SPECTROFLUORIMETRIC DETERMINATION OF TETRACYCLINE AND TERBUTALINE SULPHATE IN ITS PURE AND DOSAGE FORMS USING EOSIN Y REAGENT

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Keywords: Tetracycline; terbutaline sulphate; Eosin Y; fluorimetry.

A spectrofluorimetric method has been developed for the determination of tetracycline hydrochloride and terbutaline sulphate in different dosage forms. The method is based on the quantitative quenching effect of tetracycline hydrochloride and terbutaline sulphate on the native fluorescence of Eosin Y at the pH 6.4 and 3.5 respectively. The quenching of the fluorescence of Eosin Y was measured at 545 nm after excitation at 350 nm. The fluorescence-concentration plots are rectilinear over the range 0.5–18 and 0.05-5.0 μg mL^{-1} with LOD of 0.531 and 0.241 μg mL^{-1} and LOQ of 1.77 and 0.806 μg mL^{-1} for above drugs respectively. The proposed method has been successfully applied to the analysis of commercial tablets and capsules containing the drug. Statistical comparison of the results with those of the reference method revealed good agreement and proved that there were no significant differences in the accuracy and precision between the two methods.

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INTRODUCTION

Terbutaline sulphate [2-(*tert*-butylamino)-1-(3,5-dihydroxyphenyl)ethanol sulphate][1] is a short-acting bronchorelaxant which can be given orally,[2] it is readily metabolized in the gut wall and liver when given orally. It has a short duration of action.[3] It has the following chemical structure (Figure 1).

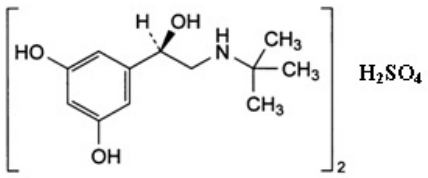

Figure 1. Structure of terbutaline sulphate.

Terbutaline sulphate is widely used as an effective bronco dilator in the management of asthma. This is used as prophylactic drug as well as to prevent acute exacerbations of asthma, chronic bronchitis, emphysema and other lung diseases. It relaxes and opens air passage in the lungs, making it easier to breathe[3,4].

Tetracycline hydrochloride [(4*S*,4a*S*,5a*S*,6*S*,12a*S*)-4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12 apentahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxa-mide hydrochloride][1] is an effective antibiotic in treating infections,[5] and its absorption is reduced by anti-acids and milk, because it can form insoluble complexes with Ca, Mg, Al and Fe.[6] It has the following chemical structure (Figure 2).

Tetracycline is an antibiotic with a broad antibacterial spectrum and bacteriostatic activity, having a good activity against acute disease caused by gram-positive and gram-negative bacteria, including the species like *Spirochete, Actinomyces, Ricketsia* and *Mycoplasma*.[7]

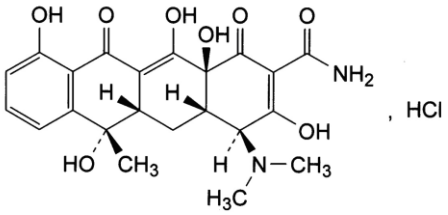

Figure 2. Structure of tetracycline hydrochloride.

Different analytical techniques have been developed for determination of terbutaline sulfate and tetracycline. HPLC,[8,9] LC–MS,[10] CE,[11] CE–MS,[12] voltammetry,[13] electrochemical method,[14,15] liquid chromatography,[16,17] capillary electrophoresis[18] and chemiluminescence[19,20] have been reported for determination of tetracycline. These methods are often time-consuming, expensive, and cumbersome.

Spectrophotometric methods have been reported for determination of both drugs using different reagents.[21-30] Spectrofluorimetry is attractive because of its sensitivity, speed, and simplicity. Most of the additives or excipients found in pharmaceutical preparations are not fluorescent in nature. The aim of this study is to develop an optimized spectrofluorimetric method for the determination of tetracycline hydrochloride and terbutaline sulphate in pharmaceutical formulations. The present spectrofluorimetric method is based on the formation of ion pair complex of tetracycline hydrochloride and terbutaline sulphate with Eosin Y at the pH 6.0 and 3.55 (sodium acetate–acetic acid buffer solution) respectively.

Experimental

Fluorescence spectral measurements were made on a RF-5301PC-Spectrofluorophotometer (Tokyo, Japan) equipped with a xenon lamp and 10 mm quartz cells. Excitation and emission wavelengths were set at 350 and 545 nm with the excitation and emission slit widths of 10 nm, respectively. All measurements were performed at 28±1 °C. Philips PW 94 supplied with CE 10-12 pH electrode was used for pH measurement.

All reagents were of analytical-reagent grade which were provided by BDH and Fluka companies. Stock solutions of terbutaline sulphate and tetracycline hydrochloride drugs were prepared in concentration of 50 μg mL^{-1} by dissolving 0.01 g of each in distilled water and made up to 200 mL in volumetric flask. The solutions were kept in refrigerator. The Eosin Y solution of 50 μg mL^{-1} was prepared by dissolving 0.01g in distilled water and made up to 200 mL in a volumetric flask. The *acetate buffer* solution was prepared with pH 6.0 and 3.55 by mixing sodium acetate and acetic acid solutions of 0.1 M and adjusted by pH meter.

General procedure

Aliquots of solution containing 0.1-3.6 mL and 0.01-1.0 mL of 50 μg mL^{-1} for tetracycline hydrochloride and terbutaline sulphate were added separately into two series of 10-mL volumetric flasks containing 2.5 mL and 2.0 mL of sodium acetate–acetic acid buffer solution of pH 6.0 and 3.5 respectively and 3.0 mL of 50 μg mL^{-1} Eosin Y. The solutions were diluted to the mark with distilled water and mixed well. The fluorescence intensity (ΔF) was recorded at 545 nm after excitation at 350 nm. The amount of the drugs was obtained either from their corresponding calibration graphs or the regression equations.

Procedure for pharmaceutical formulations

Tetracycline chloride capsule

Seven tetracycline hydrochloride capsules content (each capsule contains 250 mg tetracycline hydrochloride) were accurately weighed and pulverized. A portion of the fine and homogenized powder equivalent to one capsule was accurately weighed and dissolved in 5 mL ethanol for increasing dissolution and made up to 100 mL with distilled water. The mixture was mixing well and filtered through Whatman no.42 filter paper. The filtrate was diluted to the 250 mL with distilled water to obtain 1000 μg mL^{-1} concentration. A suitable volume was diluted, and the general procedure was followed.

Terbutaline tablet

Twenty terbutaline sulphate tablets (each tablet contains 5 mg terbutaline sulphate) were accurately weighed and pulverized. A portion of the fine and homogenized powder equivalent to one tablet was accurately weighed and dissolved in distilled water, mixing well and filtered through Whatman no.1 filter paper. The filtrate was diluted to the 100 mL with distilled water obtain a concentration of 50 μg mL^{-1}. A suitable volume was diluted, and the general procedure was followed.

Results and Discussion

In the present study, tetracycline hydrochloride and terbutaline sulphate were found to form ion pair red complexes with Eosin Y at pH of 6.0 and 3.55 respectively, with maximum absorbance at 545 nm (Figure 3). The complexes are formed mainly due to the electrostatic interaction between the studied drug and anionic functional group of Eosin Y at suitable pH. The formed ion pair complexes are not fluorescent, therefore, the decrease in the fluorescence intensity of Eosin Y upon the addition of the drugs was the basis for the spectrofluorimetric measurement at 545 nm after excitation at 350 nm (Figure 4a, 4b).

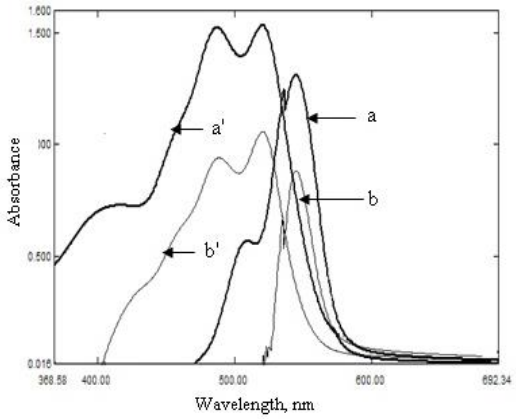

Figure 3. Absorption spectra of (a) 8 μg mL^{-1} terbutaline sulphate and (b) 40 μg mL^{-1} tetracycline hydrochloride with Eosin Y against their respective reagent blank (a', b') under optimum cond···

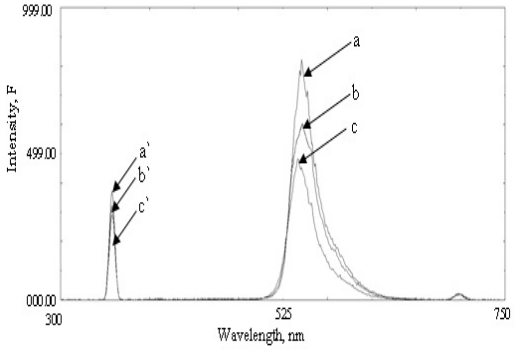

Figure 4a Excitation and emission spectra of: (a`, b`, c`) Blank Eosin Y (15 μg mL^{-1}) at pH 6.4; (a, b, c) Reaction product of Eosin Y (15 μg mL^{-1}) and tetracycline hydrochloride (0.0, 2.0 and 12.0 μg mL^{-1}).

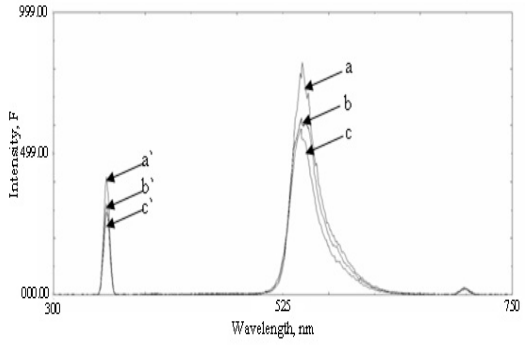

Figure 4b. Excitation and emission spectra of: (a`, b`, c`) Blank Eosin Y (15μg mL^{-1}) at pH 6.4; (a, b, c) Reaction product of Eosin Y (15 μg mL^{-1}) and terbutaline sulphate (0.0, 4.0 and 5.0 μg mL^{-1}).

Optimization of Experimental Parameters

The different experimental parameters affecting the development of the reaction products and its stability were studied and optimized for the spectrofluorimetric method. Such parameters were changed individually while others were kept constant. These parameters include selection of Eosin Y concentration**,** pH, type of buffer and its volume, temperature, reaction time and effect of solvent.

Selection of Eosin Y concentration

To select the concentration of Eosin Y for determination of the drugs, a calibration curve was prepared by addition aliquots of 50 µg mL^{-1} Eosin Y in a set of 10-mL calibrated flasks and diluted to the mark with distilled water. The fluorescence intensity was measured after 5 min. at 545 nm after excitation at 350 nm. Beer's law was obeyed in the range 0.5-15 µg mL^{-1} (Figure 5). However; 15 µg mL^{-1} of Eosin Y was selected in this study.

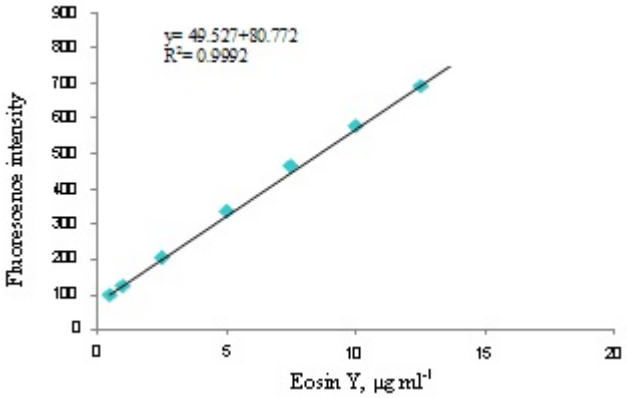

Figure 5. Calibration curve of Eosin Y.

Effect of pH and buffers

When tetracycline hydrochloride added to the Eosin Y, a quenching of the fluorescence intensity for Eosin Y was immediately observed. The final pH of the solution was measured and found 6.0. But quenching of Eosin Y by addition of terbutaline sulphate was observed in the presence of acid with pH 3.55 at final dilution. Therefore, different buffers as phthalate, acetate and citrate of pH 6 and 3.55 were prepared to obtain high ΔF for above drugs respectively. However; acetate buffer was gave maximum ΔF for both drugs and chosen as the optimum throughout the study. It was found that 2.5 and 2.0 mL of acetate buffer gave high ΔF for above drugs respectively (Figure 6).

Effect of heating time and temperature

The effect of temperature, at room temperature (27 °C) and 40 °C, and of time on the quenching of the fluorescence intensity of Eosin Y was studied. It was found that the decrease in the fluorescence intensity of Eosin Y was immediate upon addition of drug in the presence of acetate buffer solution at room temperature and remained constant for more than 90 min for tetracycline hydrochloride and more than 120 min for terbutaline sulphate (Figure 7). However, standing times of 5 and 10 min at room temperature were chosen for tetracycline hydrochloride and terbutaline sulphate, respectively.

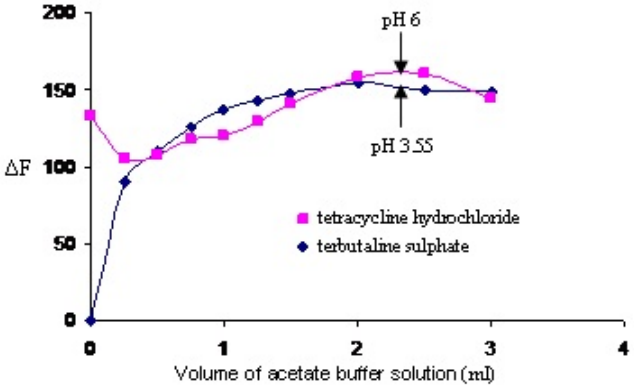

Figure 6. Effect of the volume of acetate buffer solution on ΔF of the tetracycline hydrochloride (2.5 µg mL^{-1}) and terbutaline sulphate (2.5 µg mL^{-1}) with 15 µg mL^{-1} Eosin Y.

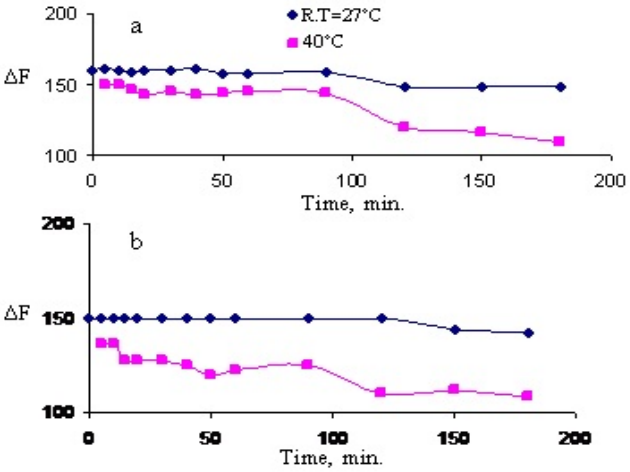

Figure 7. Effect of temperature and standing time on the fluorescence intensity (ΔF) for 2.5 µg mL^{-1} of (a) tetracycline hydrochloride and (b) terbutaline sulphate ion pair complexes with 15 µg mL^{-1} Eosin Y.

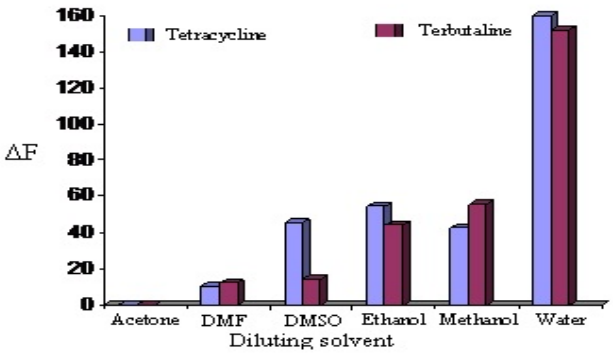

Figure 8. Effect of organic solvent and water on the fluorescence intensity (ΔF) for 2.5 µg mL^{-1} of tetracycline hydrochloride and terbutaline sulphate ion pair complexes with 15 µg mL^{-1} Eosin Y.

Effect of diluting solvents

Dilution effect with different organic solvents, such as acetone, DMF, DMSO, ethanol and methanol in addition to water, were tested on the relative fluorescence intensity. The results revealed that best solvent was water, where as the organic solvents diminished the fluorescence of Eosin Y (Figure 8). Therefore, water was used as diluting solvent.

Effect of surfactant

In order to improve fluorescence intensity (ΔF), various surfactants such as sodium dodecyl sulphate (SDS), cetyltrimethyl ammonium bromide (CTAB), Tween 80 (Tw-80), Triton X-100 (Tr-100), and cetylpyridinium chloride (CPC) were added to the tetracycline hydrochloride and terbutaline sulphate solutions, and their effect was studied. None of the studied surfactants had significant effect on ΔF for both drugs (Figure 9).

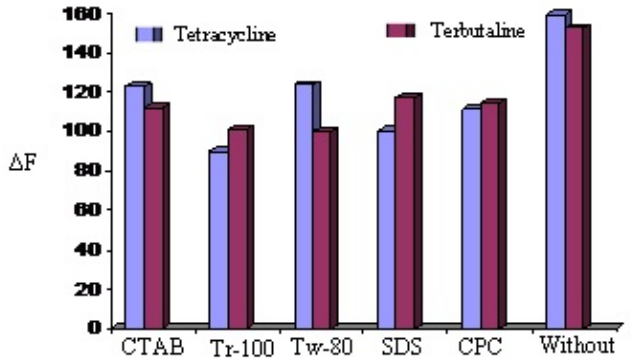

Figure 9. Effect of surfactants on the fluorescence intensity.

Effect of order of addition of reagents

Series of solutions were prepared with different orders of addition of reagents but the same concentrations of reagents, and their corresponding blank solutions were measured at λex/λem = 350 nm/545 nm. The results shown in Figure 10 indicate that addition of acetate buffer followed by addition of Eosin Y and drug gave maximum ΔF and was used in general procedure.

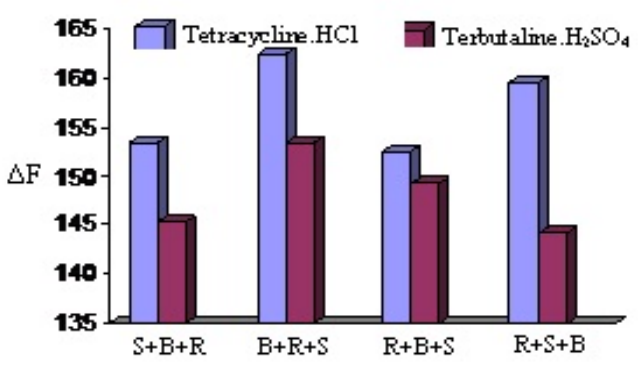

Figure 10. Effect of addition order on the ΔF for tetracycline.HCl (2.5 μg mL^{-1}) and terbutaline.H_2SO_4(2.5 μg mL^{-1}) whereas *S*= drug, *B*=buffer solution and *R*= Eosin Y.

Selectivity

The selectivity of the proposed method was evaluated by analyzing the standard solutions of tetracycline hydrochloride and terbutaline sulphate in the presence of some excipients such as cited in table 1. It was observed that these excipients did not interfere with the proposed method. The results of the recovery experiment also indicated that accuracy is not affected by the co-formulated substances.

Method validation

Linearity, limits of detection and quantitation

Under the optimized experimental conditions, the calibration graphs were constructed by plotting the difference in fluorescence intensity (ΔF) as a function of the corresponding tetracycline hydrochloride and terbutaline sulphate concentrations in μg mL^{-1} (Figure 11). The linear relationships were obtained in the concentration range 0.5–18 and 0.05-5.0 μg mL^{-1} for above drugs respectively. The linearity was represented by the regression equation and the corresponding correlation coefficient for drugs determined by the proposed method represents excellent linearity. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to the following formulae

$$LOD = 3.3\sigma/b \text{ and } LOQ = 10\sigma/b$$

where σ is the standard deviation of five reagent blank determinations and *b* is the slope of the calibration curve. The results obtained are in the accepted range below the lower limit of Beer's law range (Table 2).

Accuracy and precision

The accuracy was checked by five times analysis for three different concentrations of pure samples. The results obtained in Table 3 showed the close agreement between the measured and true values indicating good accuracy of the proposed method. The calculated relative standard deviation (RSD) values were found to be ≤ 2.58 % for tetracycline hydrochloride and ≤ 3.87 % for terbutaline sulphate indicating good repeatability and reliability of the proposed methods (Table 3).

Method validation and applications

To evaluate the analytical applicability of the proposed method, it was successfully applied to determine tetracycline hydrochloride and terbutaline sulphate in some pharmaceutical preparations. The obtained recovery % values cited in Table 4 indicated high accuracy and there is no serious interference in the determination of above drugs in such samples. The results obtained by the proposed method were compared with British Pharmacopoeia (BP) method (Table 4), by applying the F-test and the t-test at 95% confidence level with five degrees of freedom. The calculated values for F and t tests for proposed method did not exceed the theoretical values (F = 5.05, t = 2.571). This confirmed that there are no significant differences between the proposed method with BP method for tetracycline HCl and terbutaline sulphate.

Table 1. Effect of excipients for assay of tetracycline. HCl and terbutaline. H_2SO_4.

Excipient	Recovery % of 2.5 µg mL^{-1} tetracycline.HCl [Exciepient] µg mL^{-1}			Recovery % of 2.5 µg mL^{-1}terbutaline.H_2SO_4 [Exciepient] µg mL^{-1}		
	100	500	1000	100	500	1000
Starch	97.34	96.65	95.54	95.92	95.54	95.43
Glucose	100.39	97.75	95.90	98.97	97.93	95.69
Lactose	98.95	98.00	97.20	97.92	98.34	98.44
Sucrose	95.53	96.39	97.24	97.72	98.23	97.41
KCl	102.35	103.21	103.25	98.99	99.73	98.54
NaCl	98.78	99.85	97.39	100.89	102.00	99.99
Na_2SO_4	94.23	94.54	95.02	95.00	95.09	95.85
Mg-stearate	98.54	97.07	97.32	99.21	99.39	99.91

Stoichiometry

The stoichiometric ratio between tetracycline hydrochloride and Eosin Y at pH 6.4 and terbutaline sulphate and Eosin Y at pH 3.55 were evaluated by mole ratio method[32] according to following equation: A_{max} = f ([Eosin Y] / [drug]), Where the concentration of drugs and Eosin Y are identical (1.04×10^{-4}M for tetracycline hydrochloride and 9.11×10^{-5}M for terbutaline sulphate). By the change of Eosin Y volume and keeping the volume of drug constant (0.5 mL) in final volume of 10 mL, the procedures were completed using the optimum conditions for each drug. The results confirm that the ratio of complexes Eosin Y : drug are equal to 2:1 (Figure 12).

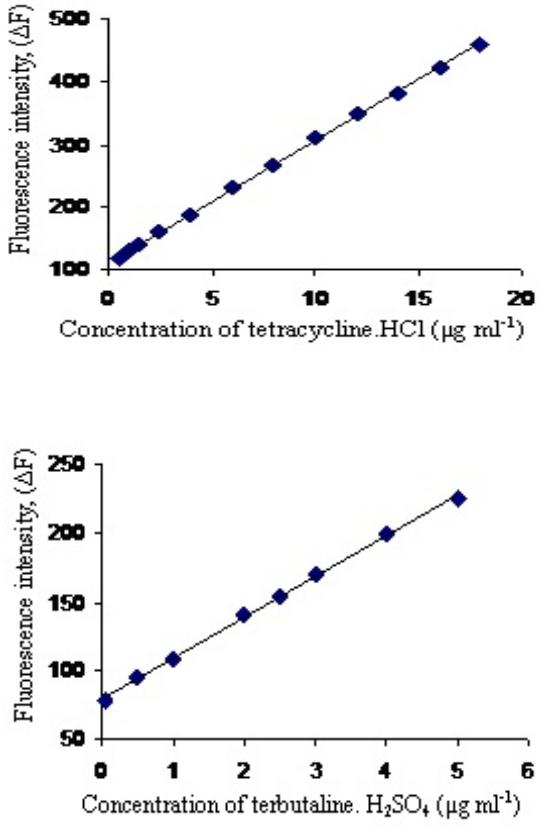

Figure 11. Calibration graphs for determination of tetracycline hydrochloride and terbutaline sulphate

Table 2. Statistical parameters for the determination of tetracycline hydrochloride and terbutaline sulphate.

Parameters	Tetracycline hydrochloride	Terbutaline sulphate
λ_{ex} (nm)	350	350
λ_{em} (nm)	545	545
Linear range (µg mL^{-1})	0.5–18	0.05-5.0
Intercept, *a*	110.9	79.46
Slope, *b*	19.45	29.6
Correlation coefficient (R^2)	0.9995	0.9990
LOD (µg mL^{-1})	0.531	0.241
LOQ (µg mL^{-1})	1.77	0.806

Table 3. Accuracy and precision of the proposed method

Drug	Amount added (µg mL^{-1})	Recovery* %	RSD*
Tetracycline hydrochloride	5	103.99	1.19
	10	101.05	2.58
	15	97.80	0.79
Terbutaline sulphate	1	102.80	0.93
	2.5	98.38	3.87
	4	97.69	1.98

Mechanism of the reaction

The stoichiometry of the reaction was found as 1:2 ratios (drug/Eosin Y), confirming that one molecule of drug reacts with two molecule of Eosin Y. As seen in the chemical structures of drugs, tetracycline hydrochloride have two basic centres of primary and tertiary aliphatic amino groups which are involved in nucleophilic reactions. Thus the carboxylate group of Eosin Y can be attacked by these nucleophilic groups. Terbutaline sulphate structure composed of two molecules of terbutaline combined with sulphuric acid and each molecule have one basic centre of secondary primary amino group which are attacked by the carboxylate group of Eosin Y. Based on all these facts, the proposed mechanisms of these reactions pathway are shown in Figure 13.

Table 4. Assay of tetracycline hydrochloride and terbutaline sulphate drugs in tablet pharmaceutical formulations by the proposed and British Pharmacopoeia methods. ([a] Every reading is an average of five determinations for the proposed method and average of three determinations for British pharmacopoeia method. [b] provided from SDI Co. Iraq. [c] Manufactured by Mediotic labs Homs-Syria. [d] The results obtained by Ref. 31).

Procedure applied	Pharmaceutical preparation	Drug amount taken (μg mL^{-1})	Recovery [a] (%)	Drug constant found, mg	Average recovery content	Certified value, mg
	Tetracycline [b]	5	102.49	256.22	248.06	
	Capsule	10	98.79	246.97	t-test =1.61	250
Proposed		15	96.40	241.00	F-test= 0.102	
method	Asmanol [c]	1	105.68	5.28	5.07	
	tablets	2.5	98.83	4.94	t-test =1.12	5
		4	99.97	4.99	F-test= 1.34	
British	Tetracycline	250 mg	97.92	244.80	-	
Pharmacopoei	Capsule					250
a	Asmanol [d]	15 mg	99.21	14.88 mg	-	
	tablets					15

[a] Every reading is an average of five determinations for the proposed method and average of three determinations for British pharmacopoeia method. [b] provided from SDI Co. Iraq. [c] Manufactured by Mediotic labs Homs-Syria. [d] The results obtained by Ref. 31

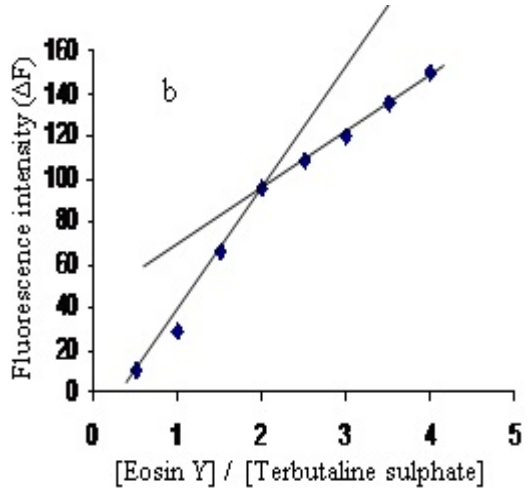

Figure 12a. Molar ratio plot for terbutaline sulphate (0.5 ml of 9.11×10^{-5}M) complexes with Eosin Y.

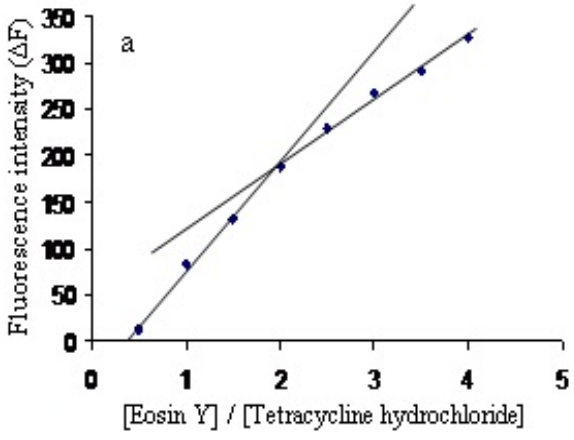

Figure 12b. Molar ratio plot for tetracycline hydrochloride (0.5 ml of 1.04×10^{-4}M)

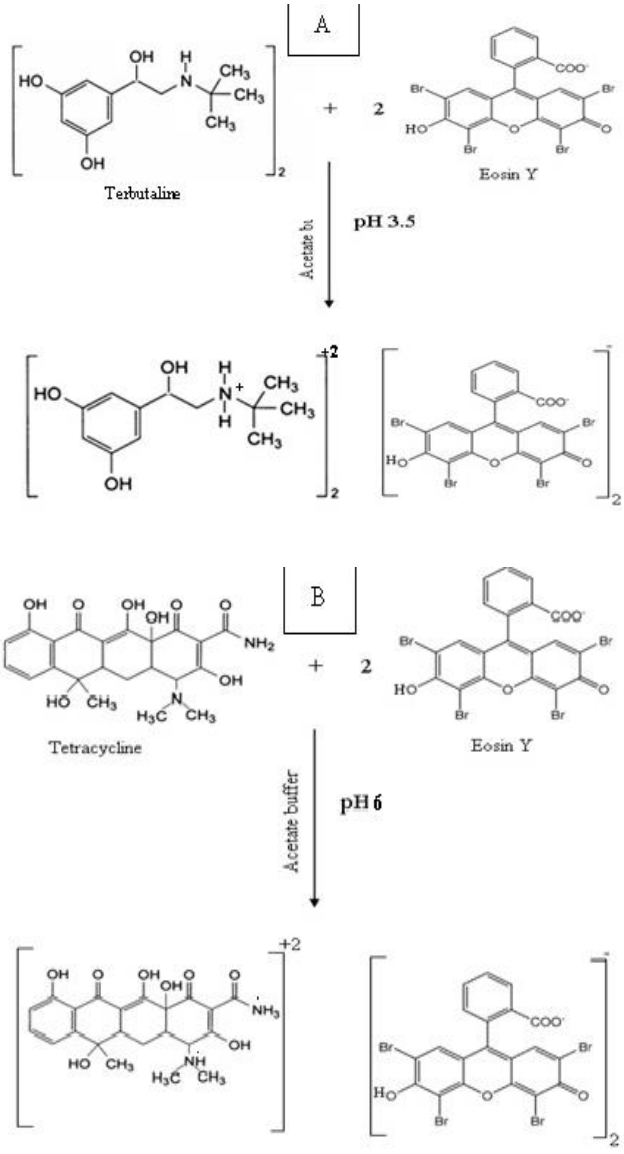

Figure 13. Proposed mechanisms for the reaction between terbutaline sulphate and tetracycline hydrochloride with Eosin Y.

Conclusion

In this study, direct, simple, and sensitive spectrofluorimetric procedure was developed and validated for determination of two drugs; tetracycline hydrochloride and terbutaline sulphate without interference from common excipients. The most important advantage of the method is rapid and inexpensive that the ion-pair formed is measured directly without need for pretreatment of the drug and extraction with organic solvent beside the use of water as diluting solvent. Hence, it can be applied for the routine quality control of the studied drug in its dosage forms.

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