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RP-HPLC Method Development and Validation for the Determination of Gemifloxacin in Pharmaceutical Dosage Form

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ABSTRACT

A rapid, accurate, specific, linear, and sensitive reverse phase-HPLC method has been developed and validated for the determination of Gemifloxacin Mesylate (GEM) in pharmaceutical dosage form. The chromatographic separation was performed on Thermo-scientific Hypersil BDS C18 Column (250mm×4.6mm, 5µm particle size) using a mobile phase A: 10g/liter Ammonium acetate and Trifluoroaceticacid(1000:4v/v) and mobile phase B: Acetonitrile, Ammonium Acetate, Methanol and trifluoroaceticacid (45:45:10:4 v/v), at a flow rate of 1.5 ml/m in at 25°C column temperature with the detection wavelength at 267nm. The retention times of GEM was 9.8 minutes. The linearity was performed in the concentration range of 20.0-30.0µg/ml with a squared correlation coefficient of 0.9996. The percentage purity of GEM was found to be >99.5%. The Proposed method has been validated for specificity, linearity, precision, accuracy, ruggedness and robustness and were within the acceptance limit according to ICH guidelines and the developed method was successfully employed for routine quality control analysis in the pharmaceutical dosage forms.

Key words: *Gemifloxacin, RP-HPLC, Validation.*

INTRODUCTION

Gemifloxacin (GEM) 7-[(4Z)-3-(aminomethyl)-4-methoxyimino-pyrrolidin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic acid (**Fig. 1**) is a new fluroquinolone antibacterial compound with enhanced affinity for bacterial topoisomerase IV. It uses similar to those of ciprofloxacin. It is used for the treatment of respiratory and urinary tract infection. It is given orally, as the mesylate, for the treatment of community acquired pneumonia and acute bacterial exacerbations of chronic bronchitis. The compound has broad spectrum of activity against gram-positive and gram-negative bacteria [1]. Doses are expressed in terms of the base; 399 mg of gemifloxacin mesylate is equivalent to about 320 mg of gemifloxacin. The usual dose is 320 mg once daily for 5 days in patients with bronchitis or for 7 days in those with pneumonia. The length of time it needs to be taken and the dosage will depend on how the patient responds and what bacterial infection they have. (**Fig. 1**) [1-2].

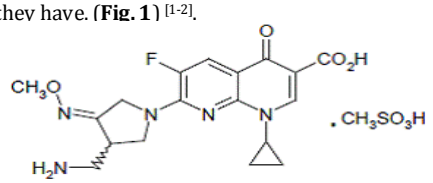

Fig. 1: Chemical Structure for Gemifloxacin Mesylate

Literature survey reveals that few HPLC methods [1-6], have been reported for the estimation of Gemifloxacin. The aim of the present study is to develop a simple, precise, linear and accurate reversed-phase HPLC method for the estimation of Gemifloxacin in pharmaceutical dosage form [7-8].

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MATERIALS AND METHODS

Instrumental and Analytical Conditions:
Reagents and Chemicals:

Gemifloxacin was purchased from Maithry Laboratories LTD. All chemicals used of HPLC grade: Acetonitrile and Methanol were purchased from J.T. Baker, and Trifluoroacetic acid which was purchased from Alfa Aesar. Water used was freshly prepared by Sama Pharmaceuticals Manufacturing Co.

Equipment:

A Dionex UltiMate 3000 HPLC system with Chromelen software "version 1.1", Photodiod Array Detector and Autosampler was used. It was manufactured by Dionex Corporation Company, USA.

Chromatographic Conditions:

The column Thermo-scientific Hypersil BDS C18 Column (250mm×4.6mm, 5µm particle size) was used for analytical separation. The mobile phase consisted of mobile phase A: 10g/liter Ammonium acetate and Trifluoroaceticacid (1000:4v/v) and mobile phase B: Acetonitrile, Ammonium Acetate, Methanol and Trifluoroaceticacid (45:45:10:4 v/v) with a gradient program as described in **Table 1**. The flow was adjusted to 1.5ml/min. The instrument was operated at 25°C temperature. The UV detection was achieved at 267nm and purity analysis was performed over a wavelength range of 200-400nm. The injection volume was 10µL.

Table No. 1: Gradient program for mobile phase

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0	80	20
7	40	60
14	40	60
14.1	80	20
17	80	20

Preparation of Analytical Solutions:

Preparation of mobile phase A:

To 1000ml of 10g/L Ammonium Acetate, add 4.0ml of Trifuoroaceticacid and degas in ultrasonic water bath for 5 minutes. Filter through 0.45µ filter under vacuum filtration.

Preparation of mobile phase B:

Mix 450ml of Acetonitrile, 450ml of Acetonitrile, 100ml of Methanol and 4ml of Trifluoroaceticacid and degas in ultrasonic water bath for 5 minutes. Filter through 0.45µ filter under vacuum filtration.

Preparation of diluent:

Mix 500ml of Acetonitrile and 500ml of Water and degas in ultrasonic water bath for 5 minutes. Filter through 0.45µ filter under vacuum filtration.

Preparation of Gemifloxacin (Mesylate) standard solution:

The Gemifloxacin(Mesylate) standard solution was prepared by dissolving Gemifloxacin Mesylate equivalent to 25.0mg Gemifloxacin as (Mesylate) standard in 100ml diluent, dissolved using sonicator, cooled to room temperature. Dilute 5ml of the resulting solution to 50ml with diluent, mix and filtered using 0.45µ filter to obtain a solution having a concentration of 25.0 µg/ml of Gemifloxacin as (Mesylate).

Preparation of sample solution:

10 tablets were weighed and the average weight (537mg) was calculated and finely powdered. 83.9 mg of powdered tablets (equivalent to 50 mg of Gemifloxacin as Mesylate) were transferred to 200 ml volumetric flask; 150 ml of diluent were added and mixed to dissolve the active ingredient by the aid of sonicator for 10 minutes. Cooled and the volume was completed with diluent. 5.0 ml of the resulting solution was diluted to 50.0 ml with diluent, mixed well and filtered using 0.45µ filter to obtain a solution having a concentration of 25.0 µg/ml of Gemifloxacin as (Mesylate)). Filter through 0.45µ filter.

Method Development and Validation of HPLC Method:

The suggested analytical method was validated according to ICH guidelines with respect to certain parameters such as specificity, linearity, precision, accuracy, and system suitability.

Specificity:

The specificity was carried out to determine whether there are any interference of any impurities (presence of components may be unexpected to present) in retention time of analytical peak. Forced degradation studies are carried out by using 0.1M NaOH, 0.2M HCl, thermal, hydrogen peroxide degradation and natural light.

Linearity:

Express ability to obtain test results where directly proportional to the concentration of analyte in the sample. The linearity of the method was established by a spiking a series of sample mixtures of GEM, the solutions of five different concentration levels 20.0-30.0 µg/ml of Gemifloxacin as Mesylate are injected into the HPLC system. Construct the calibration curves for the standard solutions by plotting their response ratios (ratios of the peak area of the analytes) against their respective concentrations linear regression was applied and slope-a, intercept-b, and correlation coefficient-R^2 were determined.

Precision:

Express the closeness of agreement between the series of measurement obtained from multiple sampling of same homogeneous sample under the prescribed conditions.

Method precision was determined both in terms of repeatability (injection and analysis) and intermediate precision/Ruggedness (It shows the degree of reproducibility of test results obtained by analyzing the sample under variety of normal test conditions such as analyst, instruments).

In order to determine precision, six independent sample solution preparations from a single lot of formulation 20µg/ml Gemifloxacin as Mesylate were injected in to HPLC system, the retention time and peak area was determined and expressed as mean and %RSD calculated from the data obtained which are found to be within the specified limits.

Accuracy:

Accuracy was determined in terms of percentage recovery the accuracy study was performed for 80%, 100% and 120 % for GEM. Standard and sample solutions are injected into HPLC system in triplicate and percentage recoveries of GEM were calculated. The area of each level was used for calculation of % recovery.

Robustness:

Robustness of the developed method was investigated by evaluating the influence of small deliberate variations in procedure variables like flow rate (± 6.6%), change in column temperature (± 5°C) and change in wave length (±5nm). The robustness was performed for the flow rate variations from 1.5ml/min to 1.6ml/min and 1.4ml/min and the method is robust even by change in the mobile phase B ±5%.

System suitability:

System suitability test was carried out on freshly prepared standard solution of GEM. Tailing factor, capacity factor and theoretical plates were calculated by injecting solution in six replicates and the values were recorded.

RESULTS AND DISCUSSION

The present investigation reported is a new RP-HPLC method development and validation of GEM estimation. The method developed was proceeding with wavelength selection. The optimized wavelength was 267nm.

In order to get the optimized RP-HPLC method various mobile phases and columns were used. From several trials final method is optimized with the following conditions:

The mobile phase consisted of mobile phase A:10g /liter Ammonium acetate and Trifluoroaceticacid1000:4v/v) and mobile phase B: Acetonitrile, Ammonium Acetate, Methanol and Trifluoroaceticacid (45:45:10:4 v/v) and the column used was Thermo scientific Hypersil BDS C18 Column (250mm×4.6mm, 5µm particle size). The flow rate was adjusted to 1.5ml/min. The instrument was operated at 25°C temperature. The UV detection was achieved at 267nm and purity analysis was performed over a wavelength range of 200-400nm. The injection volume was 10µL. The specificity of the method was to determine whether there are any interference of any impurities (the presence of components may be unexpected to present) in retention time of analytical peak. The linearity was determined as linearity regression of the claimed analyte concentration of the range 20.0-30.0µg/ml of Gemifloxacin as Mesylate . The calibration curve obtained by plotting peak area versus concentration and presented in **Table 2** was linear and the squared correlation coefficient was found to be 0.9996. The precision of the method was ascertained from determinations of peak areas of six replicates of sample solution. The %Relative Standard Deviation for system precision presented in **Table 3** was found to be 0.273 and the % Relative Standard Deviation for method precision presented in **Table 4** was found to be 0.296. The % Relative Standard Deviation for ruggedness presented in **Table 5** was found to be 0.217.

The accuracy study was performed in 80%, 100% and 120% .The percentage recovery was determined and was found to be 100.0% presented in **Tables 6**

The robustness were carried out with minor but deliberate changes in parameters i.e., detection wavelength, column temperature, and flow rate as presented in **Table 7**. Theoretical plates and tailing factor were observed and were found to be 109029 (theoretical plates) and -1.1 (tailing factor). And the Relative Standard Deviation in retention time were found to be- 0.08 in six replicate injections of standard solution

The system suitability parameters like theoretical plates, tailing factor and capacity factor were calculated and were found to be more than 2000, not more than 2 and more than 4 respectively

ascertained that proposed RP-HPLC method was accurate and precise as presented in **Table 8**.

Table No. 2: Linearity results for Gemifloxacin (Mesylate)

Concentration (µg/ml)	Area
20	12.846
22.5	14.587
25	16.313
27.5	17.948
30	19.537

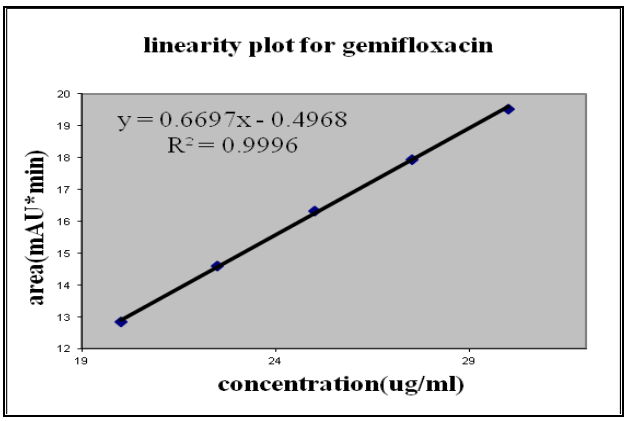

Fig. 2: Linearity plot for GEM

Table No.3: System precision for GEM

Injections	RT	Area
1	9.78	16.061
2	9.79	16.193
3	9.79	16.167
4	9.8	16.149
5	9.8	16.133
6	9.8	16.13
Average	9.793	16.139
Std. Dev.	0.0082	0.045
%RSD	0.083	0.277

Table No.4: Method precision for GEM

Injections	RT	Area
1	9.84	16.312
2	9.84	16.33
3	9.84	16.284
4	9.84	16.34
5	9.84	16.28
6	9.84	16.412
Average	9.84	16.3263
Std. Dev.	0	0.048
%RSD	0	0.296

Table No.5: Ruggedness values for GEM

Injections	RT	Area
1	9.81	16.203
2	9.81	16.124
3	9.81	16.135
4	9.81	16.128
5	9.81	16.147
6	9.82	16.099
Average	9.812	16.139
Std. Dev.	0.004	0.035
%RSD	0.042	0.217

Table No.6: %Recovery for GEM

Concentration (at spesific level)	Active drug sol added (µg/ml)	Recovery amount (µg/ml)	Mean Recovery
80%	20	19.99	
100%	25	25.04	100.00%
120%	30	29.99	

Table No.7: Robustness values for GEM

Parameter	Adjusted to	GEM	
		RT	Area
Flow rate	1.4	10.15	17.061
	1.5	9.78	16.313
	1.6	9.46	14.964
Mobile B started	-5%		
	no change	9.78	16.313
	+5%		
Column Temp.	20 C°	10.35	14.952
	25 C°	9.78	16.313
	30 C°	8.95	
Wavelength	262nm	9.84	13.862
	267nm	9.84	16.313
	272nm	9.84	17.549

Table No.8: System suitability values for standard solution

RT	Peak Area	Theoretical Plate	Tailing Factor	K-Factor
9.79	16.139	109029	1.1	4

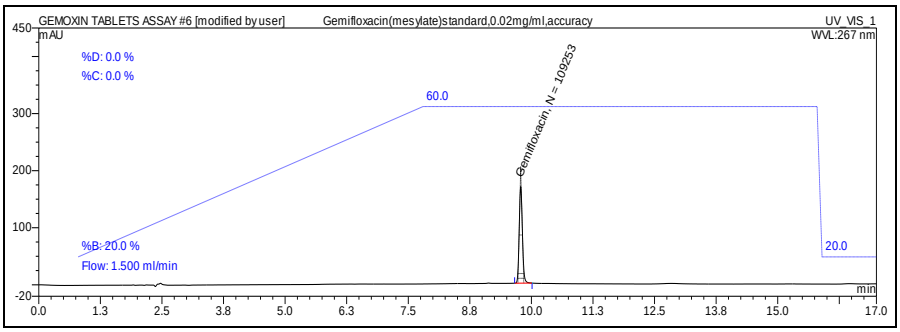

Fig. 3: Chromatogram for system suitability solution (standard solution)

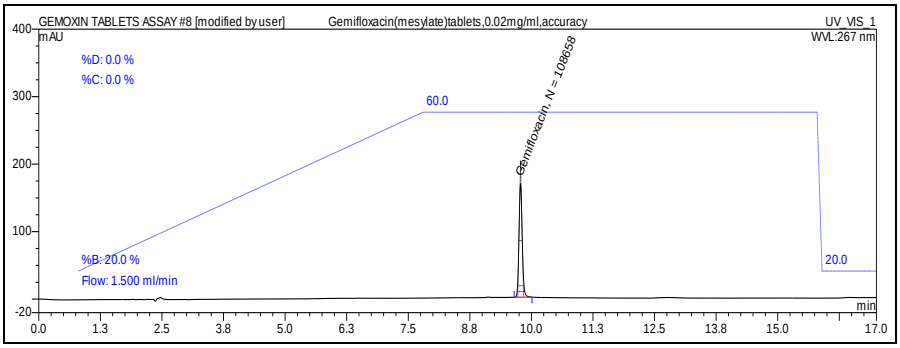

Fig. 4: Chromatogram for Gemifloxacin tablets test solution

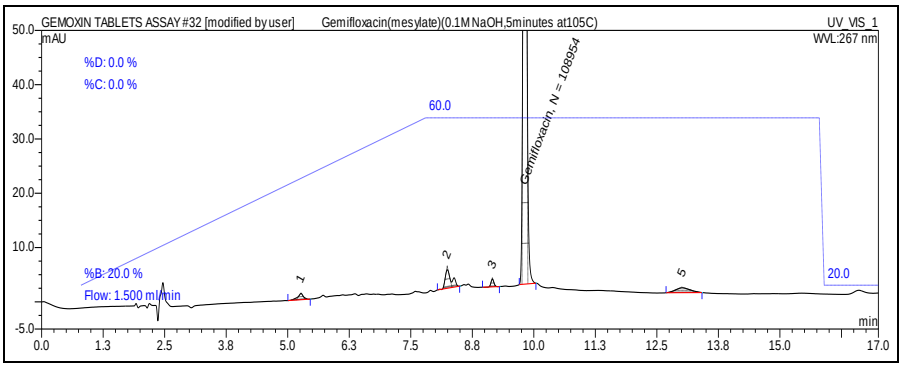

Fig. 5: Chromatogram for 0.1M NaOH Degradation for 5minutes at105°C

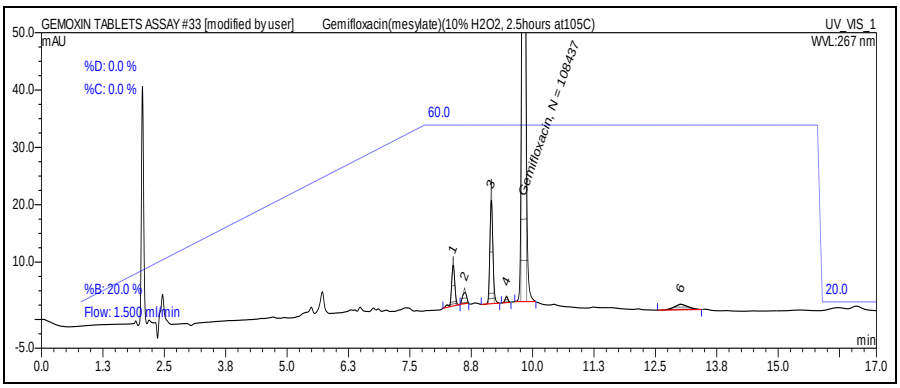

Fig. 6: Chromatogram for 10% H2O2 Degradation (2.5 hrs) at105°C

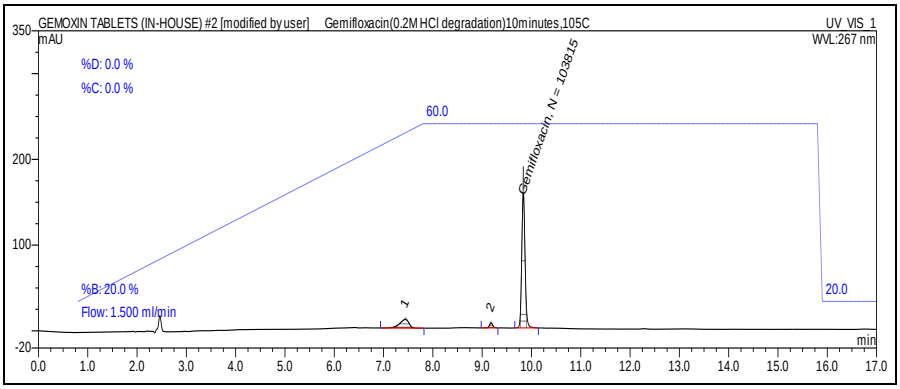

Fig. 7: Chromatogram for 0.2M HCl Degradation, 10minutes at105°C

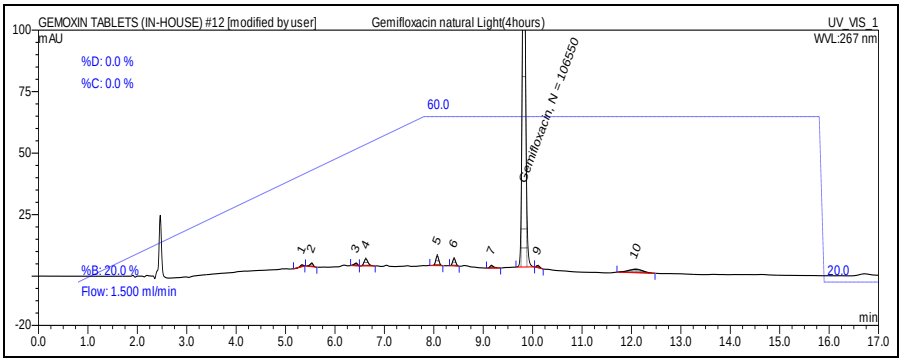

Fig. 8: Chromatogram for Light Degradation

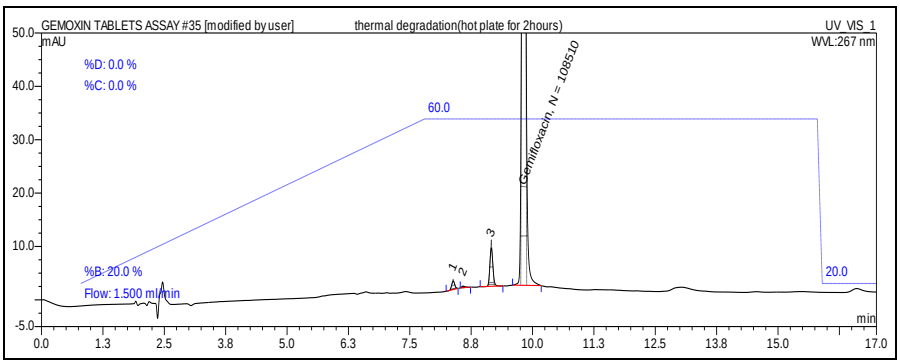

Fig. 9: Chromatogram for Thermal Degradation (heated 2 hours under reflux)

SUMMERY AND CONCLUSION

The method was found to be precise accurate and linear for determination of GEM. The method was developed and validated for system suitability linearity, specificity, accuracy, robustness and ruggedness. All parameters tested were found to be within limits. The study indicates that the method has a significant advantage in term of shorter analysis time, good resolution between active drugs and there degradation products and other system suitability parameters, high purity of active drug peaks, accuracy and precision.

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