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COST EFFECTIVE SPECTROPHOTOMETRIC METHODS FOR THE ANALYSIS OF GEMIFLOXACIN IN PURE AND PHARMACEUTICALS

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ABSTRACT

Two simple, accurate, rapid and sensitive methods (**Method M_1** and **Method M_2**) have been developed and validated for the assay of gemifloxacin pure and in its pharmaceutical dosage form. The Method M_1 and Method $M_2$are based on the formation of condensed product of gemifloxacin with 2-CPH (Method M_1) and 2,4-DNPH (Method M_2), which showed an absorbance maxima at 550 nm and 460 nm respectively. The absorbance-concentration plot is linear over the range of 2.0-10mcg/ml for Method M_1 and M_2 respectively. Results of analysis for all the methods were validated statistically and by recovery studies. The proposed methods are economical and sensitive for the estimation of gemifloxacin in pure and in its tablet dosage form

KEY WORDS: Gemifloxacin,Visible spectrophotometry,Validation

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INTRODUCTION

Gemifloxacin, 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.This mesylate salt is a white to light brown solid with a molecular weight of 485.49. Gemifloxacin is considered freely soluble at neutral pH (350 µg/mL at 37°C,pH-7.0). Its empirical formula is $C_{18}H_{20}FN_5O_4 \bullet CH_4O_3S$. Its chemical structure is represented in Figure.1. Several methods[1-21] have been reported for quantitative determination of gemifloxacin. As the analytically useful functional groups present in gemifloxacin have not been fully exploited for designing suitable visible spectrophotometric methods and therefore still offer a scope to develop more number of new visible spectrophotometric methods with better sensitivity, selectivity, precision and accuracy. The author has made some attempts in this direction and succeeded in developing six new visible spectrophotometric methods for the quantification of Gemifloxacin in pure and tablet dosage forms.

EXPERIMENTAL

INSTRUMENTS USED: Genesys 10 UV-Spectrophotometer 10mm matched quartz cells procured from Thermo Scientific company with was used for all spectral measurements. A Systronics digital pH meter [Model-362]was

used for pH measurements. All weighing were done on electronic balance (Model Shimadzu AUW-220D) respectively. Calibrated glassware was used throughout the experiment.

PREPARATION OF REAGENTS: All the chemicals and reagents used were of analytical grade and solutions were prepared in doubled distilled water.

Method - M_1[2-chlorophenyl hydrazine, (Loba;0.2%) reagent]: Prepared by dissolving 0.25g of 2-chlorophenyl hydrazine in 100mL methanol.

Method – M_2[0.2%,2,4-Dinitro Phenyl Hydrazine Reagent]: Prepared freshly by dissolving 100mg of 2,4-Dinitro Phenyl Hydrazine in a mixture of 10mL of methanol and 0.5mL of Conc.HCl was added and finally diluted to 100mL with methanol.

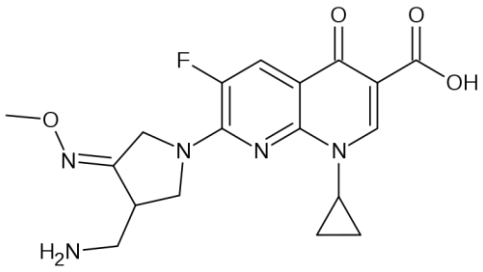


Figure.1.Structure of Gemifloxacin

PREPARATION OF STANDARD DRUG SOLUTION:

0.1% stock solution of Gemifloxacin was freshly prepared by transferring accurately weighed 100mg of Gemifloxacin into 100mL volumetric flask containing 50.0mL distilled water and later made up to the mark. Then working standard solutions of concentration 40µg.mL^{-1} are prepared by transferring 4.0mL of the stock solution into two 100mL standard flasks respectively and made up to the mark with distilled water.

PROCEDURE FOR DOSAGE FORMS: Ten tablets(FACTIVE;320mg of gemifloxacin mesylate) procured from local pharmacy were weighed and powdered finely. A quantity of tablet powder equivalent to 100mg of gemifloxacin mesylate was accurately weighed and transferred into a 100ml volumetric flask and dissolved in about 25.0mL of distilled water and was filtered through a Whatman No.42 filter paper. After mixing this filtrate was made up to mark with distilled water. Suitable volumes of this filtrate was pipetted and diluted with distilled water to get a sample concentration of 40µg.mL^{-1} for the method M_{11} & M_{12}. Aliquots of these solutions were used for the determination of each gemifloxacin drug as per the procedures described.

Fig.2: Absorption spectrum of GFX with 2-CPH (M_1)

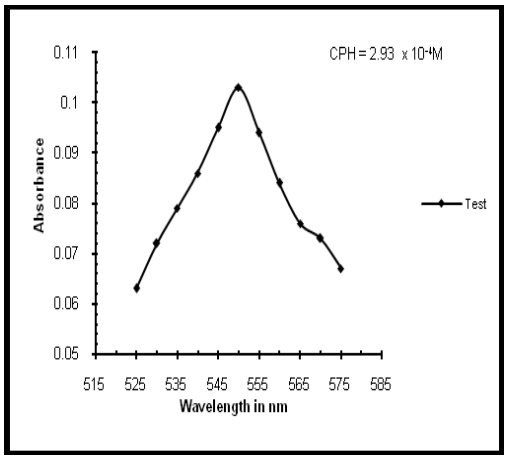


Fig. 3: Absorption spectrum of GFX with 2,4-DNPH (M_2)

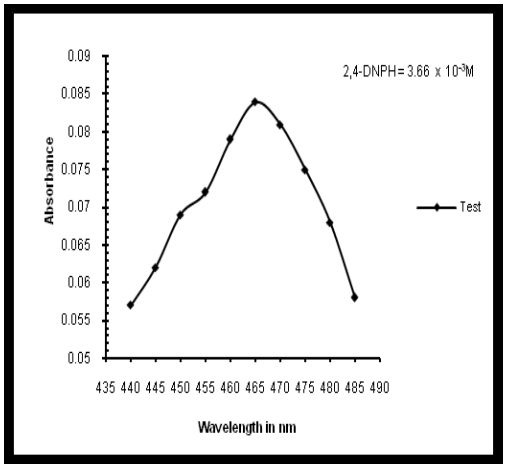


Fig.4: Beers Law plot of GFX with CPH (M_1)

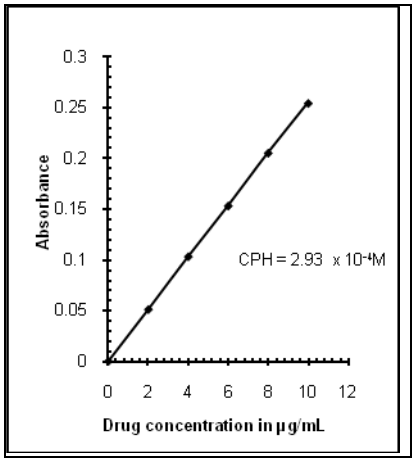


Fig.5:Beers Law plot of GFX with 2,4-DNPH (M_2)

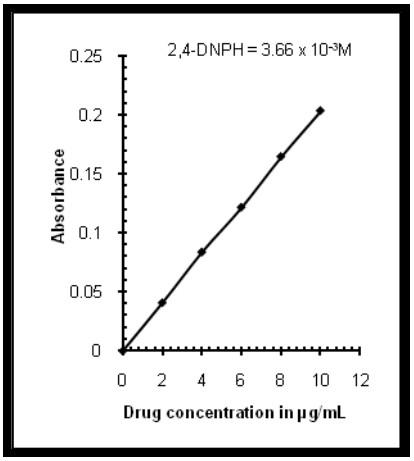


Table - 1:Optimum conditions established in method M_1 for GFX

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm) for 2-Chlorophenyl hydrazine (M_1)	530 – 570nm	550nm	
Effect of volume (0.2%) of CPH in MeOH and waiting time.	2 - 4mL ,40min	2.0mL,30min	2.0mLof CPH and 30 min waiting time were preferred for covering broad range in Beer's law limits.
Stability period after final dilution.	-------	10hrs	After 10hrs the absorbance of colored species diminishes slowly with time.

Table - 2:Optimum conditions established in method M_2 for GFX

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm) for 2,4-DNPH (M_2)	440 – 450nm	460nm	
Effect of volume Conc.HCl and waiting time.	2 - 4mL ,10min	2.0mL,10min	2.0mL of Conc.HCl and 10 min waiting time were preferred.
Stability period after final dilution.	-----	12hrs	After 12hrs the absorbances of colored species diminish slowly with time.

Table.3: Optical and Regression characteristics, Precision and accuracy of the proposed methods for Gemifloxacin

Parameter	M_1	M_2
λ_{max} (nm)	550	460
Beer's law limits (µg/mL)	2.0 - 10.0	2.0 - 10.0
Molar absorptivity (1 $mol^{-1}.cm^{-1}$)	4.945×10^3	4.465×10^3
Sandell's sensitivity (µg.cm^{-2}/0.001 absorbance unit)	0.01838	0.08474
Optimum photometric range (µg/mL)	3.0 - 9.0	2.5-4.5
Regression equation (Y=a+bc);slope (b)		0.0242
Intercept (a)	0.0137	0.0099
Correlation coefficient (r)	0.9998	0.9997
Relative standard deviation (%)*	0.9993	0.8941
% Range of error (confidence limits)	1.807	
0.05 level		0.9655
0.01 level	2.07	1.6730

* Average of six determinations

Table.4:Assay and Recovery of Gemifloxacin in dosage forms

Method	Pharmaceutical Formulation	Labeled Amount (mg)	Proposed Method			Found by reference method[14] ±S.D	%Recovery by proposed method**
			Amount found* (mg) ±S.D	t (value)	F (Value)		
M_1	Tablet	100	99.91±0.08	0.72	4.0	99.96±0.16	97.44±0.81
M_2			99.95±0.10	0.13	2.56		99.49±0.21

Average ± standard deviation of six determinations the t and F- values refer to comparison of the proposed methods. Theoretical values at 95 % confidence limits t = 2.365 and F = 4.88.

**Average of six determinations.

RESULTS AND DISCUSSIONS

PARAMETERS FIXATION (OPTIMIZATION STUDIES): The Optimization studies for the proposed procedures for the assay of gemifloxacin involve the study of the influence of various factors on the color development [optimal conditions] such as reagent concentration, order of addition of reagents, time, temperature and choice of solvent for maximum color development.

Method – M_1 [2-CPH]: The optimum conditions for the proposed method was made basing on the study of the effects of various parameters such as volume of 2-CPH solution, volume of solvents solution used initially and subsequently for final dilution. The optimum conditions developed and actual conditions chosen for the procedure are recorded in Table.1.

Method – M_2 [2,4-DNPH]: The optimum conditions in this method were established basing on the study of the effects of various parameters such as volume of 2,4-DNPH solution, volume of solvents solution used initially and subsequently for final dilution and the stability of colored species after final dilution. The optimum conditions developed for the proposed method is recorded given in Table.2.

PROPOSED PROCEDURES

Method – M_1: Aliquots of (0.5-2.5mL, 40µg.mL^{-1}) drug were transferred into a series of 10.0mL volumetric flasks. To each of the above aliquots, 2.0mL of 2-Chloro phenyl hydrazine was added followed by one drop of concentrated hydrochloric acid and heated to 50-55°C for color development. The absorbance of the color

derivatives were measured at 550nm for 2-chlorophenyl hydrazine against reagent blank. The amount of gemifloxacin was computed from its calibration graph (Fig. 4).

Method – M_2: Aliquots of working standard gemifloxacin solution ranging from 0.5-2.5mL (40µg.mL^{-1}) were transferred into a series of 10.0mL calibrated test tubes. To this 2.0mL of 2,4-DNPH reagent was added followed by one drop of Conc.HCl. The mixtures were placed on a boiling water bath for 10min and were cooled to room temperature. The contents of the tubes were mixed thoroughly and allowed to stand for 10minutes with occasional shaking at room temperature and the final volume in each tube was made up to mark with distilled water. The pink colored chromogen was measured spectrophotometrically at 460nm against a reagent blank (Fig. 5).

b. METHOD VALIDATION:

SPECTRAL CHARACTERISTICS: The absorption maxima (λ_{max}) of the colored species formed in the above methods, by following the above procedures with the specified amounts of gemifloxacin and the absorption spectra were scanned on a spectrophotometer in the wavelength region of 340 to 900nm against similar reagent blank or distilled water and the results were graphically represented in Fig.2 & 3.

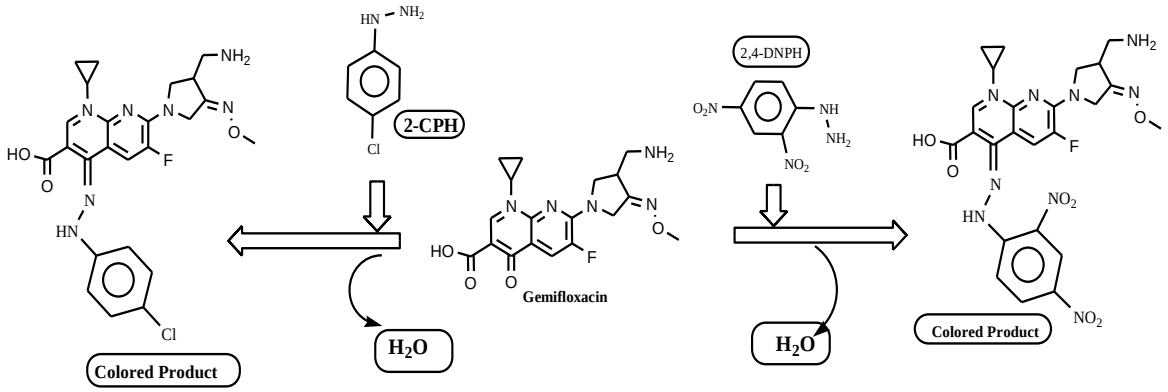
OPTICAL CHARACTERISTICS: In this study the absorbance's at appropriate wavelengths a set of solutions containing varying amounts of gemifloxacin and specified amounts of reagents (as given in the recommended procedures for each method) were recorded against the corresponding reagent blanks for each proposed method and the Beer's law plots against the corresponding reagent blanks of these systems were recorded graphically (Figs.4&5). Beer's law limits, molar absorptivity, Sandell's sensitivity and optimum photometric range (Table.3) for gemifloxacin in each method developed. Least square regression analysis was carried out for getting the slope, intercept and correlation coefficient values (Table.3).

PRECISION: The precision of each proposed method was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of gemifloxacin in total solution. The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods (Table.3).

ACCURACY: The accuracy of each proposed method was determined with different amounts of bulk samples of gemifloxacin within the Beer's law limits and analyzed by the proposed method. The results of the proposed methods are summarized in (Table.3).

ANALYSIS OF FORMULATIONS: Commercial formulations (tablets) containing gemifloxacin were successfully analyzed by the proposed methods. The values obtained by the proposed and reference methods for formulations were compared statistically with F and t tests and found not to different significantly. The results of the proposed methods are summarized in (Table.4).

NATURE OF THE COLORED SPECIES: An attempt has been made by the author to indicate the nature of colored species in each of the proposed methods for gemifloxacin is based on analogy [reactive functional moiety (Keto group) in drug, reagents nature.



Scheme.1.Reaction of Gemifloxacin with 2,4-DNPH

Method –M_1: The proposed methods developed by the author were based on condensation reaction of the keto group of gemifloxacin with 2-CPH(M_{11}) forming various colored chromogens. The predicted reaction mechanism is represented in Scheme.1 for method (M_1 respectively).

Method – M_2: The proposed method is based on the interaction of the drug with 2,4-dinitrophenylhydrazine in the presence of an acid catalyst, followed by treatment with a methanolic solution of potassium hydroxide forming an stable colored chromogen measured at 460nm.

CONCLUSIONS

The proposed visible spectrophotometric methods developed by the author were advantageous with respect to their high reproducibility, high sensitivity, less time consuming and using simple and inexpensive reagents. The proposed methods developed by the author are superior to the previously reported method[14], as the measurements are performed in the visible region, away from the UV-absorbing interfering excipients that might be co-extracted from gemifloxacin containing dosage forms. The proposed visible spectrophotometric methods can be used as alternative methods in quality control laboratories for the analysis of gemifloxacin in pure forms as well as in formulations as they are simple, rapid, sensitive and accurate.

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