Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue ?, 2012

Research Article

NEW VISIBLE SPECTROMETRIC DETERMINATION OF GEMIFLOXACIN IN ITS PURE FORM

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Received: 12 Jun 2012, Revised and Accepted: 25 July 2012

ABSTRACT

Three simple, economical, precise and reproducible Visible Spectrophotometric methods have been developed for the estimation of Gemifloxacin (GMF) in pure form. The developed methods are based on the formation of products by the reaction of GMF with Fast Green dye (FGFCF), Brucine and Vanillin. The maximum absorbances observed for the three methods are at 625nm, 520nm and 500nm respectively and linearity in the concentration range of 30-100µg/ml, 40-80 µg/ml and 10-40 µg/ml. The results of analysis for all the methods were validated statistically and by recovery studies.

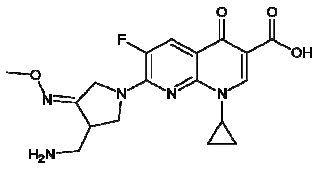
Keywords: Gemifloxacin, Fast green FCF, Brucine, Vanillin, Antibacterial and Spectrophotometer

INTRODUCTION

Fluoroquinolones are used for the treatment of various infections, as they are active against both gram-positive, and gram-negative bacteria.[1-3] However, resistance to the second-generation fluoroquinolones has been increased against many bacterial species.

Gemifloxacin chemically known as 7-[(4*Z*)-3-(aminomethyl)- 4-methoxyimino-pyrrolidin-1-yl]- 1-cyclopropyl-6-fluoro-4-oxo- 1,8-naphthyridine-3-carboxylic acid, has its molecular formula and weight as $C_{18}H_{20}FN_5O_4$, 389.381. It is a new fourth generation fluoroquinolone, antibacterial compound with enhanced affinity for topoisomerase IV, and DNA gyrase. Recently U.S. Food and Drug Administration approved for the treatment of the upper respiratory and urinary tract infections.[4-9] It is also used for acute bacterial exacerbation of chronic bronchitis and mild-to-moderate pneumonia.[10] The compound has a broad spectrum of activity against Gram-positive and Gram-negative bacteria. It has shown potent antibacterial activity against clinical isolates and reference strains in both in vitro studies and experimental models of infection in animals.[11]

The chemical structure of GMF is



Literature survey reveals that UV-Spectrophotometric method for gemifloxacin mesylate in pharmaceutical tablet dosage form,[12] Spectrophotometric determination of gemifloxacin in bulk and pharmaceutical formulations,[13-14] Spectrofluorimetric method for the determination of gemifloxacin in tablet form,[15] Development and validation of gemifloxacin by RP-HPLC method in tablet dosage form,[16] Validated stability-indicating HPLC method for analysis of gemifloxacin in tablet formulations,[17] RP-HPLC method for the determination of gemifloxacin mesylate in bulk and pharmaceutical formulation[18] were developed.

OBJECTIVE

The main objective of the present investigation was to develop simple, accurate and economical Visible Spectrophotometric methods for the estimation of Gemifloxacin in pure form.

MATERIALS AND METHODS[19-20]

Instrumentation

SL 177 model (Elico) Visible spectrophotometer wavelength accuracy of ± 0.3 nm and 1.0 cm matched quartz cell is used for analytical method development.

Experimentation

Pure sample of Gemifloxacin was purchased from Zen Pharma Gujarat. 50 mg of pure GMF is weighed accurately and dissolved in 50 ml of double distilled water. The concentration is 1000 ppm. This was treated as stock solution used for development of two methods. On further dilution to 500 ppm one method was developed.

Method-1

1 ml of 0.0316% $KMnO_4$ is added to the standard drug solution of (1.5ml-5.0ml, 500µg/ml) in a series of 25ml volumetric flasks and adjusted the volume to 10 ml with double distilled water, kept aside for 15 minutes at room temperature. Then 4.0 ml of FGFCF solution and 1.0 ml of 14.2 % Na_2SO_4 solution are added, waited for 10 minutes and made up to 25ml with distilled water and measured its optical density at 625 nm against double distilled water. A similar reagent blank was prepared without the drug. An increase in absorbance is observed. The amount of drug present was deduced from the calibration curve.

The proposed reaction is

GMF + excess $KMnO_4 \rightarrow$ oxidized product of drug + $KMnO_4$ (unreacted)

$KMnO_4$ (unreacted) + FCF$\rightarrow Mn^{2+}$ + Unreacted dye (coloured)

Method-2

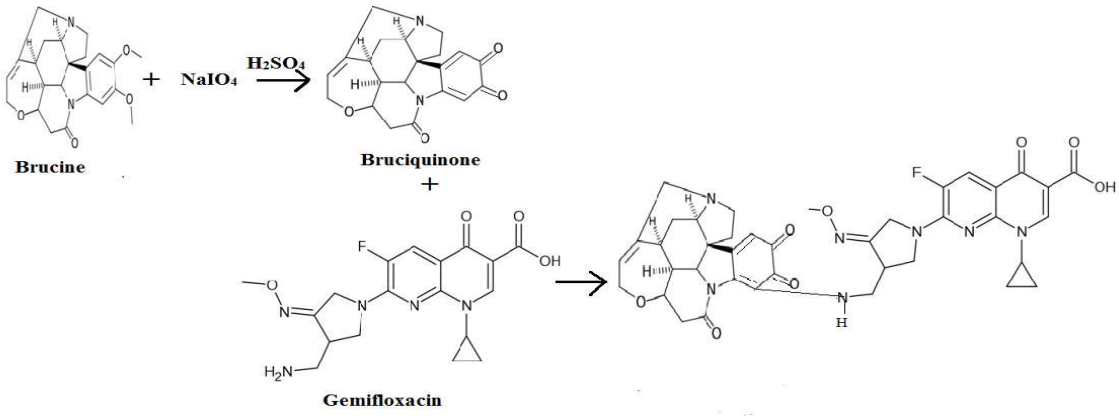
Standard drug solution of (0.75 ml-3.0 ml, 1000 µg/ml) were delivered into a series of 25 ml calibrated test tubes, 3mL of $5.067X10^{-3}$M solution of Brucine, 1.5ml of Sodium meta per iodate ($9.35X10^{-3}$M) and 2.0ml of 2.3N Sulphuric acid are added and boiled for 20 minutes, cooled and made up to the mark using double distilled water and measured the absorbance after 10 minutes at 520 nm respectively. Similarly the absorbance of the reagent blank was also measured against double distilled water. The increase in the absorbance was noted and concentration of the drug was identified from the Beer's law plot.

The expected reaction mechanism of GMF with Brucine is

Brucine is an effective reagent used for quantitative determination. On combination with a good oxidant like sodium metaperiodate the coupling reaction is feasible. Periodate converts Brucine into Brucine quininone in the presence of sulphuric acid and undergoes nucleophilic attack by electron rich coupler of aliphatic amine primary or secondary to yield 1-mono-substituted bruciquinone derivative of coloured form shows maximum absorption at 520 nm.[21-23]

In the present study, bruciquinone formed undergoes nucleophilic attack on electron rich portion of GMF containing primary amine group to give mono substituted bruciquinone derivative may be considered as oxidative coupling reaction.

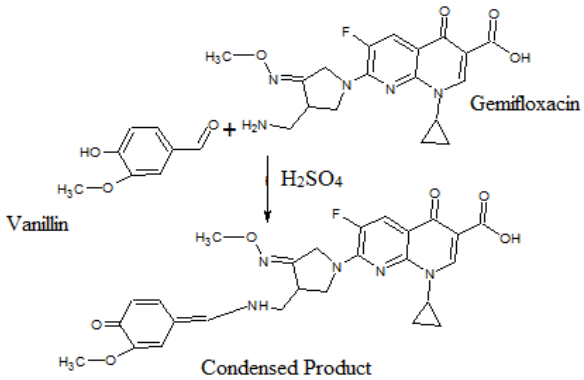
The reaction is as follows:



Method-3

To each of 25 ml calibrated tubes, aliquots of (0.5 – 2.0 ml, 500 µg/ml) standard drug solution, 2 ml of Vanillin and 3 ml of concentrated sulphuric acid were added successively and the total volume in each flask was brought to 20 ml by the addition of methanol and maintained at 50 ^{0}C for 15 minutes. Then the flasks were cooled and made up to the mark with methanol and the absorbance were measured at 500 nm against a reagent blank prepared in a similar way. The increase in the absorbance was noted and concentration of the drugs was identified from the Beer's law plot.

Aromatic aldehydes form coloured condensation product (schiff's base) with primary alkyl amines.[24] The condensation of cyclic amine with aromatic aldehyde is reported.[25]The probable sequence of the reaction between vanillin and GMF in presence of sulphuric acid is as follows:



RESULTS AND DISCUSSION

The proposed methods for the GMF by visible spectrophotometer are simple, accurate, economical and superior. The methods developed were studied for the validation of the different parameters like linearity, molar absorptivity, sandell's sensitivity, recovery studies, limit of detection and limit of quantification. The following parameters are summarized below.

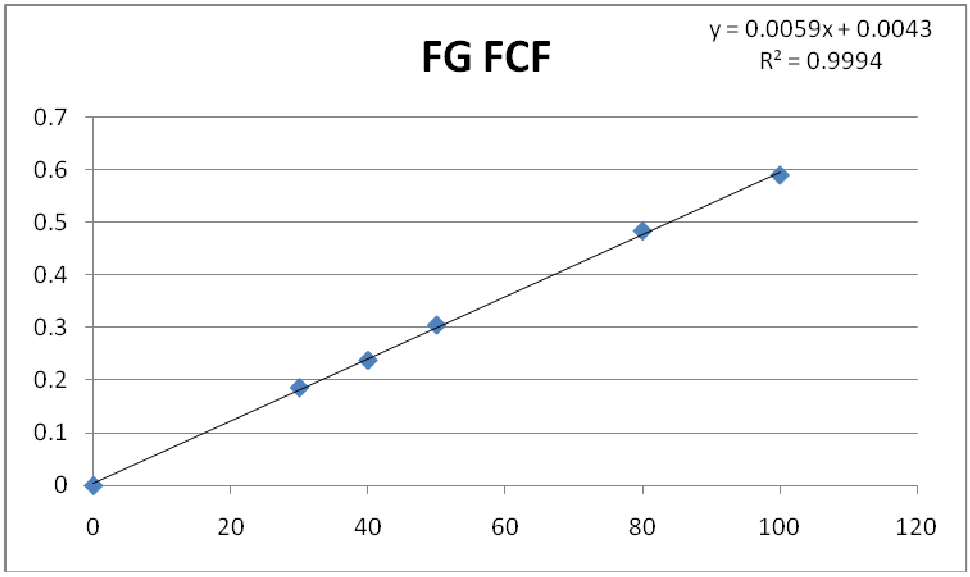


Fig. 1: Calibration curve of GMF by FG FCF method

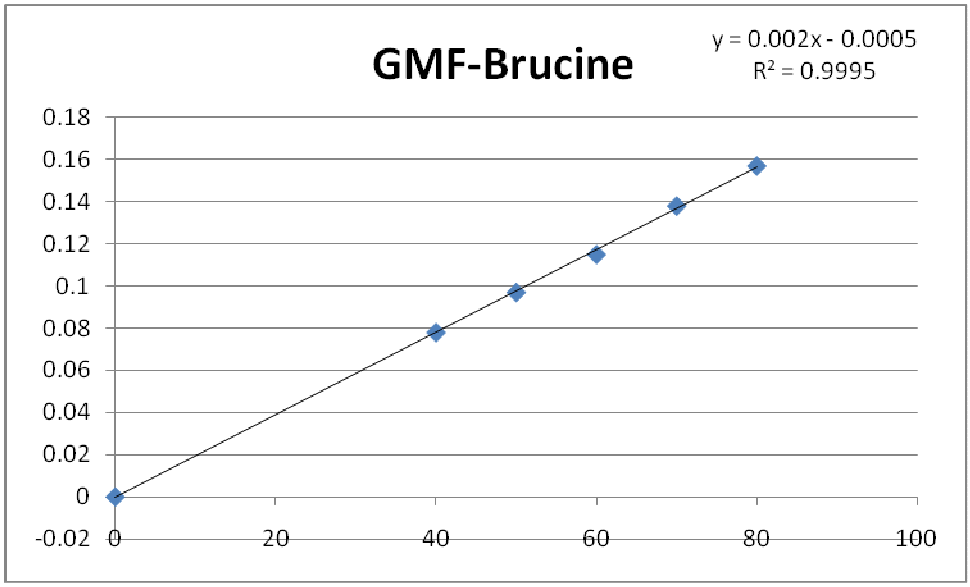


Fig. 2: Calibration curve of GMF by Brucine

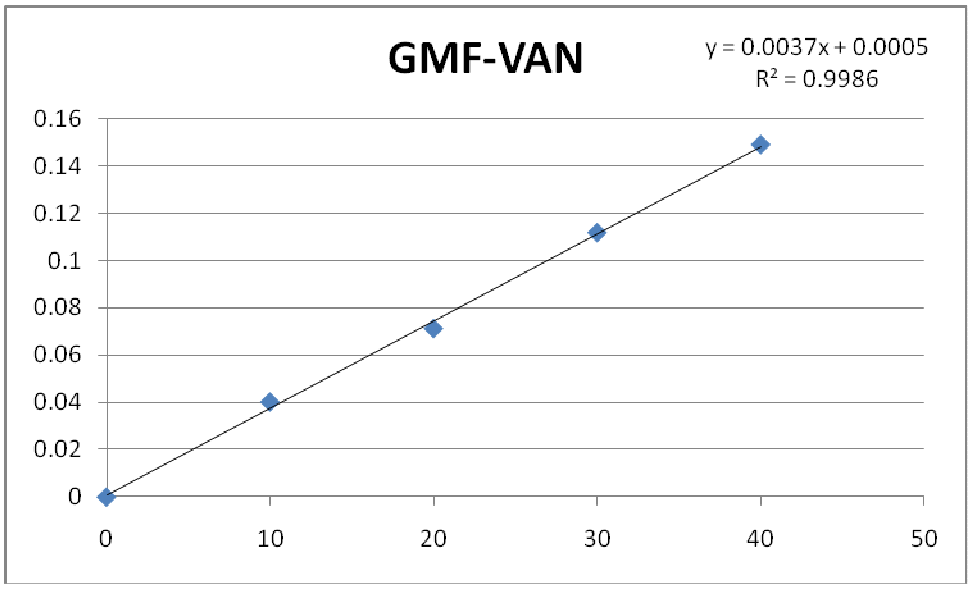


Fig. 3: Calibration curve of GMF by Vanillin

Table 1: Validation parameters for GMF developed by three methods

Parameter	FG FCF	Brucine	Vanillin
λ Max	625	520	500
Beer's Law limits (mg/L)	30-100	40-80	10-40
Molar Absorptivity	$2.37X10^{3}$	$0.746X10^{3}$	$1.38X10^{3}$
Sandell's Sensitivity (µg/cm2/0.001 Absorbance unit)	$1.64X10^{-1}$	0.521	$2.83X10^{-2}$
Regression equation (Y=ax+b)	Y=0.0059x +0.0042	Y=0.002x -0.0005	Y=0.0037x +0.0005
Slope (a)	0.0059	0.002	0.0037
Intercept (b)	0.0042	-0.0005	0.0005
Correlation Coefficient (r)	0.9994	0.9995	0.9986
Standard deviation of Intercept (S_b)	0.000467618	0.000344	0.000568331
Standard deviation of Slope (S_a)	0.000638749	0.000516	0.000906458
Limit of Detection LOD(mg/L)	0.2612	0.5676	0.5065
Limit of Quantification LOQ(mg/L)	0.7915	1.72	1.535

CONCLUSION

The proposed methods obey Beer's law in the above concentration range. The recovery studies were carried out by standard addition method and were found close to 100 % for the above methods. Hence these developed methods could be used for the routine estimation of GMF in pure form.

ACKNOWLEDGEMENT

The authors convey thanks to chemistry department of GIT, Gitam University for providing necessary facilities and their kind co-opeartion.

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