Reverse phase high performance liquid chromatography and UV-Visible method for the simultaneous estimation of gemifloxacin

Senthilraja. M

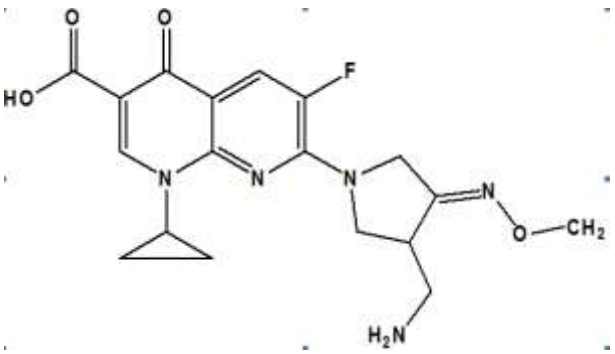
Professor, Dept. of Pharmaceutical Chemistry, J. K. K. Nattraja College of Pharmacy, Komarapalayam, Namakkal, Tamil Nadu, India

***Corresponding Author: Senthilraja. M**
Email: rajdanish2k@gmail.com

Abstract
A novel UV-Visible and reversed phase High Performance Liquid Chromatography method has been developed and validated for the simultaneous analysis of gemifloxacin and their formulation. The UV-Visible method was carried with folin reagent and reverse Phase HPLC method with acetonitrile: phosphate buffer (40:60%v/v) mobile phase, having a pH of 3.0 at a wavelength of 272 nanometer. The linearity range starts from 2 to10µg/ml and 1 to 5µg/ml, percentage recovery was found to be 98.55% and 99.68%, limit of detection 0.2651µg and 0.1588µg and 0.8033µg and 0.4811µg for the UV-Visible and RP-HPLC methods respectively.

Keywords: Reversed phase-HPLC, UV-Visible, Gemifloxacin, Folin-Ciocalteu phenol reagent, Phosphate buffer.

Introduction
Gemifloxacin is one of the flouroquinolone antibiotic, chemically it is 7-[(4E)-3-(aminomethyl)-4-methoxyiminopyrrolidinyl] -1-cyclopropyl-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic acid[1] having an empirical formula C18H20FN5O4 and having a molecular weight of 388.37.



Chemical Structure of Gemifloxacin

Two methods such as UV-Visible and reversed phase HPLC methods have been developed for an estimation of gemifloxacin in pure and in tablet form. From the literature, it was found that there were no methods has been developed for determination of standard drug and its formulation by combined UV and HPLC methods[3-6].

The main objective of the study was to develop simple, accurate and less cost new UV-Visible and reversed phase HPLC method for estimation of gemifloxacin in standard as well as in tablet form and to get validation of the proposed method.

Materials and Methods
Gemifloxacin standard and tablet gembax tablet-320mg, distilled water, folin Ciocalteu phenol reagent. The following instrument has been employed - UV-VIS double beam spectrophotometer, Lab India, Model UV-3600 with 1cm matched pair quartz cells. Distilled water as solvents, HPLC Prominence/Shimadzu, Phenomenex Luna C18 column.

UV, Visible and Reversed Phase High Performance Liquid Chromatography Method

Method Development

Physical Test
Gemifloxacin was dissolved by using solvents such as Distilled water, methyl alcohol, ethyl alcohol, acetone, HCL (0.1Molar), NaOH (0.1Molar) and chloroform. Hence, water has been selected as a solvent for this method[3].

Preparation of Stock Soution
10 mg of gemifloxacin mesylate was dissolved in 10 milli litter of distilled water and volume was made up and it gives a concentration of 1000 µ g / ml. From this, 1ml was pipette out and made up the volume to 10ml using distilled water gives a concentration of 100µg/ml.

Preparation of Working Standard Solution
From the standard stock solution, 1ml was transferred into a 10ml standard flask and the volume was made up to 10 ml with distilled water gives a 10µg/ml concentration. Later, the solution has been measured in UV-VIS Spectrometer using 200-400 nanometer against distilled water as blank and the wavelength of maximum absorbance (λmax) was found to be 272 nanometer.

Preparation of Calibration Curve
Aliquots of series of drug solution such as 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10µg/ml concentration were prepared and their absorbance's were observed and the calibration curve has been plotted using a concentration at X-axis and absorbance on Y-axis. From the Calibration curve, linearity was obtained from the concentration range of 2-10µg /milliliter. The correlation coefficient (R^2) of the method was 0.999.

Method Validation
Method validation is a analytical process of creating evidence, which gives a high degree of assurance that a

specific activity will consistently produce a desired result which meets its predetermined specifications and quality characteristics. The method has been validated to get different parameters such as linearity[2], accuracy, precision, specificity, robustness, ruggedness, limit on detection and limit of quantification (Table 1)

Linearity
Different series of solutions were prepared from the standard solution having a concentration of 100µg / milliliter from 2-10µg/ml. The series of solutions were measured in UV-VIS Spectrometer against distilled water as blank. It gives a linearity range from 2 -10µg/milliliter.

Accuracy
The accuracy of this method has been estimated from a solutions having a concentrations of 80%, 100% and 120% from which the amount of marketed formulation is kept constant (10mg) and the amount of pure drug is different such as 8mg, 10mg and 12mg for 80%, 100% and 120% respectively. All the solutions have been prepared in triplicate and their accuracy has been indicated by percentage recovery.

Limit of Detection (LOD)
The limit on detection of gemifloxacin was found to be 0.2787µg/milliliter.

Limit of Quantitation (LOQ)
The limit of quantification of gemifloxacin was found to be 0.8445 µg /milliliter.

Precision
Precision was performed by intraday and interday variation studies. In this intraday variation study, nine various solutions of same concentration such as 10µg/milliliter was prepared and measured for three times in a day like forenoon, afternoon and evening and the absorbance was observed. The result produced as percentage RSD. In this interday variation study, same concentration of solutions such as 10µg/milliliter was prepared and measured 3 times for three consecutive days and the absorbance was produced. These result too produced as percentage RSD.

Robustness
Robustness was carried out by analysis at two different temperatures such as at room temperature and at 18°Celcius. The absorbance of 10µg/milliliter was observed and the result has been shown by percentage RSD.

Ruggedness
Ruggedness was carried out by analysis by two different analysts and the absorbance of 10µg/milliliter was observed. The result has been shown by percentage RSD.

Spectrophotometric Method
Preparation of Working Standard Solution
The solution has been measured in UV-VIS Spectrometer using 400-800 nanometer against distilled water as blank and the wavelength of maximum absorbance (λmax) was found to be 450 nanometer.

Validation of Spectrophotometric Method
The results are given in table 2.

Limit of Detection (LOD)
The limit on detection of gemifloxacin was found to be 0.2515µg /milliliter.

Limit of Quantitation (LOQ)
The limit of quantification of gemifloxacin was found to be 0.7622 µg /milliliter.

Reverse Phase-HPLC METHOD
Preparation of Calibration Curve
Aliquots of Five solutions from 1 to 5µg/mL of gemifloxacin were prepared from the standard stock solution by make up the volume to 10ml in volumetric flasks using mobile phase. At starting the mobile phase was pumped for 30 min to saturate the column to get the baseline corrected. The solutions were filtered through 0.45 micron membrane filter and 20 microliter of the filtrate was injected into the column at a flow rate of 1.2 ml/min each time. Evaluation of the drug was determined with UV-visible detector at 272nm. Peak area was observed for all the peaks and peak area verses drug concentration plotted which gives the calibration curve. The curve showed linearity in the concentration range of 1 to 5µg/ml. The correlation coefficient (r^2) was found to be 0.999.

Validation of HPLC Method.
The results are given in table 3.

Limit of Detection (LOD)
The Limit of Detection of gemifloxacin was found to be 0.1588 µg /mL.

Limit of Quantification (LOQ)
The Limit of Quantification of gemifloxacin was found to be 0.4811µg /mL.

Precision
Precision was determined by system and method precision. In this study, five different solutions of same concentration such as 5µg /mL were prepared and the values were analyzed by injection of 20µL and peak area was recorded. The result of the study was done by percentage RSD.

Robustness
Robustness was carried by changing the flow rate and their respective retention time of the solution was observed.

Ruggedness

Ruggedness was carried out by analysis by two different analysts and their respective peak areas of 5µg/mL was recorded and their results were shown by percentage RSD.

Stability study

Stability of the method was carried out by solution of 5µg/mL was prepared and the peak area was observed at a interval of 0, 8, and 16th hour.

Assay

A series of six different solutions of 5µg/mL were prepared and the their peak area and standard peak area were observed. From this study, the amount of drug present in the sample was calculated. The result of this study was shown by percentage purity of the sample.

Results and Discussion

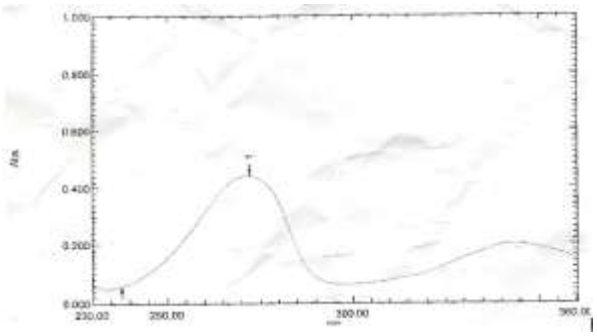


Fig. 1: Graph of Absorption maxima

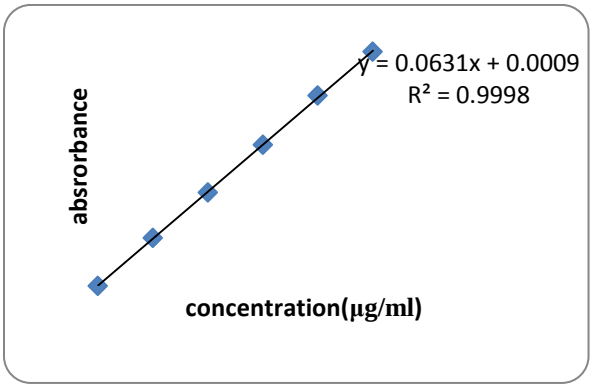


Fig. 2: Graph of Linearity

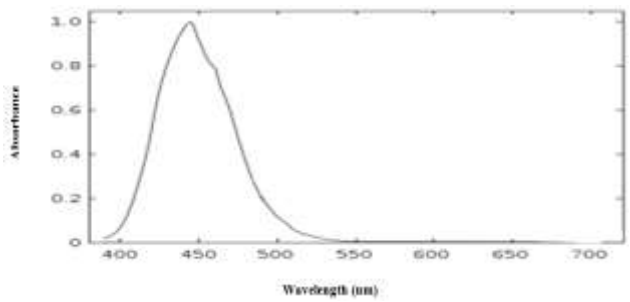


Fig. 3: Graph of Absorption Maxima with a reagent

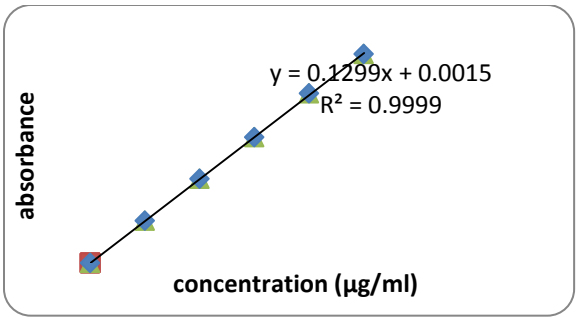


Fig. 4: Graph of Linearity with reagent

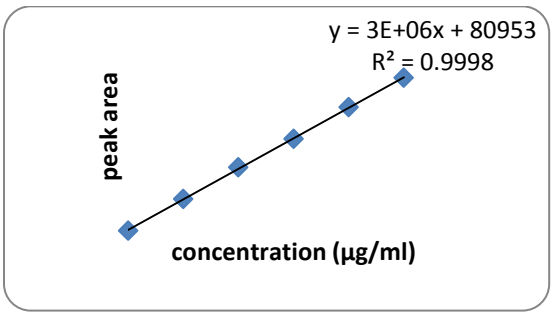


Fig. 5: Graph of Linearityb Graph

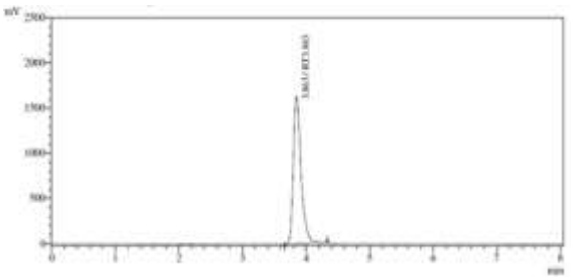


Fig. 6: Standard chromatogram

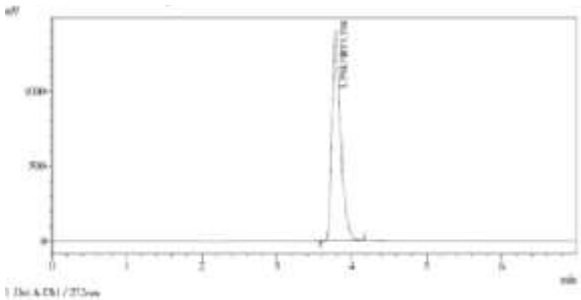


Fig. 7: Chromatogram in formulation

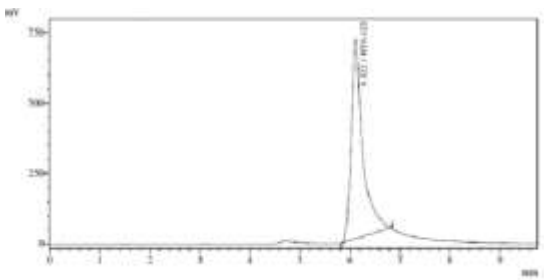


Fig. 8: Chromatogram of degradation study

Table 1: Validation summary of gemifloxacin

Particulars of Parameter	Results obtained
Linearity by correlation coefficient	0.999
Precision by Percentage RSD	0.4142
Accuracy indicated by Percentage recovery	0.5219
Limit of Detection	0.2787 µg/mL
Limit of Quantification	0.8445 µg/mL
Range	2 to 10 µg/mL
Linear regression equation	Y = 0.063X + 0.000
Robustness & Ruggedness by percentage RSD	0.4513 & 0.3539
Molar extinction coefficient(molar absorptivity)	0.0634
Sandell's sensitivity	0.01572
Slope (a)	0.063
Intercept (b)	0.000

Table 2: Validation Summary of gemifloxacin

Particulars of Parameter	Results obtained
Linearity by correlation coefficient	0.999
Precision by% RSD	1.0365
Accuracy by percentage recovery	99.31%
Limit of Detection	0.2515µg/mL
Limit of Quantification	0.7622 µg/mL
Range	2-10 µg/mL
Linear regression equation	y = 0.129x + 0.001
Robustness & Ruggedness indicated by percentage RSD	1.025 & 1.255
Molar extinction coefficient(molar absorptivity)	0.1303
Sandal's sensitivity	$7.608x10^{-3}$
Slope (a)	0.129
Intercept (b)	0.001

Table 3: Validation summary of gemifloxacin (RP-HPLC Method)

Particulars of Parameter	Results obtained
Linearity by correlation coefficient	0.999
Range	1 to 5µg/ml
Accuracy by percentage recovery	99.68
Limit of Detection	0.1588µg/mL
Limit of Quantification	0.4811µg/mL
Linear regression equation	Y = 3e+ 06X+ 80953
Ruggedness indicated by%RSD	0.1901
System precision by percentage RSD	0.7439
Method precision by percentage RSD	0.8290
Slope (a)	6
Intercept (b)	80953
Assay	99.54

Table 4: Data of validation of UV-Visible and HPLC methods

S. No.	Particulars of parameters	UV method	Visible method	HPLC method
1	Linearity range	2 to10 µg/mL	2 to10 µg/mL	1 to 5 µg/mL
2	Standard deviation	0.1993	0.4102	4583854
3	Slope	0.0630	0.129	6.0
4	Intercept	0.0001	0.000	3.0
5	Correlation coefficient	0.999	0.999	0.999
6	Molar absorptivity	0.0634	0.1303	----------
7	Sandell's sensitivity	0.0157	$7.6808x10^{-3}$	----------
8	% Recovery	99.68	98.31	99.68
9	Repeatability%RSD	0.3776	1.381	---------

10	Intraday%RSD	0.6030	1.112	---------
11	Interday%RSD	0.262	0.616	---------
12	LOD	0.2787 mcg/mL	0.2515 µg/m.	0.1588 µg/mL.
13	LOQ	0.8445 mcg/mL	0.7622 µg/Ll.	0.4811 µg/mL.
14	Robustness% RSD	0.4513	1.025	----------
15	Ruggedness% RSD	0.3539	1.255	0.1901
16	System precision% RSD	----------	----------	0.7439
17	Method precision% RSD	----------	----------	0.8290
18	Assay	----------	----------	99.54%

Conclusion

Based on the data the developed methods were more reliable, precise, simple, sensitive, robust and in cost effective way and this may be applied for the determination of gemifloxacin in bulk and other pharmaceutical formulations in future.

The three methods were found to be more precise as the percentage RSD values of intraday and inter-day was found to be less than two percentage. There was a good recovery of the drug at each added concentration and this reveals that the method was found to be accurate. The limit of detection and limit of quantification was found to be in low miligram level which provides the sensitivity of the method. These are all methods were found to be more reliable observed by percentage related standard deviation values which was found to be than 2 percentage. The assay results has shown that an amount of drug was in good agreement with the label claim of the formulation as indicated by percentage recovery. validation summary parameters of proposed uv method has shown in table 1, visible method has shown in table 2 and reversed phase high performance liquid chromatography method has shown in table 3. The degradation study of the drug by reversed phase high Performance Liquid chromatography method gives extra peaks in a chromatogram which results the degradation of drug takes place in the solution.

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Conflict of Interest: None.

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