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LC Determination of Gemifloxacin in Bulk and Pharmaceutical Formulation

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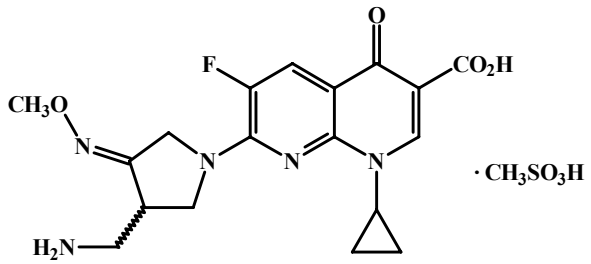
An isocratic reversed phase liquid chromatographic (RP-LC) method has been developed and subsequently validated for the determination of gemifloxacin in bulk and pharmaceutical formulation. Separation was achieved with a develosil ODS UG-5 250 × 4.6 mm; 5 µ column and triethylamine buffer (pH adjusted to 3.0 ± 0.05 with orthophosphoric acid):acetonitrile:methanol (65:10:25 v/v) as eluent at a flow rate 1.8 mL/min. UV detection was performed at 273 nm. The method is simple, rapid, selective and stability indicating. The described method is linear over a range of 10.341-82.725 µg/mL. The method precision for the determination of assay was below 0.5 % RSD. The percentage recoveries of active pharmaceutical ingredient (API) from dosage forms ranged from 98.0 to 100.1. The method is useful in the quality control of bulk manufacturing and also in pharmaceutical formulations.

Key Words: LC Determination, Gemifloxacin.

INTRODUCTION

Gemifloxacin mesylate is a synthetic broad-spectrum antibacterial agent for oral administration. Gemifloxacin, a compound related to the fluoroquinolone class of antibiotics, is available as the mesylate salt in the sesquihydrate form. Chemically, gemifloxacin is (R,S)-7-[(4Z)-3-(aminomethyl)-4-(methoxyimino)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-1, 8-naphthyridine-3-carboxylic acid[1,2].

The mesylate salt is a white to light brown solid with a molecular weight of 485.49. Gemifloxacin is considered freely soluble[3] at neutral pH (350 µg/mL at 37 ºC, pH 7.0). Its empirical formula is $C_{18}H_{20}FN_5O_4 \cdot CH_4O_3S$ and its chemical structure is



Structure of gemifloxacin

It is not official in any pharmacopoeia and till now, few liquid chromatography procedures have been reported for the determination of gemifloxacin and their metabolites in biological fluids. However there are no publications concerning the analysis of gemifloxacin in bulk and pharmaceutical dosage forms[4-8]. So it is felt necessary to develop a liquid chromatographic (LC) procedure which would serve as a rapid and reliable method for the determination of gemifloxacin in respective related impurities in bulk and pharmaceutical dosage forms. In the proposed method, related impurities were well separated and eluted before 10 min. Finally the method was thoroughly validated for the assay determination of gemifloxacin.

EXPERIMENTAL

The waters LC system equipped with 2489 pump and 2996 photodiode detector was used. The output signal was monitored and integrated using waters Empower 2 software.

Preparation of pH 3.0 buffer solution: 10 mL of triethylamine was taken in 1000 mL of milli-Q water and mixed well, pH of the solution was adjusted to 3.0 ± 0.05 with orthophosphoric acid.

Preparation of mobile phase: A mixture of pH 3.0 buffer, acetonitrile and methanol in the ratio 65:10:25 (v/v/v) was prepared and filtered through 0.45 µm nylon membrane filter prior to use.

Diluent:Milli Q water

Preparation of standard solution (50 µg/mL): About 67 mg of gemifloxacin (GEMI) working standard was accurately weighed and transferred in to a 100 mL volumetric flask and dissolved in 70 mL of diluent and diluted to volume with diluent and mixed well. Further 5 mL of the resulting solution was taken into 50 mL volumetric flask and made up to volume with diluent and mixed well. Solution was filtered through 0.45 µm nylon membrane filter prior to use.

Preparation of test solution: Twenty tablets were taken weighed and powdered. Sample equivalent to about 50 mg of gemifloxacin (GEMI) was accurately weighed and transferred in to a 100 mL volumetric flask and dissolved in 70 mL of diluent and sonicated for 0.5 h and diluted to volume with diluent and mixed well. Few mL was taken and centrifuged at 2500 rpm. 5 mL of the clear centrifuge is taken in to 50 mL volumetric flask and made up to volume with diluent. Solution was filtered through 0.45 µm nylon membrane filter prior to use.

Preparation of degradation samples for specificity study[9-12]

For acid degradation: gemifloxacin sample was stressed with 0.1 N HCl on bench top for 0.5 h and then neutralized by adjusting pH to 7.0 with 0.1 N NaOH. The solution was further diluted to required concentration with diluent.

For basic degradation: Gemifloxacin sample was stressed with 0.1 N NaOH on bench top for 10 min and then neutralized by adjusting pH to 7.0 with 0.1 N HCl. The solution was further diluted to required concentration with diluent.

For oxidative degradation: Gemifloxacin sample was stressed with 1 % H_2O_2 by heating on mantle at 40 ºC for 2 h. The solution was further diluted to required concentration with diluent.

For photolightic stress: The samples were exposed to UV at 254 nm for 63 h and visible light for 288 h meeting the specification of ICH *i.e.* UV(200 watt/m^2) and visible (1.2 million Lux hours).

For thermal degradation: Samples were exposed to temperature at 120 ºC for 24 h. The above stressed samples *i.e.* photolightic and thermal stress samples solutions were prepared to required concentration with diluent.

Chromatographic conditions: A develosil ODS UG-5 (250 × 4.6 mm; 5 µm packing) column was used for analysis at column temperature 35 ºC. The mobile phase was pumped through the column at a flow rate of 1.8 mL/min. The sample injection volume was 10 µL. The photodiode array detector was set to a wavelength of 273 nm for the detection.

RESULTS AND DISCUSSION

Method development[13]

Separation of known degradant impurities: To develop a suitable and robust LC method for the determination of gemifloxacin different mobile phases and columns were employed to achieve the best separation and resolution. The method development was started with a C18 column using a mobile phase of pH 3.0 buffer: acetonitrile: methanol in the ratio 75:10:15. In the above condition elution was very broad for gemifloxacin. Early elution with little separation was observed with mobile phase consisting of pH 3.0 buffer:acetonitrile:methanol in the ratio 50:35:15. Finally, the mobile phase consisting of pH 3.0 buffer:acetonitrile:methanol in the ratio 65:10:25 was found to be appropriate, allowing good separation and symmetrical peak at a flow rate of 1.8 mL/min using develosil ODS UG-5, 250 mm column. In the above condition all related compounds are eluted prior to gemifloxacin. The chromatogram of gemifloxacin sample spiked with the related compounds using the proposed method is shown in Fig. 1. In the proposed method the resolution is more than 2 between the gemifloxacin and related compound B. System suitability results of the method are presented in Table-1. Gemifloxacin and its related compounds show significant UV absorbance at wavelength 273 nm. Hence this wavelength has been chosen for detection in the analysis of gemifloxacin.

TABLE-1
SYSTEM SUITABILITY REPORT

Compound	Tailing factor*	Theoretical plates*	Resolution*	% RSD*
Gemifloxacin	1.1	12951	–	0.8
B	1.2	11123	3.1	1.2

*Number of samples analyzed are six.

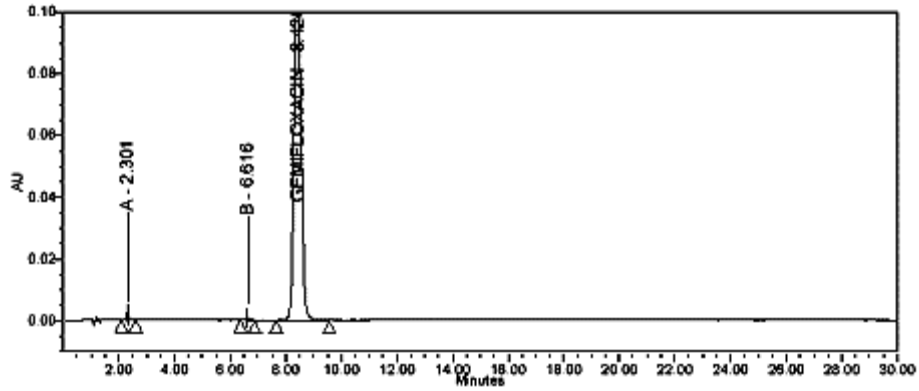


Fig. 1. HPLC chromatogram of gemifloxacin and its related compounds

Column selection: Based on the retention and separation of the compounds develosil ODS UG-5 (250 × 4.6 mm; 5 µm) column was selected as suitable column for the analysis of gemifloxacin.

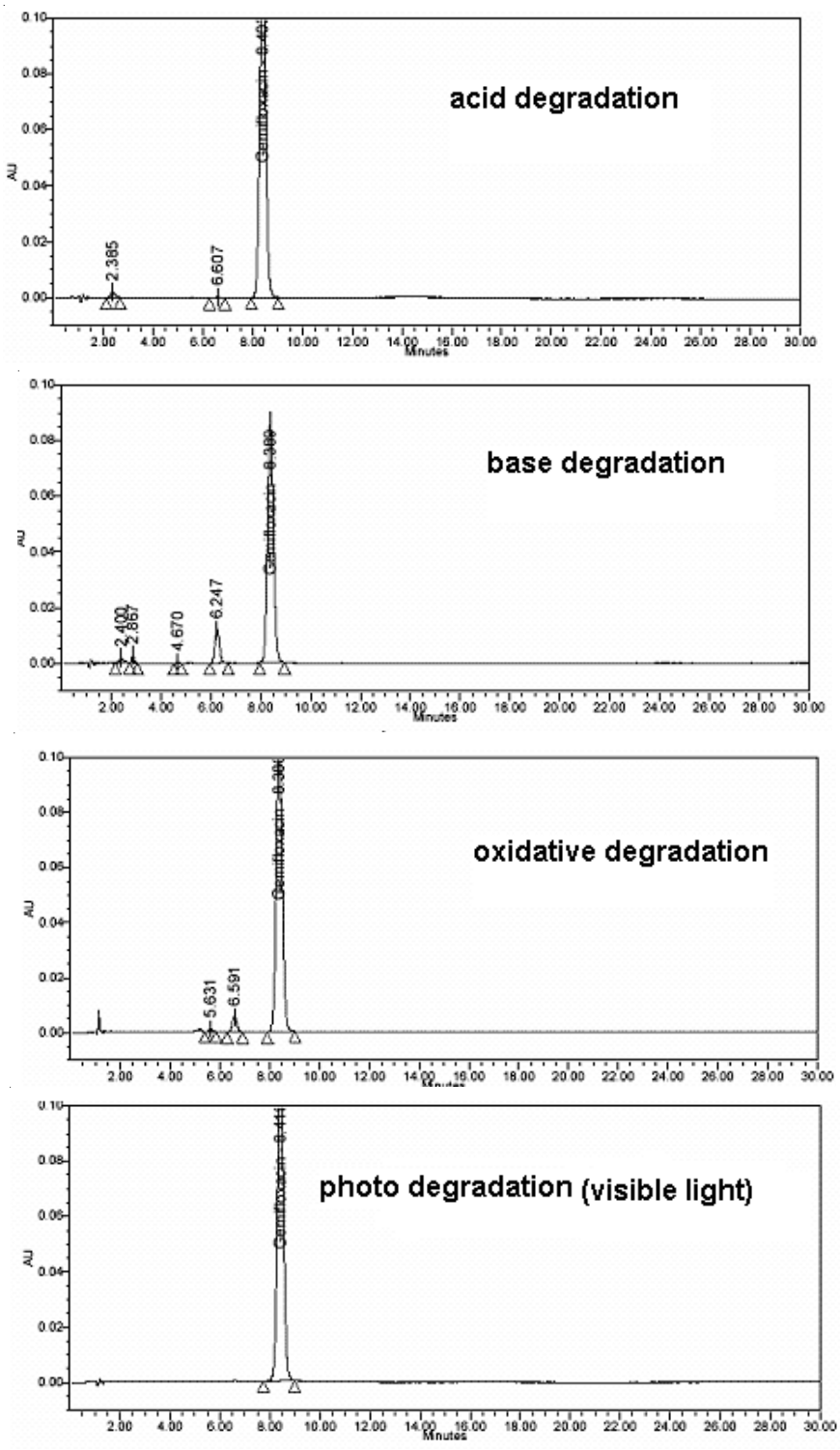
Method validation[14]: The developed LC method extensively validated for assay of gemifloxacin using the flowing parameters.

Specificity

Placebo interference: A study to establish the interference of placebo was conducted. Assay was performed on placebo in triplicate equivalent to about the weight of placebo in portion of test preparation as per test method. Chromatograms of placebo solutions showed no peaks at the retention time of gemifloxacin peak. This indicates that the excipients used in the formulation do not interfere in estimation of gemifloxacin in gemifloxacin mesylate tablets.

Interference from degradation products: A study was conducted to demonstrate the effective separation of degradants from gemifloxacin peak. Separate portions of drug product, drug substance and placebo were exposed to following stress conditions to induce degradation. Stressed samples were injected into the HPLC system with diode array detector by following test method conditions. All degradant peaks were resolved from gemifloxacin peak in the chromatograms of all samples. The chromatograms of the stressed samples were evaluated for peak purity of gemifloxacin using empower software. In all forced degradation samples, gemifloxacin peak purity angle is less than purity threshold. From the above results it is clear that the method can be used for determining the stability of gemifloxacin as bulk and pharmaceutical formulations. Fig. 2 shows the separation of gemifloxacin from its degradation products.

Linearity of detector response: Linearity of detector response was established by plotting a graph to concentration *versus* average area and determining the correlation coefficient. A series of solutions of gemifloxacin mesylate standard were


acid degradation
2.385
6.607
base degradation
2.400
2.867
4.670
6.247
oxidative degradation
5.631
6.591
photo degradation (visible light)
AU
Minutes

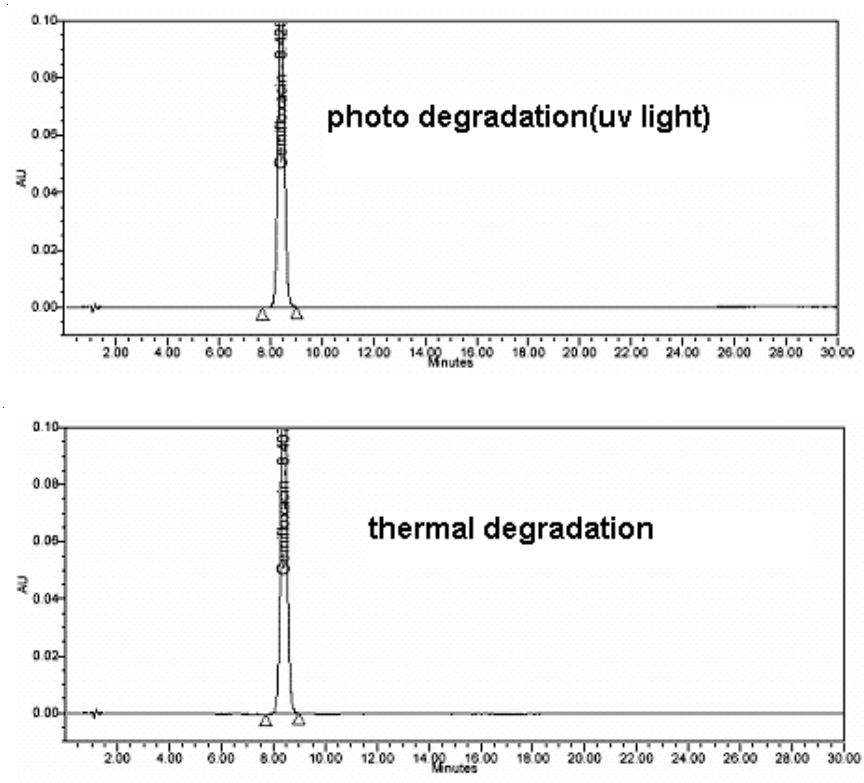


Fig. 2. HPLC chromatograms of gemifloxacin and its degradation products

prepared in the concentration range of about 10.341 µg/mL to 82.725 µg/mL. A graph was plotted to concentration in µg/mL on X-axis *versus* response on Y-axis. The detector response was found to be linear with a correlation coefficient of 0.999. Linearity graph is shown in Fig. 3.

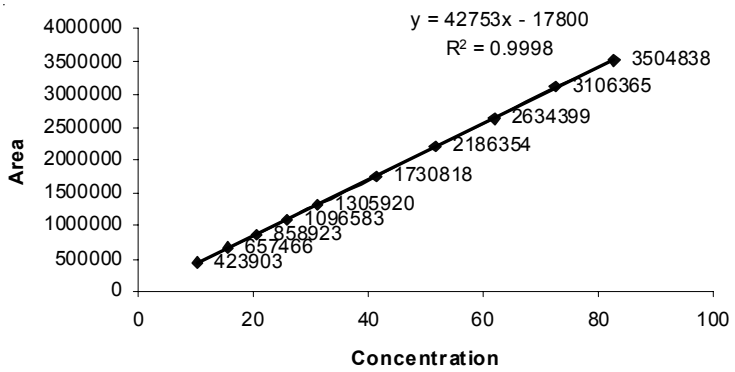


Fig. 3. Linearity of detector response graph

Precision of test method: The precision of test method was conducted by assaying six samples of gemifloxacin mesylate tablets. The average % assay of gemifloxacin in gemifloxacin mesylate tablets was found to be 98.8 % with an RSD of 0.5 %. The results were given in Table-2. A typical LC chromatogram is shown in Fig. 4.

TABLE-2
RESULTS FOR PRECISION OF TEST METHOD

Sample No.	% Assay
1	99.9
2	99.8
3	100.1
4	99.7
5	100.3
6	99.4
MEAN	99.9
% RSD	0.3

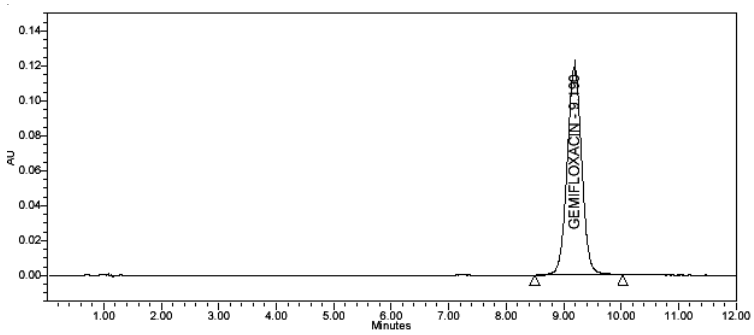


Fig. 4. Typical LC chromatogram of formulated gemifloxacin 320 mg

Accuracy: A study of recovery of gemifloxacin from spiked placebo was conducted at six different spike levels *i.e.* 25, 50, 75, 100, 125 and 150 %. Samples were prepared by mixing placebo with gemifloxacin mesylate raw material equivalent to about of the target initial concentration of gemifloxacin mesylate. Sample solutions were prepared in triplicate for each spike level and assayed as per proposed method. The slope, intercept, % recovery and correlation coefficient were calculated and given in Table-3. The mean recoveries of gemifloxacin from spiked were found to be in the range of 98.6-100.0 %.

Ruggedness: A study to establish the stability of gemifloxacin in standard and test solutions were conducted on bench top and refrigerator at initial, 1 day and 2 days. The assay of gemifloxacin in standard and test solutions were estimated against freshly prepared standard each time. The difference in % assay of standard and test solutions from initial to 1 day and 2 days was calculated and given in Table-4. From the above study, it was established that the test preparation is stable for one day on

TABLE-3
ACCURACY IN THE ASSAY DETERMINATION OF GEMIFLOXACIN

Sample No.	Spike level (%)	Added (mg)	Found recovered (mg)	Recovery (%)	Average recovery (%)
1		12.51	12.33	98.6	
2	25	12.54	12.29	98.0	98.6
3		12.53	12.43	99.2	
4		25.04	24.76	98.9	
5	50	25.04	25.04	100.0	99.5
6		24.95	24.85	99.6	
7		37.55	37.19	99.0	
8	75	37.55	37.43	99.7	99.3
9		37.71	37.39	99.2	
10		49.97	50.01	100.1	
11	100	50.03	50.06	100.1	100.0
12		49.97	49.86	99.8	
13		62.77	62.76	100.0	
14	125	62.65	62.54	99.8	99.9
15		62.71	62.68	100.0	
16		75.06	74.86	99.7	
17	150	75.18	74.88	99.6	99.5
18		75.18	74.64	99.3	

TABLE-4
STABILITY DATA OF GEMIFLOXACIN IN STANDARD AND TEST SOLUTIONS

Bench top stability						
Time (d)	% Assay of standard preparation	Difference from initial	% Assay of test preparation		Difference from Initial	
			Test-1	Test-2	Test-1	Test-2
Initial	75.7*	NA	99.5	99.3	NA	NA
1	74.4	1.3	97.7	96.8	1.8	2.5
2	72.7	3.0	94.8	94.5	4.7	4.8
Refrigerator stability						
Time (d)	% Assay of standard preparation	Difference from initial	% Assay of test preparation		Difference from Initial	
			Test-1	Test-2	Test-1	Test-2
Initial	75.7*	NA	99.5	99.3	NA	NA
1	75.1	0.6	98.0	97.4	1.5	1.9
2	74.4	1.3	97.3	97.1	2.2	2.2

*Potency of gemifloxacin on as is basis.

bench top and 2 days in refrigerator. Standard solution is stable for a period of 2 days on bench top and refrigerator.

Robustness: A study to establish the effect of variation in mobile phase composition, flow, temperature and pH of buffer in mobile phase was conducted. Standard and test solutions prepared as per proposed method were injected into HPLC system.

The system suitability parameters and % assay were evaluated. From the above study the proposed method was found to be robust.

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