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Research Article

Reverse Phase Ultra Fast Liquid Chromatographic Method for Determination of Gemifloxacin Mesylate in Tablet Dosage Form

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Abstract

A novel, accurate and precise reverse phase ultra fast liquid chromatographic method for determination of gemifloxacin mesylate has been developed and validated. Separation was achieved on an Enable C18G column (250mm × 4.6mm i.d., 5µm) using methanol: 10mM TBAHS (70:30, v/v) as mobile phase at a flow rate of 1.0ml/min and PDA detection at 271nm. Linearity was observed in the concentration range of 1.0-200 µg/ml (r2=1). The method was validated for accuracy, precision, stability, specificity, robustness and system suitability. Forced degradation was performed by using HCl, NaOH, H_2O_2, thermal and UV radiation. The method was used successfully for the determination of gemifloxacin mesylate in tablet dosage form.

Keywords **:** Gemifloxacin mesylate, RP-UFLC, TBAHS, stability

Introduction

Gemifloxacin mesylate is fluoroquinolone antibacterial having actions similar to that of ciprofloxacin1.Chemically, it is (±)-7-[3-(Aminomethyl)-4-oxo-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid 74-(Z)-(O-methyloxime) methanesulfonate(Fig.1).[1] As per literature surveys, there are only a few analytical methods reported for gemifloxacin mesylate. It includes spectrophotometric[2-10], chemiluminescence[11], spectrofluorimetry[12], HPLC[13-21], HPTLC[14], LC-MS[22-25] and microbiological assay[26] methods. However, no RP-UFLC method is reported till the literature survey for determination of gemifloxacin mesylate in pharmaceutical dosage form and in presence of its degradation products. So a successful attempt was made to develop and validate a fast, simple, precise and accurate reverse phase ultra fast liquid chromatographic method for determination of gemifloxacin mesylate in tablet dosage form. Stability parameters for the drug were assessed by subjecting the drug to forced degradation conditions like acid-alkali hydrolysis, oxidation, thermal and UV radiation.

Materials and Methods

Chemicals and reagents

Analytical grade gemifloxacin mesylate (purity > 99.8%) was received from MSN Laboratories Ltd, India. Methanol (Merck Ltd., Mumbai, India) was of HPLC grade. Analytical grade sodium hydroxide, hydrochloric acid and hydrogen peroxide of were procured from S.D. Fine Chem. Ltd., Mumbai, India. The water for HPLC was obtained by using TKA Water Purification System, Germany. Tetra butyl ammonium hydrogen sulfate (TBAHS); Himedia Laboratories Ltd., Mumbai, India) was of AR grade. The marketed tablet formulation (Zemi Tablet, FDC Ltd., India) containing 320 mg of gemifloxacin mesylate was purchased from the local market.

UFLC instrumentation and chromatographic conditions

Quantitative UFLC was performed on a binary gradient UFLC with two Shimadzu Prominence UFLC LC-20AD pumps, with a 20µl sample injection loop (manual) and SPD M20A PDA detector. The output signal was monitored and integrated using Shimadzu LC Solution Software. An Enable C18G column (250 mm × 4.6 mm i.d., particle size 5 μm) was used for separation. Chromatographic

analysis was carried out at the ambient temperatures on the column using the methanol: 10mM TBAHS (70:30, v/v) as mobile phase at a flow rate of 1.0 ml/min in isocratic mode. The 10mM TBAHS solution was prepared by accurately weighing 3.3954 g of TBAHS salt and dissolving it in 1000ml of HPLC grade water. Afterwards, both the methanol and TBAHS were ultrasonicated (Enertech, India) up to 20 min for degassing prior to use. The PDA detection was set at 271nm. Water bath (Thermolab, India) and UV Chamber (Jain Scientific Glass Works, Ambala, India) were used for forced degradation study of the drugs. Analytical balance, Model-GR-202 (AND Instrument India Pvt. Ltd., Gurgaon, India) of sensitivity 0.1mg was used to weigh the chemicals and reagents.

Preparation of standard and sample solution

Standard stock solution of the drug was prepared by dissolving accurately weighed 25mg of the drug in mobile phase up to 25ml. The volumetric flask having 10ml of mobile phase was ultrasonicated for 5 min. Finally, the volume was made up to the 25ml mark with mobile phase, which gave 1000μg/ml standard stock solution.

Preparation of calibration curve

Different aliquots(10,50,100,250,500,750,1000,1500 and 2000μl) were taken from the standard stock solutions in to separate 10ml volumetric flasks using a micropipette and finally diluted upto the mark with mobile phase to prepare a series of concentrations ranging from 1.0-200 µg/ml. A volume of 20 μl from each solution was injected into the UFLC, and the chromatograms were recorded. Calibration curve was obtained by taking the peak area on y-axis and concentration (μg/ml) on x-axis in a concentration range from 1.0 to 200 μg/ml.

Analysis of tablet dosage form

Twenty tablets were weighed and powdered finely. A quantity of tablet powder equivalent to 25 mg of gemifloxacin mesylate was accurately weighed and transferred into a 25 ml volumetric flask, containing 10 ml of mobile phase and ultrasonicated for 20 min; the volume was made up to the mark and mixed well. The solution was filtered through a 0.2µm filter to ensure the absence of particulate matter. The filtered solution was appropriately diluted with the mobile phase to finally produce a concentration 50μg/ml for analysis as already described. The amount of drug present in the sample solution was calculated by using the calibration curve of standard drug.

Method validation

Developed method was validated statistically for specificity, linearity, precision and accuracy. The specificity of the method was determined by checking the interference of any of the possible degradation products generated during the forced degradation study of the drug. The forced degradation of the drug was carried out with 0.1M HCl, 0.001M NaOH, 3% v/v hydrogen peroxide, thermal (80ºC) and photolysis (365nm) for determining the stability nature of the drug. The degradation samples were prepared by taking suitable aliquots of the drug solution, and then subjecting each of these solutions to different stress conditions. After the fixed time period, the treated drug solutions were diluted up to the mark with mobile phase. For every stress condition solution of concentration 50 μg/ml of gemifloxacin mesylate were prepared. The various stress conditions are described as follows.

A] *Acidic degradation condition:* Acidic degradation was carried out by adding 1 ml of 0.1M HCl, and after 45min neutralizing, the mixture by adding 0.1M NaOH.

B] *Alkali degradation condition:* Alkali degradation was carried out by adding 1 ml of 0.001M NaOH, and after 45min neutralizing, the mixture by adding 0.001M HCl.

C] *Oxidative degradation condition:* Oxidative degradation was performed by exposing the drug to 1 ml of 3% (v/v) H2O2 for 45min.

D] *Thermal degradation condition:* Thermal degradation was performed by heating the drug content at 80ºC on a thermostatically controlled water bath for 45min.

E] *Photolytic degradation condition:* Photolytic degradation was carried out by exposing the drug content to UV light (365nm) inside an UV chamber for 30min.

Linearity of the method was ascertained by plotting a nine-point (1.0-200μg/ml) calibration curve. The results obtained were used to calculate using the linear regression equation of the line by least-squares regression method. The repeatability (intra-day precision) of the method was ascertained from the peak areas obtained by actual determination of six replicates of a fixed concentration (50μg/ml) of drug. For intermediate precision (inter-day precision) of the method, the above same procedure was carried out by a different analyst on a different day under similar experimental conditions. Six replicates of a 50μg/ml solution of gemifloxacin mesylate were injected into the liquid chromatograph for determining the system precision. The percent relative standard deviation values were calculated. To check the accuracy of the developed method, recovery studies were carried out at 80,100 and 120 % of the test concentration as per ICH[27] guidelines. Aliquots of sample solutions containing gemifloxacin mesylate at 50µg/ml were transferred to three 10ml volumetric flasks containing, respectively, 400, 500, and 600 μl gemifloxacin mesylate reference solution (1000µg/ml).

The contents were mixed and diluted to volume in order to obtain final concentrations of 90, 100, and 110µg/ml, respectively. The recovery study was performed three times at each concentration level.

Robustness of the method was studied by deliberately changing method parameters like flow rate of mobile phase, detection wavelength and organic phase composition. The system suitability parameters like retention time, theoretical plates and tailing factor were determined for each modified condition. Solution stability of the drug in the mobile phase was determined by keeping the drug solution at ambient conditions for 24h. The limit of detection (LOD) and limit of quantitation (LOQ) were determined based on the 3.3 and 10 times the standard deviation of the response, respectively, divided by the slope of the calibration curve.

Table 1. Forced degradation study

Stress Applied/Time (min.)	% Degradation	Peak Purity of drug[a]
Untreated	-------	1.00000
0.1M HCl/45	31.11	0.99961
0.001M NaOH/45	11.55	0.99999
3% H2O2/45	10.14	0.99933
80ºC/45	9.58	1.00000
UV radiation at 365nm/30	32.92	1.00000

[a]*Peak purity 0.999-1.00000 indicates peak homogeneity*

Table 2. Intraday, interday and system precision (n=6)

Study	Concentration (µg/ml)	Peak Area[b] ±SD, (%RSD)
Intraday precision	50	522114718000.34, 0.34
Interday precision	50	5214324 21982.74, 0.42
System precision	50	5233736 21878.2, 0.42

[b]*average of six determinations*

Table 3. System suitability results for robustness study

Parameter	Retention Time (min)	Theoretical Plates	Tailing Factor
Flow rate (ml/min)			
0.9	3.673	8974	1.290
1.0	3.164	8340	1.303
1.1	3.016	7653	1.355
Wavelength (nm)			
266	3.164	8420	1.304
271	3.164	8340	1.303
276	3.164	8260	1.302
Methanol (%)			
68	3.398	8415	1.342
70	3.164	8340	1.303
72	3.164	7477	1.432
	Initial time	Final time	Recovery (%)
Solution stability	0 h	24 h	99.16

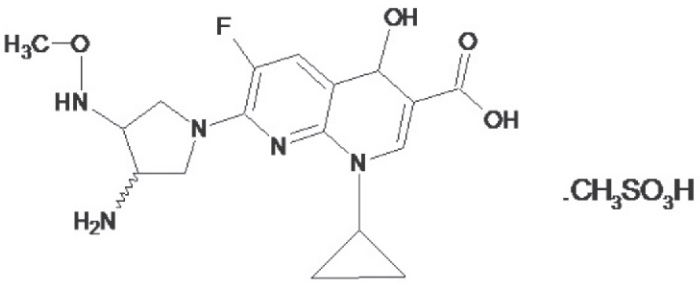


Fig. 1: Chemical structure of Gemifloxacin mesylate

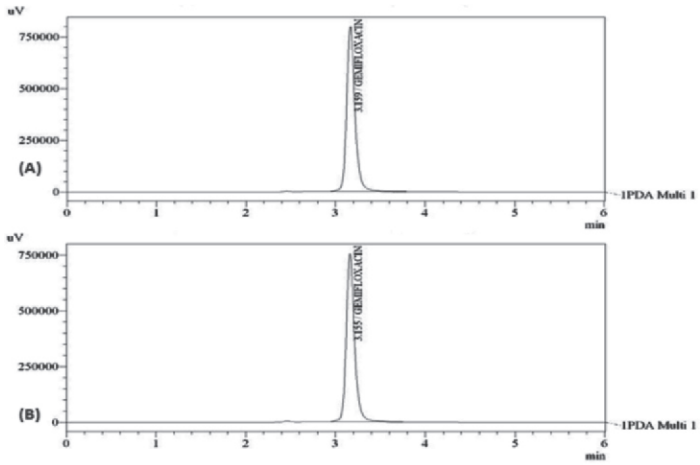
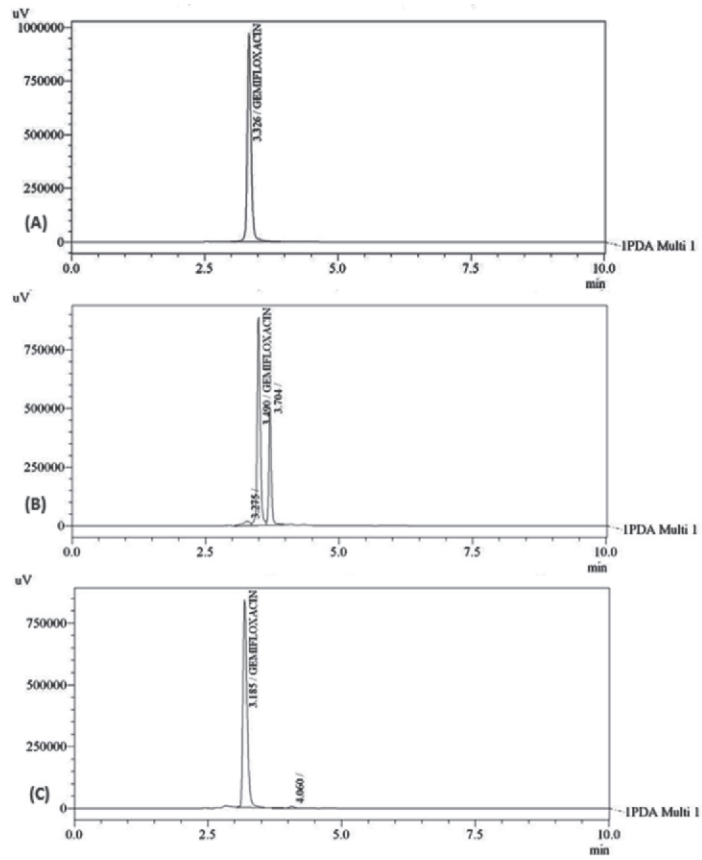


Fig. 2: Representative chromatograms of Gemifloxacin mesylate (A) standard drug, (B) tablet formulation



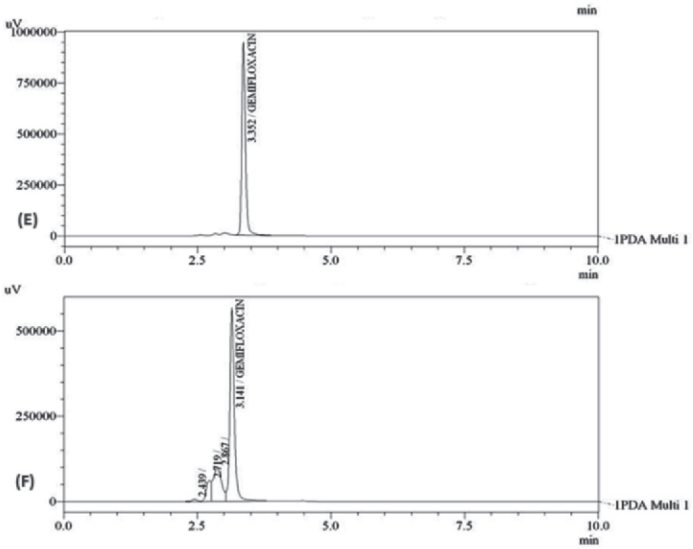


Fig. 3: Representative chromatograms of Gemifloxacin mesylate (A) untreated drug, (B) acid degraded drug, (C) alkali degraded drug, (D) H_2O_2 degraded drug, (E) thermally degraded drug, (F) photolysis degraded drug.

Results and Discussion

A reverse phase ultra fast liquid chromatographic method was developed for determination of gemifloxacin mesylate in tablet dosage form. Optimization of mobile phase was carried out basing on tailing factor and theoretical plates obtained for gemifloxacin mesylate. During the trial runs different mobile phase compositions like methanol: water, methanol: 10mM TBAHS, methanol: buffer, at various compositions (50:50, 70:30, 60:40, v/v) and flow rates (0.8, 1.0 and 1.2ml/min) were tested for selection. The mobile phase consisting of methanol: 10mM TBAHS (70:30, v/v) at a flow rate of 1.0ml/min was selected, which gave a sharp, symmetric peak with a tailing factor of 1.303 for gemifloxacin mesylate. The retention time for gemifloxacin mesylate was found to be 3.159min. The run time was set 6 min for time efficient and cost effective drug analysis. PDA detection was set at 271nm. The separation was carried out at room temperature. The chromatograms of gemifloxacin mesylate standard drug and tablet formulation show no significant difference in retention times (Fig.2A- B).

The calibration curve (9 point) was found to be linear over a concentration range of 1.0-200 μg/ml for gemifloxacin mesylate. The linear regression equation was $y = 103858x+4059$ ($r^2=1$). Specificity of the method was ascertained by checking any interference due to excipients or degradation products. PDA detector was applied to determine the peak purity of the stress treated drug solutions. Gemifloxacin undergoes complete

degradation in the applied alkaline (0.1M) and photolytic (UV radiation exposure for 1h) degradation condition. So both the stress conditions were modified to decrease the extent of degradation. The modified alkaline stress was applied by using 0.001M NaOH solution. In case of photolytic degradation, the duration of UV radiation exposure was decreased from 1h to 30min. The drug shows some significant degradation in the applied acidic and photolytic stress conditions, and moderate degradation in alkaline, oxidative and thermal stress conditions. The run time for each stressed drug solution was increased from 6 min to 10 min in order to find out presence of any extra peak due to degradation of gemifloxacin. The chromatograms of untreated drug, acid, alkali, hydrogen peroxide, thermal and photolysis degraded drug, indicates the method specificity (Fig.3A-F). The results for forced degradation study are satisfactory (Table 1).

The assay result (n=3) for the tablet dosage form yielded 100.79% (SD= ±0.8649) of gemifloxacin mesylate. The method was found to be precise as the results for % RSD values (Table 2) were well below 2%. Accuracy of the method was determined by recoveries of gemifloxacin mesylate by standard addition methods. The average recovery was in the range of 100.13-102.36%. The values show high levels of accuracy of the method.

The method was found to be robust in accordance with deliberate changes in mobile phase flow rate (±0.1ml/min), detection wavelength (±5nm) and organic phase composition (±2%). The solution stability study shows that drug solutions were stable for 24 h at ambient conditions without any degradation of the analyte. The result for robustness study was evaluated in terms of the different system suitability parameters (Table 3). The limit of detection and limit of quantification values were 0.33μg/ml and 0.98μg/ml, respectively.

Conclusion

A validated RP-UFLC method has been developed for determination of gemifloxacin mesylate in tablet dosage form. The results obtained by the forced degradation of the drug revealed that gemifloxacin undergoes degradation in the order of photolysis (365nm) > acid (0.1M HCl) > alkali (0.001M NaOH) > oxidation (3% H_2O_2) > thermal (80ºC).

Validation study revealed that the developed analytical method is linear as well as accurate, precise and specific. The method was found to be robust with respect to deliberate changes in flow rate, detection wavelength and organic phase composition. The method was successfully applied for the determination of gemifloxacin in tablet dosage form. The commonly used excipients do not interfere in the estimation of gemifloxacin. Further this method may be applied for routine analysis of gemifloxacin mesylate in API, pharmaceutical formulations, dissolution medium and biological fluids.

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