

Fluorometric Method for the Determination of Gemifloxacin Mesylate in Bulk and Pharmaceutical Formulations Using Tb³⁺ Ions in the Presence of Hexamine

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Abstract: A simple and highly sensitive method for the determination of Gemifloxacin Mesylate in bulk drug and pharmaceutical formulations. The method is based on the reaction of Gemifloxacin Mesylate with Tb³⁺ in the presence of hexamine at pH 7.2. The fluorescence was measured at 545 nm after excitation at 271 nm. The calibration graph was linear over the range 0.05–1.3 µg mL, with minimum quantification limit (LOQ) of 0.024 µg mL and minimum limit of detection (LOD) of 7.65×10⁻³ µg mL. The proposed method was successfully applied for the determination of this drug in its dosage forms.

Keywords: Gemifloxacin Mesylate, Spectrofluorometry, Terbium(III), Hexamine, Ternary Complex

1. Introduction

Gemifloxacin Mesylate (GEM) is a broad-spectrum fluoroquinolone antibacterial agent. Its bactericidal activity depends on inhibition of DNA synthesis. This mode of action involves dual targeting of two bacterial enzymes: DNA gyrase and topoisomerase IV, which are essential for bacterial DNA replication and transcription [1]. Chemically, it is designated as (±)-7-[3-(aminomethyl)-4-(methoxyimino)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid methanesulfonate [1, 2] (Figure 1).

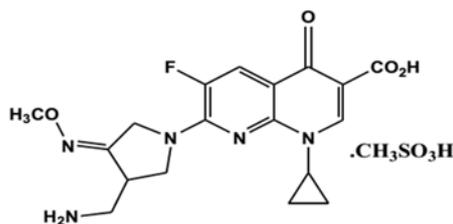


Figure 1. Chemical Structures of Gemifloxacin Mesylate.

GEM is not official in any pharmacopoeia. The literature survey revealed several reported analytical approaches for its determination in pharmaceutical dosage forms and in biological fluids. Various spectrophotometric [3 – 11] and spectrofluorimetric [12, 13] methods were described. Chromatographic methods were also reported and include capillary electrophoresis [14], HPTLC [15] and HPLC [16].

The present paper describes a specific and highly sensitive method for the determination of Gemifloxacin Mesylate that can be adopted for its determination in tablets. The method is based on the reaction of Gemifloxacin Mesylate with Tb³⁺ in the presence of hexamine at pH 7.2.

2. Materials and Methods

2.1. Materials and Reagents

All chemicals used were of analytical reagent grade, and the solvents were of spectroscopic grade. Pure grade Gemifloxacin Mesylate and its tablets (Factive® 320 mg/tablet) were supplied from Tabuk pharmaceutical. MFG.

CO., Saudi Arabia.

Terbium (III) chloride hexahydrate solution, An aqueous solution of 1×10^{-3} M Terbium (III) chloride hexahydrate (TbCl₃.6H₂O, 99.9%; Sigma, ALdrich, Germany), was prepared by dissolving 0.037g in 100 mL of distilled water. Hexamine solution, An aqueous solution of 1% (W/V) hexamine (Sigma, ALdrich, Germany), was prepared and its pH was adjusted to 7.2 with 1 M HCL.

2.2. Instrument

The fluorescence intensity was measured on a Perkin-Elmer model RF-5301PC Spectrofluorophotometer (SHIMADZU), equipped a 150 W xenon arc lamp, grating excitation and emission monochromators and a Perkin-Elmer recorder. Slit widths for excitation and emission monochromators were set at 3.0 and 3.0 nm, respectively. A 1 cm quartz cells was used for all measurements. pH was measured on a HANNA pH meter (Romania).

2.3. Preparation of Standard Solution

A stock solution containing 10.0 mg/mL of Gemifloxacin Mesylate was prepared in distilled water. This solution was found to be stable for two week without alteration when kept in the refrigerator. Working standard solution were prepared from stock solution further dilution with distilled water to cover the working range of 0.05 – 1.3 µg/mL.

2.4. Procedures

2.4.1. Construction of the Metal Complexes Calibration Graphs

Transfer 2.0 ml of TbCl₃.6H₂O (1×10^{-3} M) into a series of

10 mL standard flasks. Add Gemifloxacin Mesylate covering the working range (0.05 – 1.3µg/mL) then add 2 ± 0.1 ml of hexamine solution of pH 7.2 ± 0.2 . Complete to volume with distilled water. Measure the fluorescence intensity at 545 nm using 271 nm as an excitation wavelength. Plot the concentration versus the fluorescence Intensity (FI) to obtain the standard calibration graph. Alternatively one could calculate the linear regression equation.

2.4.2. Application of the Proposed Method to the Analysis of Gemifloxacin Mesylate in Its Tablets

An accurately weighed amount of ten powdered tablets equivalent to 10.0 mg of Gemifloxacin Mesylate was transferred into a 100 mL volumetric flask and completed to volume with distilled water. The flask with its contents was sonicated for 30 min and then filtered. The above procedure was followed. The nominal content was calculated either from the previously plotted calibration graph or using the corresponding regression equation.

3. Results and Discussion

The Gemifloxacin Mesylate-Tb³⁺-hexamine ternary complex (GFX-Tb(III)-N₄(CH₂)₆) was found to exhibit intense fluorescence at 545 nm after excitation at 271 nm (Figure. 2). The fluorophore was formed instantaneously and remained stable for more than 1 h. Compared to the native fluorescence of Gemifloxacin Mesylate, the product is 2.5 times more intense (Figure. 3).

Moreover, the effect of different concentration of Gemifloxacin Mesylate-Tb³⁺-hexamine ternary complex on the fluorescence intensity was studied (Figure 4).

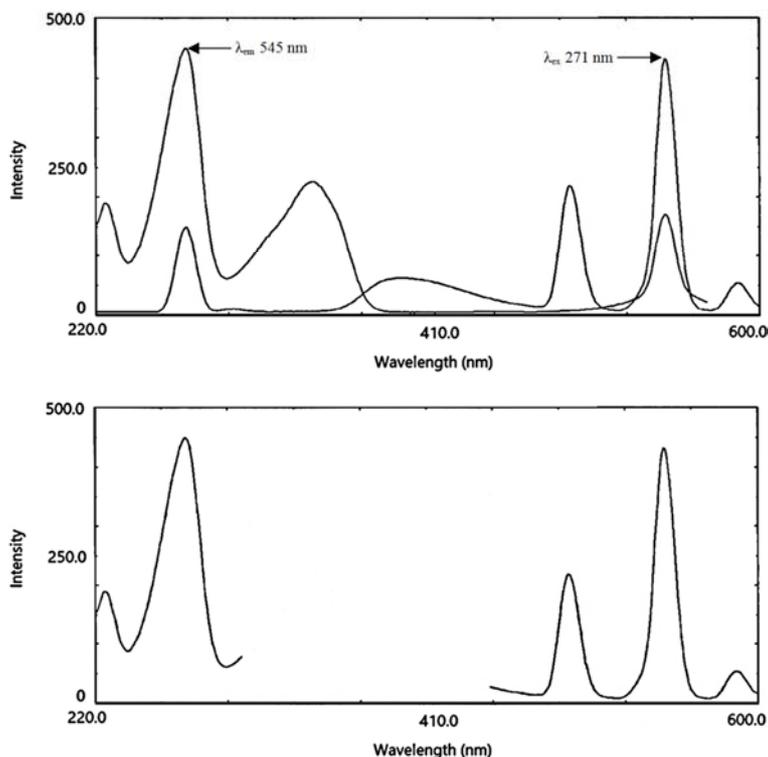


Figure 2. Fluorescence spectra of Gemifloxacin Mesylate-Tb³⁺-hexamine ternary complex (Excitation spectra 261nm)(Emission spectra 545 nm).

3.1. Optimization of the Reaction Condition

The factors affecting the reaction conditions were carefully studied and optimized. Such factors were changed individually while the others were kept constant. These factors included reagents concentration, nature of the solvent, reaction time and stability.

3.1.1. Effect of pH and Buffer Solution

It was found that upon using 1% (W/V) hexamine solution covering the pH range from 5 to 10, maximum fluorescence intensity was obtained at and above pH 7.0. Higher pH values did not affect the fluorescence intensity (Figure 5). Therefore, $\text{pH } 7.0 \pm 0.2$ was recommended for the determination. At the same time, some buffer was studied as follows: Tris-HCl, Borate buffer, phosphate buffer, acetate buffer. It was not effected on the fluorescence intensity; the highest fluorescence intensity was obtained in hexamine.

3.1.2. Effect of Hexamine Concentration

The effect of hexamine concentration on the fluorescence intensity was studied using different volumes (0.1–4.0 mL) of hexamine. Figure 6 show the maximum fluorescence intensity was attained using 1.5 mL, after which hexamine has no effect on the fluorescence intensity. Thus 2 mL of hexamine was used for further studies.

3.1.3. Effect of Terbium Concentration

The effect of Terbium (III) chloride hexahydrate concentration on the fluorescence intensity was studied using different volumes (0.1 – 4.0 mL) of $\text{TbCl}_3 \cdot 6\text{H}_2\text{O}$. Figure 7 show the maximum fluorescence intensity was attained using 1.0 mL, after which $\text{TbCl}_3 \cdot 6\text{H}_2\text{O}$ has no effect on the fluorescence intensity. Thus 2 mL of 1×10^{-3} M $\text{TbCl}_3 \cdot 6\text{H}_2\text{O}$ was used for further studies.

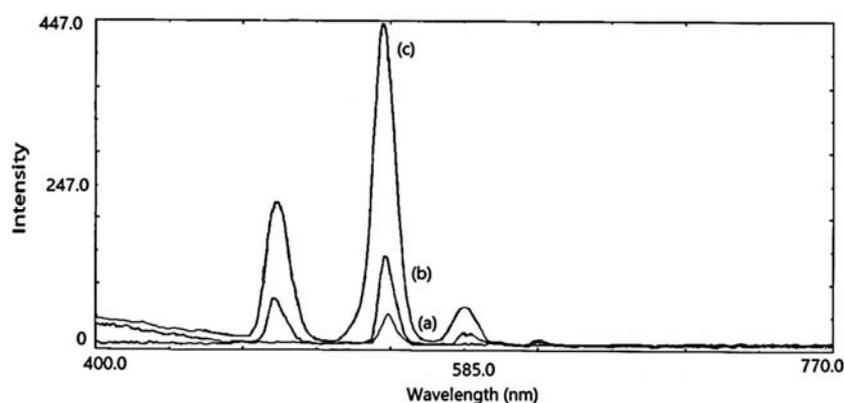


Figure 3. Fluorescence emission spectra of: (a) Blank, (b) Gemifloxacin Mesylate and hexamine, (c) Gemifloxacin Mesylate- Tb^{3+} -hexamine ternary complex.

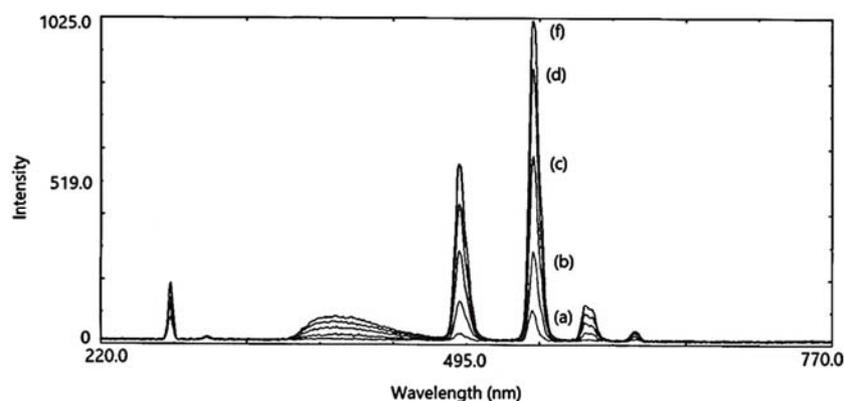


Figure 4. Fluorescence emission spectra of Gemifloxacin Mesylate- Tb^{3+} -hexamine ternary complex. (a) $0.05 \mu\text{g/mL}$, (b) $0.3 \mu\text{g/mL}$, (c) $0.7 \mu\text{g/mL}$, (d) $1.1 \mu\text{g/mL}$, (e) $1.3 \mu\text{g/mL}$.

3.1.4. Effect of Reagent Addition Order

The effect of the order of reagent addition on the fluorescence intensity was also examined. The results indicated that an addition order of Tb^{3+} , GFX and $\text{N}_4(\text{CH}_2)_6$ colloidal solution offered the maximum intensity which was

chosen for this study.

3.1.5. Stability Test

The experiments indicated that the fluorescence intensity of the system reached maximum in 30 min after all the reagents had been added and remained stable for more than 1 h.

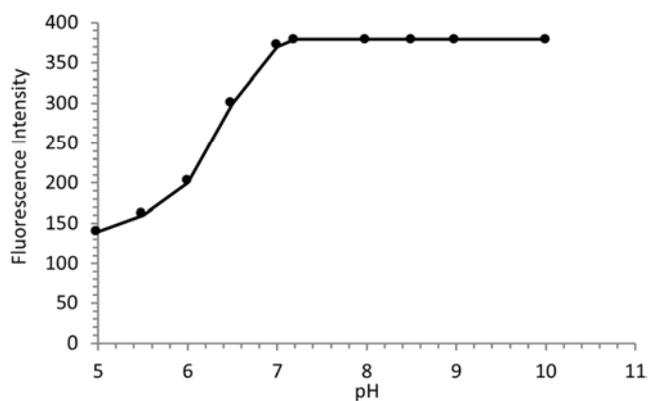


Figure 5. Effect of p H on the fluorescence product of gemifloxacin mesylate (0.5µg/ml) and Tb^{3+} .

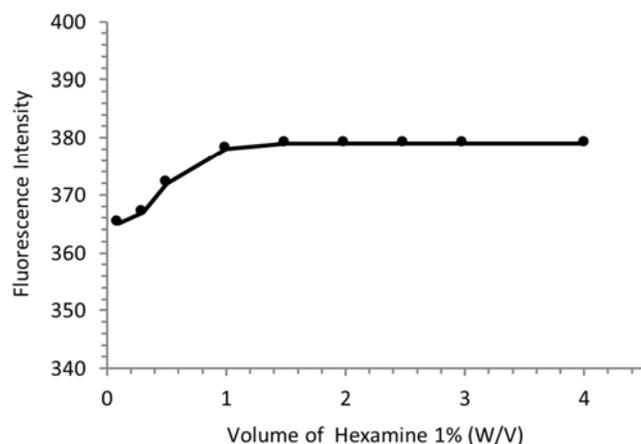


Figure 6. Effect of volume of 1%(W/V) of hexamine (PH=7.2) on its reaction with gemifloxacin mesylate (0.5µg/ml) and Tb^{3+} .

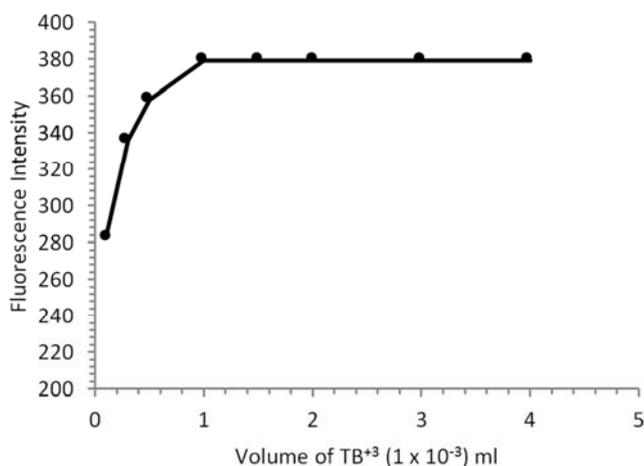


Figure 7. Effect of volum of (1×10^3) Tb^{3+} on its reaction of gemifloxacin mesylate (0.5µg/ml) and hexamine.

3.2. Stoichiometry and Reaction Mechanism

The stoichiometry of the reaction was studied by the molar

ratio method [17] and was found to be 1: 3 [Tb^{3+} -Gemifloxacin Mesylate] as shown in Figure 8. The reaction pathway was postulated to proceed as shown in Figure 9.

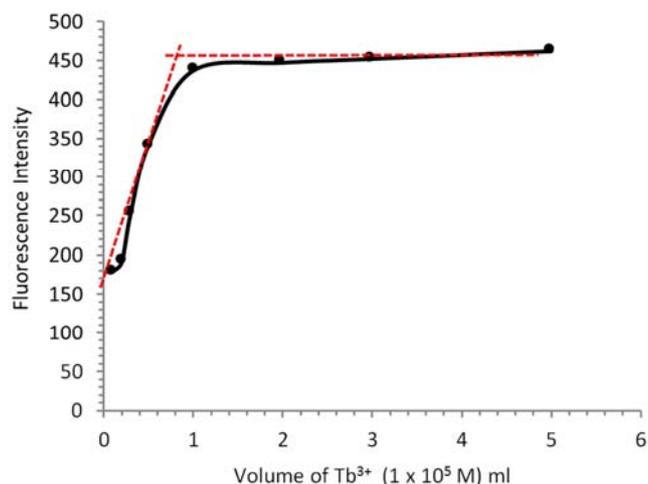


Figure 8. The determination of Molar Reactivity of gemifloxacin mesylate (3 mL of 1×10^5) complex at pH 7.2.

3.3 Validation

3.3.1. Linearity and Range

After optimization the reaction conditions, calibration graph was constructed by plotting the fluorescence intensity vs Gemifloxacin Mesylate concentration. A straight liner was obtained over the concentration range 0.05-1.3µg/m L (Figure. 10).

Linear regression analysis of the data using the method of least squares [18] gave a high value of the correlation coefficient (r), small values of standard deviation of intercept (S_a), and standard deviation of slope (S_b). These data proved the linearity of the calibration graph. Table 1 shows the performance data for the determination of Gemifloxacin Mesylate with Tb^{3+} in the presence of hexamine at pH 7.2.

3.3.2. Accuracy and Precision

To prove the accuracy of the proposed methods, the results of the assay of Gemifloxacin Mesylate in pure form were compared with those of the published spectrophotometric method [8]. Statistical analysis of the results obtained by the proposed and published methods using Student's t -test and variance ratio F -test, showed no significant difference between them regarding accuracy and precision, respectively (Table 2) [18].

Intraday and interday precisions were assessed using three concentration and three replicates of each concentration. The standard deviations were found to be very small indication reasonable, repeatability and intermediate precision of the proposed methods (Table 3).

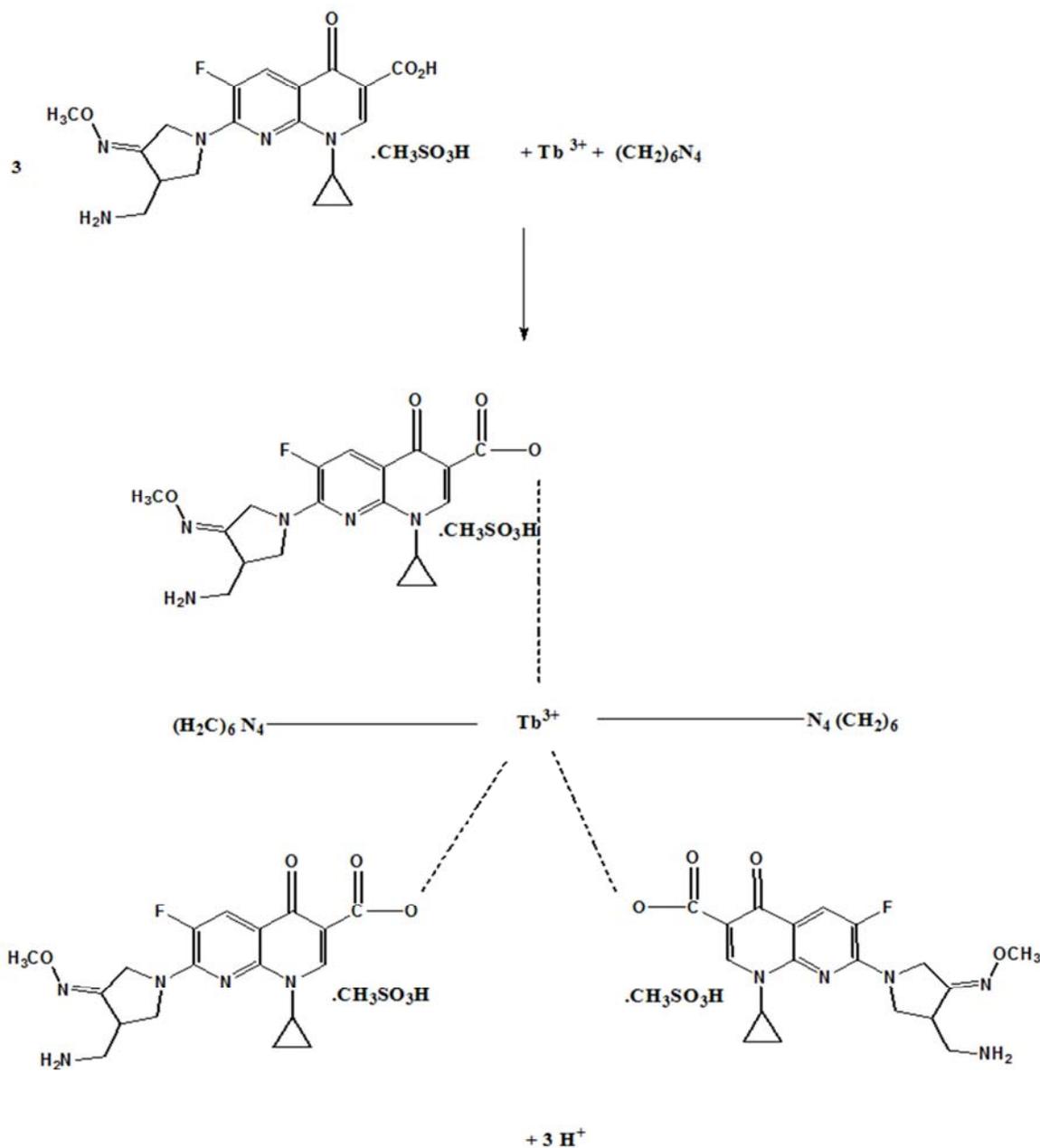


Figure 9. The Proposed pathway for the reaction of Gemifloxacin Mesylate with Tb^{3+} in the presence of hexamine.

3.3.3. Selectivity

The selectivity of the optimized procedures for the assay of Gemifloxacin Mesylate was excipients present in Factive® tablets, e.g. crospovidone, hydroxypropyl methylcellulose, magnesium stearate, microcrystalline cellulose, polyethylene glycol, povidone, and titanium dioxide [19]. It was found that there is no significant interference from excipients. Accordingly, the proposed procedure can be considered as a selective method.

3.3.4. Limited of Detection (LOD) and Limited of Quantitation (LOQ)

LOD and LOQ were determined according to the ICH [20] guidelines, LOD was determined by establishing the minimum level at which the analyte can reliably be detected,

using the relation $3.3 (S_a)/b$ [20], LOQ is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions, using the relation $10 (S_b)/b$ [20]. The results are illustrated in Table 1.

3.3.5. Ruggedness

To examine the ruggedness of procedures, the interday precisions were evaluated as shown in Table 3. The precision of the proposed method was found to be fairly high, as indicated by the low values of S.D.

3.3.6. Robustness

The robustness of the methods was demonstrated by the constancy of the fluorescence intensity with minor changes in the experimental parameters such as 2.0 ± 0.2 mL of 1×10^{-3} M

TbCl₃.6H₂O and 2.0 ± 0.2 mL of 1% (W/V) hexamine (pH=7.2) These minor changes that may take place during the experimental operation did not affect the fluorescence intensity.

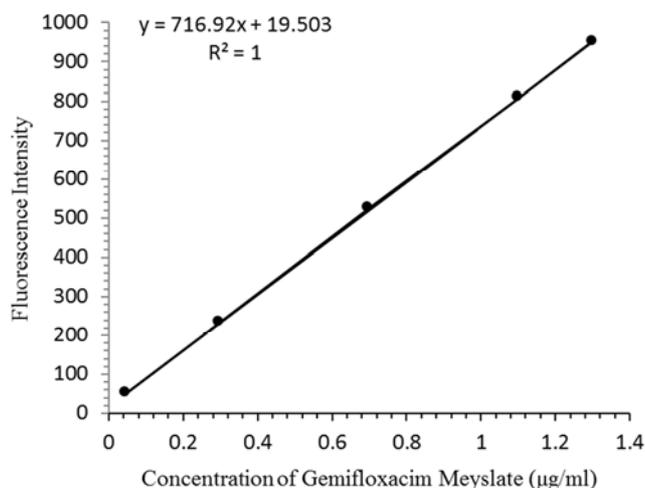


Figure 10. Calibration curve for the determination of gemifloxacin mesylate with Tb³⁺ in the presence of hexamine at 545 nm after excitation at 271 nm.

Table 1. Analytical performance data for the fluorometric determination of Gemifloxacin Mesylate with Tb³⁺ in the presence of hexamine.

parameter	value
Wavelength [λ_{ex} / λ_{em}] (nm)	271 / 545
Linearity range ($\mu\text{g/ml}$)	0.05-1.3
Intercept	19.503
slope	716.923
Correlation coefficient (r)	0.9999
S.D. of Intercept (S_a)	1.663
S.D. of slope (S_b)	1.993
S.D.	0.702
Relative standard deviation % RSD	0.700
SE	0.313
Limit of detection LOD ($\mu\text{g/ml}$)	7.65×10^{-3}
Limit of quantitation LOQ ($\mu\text{g/ml}$)	0.024
Molar ratio (drug/reagent)	1:03

Table 2. Analytical of Gemifloxacin Mesylate in pure form by the proposed fluorometric method using Tb³⁺ in the presence of hexamine and published methods.

Concentration found ($\mu\text{g/mL}$)	Concentration taken ($\mu\text{g/mL}$)	(%) Found ^a	
		Published method ⁽⁸⁾	Proposed method
98.00	98.30	0.049	0.05
99.75	100.05	0.300	0.3
99.91	100.607	0.704	0.7
99.60	99.83	1.098	1.1
99.82	99.94	1.299	1.3
Mean ± S. D	-	99.74± 0.86	99.41± 0.79
Student's t-value	-	0.638	-
Variance ratio F-test	-	(2.306) ^b	-

^aEach result is the average of three separation determinations.

^bthe figures between parentheses are the tabulated values of *t* and *F* at P = 0.05 [18].

Table 3. Precision data for the determination of Gemifloxacin Mesylate by the proposed fluorometric method using Tb³⁺ in the presence of hexamine.

Parameter	gemifloxacin mesylate ($\mu\text{g/mL}$)			
	0.05	0.70	1.30	
Intraday	% Found ^a	98.30	100.05	100.10
	Mean	98.85	100.90	99.04
	S.D.	99.19	100.22	100.58
	S(mean) ^b	98.78	100.39	99.90
		0.45	0.45	0.78
Interday	% Found ^a	0.25	0.25	0.45
	Mean	98.30	101.00	100.10
	S.D.	99.90	100.00	98.51
	S(mean) ^b	98.53	99.63	99.74
		98.91	100.21	99.45
	0.86	0.71	0.83	
	0.49	0.41	0.48	

^a Each result is the average of three separation determinations.

^b Calculated as (S.D/ \sqrt{N})

3.4. Application on the Analysis of Dosage Form

In order to evaluate the analytical usefulness of the proposed Spectrofluorophotomete method, Gemifloxacin Mesylate was determined in its tablets. The recoveries of the different concentration of Gemifloxacin Mesylate were based on the average of three replicate determinations. The results obtained were in good agreement with those obtained by the published spectrophotometric method [8] (Table 4). Statistical analysis [18] of the results obtained by the proposed and the comparison methods shows no significant difference between the two methods as regards to accuracy (*t*-test) and precision (*F*-test).

Table 4. Analytical of Gemifloxacin Mesylate in tablets from by the proposed fluorometric method using Tb³⁺ in the presence of hexamine and published methods.

Preparations	Concentration taken ($\mu\text{g/mL}$)	(%) Found ^a	
		Proposed method	Published method
Factive® tablets	0.05	98.43	98.29
(320 mg	0.3	100.21	99.70
gemifloxacin	0.7	101.30	100.39
mesylate) ^b	1.1	100.01	99.09
	1.3	99.82	101.22
Mean ±S.D.		99.95 ± 1.02	99.73 ± 1.13
Student's t-value		0.327 (2.306) ^c	-
Variance ratio F-test		1.227 (6.39) ^c	-

^aEach result is the average of three separation determinations.

^bproducts of Tabuk Pharma. MFG. CO., Saudi Arabia. ^c the figures between parentheses are the tabulated values of *t* and *F* at P = 0.05 [17].

4. Conclusion

The proposed fluorometric method could be successfully used for the determination of Gemifloxacin Mesylate in the pharmaceutical formulation. The proposed procedure is a simple, sensitive, reliable, and rapid. It could be regarded as a useful technique for the routine quality control of pharmaceutical formulations with a relatively inexpensive instrumentation. In addition, the proposed method is very suitable to be applied in content uniformity testing.

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