Development and Validation of RP-HPLC Method for the Estimation of Gemifloxacin Mesylate in Bulk and Pharmaceutical Dosage Forms

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Abstract: A simple, rapid, accurate, precise and reproducible reverse phase high performance liquid chromatographic method has been developed for the estimation of gemifloxacin mesylate in bulk and pharmaceutical formulations. The quantification was carried out using cyberlab capcell pak, ODS C₁₈ (250 × 4.6 mm i.d., 5 µm particle size) column in an isocratic mode, with mobile phase comprising Buffer (KH₂PO₄ with pH 6.8): acetonitrile in the ratio of 80:20 (%v/v). The flow rate was at 1.2 mL/min and the detection was carried out at 265 nm. The retention time of the drug was found to be 7.47 min and the method produced linear response in the concentration range of 25-150 µg/mL (R~0.99986). The recovery studies were also carried out and % RSD from reproducibility was found to be 0.82. The proposed method was statistically evaluated and can be applied for routine quality control analysis of gemifloxacin mesylate in tablets.

Keywords: RP-HPLC, Gemifloxacin mesylate, Tablets, Estimation.

Introduction

Gemifloxacin mesylate is chemically (*R, S*)-7-[(4*Z*)-3-(amino methyl)-4-(methoxy imino)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-1, 8-naphthyridine-3-carboxylic acid (Figure 1) and its empirical formula is C₁₈H₂₀FN₅O₄•CH₄O₃S and molecular weight is 485.49. It is a synthetic broad-spectrum antibacterial agent. Gemifloxacin, a compound related to the fluoroquinolone class of antibiotics, is available as the mesylate salt in the sesquihydrate form. It is used to treat infections that are proven or strongly suspected to be caused by susceptible gram-positive and gram-negative bacteria¹⁻³.

Figure 1. Chemical structure of gemifloxacin mesylate

Literature survey reveals that the drug can be estimated only by LC-MS/MS⁴⁻⁵ and Chiral HPLC⁶ in biological fluids and also by microchip electrophoresis⁷, chiral counter-current chromatography⁸ and colorimetric method⁹ but no UV spectrophotometric method and HPLC method in pharmaceutical dosage forms have been reported so far. The present study describes a simple, sensitive, economical, accurate and precise HPLC method for the estimation of gemifloxacin mesylate in bulk and tablet formulation.

The objective of this study is to develop a simple, fast, selective, accurate, precise and sensitive RP-HPLC-UV method for the determination of gemifloxacin mesylate in bulk and in pharmaceutical dosage forms (tablets) suitable for routine quality control analysis.

Experimental

Chemicals and reagents

Gemifloxacin mesylate bulk drug was received as gift sample from Orchid Chemicals and Pharmaceuticals Ltd., Chennai, India. G-cin-320 mg tablets, manufactured by Lupin Laboratories Ltd., Mumbai and Gemez-320 mg tablets, manufactured by Glenmark Pharmaceuticals Ltd., Mumbai, India were procured from local Pharmacy. HPLC grade water, methanol and acetonitrile were purchased from Merck, Mumbai, India. Potassium dihydrogen phosphate from S.d.fine chemicals ltd., India.

Instrumentation

The method development study was carried out isocratically on a high performance liquid chromatograph using Cyber Lab LC-100 separation module equipped with a rheodyne injector 7725i, single pump, 20 μ L fixed sample loop, 25 μ L Hamilton syringe and detection was carried out using ultraviolet detector. Cyberlab digital balance was used for weighing purpose.

Optimized chromatographic conditions

Chromatographic separation was carried out at room temperature with Capcell Pak ODS C₁₈ (250 $\times$ 4.6 mm with 5 μ m particles) column. Mobile phase containing buffer (KH₂PO₄ adjusted with pH 6.8): acetonitrile in the ratio of 80:20 (%v/v), were filtered through 0.45 μ m membrane filter and degassed in a sonicator for 10 min before use. The flow rate of mobile phase was maintained at 1.2 mL/min and detection was done using UV detector at 265 nm. The injection volume of both standards and samples were 10 μ L (100 μ g/mL).

Procedure

Preparation of standard

A standard stock solution containing 1 mg/mL of gemifloxacin mesylate was prepared by completely dissolving 50 mg of pure drug of gemifloxacin mesylate in 50 mL of methanol in a 50 mL volumetric flask. A working standard solution containing 100 μ g/mL was prepared by diluting 2.5 mL of stock solution (1000 μ g/mL) into a 25 mL of mobile phase.

Calibration curve of gemifloxacin mesylate

Linearity solutions ranging of 25-150 $\mu\text{g/mL}$ of gemifloxacin mesylate were prepared from the above standard stock solution (1000 $\mu\text{g/mL}$) by diluting into 10 mL volumetric flasks with mobile phase. Initially the mobile phase was pumped for 30 min to saturate the column there by to get the baseline corrected as shown in Figure 2. Then solutions prepared as above were filtered through 0.45 μ membrane filter and then 10 μL of the filtrate was injected each time into the column at a flow rate of 1.2 mL/min. Evaluation of the drug was performed with UV-visible detector at 265 nm after the drug solution of 10 $\mu\text{g/mL}$ in methanol was scanned in UV-visible spectrophotometer SL-164 in the range of 200-370 nm against methanol as blank and found λ_{max} at 265 nm as show in Figure 3. Peak area was recorded for all the peaks. The plot of peak area vs. the respective drug concentration gives the calibration curve. The retention time of gemifloxacin mesylate standard was found to be 7.47 minutes as shown in Figure 4.

Figure 2. Chromatogram of gemifloxacin mesylate blank

Figure 3. Absorption spectrum of gemifloxacin mesylate (10 $\mu\text{g/mL}$) in methanol

Figure 4. Chromatogram of gemifloxacin mesylate standard

Analysis of gemifloxacin mesylate in tablet dosage forms

Twenty tablets each containing 320 mg gemifloxacin mesylate were accurately weighed and powdered. A quantity of the powder equivalent to 100 mg was taken into a 100 mL volumetric flask and 60 mL methanol was added. Then solution was sonicated for 10 min, dissolved and then made up to the volume with the methanol and filtered through a 0.45 μ membrane filter. Then 10 mL of the above filtrate was transferred into a 100 mL volumetric flask and diluted to the mark with mobile phase to obtain working standard solution of 100 μ g/mL. Then 10 μ L of the above solutions were injected each time into the column at a flow rate of 1.2 mL/min. The retention time of gemifloxacin mesylate samples were found to be 7.50 and 7.58 minutes as shown in Figure 5 and 6.

Figure 5. Chromatogram of gemifloxacin mesylate sample 1 (in tablet)

Figure 6. Chromatogram of gemifloxacin mesylate sample 2 (in tablet)

Results and Discussion

The present study was carried out to develop a simple, fast, accurate and precise RP-HPLC method for the analysis of gemifloxacin mesylate in bulk and in tablet dosage forms. For the determination of gemifloxacin mesylate, different compositions of mobile phases were employed. Initially, a mobile phase consisting of Methanol and Water in the ratio of 70:30 (%v/v) was tried where broad peak shape and more tailing was observed and a mobile phase consisting of acetonitrile and water in the ratio of 60:40 (%v/v) was tried where broad peak shape and less retention time was observed. Then the composition of mobile phase was changed to Phosphate buffer adjusted to pH 6.8 and methanol in the ratio of 70:30 (%v/v) but in these condition less retention time and broad peak shape was observed and a mobile phase consisting of phosphate buffer adjusted to pH 6.8 and acetonitrile in the ratio of 20:80 (%v/v) where less retention time and more tailing was observed. Similarly different trails were tried by adjusting compositions of buffer and acetonitrile but finally the ratio was changed to phosphate buffer adjusted to pH 6.8 and acetonitrile in the ratio of 80:20 (%v/v), where gemifloxacin mesylate was eluted at around 7.47 min with symmetric peak shape and shorter retention time. The results of system suitability parameters were given in the Table 1.

Table 1. Results of system suitability parameters of gemifloxacin mesylate in standard and in tablet formulations

S.No.	Parameter	Standard	Sample 1	Sample 2
1	Retention Time, Min	7.47	7.50	7.58
2	Peak area response	295944.4	294872.6	296208.3
3	Theoretical plates(n)	3922.3	4623.2	5656.5
4	Tailing factor(t)	1.76	1.89	1.74

Method validation

Linearity was determined from calibration graph plotted using peak area response *versus* concentration of the standard solutions and it was found to be obeyed in the concentration range of 25-150 µg/mL with a good linear relationship ($r=0.99986$) as shown in Figure 7. The regression curve was constructed by linear regression fitting and its mathematical expression was $y=2984.56x-1864.33$ (where y is the peak area and x is the concentration of gemifloxacin mesylate).

Precision of the developed method was studied by repeatedly injecting gemifloxacin mesylate standard and sample solutions for six times (n=6). The % RSD was found to be 0.82 and 0.78 respectively.

Figure 7. Calibration curve of gemifloxacin mesylate.

The drug content (Assay) in the tablets was quantified using the proposed RP-HPLC method. The amount (%Assay) of gemifloxacin mesylate in two different brands of tablet dosage forms is shown in Table 2. The tablets were found to contain 99.64% and 100.09% of the drug. It can be concluded that the proposed RP-HPLC method is sufficiently sensitive and reproducible for the analysis of gemifloxacin mesylate in tablet dosage forms within a short analysis time.

Table 2. Results of assay in marketed formulation

S. No.	Brand	Standard Peak Area	Sample Peak Area	Labelled amount (mg/tab)	Amount found (mg/tab)	% Assay
1	G-CIN	295944.4	294872.6	320	318.85	99.64
2	GEMEX	295944.4	296208.3	320	320.29	100.09
Mean					99.87	
% RSD					0.32	

The recovery experiment was carried out by spiking the already analyzed sample of the tablets with their different known concentration of standard. The average percentage recovery was calculated and results are summarized in Table 3.

Table 3. Results of Accuracy (Recovery studies, n=3)

S. No.	Test concentration, $\mu\text{g/mL}$	Amount added, $\mu\text{g/mL}$	Amount Recovered, $\mu\text{g/mL}$	Average % Recovery	% RSD
1	50	5	54.87	99.76	0.47
2	100	10	110.02	100.02	0.29
3	150	15	149.92	99.95	0.56

The developed method was validated according to the standard procedure and the summary of results obtained is presented in Table 4.

Table 4. Summary of Validation Parameters

S.No.	Parameters		Results
1	Linearity	25-150 $\mu\text{g/mL}$	Range, $\mu\text{g/mL}$
		0.99986	Correlation Coefficient (r)
		2984.56	Slope
		-1864.33	Intercept
		$Y=2984.56x-1864.33$	Regression Equation
2	System Precision (n=6)	0.82	% RSD
3	Method Precision (n=6)	0.78	% RSD
4	Accuracy	99.95	Mean % Average Recovery
5	Assay	99.87	Mean % Assay
6	Specificity	No interference of other peak	Specific

Conclusion

In this present study an attempt has been made to develop RP-HPLC method for the determination of gemifloxacin mesylate in tablet dosage form. The results obtained were reproducible and reliable. The validity and precision of the methods were evident from the statistical and analytical parameters obtained. From the forgoing it is concluded that the method developed is simple, rapid, selective, economical, accurate and precise and hence suitable for application in routine quality control analysis of Pharmaceutical preparations.

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