

Spectrophotometric method for the determination of *Gemifloxacin mesylate* in pure and tablet dosage form

Syed Saeed Ul Hassan^{1*}, Uzma Hayat¹, Imran Tariq¹ Irshad Ahmad²,
Muhammad Munawar Hayat³, Muhammad Uzair³ and Muhammad Tayyab Ansari³

¹University College of Pharmacy, University of the Punjab, Lahore, Pakistan

²Department of Pharmacy, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

³Department of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan

Abstract: A spectrophotometric method for the determination of Gemifloxacin mesylate (GFX) is developed and validated according to ICH guidelines. GFX is a fluoroquinolone that is used in the treatment of pneumonia. The analysis of the pure drug was carried out at its λ_{\max} 270 nm. The method was linear from 0.5-5 μ g/mL, r^2 0.999 and equation is 0.102-0.000. The % RSD for inter-day (0.969%) and intra-day (0.714%) assuring a good precision and accuracy was close to 100%. Limit of detection and Limit of quantification were 0.197 and 0.599 μ g/mL, respectively. The validation results and statistical data demonstrate that the method is accurate, sensitive, cost effective and reproducible and has an importance in quality assurance of GFX analysis. The developed method was proved suitable for analysis of GFX in the pure and tablet dosage forms without interference of exceptions.

Keywords: Gemifloxacin mesylate, spectrophotometer, tablet dosage form, validation.

INTRODUCTION

Gemifloxacin mesylate (fig. 1) is a newer fluoroquinolone and an effective treatment of pneumonia (Sweetman, 2009). It is chemically it is (R,S)-7[(4Z)-3-(aminomethyl)-4-(methoxyimino)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxol, 8-naphthyridine-3-carboxylic acid) with formula $C_{19}H_{24}FN_5O_7S$. It is a white to light brown powder, available only in tablet dosage form and absorbed well orally with fewer side effects (Thomas, 2006). It is a novel fluoroquinolone broad spectrum antibacterial agent mostly used in the treatment of pneumonia and acute bacterial exacerbation of chronic bronchitis (AECB). It is effective against streptococcus pneumonia. It inhibits DNA synthesis by promoting bacterial DNA cleavage in the DNA-enzyme complex of topoisomerase IV and gyrase. GFX is bactericidal with minimum bactericidal concentration (MBCs). Oral bioavailability is 71% and plasma protein binding is 60-70%. It is metabolized to a small extent (about 10%) in liver to N-acetyl GFX and carbamyl glucuronide of GFX, both excreted in urine and feces. About 25-40% excreted unchanged in urine and 60-70% in feces (Oliphant *et al.*, 2002). It is available as 320 mg tablets in Pakistani market and is given one tablet daily for 5 to 7 days.

There would be several previously published methods for assay of GFX using different techniques but very few UV methods. There is no monograph for Gemifloxacin mesylate in British Pharmacopeia (B.P.), United State Pharmacopeia (U.S.P.), International Pharmacopeia (I.P.)

and U.S.P Salmous Standard. All ready reported methods were require expensive reagents and instruments like LC-MS method (Gandhimathi *et al.*, 2010) RP-HPLC method (Sugumaran *et al.*, 2011, Nagavalli *et al.*, 2011) and LC fluorescence method (Badraddin *et al.*, 2010) are reported for the determination of GFX. The literature survey also showed that there was few spectrophotometric methods (Panda *et al.* 2011, Jyothirmayee *et al.* 2010) Spectrophotometric determination through ion pair complex formation (Krishna *et al.*, 2008). Derivative spectrophotometric determination by chelation with Palladium (II) 12, derivative spectrophotometric determination by chelation with Cr (III) (Madhuri *et al.*, 2010) and derivative spectrophotometric determination using π receptors I (Madhuri D *et al.*, 2010). The existing methods were indirect, lengthy, expensive or involving costly equipments and tedious. This research work aims to make a contribution to the assessment of GFX and its tablet dosage form available in market. Therefore, it is need to develop a direct, less expensive and fast spectrophotometric method for the determination of GFX in pure and pharmaceutical tablet dosage forms.

MATERIALS AND METHODS

Spectrophotometric measurements were taken on (Shimadzo, Japan) model- UV-1700 spectrophotometer.

Reagents

All kinds of chemicals and reagents were of analytical grade. Methanol was of HPLC grade purchased from Sigma-Aldrich. Gemifloxacin mesylate (Batch No.GMF 0520811 and GMF 0781111) used for the present study was supplied by Hetero Drugs Ltd. Lahore. Five brands of

*Corresponding author: e-mail: saeedulhassan8@hotmail.com

GFX are Qflox (Schazoo), Gemglow (Searle), Gemicid (Himont), Gemiflet (Pulse) and Qupric (Wilshire).

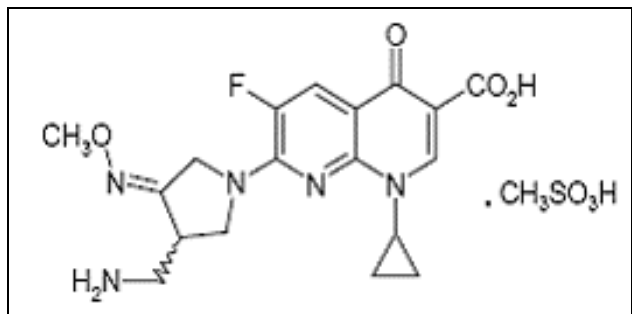


Fig. 1: Structure of Gemifloxacin mesylate (R, S)-7[(4Z)-3-(aminomethyl)-4-(methoxyimino)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo,8-naphthyridine-3-carboxylic acid).

Method development

A direct method was developed for the determination of GFX in pure and tablet dosage forms. The drug was dissolved in methanol, UV spectrum of GFX exhibits the maximum absorption at 270 nm was selected, as it had the least interference from the other solvents.

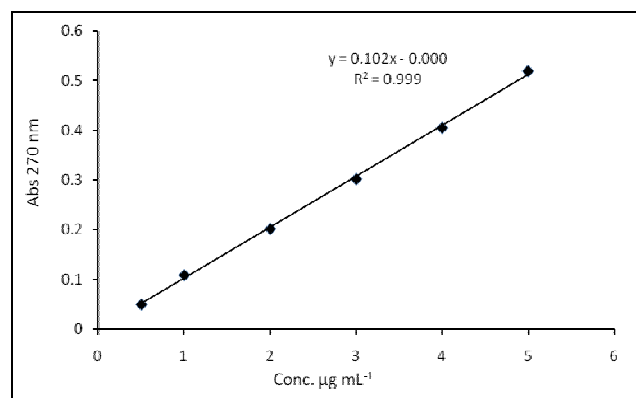


Fig. 2: Calibration curve of GFX at 270 nm.

Preparation of standard stock solution

Standard Stock solution of GFX was prepared by dissolving an accurately weighed 62.3 mg of standard GFX as mesylate (equivalent to 50 mg of GFX) in 100 ml of methanol in volumetric flask, to get a concentration of 0.5 mg/ml which was further diluted to get the different working concentrations. Fresh solution was prepared and sonicated.

Preparation of sample solution

Five commercial brands of GFX tablets; Qflox (Schazoo), Gemglow (Searle), Gemicid (Himont), Gemiflet (Pulse) and Qupric (Wilshire) were taken and various concentrations of solutions were made for these studies. Weighed and powder 20 tablets of GFX and taken an equivalent amount of 50mg of GFX, dissolved in methanol in 100ml volumetric flask. Fresh solution was prepared and sonicated.

Method validation

The developed method for spectrophotometric determination of GFX in pure and tablet dosage forms was validated according to International Conference of harmonization (ICH) guidelines.

Linearity and range

Linearity is a parameter which is used to see that the test results are within given range. The drug was dissolved in methanol and calibration curve was constructed in the range 0.5-5 $\mu\text{g/ml}$ (table 1 and fig.2).

Table 1: Validation and sensitivity parameters of method of GFX

Parameter	Value
Wavelength (λ_{max})	270 nm
Range	0.5-5 $\mu\text{g/ml}$
LOD	0.197 $\mu\text{g/ml}$
LOQ	0.599 $\mu\text{g/ml}$
r^2	0.999
Slope	0.102
Intercept	0.000
Accuracy	100.52-101.12% (N=9)
Specificity	99.6%
Robustness indicated by %RSD	1.18%
Precision indicated by RSD (%)	Intra-day = 0.714% (N=6) Inter-day = 0.969% (N=18)

Precision

Precision of this developed method was 0.969% RSD for inter-day and 0.714% RSD for intra-day sampling and the values within the range of ICH guidelines (table 1).

Accuracy

Accuracy was determined by selecting three different concentrations of GFX in triplicate. The mean values low, medium and high concentrations for pure drug GFX, the accuracy were 100.52%, 100.13% and 101.12%, respectively (table 1).

Specificity

In validation process, specificity test is a parameter used for the determination of impurity, degradants or excipients. This test was performed by changing the percentage of excipients with fix concentration of GFX (table 1).

Sensitivity: Limit of detection & limit of quantification

The limit of detection (LOD) was calculated by formula ($\text{LOD}=3.3 \sigma/S$), where σ is SD and S is slope of calibration curve. Limit of quantification (LOQ) was calculated by formula ($\text{LOQ}=10 \sigma/S$), where σ is SD and S is slope of calibration curve. LOD and LOQ were 0.197 $\mu\text{g/ml}$ and 0.599 $\mu\text{g/ml}$, respectively (table 1).

Robustness

It is ability of the analytical method to remain unaffected when small variations are made in different parameters and reflex the reliability of the method. The developed method was checked by change the wavelength (± 3), using AR grade methanol and allowing solution to stand for period (2-20 hours). RSD of Robustness is 1.18% that shows method is reliable.

Stability studies

The effect of the temperature (40 °C) and humidity (74%) on %age contents of six different brands of tablet dosage forms was studied (table 2).

Table 2: Effect of temperature (40 °C) and humidity (74%) on % age contents of GFX tablets

Serial No.	Time in months	% age contents
1	1	101.87
2	2	99.97
3	3	100.75
4	6	101.55

RESULTS

The developed method has linearity range between 0.5-5 $\mu\text{g/ml}$ ($r^2=0.999$), accuracy 100.52-101.12 %, specificity 99.6 % and LOD and LOQ were 0.197 $\mu\text{g/ml}$ and 0.599 $\mu\text{g/ml}$, respectively (table 1). This method has been validated successfully as shown in the five different brands of tablet dosage forms of different manufacturers. It has also been applicable in the determination of drug in both pure and pharmaceutical tablet dosage forms (table 3).

Table 3: Determination of the concentration of the five brands of different manufacturers of GFX tablet dosage form the forecast formula ($Y = A + B X$).

S. No.	File name	Quantity quoted in dosage forms (mg)	Contents (%)
1	Brand-1	320	101.95
2	Brand-2	320	97.35
3	Brand-3	320	101.23
4	Brand-4	320	96.09
5	Brand-5	320	100.19

DISCUSSION

The validation of the developed method was studied according to guidelines of International Conference of Harmonization (ICH) which are linearity, accuracy, precision, sensitivity, specificity, robustness and system suitability in pure and tablet dosage forms, statistical data (table 1) shows that the developed method is reliable, specific, precise, accurate and sensitive. This method has an importance in the quality assurance of the

pharmaceutical dosage forms especially tablets in the drug testing laboratories.

There is no monograph for the GFX in pure and formulations in B.P and U.S.P Literature survey has shown D. Jyothirmayee *et al.* (2010) developed a spectrophotometric method based on reduction reaction which has less linearity then the developed method and not determined the sensitivity and specificity of method. Madhuri *et al.* (2010) developed a derivative spectrophotometric method based on chellate formation with palladium (II) and UV detection at 430 nm for 1st derivative and 480 nm for 2nd derivative. Same author in another study spectrophotometric estimation of GFX by chellation with Cr (III) and showed maximum UV absorption at 620 nm for 1st derivative and 660 nm for 2nd derivative. These both reported methods are indirect and derivative, quit different from this study. Panda *et al.* 2011 developed a UV method for determination of GFX in tablets. They studied that GFX shows two different forms in acidic (0.1 M-HCl) and basic (0.1M-NaOH) medium that differ in absorption spectra and measures absorption between 278 nm and 320 nm.

CONCLUSION

The present method was found direct and economical compared to the published methods. This method is too sensitive with LOD and LOQ were 0.197 and 0.599 $\mu\text{g/ml}$, respectively and shows RSD value low then 2% (intra-day and inter-day) assuring a good precision. The all validation parameters given in table 1 indicate the accuracy and reproducibility of the developed method. The method was successfully applied on five brands of tablets shows the accuracy about 100% and has an importance in quality assurance of solid pharmaceutical dosage forms. It is concluded that the developed method is direct, specific, cheap, rapid and precise.

REFERENCES

- Badraddin MH, Al-Hadiyaa AA and KhadybGamal AE (2010). Validated liquid chromatographic-fluorescence method for the quantitation of Gemifloxacin in human plasma. *Talanta*, **83**: 110-116.
- Gandhimathi M, Nair NBDK and Ravi TK (2010). Study hydrolytic and oxidative behavior of gemifloxacin mesylate in aqueous solution by LC-MS. *J Global Pharm. Tech.*, **2**(1): 81-85.
- Jyothirmayee D, Sudhakar Sai Babu G and Devala Rao G (2010). Spectrophotometric determination of gemifloxacin in pharmaceutical formulation. *Asian J Chem.*, **22**(2): 1634-1636.
- Krishna MV and Sankar D (2008). Direct and derivative spectrophotometric estimation of Gemifloxacin mesylate by chelation with Cr(III) ion. *E-Journal of Chem.*, **5**(3): 515-520.

- Madhuri D, Chandrasekhar KB, Devenna N and Somasekhar G (2010). Direct and derivative spectrophotometric estimation of Gemifloxacin by chelation with palladium (II) ion. *Rasayan J Chem.*, **3**(1): 159-165.
- Madhuri D, Chandrasekhar KB, Devenna N and Somasekhar G (2010). Direct and derivative spectrophotometric estimation of Gemifloxacin mesylate by chelation with Cr(III) Ion. *Rasayan J Chem.*, **3**(1): 9-15.
- Madhuri D, Chandrasekhar KB, Devenna N and Somasekhar G (2010). Direct and derivative spectrophotometric determination of Gemifloxacin mesylate in pure form and pharmaceutical preparations using π acceptors. *Int. J Pharm. Sci. Res.*, **1**(4): 222-231.
- Nagavalli D, Abirami G and Kumar KS (2011). Validated HPLC method for the simultaneous estimation of gemifloxacin mesylate and ambroxol hydrochloride in bulk and tablet dosage form. *J Pharm. Res.*, **4**(6): 1701-1703.
- Oliphant CM and Green GM (2002). Quinolones: A Comprehensive Review. *J AM Fam. Physician*, **65**(3): 455-64.
- Panda SS, Humar RBVV, Rao KS, Kumar RV and Patanaik D (2011). Difference spectrophotometric determination of Gemifloxacin mesylate in tablet formulation. *Asian J Biochem. Pharm. Res.*, **1**(3): 442-447.
- Sugumaran M and Jotheeswari D (2011). RP-HPLC method for the determination of gemifloxacin mesylate in bulk and pharmaceutical formulation. *Int. J Pharm. Sci Rev. Res.*, **6**(1): 18-20.
- Sweetman SC (2009). Martindale, the complete Drug Reference, 36th Edition, pp.181.
- Thomas Health Care (2006). Physician Desk Reference, 60th Edition, p.2464.