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DETERMINATION OF TOLTERODINE TARTRATE IN PHARMACEUTICAL PREPARATIONS USING EOSIN, APPLICATION TO STABILITY STUDY

M. I. Walash, F. Belal, N. El-Enany* and H. Elmansi

Department of Analytical Chemistry, Faculty of Pharmacy, University of Mansoura, 35516, Mansoura, Egypt

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Correspondence to Author:

N. El-Enany

Department of Analytical Chemistry, Faculty of Pharmacy, University of Mansoura, 35516, Mansoura, Egypt

ABSTRACT

Spectrophotometric method has been developed for the determination of Tolterodine tartrate (TOL) which is based on formation of a binary complex between TOL and eosin at 545 nm using acetate buffer at pH 3.8.The absorbance-concentration plot is rectilinear over the range (1-10µg/ml) with LOD of 0.1µg/ml and LOQ of 0.31µg/ml. The proposed method was successfully applied to the analysis of commercial tablets containing the drug and the results were in good agreement with those obtained with the reference method. The method was also utilized to investigate the kinetics of alkaline, acid and oxidative degradation of the drug .It was found to be stable upon acidic and alkaline degradation and only undergo oxidative degradation using 33.3%H_2O_2. The apparent first order rate constant and $t_{1/2}$ of the oxidative degradation reaction were calculated.

INTRODUCTION: Tolterodine tartrate 2-[(1R)-3-[Bis(1-methylethyl)amino]-1-phenylpropyl]-4-methylphenol (**Fig. 1**) [1] is a tertiary anti-muscarinic with actions similar to those of atropine. It is used in the management of urinary frequency, urgency, and incontinence in detrusor instability [2].

The few analytical methods published for its determination include use of UV detection for quantification of enantiomers of tolterodine using chiral LC [3], also there is a few spectrophotometric methods [4, 5], the other reported methods are applicable for biological matrices using complex analytical instruments such as mass spectrophotometer [6-10].

The method suggested in this paper based on the reaction of eosin with the drug measuring the formation of binary complex in an attempt to develop a simple, sensitive, and accurate stability indicating method for the determination of the studied drug either in pure form or in pharmaceutical preparations.

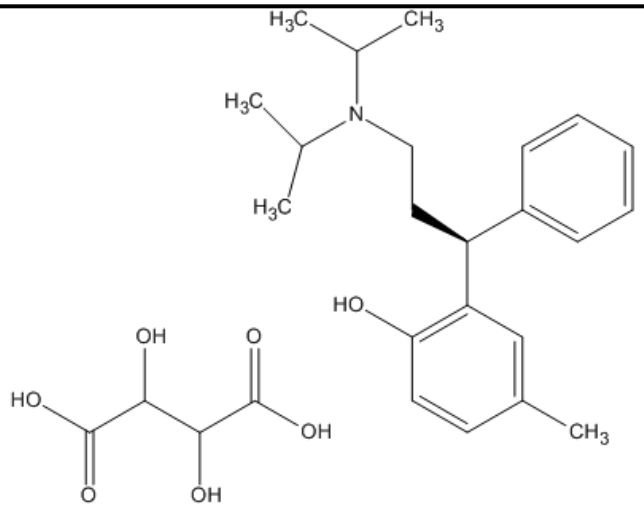


FIG. 1: STRUCTURAL FORMULAE OF TOLTERODINE TARTRATE

Experimental:

Apparatus: The spectrophotometric measurements were established using Shimadzu UV- Visible 1601 recording Spectrophotometer (P/N 206-67001), Recording range, 0-1.0; wavelength 545 nm.

MATERIALS AND REAGENTS: All materials and reagents were of analytical grade.

- Tolterodine tartrate was kindly supplied by El-Kahira, Cairo, Egypt. The purity of the drug was found to be 99.55% according to the reference method [5].
- Detrusitol tablet contains 2mg tolterodine-L-tartrate. (B.No.- H885A, manufactured by Pharmacia & Upjohn) was obtained from commercial sources.
- Eosin (Riedel-De-Haen AG-D-3016 Seeize 1) $4x10^{-3}$ M aqueous solution. The solution was freshly prepared in distilled water and further diluted with the same solvent to the appropriate concentration.
- Acetate buffer Buffer solution 0.4M (pH range from 2.5 to 4.2) was prepared by mixing appropriate volumes of 0.4 M sodium acetate and 0.4 M acetic acid.

Standard Solutions: A stock solution of (TOL) was prepared by dissolving 20.0 mg of (Tol) in 100.0 mL of water and was further diluted with the same solvent as appropriate. The standard solution was stable for 2 weeks when kept in the refrigerator.

General Procedure:

Construction of the Calibration Curve: Accurately measured aliquots of (TOL) in the concentration range shown in **Table 1** were transferred into a series of 10 ml volumetric flasks and diluted to about 7 ml with distilled water. A volume of 0.8ml of $4x10^{-3}$ M eosin solution was added to each flask, and the solutions were mixed well before the addition of o.7 ml 0.4M acetate buffer (pH 3.8). The mixtures were diluted with distilled water to 10 ml and mixed well. The absorbance was measured at 545 nm against an appropriate blank prepared simultaneously. The measured absorbance vs the final concentration in µg/mL were plotted to get the calibration graph. Alternatively, the regression equation was derived.

Applications:

Procedure for tablet: Weigh and pulverize 20 tablets. Transfer a weighed quantity of the powder equivalent to 20.0 mg of tol into a small conical flask. Extract with 3 x 30 ml of water on three successive times each with 30 ml. Filter the extract into a 100 ml volumetric flask. Wash the conical flask with few mls of water. Pass the washings into the same conical flask and complete to the mark with the same solvent. Transfer aliquots covering the working concentration range into 10 ml volumetric flasks. Proceed as described under "General Procedure", adopting the method. Determine the nominal content of the tablets either from the calibration curve or using the corresponding regression equation.

Procedure for stability study: For the kinetic study, aliquot volumes of TOL stock solution equivalent to 400 µg/ml were transferred to 50 ml volumetric flasks, then aliquot volumes of H_2O_2 (2ml 33.3% H_2O_2), then aliquot volumes of this solution equivalent to 8 µg/ml were transferred to 10 ml volumetric flasks every 20 min. and then the reaction described under (General procedure) was performed.

RESULTS AND DISCUSSION: Eosin (a tetrabromofluorescein derivative) is a yellowish red dye with green fluorescence. It has been utilized for the determination of many pharmaceutical compounds of interest either through spectrophotometric measurment such as, carbinoxamine maleate [11], fluoroquinolone antibacterials [12], gliclazide [13], ramipril and enalapril [14], lansoprazole and pantoprazole sodium sesquihydrate [15].

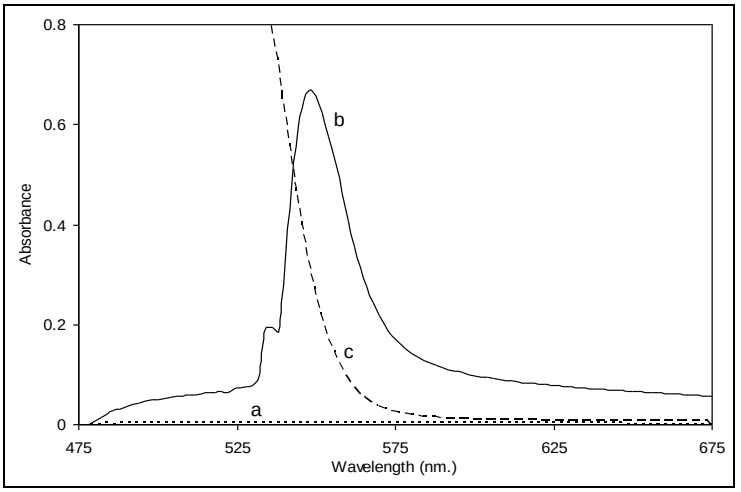


FIG. 2: a) TOL only (6.0 µg/mL); b) Absorption spectrum of the reaction product of TOL (6.0 µg/mL) with (4×10^{-3}) M eosin at pH 3.8. c) Blank.

Optimization of Experimental Conditions: The spectrophotometric properties of the product as well as the different experimental parameters affecting its development and stability were carefully studied and optimized. Such factors were changed individually while the others were kept constant. These factors include; the pH, type of buffer, volume of buffer, volume of eosin and time.

In spectrophotometric measurement, due to the slight solubility of the complex formed with eosin in aqueous solutions, it is difficult for the produced color to be accurately and precisely measured. Therefore, studies for solving this problem were done. Extraction with organic solvent has been an approach [16, 17]. Another method was the addition of non ionic surfactants, such as methyl cellulose and Tween 80 to solubilize and stabilize this type of complex [13, 18]. In this study non ionic surfactants were tried to prevent complex precipitation.

However, the reproducibility upon these surfactants was found to be adversely affected; therefore, El-Brashy *et al*., method [12] was followed which based on keeping the sample concentration at maximum dilution before adding the dye solution, and mixing well before the addition of acidic buffer. By this procedure, the complex stability was greatly increased, with complete prevention of precipitate formation, and maximum precision was achieved, so it has the advantage of being simple and rapid and it can be used for the determination of the studied drugs in pure form and pharmaceutical preparations.

A. Effect of pH and volume of Acetate buffer:

a. Effect of pH: The pH is a critical factor, since it affects the ionization of eosin. Depending on the pH of the solution, eosin can exist in any of the following forms:

$$H_3R^+ \xleftrightarrow{Ka_1} H_2R^+ \xleftrightarrow{Ka_2} HR^- \xleftrightarrow{Ka_3} R^{2-}$$

Equation 1: Effect of pH on dissociation of eosin

Where; R denotes the ionic part of eosin.

The values pK_{a1}, pK_{a2} and pK_{a3} of eosin were reported to be 2.1, 2.85 and 4.95, respectively. At pH 3.8, eosin is found in the form of HR^- [19].

The influence of pH of Acetate buffer on the absorbance value of the binary complex was studied over the pH range 3.5-4.5. It was found that optimum absorbance values were achieved at pH 3.8 ±0.2 (**Fig. 4**)

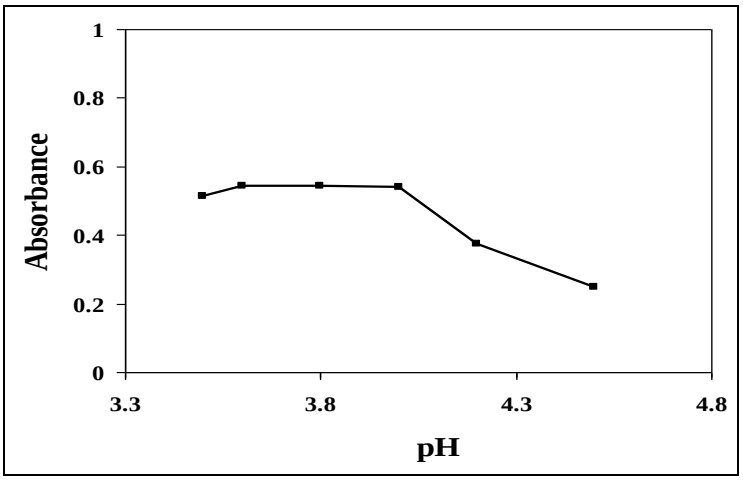


FIG. 3: EFFECT OF PH OF 0.4 M ACETATE BUFFER ON THE ABSORBANCE VALUE OF 5µg/ml TOL.

b. Effect of volume of Acetate buffer: Increasing the volume of the buffer resulted in gradual increase in the absorbance value of the complex up to 0.5 mL and further increase resulted in negligible increase in the absorbance till 2.0 mL therefore, 0.7±0.2 mL of acetate buffer of pH 3.8 was used through this study for (TOL) (**Fig. 5**)

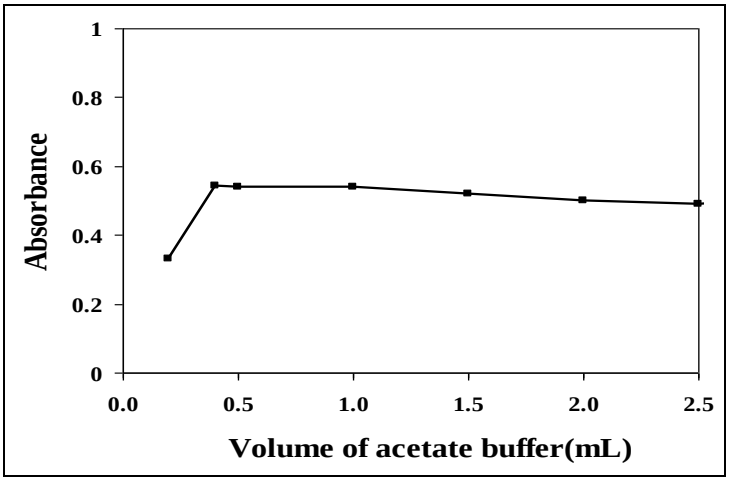


FIG. 4: EFFECT OF VOLUME OF 0.4 M ACETATE BUFFER ON THE ABSORBANCE VALUE OF 5 µg/mL TOL

B. Effect of volume of eosin: The optimum volume of the reagent was determined for the drug. For this method 0.8 mL of eosin (4×10^{-3}) was suitable to develop the absorbance to its maximum intensity (**Fig. 6**)

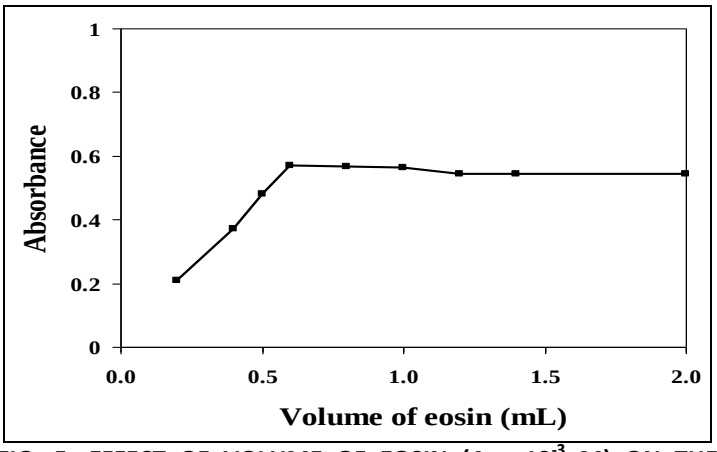


FIG. 5: EFFECT OF VOLUME OF EOSIN (4 × 10^{-3} M) ON THE ABSORBANCE VALUE OF 5 µg/mL TOL

C. Effect of time: The effect of time on the absorbance value of the complex was also studied. It was found that the formation of the binary complex was immediate and the intensity of the final color was stable for 48 h with no precipitation of the complex.

Analytical Performance: The absorbance-concentration plot was found to be linear over the range of 1-10 µg mL^{-1}. Linear regression analysis of the data is shown in **table 1**.

Linear regression analysis of the data gave the following equation

$$A = -0.026 + 0.112C \qquad (r=0.9999)$$

Equation 2: Linear regression analysis of the data

Where; A is the absorbance in 1-cm cell, C is the concentration of the drug (µg/mL) and r is the correlation coefficient.

The limit of quantitation (LOQ) was calculated according to ICH Q2 Recommendation [20] by establishing the lowest concentration that can be measured, below which the calibration graph is non linear and was found to be 0.31 µg/mL for the method. LOQ was calculated from the following equation [21]:

$$LOQ = 10\ S_a / slope$$

Equation 3: Calculation of limit of quantitation

The limit of detection (LOD) was calculated according to ICH Q2 Recommendation [20] and was found to be 0.10 µg/mL for the method. LOD was calculated from the following equation [20]:

$$LOD = 3.3\ S_a / slope$$

Equation 4: Calculation of limit of detection

The proposed method was evaluated by calculating the accuracy as percent relative error and precision as percent standard deviation (RSD %) (Table 1).

TABLE 1: PERFORMANCE DATA OF THE PROPOSED METHOD

Parameter	Proposed Method
-concentration range (µg/ mL).	1-10
-LOD (µg/mL).	0.102
-LOQ (µg/ mL).	0.309
-Correlation coefficient (r).	0.9999
-Slope	0.112
-Intercept	-0.026
$-S_{y/x}$	4.55×10^{-3}
$-S_a$	3.48×10^{-3}
$-S_b$	5.87×10^{-4}
-% Error	0.34
-%RSD	0.84
-No.of Experiments.	6
-Mean found (%)	100.48
± SD.	0.84
-Student's t-value.	1.27 (2.26)
-Variance ratio F-test.	2.14 (5.19)
-Applications.	Tablet preparations

N.B. $-S_{y/x}$ =standard deviation of the residuals; $-S_a$ = standard deviation of the intercept of regression line; $-S_b$ = standard deviation of the slope of regression line; -% Error = RSD% / √ n.

- Figures between parentheses are the tabulated t and F values respectively, at p = 0.05 [20] .

Validation of the method: The proposed method was tested for linearity, specificity, accuracy and precision.

Linearity: Under the described experimental conditions, the calibration graph for the method was constructed by plotting absorbance versus concentration in µg/mL**.** The regression plot showed a linear dependence of absorbance values on the drug concentrations over the range cited in table 1. Regression equations, intercepts, slopes and correlation coefficients for the calibration data were presented in table 1. The validity of the method was evaluated by statistical evaluation of the regression lines regarding standard deviation of the residual ($S_{y/x}$), standard deviation of the intercept (S_a) and standard deviation of the slope (S_b). The small values of the figures point out to the low scattering of the points around the calibration graphs (Table 1).

Accuracy: Statistical analysis [21] of the results, obtained by the proposed and the reference methods for TOL using Student's t-test and variance ratio F-test, shows no significant difference between the performance of the method regarding the accuracy and precision, respectively (Table 1). The reference method [5] is based on spectrophoto- metric measuring of (Tol) in pure and pharmaceutical formulations. This method obeys Beer's law limits in the concentration range of 10-90 µg/mL exhibiting maximum absorbance at 280 nm.

Precision:

a. **Repeatability:** The repeatability was performed by applying the proposed method for the determination of two concentrations of (Tol) in pure form on three successive times, and the results are shown in **table 2**.

b. **Intermediate precision:** It was performed through repeated analysis of (Tol) in pure form, using the concentrations abridged in table 2 for a period of three successive days.

Robustness of the method: The robustness of the method adopted for the method was demonstrated by the constancy of the absorbance value with the minor changes in the experimental parameters such as pH 3.8 ± 0.2 and change in the volume of eosin, (4×10^{-3} M), using 0.8 ± 0.2 mL. These minor changes that may take place during the experimental operation didn't greatly affect the absorbance value.

TABLE 2: VALIDATION OF THE PROPOSED METHOD FOR THE DETERMINATION OF TOL IN PURE FORM

Sample concentration	% recovery (repeatability)	% recovery Intermediate precision
3 µg/mLs	99.9	100.70
	97.6	101.12
	98.5	99.82
X`	98.67	100.55
± SD	0.99	0.92
%RSD	0.99	0.92
% Error	0.58	0.53
8 µg/mL	98.92	102.13
	99.43	101.12
	100.87	100.87
X`	99.74	101.37
± SD	1.02	0.18
%RSD	1.02	0.18
% Error	0.59	0.10

Pharmaceutical Applications: The proposed method was applied to the determination of the studied drug in its tablet preparation.

Specificity: The specificity of the method was investigated by observing any interference encountered from the common tablets excipients, such as talc, lactose, starch, avisil, gelatin, and magnesium stearate. These excipients didn't interfere with the proposed method.

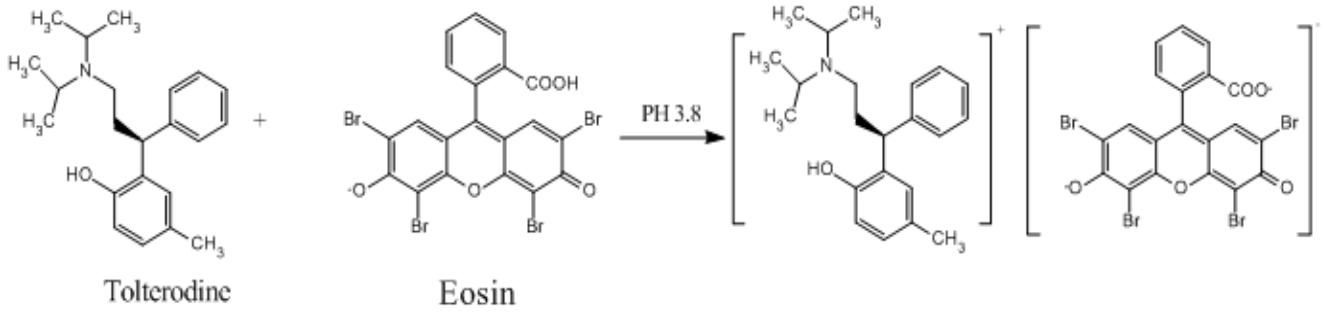
Accuracy: The results of the proposed method were compared with those obtained using the reference method. Statistical analysis [21] of the results obtained using Student's t-test and variance ratio F-test revealed no significant difference between the performance of the method regarding the accuracy and precision, respectively (Table 3).

TABLE 3: APPLICATION OF THE PROPOSED SPECTROPHOTOMETRIC METHOD TO THE DETERMINATION OF TOL IN COMMERCIAL TABLETS

Preparation	Spectrophotometric method		Reference method	
	Amt. Taken (µg/ml)	% found	Amt. Taken (µg/ml)	% found
Detrusitol tablets	3.0	100.53	10.0	101.93
(2.0 mg tolterodine-L-	7.0	99.81	30.0	99.34
tartrate/Tablet)	10.0	101.08	50.0	99.56
$\bar{X}$ ± SD		100.47±0.90	100.28 ± 1.44	
Student's t test		0.22		
Variance ratio F test		5.00		

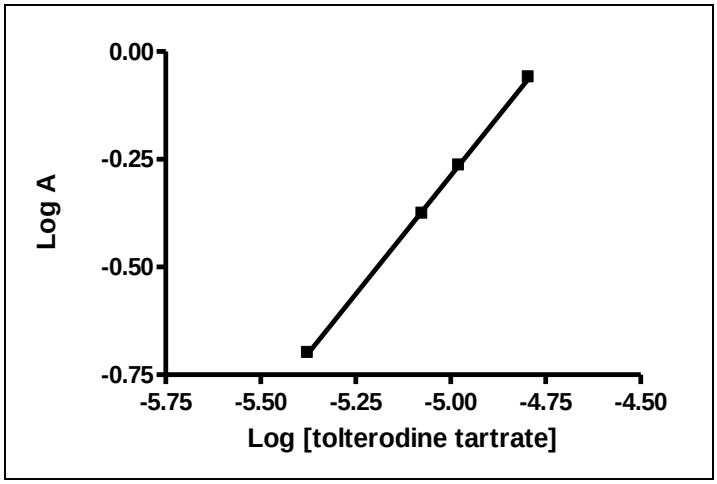
N.B.: The tabulated values of t and F are (2.78) and (19.00) respectively, at p=0.05 [21]. Each result is the average of three separate determinations.

Mechanism of the reaction: The stoichiometry of the reaction between the studied drug and eosin was studied adopting the limiting logarithmic method [22]. The absorbance value of the reaction product was alternatively measured in the presence of either eosin or the studied drug. Plots of log [drug] vs log A and log [eosin] vs log Δ A gave two straight lines, the values of the slopes were 1.1: 1.15 for TOL: eosin (**Fig. 6**). Hence, it is concluded that, the molar reactivity of the reaction is 1: 1. Based on the obtained molar ratio, a schematic proposal for the reaction between the studied drug and eosin is shown in the following **scheme 1**:



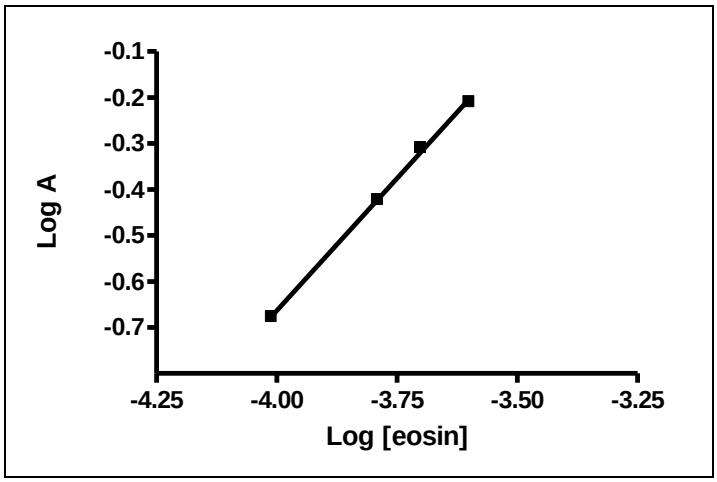
SCHEME 1: THE PROPOSAL MECHANISM FOR THE REACTION BETWEEN TOL AND EOSIN

A-



(A) Log [drug] *vs* log A.

B-



(B) Log [eosin] *vs* log A.

FIG. 6: STOICHIOMETRY OF THE REACTION BETWEEN TOL AND EOSIN (4×10^{-3}M) ADOPTING LIMITING LOGARITHMIC METHOD

Stability indication of the method:

Forced Degradation Studies of TOL: In order to establish whether the analytical method was stability indicating or not, TOL was exposed to different forced degradation studies. Solutions for alkaline degradation were prepared by dissolving 100 mg TOL in the least amount of water, then the volume was completed to 100 ml in a volumetric flask with 5.0 M sodium hydroxide, then the solution was heated in a boiling water bath for three hours. The drug was found to be highly stable under these conditions as revealed from the constant absorbance values. The same method was followed using 5.0 M hydrochloric acid instead of 5.0 M sodium hydroxide. Similarly, the drug showed a high stability as indicated from the constant absorbance values.

Oxidative Degradation Studies:

Degradation Kinetics Study: For the kinetic study, 33.3% (v/v) of hydrogen peroxide was used for the oxidative degradation of the drug. The degradation was found to proceeding at room temperature (**Fig. 7**). The apparent first order degradation rate constant was 11.3×10^{-3} min^{-1} and the half life time at room temperature was 82.5 min. It was proposed that the main pathway of H_2O_2 degradation was via oxidative degradation with the formation of N-oxide A decrease in the difference of absorbance value with the time indicates first order reaction.

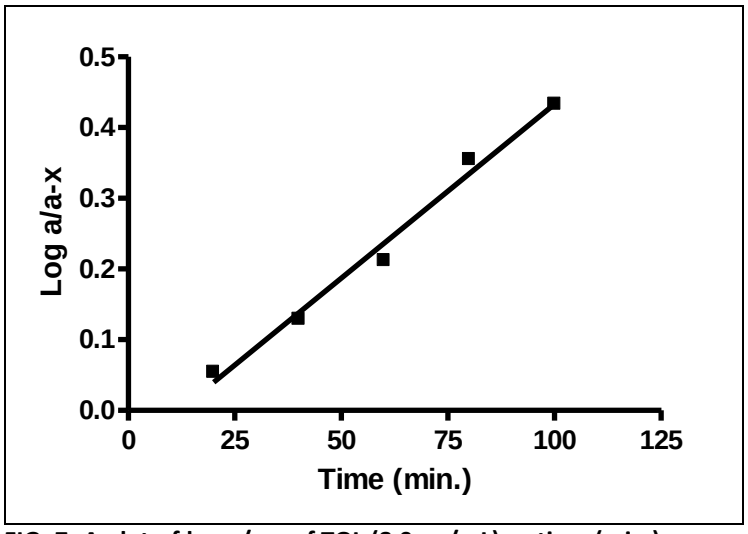


FIG. 7: A plot of log a/a-x of TOL (8.0 µg/mL) *vs* time (min.) upon degradation with 33.3% H_2O_2 at room temperature

CONCLUSION: The present study describes a sensitive stability indicating method for the determination of the studied drug without interference from common tablet excipients. Hence, it can be applied for the routine quality control of the studied drug either in bulk or in its corresponding dosage forms especially that there are only few methods for its spectrophotometric measurements and they are less sensitive than this method. From economic point of view, the proposed method is simple, rapid and inexpensive besides the use of water as diluting solvent. So, it is a good alternative to the other few reported methods and to high cost HPLC methods.

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