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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF GEMIFLOXACIN MESYLATE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

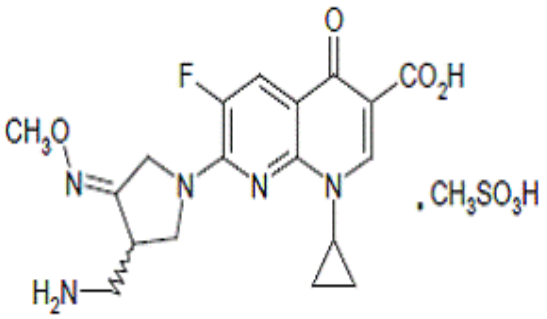
A simple, rapid reversed phase high performance liquid chromatographic method was developed and validated for estimation of Gemifloxacin Mesylate in bulk and pharmaceutical dosage form. Optimum separation was achieved at 8.58±0.03 min using an end-capped C18 column (250 mm × 4.6 mm i.d, 5µ particle size) by isocratic elution with a mixture of citric acid-sodium citrate buffer (pH 2.8): acetonitrile in the ratio of 70:30:(v/v) as mobile phase. Column effluents were monitored at 267nm at a flow rate of 1mL/min.The method was validated for linearity, accuracy, precision and specificity as per ICH guidelines. The developed and validated method was successfully used for the quantitative analysis of commercially available dosage form. The calibration curve was linear over the concentration range of 0.096-0.144 mg.mL^{-1} for Gemifloxacin Mesylate. The LOD and LOQ values were found as 0.628 µg.mL^{-1} and 1.90 µg.mL^{-1} respectively. The high percentage of recovery confirms the suitability of the method for the estimation of Gemifloxacin Mesylate in pharmaceutical dosage form.

KEYWORDS: Gemifloxacin Mesylate (GFM) tablets, Potency, RP-HPLC, Method validation.

INTRODUCTION

Gemifloxacin Mesylate (**Fig. 1**) is a synthetic broad-spectrum antibacterial of fluoroquinolone class of antibiotics is available as the mesylate salt in the sesquihydrate form.[1] It is chemically described as (R,S)-7[(4Z)-3-(aminomethyl)-4-(methoxyimino)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo1,8-naphthyridine-3-carboxylic acid. It is used for the treatment of acute bacterial exacerbations of chronic bronchitis caused by susceptible *Streptococcus pneumoniae*, *Haemophilusinfluenzae*, *H. parainfluenzae*, or *Moraxella catarrhalis*.[2,3]

The literature survey revealed only a few analytical methods reported for Gemifloxacin Mesylate. It includes spectrophotometric,[4-10] chemiluminescence,[11] spectrofluorimetry,[12] HPLC,[13-19] HPTLC,[14] LC-MS,[20-23] and microbiological assay[24] methods. Also Sugumaran and Jotheeswari[25] developed a RP-HPLC method where they used acetonitrile and phosphate buffer in mobile phase. And Gumustas and Ozkan[26] developed another LC-DAD method using HPLC grade methanol and water in mobile phase. Again Panda *et al.*[27] developed and validated a reverse phase Ultra Fast Liquid Chromatographic method using methanol and *tetra*butylammonium hydrogen sulfate (TBAHS) in mobile phase. So in case of all above HPLC methods, methanol of HPLC grade were used sufficiently which are very costly. In the present study, a successful attempt was made to develop and validate a fast, simple, precise, accurate and cost effective reversed phase HPLC method using HPLC grade water in mobile phase instead of methanol to quantify GFM in bulk, and its pharmaceutical tablet dosage form.



Molecular formula $C_{19}H_{24}FN_5O_7S$. Molecular weight 485.49g

Fig. 1: Structure of Gemifloxacin Mesylate (GFM)

MATERIALS AND METHODS

Chemicals and reagents

Pharmaceutical grade of Gemifloxacin Mesylate INN was obtained from Maithri Laboratories Pvt. Ltd.,

(India). Excipients used in tablet formulation were Microcrystalline Cellulose (PH 101),Maize Starch, Povidon (K-30), Polacriline Potassium (KyronT-314), Colloidal Silicon Dioxide, Magnesium Stearate and Talcum Purified and they were of BP and/USP grade. Water was obtained from double distillation in glass and passage through a Milli-Q® System, Millipore, Milford, MA, USA.

Instrumentation and Chromatographic condition

The analysis of the drug was carried out on Shimadzu HPLC (Prominence LC 20) equipped with gradient pump, PDA Detector, auto sampler and column oven. The analysis was performed using end-capped ODS C-18 column with 250 × 4.6 mm internal diameter and 5 µm particle size. Sartorius electronic balance (CPA224S) was used for weighing. Isocratic elution with citric acid-sodium citrate buffer (pH-2.8):acetonitrile 70:30 (V/V) was selected with a flow rate of 1.0 mL/min. The detection wavelength was set at 267 nm with a runtime 12 min. The mobile phase was prepared freshly and it was degassed by sonicating for 5 min before use. The column was equilibrated for at least 30 min with the mobile phase flowing through the system. The column and the HPLC system were kept at ambient temperature.

Preparation of Standard Solution

Accurately weighed 60 mg of GFM working standard was transferred into 50mL volumetric flask, and it was sonicated to dissolve with mobile phase and volume was made up to the mark with mobile phase. This solution was treated as stock solution which contained 1.2 mg GFM in each mL. Five (5) milliliter of GFM stock solution was transferred to a 50 mL clean volumetric flask and volume was made up to the mark with mobile phase. The solution was then filtered through 0.45µm nylon membrane filter and degassed. Twenty microliter (20 µL) of final solution was injected into the HPLC system and chromatograms were recorded.

Analysis of tablets

Twenty (20) GFM tablets were grinded well to get fine powder. A portion of the powder equivalent to 60 mg of GFM was accurately weighed and transferred into a 50 mL volumetric flask. About 50 mL of mobile phase was added and sonicated for 10 minute to dissolve it completely and made up to the mark with mobile phase. It was mixed well and filtered through 0.45µm nylon membrane filter. Further, 5 mL of the above solution was pipetted into a 50 mL volumetric flask and diluted up to the mark with mobile phase. The solution was filtered through 0.45µm nylon membrane filter and degassed. Twenty microliter (20 µL) of final solution was injected into the HPLC system and chromatograms were recorded.

Method development

The chromatographic condition was analysed with a view to develop an assay method for GFM in pharmaceutical dosage form. Detection was performed at 267 nm which was based on UV scan of sample. Using end-capped ODS C-18 column different mobile phase ratios of citric acid-sodium citrate buffer and acetonitrile were run but the most selective peak was arrived by using them in the ratio of 70:30 respectively. The final chromatographic system optimized is shown in **Table 1**.

Table 1: Optimized chromatographic conditions for GFM

Test	Condition
Mobile Phase	Citric acid-sodium citrate (pH-2.8): Acetonitrile (70:30 V/V), Isocratic
Diluent	Mobile phase
Column	End-capped ODS C-18 column with 250 × 4.6 mm internal diameter and 5µm particle size
Column oven	Ambient temperature
Flow rate	1.0 mL/min
Detector	PDA
Wavelength	267 nm
Injection volume	20 µL
Run time	12 min

Chromatogram with working standard

GFM (60 mg) was weighed accurately, transferred into a 50 mL volumetric flask, sonicated to dissolve with mobile phase and volume was made up to the mark with mobile phase. It was treated as stock solution which contained 1.2 mg of GFM in each mL of solution. From this stock solution, working standard solutions 0.096, 0.108, 0.120, 0.132, 0.144 $mg.mL^{-1}$ of the drug were prepared by diluting respectively to 50 mL with mobile phase. Each of the diluted solution (20 µL) was injected by auto injector into the column at a flow rate 1.0 mL/min of mobile phase and the corresponding chromatogram was (**Fig. 2**) recorded. It is evident from the **Fig. 2** that the chromatogram was quite good and it could be used for qualitative and quantitative analysis of GFM in bulk and its tablet dosage form. Retention time of the chromatogram was ascertained from the replicates and it was found as 8.58±0.03 min.

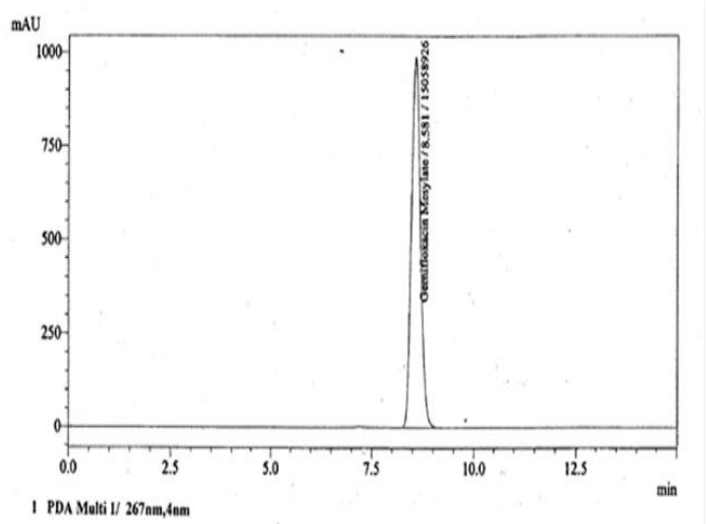


Fig. 2: A Representative chromatogram of GFM (R_T=8.58±0.03 min) under optimized conditions

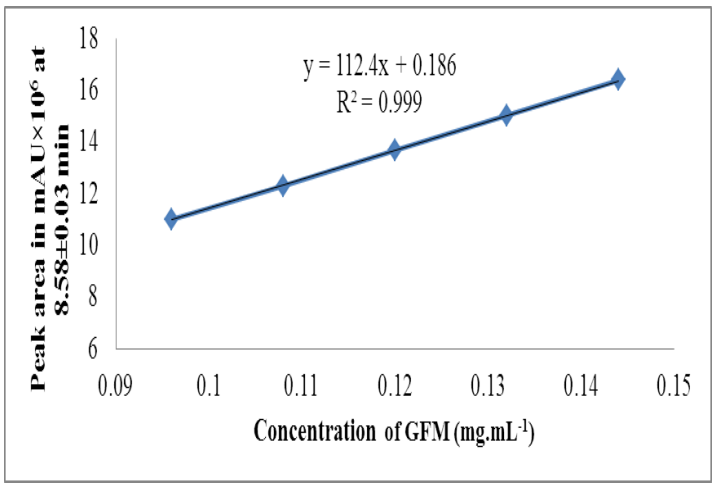


Fig. 3: Calibration curve for GFM only (Working standard)

Calibration Plot

The calibration curve was constructed by plotting concentrations of the drug against peak area (mAU) of the chromatogram (**Fig. 3**) at R_T=8.58±0.03 min and it was found linear. However, it is important to mention here that the linearity was not observed below the concentration of 0.096 mg.mL^{-1} nor above the concentration of 0.144 mg.mL^{-1}.The regression equation for the curve was found as y=112.4x+0.186 with correlation coefficient (R^2) 0.999. It was used to estimate the amount of GFM in bulk and tablet dosage form.

RESULTS AND DISCUSSIONS

Validation of the proposed method

System suitability

Performance of the analytical method was confirmed by system suitability test where % RSD of peak area was calculated as 0.503 (**Table 2**). It complied with the recommended range (NMT 1%) of CDER.[28]

Table 2: Results of System Suitability test

No. Sample (Replicates)	Peak Area (mAU)	RSD (%) of Peak area		Remarks
01	11713340	**Result**	**CDER Limit**	**Complied and the system is suitable to carry out the analysis**
02	11804958	0.503	NMT 1	
03	11711716			
04	11803200			
05	11647785			
06	11745162			
07	11801744			
08	11743445			

09	11645162			
10	11711716			

Table 3: Results of Specificity test

	Content	Retention Time of API (min)	Content of GFM (%)			Remarks
			Theoretical	Observed	ICH Limit	
Blank	Mobile phase only	_	_	_	-	Complied and method is specific
Control	Mobile phase + excipients	_	_	_	-	
Standard	Mobile phase + GFM INN (0.12 mg.mL^{-1})	8.342	99.93	99.10	98 -102	
Tablet	Mobile phase + GFM INN (0.12 mg.mL^{-1}) + excipients (0.139mg.mL^{-1})	8.353	100.76	101.23	-	

Table 4: Percent recovery of GFM from simulated tablet contents

GFM (mg.mL^{-1})	% of test conc.	Peak area (mAU)	Recovery from sample (mg.mL^{-1})	Recovery (%)	Average Recovery (%)	ICH Limit (%)	Remarks
0.096	80	10979754	0.0957	99.69	100.56	98-102	Complied and the method is accurate
0.096		10999071	0.0965	100.52			
0.096		11001744	0.0972	101.25			
0.120	100	13672319	0.121	100.83			
0.120		13676249	0.119	99.17			
0.120		13672854	0.121	100.83			
0.144	120	16379755	0.144	100.00			
0.144		16390730	0.145	101.39			
0.144		16405485	0.146	101.39			

Table 5: Relative standard deviation of six determinations of GFM contents in simulated tablet amount

Sample	Concentration (mg.mL^{-1})	Peak area (mAU)	Result (%)	RSD (%)	ICH Limit of RSD (%)	Remarks
01	0.12	13676103	100.82	0.784	NMT 2	Repeatability of GFM measurement complied
02	0.12	13214047	99.98			
03	0.12	13214047	99.98			
04	0.12	13587099	100.08			
05	0.12	13676038	100.19			
06	0.12	13677891	101.98			

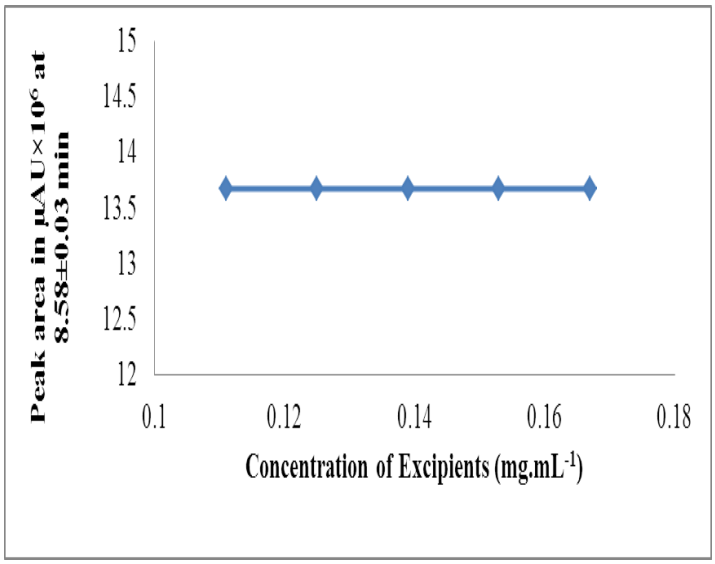


Fig. 4: Peak area of GFM at various concentrations of excipients

Linearity

Plot of peak area versus concentration of GFM **(Fig. 3)** of regression analysis resulted in the linear regression equation y=112.4x+0.186 (R^2=0.999). It is clear from the **Fig. 3** that the response was linearly dependant on the concentration of GFM. The linearity of the regression line also evident from correlation coefficient R^2=0.999. Similar dose-response relationship of GFM was observed even in presence of excipients (data are not shown). And with fixed concentration of API, the response for GFM (area) was not changing (**Fig. 4**) with the increase of excipient concentration. It means that there is no interference on GFM response from the excipients.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were determined from standard deviation of y-intercept of regression line and slope method as per ICH guidelines. For GFM, LOD was found as 0.628 µg.mL^{-1} and LOQ was found as 1.90 µg.mL^{-1}.

Range

The proposed RP-HPLC method for GFM estimation was found linear in the range of 0.096-0.144 mg.mL^{-1} (**Fig. 3**) but beyond that range linearity was not found (data are not shown). Lower limit of quantification (LLOQ) is therefore 0.096 mg.mL^{-1} while upper limit of quantification (ULOQ) is 0.144 mg.mL^{-1}.

Specificity

The specificity of the method was checked by monitoring a standard (raw material) API solution, its tablet, blank sample and placebo (excipients) materials. Sample of standard and tablets showed peak at retention time R_T=8.34±0.03 min when run separately in RP-HPLC while blank and placebo did not show any peak at that R_T value. These results indicate that GFM can be detected by the present method and it is also able to separate GFM from its excipients quantitatively (**Table 3**). Percent recovery of GFM in the absence and in the presence of excipients was found within the limit of ICH guidelines (**Table 3**) and thus it means that the developed method is specific for quantification of GFM.

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. Accuracy was assessed using nine determinations over three different concentration levels covering the predetermined range (0.096-0.144 mg.mL^{-1}) of analysis. And there were three replicates of each concentration (**Table 4**). From these determinations, it was found that the values of recovery for each estimation were within the range (98%-102%) of ICH percentage recovery guidelines.[29] Thus, it indicates that the proposed method is accurate enough for the analysis of the drug GFM.

Precision

Repeatability precision was carried out by six independent determinations of a fixed test concentration (0.12 mg.mL^{-1}) of a solution (**Table 5**) of GFM. Values of %RSD were calculated from these determinations and the obtained RSD value was checked to see whether it was within the limit (NMT 2%) of ICH guideline.[29] In the present case, RSD was found as 0.784% (**Table 5**) which was within the limit (NMT 2%) of ICH guideline and hence the repeatability precision was complied for the present method of analysis of GFM. Similarly, it was found that the intermediate precision and reproducibility criteria were as per ICH guideline (data are not shown).

Solution Stability

The sample solution was allowed to stand at ambient temperature (25ºC) for different time intervals (0, 12, 24 hrs) to see the stability of GFM. The obtained relative standard deviation was a measure of the stability of sample solution over a period of 24 hours. In the present study, the %RSD for sample solution was found as 0.790% (ICH limit NMT 2%) which indicates that the working sample solution was stable for at least 24 hours.

Robustness

Robustness (or Ruggedness) of the method was determined by making small deliberate change in column temperature (± 2ºC), mobile phase (± 2%) and flow rate (± 0.1 mL) of the operating parameters of the method and found no remarkable change in the test results. Percentage (%) of RSD of the test results of the three parameters at different condition was calculated and found as 0.189%, 0.432% and 0.423% respectively which was within the ICH limit (NMT 2%), indicating that the method is sufficiently robust to analyze GFM.

In the light of validation parameters results, the developed method can be used successfully for the estimation of GFM from the bulk and its tablet formulation.

CONCLUSION

A RP-HPLC method was developed and validated for the analysis of GFM in bulk and formulated tablet. The developed method is less costly than the methods reported so far.

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