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Spectrophotometric Determination of Gemifloxacin in Bulk and Pharmaceutical Formulation

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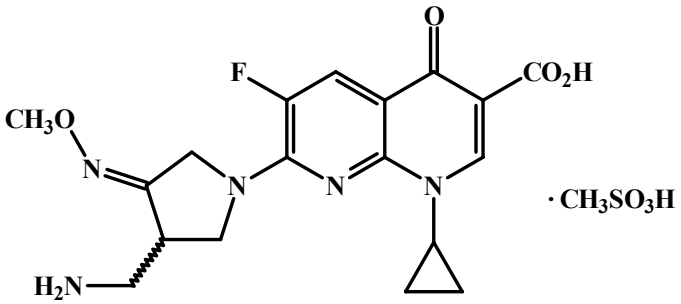
A three simple, sensitive and reproducible visible spectrophotometric methods (methods **A**-**C**) are developed for the determination of gemifloxacin in pure and dosage forms. Method **A** is based on the formation of coloured species on treatment of reduced gemifloxacin with Folin-Ciocalteu reagent in presence of 4 % NaOH solution. Method **B** is based on the formation of coloured species on treatment of gemifloxacin with 3-methyl-2-benzo thiazolinone hydrazone and ferric chloride. Method **C** is based on the formation of coloured species on treatment of gemifloxacin with $FeCl_3$ and 1,10-phenanthorline.

Key Words: Spectrophotometry, 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.

The mesylate salt is a white to light brown solid with a molecular weight of 485.49. Gemifloxacin is considered freely soluble[1] at neutral pH (350 µg/mL at 37 ºC, pH 7.0). Its empirical formula is $C_{18}H_{20}N_5O_4F \cdot CH_3SO_3H$ and its chemical structure is



Structure of gemifloxacin

It is not official in any pharmacopoeia and no visible spectrophotometric analytical reports are found in literature for its quantitative estimation in bulk and tablet dosage forms[2-4]. In the present investigation a three simple, sensitive and reproducible visible spectrophotometric methods are developed for the determination of gemifloxacin in their dosage forms. using Folin-Ciocalteu (FC) reagent in presence of 4 % sodium hydroxide solution, 3-methyl-2-benzo thiazolinone hydrazone (MBTH)-$FeCl_3$ and $FeCl_3$-1,10-phenanthorline. In method **A**, the formation of coloured species is based on the reduction of phosphomolybdotungstic acid (FC reagent), by gemifloxacin in presence of 4 % sodium hydroxide solution, thereby producing reduced species having characteristic blue colour with maximum absorption at 685 nm. In method **B**, the formation coloured species is due to the oxidative coupling reaction with MBTH in presence of $FeCl_3$ to form a green coloured complex having λ_{max} at 617 nm. In method **C**, the formation of coloured species having λ_{max} at 466 nm is due to reaction of reduced ferric ion to ferrous ion with 1,10-phenanthorline.

EXPERIMENTAL

A Shimadzu UV-Vis spectrophotometer was used for absorbance measurements. All the chemicals used were of analytical grade

Method A

Folin-Ciocalteu reagent: 50 mL of Folin-Ciocalteu reagent (2 N) was diluted to 100 mL with distilled water.

NaOH (4 %): 4.0 g of sodium hydroxide was dissolved in 100 mL of distilled water.

Diluent: Water.

Preparation of standard solution (100 µg/mL): About 125 mg of gemifloxacin working standard was accurately weighed and dissolved in 20 mL of distilled water and treated with 10 mL of 2 N HCl and 1 g zinc dust was added in portions and kept aside for 1 h. After standing for 1 h at room temperature the solution was filtered through cotton wool in to a 100 mL volumetric flask and the residue was washed with 3 × 10 mL portions of distilled water and the total volume is made up to volume with diluent. 5 mL of the above stock is taken into 50 mL volumetric flask and made up to volume with diluent.

Preparation of test solution: Twenty tablets were taken weighed and powdered. Sample equivalent to about 100 mg of gemifloxacin was accurately weighed and dissolved in 20 mL of distilled water and treated with 10 mL of 2 N HCl and 1 g zinc dust was added in portions and kept aside for 1 h. After standing for 1 h at room temperature the solution was filtered through cotton wool in to a 100 mL volumetric flask and the residue was washed with 3 × 10 mL portions of distilled water and the total volume is made up to volume with diluent. 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.

Method B

MBTH (0.2 %): 200 mg of MBTH was dissolved in 100 mL of distilled water.

$FeCl_3$ (0.9 %): 900 mg of ferric chloride ($FeCl_3.6H_2O$) was dissolved in 100 mL of distilled water.

Diluent: Water.

Preparation of standard solution (1000 µg/mL): About 125 mg of gemifloxacin working standard was accurately weighed and dissolved in 20 mL of distilled water and treated with 10 mL of 2 N HCl and 1 g zinc dust was added in portions and kept aside for 1 h. After standing for 1 h at room temperature the solution was filtered through cotton wool in to a 100 mL volumetric flask and the residue was washed with 3 × 10 mL portions of distilled water and the total volume is made up to volume with diluent.

Preparation of test solution: Twenty tablets were taken weighed and powdered. Sample equivalent to about 100 mg of gemifloxacin was accurately weighed and dissolved in 20 mL of distilled water and treated with 10 mL of 2 N HCl and 1 g zinc dust was added in portions and kept aside for 1 h. After standing for 1 h at room temperature the solution was filtered through cotton wool in to a 100 mL volumetric flask and the residue was washed with 3 × 10 mL portions of distilled water and the total volume is made up to volume with diluent. 10 mL was taken and centrifuged at 2500 rpm.

Method C

$FeCl_3$ (0.9 %): 900 mg of $FeCl_3$ was dissolved in 100 mL of distilled water.

1,10-Phenanthorline (0.125 %): 125 mg of 1,10-phenanthorline was dissolved in 100 mL of distilled water.

Diluent: Water.

Preparation of standard solution (1000 µg/mL): About 100 mg of gemifloxacin working standard was weighed and transferred in to a 100 mL volumetric flask, dissolved in 70 mL of diluent and made up to volume with diluent.

Preparation of test solution: Twenty tablets were taken weighed and powdered. Sample equivalent to 100 mg of gemifloxacin was accurately weighed and transferred in to 100 mL volumetric flask, 70 mL of diluent was added sonicated for 20 min and made up to volume with diluent. 10 mL was taken and centrifuged at 2500 rpm.

Assay procedures: For method **A**, 1 mL portion of Folin-Ciocalteu reagent was taken in a series of 10 mL graduated volumetric flasks. Volumes of standard solution ranging from(1-5 mL; 1 mL = 100 µg) were transferred into each flask and shaken gently for 5 min. Then 2 mL of NaOH solution was added to each flask and kept aside for 15 min for maximum colour development. Appropriate volume of distilled water was added to each flask to bring the total volume 10 mL. The absorbance was measured at 685 nm against reagent blank. Test solution was treated in the same manner as standard and measured the absorbance at 685 nm.

For method **B**, volumes of standard solution ranging from (0.1-1 mL; 1 mL = 1000 µg) were transferred into a series of 10 mL graduated volumetric flasks. A 1 mL portion of $FeCl_3$ was added to each flask and shaken gently for 5 min. Then 2 mL of MBTH solution was added to each flask and kept aside for 15 min for maximum colour development. Appropriate volume of distilled water was added to each flask to bring the total volume 10 mL. The absorbance was measured at 617 nm against reagent blank. The test solution was treated in the same manner as standard and measured the absorbance at 617 nm.

For method **C**, volumes of standard solution ranging from (0.4-2 mL; 1 mL = 1000 µg) were transferred into a series of 10 mL graduated volumetric flasks. Then 1 mL portion of $FeCl_3$ and 2.0 mL of 1,10-phenanthorline were added successively and set aside for 10 min and the total volume made up to 10 mL with distilled water. The absorbances of the coloured complex solutions were measured at 466 nm against reagent blank prepared similarly. Test solution was treated in the same manner as standard and measured the absorbance at 466 nm.

The amount of gemifloxacin present in the sample solutions (X) using the proposed methods was calculated by formula given below and the results are given in Table-2.

$$X = \frac{A_T}{A_s} \times \frac{D_s}{D_T} \times \frac{A_w}{L} \times \frac{P}{100} \times 100$$

where A_T = Absorbance of sample solution, A_s = Absorbance of standard solution, D_s = Dilution factor for standard, D_T = Dilution factor for test solution, A_w = Average weight of sample in mg, L = Label claim, P = Potency of standard on as is basis.

RESULTS AND DISCUSSION

The optical characteristics such as Beer's law limits (µg/mL), Sandell's sensitivity (µg/cm^2/0.001 A.U), correlation coefficient (r), % relative standard deviation (calculated from six determinations), % range of error (0.05 confidence limits and 0.01 confidence limits), regression equation (I+ac) for the proposed method is calculated and summarized in Table-1.

TABLE-1
OPTICAL CHARACTERISTICS AND PRECISION

Method parameters	Method **A**	Method **B**	Method **C**
λ_{max} (nm)	685	617	466
Beer's law limits (µg/mL)	10-50	10-100	40-200
Sandell's sensitivity (µg/cm^2/0.001 AU)	0.07741	0.18541	0.21642
Regression equation (I + ac)			
Slope (a)	0.01314	0.00550	0.00449
Intercept (I)	-0.01239	-0.00661	0.01524
Correlation coefficient(r)	0.999	0.999	0.999
%RSD	0.1870	0.1890	0.060
% Range of error			
Confidence limits with 0.05 level	0.156	0.158	0.050
Confidence limits with 0.01 level	0.231	0.234	0.074

*Mean of six determinations.

The results obtained with the proposed methods for dosage forms is compared with the results obtained with HPLC method and presented in Table-2. The proposed method is validated and found to be specific, accurate, precise and linear. Interference studies revealed that the common excipients used in the dosage forms do not interfere in the estimation of gemifloxacin using the proposed methods. To check the recovery using the proposed method known amounts of pure drug was added to the placebo used in the pharmaceutical preparation of gemifloxacin and the mixtures were analyzed by the proposed method and the % recoveries are given in the Table-2. In conclusio, the proposed methods are simple, sensitive and accurate and can be used for the routine determination of gemifloxacin in pharmaceutical preparations.

TABLE-2
EVALUATION OF GEMIFLOXACIN IN PHARMACEUTICAL PREPARATIONS

Label claim (mg/tab)	Amount obtained (mg)*				Recovery (%)**		
	Proposed method			Reference method (HPLC)			
	Method **A**	Method **B**	Method **C**		Method **A**	Method **B**	Method **C**
320	319.3± 0.605	319.9± 0.455	320.1± 0.194	319.7 ± 0.314	99.3± 0.561	99.9± 0.163	99.5± 0.501

*Mean ± SD of six determinations,
**Mean ± SD of six determinations (100 mg of gemifloxacin was added and recovered).

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