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Green Spectrophotometric Method for the Determination of Tigecycline in Different Matrices

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Abstract

This work presents a green, simple, validated colorimetric method for the determination of tigecycline in lyophilized powder and laboratory prepared pharmaceutical wastewater. The suggested method depends on the reaction of tigecycline with Eosin-Y targeting its tertiary amino group, forming a reddish-orange ion pair complex with a wavelength of maximum absorbance of 555 nm. The greenness of this method was assessed by four methods; 1-National Environmental Methods Index assessment (NEMI), 2-Analytical Eco-Scale assessment, 3-Green Analytical Procedure Index assessment (GAPI), and 4-The Analytical Greenness Metric assessment (AGREE). They showed the significant greenness of the proposed method. The analyte followed a linear pattern over the concentration range of 1.0-14.0 µg/mL, with a lower detection limit of 0.21 µg/mL and a quantitation limit of 0.64 µg/mL. The suggested method was completely validated according to the International Conference on Harmonization (ICH) guidelines regarding linearity, accuracy, precision, specificity, and robustness, which permits its application for the intended purpose.

Keywords: Tigecycline; Eosin-Y; colorimetry; wastewater.

1. Introduction

Tigecycline (TGC) (4 S,4 aS,5 aR,12 aS)-9-[2-(tert-butylamino) acetyl] amino]-4,7-bis(dimethylamino)-1,10,11,12 a-tetrahydroxy-3,12-dioxo-4a,5,5a,6-tetrahydro-4H-tetracene-2-carboxamide (Marot et al., 2012). (Fig. 1A) belongs to the new class of antibacterial ″Glycylcyclines″ (Yaghoubi et al., 2022). This minocycline derivative offers physicians a novel, broad spectrum antibiotic with activity against difficult-to-treat pathogens like methicillin-resistant Staphylococcus aureus, vancomycin-resistant Enterococcus spp., Acinetobacter baumannii, and Gram-negative bacterial strains that produce extended-spectrum β -lactamases (Remash et al., 2024). It is indicated for the treatment of complicated skin and intra-abdominal infections (Pankey, 2005).

A literature survey revealed that liquid chromatography was the prevalent analysis tool of TGC (Barco et al., 2020, Ma et al., 2024, Xie et al., 2022, Yang et al., 2021). while few reports were recorded for the spectrophotometric analysis of the concerned analyte (Silva et al., 2012). The simplicity, ease of operation, availability in most laboratories, and cost effectiveness of spectrophotometry render it an appealing tool for analysis. Despite its distinct advantages, liquid chromatography needs experienced analysts for its operation, requires continuous maintenance, uses expensive high-grade reagents, and consumes large volumes of organic solvents depriving it from greenness. These facts enthusiast the authors to adopt colorimetry for the assay of TGC, where the proposed method was more sensitive (as revealed from LOD and LOQ values) and offered a wider linearity range than the reported assay (Silva et al., 2012).

Eosin Y (Fig. 1B) is a xanthene dye capable of forming ion-pair complexes with drugs carrying tertiary amino groups(Ahmed et al., 2019). And it was previously reported for the analysis of several pharmaceuticals (Barghash et al., 2021, Derayea et al., 2024).

Recently, manufacturing sites have begun to be considered a source of antibiotic resistance, due to a much higher discharge level than any other source. As hot spots for antibiotic-resistance development, the control of antibiotic concentrations in full-scale pharmaceutical wastewater should be given more attention (Babić et al., 2013). Therefore, TGC was determined in laboratory prepared pharmaceutical wastewater in this work.

2. Experimental

2.1. Instrument

Shimadzu (Kyoto, Japan) UV-1900i PC, UV-Visible double-beam spectrophotometer. HANNA pH meter (Romania).

2.2. Materials and reagents

TGC was kindly provided by Amoun pharmaceutical company, El-Obour City, Cairo, Egypt (purity 99.97%). Standiga vial (Batch # 304560), containing 50 mg of TGC (lyophilized powder) was obtained from local pharmacies. Eosin Y, anhydrous sodium acetate, and acetic acid (90%) were purchased from (Merck, Darmstadt, Germany).

2.3. Standard solution

A standard stock solution of TGC was freshly prepared as 200.0 µg/mL in deionized water, then dilution with the same solvent was carried out to obtain working standard solutions. A solution of Eosin ($5x10^{-3}$ M) was prepared by dissolving 346mg in 100 mL deionized water. The required pH value was obtained by mixing appropriate volumes of (0.2 M) sodium acetate and (0.2 M) acetic acid solutions to prepare 0.2 M acetate buffer.

2.4. Procedures

2.4.1. Construction of the calibration curve

Appropriate volumes of TGC standard stock solution were transferred into a set of 10.0 mL volumetric flasks to cover the concentration range of (1.0-14.0 µg/mL). To each flask, 2 mL of ($5x10^{-3}$ M) eosin Y was added followed by 2 mL of 0.2M acetate buffer (pH 3.5) and mixed well, then dilution to the volume with deionized water was carried out. The absorbance of each concentration was measured at 555 nm against the blank, and the resultant responses were plotted *versus* the final drug concentration in µg/mL, followed by the derivation of the regression equation.

2.4.2. Application to dosage form

An accurately weighed amount of Standiga vial was dissolved in deionized water with the aid of sonication to prepare a stock solution equivalent to 200.0 µg/mL TGC.

The procedures previously mentioned under *"Construction of Calibration curve"* were then followed to estimate the content of TGC in its vials by applying the regression equation.

2.4.3 Application to wastewater

Pharmaceutical wastewater (PWW) was prepared to resemble the composition of wastewater resulting from pharmaceutical companies. Based on a previous article(Guo et al., 2018), it was prepared in tap water to contain 30 mg ascorbic acid, 50 mg citric acid, 100 mg saccharose, 230 mg disodium hydrogen phosphate, and 1 gm sodium chloride, adjusting the pH to 7 by 0.1 M HCl and 0.1 M NaOH.

The steps mentioned under the section *"Construction of Calibration curve" were* performed, and the nominal content of TGC in PWW was estimated using its regression equation.

3. Results and Discussion

A simple spectrophotometric method aiming to assay TGC in its vials and in PWW is developed, without the need for any prior preparation or extraction steps. To achieve maximum sensitivity parameters, optimization of reaction conditions was carried out by changing one factor and keeping the others constant. The reaction depends on forming an ion pair complex

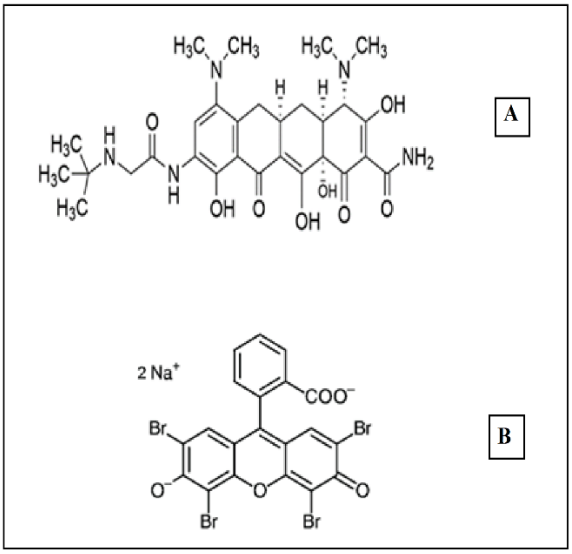


Figure 1: Structural formulae of TGC (A) and eosin Y (B).

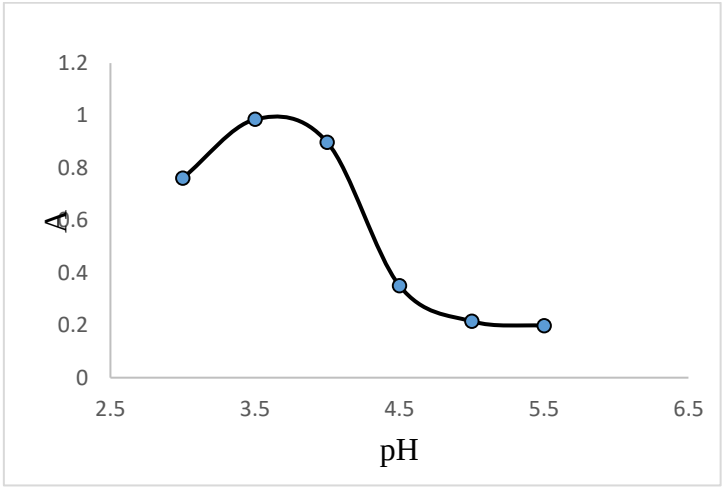


Figure 2: Effect of buffer pH on the absorbance readings of 10.0 µg/mL TGC.

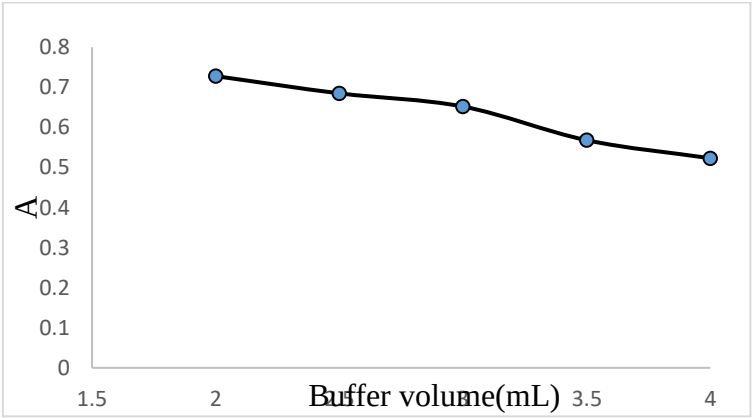


Figure 3: Effect of buffer volume on the absorbance readings of 10.0 µg/mL TGC.

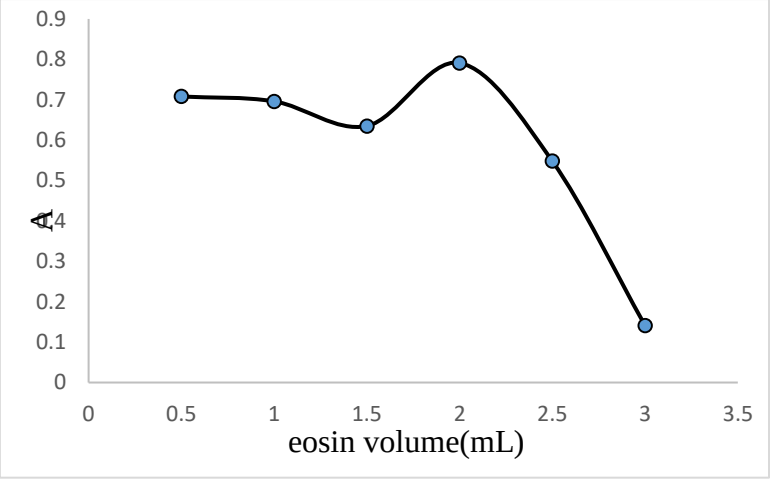


Figure 4: Effect of eosin volume on the absorbance readings of 10.0 µg/mL TGC.

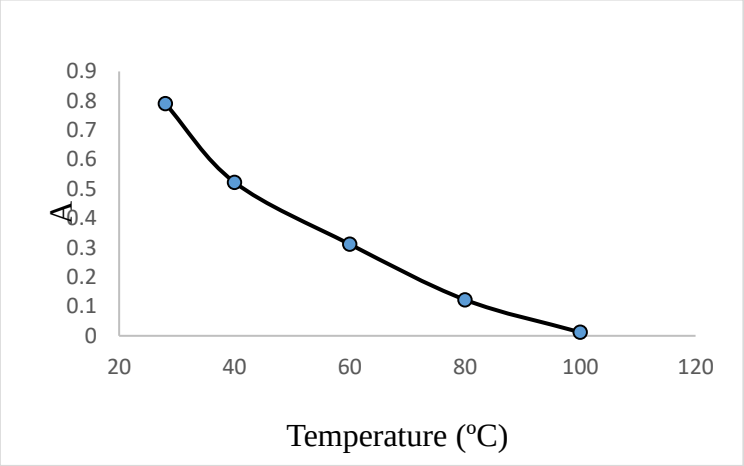


Figure 5: Effect of temperature on the absorbance readings of 10.0 µg/mL TGC.

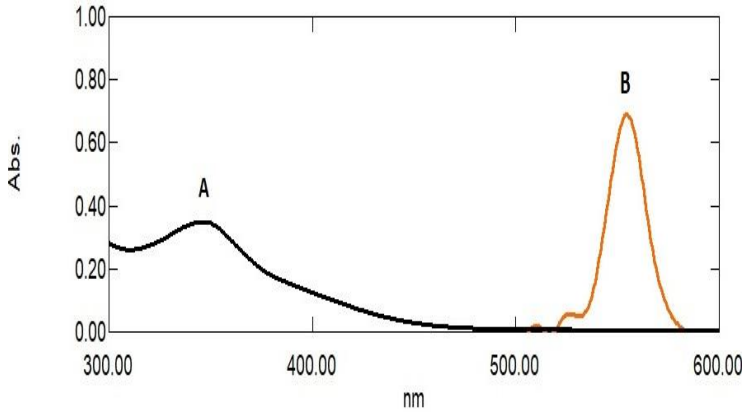


Figure 6: Absorption spectrum of 10.0 µg/mL TGC before (A) and after (B) reaction.

between the tertiary amino group of TGC and eosin Y in an acidic medium, where the formed colored complex was measured at 555 nm.

3.1. Optimization of the reaction conditions

3.1.1 Effect of pH

The effect of pH on the absorbance readings of the formed complex was studied over the pH range of 3-5.5 using 0.2 M acetate buffer. Maximum absorbance readings were obtained at pH 3.5, while a further increase in pH causes a gradual decrease in the measured responses (Fig. 2). Accordingly, an acetate buffer of pH 3.5 was used throughout this study.

3.1.2 Effect of buffer volume

Different volumes of 0.2 M acetate buffer (pH 3.5) were added to the reaction mixture to assess the optimum value. After a detailed investigation, 2 mL of the buffer was found suitable, yielding maximum absorbance readings for the formed complex. It is to be noted that a gradual increase in the buffer volume results in a decrease in the obtained responses (Fig. 3).

3.1.3 Effect of eosin Y volume

The influence of (5×10^{-3} M) eosin Y solution volume on the absorbance values was thoroughly investigated. Volumes ranging from 0.5 mL to 1.5 mL yielded almost the same responses, by increasing the volume to 2 mL, an enhancement of the absorbance readings occurred. Gradual increase in the volume of the reagent (2-3 mL) resulted in lower responses of the formed complex, hence, 2 mL of (5×10^{-3} M) eosin Y was used (Fig. 4).

3.1.4 Effect of reaction temperature

The effect of different temperature settings on the absorbance of the formed complex was studied over the range of (25-100º C). Maximum responses were obtained at room temperature, while the absorbance readings decrease gradually by increasing the reaction temperature (Fig. 5). This behavior could be attributed to a decrease in the stability of the formed complex by elevating the temperature.

3.1.5 Effect of order of addition

The effect of the order of addition on the absorbance readings of the formed complex was also studied and revealed that there was an insignificant difference upon the addition of either eosin Y or buffer solution first.

3.1.6 Effect of reaction time

The effect of time on the formation of the complex was studied by measuring the absorbance of the reaction mixture every 10 minutes. It was found that the reaction occurred immediately, and the formed complex was stable for more than 2 hours.

A representative spectrum of the obtained complex using the optimized experimental conditions is illustrated in (Fig. 6).

3.2. Stoichiometry of the reaction

The reaction stoichiometry between TGC and eosin Y was studied using the limiting logarithmic method (Tobey, 1965). Straight lines with slopes of 1.041/0.5765 were produced by plotting log absorbance against either log [eosin Y] or log [TGC] (Fig. 7). Thus, it is postulated that the reaction takes place in a 2:1 ratio between TGC and eosin Y respectively (Scheme 1). It is suggested that the complex is formed by the reaction of the basic tertiary amino groups of TGC, and both the carboxylate and carbonyl anions of eosin Y obtained at the acidic pH of the reaction. This postulated reaction mechanism is under the guidance of a previous report (Ahmed et al., 2019).

3.3. Validation of the developed method

The suggested method was validated regarding the guidelines stated by the International Conference of Harmonization (ICH) Q2 (R1) (Borman and Elder, 2017).

The linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample. A linear relationship was found to exist between the absorbance values and the corresponding drug concentration over the range of (1.0-14.0) µg/mL for TGC (Table 1).

The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be detected, not quantitated. LOD was found to be (0.2114 µg/mL) for TGC (Table 1).

The Limit of Quantification (LOQ) is defined as the lowest concentration of an analyte that can be quantitatively detected. LOQ was found to be (0.6405 µg/mL) for TGC (Table 1).

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The evaluation of the intra-day or inter-day precision of the present method was carried out by analyzing three concentrations of TGC on the same day or over three consecutive days, respectively. The small values of (% RSD) ranged from 0.18 to 1.11%, indicating reasonable repeatability, high precision, and accuracy of the present method (Table 2).

Table (1): Analytical performance data for the determination of TGC by the proposed method.

Parameter	Tigecycline
Concentration range (µg/mL)	1.0-14.0
Limit of Detection LOD (µg/mL)	0.2114
Limit of Quantitation LOQ (µg/mL)	0.6405
Regression equation (Y=a + bX)	Y=0.0765X+0.1085
Correlation coefficient	0.9996
S y/x, S.D. of the residuals	0.0073
Sa, S.D. of the intercept	0.0049
Sb, S.D. of the slope	0.00059

Table (2): Precision data for the determination of TGC by the proposed method.

	Intra-day precision			Inter-day precision		
	Conc. Taken (µg/mL)	Mean{%found} ±SD	%RSD	Conc. Taken (µg/mL)	Mean{%found} ±SD	%RSD
Tigecycline	2	100.33±0.53	0.53	2	99.89±1.11	1.11
	6	100.11±0.18	0.18	6	99.89±0.18	0.18
	12	100.13±.22	0.22	12	100.20±0.18	0.18

* Each result is the average of three separate determinations.

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. To validate the accuracy of this method, the results of the proposed method were compared to those obtained using the reference method(Silva et al., 2012). For the determination of TGC in the reference method, a colorimetric spectrophotometric method was used, using copper acetate reagent under acid conditions (acetate buffer pH 3), forming a greenish-colored solution with an absorption maximum of 378 nm. But the proposed method was more sensitive (as revealed from LOD and LOQ values), offered a wider linearity range than the reported assay, and was applied to pharmaceutical wastewater. So, the proposed method was preferred over the reference method.

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Method robustness was determined by the resistance of the proposed method to deliberate minor changes in the parameters of the experiment, such as the change in buffer pH (3.5 ± 0.3), buffer volume (2±0.3), and eosin volume (2±.0.3). The absorption intensity was not affected by these minor changes, proving the good robustness of the developed method.

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. Typically, these might include impurities, degradants, and matrix. Method specificity was assessed by the absence of interference from the common excipients of the dosage form during analysis (Table 3).

3.4. Applications

The developed method was successfully used to assay TGC in its dosage form and in laboratory prepared pharmaceutical wastewater. Statistical comparison of the obtained results to the reference method (Silva et al., 2012) was performed by applying the t-test and F-test (Miller and Miller, 2005). The results obtained proved the excellent accuracy and precision of the developed method (Table 3,4).

3.5. Greenness assessment of the proposed method

3.5.1 National Environmental Methods Index assessment (NEMI)

NEMI uses a pictogram with four quadrants to assess a method's greenness (Keith et al., 2007). These quadrants represent; 1- PBT (persistent, bio accumulative, and toxic), 2- Hazardous, 3- Corrosive, and 4- Waste. The corresponding quadrant is shaded green if the following conditions are met; 1- the used reagents are not defined as PBT by the Environment Protection Agency's Toxic Release Inventory (EPA-TRI) (Evans and Martin, 1996), 2- the chemicals used are not hazardous and are therefore not listed on the TRI list (Minnick and Wachter, 2010), 3- the medium's pH falls in the range of 2-12, and 4- the generated waste is less than 50g. The NEMI pictogram for the proposed method was created as displayed in (Fig. 8). From the first look at the pictogram, which showed that all four quadrants were shaded green, the proposed method met all four of the NEMI's requirements and was environmentally friendly. Since the used solvents and reagents are not on the TRI list, they are neither reported as hazardous nor PBT (Evans and Martin, 1996, Minnick and Wachter, 2010). The method is also regarded as non-corrosive because the pH was 3.5 and the amount of waste generated was less than 50g.

3.5.2 Analytical Eco-scale assessment

The analytical Eco-scale proposed by Gauszka et al (Gałuszka et al., 2012), which includes all steps involved in analytical methodologies starting from sample preparation, extraction, transport, and storage, followed by analysis, and finally subjected to calibration and validation (Gałuszka et al., 2012), was used to evaluate the greenness of the proposed method. The four main parameters found in every analytical tool that have a negative impact on environmental safety, according to the analytical Eco-Scale, are: reagents, instrumentation, consumed energy, and produced waste. These parameters were assigned by penalty points PP based on their hazardous effect, which were then subtracted from a total score of 100 to describe the degree of greenness of analytical methods (Gałuszka et al., 2012). The proposed method proved to be an excellent green analysis tool being scored by 99%(100-1PP) as shown in (Table 5).

3.5.3 Green Analytical Procedure Index assessment (GAPI)

The Green Analytical Procedure Index (GAPI) is the most recent method used to assess the greenness of analytical methodologies (Płotka-Wasylka, 2018), tracing all steps involved in assay procedures. This useful method utilizes pictograms for illustrating the obtained output (Płotka-Wasylka, 2018), with each pictogram describing a

Table (3): Assay results for the determination of TGC in its dosage form (Standiga) by the proposed and reference method.

Parameters	Proposed method		Reference method	
	Conc. taken (µg/mL)	%found	Conc. taken (µg/mL)	%found
	1.0	98.73	10.0	99.09
	2.0	99.86	20.0	100.9
	4.0	99.75	40.0	100.0
	6.0	101.38		
	8.0	99.35		
	10.0	99.41		
	12.0	100.28		
Mean ±SD		99.82±0.77		99.996±0.7389
T	0.873(2.447)			
F	1.087(19.33)			

Table (4): Assay results for the determination of TGC in waste pharmaceutical water by the proposed and reference methods.

Parameters	Proposed method		Reference method	
	conc taken (ug/ml)	%found	conc taken (ug/ml)	%found
	1.0	99.44	10.0	98.05
	2.0	100.56	20.0	101.68
	4.0	98.02	40.0	99.87
	6.0	100		
	8.0	99.58		
	10.0	102.03		
	12.0	100.0		
	14.0	99.1		
Mean ±SD		99.84±1.086		99.86±1.48
t	0.9193 (2.365)			
F	1.857 (19.35)			

Table (5): Analytical Eco-Scale penalty points of the proposed method.

Parameter				Subtotal PP*	Total PP
Reagent	Water	Amount	10-100 ml	2	0
		Hazard	None	0	
	Acetic acid	Amount	< 10 ml	1	0
		Hazard	None	0	
Instrumentation	Spectrophotometry	Energy used	< 0.1 kWh /sample	0	0
Occupational hazard	None			0	0
Waste			< 10 ml, degradable		1
Total PP = 1					1

*Subtotal PP is calculated according to Analytical Eco-Scale (Gałuszka et al., 2012).

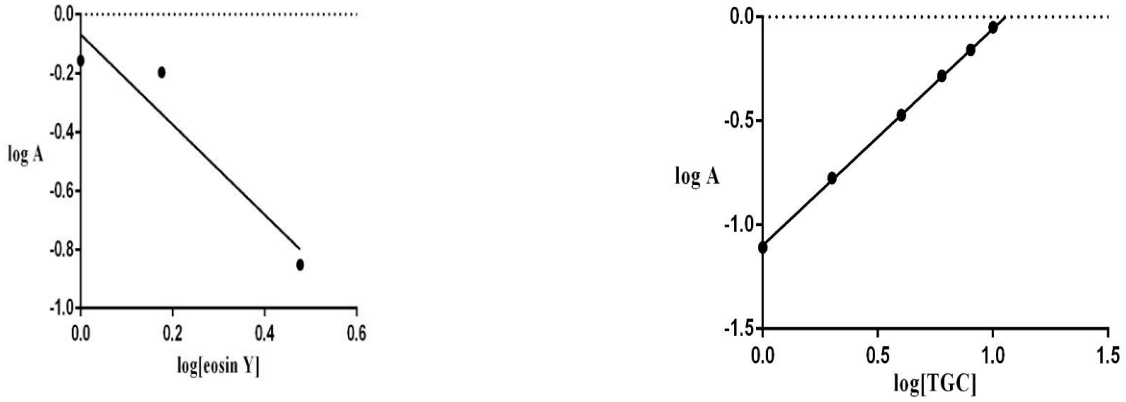


Figure 7: Reaction stoichiometry between TGC and Eosin Y (5.0×10^{-3} M) by limiting logarithmic method.

Figure 8: NEMI pictograms for greenness evaluation of the proposed method.

distinct category. The first category (steps 1-4) is concerned with sample collection, preservation, storage, and transportation, while the second category (step 5) is concerned with the analysis method. Category 3 (steps 6-8) studies sample preparation, while category 4 (steps 9-11) looks into the solvents and reagents consumed. Finally, category 5 (steps 12-15) demonstrates the impact of instrumentation on the ecosystem. The proposed method's greenness was assessed by evaluating the impact of each step under the guidance of GAPI (Płotka-Wasylka, 2018), imparting it either a green, yellow or red color, describing it to be green, moderately green, or hazardous analytical tool respectively (Płotka-Wasylka, 2018). The GAPI index of the suggested assay is illustrated in (Table 6), where its greenness is demonstrated. Meanwhile, (Fig. 9) depicts GAPI pictograms illustrating the significant greenness of this work.

3.5.4 The Analytical Greenness Metric assessment (AGREE)

AGREE is the most recent and automated tool for greenness assessment. AGREE assesses the greenness of any analytical approach, considering the 12 GAC (Green Analytical Chemistry) principles to be significant among the greenness assessment tools (Pena-Pereira et al., 2020). As a result, the new Spectrophotometric methodology's greenness properties were identified utilizing the AGREE approach. The different AGREE scores for each criteria of GAC were assigned by the AGREE calculator. The assigned scores ranged from 0.0 to 1.0. The AGREE report sheet and AGREE score for each GAC concept are illustrated in (Table 7). The expected overall AGREE score using the twelve distinct GAC principles is summarized in (Fig. 10). The proposed method proved to be an excellent green analysis tool, scoring 0.85 as shown in (Fig. 10).

Table (6): GAPI parameters description of the proposed method.

Category	Step	Description	Green	Yellow	Red
Sample collection	1	at-line		√	
Sample preservation	2	none	√		
Sample transport	3	none	√		
Sample storage	4	none	√		
Method type	5	direct	√		
Extraction	6	none	√		
Used solvents	7	water and acetic acid		√	
Sample treatment	8	none	√		
Solvent volume	9	10-100 ml		√	
Health hazard	10	non-irritant, nontoxic	√		
Safety hazard	11	no safety hazard	√		
Consumed energy	12	≤0.1 kWh per sample	√		
Occupational hazard	13	none	√		
Waste volume	14	1-10 ml		√	
Waste treatment	15	biodegradable		√	

Table (7): AGREE assessment parameters description of the proposed method.

Criteria	Description	Score	Weight
1-Direct Analytical techniques should be applied to avoid sample treatment.	At-line analysis.	0.60	2
2-Minimal sample size and minimal number of samples are goals.	Semimicro analysis.	1.0	2
3-If possible, measurements should be performed in situ.	In-line.	1.0	2
4-Integration of analytical processes and operations saves energy and reduces the use of reagents.	No derivatization or extraction are needed.	1.0	2
5-Automated and miniaturized methods should be selected.	Semi-automatic, and miniaturized.	0.75	2
6-Derivatization should be avoided.	No derivatization.	1.0	2
7-Generation of a large volume of analytical waste should be avoided, and proper management of analytical waste should be provided.	< 10 ml.	0.4	2
8-Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time.	50 analytes determined per hour.	0.9	2
9-The use of energy should be minimized.	≤0.1 kWh per sample	1.0	2
10-Reagents obtained from renewable sources should be preferred.	Some reagents are from bio-based source.	0.5	2
11-Toxic reagents should be eliminated or replaced.	No toxic reagents are used.	1.0	2
12-Operator's safety should be increased.	No threats are found.	1.0	2

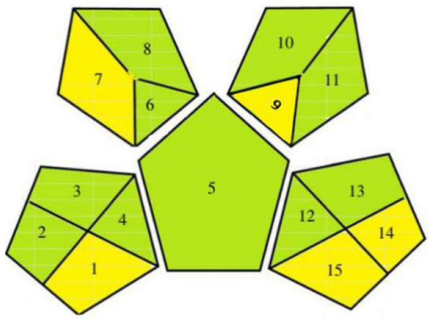


Figure 9: GAPI pictogram for greenness evaluation of the proposed method.

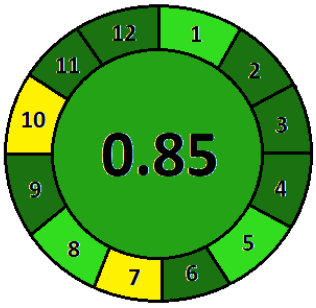
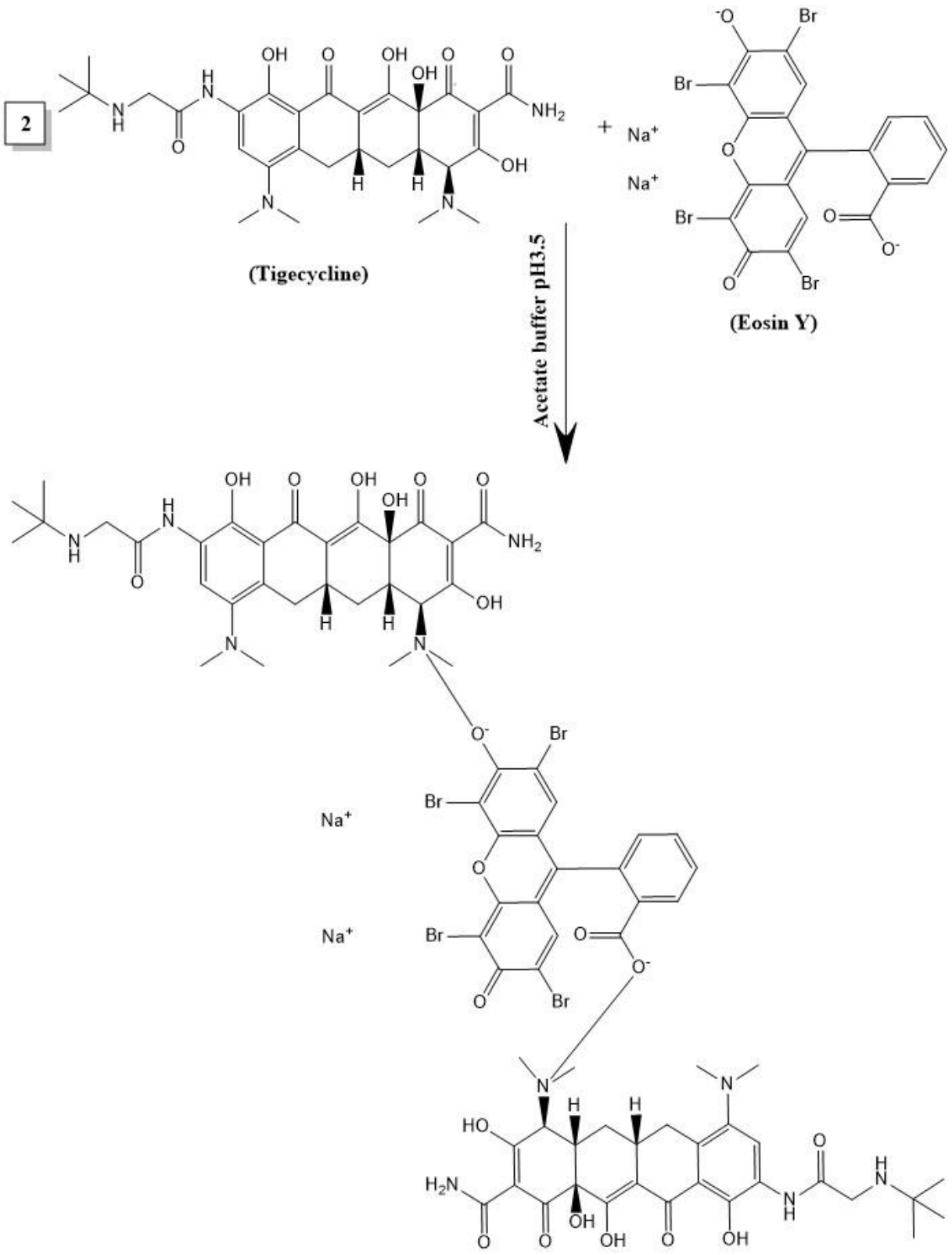


Figure10: AGREE pictogram for greenness evaluation of the proposed method.



Scheme 1: Proposal for the reaction mechanism between TGC and eosin Y.

4. Conclusion

Tigecycline was determined in its dosage form and in pharmaceutical wastewater using an accurate and simple visible spectrophotometric method depending on water soluble ion- pairing complex with eosin Y. No organic solvents were used, which renders the proposed method environmentally friendly. The proposed method carries many advantages being simple, time saving, does not require elaborate treatments or tedious extraction methods, and fully validated which qualifies it to be appropriate in quality control laboratories for routine analysis.

5. Conflict of interest

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

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