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Original Article

Spectorphotometric Methods for Determination of Terbutaline Sulphate in Pure and Pharamaceutical Formulation

Intisar Adil Shihab Al-Hammoodi[1]*, Mohammed Salim Al-Enizz[1], Abdussamed M. A. Saeed[2]

[1]*Department of Chemistry, Colleague of Education for Girls, University of Mosul, Iraq*
[2]*Section of Basic Sciences, College of Agriculture and Forestry, University of Mosul, Iraq*

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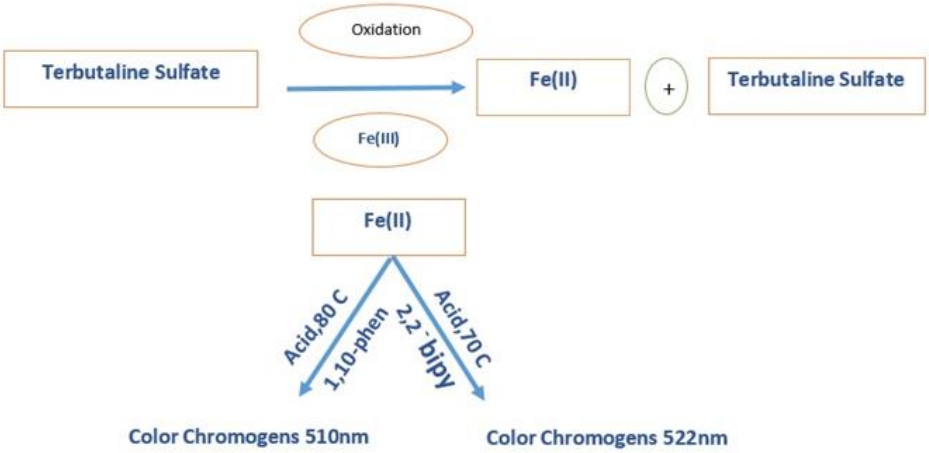
ABSTRACT

Two simple, sensitive, and accurate spectrophotometric methods are described to determine terbutaline sulfate in pure and pharmaceutical formulations. They are primarily based totally on the oxidation of Terbutaline sulfate with Fe(III) in the medium of nitric acid and the resulting Fe(II) subsequent chelation with 1,10-phenanthroline (1,10-Phen) in method A and with 2,2´-bipyridyl (2,2'-bipy) in method B. The resulting color chromogens were measured at 510 and 522 nm in the above methods, respectively. The two methods A and B were obeyed Beer's law in the ranges of 0.1 - 2.0 and 0.1 - 1.2 μg mL^{-1} with molar absorptivity values of 2.44×10^5 and 3.27×10^5 L mol^{-1}. cm^{-1}, respectively. The relative standard deviation (RSD) values were found ≤5.53% and ≤ 3.15%, respectively. The reaction mechanisms have been established. The suggested methods were applied successfully for the assay of Terbutaline sulfate in its commercial formulations as syrup and tablet and the obtained results were compared statistically with the official method.

GRAPHICAL ABSTRACT

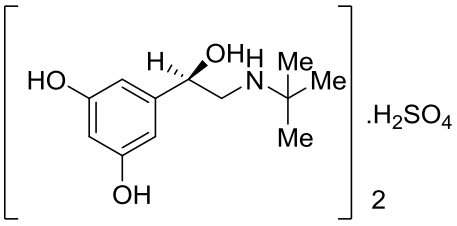


* Corresponding author: Intisar Adil Shihab Al-Hammoodi
✉ E-mail: Email: entesar@uomosul.edu.iq

Introduction

Terbutaline sulphate (±)-α-[(tert-butylamino)methyl]-3,5-dihydroxybenzyl alcohol sulfate (I) (Scheme 1) is a beta-adrenergic radioactive substance that can be taken orally [1]. It was used in bronchitis, breathing problems, sinusitis, obstructive airway diseases, uncomplicated preterm labor, and other conditions [2]. In addition to what has been mentioned, it is a muscle relaxation that opens the air passage in the lungs to facilitate [3, 4].

To determine terbutaline sulfate, various analytical techniques have been reported such as HPLC [5-8], LC–MS, CE [9], CE–MS [10], chemiluminscense [11-13], and voltammetry [14, 15]. These techniques are cumbersome, time-consuming, and expensive. The spectrophotometry has been used for its low cost, simplicity, and versatility. Different spectrophotometric methods using various reagents are described for assay of Terbutaline sulfate, such as. 4-amino antipyrine [16] and iron [17] in the presence of potassium ferricyanide, eosin y [18], sodium borate, and treatment acetylacetone [19], p-aminophenol in basic medium of sodium hydroxide [20], 3-methyl-2-Benzothiozolone hydrochloride hydrochloride in the presence of ferric chloride [20], and p-chloranilic acid [21]. This paper describes sensitive, fast, and simple methods to determine terbutaline sulfate based on the redox reactions.



(I)

Scheme 1: Terbutaline sulphate (±)-α-[(tert-butylamino)methyl]-3,5-dihydroxybenzyl alcohol sulfate (I)

Materials and Methods

Apparatus

Perkin-Elmer and Lambda 25 double-beam UV-visible and Genway 6300 were used as a single-beam UV-visible spectrometer, with identical 1 cm silica cells. The PGE453e type sensor scale with four digital weighing numbers was used. With a Cyber Scan 510 computer, pH measurements were performed using PH-meter with built-in glass electrode. The solutions are heated in a water bath 1003 Germany.

Reagents

1,10-Phenanthroline solution (0.025 M) was prepared by dissolving of 0.450 g of 1,10-phenanthroline with distilled water and completing the volume to 100 mL in a calibrated flask. This solution was prepared daily and used immediately.

2,2'-bipyridyl solution (0.025 M) was prepared by dissolving 0.390 g of 2,2'-bipyridyl with distilled water and completing the volume to 100 mL in a calibrated flask. This solution was prepared daily and used immediately.

Standard solution of terbutaline sulfate (100 µg mL^{-1}) was prepared by dissolving 0.01 g of pure terbutaline sulfate, which is supplied by Samara Pharmaceutical Industries (SDI), with distilled water and the volume was completed to 100 mL in a calibrated flask with distilled water and keeping in a refrigerator, and then, the solution was diluted as needed.

Fe(III) solution (0.025M) was prepared by dissolving 1.1945 g of ferric ammonium sulfate ($Fe(SO_4)_2NH_4$) in a mixture of 5 mL of distilled water and 5 mL of 0.05 M HNO_3, and then and diluted to 100 mL with distilled water in a calibrated flask.

Surfactant solutions, 0.1%: 0.1 g of various surfactants (neutral, positive, and negative) dissolved in 100 mL of ethanol.

Nitric acid solution (0.1 M) was prepared by diluting the concentrated solution with distilled water. The chemicals used are of the highest purity supplied by Lab Pak Chemicals (LTD), UNI-Chem, and 1,10-phenanthroline from Fluka Co.

General procedure

In two series of 25 mL volumetric flasks, 0.1-2.0 and 0.1-1.2 µg mL^{-1} terbutaline sulfate were

added separately followed by addition of 0.3 mL of $Fe(SO_4)$ $2NH_4$ and 2.4 mL of 1,10-Phenanthroline solutions in method A, 0.2 mL of $Fe(SO_4)$ $2NH_4$, 2.8 mL of 2,2'-bipyridyl solutions were added and followed by the addition of 0.4 mL of HNO_3 in method B. The volumes were completed with distilled water to the mark, then mixed and placed in a water bath its temperature is set at 80 °C for 40 min in method A and at 70 °C for 80 min in method B, and then cooling and the absorbance of the complexes was measured at 510 nm and at 522 nm against corresponding reagent blank for the two methods, respectively.

Analysis of dosage forms

Tablets

10 tablets were carefully powdered (each including 5 mg Terbutaline sulfate). The quantity of powder equivalent to one tablet was precisely transferred into a 50 mL beaker and dissolved in 20 mL pure water. After that, the solution was filtered through Whatman filter paper no.41. The filtrate was completed to 100 mL by pure water in a calibrated flask to obtain 50 µg mL^{-1} of Terbutaline sulfate in the final dilution. The analysis was performed due to the general procedure.

Syrup

A bottle containing 20 mL of syrup (30 mg/100 mL terbutaline sulfate) was diluted to 100 mL with distilled water in a calibrated flask to get 60 µg mL^{-1} Terbutaline sulfate. Then, the stock solution was diluted and followed the general procedure.

Results and Discussion

Absorption spectra

In general, in the spectroscopic determination of many drugs, Iron salts play an important role [22-24], as they act as an oxidizing agent, the ferric ion is reduced to an amount of ferrous ion corresponding to the drug concentration. The amount of resulting Fe(II) can be estimated by complexation with 1,10-Phenanthroline in method A and 2,2'-bipyridyl. in method B. However, methods A and B are depended on the oxidation of Terbutaline sulfate drug with Fe(III) in an acidic medium and Fe(II) librated. Fe(II) interacts with 1,10-phen. to produce a red colored compound of tris-1,10-phen-Fe(II) chelate (ferroin) with maximum absorbance at 510 nm in method A, and interacts with 2,2'-bipyridyl. to produce a red-colored compound of tris-2,2'-bipy-iron(II) chelate $[Fe(bipy)_3]^{+2}$, with maximum absorbance at 522 nm in method B (Figure 1).

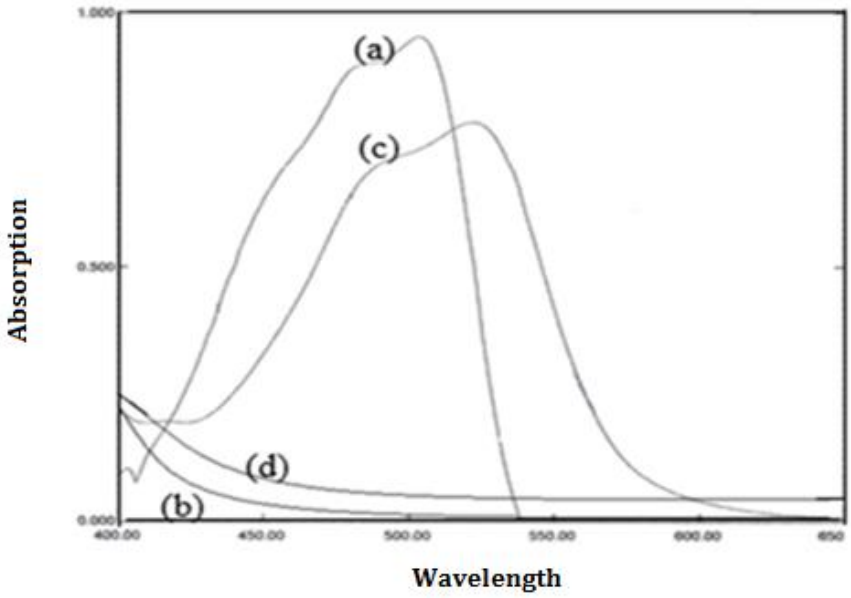


Figure 1: Absorption spectra of a) Fe(III)-o-phen with terbutaline sulphate (2 µg. mL^{-1}) against, b) reagent blank, c) Fe(III)- 2,2'-bipyridyl with terbutaline sulphate (2 µg. mL^{-1}) against, d) reagent blank

Optimization of reaction conditions

Several variables that affect the absorption intensity of the complexes formed, in both methods, have been studied to reach the best conditions for the estimation of Terbutaline sulfate.

Effect of temperature and reaction time

The time required for the reaction was determined by following the formation of the complexes color at different temperatures for both methods A and B. The absorbance was measured at 5- and 10-min intervals against the similarly treated blank reagent. It was found that these colored complexes form slowly at laboratory temperature and demanded a longer time for completion. The reaction was accelerated by performing the reaction at greater temperature levels. It was observed that the maximum absorption was obtained when the reaction mixture heated at 80 °C for 40 min and remained constant for 20 minutes in method A and at 70 °C for 80 min and remained constant for 80 min in method B (Figure 2). The complexes were found steady after being cooled at room temperature for more than 24 hours.

Effect of acid, pH, and buffer solution

The effect of acid on the color intensity was studied by examining 0.1 M of different acids including HNO_3, HCl, H_2SO_4, and CH_3COOH. The results indicate that the complexes in both methods A and B are affected by the addition of nitric acid, and as for the uptake it is high, as shown in Figure 3.

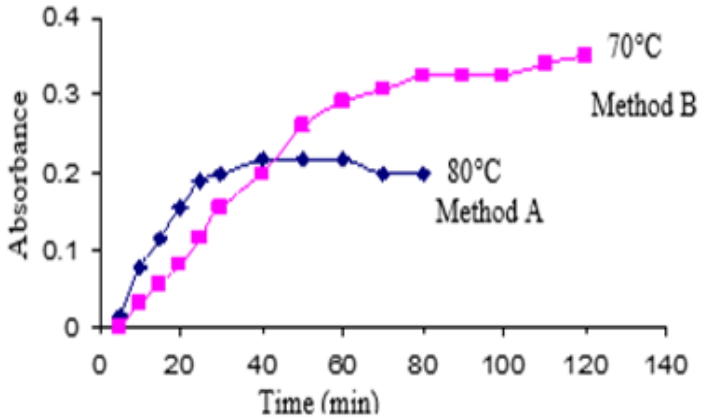


Figure 2: Effect of the time and temperature on the absorbance of 1 µg. mL^{-1} terbutaline sulphate in methods A and B

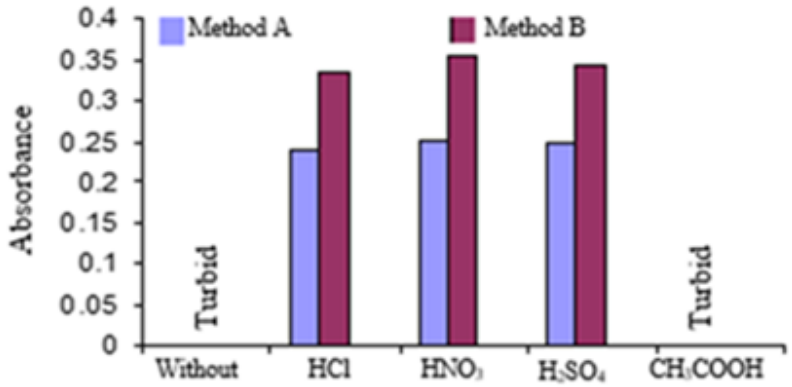


Figure 3: Effect of acid on the absorbance of complexes in the presence of 1 µg. mL^{-1} terbutaline sulphate

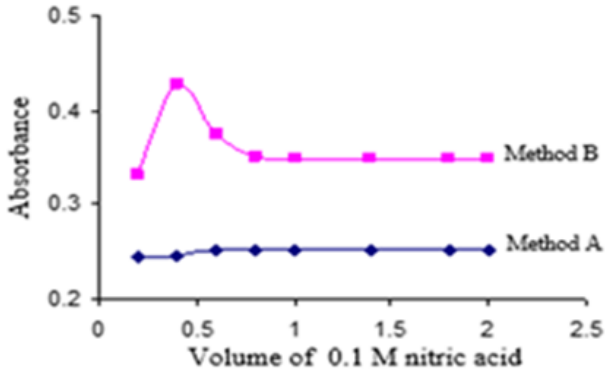


Figure 4: Effect of nitric acid volume of 0.1 M concentration on the absorbance of complex in the presence of 1 µg. mL^{-1} terbutaline sulphate

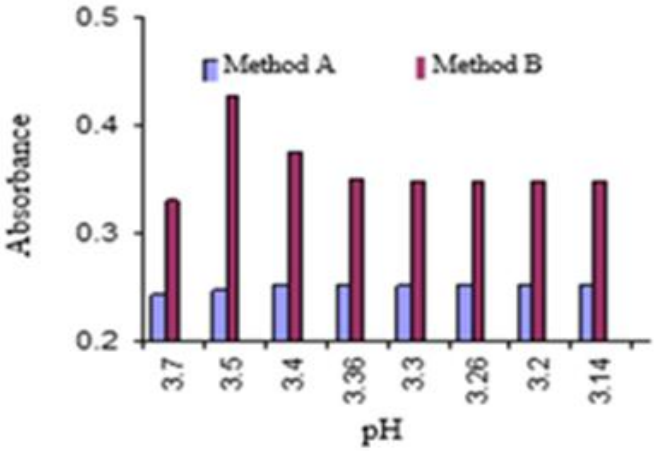


Figure 5: Effect of pH on the absorbance of complexes in method A and B

The effect of pH in the presence of different concentrations of nitric acid on the absorption of the complexes has been studied. The results indicated that 0.6-2.0 mL and 0.4 mL at pH 3.43-3.14 and 3.51 gave it maximum absorption in method A and B. respectively (Figure 4 and 5). The effect of buffer solution is investigated via testing different types of the regular function of pH 3.4 and 3.5 for method A and B, respectively. However, the solution became turbid.

Effect of ferric ammonium sulphate

The effect of 1 mL of different concentrations of ferric ammonium sulfate solution, while keeping a fixed concentration of Terbutaline sulfate and 1,10-Phenanthroline or 2,2'-bipyridyl were investigated on the absorption of the compounds in both methods A and B. As depicted in Figure 6, it was found that 0.3 and 0.2 mL of 0.025 M concentration of ferric ammonium sulfate gave the maximum absorption as the solution became cloudy for both methods, respectively. Therefore, these quantities were approved as ideal.

Effect of 1,10-Phenanthroline and 2,2'-bipyridyl reagents concentration

The effect of 1,10-Phenanthroline and 2,2'-bipyridyl concentrations on the absorbance of the complexes in both methods A and B, respectively, were investigated. It was found that 2.4-3.0 mL and 2.6-3.0 mL of 0.025 M of 1,10-Phenanthroline and 2,2'-bipyridyl gives, respectively, the maximum absorption. Above the mentioned concentrations, there is a decrease in the absorbance, as demonstrated in Figure 7.

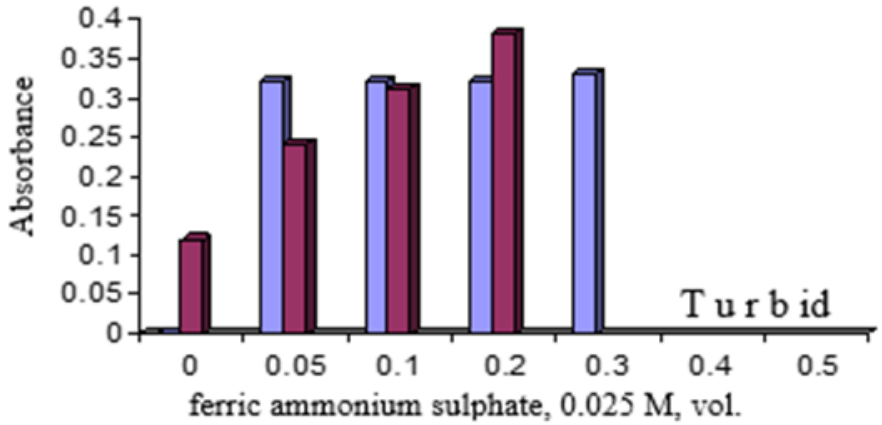


Figure 6: Effect of ferric ammonium sulphate on the absorbance of complexes in method A and B in the presence of 1 µg. mL^{-1} terbutaline sulphate

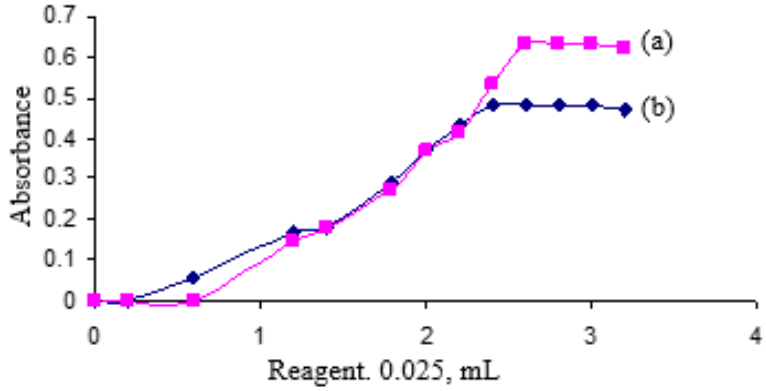


Figure 7: Effect of reagent concentration of a) 2,2'-bipyridyl and b) 1,10-phen in the presence of terbutaline sulphate (1 µg. mL^{-1})

Effect of solvent dilution

The dilution effect with various types of solvents (water, methanol, ethanol, and acetonitrile) are used for the final dilution to obtain the maximum sensitivity to the compound and it has been observed that using distilled water for dilution gives the maximum absorption (Figure 8).

Effect of surfactant

Surfactant effect different surfactants (positive, negative, and neutral) affect the color intensity. The results revealed a negative effect on the absorption of the complexes in both methods A and B.

Quantification

By plotting the absorptions against the concentrations, the standard curves displayed in Figure 8 were obtained, which indicate that the method follows Beer's law within the ranges reported in Table 1 for Terbutaline, which shows the possibility of estimating infinitesimal quantities, and that there is a deviation negatively about Beer's law after discretionary upper limit. The molar absorptivity, detection limit, quantitative limit, recovery %, and relative standard deviation (RSD) values indicating good sensitivity, and reproducibility. The values of the square correlation coefficient statistically, which are greater than 0.99, listed in Table 1, indicate that the standard curves have the excellent linear characteristics.

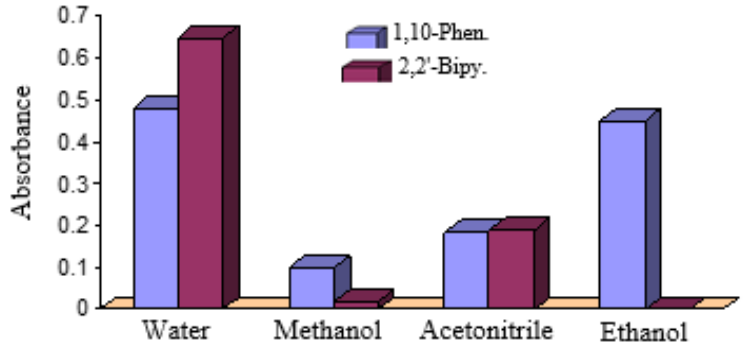


Figure 8: Effect of solvent on the absorption of the complexes in the presence of 1 µg. mL^{-1} terbutaline sulphate

Table 1: Analytical information of the suggested methods

Parameter	Values of	
	Method A	Method B
Linearity range (µg/mL)	0.1-2.0	0.1- 1.4
Molar absorptivity (L.mol^{-1} cm^{-1})	2.446×10^5	3.313×10^5
Detection limit (µg mL^{-1})	0.019	0.307
Quantitative limit (µg mL^{-1})	0.058	0.933
Average recovery %	97.77	97.77
RSD**	≤5.53	≤3.15
Correlation coefficient	0.9967	0.9901
Regression equation (*Y*)*		
Slope, *a*	0.446	0.604
Intercept, *b*	0.0356	0.0539

**Y = aC + b, where C is the concentration of Terbutaline sulfate in µg mL^{-1}.*
*** Average of six determinations.*

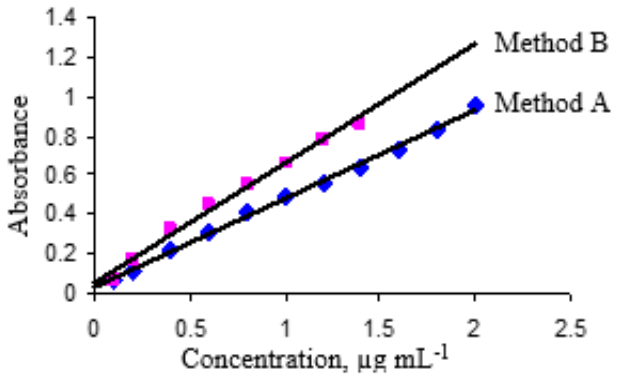


Figure 9: Calibration graphs for the determination of terbutaline sulphate

Validity of the method

The proposed methods were applied successfully for the determination of terbutaline sulfate in its dosage forms as tablet and syrup. As presented in Table 2, the results were in agreement with the certified values indicating good recovery. To prove the efficiency of the developed methods, the standard addition procedure had been applied to the pharmaceutical preparations for Terbutaline sulfate as tablet and syrup. The method was summarized by adding increasing amounts of the pure drug standard solution, in the range of Beer's law, to the known amount of the pharmaceutical preparation. By following the general procedure, the absorption was measured at the wavelength of 510 nm and 522 nm for methods A and B, respectively, and the obtained results were included in Figure 9 and Table 2, which indicate that the method has a good selectivity.

To know the reliability of the developed methods, both methods were compared statistically by a Student's t-test for accuracy and a variance ratio F-test for precision with the official method [26] at the 95% confidence level with six degrees of freedom. It was found from the results presented in Table 2, that the experimental t and F values are less than the tabular values (t=2.45 and F=6.39) at the 95% confidence level and for six degrees of freedom, indicating there was not much difference between the proposed methods and the official method.

Stoichiometry and mechanism

One of the most commonly used methods were that of continuous variation presented by Job's and molar ratio methods [25]. In the proposed methods, the stoichiometric ratio for the oxidation of Terbutaline sulfate by Fe(III) was investigated applying the above methods using identical molar concentrations (2×10^{-3} M) of the drug and Fe(III) in methods A and B. The results indicated that the product was formed in the ratio of 1:4 for Terbutaline sulfate: Fe(III) in both methods. This indicates that number of moles of ferric ion depend on the number of aromatic hydroxyl groups present in the drug structure. Accordingly, the proposed mechanisms can be explained in Scheme 2.

Table 2: Assay of Terbutaline sulfate in pharmaceutical preparations using the proposed method, standard addition procedure, and comparison with the official method

Applied Procedure	Dosage form	Drug taken (μg/mL)	Recovery[b] (%)	Drug found (mg)	Average Drug found[c] (mg)	Certified value (mg)
1,10-Phen.	Tablet[a]	0.5	92.415	4.620	4.686 (1.31, 0.25)	5 mg
		1.0	91.246	4.562		
		1.5	97.512	4.875		
	Syrup[a]	0.5	98.418	29.525	29.029 (1.02, 0.21)	30 mg/100 mL
		1.0	94.825	28.447		
		1.5	97.048	29.114		
2,2'-Bipy.	Tablet	4.780	95.601	0.5	4.753 (1.56, 0.29)	5 mg
		4.688	93.769	0.7		
		4.791	95.833	1.0		
	Syrup	29.043	96.811	0.5	27.998 (1.31, 0.33)	30 mg/100 mL
		27.618	92.063	0.7		
		27.332	91.107	1.0		
Standard addition procedure	Tablet	0.5	99.6	4.98	4.875	5 mg
		0.8	101.25	5.062		
		1.2	91.66	4.583		
	Syrup	0.5	99.8	29.94	29.021	30 mg/100 mL
		0.8	98.75	29.625		
		1.2	91.66	27.498		
British Pharmacopoeia	Pure form	0.4 g	99.5	-	0.398 g	0.4 g

[a] *Manufactured by Mediotic Labs, Homs-Syria.*
[b] *Average of five determinations.*
[c] *Values in parenthesis are the experimental values for t, and F, respectively*

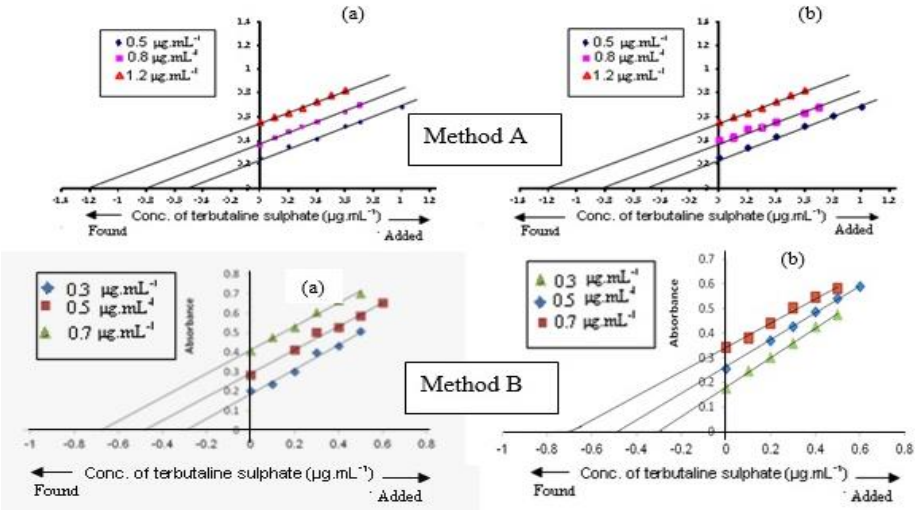
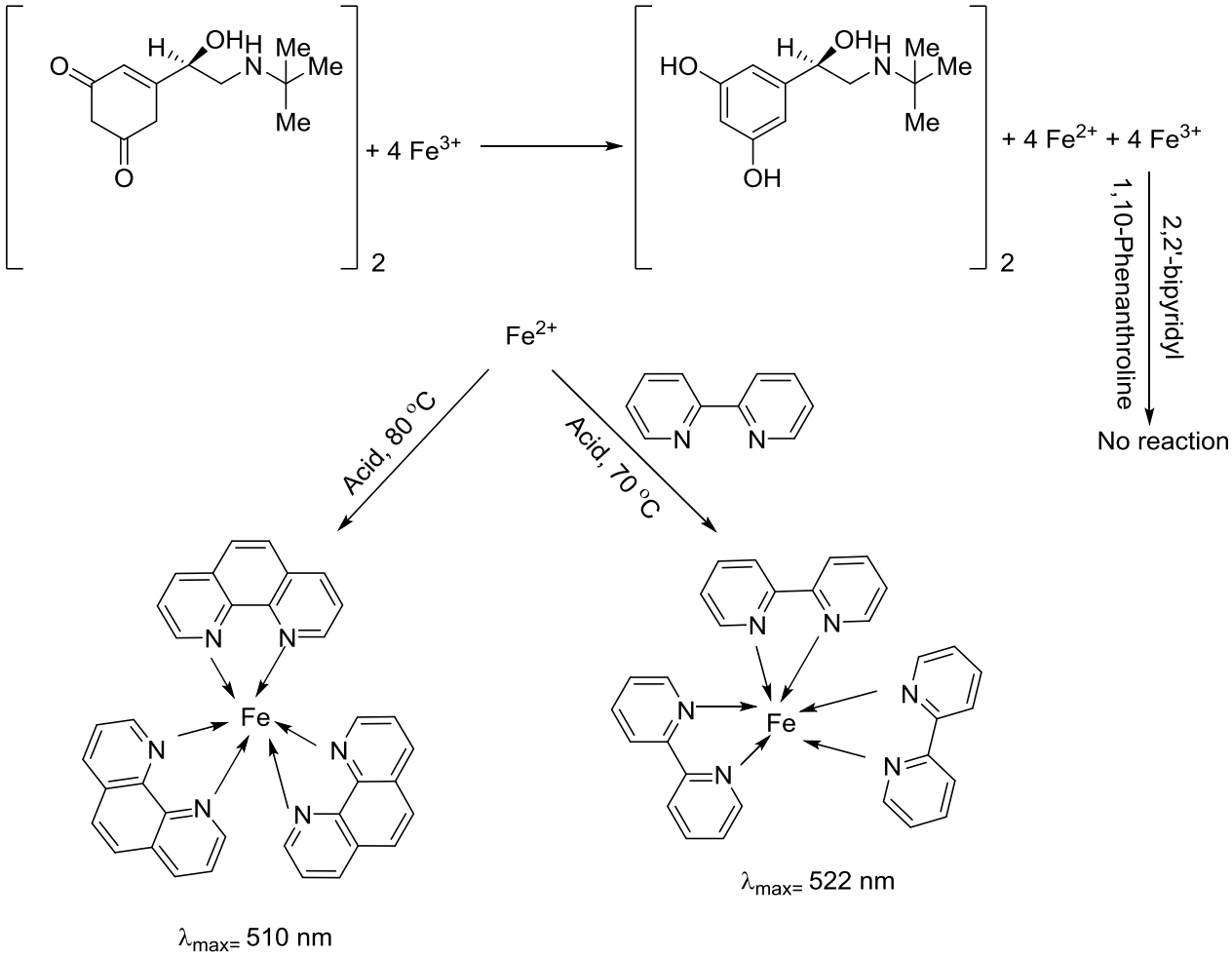


Figure 10: Standard addition plots of terbutaline sulphate as tablet a) and syrup b) in methods A and B

Comparison of the present methods with some literature spectrophotometric methods

Table 3 presents the comparison among a number of the analytical variables for the proposed methods with that of different literature spectrophotometric methods. As listed in Table 3, the sensitivity of the proposed methods is superior to those reagents in the other reported methods that were carried in an aqueous medium. Heating was used also to prove the sensitivity.



Scheme 2: Probable mechanism of the redox reactions of terbutaline sulphate with ferric ion and complexation with 1,10-Phenanthroline and 2,2'-bipyridyl

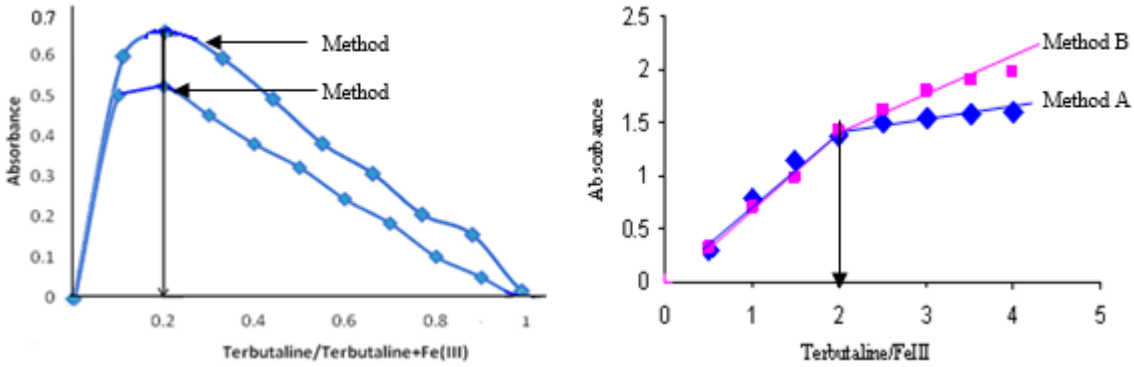


Figure 11: The stoichiometry reaction between terbutaline and Fe(III) by a) Job's and b) mole ratio methods

Table 3: Comparison of the proposed methods with other spectrophotometric method in the determination of Terbutaline sulfate

Analytical parameters	Present methods		Literature method			
	1,10-Phen.	2,2'-Bipy.	p-Chloranilic acid		Eosin Y	Antipyrine
λ_{max} (nm)	510	522	319	529	545	550
pH	3.43-3.14	3.51	2.65	2.65	3.2	9.5
Solvent	Water	Water	Ethanol	Ethanol	Water	Water
Temp. (°C)	70	80	25	25	R.T	R.T
Development time (min)	40	80	15	15	Immediately	3
Stability period (min)	20	20	60	60	>24 hrs	No stability
Linearity ($\mu g.mL^{-1}$)	0 – 2	0 – 1.2	0-70	0- 70	0.5-10	4 - 20
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	2.615×10^5	3.36×10^5	4.05×10^2	3.041×10^3	$3.169\ 10^3$	4.05×10^2
Recovery (%)	97.7	97.7	96.2	95.0	101.4 %	101.6
RSD (%)	≤ 5.53	≤ 3.15	≤ 3.51	≤ 0.69	≤ 0.72	0.93
Type of reaction	Oxidation-Reduction		Charge transfer		Ion pair	Oxidative coupling
Application	Asmanol tablet Asmanol syrup		Asmanol tablet		Terbutaline sulfate tablet	Terbutaline sulfate tablet

Conclusion

It can be concluded that the suggested methods are sensitive, accurate, and precise. Method A (used 1,10-Phenanthroline) was found to be more sensitive compared with Method B (used 2,2'-bipyridyl) for the Terbutaline sulfate assay. By analyzing the dosage form of Terbutaline sulfate (tablet and syrup); the correctness of the proposed methods had been well-demonstrated. Moreover, the methods are free from interference by the common additives and excipient.

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Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

The author declared that they have no conflict of interest.

ORCID:

Intisar Adil Shihab Al-Hammoodi
https://www.orcid.org/0000-0002-8415-3113
Mohammed salim Al-Enizzi
https://www.orcid.org/0000-0002-5910-2322
Abdussamed M. A. Saeed
https://www.orcid.org/0000-0002-8064-7458

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