Al-Kimia, 13-2-2025, 45-54
Available online at: http://journal.uin-alauddin.ac.id/index.php/al-kimia

5-Aminosalicylic Acid as a Spectrophotometric Reagent for the Determination of the Drug Terbutaline Sulfate

Ashraf Mustafa Abusniena[1], Abdussalam Salhin Mohamed[1*], Moftah Ali Mohmed[2], Antesar Jumah Elmagirbi[3]

[*1]Chemistry Department, Faculty of Science, Sirte University, Sirte, Libya
[2]Department of Medical Laboratories, Faculty of Health Sciences, Sirte University, Sirte, Libya
[3]Department of Chemistry, Faculty of Science, Al-Zaytouna University, Tarhuna, Libya

**Corresponding Author: salam_salhin@su.edu.ly*

Received: November, 12, 2025/Accepted: December, 27, 2025
doi:10.24252/al-kimia.v13i2.62620

Abstract: *A new, simple, and highly sensitive spectrophotometric method for determining terbutaline sulfate using 5-aminosalicylic acid (5-ASA) as a reagent has been developed. This method is based on the coupling reaction between the drug and the 5-ASA reagent in a basic medium, resulting in the formation of a blue-colored complex at 25°C, with maximum absorption at 608 nm. The applicability of the method was extensively studied and evaluated based on pH, drug and reagent concentrations, time, and temperature. Beer's law is valid within the concentration range of 2.0 to 37.3 mg ml^{-1}, demonstrating a correlation coefficient (R^2) of 0.9963. The detection limit (LOD) is 0.6 mg ml-1, and the limit of quantitation (LOQ) is 2.0 mg ml^{-1}. The method's precision and accuracy were evaluated, yielding a relative standard deviation (RSD) of 0.15 for a concentration of 27.0 mg ml^{-1} and an average recovery of 99.87%. The molar absorptivity was determined to be 1.2×10^4 L mol^{-1} cm^{-1}, and Sandell's sensitivity index was measured at 45.8 µg cm^2. A 1:2 mole ratio of the complex was established using Job's method, resulting in a complex formation constant (K) of 8.53×10^5. This method was successfully applied to determine the drug in both pure and pharmaceutical formulations.*

Keywords*: Terbutaline Sulfate, 5-aminosalicylic Acid, Mesalamine, Coupling Reaction*

INTRODUCTION

Spectrophotometric methods are considered as one of the most evaluable methods used by chemists for a quantitative analysis, because of their simplicity, fastness and sensitivity towards targeted species. Accordingly, many organic compounds used as complexing reagents in a colorimetric methods have been developed (Blaed et al, 1973) . Results proved the dependence of complex formation mainly on pH and temperature. Attention have been paid to the organic oxidative-coupling reactions, which involved the coupling between two organic materials. Partially, on the oxidative-coupling of phenol derivatives with amine in the presence of a suitable oxidizing reagent, or between amine derivative and another amine compound (Panchumarthy et al, 2017 ; Ramadan et al, 2014). Spectrophotometric methods are considered one of the most valuable techniques for quantitative analysis in chemistry due to their simplicity, speed, and sensitivity towards targeted species. As a result, many organic compounds have been developed as complexing reagents for colorimetric methods (Panchumarthy et al, 2017; Ramadan et al, 2014).

Results have demonstrated that complex formation primarily depends on pH and temperature. Attention has been focused on organic oxidative-coupling reactions, which involve the coupling of two organic materials. This includes the oxidative coupling of phenol derivatives with amines in the presence of a suitable oxidizing reagent, or the coupling between amine derivatives and other amine compounds(Panchumarthy et al, 2017 ; Ignacio, 2018). The mechanism is primarily driven by free radical formation. Michael and Kamala 1988, reported on the determination of aromatic amines in industrial and pharmaceutical

products. They successfully detected phenothiazine using 3-methylbenzothiazolin-2-hydrazone (MBTH) in the presence of ferric (Fe^{3+}) ions as an oxidizing reagent. The same technique was applied to determine trifluoperazine hydrochloride (TFPH) in pharmaceutical formulations(Maadh et al 2016).

Especially medical and pharmaceutical specialists have done much work on the determination of terbutaline sulfate (TBS) drug. (Faiyazuddin et al , 2011; Shinzo et al, 1989). Accordingly, several techniques, including capillary electrophoresis (Shuting et al 2009) and liquid chromatography (Daraghmeh et al 2002; Faiyazuddin et al 2011), have been employed for the determination of terbutaline sulfate (TBS), utilizing various reagents such as fast red-B salt (Afaf et al 2008), 3-methylbenzothiazolin-2-one hydrazine (Revanasiddappa et al 1999), and permanganate Zhouping et al, 2004 have been imployed.

As the application of the oxidative coupling technique (under study), the present work is devoted to determining the TBS drug in its pure and mixed form in some pharmaceutical formulations.

TBS is chemically known as 5-[2-(tert-butylamino)-1-hydroxy-ethyl]benzene-1,3-diol; sulfuric acid $(C_{12}H_{19}NO_3)_2.H_2SO_4$ (trade names: Bronclyn, Brethine, Bricanyl, Brethaire, or Terbuli). It is a synthetic drug, widely used as a respiratory medicine and for other chronic obstructive diseases. It is a short-acting bronchodilator, which can be administered orally, parenterally, or by suitable inhalation systems or nebulization (Daraghmeh et al 2002).

The drug exists as a 1:1 racemic mixture (±TBS). The (-)TBS enantiomer is responsible for β-agonist activity, while the second enantiomer (+TBS) is responsible for some pharmacological activities. TBS is incompletely absorbed from the gastrointestinal tract, with a very low bioavailability (10-15%) and stereoselectivity. It is subject to fairly extensive first-pass metabolism by sulfate conjugation in the liver and possibly in the gut wall (Bruchhausen et al 2014; Satinder et al, 1990). Therefore, its determination in biological fluids and detection (at tiny levels) is very important nowadays. The aforementioned methods are highly expensive, require a very high level of expertise, and limit routine analysis. Therefore, in the present work, a very low-cost, simple, and highly selective spectrophotometric method for the determination of TBS, based on the oxidative coupling reaction using 5-aminosalicylic acid (5-ASA), is discussed. 5-ASA is primarily utilized as an analytical reagent, serving as a peroxidase substrate. It produces a soluble colored product for spectrophotometric analysis and functions as a chromogenic agent for nitrate coupling.

RESEARCH METHODS

Materials and Tools

Spectroscopic measurements were conducted using a UK-Spectrometer-Unicom (Model 929AA) UV-Vis spectrophotometer, which was equipped with a 1.0 cm path length quartz cell. Additionally, a Herison pH meter from Metrohm Ion-Analysis in Swaziland was utilized for pH measurements.

Chemicals and reagents

All chemicals and solvents used were of analytical grade and were used as received without further purification. Terbutaline sulfate (TBS) and 5-aminosalicylic acid were purchased from Jordan Pharmaceutical Manufacturing Co. Ltd. Potassium periodate, potassium permanganate, and acetone were obtained from Canadian Chemical Company,

GCC. Potassium dichromate, sodium hydroxide, and dimethyl sulfoxide (DMSO) were supplied by Fluka, Surechem Products Ltd., and LAB-SCAN, respectively.

Standard solutions

Terbutaline sulfate (1.89×10^{-3} M): A standard solution of TBS was prepared by dissolving 518.6 mg in 50 mL doubled distilled water and made up to 500 mL flask. The flask was wrapped with aluminum foil and stored in a dark and dry place. The working solution was then prepared by dilution.

5-aminosalicylic acid (5.74×10^{-3} M): A standard solution of the reagent was prepared by dissolving 439.6 mg in 100 mL DMSO, and made up to 500 mL flask with doubled distilled water. The flask was then treated as above solution.

All other standard solutions; 5M (NaOH, KOH, NH_4OH); 4.3M (KIO_4, $KMnO_4$, $K_2Cr_2O_7$, $K_3Fe(CN)_6$) and 2.5M Na_2CO_3, were prepared by dissolving the proper weight of each in a doubled distilled water.

Sample preparation: Stock solution of TBS (50 µg.mL^{-1}) was prepared by dissolving two TBS drug tablets (commercially purchased from local pharmacy, which contains 2.5 mg TBS) in 100 mL doubled distilled water. The flask was wrapped with aluminum foil and stored in a dark and dry place. Three standards (5, 10 and 15 µg.mL^{-1}) of TBS were prepared by the proper dilutions of the stock solution in 25 mL flask using a doubled distilled water.

UV-Vis Analysis

Wavelength Selection

The optimal wavelength was determined by measuring the absorbance of a complex formed from a mixture of a 1.0 mL stock solution of TBS (1.89×10^{-3} M) in a 25 mL flask, along with 2.0 mL of individual standard solutions of NaOH (5 M), KIO_4 (4.3×10^{-3} M), and 5-ASA reagent (5.74×10^{-3} M). The mixture was then brought up to the mark with double-distilled water. The solution was allowed to stand at room temperature for 7 minutes to ensure complete complex formation. The absorbance of the resulting blue mixture was measured against a blank solution prepared in the same way, but without TBS. Figure 1 displays the UV-Vis spectrum of the obtained complex.

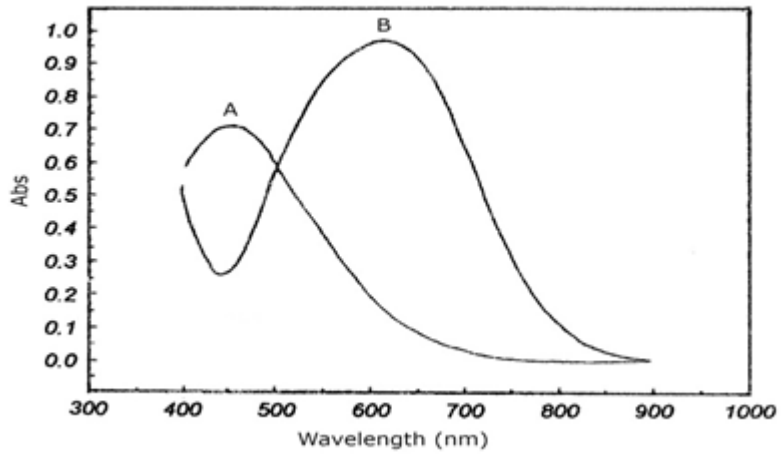

Figure 1: The *UV-Vis* spectrum of the *TBS* complex with *5-ASA*. (A) is the pure ligand, and (B) is *TBS:5-ASA complex.*

The calibration curve

Under optimal conditions, linear regression analysis was conducted on the absorbance of the TBS-5-ASA complex in relation to TBS concentration. Working solutions of TBS, ranging from 3.8 to 7.6 × 10^{-6} M, were prepared in a 25 mL flask by diluting the stock solution with double-distilled water. Each solution was mixed with 3.0 mL of KIO_4 (4.3 × 10^{-3} M), 4.0 mL of NaOH (5 M), and 2.5 mL of 5-ASA reagent (5.74 × 10^{-3} M). To ensure complete complex formation, all standard solutions were allowed to stand for 7 minutes at room temperature in a cool, dry place. The absorbance of each standard was then measured at the maximum wavelength against a blank solution. A linear curve was plotted and subsequently used to calculate the concentrations of unknown samples.

RESULTS AND DISCUSSION

Oxidative coupling of TBS

When 5-ASA is treated with KIO_4 in a basic medium, it reacts with a TBS solution, resulting in a blue color that exhibits maximum absorbance at 608 nm, as shown in Figure 1. Figure 2 illustrates the proposed reaction and the complex formed between the 5-ASA reagent and TBS in an alkaline medium(Suzan et al, 2023; Salih et al, 2020).

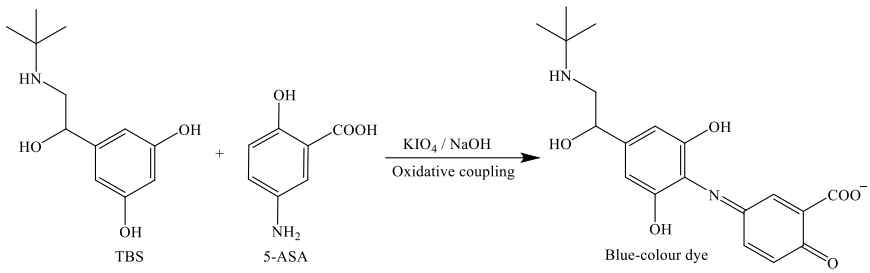

Figure 2: The proposed mechanism of *5-ASA-TBS* reaction

Optimizing conditions

Reaction time and temperature on stability of the color

Under the optimum conditions, the best time required for complex formation was studied. The oxidation of the drug (TBS) with 5-ASA was tested during different periods (1:00-30:00 min). It was observed that the best complexation occurs between (4:00-12:00 min). Therefore, 6:00 min was selected for the subsequent experiments. A slight decrease in the color stability of the complex was observed after 12 minutes (Fig. 3). In conjunction with that, the effect of temperature on color stability was also studied between 20-50°C. The highest absorbance was achieved in the temperature range from 20-30°C. Therefore, 25°C (room temperature) was chosen for the subsequent experiments.

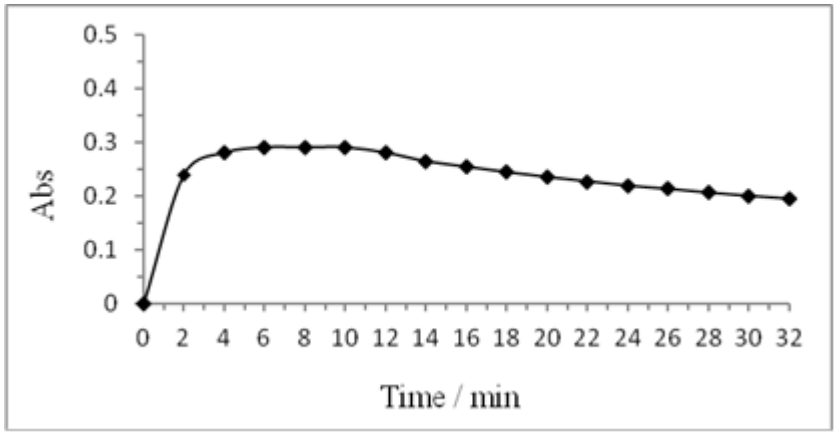

Figure 3: *Reaction time and stability of the complex. ʎ max= 608nm.*

Effect of base

The effect of the medium of interaction on complex formation was also studied. The color stability of the resulting complex was examined in acidic, basic, and neutral mediums, with the best results observed in the basic medium. Accordingly, the effects of different bases, including NaOH, KOH, NH_4OH, and Na_2CO_3, were examined under the general conditions applied for the complex. Table 1 displays the changes in color and the pH of the resulting complex upon the addition of various types and concentrations of the bases. NaOH (pH 13.57) was found to be the most effective among the studied bases, and it was therefore used for further investigation.

Table 1: *The effect of base used on the complex formation.*

Base	Amount added (mL)	Color observed		Absorbance		pH
		Free solution	Complex	Free solution	Complex	
NaOH	4.0	Orange	Dark blue	0.079	0.715	13.6
Na_2CO_3	7.0	Orange	Light Orange	0.013	0.165	11.6
KOH	5.0	Orange	Blue	0.052	0.380	14.1
NH_4OH	5.0	Yellow	Light Orange	0.000	0.027	12.0

Solution concentrations: NaOH, 5M; Na_2CO_3, 2.5M; KOH, 5M; NH_4OH, 5M. Free solution= absence of base.

Effect of the amount of 5-ASA

The effect of the 5-ASA reagent, ranging from 0.5 to 5.0 mL of 5.74×10^{-3} M, on the color stability and absorbance of the complex was also studied. It was determined that 2.5 mL of the 5-ASA reagent (Fig. 4) produced the optimum absorbance, and this volume was used for further investigation.

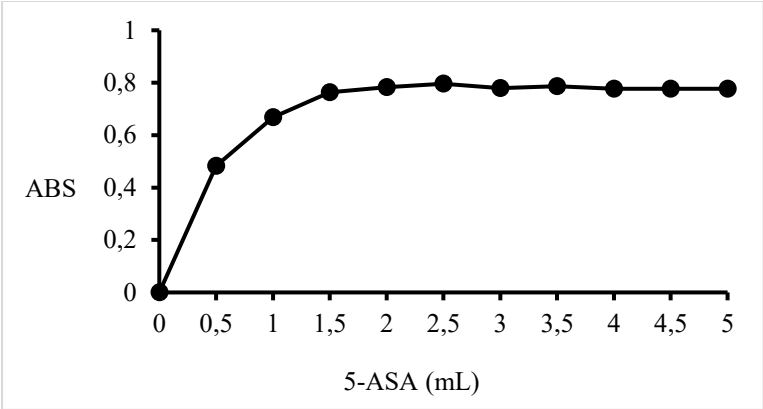

Figure 4: Effect of the amount of *5-ASA* reagent on the *complex* formation

Effect of oxidizing agent

The effect of oxidizing agents (KIO_4, $KMnO_4$, $K_2Cr_2O_7$, and $K_3Fe(CN)_6$) on the complex formation was also tested under the optimum condition. Results (Fig 5) indicated that potassium periodate (KIO_4) gave the best absorption maxima (lmax: 608 nm) at a very short time. Therefore, it is used in for subsequent experiments. Additionally, the most effective concentration of KIO_4 was determined to be 3.0 mL of a 4.3×10^{-3} M solution, which was deemed optimal for complex formation.

Order of Addition

The impact of the order of addition on the sensitivity of the method was also examined. The results demonstrated that the sequence of adding the oxidizing agent, followed by the reagent, TBS, and base (Ox + R + D + B), resulted in a more intense color compared to other combinations (Table 2). Consequently, this order was adopted for all subsequent experiments.

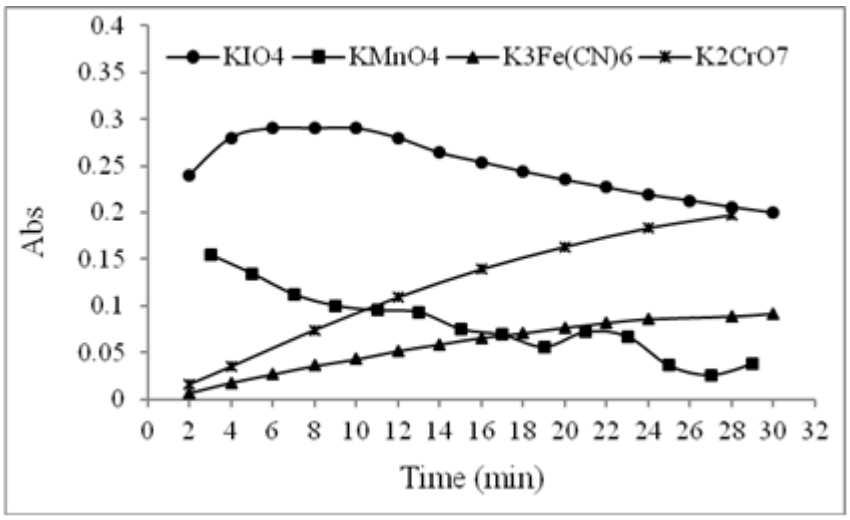

Figure 5: Effect of oxidizing agent *($KMnO_4$, $K_2Cr_2O_7$, and $K_3Fe(CN)_6$)* on the complex formation. *λmax= 652, 620, 660nm,* for the three reagents, respectively.

Table 2: The effect of addition order on the complexation of TBS with *5-ASA*

Order	Reaction component*	Absorbance	Order	Reaction component*	Absorbance
1	*D+B+Ox+R*	0.874	6	*B+R+Ox+D*	0.561
2	*D+Ox+B+R*	0.871	7	*Ox+D+R+B*	0.945
3	*D+R+Ox+B*	0.904	8	*Ox+B+R+D*	0.558
4	*B+D+R+Ox*	0.877	9	*Ox+R+D+B*	0.963
5	*B+Ox+D+R*	0.871	10	*R+B+D+Ox*	0.831

*: *D*= TBS (1.0 mL, 1.89×10^{-3}M); *R*= 5-ASA reagent (2.5 mL, 5.74×10^{-3}M); *Ox*= KIO_4 (3.0 mL, 5.74×10^{-3} M) and *B*= NaOH (4.0 mL, 5.0 M).

Accuracy and precision of the method

In this study, the absorbance of three different concentrations of the TBS drug (24.9, 27.0, and 37.3 µg mL^{-1}) was measured. Table 3 indicates good accuracy and precision, with an average recovery rate approaching 99.867% and a relative standard deviation (RSD) of ≤ 0.5%.

Table 3: The accuracy and precision of the method (n=5)

Sample	TBS concentration (µg.mL^{-1})		Recovery (%)	R.S.D (%)
	Used	Found		
1	24.9	24.8	99.6	0.244
2	27.0	27.2	100.7	0.147
3	37.3	37.0	99.2	0.151

R.S.D= *Relative standard deviation.*

Calibration Graph

Under the optimum experimental conditions and following the recommended procedure, a calibration graph was obtained as explained previously. A straight-line calibration graph was plotted (Fig. 6).

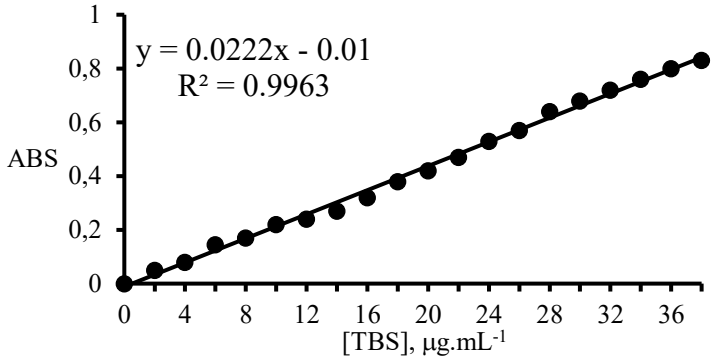

Figure 6: Calibration curve for the determination of *TBS* by oxidative coupling with *5-ASA* reagent in a basic medium.

The graph adhered to Beer's law within the concentration range of 2.0 to 37.3 mg mL^{-1} of TBS, exhibiting a correlation coefficient of 0.9963 and a detection limit of 0.60 µg mL^{-1}. The molar absorptivity, Sandell's index, and limit of quantification (LOQ) were determined to be 1.20×10^4 L mol^{-1} cm^{-1}, 45.8 µg cm^{-2}, and 2.0 mg mL^{-1}, respectively.

Job's Method

An investigation into the stoichiometry between the TBS drug and the 5-ASA reagent was conducted using the continuous variations method. The data related to Job's method is presented in Fig. 7. Analysis of the Job's plot indicates that the stoichiometry of the complex formed between TBS and 5-ASA in aqueous solution is 1:2. The stability constant ((Ks)) of the complex is calculated using the following formula (Reddy et al, 2011; Purohit et al, 2006).

$$K_s = \frac{1-\alpha}{\alpha^2 c}$$

Where α = (Em-Es/Em). Em is the maximum absorbance obtained at the intersection of two straight lines. Es is the maximum absorbance from stoichiometric ratio of the complex formed. Both were found from the graph as 1.22, 1.19, respectively. C is the concentration of the ligand, 1.89×10^{-3}M.

Hence: KS = $(1 - 0.0246) / (0.0246)^2 (1.89 \times 10^{-3}) = 8.53\times10^5$.

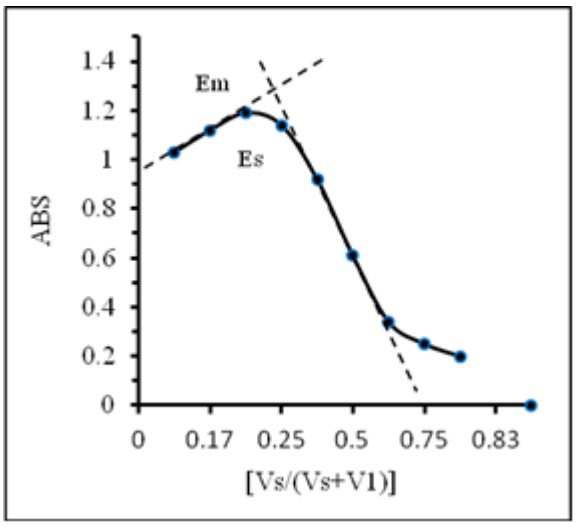

Figure 7: Job continuous variation plots for TBS with *5-ASA* reagent at optimum condition, *λ*= 608 nm.

Validity of the proposed method

To evaluate the applicability of the proposed method, a commercial drug which is real sample (contains 2.5 mg TBS) was analyzed. Three sample of the drug (5, 10, 15 µm.mL^{-1}) were prepared following the recommended procedure. Results are summarized in Table 4.

Table 4: Recoveries of TBS from commercial tablets determined using the proposed method (n= 5)

Sample No.	TBS Concentration (µm.mL^{-1})		% Recovery
	Used	Found (±SD)	
1	5	5.12 (2.4)	102.4
2	10	9.96 (0.4)	99.6
3	15	15.10 (0.7)	100.7

The good recovery obtained for the analysis of the pharmaceutical product to the claimed values supports the accuracy of the method. The proposal method showed good sensitivity and precision (RSD 0.15%) for the determination of TBS.

CONCLUSION

The determination of TBS in pharmaceutical products can be performed using the proposed spectrophotometric method. This method offers adequate sensitivity and is rapid, simple, and easy to handle, utilizing low-cost instruments compared to more sophisticated techniques. It does not require the removal of excipients, sample pretreatment, or expensive reagents and solvents. The method adheres to Beer's law over a concentration range of 2.0–37.3 mg mL^{-1}, with a correlation coefficient (R^2) of 0.9963 and a detection limit of 0.6 mg mL^{-1}. The relative standard deviation (RSD) for most analyzed samples was less than 1.0%, indicating good reproducibility for TBS analysis in various sample matrices. This method is recommended for routine analysis in quality control laboratories.

REFERENCES

Blaed, W. J., & Meloche, V. W., 1973. Elementary quantitative analysis, Theory and practice, *Harper and Row, Publishers*, 156.

Panchumarthy R. MD. Shaheem S. P. S. Babu, SK. Afzal Basha, R. Aswini, V. Swathi, SK. Mahamuda Sultana, M. Sri Lakshmi P. N. & Navyasri, I. M. T., 2017. Comprehensive review of important analytical reagents used in spectrophotometry. *Indo American Journal of Pharmaceutical Research*, 2017. 7(5), 8716-8744.

Zygmunt M., & Maria B. 2000. Chapter 3 - Spectrophotometric methods. *Analytical Spectroscopy Library*. 10, 39-52

Alhaji N. M. I., Ayyadurai G. K. & Shajahan A. 2013. Kinetics and mechanism of oxidation of aniline by N-bromophthalimide. *Chem. Sci. Trans*., 2(2), 467-472.

Ramadan A. B. & Ebtisam M. A. 2014, *J. Chem. Eng. Chem. Res*. 1(4), 246-252.

Maadh T. A. & Kamal M. M. 2016. Spectrophotometric determination of trifluoperazine hydrochloride using oxidative coupling reaction. *International Journal of Innovative Research in Technology & Science (IJIRTS)*. 4(6), 23-27.

Ignacio F.-A. & Feliu M. 2018. Oxidative coupling mechanisms: *Current State of understanding. ACS Catalysis*. 8, 1161−1172.

Michael E., & Kamla M., 1988. *Analyst*, 113, 1267-1271.

Maadh T. A., & Kamal M. M., 2016. *International Journal of Innovative Research in Technology and Science*, 4(6). 23-27.

Faiyazuddin, Md. Abdul Rauf, Niyaz A., Sayeed A., Zeenat I., Sushma T., Aseem B., Roop K. K., & Farhan J. A. 2011. A validated HPTLC method for determination of terbutaline sulfate in biological samples: Application to pharmacokinetic study. *Saudi Pharmaceutical Journal*. 19(3), 185–191.

Selek, H., Fiahin, S., Ercan, M.T., Sargon, M., Hncal, A.A., & Kafl, H.S., 2003. Formulation and in vitro/in vivo evaluation of terbutaline sulphate incorporated in PLGA (25/75) and L-PLA microspheres. *J. Microencap*. 20, 261–271.

Chiap, P., Rbeida, O., Christiaens, B., Hubert, P., Lubda, D., Boos, K.S., & Crommen, J., 2002. Use of anovelcation-exchange restricted access material for automated sample clean-up prior to the determination of basic drugs in plasma by liquidchromatography. *J. Chromatogr.A*., 975,145–155.

Intisar A. A., Mohammed S. A., & Abdussamed M. A. S. 2023. Spectorphotometric methods for determination of terbutaline sulphate in pure and pharamaceutical formulation. *Journal of Medicinal and Chemical Sciences* 6,1032-1043.

Lv Y., Zhang Z., Hu Y., He D., & He S., 2003. A novel chemiluminescence method for determination of terbutaline sulfate based on potassium ferricyanide oxidation sensitized by rhodamine 6G, *Journal of pharmaceutical and biomedical analysis*, 32, 555-561.

Shinzo T., & Koji K., 19289. Colorimetric determination of terbutaline sulfate in pharmaceutical preparatinos using phenanthro [9,10-d]imidazole-2-N-chloroimide. *Chemical and Pharmaceutical Bulletin*. 37(11), 3131-3133.

Shuting L., Janshi W. & Shulin Z., 2009. Journal of Chromatography B, 877(3). 155-158.

Daraghmeh N., Al-Omari M. M., Sara Z., Badwan A. A., & Jaber A. M. 2002. *J. Pharm Biomed Anal*. 29(5). 927-37.

Faiyazuddin M. d. , Abdul R., Niyaz A., Sayeed A., Zeenat I., Sushma T., Aseem B., Roop K. & Khar F. J. Ahmad. A 2011. Saudi Pharmaceutical Journal, 19(3). 185-191.

Afaf A. K., Magda M. E. & Mana S. E. 2008. *E-Journal of Chemistry*, 5(S2), 1087-1097 .

Revanasiddappa H. D. & Manju B. 1999. *Eu. Journal of Pharmaceutical Sciences*. 9, 221–225.

Zhouping W., Zhujun Z., Zhifeng F., & Xiao Z., 2004. *Analytical and Bioanalytical Chemistry*. 378(3), pp 834–840.

Bruchhausen F. V., Dannhardt G., Ebel S., Frahm A. W., Hackenthal E., & Holzgrabe U.: 2014. Hagers Handbuch der Pharmazeutischen Praxis: Band 9: Stoffe P-Z*, Springer Verlag, Berlin, Aufl*. 5, 804, ISBN 978-3-642-63389-8.

Satinder A. J. Ashman, (1990). Analytical Profiles of Drug Substances, 9. 601-625.

Suzan S. T. & Diyar S. A. 2023. *Hindawi Journal of Spectroscopy. Article ID* 4583013, 1-9.

Salih E. S. & Al-Enizzi M. 2020. Journal of Education and Science, 29(1), 279–292.

Reddy, K. V., Reddy D. N. & Reddy K., 2011. *Journal of Chemical and Pharmaceutical Res*., 3(2), 234-244.

Ambily P. N., Christine J., & Desai K. K., 2008. *Oriental Journal of Chemistry*, 24(2), 693-696.

Purohit M. K., & Desai K. K., 2006, *J. Saudi Chem. Soc*., 10(1), 129-134.