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Spectrophotometric Determination of Terbutaline Sulphate and Tetracycline Hydrochloride via Ion pair Complex Formation Using Eosin Y

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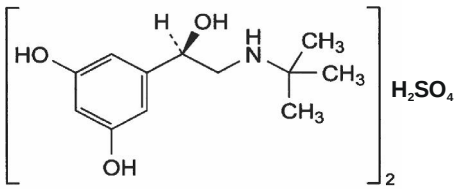
Abstract

A simple, sensitive and rapid spectrophotometric method was developed and validated for the determination of terbutaline sulphate and tetracycline hydrochloride drugs in pure form and pharmaceutical formulations. The proposed method is based on the formation of binary complexes between these drugs and eosin Y in aqueous acetate buffered medium. Under the optimum conditions, the binary complexes showed absorption maxima at 545 nm. Beer's law was rectilinear over concentration range of 0.5-10 and 5-45 µg/mL, R^2 were 0.9984 and 0.9988, RSD were ≤ 0.72 and ≤ 0.19 (n=5) with average recovery % 101.42 % and 100.08 % and the average recovery values of pharmaceutical formulations 101.48 and 98.01 for above drugs respectively. The limit of detection (LOD) were 0.030 and 0.613 µg/mL and limit of quantitation (LOQ) were 0.103 and 2.00 µg/mL with molar absorptivity values 3.169×10^3 and 6.347×10^3 l. mol^{-1}. cm^{-1} and the relative standard deviation values ≤0.720 and ≤ 0.19 for both drugs respectively. No interference was observed from the excipients that are commonly present in pharmaceutical formulations. The proposed method was successfully applied to the analysis of terbutaline sulphate tablet and tetracycline hydrochloride capsule in their dosage forms.

***Keywords*:** Ion association; Spectrophotometry; Terbutaline; Eosin Y.

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:



Terbutaline sulphate ($C_{24}H_{40}N_2O_{10}S$)
M.Wt =548.7

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, 12apentahydroxy-6-methyl – 1, 11 -dioxonaphthacene-2-carboxamide hydrochloride] [1] is an effective antibiotic in treating infections [5], 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

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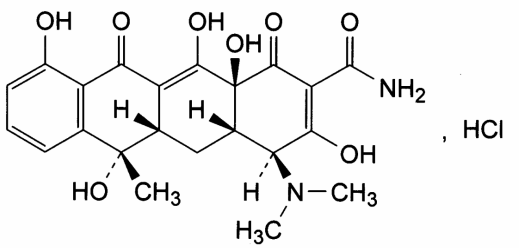
following chemical structure:



Tetracycline hydrochloride ($C_{22}H_{24}N_2O_8$.HCl)
M.Wt=480.9

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 *Spirochete, Actinomyces*, *Ricketsia* and *Mycoplasma* [7].

Different analytical techniques have been developed for determination of terbutaline sulfate and tetracycline, including HPLC [8,9], LC–MS [10], CE [11], CE–MS [12], and voltammetry [13]. Usually, HPLC and CE are applied to the determination of terbutaline sulfate in real samples. Fluorimetry [13,14], electrochemical method [15,16], liquid chromatography [17,18], capillary electrophoresis [19] and chemiluminescence [20,21] have been reported for determination of tetracycline. These methods are often time-consuming, expensive, and cumbersome. Spectrophotometry continues to be very popular, because of its simplicity, versatility and low cost. Several spectrophotometric methods using different reagents have been reported for determination of terbutaline sulphate. 4-Aminoantipyrine in the presence of potassium ferricyanaide [22], Fe (III) in the presence of potassium ferricyanide [23], sodium periodate in the presence of acetylacetone [24], p-aminophenol-molecular oxygen [25], 3- methyl-2-benzothiozolone hydrazone hydrochloride in the presence of ferric chloride [26] and phenanthro[9,10-d]imidazole-2-N-chloroimide [27] reagents were used for determination of terbutaline sulphate.

Chloramine-T [28], ammonium vanadate in sulphuric acid medium [29], p-N,N-dimethyl phenylenediamine [30], hydroxylamine in the presence of ferric ions, chloranil in the alkaline solution and Au (III) and Hg (II) ions [31] reagents were used for determination of tetracycline.

Hence we report a new simple, sensitive, rapid and precise spectrophotometric method for determination of terbutaline sulphate in pure form and pharmaceutical formulations.

Experimental

Apparatus

Shimadzu UV-1650 PC UV-Visible spectrophotometer equipped with a 1.0-cm path length silica cell**,** Philips PW (9421) pH-meter with a combined glass electrode was used for pH measurements**.** All calculations in the computing process were performed in Microsoft Excel for Windows.

Reagents

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 100 $\mu g/mL^{-1}$ by dissolving 0.01 g of each in distilled water and making the volume up to 100 mL in volumetric flask. The solutions were kept in refrigerator. Working standard solutions, for calibration graph, were prepared by suitable dilution in 10 mL volumetric flasks.

The solution of *eosin Y* (4×10^{-3}M) was prepared by dissolving 1.3837 g in distilled water, mixing well and making the volume up to 500 mL in volumetric flask.

The acetate buffer solution was prepared with pH 3.2 and 3.5 by mixing sodium acetate and acetic acid solutions of 0.1 M and adjusted by pH meter.

General procedure

Into two series of 10mL volumetric flasks aliquots of solution containing 5-45 and 0.5-10 $\mu g/mL^{-1}$ terbutaline sulphate and tetracycline hydrochloride, respectively, were added separately. 0.5 ml of 4×10^{-3}M and 3.0 mL of 8×10^{-3}M eosin Y followed by addition 0.6 mL of pH 3.2 and 0.3 mL

of pH 3.5 acetate buffer solution to terbutaline and tetracycline respectively. The solutions were diluted to the mark with distilled water and the absorbance was measured at 545nm for terbutaline and tetracycline at room temperature respectively.

Procedure for pharmaceutical formulations
Terbutaline tablet

Twenty terbutaline tablets (each tablet contains 5 mg) 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, mixed well and filtered with Whatman filter paper No.1. The filtrate was diluted to the 100 mL with distilled water in a volumetric flask to obtain 50 $\mu g/mL^{-1}$. A suitable volume was diluted, and the general procedure was followed.

Tetracycline chloride capsule

Ten tetracycline hydrochloride capsules content (each capsule contains 250 mg) were accurately weighed and pulverized. A portion of the fine and homogenized powder equivalent to one capsule was accurately weighed and dissolved in distilled water, mixed well and filtered with Whatman filter paper No.1. The filtrate was diluted to the 250 ml with distilled water in a volumetric flask to obtain 1000 μgml^{-1}. A suitable volume was diluted, and the general procedure was followed.

Results and Discussion

Terbutaline and tetracycline react with eosin Y through an ion-pair salt formation, forming a reddish orange complex in acidic medium with λ_{max} at 545, (Fig. 1). These complexes were probably formed via electrostatic interaction between the most basic center in the drug molecule (amino group) and the carboxylate anion of the dye. This primarily occurs in an acidic solution, increasing the electron delocalization of eosin and producing a bathochromic shift of the dye about 30 nm [32].

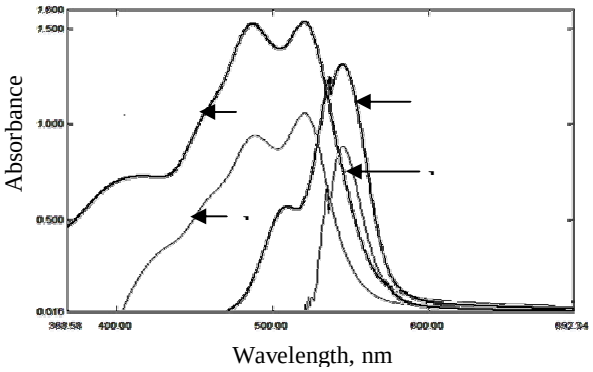


Figure 1. **Absorption spectra of 8$\mu g/mL^{-1}$ terbutaline sulphate and 40$\mu g/mL^{-1}$ tetracycline hydrochloride with eosin Y against their respective reagent blank (a', b') under optimum conditions**

Optimization of reaction conditions

The influence of different parameters on the color development was studied to determine optimum conditions for the assay procedure.

Effect of eosin Y concentration

Various concentrations of eosin Y were added to the studied drugs (1×10^{-4}-1×10^{-3} M), as shown in (Fig. 2), it was found that the ion-pair formation was optimized using 0.5 mL of 4×10^{-3} M and 3 ml of 8×10^{-4} M eosin Y for terbutaline sulphate and tetracycline HCl, respectively. These concentrations were used in subsequent experiments.

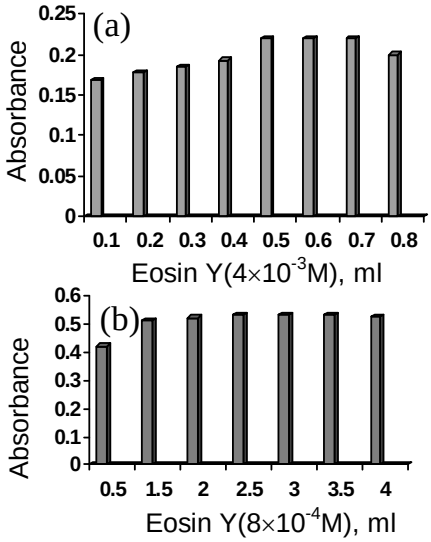


Figure 2. **Effect of eosin concentration on the absorption of: (a) 2 $\mu g/mL^{-1}$ terbutaline sulphate in the presence of 0.25mL of 2 M acetic acid. (b) 20 $\mu g/mL^{-1}$ tetracycline HCl in the presence of 0.5 ml of 2 M acetic acid**

Effect of acid, pH and buffer solution

As mentioned above, the complexes were formed in acidic medium, therefore different acids were tested to obtain high sensitivity. As shown in (Fig. 3), acetic acid gave highest absorbance value for both drugs. It was found that these complexes are formed at pH 3.2 and 3.5 in the presence of 0.5 and 0.3 ml of 2 M acetic acid for terbutaline and tetracycline, in final dilution, respectively. However; different buffers with above pHs were prepared and tested. Acetate buffer gave stable complexes and considered as the optimum for both drugs with amounts of 0.6 and 0.3 mL for terbutaline and tetracycline respectively, (Fig. 4).

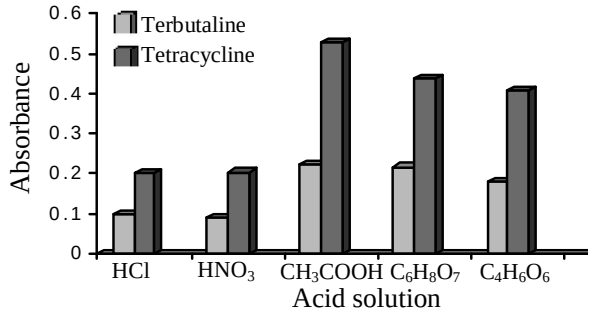


Figure 3. **Effect of 0.5 mL and 0.25 mL of 2 M acid on the absorption of 2 and 20 µg/mL^{-1} terbutaline and tetracycline respectively in the presence of 0.5 mL of 4×10^{-3} M and 3 mL of 8×10^{-3} M eosin Y respectively**

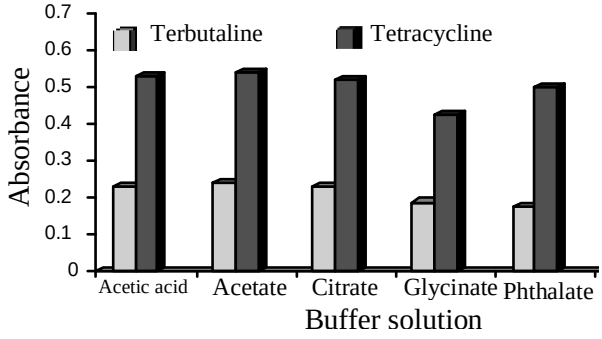


Figure 4. **Effect of buffer solutions on the absorption of ion pair terbutaline and tetracycline complexes with eosin Y**

Effect of temperature and developing time

The optimum reaction time was determined by following the color development at ambient temperature (26±1°C) and 40±1°C. It was found that both complexes are formed with maximum absorption immediately at room temperature and remain constant for more than 24 hr. However; the absorbance of both complexes were decreased gradually at 40°C, indicating the destruction of ion pair complexes, (Fig. 5). Then, room temperature was considered the optimum and used in all subsequent experiments.

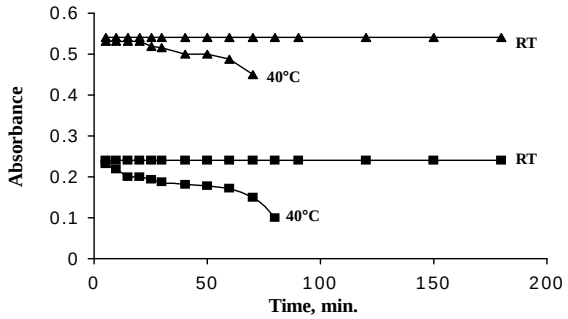


Figure 5. **Effect of temperature and developing time on the absorption of ion pair complexes for terbutaline (■) and tetracycline (▲) with eosin Y**

Effect of order of addition of reactants

The effect of variation of the order of addition of reactants on the absorption of both complexes was not observed.

Composition and stability constant of the ion-pair complexes

The composition of the ion-pair was studied by Job's of continuous variation and mole ratio methods [33] and was found to be 1:2 drug: eosin Y in both complexes, (Fig. 6).

The apparent stability constant was estimated by comparing the absorbance of a solution containing stoichiometric amounts of the drug and eosin Y (As) to one containing an excessive amount of eosin Y reagent (Am). The average conditional stability constant of the complexes were calculated, according to the 1:1 ratio, by the following equation:

$$Kc=1-\alpha/ 4\alpha^3 C^2$$

$$\alpha=Am-As/Am$$

where Kc is the stability constant ($l^2.mol^{-2}$), α the dissociation degree and C the concentration of the complex which is equal to the concentration of drug. However; the average of stability constant for three different concentration was found 4×10^9 and 1.2×10^{10} $l^2.\ mol^{-2}$ for terbutaline and tetracycline respectively indicating the good stabilities.

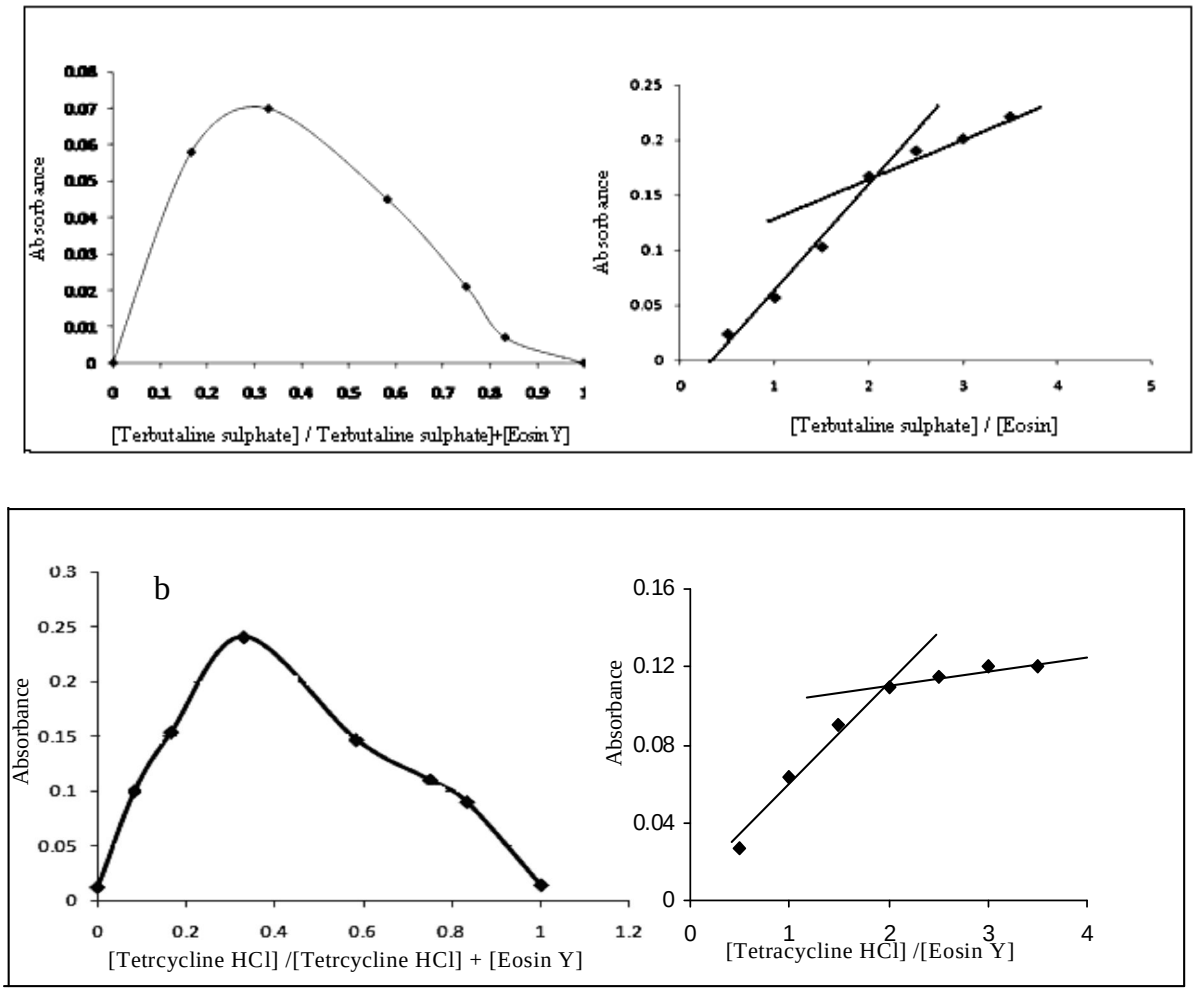
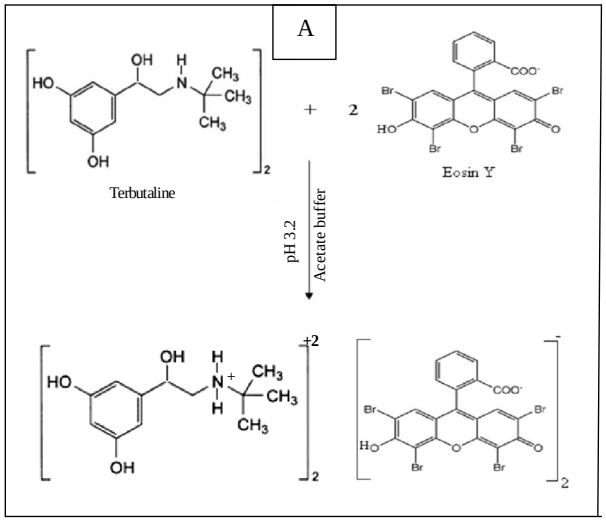


Figure 6. **Continuous variation and mole ratio plots for terbutaline-eosin Y complex of 1.822×10^{-4}M (a) and tetracycline-eosin Y complex of 2.07×10^{-4}M (b)**

Mechanism

The proposed method is based on a binary complex formation between the studied drugs and eosin Y. These complexes were probably formed via electrostatic interaction between the most basic center in the drug molecule (amino group) and the carboxylate anion of the dye. This primarily occurs in an acidic solution, increasing the electron delocalization of eosin Y and producing a bathochromic shift of the dye about 30 nm [32] (Fig. 1), Applying Job's and mole ratio methods (Fig. 6), it was found that, the reaction proceeds in the ratio of 1:2 of drug to eosin for both drugs, as seen in their chemical structures, they have two basic centers, the proposed mechanism of the reaction pathway is shown in (Fig. 7).



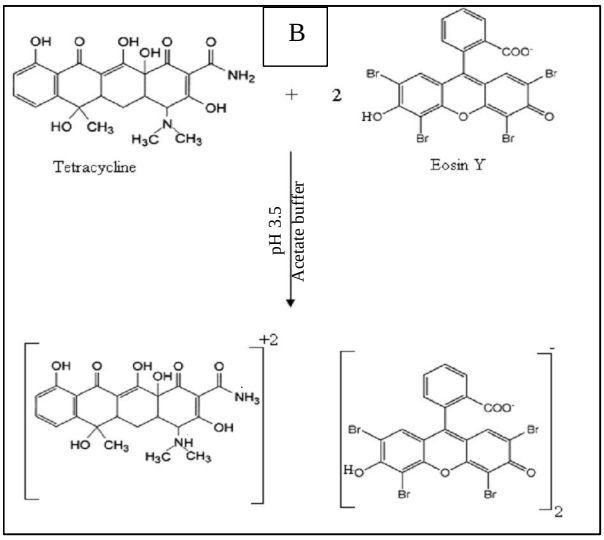


Figure 7. **Proposed mechanisms for the reaction between terbutaline sulphate (A) and tetracycline hydrochloride (B) with eosin Y**

Quantitation

Under the described experimental conditions, standard calibration curves for, terbutaline sulphate and tetracycline HCl with eosin Y were constructed by plotting absorbance against concentration (Fig. 8). The Beer's law limits and molar absorptivity values were evaluated and given in Table1, which indicated that the method is sensitive. The linearity was represented by the regression equation and the corresponding correlation coefficient for drugs determined by the proposed method represents excellent linearity. The relative standard deviation (RSD) and accuracy (average recovery %) for the analysis of five replicates of each three different concentrations for terbutaline sulphate and tetracycline HCl indicated that the method is precise and accurate. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to the following equations:

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

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 1).

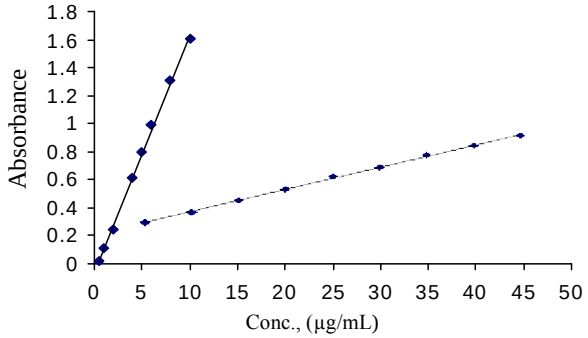


Figure 8. **Calibration graphs for determination of terbutaline sulphate (●) and tetracycline HCl (♦) drugs**

Table 1. **Summary of optical characteristics and statistical data for the proposed method.**

Parameter	Terbutaline	Tetracycline
λ_{max} (nm)	545	545
Linear range (µg/mL)	0.5-10	5-45
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	$3.169x10^3$	$6.347 x10^3$
LOD (µg/mL)	0.030	0.613
LOQ (µg/mL)	0.103	2.00
Average recovery (%)*	101.42	100.08
Correlation coefficient	0.9984	0.9988
Regression equation (*Y*)**		
Slope, *a*	0.1698	0.0134
Intercept, *b*	0.0623	0.2726
RSD*	≤ 0.72	≤ 0.19

* Average of five determinations
** $Y = a X + b$, where X is the concentration of terbutaline sulphate and tetracycline HCl in $\mu g/mL^1$.

Specificity

The specificity of the method was investigated by observing any interference encountered from the common excipients of the pharmaceutical formulations by measuring the absorbance of solutions containing 2 $\mu g/mL^{-1}$ terbutaline sulphate and 20 $\mu g/mL^{-1}$ tetracycline HCl separately, and various amounts of diverse species, up to 100 fold excess, in a final volume of 5 mL. It was found that the studied excipients did not interfere seriously (Table 2).

Table 2. **Effect of excipients for assay of terbutaline sulphate and tetracycline HCl.**

Excipient	Recovery %					
	2 µg ml^{-1} of terbutaline sulphate per fold excess foreign added			20 µg ml^{-1} of tetracycline HCl per fold excess foreign added		
	10	*50*	*100*	*10*	*50*	*100*
Glucose	98.50	98.01	98.00	99.20	98.56	97.64
Lactose	100.01	98.42	97.87	99.22	98.33	96.50
Acacia	99.70	98.21	95.01	97.30	95.63	94.22
Starch	97.31	95.09	93.21	96.33	95.06	95.11
NaCl	99.70	98.88	98.42	98.10	97.71	95.54
Urea	98.82	98.80	97.80	98.91	99.90	98.81
$MgCl_2$	97.55	95.50	94.94	98.01	96.00	96.53
Na_2SO_4	97.7	95.53	93.94	98.10	96.51	96.72

Method validation and applications

To evaluate the analytical applicability of the proposed method, it was successfully applied to determine terbutaline sulphate and tetracycline HCl in some pharmaceutical preparations. The obtained recovery % values cited in Table 3 indicated high accuracy and there is no serious interference in the determination of above drugs in such samples. However; standard addition procedure was applied for determination of terbutaline sulphate in tablets (Fig. 9). The results, cited in Table 3, indicated good recovery. The results obtained by the proposed method were compared with British Pharmacopoeia (BP) method (Table 3), by applying the F-test and the t-test at 95% confidence level. The calculated values for F and t tests for proposed method did not exceed the theoretical values. These confirming that there are no significant differences between the proposed method with BP method for terbutaline sulphate [33,34] and tetracycline HCl.

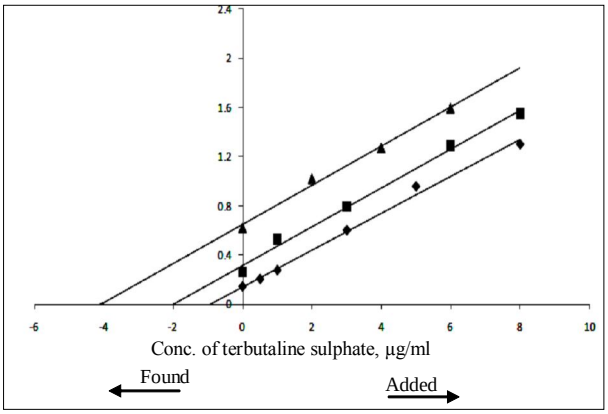


Figure 9. **Standard addition plots for the recovery of 1 (♦), 2 (■) and 4 (▲) µg/mL of terbutaline sulphate in the dosage forms**

Table 3. **Statistical analysis of results obtained by the proposed eosin method compared with the official BP and standard addition methods.**

Procedure applied	Pharmaceutical preparation	Drug amount present (µg/mL)	Recovery[a] (%)	Drug content found (mg)	Average recovery (mg)	Certified value (mg)
Proposed eosin method	Samabutaline[b] tablet	3	99.99	4.99	5.06 (0.50,7.16)[c]	5
		6	103.33	5.16		
		8	101.12	5.05		
	Samacycline[b] capsule	15	97.12	242.80	245.02 (1.05,1.02)	250
		25	98.33	245.82		
		35	98.59	246.45		
British Pharmacopoeia[e]	Bricanyl[d] tablet	15mg	99.21	14.88mg	-	15
	Samacycline capsule	250mg	97.92	244.80	-	250
Standard addition method	Samabutaline tablet	1	96.39	4.82	4.99	5
		2	101.20	5.06		
		4	101.80	5.09		

[a] Average of three determinations.
[b] provided from SDI Co. Iraq.
[c] Figures in parenthesis are the calculated values for *t*, and *F* respectively. Theoretical values for *t* and *F* at 95% confidence limit are 2.36 and 8.941, respectively.
[d] Astra Zeneca UK Limited.
[e] The results obtained by Ref. 34 for terbutaline.

Comparison of the proposed method with reported methods

The proposed method compared favorably with other reported spectrophotometric methods. As shown in Table (4), the present method is simpler than other methods as no need an extraction step, and have stability period but less sensitive than some methods.

Table 4. **Comparison of the proposed method with other spectrophotometric methods.**

Analytical parameters	**Tetracycline**	**Terbutaline**	**Tetracycline**			**Terbutaline**		
Reagent	Eosin Y		Au (III)	Hg (II)	DMPD[a]	PI-NCl[b]	p-Aminophenol	L-amino antipyrine
λ_{max} (nm)	545		425	320	625	525	590	550
pH	3.5	3.2	4	2-4	7.0	8.0	Alkali (0.1 N NaOH)	9.5
Solvent	Water		ethyl acetate		Water & n-butanol	50% Ethanol	Water	Water
Temp. (°C)	RT[c]		75		RT	40	RT	RT
Development time (min)	Immediately		15		30	20	Immediately	Immediately
Stability period (min)	>24 hrs		-		120	50	5	3
Beer's law (µg/mL)	5-45	0.5-10	5–70		5-50	0.7-270	20-160	4-20
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	6347	3169	3482	6965	6120	73800	14600	11905
Recovery (%)	98.01	101.48	100.78	100.95	98.50 - 98.90	99.04	100.97	99.87
RSD (%)	≤ 0.19	≤ 0.72	0.961		≤2.20	≤ 0.62	1.23	0.937
Applications	Capsule	Tablet	Capsule		Tablet, Capsule	Tablet, injection	Tablet, syrup	Tablet
Disadvantages	Less sensitive than some cited methods		The methods need extraction, heating and complexes have no stability period		Tedious and need extraction	Using of mixed solvent, sensitivity and stability affected by pH	No stability for the product and measurements must be carried within 5 min.	No stability for the product, measurements must be carried within 3 min and dilution with buffer
Reference	Present method		[31]		[30]	[27]	[25]	[22]

[a] DMPD= p-N,N-dimethyl phenylenediamine
[b] PI-NCl= phenanthro[9,10-d]imidazole-2-N-chloroimide
[c] RT= Room temperature

Conclusion

A simple, sensitive, fast, accurate and precise spectrophotometric method was developed for the determination of terbutaline sulphate and tetracycline HCl in some of their pharmaceutical formulations. The statistical parameters and the recovery test data indicated the high reproducibility and accuracy of the proposed method. Analysis of authentic samples containing the studied drugs showed no interference from common additives and auxiliary substances in general. The advantage of the method being less time consuming and do not require various elaborate treatments and tedious extraction procedures. In addition to the satisfactory sensitivity and reproducibility method is convenient and simple as well.

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