the routine quality control analysis of Gemifloxacin to quantify the drug in the pharmaceutical dosage form using C_{18} X terra column and simple mobile phase and validate the above method as per ICH guide lines[26, 27].

Experimental

Chemicals and reagents

Standard gemifloxacin was obtained as a gift sample from Glenmerck Generics India Ltd. and was used as reference standard without further purification. The pharmaceutical dosage forms used in the study was EG1 (Cipla Ltd.) tablet containing 320 mg of gemifloxacin from retail shop.

All solvents were of HPLC grade and reagents were of analytical grade. Potassium di-hydrogen phosphate and dipotassium hydrogen phosphate of AR grade and acetonitrile of HPLC grade were purchased from Merck Ltd., Mumbai. HPLC grade water was used for the preparation of buffer and other solutions obtained from Aurium 611 UV water purification system of Sartorius, Germany.

Equipments

Quantitative HPLC determination was performed on a Waters Alliance e2695 separation module and Waters 2489 dual lambda absorbance detector. The separation was carried out on a C_{18} (150 mm × 4.6 mm, 5 µm) X terra column. Chromatograms were analyzed by using Empower-3- software.

Chromatographic conditions

Mobile phase was prepared with 5.3 mM phosphate buffer solutions and acetonitrile (60: 40) v/v. The pH was adjusted to 3.5 ± 0.1 with orthophosphoric acid. All solutions, including mobile phase, were sonicated during 15 min and filtered through a 0.45 µm membrane filter (Millipore) before use. A rheodyne injector with a 10 µl loop was used for the injection of standard and sample solutions of gemifloxacin. The pump flow rate was 0.5 ml /min. The column temperature was maintained at room temperature (24 ± 2°C). The eluent was detected at 272 nm.

Preparation of standard solution

Standard solution of gemifloxacin was prepared by taking 12.5 mg of gemifloxacin in 25 ml volumetric flask with 10 ml HPLC grade water and one drop of conc. HCl followed by sonication for 15 minutes and the final volume was made by mobile phase. 1 ml of the above solution was taken in a 25 ml volumetric flask and diluted up to the mark by mobile phase in order to obtain solution with final concentration of 0.02 mg/ml. The contents of standard solution were filtered through 0.45 µm syringe filter.

Preparation of sample solution

The method was applied to the analysis of commercial sample available in the market as EG1 tablet. Twenty tablets of gemifloxacin were individually weighed, mean weight was determined and tritruanted to a homogeneous mixture. An amount of powder mass equivalent to 12.5 mg of gemifloxacin was weighed accurately and transferred to a 25 ml volumetric flask. About 10 ml of HPLC grade water and 1 drop of conc. HCl were added to the volumetric flask and sonicated for 15 minutes. The final volume was made up to the mark by mobile phase. The resulting solution was filtered through Whatman filter paper no. 1. One ml of the filtered solution was transferred to another 25 ml volumetric flask and diluted up to the mark with mobile phase in order to obtain solution with final concentration of 0.02 mg/ml of gemifloxacin (theoretical value). The content of sample solution was filtered through 0.45 µm syringe filter.

Analytical method validation

The proposed method was validated for the assay of gemifloxacin in formulation using following parameters.

System suitability

A system suitability test of the chromatography system was performed before each validation run. The standard solution of gemifloxacin was scanned in the UV range of 200-400 nm and its wave length of maximum absorbance was found to be 272 nm (Table 1). For measuring system suitability, six replicate injections of the standard solution were made and the respective chromatograms were obtained. The retention time, tailing factor, theoretical plates, area relative standard deviation were determined (Table 1).

Table 1. Sample suitability parameter

Parameters	Gemifloxacin
Wavelength of the max absorbance (nm)	272.0
Retention Time (mins)	5.164
Tailing factor	0.65
Theoretical Plate	11326
LOD (µg/ml)	0.06
LOQ (µg/ml)	0.19

Linearity

Linearity of an analytical method is the ability of that method to elicit test results that are directly proportional to the concentration of the analyte. The linearity of the proposed method was determined at six concentrations level ranging from 0.01 to 0.5 µg/ml for gemifloxacin. The calibration curve was constructed by plotting response factor against concentration of drugs. The correlation coefficient (r) for gemifloxacin was calculated (Table 2).

Table 2. Linearity parameters

Parameters	Gemifloxacin
Linearity range (µg/ml)	0.01 to 0.50
Regression coefficient	0.97
Slope	2089
Intercept	1623

Assay

For analysis of the drug in commercial formulations EG1 tablet was taken. As per the label claim, commercial tablet contain 320 mg of gemifloxacin. The quantification of the drug was done in formulation by injecting 10 µl of standard and sample solution separately on HPLC system. Chromatograms of standard solution (six replicates) and sample solution (two replicates) were recorded. A typical chromatogram of

gemifloxain was presented in Figure 2. The concentration of the drug in commercial formulation was calculated by comparing area of the sample solution with that of the standard solution. Results were recorded in Table 3.

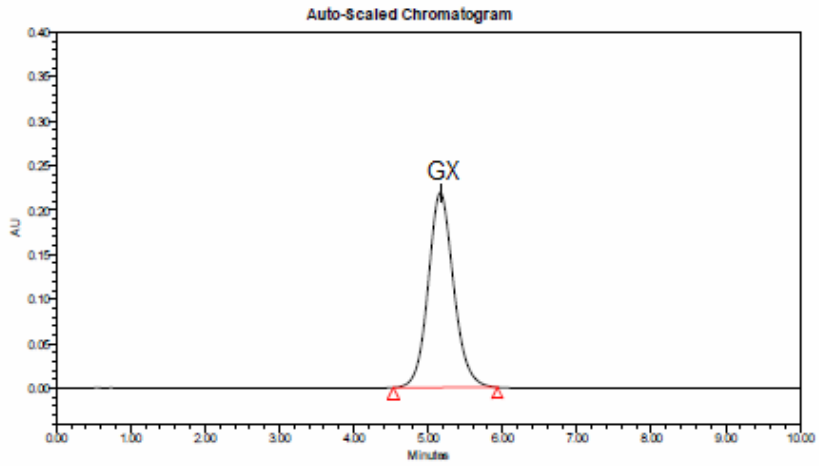

Figure 2. Representative chromatogram for Gemifloxacin (retention time = 5.164)

Table 3. Accuracy parameters (recovery studies)

Tablet Formulation	Drug	Labelled Amount of Drug (mg/tab)	Amount mg/tab found	% label claim (n =6)	% RSD	Recovery Studies (n = 3)				
						Total Amt. after spiking (mg)	Amt recovered (mg) Mean ± SD	% Recovery	% Mean Recovery	% RSD
EG1Tab (Cipla Ltd.)	Gemifloxacin	320	318.99	99.68	0.54	288 352 384	288.79±2.22 350.23±2.29 383.99±1.89	100.27 99.49 99.99	99.92	0.63

Precision

The precision of the method was demonstrated by intra-day and inter-day studies. In the intra-day precision studies, six repeated injections of standard solutions were made on the same day. In the inter-day variation studies, six repeated injections of same standard solution were made for three consecutive days. The percentage RSD with respect to the peak area, peak retention time and the amount were calculated for each case and presented in Table 4.

Table 4. Precision parameters

Parameters	Intra-day	% RSD	Inter-day			
			Day1	Day2	Day3	% RSD
Peak Area	733971	0.08	733801	733011	732871	0.06
Peak RT	5.164	0.10	5.152	5.161	5.159	0.07
Amount (mg/Tablet)	318.99	0.10	318.88	318.51	318.50	0.05

Accuracy

To ascertain the accuracy of the proposed method recovery studies were carried out by standard addition method. In this recovery test known amount of standard solutions (90%, 110%, and 120%) were added to the pre analysed sample followed by analysis using the proposed method. Recovery studies were carried out in triplicate and the percentage recovery and standard deviation of the percentage recovery were calculated and given in Table 3.

LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) decide about the sensitivity of the method. LOD is the lowest detectable concentration of the analyte by the method while LOQ is the minimum quantifiable concentration. The LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. LOD and LOQ for the drug were calculated by using the values of slopes and intercepts of the calibration curves and presented in Table 1.

Stability

Stability studies of the sample was done by keeping the sample at room temperature, 4°C and -20°C refrigeration temperature for 8 h (short-term) and refrigerated at 4°C and -20°C for 72 h (long-term). The results of stability studies were given in Table 5.

Table 5. Stability of gemifloxacin in Solution

Tablet Formulation	Drug	Labelled Amt. of Drug (mg/tab)	Room Temp Stability		Refrigeratory Stability + 4°C		Frozen Stability - 20°C	
			% Label Claim ± RSD		% Label Claim ± RSD		% Label Claim ± RSD	
EGI	Gemifloxacin	320.0	8 h	24 h	24 h	72 h	24 h	72 h
			99.32±1.7	98.92±1.2	99.42±1.9	100.12±1.2	100.33±1.0	99.51±1.9

RSD: Relative standard deviation of six replicate determinations.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variation in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was investigated under a variety of conditions including changes of composition of buffer in the mobile phase, pH of the mobile phase and flow rate.

Ruggedness

In order to demonstrate the ruggedness, analysis was performed on different days and different chemists by taking different instruments and using different columns of similar type.

Results and Discussion

The present work involves estimation of gemifloxacin in tablet dosage form using reverse phase high performance liquid chromatography. The chromatographic conditions were optimized to get best resolution and peak shape. The selection of mobile phase was based on peak parameters like symmetry, theoretical plates and capacity factor (system suitability factor, Table 1). Symmetrical peaks with good separation and with retention time, 5.164 min (Figure 2) were obtained in C_{18} X terra column (4.6 x 150 mm, 5µm). The mobile phase was used at a flow rate of 0.5 ml/min. The optimum wavelength for detection and quantification was at 272 nm, at which good detector response was obtained for the drug. The proposed method was statistically validated as per ICH guide line[26,27]. The analytical procedure was found to be linear in the concentration range of 0.01 to 0.5 µg/ml with regression factor 0.97. The results were shown in Table 2. The results show that an excellent correlation exists between peak area and concentration of drug within the concentration range. Precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). The %RSD values for peak area, peak retention, time and amount of sample solution were calculated in each case and the results were given in Table 4. The low RSD values indicate that the method was precise. From the recovery study it was clear that the method was very accurate for quantitative estimation of gemifloxacin in tablet dosage form as all the statistical results were within the range of acceptance, 99.49 to 100.27, which shows that there is no

interference with excepients. Percentage recovery values were as given in Table 3. The LOD and LOQ were found to be 0.06 µg/ml and 0.19 µg/ml respectively. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters proved that the method was robust. The method was satisfactory with respect to ruggedness also.

Conclusion

A convenient, rapid, accurate, precise and economical RP-HPLC method was developed for estimation of gemifloxacin in tablet dosage form. The assay provides a linear response across a wide range of concentrations and it utilizes a mobile phase which can be easily prepared and diluents are economic, readily available. The proposed method can be used for the routine analysis of bulk preparations of the drug and in pharmaceutical dosage forms without interference of excepients in quality control laboratories.

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