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DEVELOPMENT OF A NEW CHROMATOGRAPHIC METHOD FOR ESTIMATION OF GEMIFLOXACIN IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract:

A rapid and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Gemifloxacin, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Hypersil C18 (4.6×150mm, 5µ) column using a mixture of Methanol (100% v/v) as the mobile phase at a flow rate of 0.8ml/min, the detection was carried out at 230nm. The retention time of the Gemifloxacin was 3.515 ±0.02min. The method produce linear responses in the concentration range of 35-175µg/ml of Gemifloxacin. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: *Gemifloxacin, RP-HPLC, validation, ICH guidelines, Linearity.*

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INTRODUCTION:

Gemifloxacin is an oral broad-spectrum quinolone antibacterial agent used in the treatment of acute bacterial exacerbation of chronic bronchitis and mild-to-moderate pneumonia. Gemifloxacin acts by inhibiting DNA synthesis through the inhibition of both DNA gyrase and topoisomerase IV, which are essential for bacterial growth. Gemifloxacin is indicated for the treatment of infections caused by susceptible strains of the designated microorganisms in the conditions listed below. Acute bacterial exacerbation of chronic bronchitis caused by S. pneumoniae, Haemophilus influenzae, Haemophilus parainfluenzae, or Moraxella catarrhalis. Community-acquired pneumonia (of mild to moderate severity) caused by S. pneumoniae (including multi-drug resistant strains, Haemophilus influenzae, Moraxella catarrhalis, Mycoplasma pneumoniae, Chlamydia pneumoniae, or Klebsiella pneumoniae. (Snyder, 1997) The literature survey revealed several reported analytical approaches for its determination in pharmaceutical dosage forms and in biological fluids. Various Spectrophotometric[1-7] and Spectrofluorimetric [8,9] methods were described. Chromatographic methods such as Capillary electrophoresis[10] and LC-MS [11] were also reported. Only few methods were reported for RP-HPLC [12,13] for the estimation of this drug in bulk and in its formulation. Hence the present work targeted to develop a new precise, accurate and sensitive RP-HPLC [14-18] method for the determination of Gemifloxacin in API and formulation. The developed method validated as per ICH guidelines[19,20].

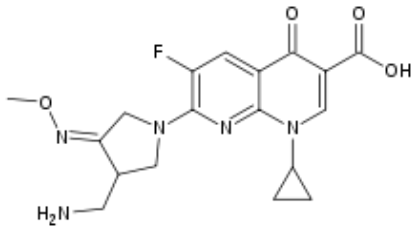

Figure 1: structure of Gemifloxacin

MATERIALS AND METHODS:

Chemicals and reagents used

Gemifloxacin as pure standard reference drug was obtained from SURA labs, Hyderabad, India. Acetonitrile, Water and Methanol used were of HPLC grade and purchased from MERCK specialties Private Limited, Mumbai, India.

Chromatographic conditions and instrumentation

HPLC analysis was performed on WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector. Chromatographic conditions were cited in Table 1.

Table.1: Chromatographic conditions for Gemifloxacin

Mobile phase ratio	Methanol (100% v/v)
Column	Hypersil C18 (4.6 x 150mm, 5µm)
Column temperature	40ºC
Wavelength	230nm
Flow rate	0.8ml/min
Injection volume	10µl
Run time	6minutes

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Gemifloxacin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 1.05ml of the above Gemifloxacin stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:

Initially the mobile phase tried was Water, Methanol: Water and Acetonitrile: Water, with varying proportions. Finally, the mobile phase was optimized to Methanol in proportion 100% v/v.

Optimization of Column:

The method was performed with various C18 columns like Phenomenex column, Xterra, and Symmetry C18 column. Hypersil C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Preparation of mobile phase:

Accurately measured 1000 ml (100%) of HPLC Methanol in a 1000ml volumetric flask.

Diluent Preparation:

The Mobile phase was used as the diluent.

Method development

Trials showed that mobile phase with Hypersil C18 (4.6 x 150mm, 5µm) column gives symmetric and sharp peaks. After the optimization of chromatographic conditions, estimation of Gemifloxacin as carried out by

the developed RP-HPLC method. Standard solution of drug was injected separately and chromatogram of Gemifloxacin was recorded in Figure 2. Now the sample solution was injected separately and chromatogram was recorded in figure 3 until the reproducibility of the peak areas were satisfactory.

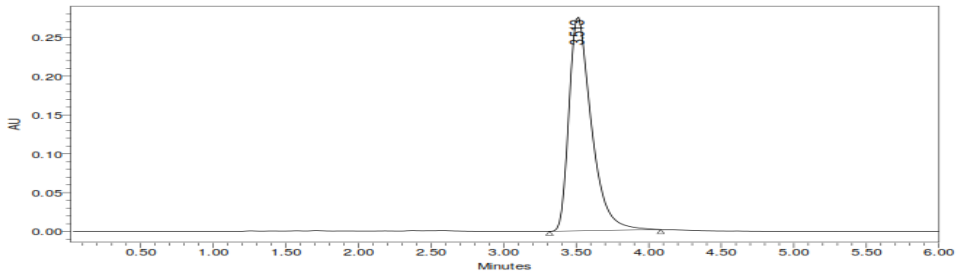

Figure 2: Standard Chromatogram of Gemifloxacin

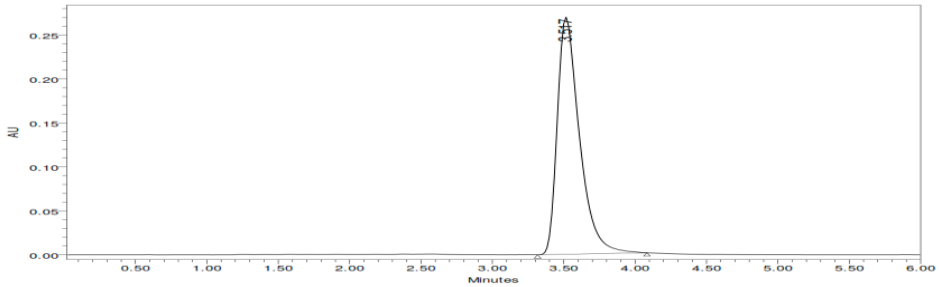

Figure 3: Sample Chromatogram of Gemifloxacin

Analytical method validation

HPLC method was validated [21] according to the International Conference on Harmonization guidelines (ICH Q2B, validation of analytical procedures, methodology). The method was validated for parameters such as linearity, precision, accuracy, system suitability limit of detection, limit of quantification and robustness.

Linearity

Inject each level (35, 70, 105, 140 and 175µg/mL) solutions (prepared from standard stock solution) into HPLC system and observed the linear relationship between concentration and peak area in the concentration range of 35 – 175µg/mL. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficients.

Precision

Repeatability

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was calculated.

Intermediate precision:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different analysts by maintaining same conditions. For intermediate precision % RSD was calculated from repeated studies.

Accuracy

Inject the three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Gemifloxacin and calculate the individual recovery and mean recovery values.

Robustness

Robustness was done by changing the actual chromatographic conditions like mobile phase ratio and flow rate. Results were determined by calculating the %RSD for injections peak area values of each change in condition.

System suitability

This parameter used to know whether the HPLC system is suitable for actual chromatographic conditions or not. System suitability was estimated by injecting five standard solutions of Gemifloxacin and from the chromatograms %RSD, theoretical plates and peak symmetry were calculated.

Specificity

Specificity of a method was determined by testing standard substances against potential interferences. The method was found to be specific when the test solution was injected.

It is calculated by the formula

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Limit of detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Quantitation limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

RESULTS AND DISCUSSION:

Linearity and range

Linearity and range estimated by constructing the calibration curve by taking concentration on X-axis and peak area on Y-axis of 35, 70, 105, 140 and 175µg/mL solutions (prepared from standard stock solution) and calculate the correlation coefficient. Correlation Coefficient (r) is 0.99, and the intercept 16821. These values meet the validation criteria as shown in Figure 4 and linearity values tabulated in Table 2.

Table 2: Chromatographic data for linearity study

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	35	1083048
66	70	1973321
100	105	2955166
133	140	4063921
166	175	5006038

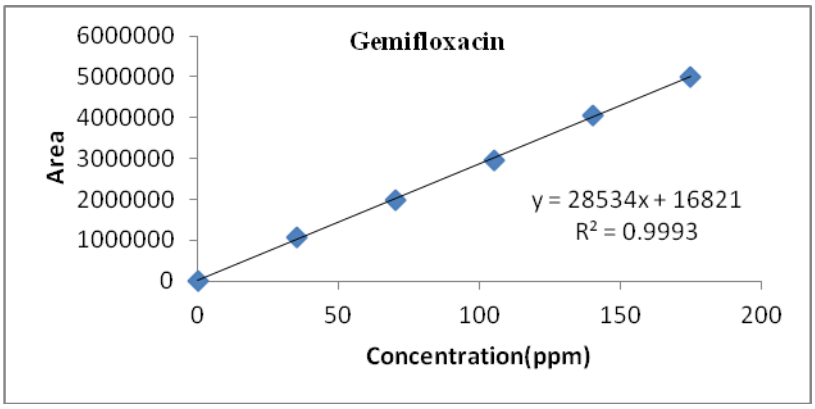

Figure 4: Calibration curve of Gemifloxacin

Precision

Intermediate precision

Analyst 1:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits. The results were reported in Table 3.

Analyst 2:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits. The results were reported in Table 4.

Table 3: Results of Intermediate precision analyst 1 for Gemifloxacin

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Gemifloxacin	3.517	2957344	275838	7194	1.1
2	Gemifloxacin	3.514	2951847	275629	8573	1.1
3	Gemifloxacin	3.517	2950834	276931	7655	1.1
4	Gemifloxacin	3.517	2957155	275623	7944	1.1
5	Gemifloxacin	3.512	2950185	275184	7562	1.1
6	Gemifloxacin	3.518	2951750	275193	7585	1.1
Mean			2953186			
Std. Dev.			3207.331			
%			0.108606			

Table 4: Results of Intermediate precision Analyst 2 for Gemifloxacin

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Gemifloxacin	3.513	2951848	275929	7937	1.1
2	Gemifloxacin	3.511	2958275	275222	7284	1.1
3	Gemifloxacin	3.516	2950185	275857	7684	1.1
4	Gemifloxacin	3.518	2957462	275163	7917	1.1
5	Gemifloxacin	3.511	2957541	275164	7585	1.1
6	Gemifloxacin	3.519	2951164	275154	7192	1.1
Mean			2954413			
Std. Dev.			3715.025			
% RSD			0.125745			

Repeatability

Preparation of Gemifloxacin Product Solution for Precision:

Accurately weigh and transfer 10 mg of Gemifloxacin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 1.05ml of the above Gemifloxacin stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The results were reported in Table 5.

Table 5: Results of Intermediate precision for Gemifloxacin

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Gemifloxacin	3.517	2957344	275838	7194	1.1
2	Gemifloxacin	3.514	2951847	275629	8573	1.1
3	Gemifloxacin	3.517	2950834	276931	7655	1.1
4	Gemifloxacin	3.517	2957155	275623	7944	1.1
5	Gemifloxacin	3.512	2950185	275184	7562	1.1
6	Gemifloxacin	3.518	2951750	275193	7585	1.1
Mean			2953186			
Std. Dev.			3207.331			
% RSD			0.108606			

Accuracy:

Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated. The results were reported in Table 6.

Table 6: The accuracy results for Gemifloxacin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1483420	52.5	52.3	99.6%	99.7%
100%	2978609	105	104.8	99.8%	
150%	4473692	157.5	157.3	99.8%	

Robustness

The robustness was performed for the flow rate variations from 0.7 ml/min to 0.9ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Gemifloxacin. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Gemifloxacin were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count. The results were reported in Table 7.

Table 7: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	2955764	3.513	7483	1.1
Less Flow rate of 0.9 mL/min	2958393	3.897	6028	1.1
More Flow rate of 1.1 mL/min	2956411	3.218	6928	1.2

System suitability

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The results were cited in table 8.

Table 8: Results of system suitability for Gemifloxacin

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Gemifloxacin	3.513	2947505	275462	7462	1.1
2	Gemifloxacin	3.516	2958475	275361	7462	1.1
3	Gemifloxacin	3.515	2965847	275144	6472	1.1
4	Gemifloxacin	3.517	2952642	275837	7183	1.1
5	Gemifloxacin	3.512	2951645	275948	7428	1.1
Mean			2955223			
Std. Dev.			7114.704			
% RSD			0.24075			

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. The % purity of Gemifloxacin in pharmaceutical dosage form was found to be 100.4%. Analytical method was tested for specificity to measure accurately quantitates Gemifloxacin in drug product. The results were reported in Table 9 and 10.

Table 9: Peak results for assay standard

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Gemifloxacin	3.518	2967593	275837	1.1	6583
2	Gemifloxacin	3.517	2967399	275922	1.1	5938
3	Gemifloxacin	3.515	2960183	271844	1.1	5883

Table 10: Peak results for Assay sample

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Gemifloxacin	3.511	2983744	275833	1.1	7584
2	Gemifloxacin	3.511	2958374	275984	1.1	6294
3	Gemifloxacin	3.514	2957262	275481	1.1	8194

Limit of detection

Limit of detection is defined as lowest concentration of analyte that can be detected, but not necessarily quantified, by the analytical method. It is determined by the analysis of sample with known concentration of analyte and by establishing the minimum level at which the analyte can be reliably detected and it was found to be 7.3µg/ml of Gemifloxacin.

Limit of quantification

Limit of quantification is the concentration that can be quantified reliably with a specified level of accuracy and precision. LOQ was found to be 22.1µg/ml of Gemifloxacin.

CONCLUSION:

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Gemifloxacin in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Gemifloxacin was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Gemifloxacin in bulk drug and in Pharmaceutical dosage forms.

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