



**UV SPECTROPHOTOMETRIC QUANTIFICATION OF GEMIFLOXACIN
IN PHARMACEUTICALS EMPLOYING MULTIVARIATE CALIBRATION
TECHNIQUE AND EVALUATION OF GREENNESS PROFILE**

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ABSTRACT

The present work proposes determining Gemifloxacin in bulk and formulations by developing a novel approach and verifying. It is an analytical technique that is sensitive, exact, and rapid. Using a UV spectrophotometer and multivariate calibration technique, the analytical method was carried out. The relation between concentration and absorbance, which has been determined at five different wavelengths, including the absorption maxima, was used to build this multivariate calibration technique. The linear regression equations, and other mathematical and statistical techniques were used to validate the process in accordance with ICH Q2 (R1) criteria. Precision, accuracy, linearity, and other validation parameters has listed in the instructions has been satisfied. The relationship between concentration and absorbance was linear for concentration levels between 3.5 and 6.5 $\mu\text{g mL}^{-1}$. This method is cost effective and has good sensitivity, and produces good findings when employed in regular analysis of drugs and formulations. The method greenness values were calculated using the analytical Eco scale, Agree metrics, and Green Analytical Procedure Index.

Keywords: Gemifloxacin, Multivariate calibration technique, UV Spectrophotometer, Pharmaceutical formulations, ICH guidelines, Validation

INTRODUCTION

Gemifloxacin, a quinolone antibacterial to treat mild to moderate pneumonia and drug with broad-spectrum activity, is used acute bacterial episodes of chronic

bronchitis. There are oral preparations available for it. The method that gemifloxacin works by preventing the bacterial growth enzymes DNA gyrase and topoisomerase IV from synthesising DNA [1].

7-[(4Z)-3-(aminomethyl)-4-methoxyiminopyrrolidin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,8-naphthyridine-3-carboxylic acid is the chemical name for Gemifloxacin. The molecular formula is $C_{18}H_{20}FN_5O_4$ and has molecular mass 389.4g/mol [2].

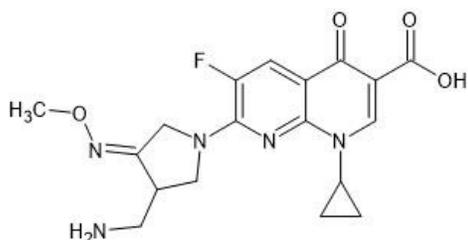


Figure 1: Structure of Gemifloxacin

In April 2003, the FDA approved gemifloxacin to treat AECB and mild-to-moderate CAP brought on by a variety of infections, including the first treatment for multidrug-resistant *S. pneumoniae* [3]. Gatifloxacin and moxifloxacin are licenced by the FDA for the treatment of acute sinusitis, chronic bronchitis, and community-acquired pneumonia. Gemifloxacin has been approved for use in the treatment of mild-to-moderate community-acquired pneumonia and acute exacerbations of chronic bronchitis [4]. Gemifloxacin has been shown to have broad spectrum activity against clinically important Gram-positive and atypical

pathogens while at the same time maintaining broad spectrum anti-Gram-negative activity [5]. Because of its simultaneous targeting of DNA Gyrase and TOPO IV, gemifloxacin is considered to be the only fluoroquinolone with the capacity to inhibit both enzyme systems in *S. pneumoniae* at clinically relevant drug concentrations [6]. The review of literature indicates that various techniques have been published for determining Gemifloxacin in pharmaceutical formulations or biological fluids. For Gemifloxacin, a few chromatographic techniques such as HPLC [7–12], spectrophotometric techniques like UV [13], HPTLC [14, 15], LC-MS/MS [16, 17]. The literature review referred to the Multivariate calibration (MVC) technique using UV spectrophotometry, which haven't reported. Hence, the present method deals with the development of the MVC technique for the estimation of Gemifloxacin. The MVC approach was used to decrease instrumental error and increase efficiency. The method is easy, inexpensive and applied to pharmaceutical dosage forms. For exact findings, MVC employs straight regression algorithms ranging between the wavelengths of 5-10nm [18]. In this study, we discussed the use of a UV spectrophotometric MVC approach with minimal mathematical content for estimating Gemifloxacin in pharmaceutical dosage forms. As a result,

to ensure the sensitivity in comparison to the traditional ultraviolet (UV) approach, five distinct wavelengths were chosen. The algorithm techniques of MVC's statistics multivariate data are converted into univariate data using the following equations [19].

If the absorbance of a sample(x) is measured at different wavelengths (λ), that is, at 263, 265, 267, 269, and 271nm, the following equations can be produced for each selected wavelength

$$A_{\lambda 221} = a \times C_x + k_1 \dots\dots\dots (1)$$

$$A_{\lambda 224} = b \times C_x + k_2 \dots\dots\dots (2)$$

$$A_{\lambda 227} = c \times C_x + k_3 \dots\dots\dots (3)$$

$$A_{\lambda 230} = d \times C_x + k_4 \dots\dots\dots (4)$$

$$A_{\lambda 233} = e \times C_x + k_5 \dots\dots\dots (5)$$

Whereas,

- A_{λ} = Absorbance of the sample;
- a, b, c, d, e = Slope of the straight regression functions of a sample;
- k_1, k_2, k_3, k_4, k_5 = Intercept of the straight regression;
- C_x = Concentration of the sample

The above five equations can be rearranged as:

$$A_T = a \times C_x + b \times C_x + c \times C_x + d \times C_x + e \times C_x + K_T \dots\dots\dots (6)$$

Equation (6) can be re-arranged as:

$$A_T = C_x (a + b + c + d + e) + K_T \dots\dots\dots (7)$$

Whereas,

- A_T = Sum of the absorbances acquired
- K_T = Sum of intercepts of regression equation

The concentration of the sample (X) in a solution can be calculated by using the equation

$$C_x = \frac{A_T - K_T}{(a + b + c + d + e)} \dots\dots\dots (8)$$

Greenness evaluation techniques

The eco analytical scale [20] is based on assigning penalty points based on the quantity of signal words connected with the pictograms as specified by "The Globally Harmonized System of Classification and Labelling of Chemicals (GHS)", along with the quantity. The analytical eco scale approach considers every reagent, including its type and quantity, potential occupational exposure, energy depletion, and waste. Penalty points are subtracted from a 100 point beginning score.

$$\text{Analytical eco-scale} = 100 - \text{total penalty points} \dots\dots\dots (9)$$

The another representation is the Green Analytical Procedure Index [21] (GAPI), which is made up of five pentagons and has a distinctive colour scheme. Three degrees of evaluation are used in the pictogram's colour coding for each step of an analytical process. Green, yellow, and red are the three colours used by GAPI to indicate the levels of low, medium, and high environmental impact associated with the

analytical technique, respectively. J. Potka-Wasyłka accurately characterised and reported GAPI in a concise manner in 2018 [21]. AGREE metrics, [22] In the second assessment process, special software is employed to quantify the greenness profile. A circular diagram with numbers on the edges that range from 1 to 12 are oriented clockwise is the result of the software. These figures represent the 12 green analytical chemistry philosophies. Based on the inputs and their weight, the results of these 12 principles are rated from 0 to 1 on a scale of 1 to 12. Red indicates zero, dark green indicates one or a value close to one, and yellow indicates a value between red and dark green on this aggregate scale. The 12 principles are added along with the core to provide a score that represents the degree of greenness.

MATERIALS AND METHODS

Materials

Analytical grade materials were used for all "chemicals and reagents" in this experiment. Each film-coated tablet in a marketed formulation contains 320mg of Gemifloxacin from various brands that were selected and purchased from a retail pharmacy.

Instrumentation

- UV-Visible double beam spectrophotometer (LABINDIA UV 3092 model)

- Soniclean Sonicator (model 106 T, Thebarton, Australia)
- Analytical balance (AS 245, Mettler Toledo, India)

Method development

Solubility

In water, Gemifloxacin was completely soluble. So, water was employed as the study's solvent throughout the research.

Reference Samples

Gemifloxacin was kindly supplied by Ideal Analytical and Research Institution (Pondicherry, India).

Preparation of Standard solution

The standard solution of Gemifloxacin reference standard were prepared by dissolve 100 mg of drug in 100 ml of the solvent to acquire $1000 \mu\text{g mL}^{-1}$ with the use of sonicator. The solution is diluted to get concentrations in the range of 3.5-6.5 $\mu\text{g mL}^{-1}$

Preparation of sample solution

Gemifloxacin tablets were weighed, added to a mortar, ground into a powder, and thoroughly combined 100 mg of Gemifloxacin, correctly measured from the tablet powder, was dissolved in 100 mL of water using a sonicator, filtered, and diluted for additional studies.

Selection of wavelength for MVC

Across the wavelength range of 200 to 400 nm, the Gemifloxacin working standard solutions were scanned against water as the blank solution., which has maximum

absorption at 267nm. Thus, the wavelength for MVC approach were around these absorption maxima, i.e., 263,265,267,269,271nm.

Stability of the solution

Solution stability studies were performed for Gemifloxacin by storing prepared sample solutions at room temperature for 0-12 hours. The absorbance was measured at regular intervals of 0, 6, and 12 hrs.

METHOD VALIDATION

The prepared method were validated per ICH guidelines [23] for accuracy, precision, linearity.

Linearity

Gemifloxacin stock solution was dissolved in the solvent to produce concentrations between 3.5 and 6.5 $\mu\text{g mL}^{-1}$. The amplitudes of these solutions were measured around 267 nm, i.e., 263, 265, 267, 269, and 271 nm, in the spectrum mode, to reduce instrumental fluctuations and increase correlation.

Limit of Detection and Limit of Quantification

Based on the calibration curve slope and standard deviation of responses for a specific wavelength, the limit of detection (LOD) and limit of quantification (LOQ) for Gemifloxacin were predicted using the following formulas.

$$\text{LOD} = \frac{3.3 \times \text{standard deviation}}{\text{Slope}} \dots\dots\dots (10)$$

$$\text{LOQ} = \frac{10 \times \text{standard deviation}}{\text{Slope}} \dots\dots\dots (11)$$

Precision

The intra-day and inter-day precisions were measured three times daily and three distinct days (inter-day precision) and (intra-day precision) accordingly, using replicate measurements on homogeneous solutions containing 5 $\mu\text{g mL}^{-1}$.

Accuracy

At 267 nm, the extracted sample solution's amplitude was captured. The estimated amount of the medication in the formulation is shown in **Table 5**.

Assay

Weigh accurately a quantity of Gemifloxacin tablets equivalent to 100 mg of Gemifloxacin, add 100 ml of water and sonicate for 20 minutes. Add sufficient water and make upto 100 mL. The solution obtained above is filtered and diluted with water to attain 5 $\mu\text{g mL}^{-1}$ concentration of Gemifloxacin. The absorbance of the resulting solution is measured at 267 nm and the content of Gemifloxacin is quantified.

RESULTS AND DISCUSSION

The maximum amplitude was recorded at 267 nm using water as the solvent (**Figure 2**).

Linearity

As demonstrated in **Figure 3**, the linearity was measured at 263, 265, 267, 269 and 271 nm for various concentrations between

3.5 to 6.5 $\mu\text{g mL}^{-1}$. Table 1 presents the observed results in tabular form. In the chosen concentration range, it was discovered that all of the standard curve

were linear. Figures 4-8 and Table 2 provide the calibration graphs, related residual plots, and regression analysis, respectively.

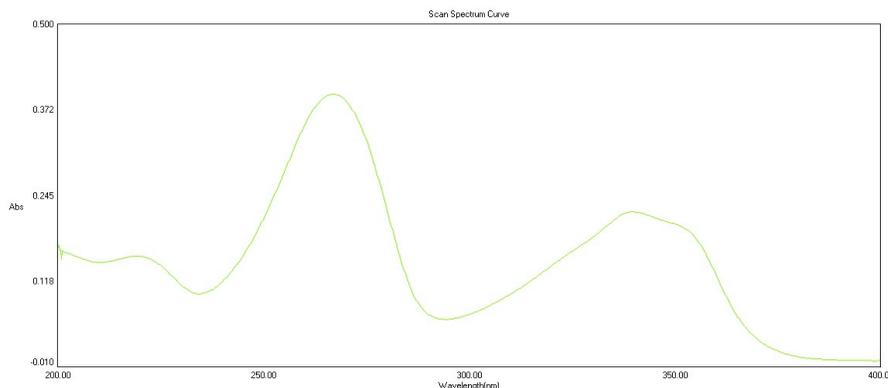


Figure 2: UV spectrum of standard Gemifloxacin ($5 \mu\text{g mL}^{-1}$) using water as blank

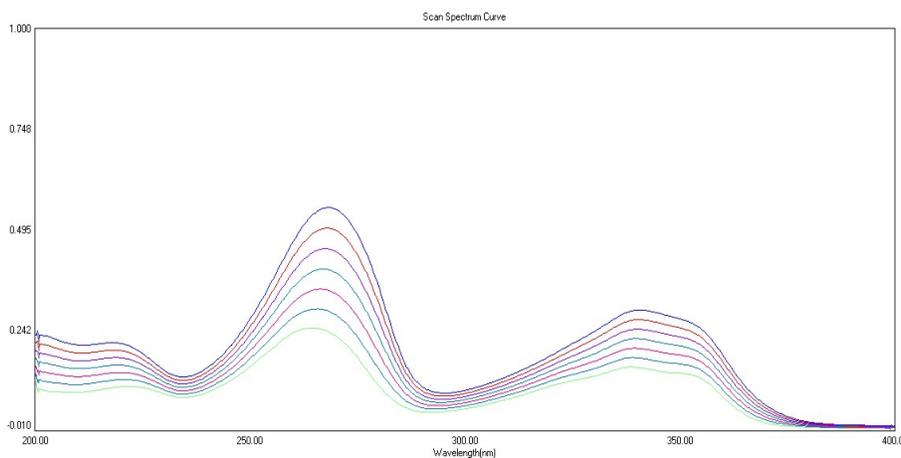


Figure 3: Linearity spectrum of Gemifloxacin ($3.5\text{-}6.5 \mu\text{g mL}^{-1}$) using water as a blank

Table 1: Multivariate UV calibration data at five selected wavelengths

Concentration ($\mu\text{g mL}^{-1}$)	263nm	265nm	267nm	269nm	271nm
3.5	0.246	0.336	0.248	0.239	0.220
4	0.293	0.379	0.297	0.287	0.282
4.5	0.335	0.432	0.345	0.341	0.327
5	0.379	0.475	0.396	0.392	0.380
5.5	0.423	0.528	0.447	0.445	0.433
6	0.468	0.576	0.497	0.497	0.487
6.5	0.512	0.624	0.548	0.551	0.540

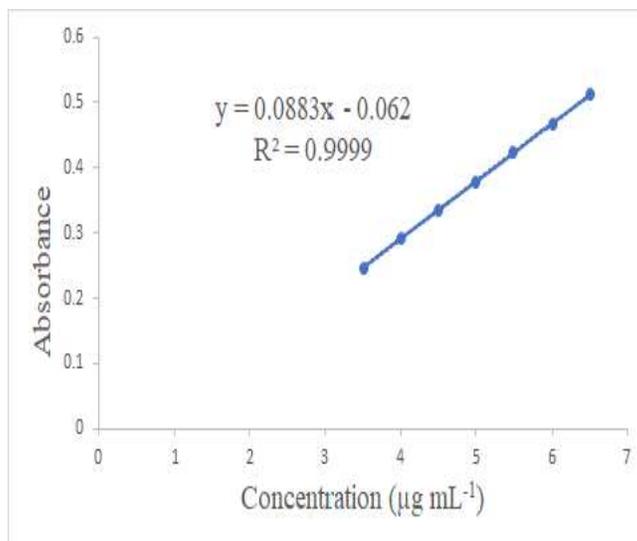


Figure 4: Calibration curve at 263 nm

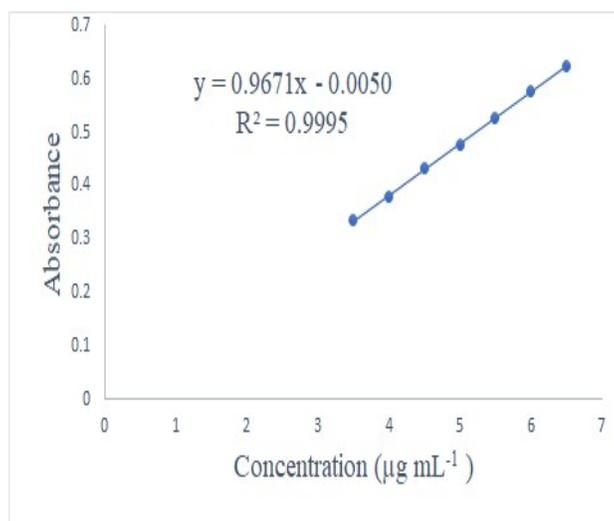


Figure 5: Calibration curve at 265 nm

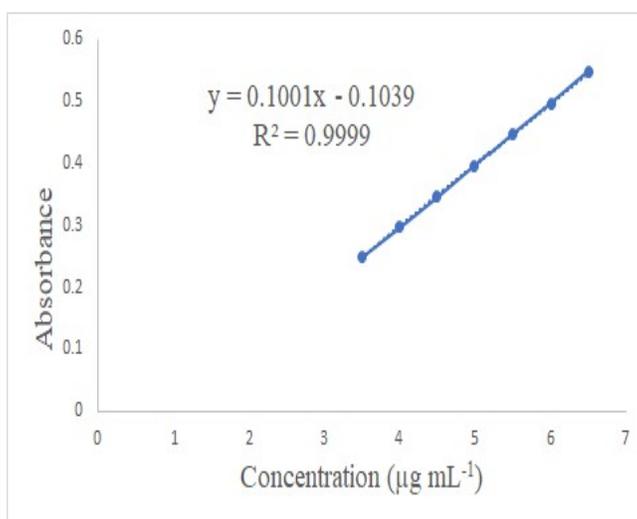


Figure 6: Calibration curve at 267 nm

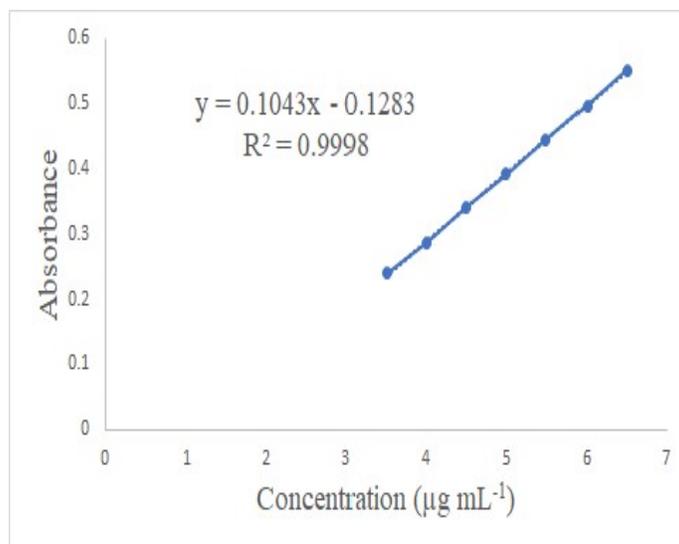


Figure 7: Calibration curve at 269 nm

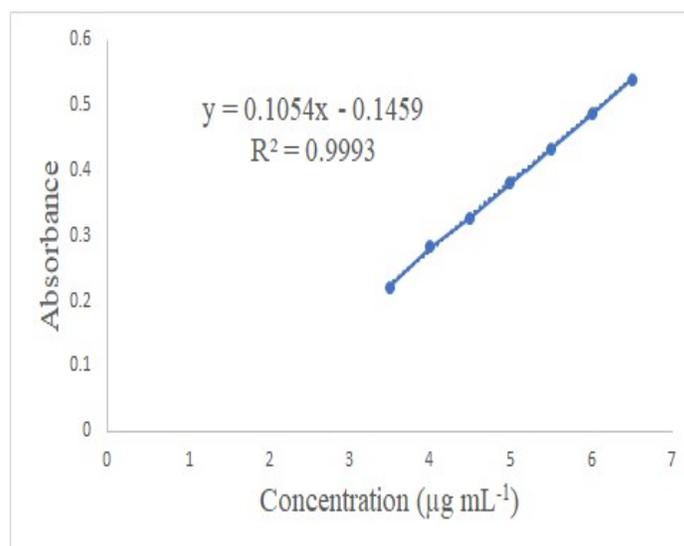


Figure 8: Calibration curve at 271 nm

Table 2: Linearity data shows statistical parameters at the selected wavelengths

Wavelength(nm)	Regression equation	Slope	Intercept	R ²	LOD ($\mu\text{g mL}^{-1}$)	LOQ ($\mu\text{g mL}^{-1}$)
263	$y = 0.0883x - 0.062$	0.0883	-0.062	0.9999	0.1932	0.5856
265	$y = 0.9671x - 0.0050$	0.9671	-0.005	0.9995	0.0441	0.1337
267	$y = 0.1001x - 0.1039$	0.1001	-0.1039	0.9999	0.0192	0.0583
269	$y = 0.1043x - 0.1339$	0.1043	-0.1339	0.9999	0.2528	0.7663
271	$y = 1.0671x - 0.1536$	1.0671	-0.1536	0.9993	0.5204	1.5771

Precision

The system precision spectra for Gemifloxacin are represented in **Figure 9**. The interday precision spectra for

Gemifloxacin are represented in **Figure 11**. The intraday precision spectra were represented in **Figure 10** for Gemifloxacin. The % RSD of system precision, interday

and intraday precision, was determined for Gemifloxacin. It was found to be lower than than 2%, which shows that the approach method is precise. **Tables 3** and **4** list the absorbance measurement made at the chosen wavelength sets. At the chosen wavelengths, the SD and % RSD values were also determined and are shown in **Tables 5** and **6**. **Figures 9** and **10**, respectively, depict the overlay UV spectra for the intraday precision and interday precision. The outcomes are represented for Gemifloxacin. The proposed method shows good precision compared to the values obtained from various precision methods.

Accuracy

Gemifloxacin's accuracy was tested at 80, 100, and 120%. **Figure 12** shows the overlay spectra for Gemifloxacin. The results for accuracy are shown in **Table 4**,

and the obtained results were found to be within limits.

Assay of marketed formulations

The recommended UV method was used to investigate the quantity of Gemifloxacin in tablet formulation. The spectrum of tablet was obtained for three replicates. After extraction and filtration, there was no appreciable decrease in the pharmaceutical formulation's excellent analytical recovery values. The results are provided in the **Table 5** for Gemifloxacin, which demonstrates that the new strategy out performs the earlier ones.

Evaluation of Greenness Profile

The results of greenness profile for the proposed methods were evaluated. The results of analytical scale is shown in **Table 6**, while the results for GAPI and agree metrics is depicted in **Figure 14** and **Figure 15**.

Table 3: System precision, Intraday and Interday precision data for the prepared method of Gemifloxacin

	System Precision	Interday and Intraday Precision		
	Amplitude of Standard (5 µg mL ⁻¹)	% Recovery of sample equivalent to 5 µg mL ⁻¹ of sample		
		Day 1	Day 2	Day 3
1	413.742	99.12	98.01	98.3
2	412.542	98.85	99.2	99.49
3	415.243	99.32	99.1	99.32
4	416.729	99.25	99.47	99.5
5	414.751	99.35	99.11	98.48
6	415.871	99.29	98.16	99.5
Mean	414.813	99.20	98.84	99.10
SD	1.502	0.19	0.60	0.56
% RSD	0.36	0.19	0.61	0.56

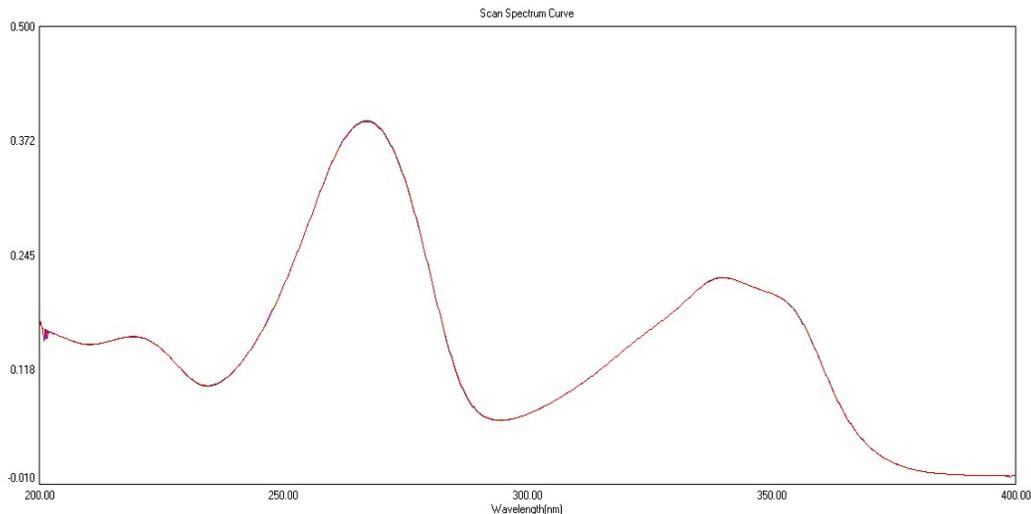


Figure 9: System precision overlay spectra of Gemifloxacin

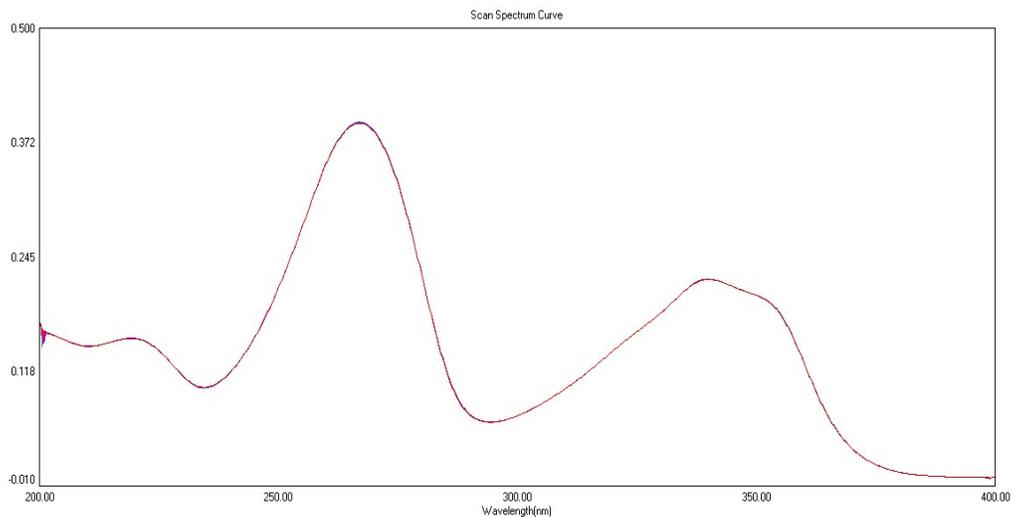


Figure 10: Interday precision overlay spectra of Gemifloxacin

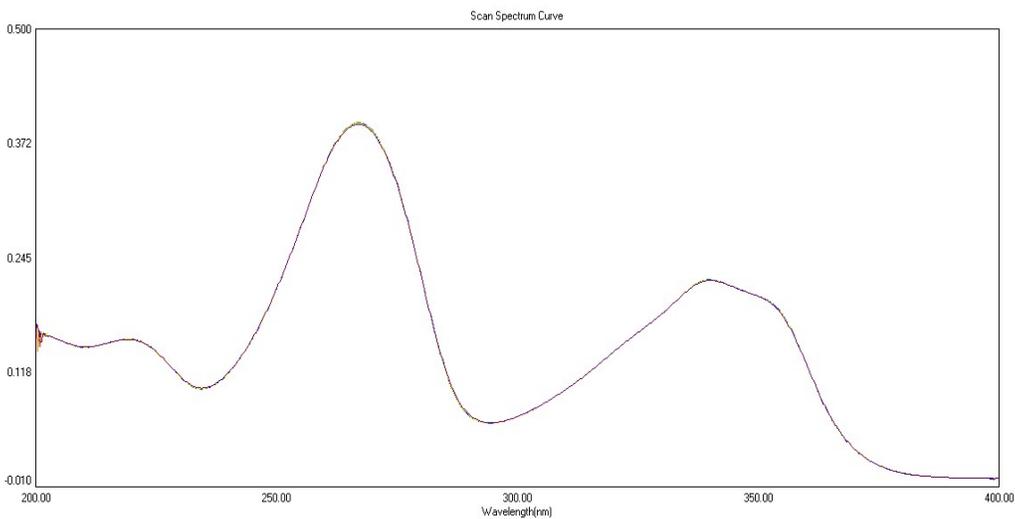


Figure 11: Intraday precision overlay spectra of Gemifloxacin

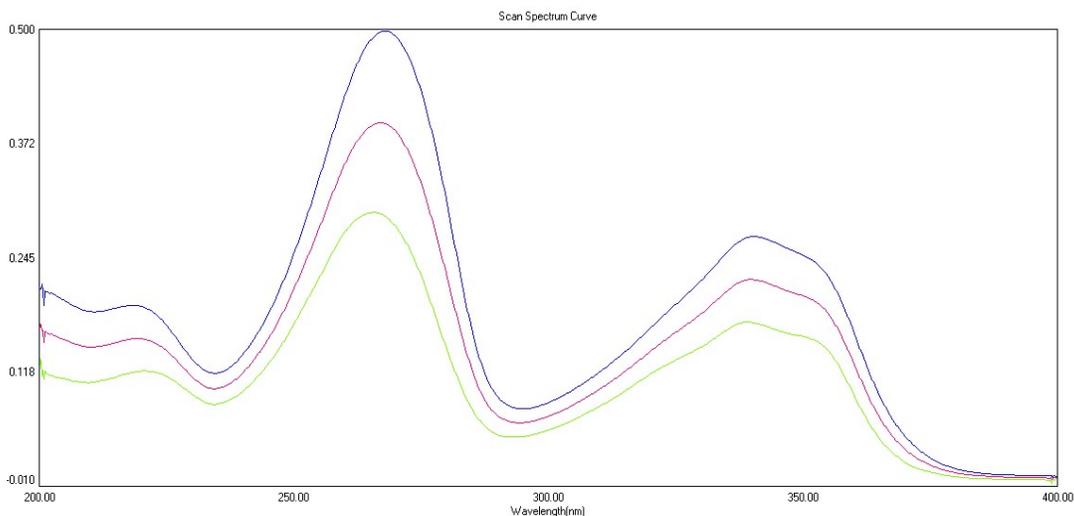


Figure 12: Overlay spectra of accuracy of Gemifloxacin at 80, 100, 120 % raising

Table 4: Accuracy data for prepared method of Gemifloxacin

Concentration levels (%)	Amount present	Amount added ($\mu\text{g mL}^{-1}$)	Amount recovered ($\mu\text{g mL}^{-1}$)	Mean % Recovery	SD
80	3	2	4.9	98	0.5773
100	3	7	9.9	99	0.4359
120	3	12	14.7	98	0.4194

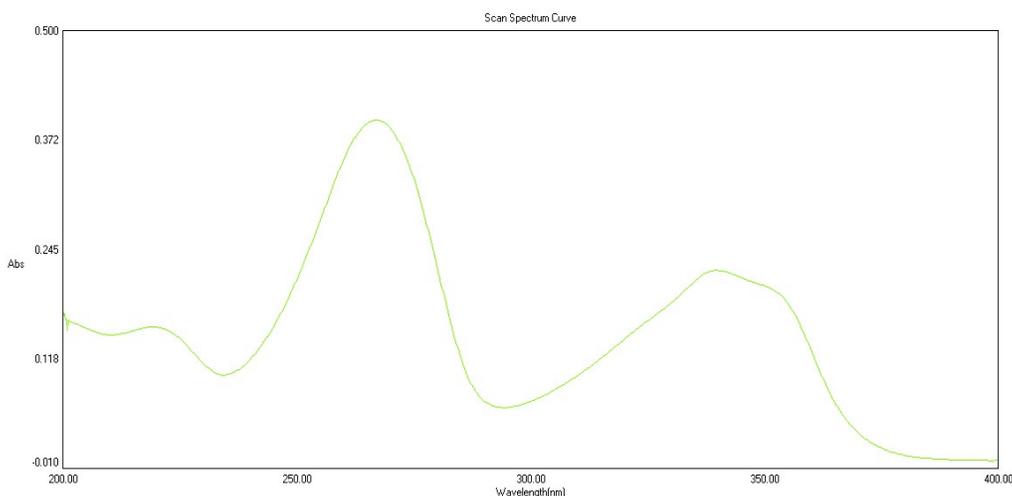


Figure 13: UV spectrum of standard Gemifloxacin ($5 \mu\text{g mL}^{-1}$) using water as a blank

Table 5: Assay results for marketed formulation of Gemifloxacin

Marketed formulation	Label claim (mg)	Mean \pm SD (n=3)	% RSD
Batch - 1	320	307.33 \pm 2.52	0.4727
Batch - 2	320	314.67 \pm 2.52	0.7997

Table 6: Summary of Eco scale penalty points for the proposed method

Description	Penalty points	Total Penalty Points	Score
Water	3	6	94
Instrument	0		
Occupational hazard	0		
Waste	3		

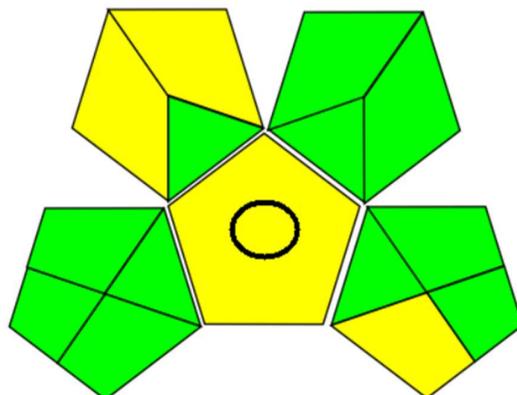


Figure 14: GAPI Pictogram for the proposed method

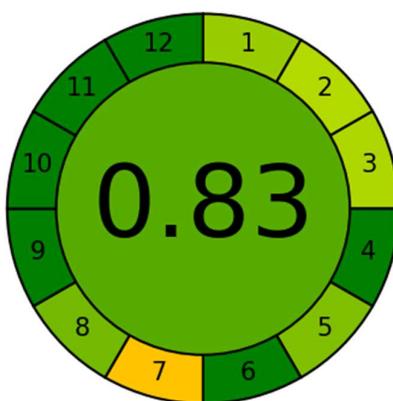


Figure 15: Agree metrics output for the proposed method

CONCLUSION

The evaluation of the validation parameters in accordance with ICH Q2 (R1) guidelines helped to validate this newly created method of multivariate calibration technique using UV spectrophotometry. The study shows this method is affordable, accurate, and non-destructive, it can be used in the analysis of Gemifloxacin in both bulk and pharmaceutical dosage form in industry as well as quality control process or even in routine analysis. The proposed method for determining

Gemifloxacin in its tablet formulation was shown to be sensitive, accurate, precise, and repeatable. We strongly advise using this method for routine analysis of Gemifloxacin in pharmaceutical formulations because it is more useful and dependable than the other UV spectrophotometric methods. The proposed method possess an ideal greenness profile assessed by analytical ecoscale, GAPI and agree metrics shall be used for routine simultaneous determination of Gemifloxacin as an alternative to time-

consuming and expensive separation techniques.

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CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article exists.

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