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Spectrophotometric Determination of Salbutamol and Terbutaline using 9-Chloroacridine Reagent

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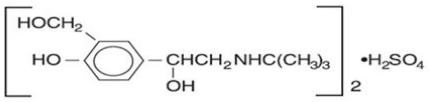
Abstract

A simple and sensitive spectrophotometric method has been described for the assay of Salbutamol sulphate (SBS) and Terbutaline sulphate (TERS) in their pure and dosage forms. The method was based on the reaction of the nucleophilic compensation by interacting of drugs with the reagent 9-chloroacridine (*9-CA*) in basic medium. The products have absorption bands with maximum absorbance at 600 nm and 588 nm for SBS and TERS respectively. Beer's law was obeyed in the range 0.5-12.0 µg.ml^{-1} and 0.1-6.0 µg.ml^{-1} with molar absorptivity values 1.913×10^4 and 1.0755×10^5 l.mol^{-1}.cm^{-1}, recoveries are 101.3% and 99.6% for above drugs respectively. The relative standard deviation (RSD) is less than 1.7% for both drugs. The probable reactions have been suggested.

Keywords: Spectrophotometry; Salbutamol sulphate; Terbutaline sulphate; 9-chloroacridine

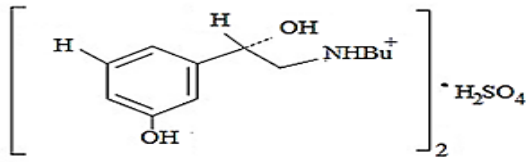
1. Introduction

SBS, also known albuterol chemically name is bis [(1RS)-2-[(1,1-dimethyl ethyl) amino]-1-[4-hydroxy-3-(hydroxyl methyl) phenyl] ethanol] sulphate [1], and have the following structure:



Mwt=576.702 g/mol

SBS is acted as its cardiovascular and bronchodilator [2]. SBS is prevalently used by athletes, anti-doping agent [3]. The drug is additionally employed in tocology for the hindrance of premature labor and as a decongestant [4]. High doses of SBS may have a lipolytic effect and residues of this compound, which are most abundant in liver and meat, can be toxic to humans [5]. TERS chemically name is 2-t-Butylamino-1-(3,5-dihydroxyphenyl)ethanol sulphate [6], and have the following structure:



M.wt = 548.6 g/mol

TERS is an orally active adrenergic β2 receptor agonist [7] and effective in bronco dilator [8]. It helps in treating wheezing and shortness of breath from lung problems, such as chronic obstructive pulmonary disease, bronchitis, asthma and emphysema. It has little effect on β1 receptors, thus direct cardiovascular stimulation occurs [9,10].

Several analytical methods are developed for quantitative estimation of SBS and TERS in pharmaceutical dosage forms. Among the analytical reported methods for SBS is the standard method of SBS determination is a potentiometric titration in a non-aqueous medium [11], and other analytical techniques are reported in the literature for the estimation of SBS such as (HPLC) [13,14], fluorescence [15] and voltammetry [16]. TERS has been determined by a range of methods, such as: HPTLC [17], flourimetry [18], HPLC [19,20], capillary electrophoresis[21], CE-MS [22] and voltammetry [23]. However, most of these methods need more complicated procedures, costly instrumentation.

Spectrophotometric techniques due to their accurate, precise, reproducible procedure and low cost are employed as the favorable method in most internal control laboratories, these methods are wide used

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techniques in pharmaceutical analysis.

The most wide used technique for the assay of SBS in pharmaceuticals is visible spectrophotometry; procedures based on such varied reactions as oxidation-reduction [24], oxidative coupling [25], diazotization and coupling [26,27], oxidation [28] and charge-transfer complex formation reaction [29] are described in the literature.

Various spectrophotometric methods using different reagents were reported for the determination of TERS, such as p-chloranilic acid [30], Eosin Y [31], cerium (IV) [32], Antipyrine reagent in the presence of ferric cyanide [33], sodium periodate in the presence of acetyl acetone [34] and 7,7,8,8-tetracyanoquinodimethane [35].

The benefit of this work lies in the development of a non-extractive, simple, sensitive and selective spectrophotometric determination of SBS and TERS using 9-chloroacridine reagent without interference with pharmaceutical additives and giving a stable product

2. Experimental part

2.1. Apparatus

Jenway 6800 uv-visible double beam spectrophotometer supplied with a 1cm silica cells, Jenway 3505 pH-meter equipped with a combined glass electrode was used for pH measurements. Heating of solutions is carried out on a water bath of frost instruments.

2.2.Reagents

All chemicals used are of the highest purity available which are provided by BDH, Fluka, and Molekula companies. Stock solutions of TERS and SBS drugs were provided from Sammara drug industries (SDI) and prepared in a concentration of (100 µg.mL^{-1}) by dissolving 0.01 g of each in double-distilled water and making the volume up to 100 mL in a volumetric flask. The solutions were stored in an amber coloured bottle in the refrigerator. The working standard solutions, for the calibration graph, are prepared by suitable dilution in 10 mL volumetric flasks. Sodium hydroxide (0.5M) and sodium carbonate (5×10^{-3}M) solutions are prepared by dissolving an appropriate weight in distilled water.

9-CA reagent (2×10-3 M) solution is prepared by dissolving 0.0107 g in small amount of methanol, to increase solubility, and diluted to the mark in a 25 ml-volumetric flask with double distilled water or with methanol for the determination of SBS or TERS respectively. The solutions are freshly prepared and used immediately.

2.3. Pharmaceutical preparations

Butadin syrup (containing 2 mg SBS per 5ml), this solution was prepared by diluting 5ml of Butadin syrup to 20 ml with double distilled water in a volumetric flask to obtain 100 µg.ml^{-1}.

Allied Salbutamol (containing 5mg SBS per 1ml), this solution was prepared by diluting 1ml of SBS vent to 50 ml with double distilled water in a volumetric flask to obtain 100 µg.ml^{-1}.

Butalin tablets (each tablet containing 2 mg of SBS). Ten tablets were weighed and finely powdered. An quantity of powder equal to one tablet was accurately weighed and transferred into a 25 ml beaker. The powder was utterly dissolved in double distilled water, and the solution was filtered through a Whatmann filter paper No.1. The filtrate was made up to 20 ml with double distilled water in a calibrated flask to get 100 µg.ml^{-1}.

Bricanyl tablets (each tablet contains 2.5 mg TERS). Six TERS tablets were weighed and small-grained. A portion of the fine and homogenized powder equivalent to one tablet was accurately weighed and dissolved in methanol, mixed well and filtered with Whattmann paper No.1. The filtrate was diluted to the 25 mL with methanol in a volumetric flask to get 100 µg.ml^{-1}. style on the drop-down menus.

2.4. General procedure

Aliquots of pure drugs solutions of SBS and TERS were transferred into two series of 10 ml calibrated flask separately. To the first series containing SBS, 0.8 ml of Na2CO3 (0.005M) was added followed by addition of 1ml of *9-CA* (1x10-3M) solutions and diluted to the mark with double distilled water. The second series containing 0.8 ml of NaOH (0.5M) followed by addition of 1ml of *9-CA* (2x10-3M) solutions then TERS was added and diluted to the mark with methanol. The absorbance was measured at 600 nm and 588 nm against reagent blank after 40 min and 10 min at room temperature respectively.

3. Results and discussion

It was found that the reagent *9-CA* interacts with both SBS and TERS in the basic medium to form colored products having maximum absorption at the wavelengths of 600 and 588 nm respectively, (Fig. 1). In order to obtain the highest sensitivity in estimating the aforementioned drugs, the optimal conditions are

studied by varying one parameter at a time and keeping the others constants.

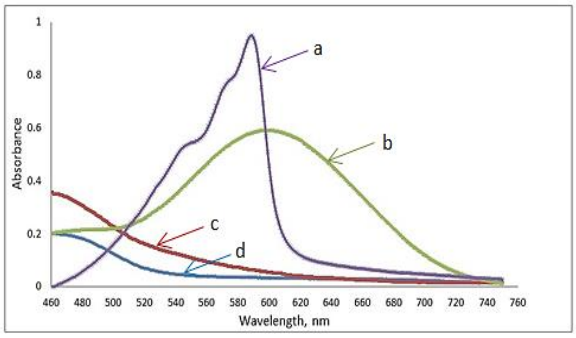


Figure1. Absorption spectra of products *9-CA* with TERS (5µg.mL^{-1}) and (b) SBS (10µg.mL^{-1}) versus their corresponding blank (c) and (d) respectively.

3.1. Effect of solvent

It was found that the reagent *9-CA* forming a stable and sensitive product with SBS but an unstable product with TERS in the basic aqueous medium. The stability of the TERS-*9-CA* product was observed in the organic solvent. Therefore; different systems of solvents were tested to achieve sensitivity and stability of the product. As seen in Table 1, methanol as a solvent for TERS (5µg.ml^{-1}) and *9-CA* (1ml of 1x10^{-3}M) in the presence of NaOH (0.5ml of 0.5 M) and dilution with the same solvent gave high absorbance and stability of the product. Therefore; water as a solvent for SBS and methanol for TERS were recommended in this method.

Table 1. solvent effect on the color intensity of TERS-*9-CA* product

TERS dissolved in	***9-CA* dissolved in**	**Dilution by**	**λ_{max} (nm)**	**Abs.**
Water	Methanol	Water	588	0.220
Water	Methanol	Methanol	588	0.297
Methanol	Methanol	Methanol	588	0.543
Water	Ethanol	Water	593	0.168
Water	Ethanol	Ethanol	593	0.287
Ethanol	Ethanol	Ethanol	593	0.417
Water	Acetonitrile	Water	593	0.098
Water	Acetonitrile	Acetonitrile	593	0.168
Acetonitrile	Acetonitrile	Acetonitrile	500	0.319
Water	Acetone	Water	593	0.124
Water	Acetone	Acetone	593	0.185
acetone	acetone	Acetone	519	0.242
water	water	water	593	0.129

3.2. Effect of pH and buffer solution

It was found the products formed in the basic medium through the reaction between drugs and *9-CA* reagent, where no reaction observed in acidic medium. Therefore various some strong and weak bases have been tested and their results in Figure 2 indicated that Na_2CO_3 and NaOH gave the highly sensitivity than other bases for SBS and TERS respectively. Also it was found that these products are formed at pH 9.95 and 12.30 for SBS and TERS respectively, so; different buffers such as carbonate, borate, borax and potassium chloride with above pH values were prepared and tested. The result in Figure 3 show negative effect on the absorbance of the products.

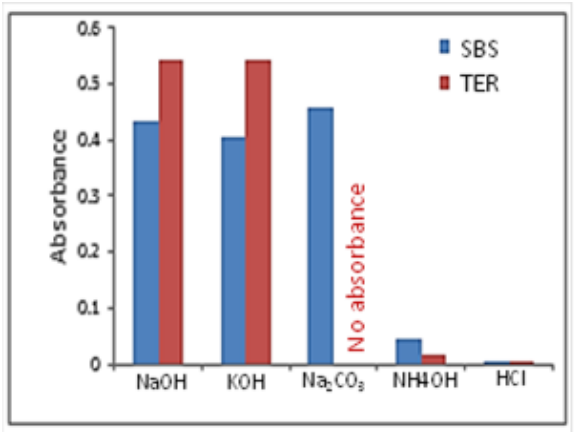


Figure 2. Effect of base and acid

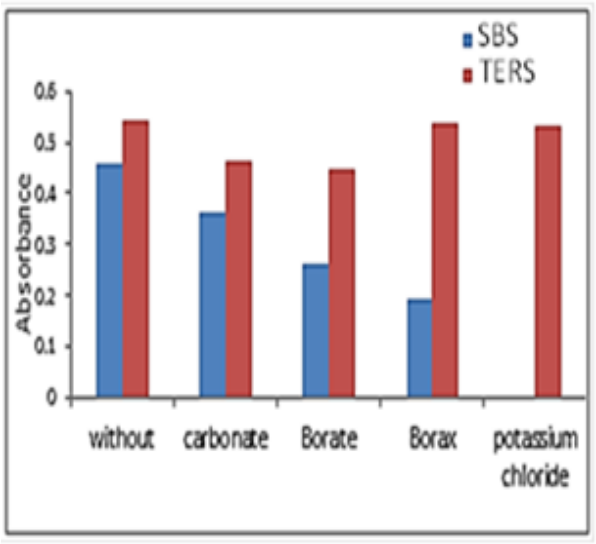


Figure 3. Effect of buffers

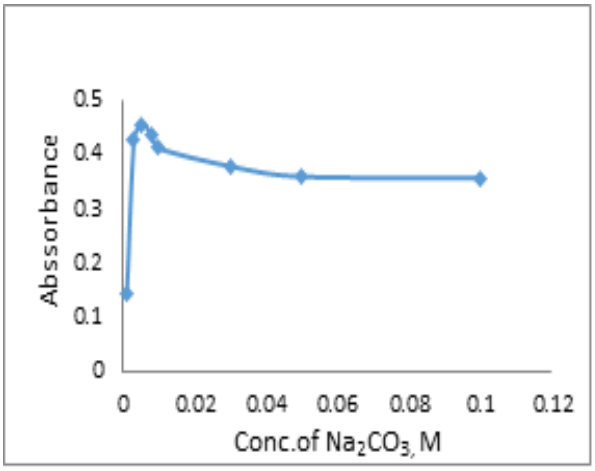


Figure 4. Effect of different conc. of Na_2CO_3

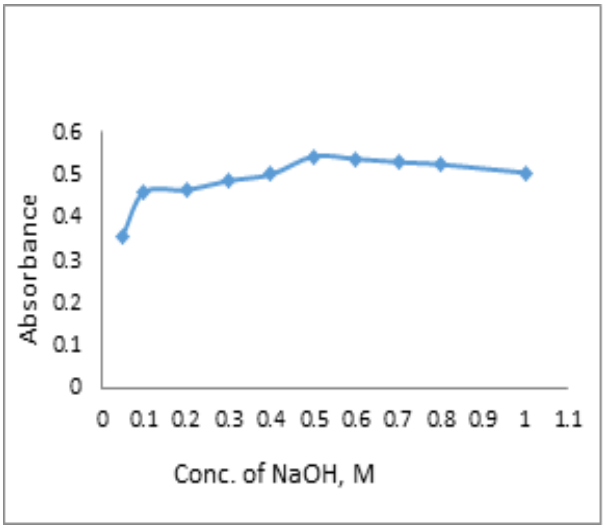


Figure 5. Effect of different conc. of NaOH

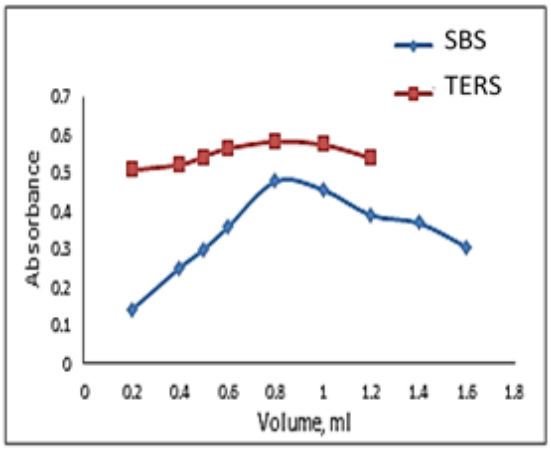


Figure 6. Effect of different volume of Na_2CO_3 and NaOH on the absorbance of the products

3.3. Effect of 9-CA reagent concentration and volume

The effect of 1ml of different concentration of *9-CA* was investigated. as shown in (Figure 7), it was found that $1x10^{-3}$ M and $2x10^{-3}$ M concentration of *9-CA* gave maximum absorbance for the SBS and TERS respectively. Also; the volumes of these concentrations were studied and found that the absorbance increased and reached maximum by using 1 ml of *9-CA* for both drugs, (Figure 8), therefore adopted as being optimal.

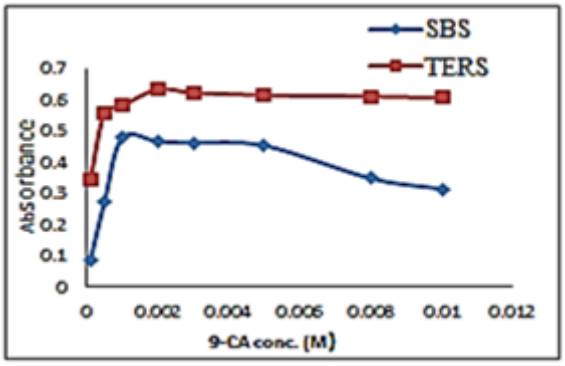


Figure 7. Effect of different conc. of 9-CA

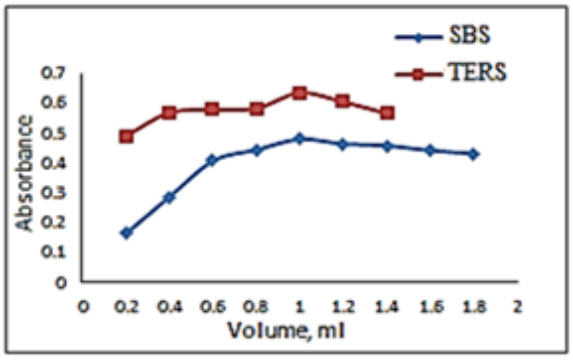


Figure 8. Effect of different addition of 9-CA

3.4. Effect of temperature on products formation time

The effect of time at different temperatures ranging from the laboratory temperature (25˚C) to 60˚C on the intensity of absorption and stability of the formed products was studied in a thermostatically controlled water bath. The absorbance was measured at 5 min intervals against the reagent blank treated similarly. it was observed that the formation of coloured products was achieved maximum after 40 and 10 min at room temperature (30ºC) for SBS and TERS respectively and stable for at least 120 min for both drugs. At higher temperatures, the absorbance was decreased indicating the decomposition of the products (Fig.9).

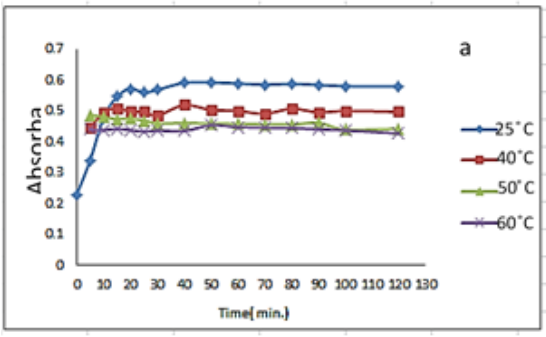


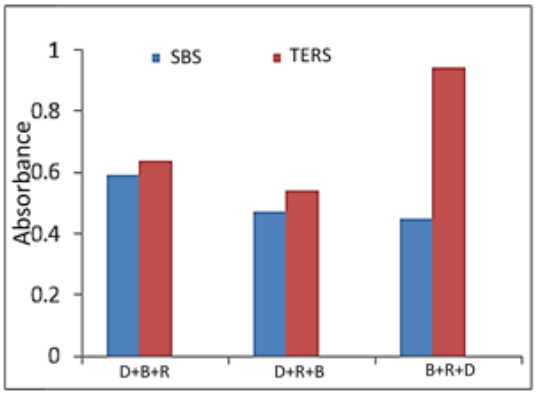
Figure 9. Temperature and developing time effect on the absorbance of SBS-9-CA (a) and TERS (b) products

3.5. Effect of surfactants

Effect of various surfactants including sodium dodecyl sulphate, cetyltrimethyl ammonium bromide, tween-80, and Triton X-100 on the absorbance intensity of the products have been investigated. It was found to decrease in absorbance.

3.6. Effect of order of addition

To obtain high sensitivity, the order of addition of reagents should be as follows: SBS+Na_2CO_3+*9-CA* and NaOH+*9-CA*+TERS, (Fig. 10).



D= drug, B=base, R= *9-CA*

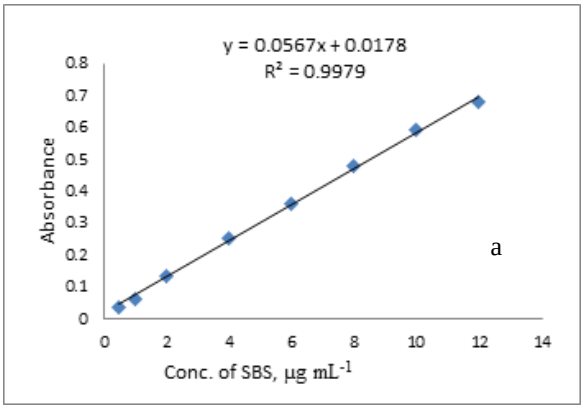
Figure 10. Effect of order of addition

4. Quantification

By plotting the absorbance against the concentrations, the standard curves shown in Figure 11 were obtained, which indicate that the method follows Beer's law within the ranges shown in Table (2) for the studied drugs, which shows the possibility of estimating infinitesimal quantities, and that there is a deviation negatively about Beer's law after discretionary upper limits. The molar absorptivity values indicating the sensitivity of the method. The detection and quantitation limits (LOD and LOQ) were calculated according to the following equations:

LOD= 3.3σ/s, LOQ = 10 σ/s

Where σ is the standard deviation for five replicates of blank reagent and s is the slope of the calibration curve. The results obtained are in the accepted range below the lower limit of Beer's law range. The values of the square correlation coefficient statistically, which are greater than 0.99, shown in Table (2), indicate that the standard curves have an excellent linear characteristics. The relative standard deviation (RSD) and accuracy (average recovery %) for the analysis of four replicates of each three different concentrations of SBS (2,6,10 µg.mL^{-1}) and precise and accurate.



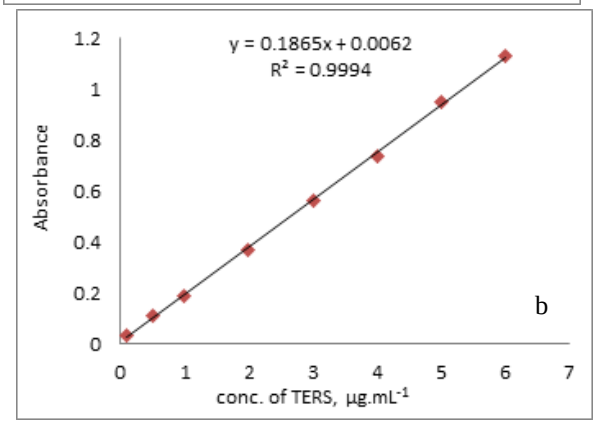


Figure 11: Calibration curve for SBS (a) and TERS (b)

Table 2. Summary of statistical data and optical characteristics for the suggested method

Parameter	Values of method	
	SBS	TERS
λ_{max}	600 nm	589 nm
Beer's law limits (µg/ml)	0.5-12.0	0.1-6
Molar absorptivity (L.mol.cm^{-1})	1.913×10^4	1.0754×10^5
Limit of detection LOD (µg.mL^{-1})	0.3074	0.0983
Limit of quantitation LOQ (µg.mL^{-1})	0.9315	0.2978
Correlation coefficient	0.9979	0.9994
Slope, a	0.0567	0.1865
Intercept, b	0.0178	0.0062
RSD	1.66	1.57
Average recovery (%)	101.3	99.6

Table 3: Analytical application of the proposed method

Pharmaceutical preparations	Amount taken (µg/ml)	Recovery (%)	Average recovery* (%)	Certified value	Amount found	RSD	t-exp
Butadin syrup	4	98.81	100.51	2 mg/5ml	2.01mg/5ml	1.66	±0.013
	6	100.15				1.84	
	10	102.57				0.89	
Butalin tablet	4	102.99	102.92	2mg	2.06 mg	3.87	±0.268
	6	102.32				0.89	
	10	103.46				0.69	
Allied Salbutamol	4	102.78	102.25	5mg/1ml	5.11mg/1ml	1.37	±0.246
	6	100.99				1.96	
	10	102.98				1.04	
Bricanyl tablet	1	96.3	97.45	2.5 mg	2.44 mg	0.70	±0.938
	3	96.53				0.72	
						1.49	

*Average of four determinations

5. Application of the method

The proposed method has been applied successfully for determination of SBS and TERS in their pharmaceutical preparations (tablet, syrup and inhalation). The results cited in Table 3 indicated a good recovery and RSD% is ≤3.87% were obtained. Also, the results of the method were examined statistically by the Student's *t*-test for accuracy by applying the following equation:

$$\pm t_{exp} = \frac{|\mu - \overline{X}| \times \sqrt{n}}{s}$$

Where µ is the certified value of the drug in its formulation, X , *s* and *n* are the average amount found, standard deviation for four replicates (n) respectively. The results in Table 4 indicated that the experimental t-test at the 95% confidence level, were less than the theoretical value (t=2.78), proving there is no significant deference between certified value µ and the amount found X.

6. Validity of the method

The validity of the method was confirmed by applying the standard addition procedure, As shown in (Fig. 12), the results showed that the proposed method was free from interferences in the determination of SBS and TERS in their pharmaceutical preparations with good selectivity.

7. Stoichiometry, stability constant and reaction equations

The two methods of continuous variations [36] and the mole ratio [37] were followed for investigation of the molar ratio of the products formed between each of SBS and TERS with *9-CA* reagent. The results, asseen in Figure 12, indicated that products were formed in the ratio of 2:1 *9-CA* : SBS or TERS which is means 1:1 *9-CA* : SB or TER (Fig. 13). The conditional stability constant (Kst) of these products was determined according to the previous ratio and found $0.754\ x10^5$ and $0.210\ x\ 10^5$ L mol^{-1} for SBS and TERS respectively, indicating good stability

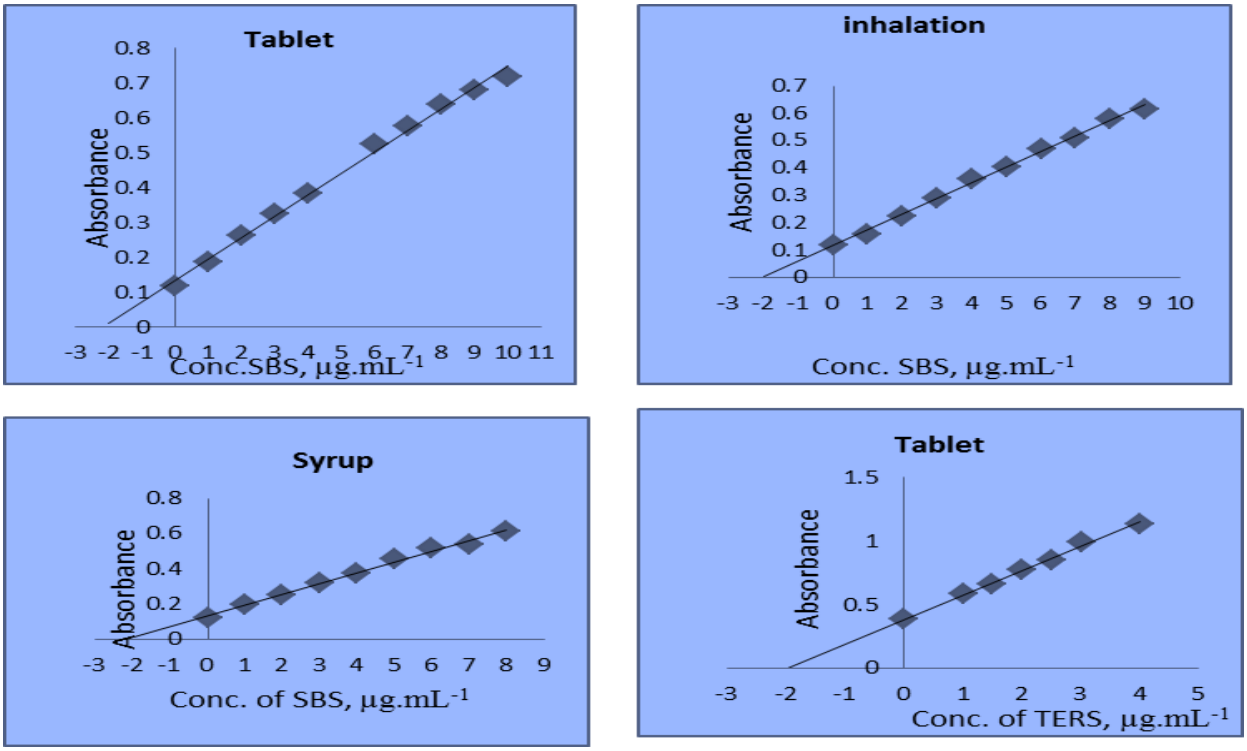


Figure 12: Standard addition plot for the recoveries of 2 µg mL^{-1} for each of SBS and TERS in dosage forms

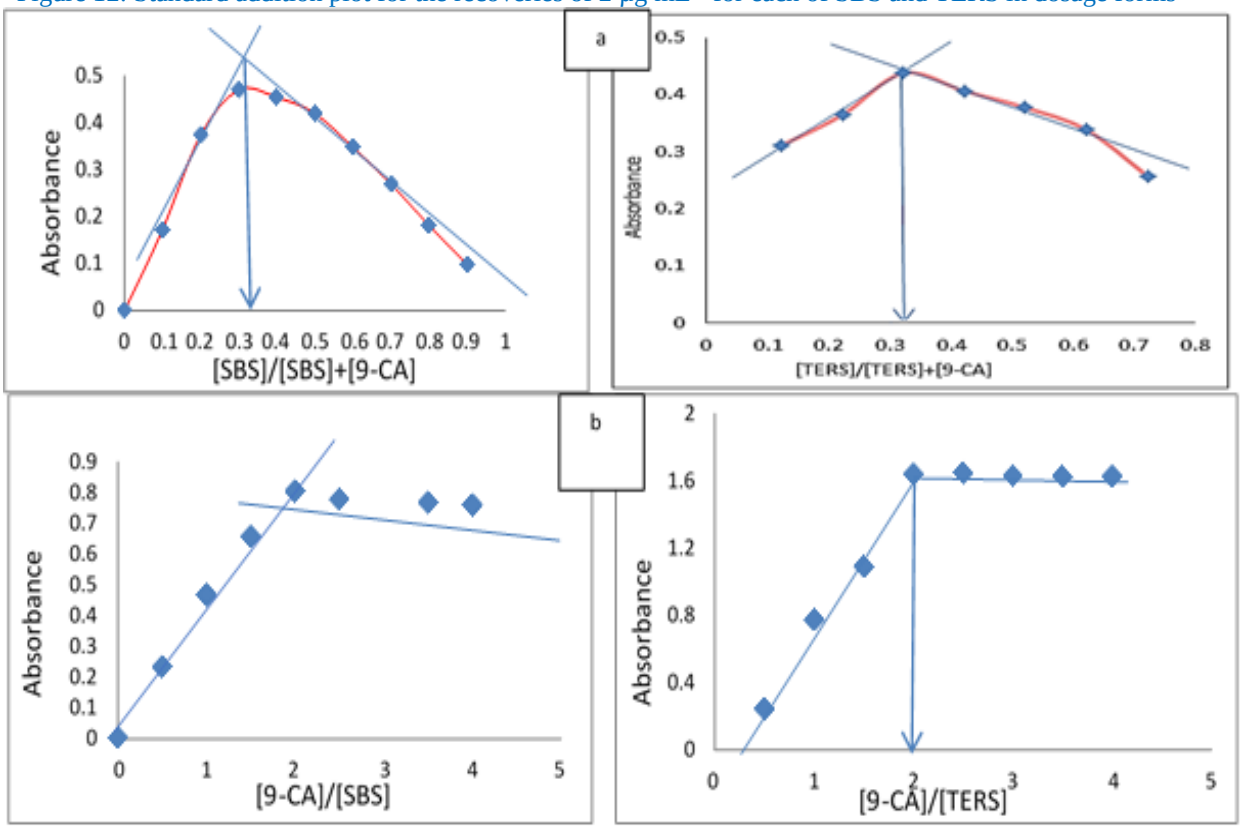
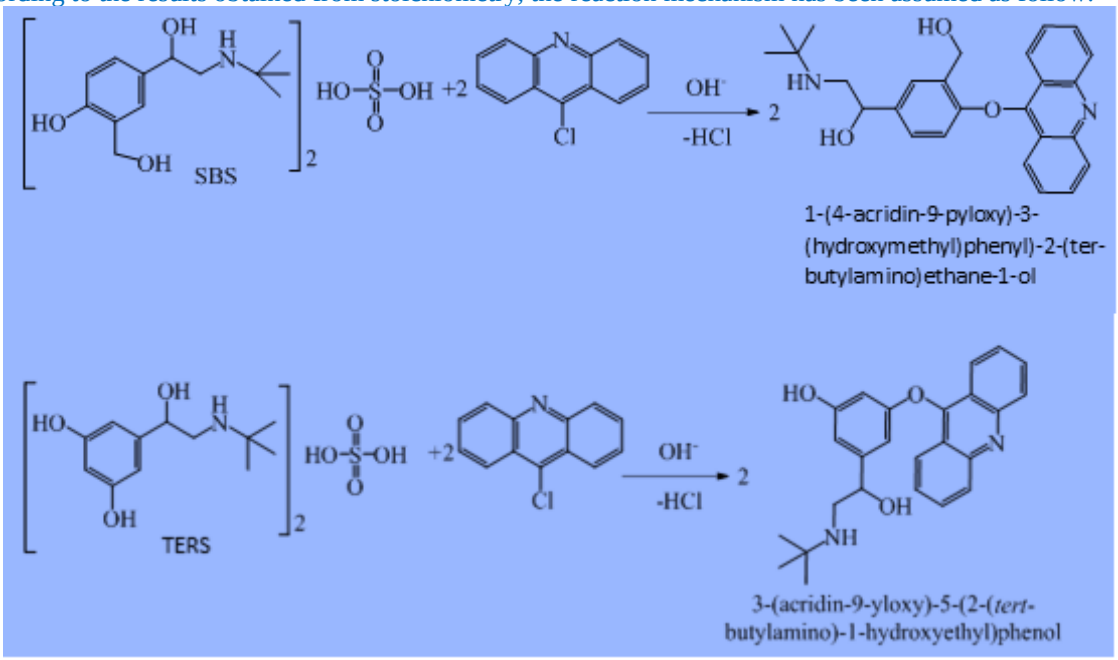


Figure 13: Plots of continuous variation (a) and mole ratio (b) for drug– *9-CA* products

According to the results obtained from stoichiometry, the reaction mechanism has been assumed as follow:



8. Comparison of the current method with other spectrophotometric methods

The current method using *9-CA* reagent has been compared with the reported spectrophotometric methods. Through Table (4) it is clear that the proposed method has a higher sensitivity than the methods mentioned in the literature.

Table 4. Comparison of the proposed method for the assay of SBS and TERS with reported spectrophotometric methods

Analytical Parameters	Current method		Literature method			
			SBS		TERS	
	SBS	TERS	Ref. [25]	Ref. [38]	Ref. [33]	Ref. [31]
Reagent	*9-CA*	*9-CA*	AMP* +KIO_3	Eosin Y	Antipyrine+Fe^{+3}	Eosin Y
λ_{max} (nm)	600	589	499	558	550	545
Linearity (µg.mL^{-1})	0.5-12.0	0.1-6	2–32	0.1-20	4-20	0.5-10
Temp. ˚C	R.T	R.T	R.T	60 ˚C	-	R.T
Recovery%	101.3	99.6	99.6	98.21	99.87-100.66	101.42
Molar absorptivity (l.mol^{-1}.cm^{-1})	1.913×10^4	1.075×10^5	9.165×10^3	4.0×10^4	1.190×10^4	3.169×10^3
RSD%	1.66	1.57	0.98	≤ 2.00	0.93	≤0.72
LOD (µg.mL^{-1})	0.3074	0.0983	0.44	0.041	0.0811	0.030
LOQ (µg.mL^{-1})	0.9315	0.2978	1.33	0.139	0.2460	0.103
Applications	Syrup, Tablets, inhalation	Tablets	Syrup, Tablets, inhalation	Tablets	Tablet	Tablet

* 4-amino-5-isopropyl-1-methyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one

9. Conclusion

A sensitive, simple and precise spectrophotometric method has been suggested for the determination of SBS and TERS based on the neucleophilic substitution reaction of drugs with *9-CA* reagent to form colored products having maximum absorption at 600 nm and 500 nm respectively in basic medium. The method was successfully applied for the assay of the drugs in their dosage forms.

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