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ORIGINAL RESEARCH ARTICLE

New spectrophotometric determination of salbutamol sulphate using p-methoxy aniline

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ABSTRACT

Salbutamol sulfate was analyzed using a straightforward, expeditious, and precise spectrophotometric approach. This method enabled the rapid and precise quantification of salbutamol sulfate within complex samples.in this method Salbutamol sulfate is coupled with a diazotized p-methoxy aniline reagent in an alkaline medium to form stable and water-soluble yellow azo dye . The resulting compound exhibited maximum absorption at 426 nm The method follows Beer's law was over the concentration range 2-12 μg mL^{-1} and Sandell's sensitivity index at 1.76×10^{-2}μg cm^{-2}, Detection limit is 0.0096 μg mL^{-1}. This approach demonstrates rising reliability (average recovery 98.75%), with a relative standard division (SD) is 0.0017 μg mL^{-1} and molar absorption coefficient 3.269×10^{4} L mol^{-1} cm^{-1}.This proposed technique has been successfully applied to determine the salbutamol sulphate with no interference from conventional pharma logical excipients. Furthermore, this procedure was effectively versus the official ones, demonstrating its reliability and potential for routine analysis.

Keywords: p-methoxy aniline; spectrophotometry; Detection limit; coupling reactions

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1. Introduction

Salbutamol (SBS), 4-[2-(tert-butyl amino)-1-hydroxyethyl]-2-(hydroxymethyl) phenol, is a white, water-soluble powder widely used as a bronchodilator and selective β2-adrenergic agonist with a short duration of action [1,2] . It is primarily employed in the management of bronchospasm associated with asthma and chronic obstructive pulmonary disease (COPD) due to its broncho dilating properties [3]. SBS exerts its effects by binding to β2-adrenergic receptors on bronchial smooth muscle, leading to activation of adenylate cyclase and an increase in cyclic adenosine monophosphate (cAMP). Elevated cAMP levels reduce intracellular calcium and inhibit myosin phosphorylation, resulting in relaxation of bronchial smooth muscles and subsequent bronchodilation [4,5] Furthermore, elevated cAMP has been shown to impede the secretion of inflammatory mediators, such as histamine, from airway mast cells. This finding contributes to the understanding of its anti-inflammatory properties. [6]. From a clinical perspective, the bronchodilator effect of SBS manifests within 5–15 minutes of administration and persists for 3–6 hours, thereby rapidly ameliorating bronchospasm [7,8] .

The precise determination of SBS in pharmaceutical formulations is imperative for ensuring the quality of these products. A plethora of analytical methodologies have been documented, encompassing high-performance liquid chromatography (HPLC) [9-11]Spectrophotometry[5,12,13] , flow-injection analysis[14,15] and derivatization techniques[16,17]. SBS is a white, water-soluble powder widely used as a bronchodilator and selective β2-adrenergic agonist with a short duration of action [1,[2] . The precise determination of SBS in pharmaceutical formulations is imperative for ensuring the quality of these products. A plethora of analytical methodologies have been documented, encompassing high-performance liquid chromatography (HPLC) [3]. SBS exerts its effects by binding to β2-adrenergic receptors on bronchial smooth muscle, leading to activation of adenylate cyclase and an increase in cyclic adenosine monophosphate (cAMP), elevated cAMP levels have been shown to reduce intracellular calcium and inhibit myosin phosphorylation [18]. This results in the relaxation of bronchial smooth muscles and subsequent bronchodilation. [4],[5] . Additionally, increased cAMP can inhibit the release of inflammatory mediators such as histamine from airway mast cells, contributing to its anti-inflammatory potential [6]. Clinically, the broncho dilatory effect of SBS begins within 5–15 minutes of administration and lasts for 3–6 hours, providing rapid relief from bronchospasm[7],[8] .

Accurate determination of SBS in pharmaceutical formulations is essential for quality control. Various analytical methods have been reported, including high-performance liquid chromatography (HPLC) [9-11] spectrophotometry[5,12,13], flow-injection analysis[14],[15] and derivatization techniques[16],[17].

Spectrophotometric methods often rely on the formation of colored products .A sensitive colorimetric assay for SBS in tablets and plant samples have been developed previously [19],[20]. Spectrophotometric methods often rely on the formation of colored products .A sensitive colorimetric assay for SBS in tablets and plant samples have been developed previously [19],[20]. Although advanced analytical platforms such as (HPLC) offer exceptional precision in quantitative assessments spectrophotometric techniques continue to hold significant analytical value owing to their rapid colorimetric response, minimal sample handling, and reduced solvent usage [21]. This merit is further reinforced by numerous studies that have effectively applied spectrophotometric and kinetic-spectrophotometric strategies across diverse chemical and pharmaceutical matrices. Investigations have demonstrated the successful determination of inorganic species using dye-based reagents [20], while other works have highlighted the suitability of kinetic spectrophotometric approaches for pharmaceutical compounds [21]. Although several analytical methods are available for determining salbutamol sulfate, many of these techniques have drawbacks, such as high operational costs, time-consuming procedures, and the need for sophisticated instrumentation. Therefore, there is still a need for a simple, rapid, and cost-effective method suitable for routine analysis.

In this context, the proposed spectrophotometric method, which is based on a reaction with p-methoxy aniline, has significant advantages due to its simplicity, sensitivity, and minimal sample preparation requirements. This method does not require expensive equipment and can easily be applied in quality control laboratories, especially in settings with limited resources.

Furthermore, this method provides reliable analytical performance, making it a practical alternative to more complex techniques for determining salbutamol sulfate in pharmaceutical formulations. and more recent advancements have introduced nanomaterial-assisted systems capable of highly sensitive detection of biomolecules [22]. Collectively, these contributions underscore the versatility, sustainability, and analytical robustness of spectrophotometric methods within modern chemical analysis [23].

A perspective further exemplified by the utilization of a p-methoxy aniline-based spectrophotometric approach has been demonstrated to be effective for the accurate, reproducible, and environmentally compatible quantification of salbutamol sulfate. This assertion is supported by the collective findings of recent studies .

In this study, p-methoxy aniline was diazotized and subsequently coupled with Salbutamol Sulphate under alkaline conditions to form a stable yellow-colored azo dye. The resulting product was quantitatively analyzed using UV spectrophotometry, a technique distinguished by its operational simplicity, rapidity, cost-effectiveness, and superior analytical sensitivity and accuracy. Furthermore, the aim of this study is to develop a simple, sensitive, and cost-effective spectrophotometric method for the determination of salbutamol sulphate using p-methoxy aninlne The novelty of the present study lies in the use of p-methoxy aniline as a new analytical reagent for the spectrophotometric determination of salbutamol sulphate. To the best of our knowledge, this reagent has not been previously reported for this purpose.

The selection of p-methoxy aniline is particularly significant due to its ability to form a stable and intensely colored product with salbutamol sulphate, which enhances the sensitivity and selectivity of the proposed method.

This approach provides an improved analytical performance compared to previously reported spectrophotometric methods, making it a valuable contribution to the development of simple and efficient techniques for pharmaceutical analysis

2. Materials and Methods

Chemicals

P-Methoxy aniline (purity 98%) was obtained from HI Media Laboratories Pvt. Ltd.(Mumbai India) . Salbutamol Sulphate (SBS) (98%) and sodium hydroxide[NaOH] (98%) were purchased from LOBA Chemistry. Hydrochloric acid [HCl] (35–38%) was obtained from Sefl ,Mumbai ,India , Sodium nitrite $NaNO_3$ (98%) from center drug house (CDH, New Delhi, India), sulfamic acid [H_3NSO_3] (purity 99%) from Eisen-Golden Laboratories (Mumbai ,India) , and ethanol [C_2H_5OH] (purity 98%) from Alpha Chemicals (Mumbai, India). All chemicals were used as received without further purification.

Apparatus

UV–visible spectra were recorded using a Shimadzu UV-1800 Double Beam UV–Visible spectrophotometer. FTIR spectra were obtained in the range 4000–450 cm^{-1} with 1 cm^{-1} resolution using two spectrometers. The products were characterized by comparison with reference samples and by spectroscopic data (FTIR and UV).

Preparation of Standard and Work Solutions

A stock solution of SBS (1000 µg mL^{-1}): Prepared by accurately dissolving 0.1 g of SBS in distilled water and diluted to 100 mL in a Volumetric flask.

Stock Sodium nitrite solution, 0.1M: Prepared by dissolving 0.69 g of sodium nitrite in distilled water and diluting to 100 mL.

Stock Sodium hydroxide solution, 0.1 N: Prepared by dissolving 0.4 g of NaOH in distilled water and completing the volume to 100 mL.

Stock Hydrochloric acid solution, 0.1 N: Prepared by diluting 0.8 g of HCl in distilled water to a final volume of 100 mL.

Stock p-Methoxy aniline solution, 1000 µg mL^{-1}: Preparing by dissolving 0.2580g of p-methoxy aniline , followed by the addition of 1.0 mL of 0.1 N HCl and 1.0 mL of 0.8% $NaNO_2$, and then completing the volume to 100 mL with distilled water.

pharmaceutical formulation analysis: syrup preparations containing salbutamol sulfate (Butarin, Vetoline, and Salutin; each 5 mL containing 2 mg SBS) were used. An aliquot of 2 mL of syrup was diluting to 6mL by distilled water, and 1 mL of this solution was further diluted to 5 mL, resulting in a final concentration of 6.4 µg mL^{-1} salbutamol sulfate

General Determination Procedure

At installed conditions, in a series of (30 mL) volumetric flasks, (2-12) µg mL^{-1} of diazonium salt solution was added and followed by 1mL from (1000 ppm) p-methoxy aniline solution. Finally, 1.00 mL from compound (1ml from 1000 µg mL^{-1} SBS and 1ml from 0.1N NaOH) were added, the color azo-dye began to form, and after 5 minutes, the color stabilized, completing the volume to mark with ethanol solution. The absorbance of the compound solution was measured against a blank containing all material reacted without SBS; it gave a maximum absorbance of 426 nm.

3. Results and Discussion

UV-Vis Spectroscopic characterization

The use of UV-Vi's spectroscopy was instrumental in the investigation of the electronic transitions and the subsequent confirmation of the formation of the azo compound. The spectra of the starting materials, SBS (A) and p-methoxy aniline (B), exhibited characteristic absorption bands in the ultraviolet region. SBS exhibited a maximum wavelength of approximately 275 nanometers (nm), while p-methoxy aniline demonstrated a maximum wavelength of approximately 300 nm [22]. These bands are attributed to $\pi \rightarrow \pi^*$ transitions of isolated aromatic rings, indicative of localized π-electron systems without significant conjugation. The molar absorptivity (ε) values for these transitions were consistent with those observed for typical aromatic compounds, thereby confirming the anticipated electronic environment of the precursors .Subsequent to diazo coupling, the synthesized azo compound (C) demonstrated a substantial bathochromic shift, exhibiting a conspicuous absorption band at 426 nm within the visible region [22]. This shift, which exceeds 100 nanometers relative to the initial materials, serves as a definitive indication of the formation of an extended conjugated system [23]. The observed transition is consistent with the excitation of $\pi \rightarrow \pi^*$ across the newly formed azo (–N=N–) linkage, which facilitates the delocalization of electrons over the conjugated aromatic system [24]. This extended conjugation is responsible for the visible coloration of the product, which is absent in the spectra of the individual precursors .The intensity of the absorption band, as indicated by its high molar absorptivity, suggests a highly allowed electronic transition and confirms the presence of a strong chromophore azo system [22]. Consequently, the spectral data provide direct evidence of successful azo bond formation and corroborate the structural assignment of the synthesized derivative .Furthermore, a comparison of the UV–Vi's spectra (Figure 2) reveal the complete absence of the 240–320 nm bands in the final product, thereby further supporting the structural transformation. The combination of the bathochromic shift and the enhanced absorptivity confirm the successful formation of the yellow chromophore product, providing reliable and reproducible spectroscopic results [24]. This observation is clearly illustrated in Figure 1, which shows the transition from the colorless drug and reagent to the final yellow compound .

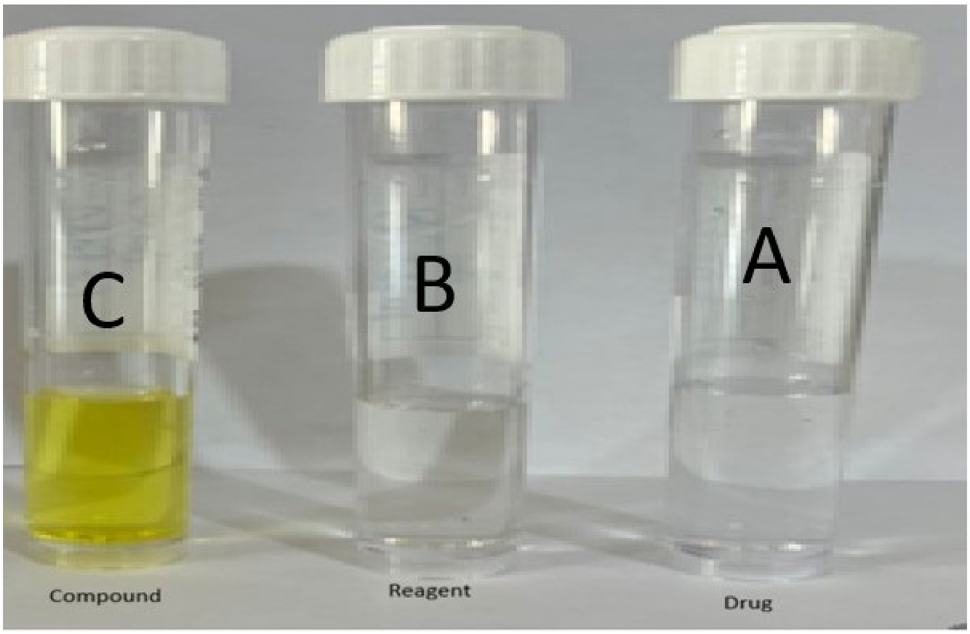

Figure 1. Color change from colorless reactants to yellow final product indicating formation of the chromophore compound used for spectrophotometric determination.

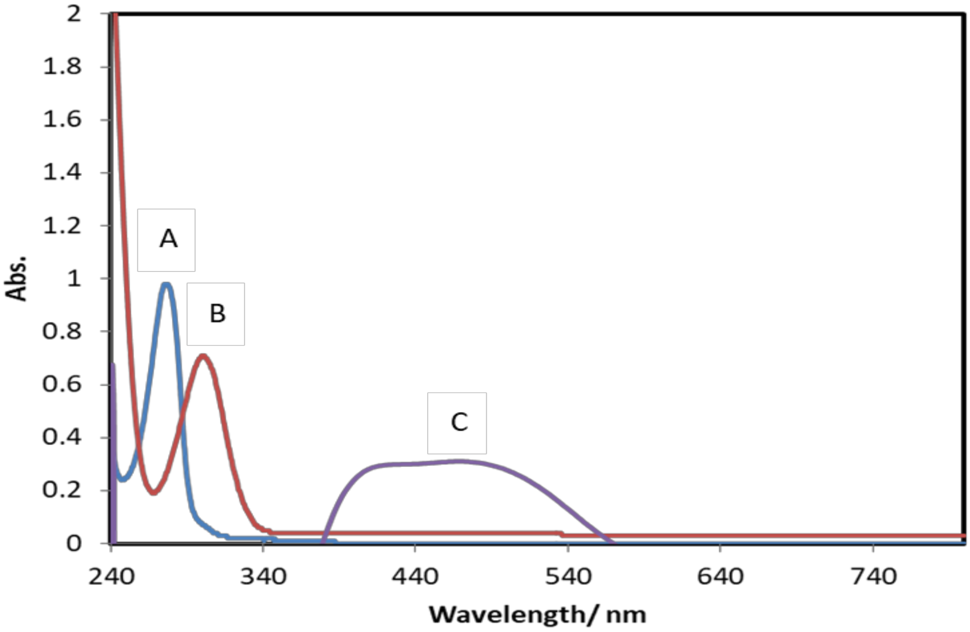

Figure 2. Absorption spectra of (A)SBS (B) Reagent p-Methoxy aniline (C) Compound

FTIR INTERPRETATION

The disappearance of the N–H stretching vibration, with a wave number ranging from 3400 to 3300 cm^{-1}, is a notable observation [25] . The FTIR spectrum of p-methoxy aniline exhibits a significant N-H stretching band within the 3400–3300 cm-1 region, which is indicative of primary amines. This peak is not present in the spectrum of the final product, which indicates the consumption of the amino group during diazo coupling. A similar set of observations has been reported in the context of azo compound studies. In these studies, the N–H stretching bands disappear upon the formation of the azo linkage [12],[13] . The emergence of the Azo (–N=N–) stretch is observed in the range of 1600–1500 cm^{-1} [26]. A novel and pronounced absorption band manifests at 1600–1500 cm^{-1} within the spectrum of the yellow azo compound. This band corresponds to the azo group (–N=N–) in conjugation with the aromatic ring, a well-documented indicator of azo formation. A multitude of

FTIR studies have previously concluded that this region is consistent with N=N and aromatic C=C stretching in azo dyes[27-29], .A negligible alteration in the O–H stretch of salbutamol has been observed. A minor alteration in the broad O–H stretching band of SBS signifies the presence of modified hydrogen bonding patterns subsequent to azo coupling. This subtle behavior is consistent with menstruate analogous alterations subsequent to coupling [17],18] The findings of related reports, which indicate that nationalized phenols and alcohols , see Figure56.The disappearance of the N–H stretching vibration, with a wave number ranging from 3400 to 3300 cm⁻¹, is a notable observation [25] . The FTIR spectrum of p-methoxy aniline exhibits a significant N-H stretching band within the 3400–3300 cm-1 region, which is indicative of primary amines. This peak is not present in the spectrum of the final product, which indicates the consumption of the amino group of azo compound studies. In these studies, the N–H stretching bands disappear upon the formation of the azo linkage [12,13] . The emergence of the Azo (–N=N–) stretch is observed in the range of 1600–1500 cm⁻¹ [26]. A novel and pronounced absorption band manifests at 1600–1500 cm⁻¹ within the spectrum of the yellow azo compound. This band corresponds to the azo group (–N=N–) in conjugation with the aromatic ring, a well-documented indicator of azo formation. A multitude of FTIR studies have previously concluded that this region is consistent with N=N and aromatic C=C stretching in azo dyes[22,23,29] .A negligible alteration in the O–H stretch of salbutamol has been observed. A minor alteration in the broad O–H stretching band of SBS signifies the presence of modified hydrogen bonding patterns subsequent to azo coupling. This subtle behavior is consistent with menstruate analogous alterations subsequent to coupling [17,19] The findings of related reports, which indicate that nationalized phenols and alcohols , see Figure 3.

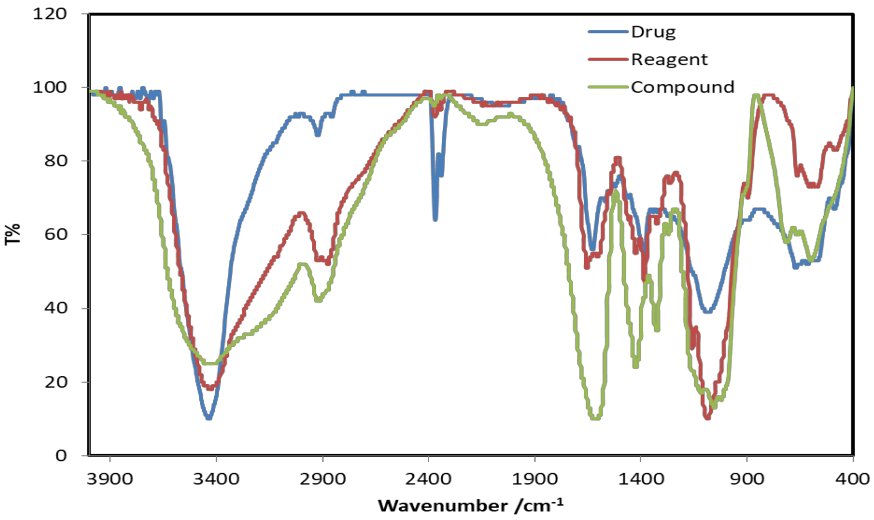

Figure 3. Result of FTIR for Drug, Reagent, Compound

Reaction Conditions' Optimization

The formation of the yellow azo dye was systematically optimized by investigating the critical experimental parameters, including the alkaline medium and the concentration of diazotized p-methoxy aniline, to establish conditions that yield maximum absorbance and reproducible results.

Effect of Diazotized p-Methoxy Aniline Concentration

The present study systematically investigated the influence of p-Methoxy aniline concentration on dye formation within a range of 2–12 µg mL⁻¹. The findings demonstrated that 1.0 mL of 8 µg mL⁻¹ p-Methoxy aniline yielded the maximum absorption, as depicted in Figure 4 (A) . This observation signifies the optimal azo coupling efficiency. Conversely, lower concentrations resulted in incomplete color development, while higher concentrations did not significantly enhance the absorption of light and could potentially lead to side reactions or self-association of the diazonium species. Consequently, this concentration was selected as the optimal value for all subsequent experiments, ensuring reproducible and stable dye formation.

Effect of H_3NSO_3volume on dye formation

The effect of H_3NSO_3volume on dye formation was investigated using volumes ranging from (0.5-2.0) mL Figure 4 (B) . Maximum absorbance (1.043) was observed when 1.0 mL of $H_3NSO_3$was applied, indicating the optimal volume for efficient dye formation. Further increases in H_3NSO_3volume (1.2–2.0 mL) resulted in relatively constant absorbance values (1.013–1.026), suggesting that the reaction had reached a saturation point, where additional reagent did not significantly enhance dye formation. Consequently, 1.0 mL of Sulfamic Acid was identified as the optimal volume for reproducible and efficient azo dye synthesis.

Effect of Acid Medium

The effect of acidic conditions on dye formation was also investigated using 0.1 N (HCl). The reaction produced maximum absorption when 1.0 mL of 8 µg mL^{-1} p-Methoxy aniline was applied under these acidic conditions. An increase in HCl concentrations led to a concomitant increase in solution turbidity and a decrease in color intensity. These phenomena suggest that the process of azo dye formation may be incomplete or unstable. Consequently, 1.0 mL of 8 µg mL^{-1} p-Methoxy aniline in 0.1 N HCl was identified as the optimal condition for efficient dye synthesis see Figure 4 (C)

Effect of Alkaline Medium

Preliminary experiments demonstrated that full color development occurs exclusively under alkaline conditions, thereby highlighting the essential role of pH in the diazo coupling reaction. To ascertain the optimal condition, different volumes of 0.1 molL^{-1} sodium hydroxide (NaOH) were evaluated. The results of this study indicated that the addition of 1.0 mL of 0.1 mol L^{-1} NaOH yielded the highest degree of absorption intensity. It was observed that volumes exceeding 1.0 mL resulted in solution turbidity, likely due to precipitation or excessive alkalinity. Consequently, the employment of 1.0 mL of 0.1 mol L^{-1} NaOH was determined to be the most effective quantity, thereby ensuring the complete and uniform development of color see Figure 4 (D).

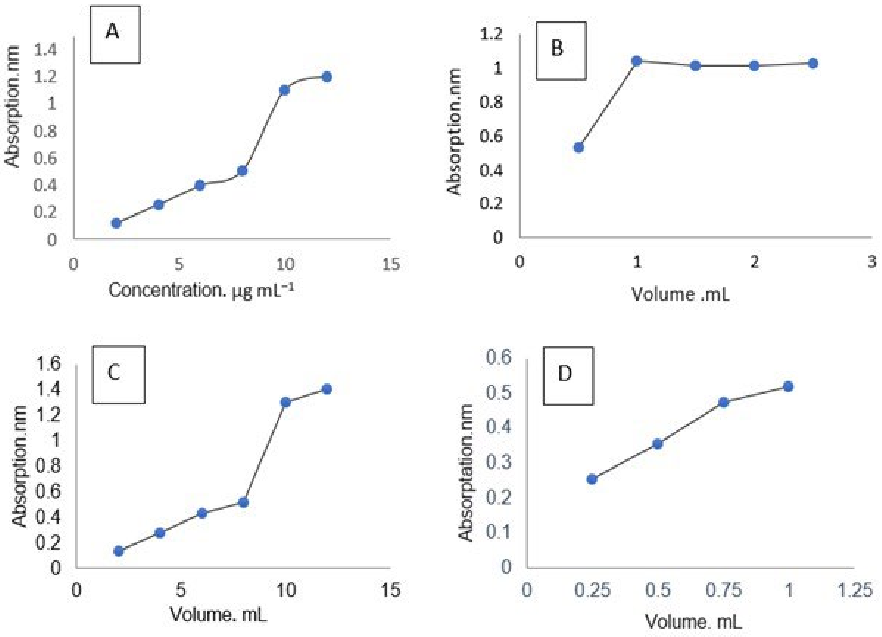

Figure 4. (A) Effect of p-methoxy aniline concentration on the absorbance of the colored product, (B) Effect of (H_2NSO_3H) concentration on the absorbance of the formed azo dye , (C) Effect of acid concentration on the absorbance of the formed complex, (D) Effect of (NaOH) concentration on the absorbance of the colored product.

Effect of Time on Color Development

The impact of time on formation of azo dye has been investigated under optimized conditions using 1000 µg mL^{-1} of (SBS). The results showed that the color developed rapidly within 20 seconds and remained stable without significant loss in absorbance for at least seven days, confirming the robustness of the developed chromogenic system as a Figure 5 (A).

Effect of Temperature on the Compound

Temperature variation between (5-50)°C was evaluated to assess its effect on absorbance. A slight increase in absorbance was observed up to 30 °C, likely due to enhanced molecular collisions and energy absorption. However, beyond 30 °C, a gradual decline in absorbance was recorded, reaching a value of 0.491 at 50 °C. This reduction may be attributed to structural alterations as shown in Figure 5 (B), partial degradation of the chromophore, or disruption of molecular interactions at elevated temperatures. These findings suggest that temperatures no affect the dye complex .

Effect of predominant acidity (PH *)*

The PH of the compound is attributable to the presence of HCl, sulfamic acid, and NaNO2, which are the dominant components in the aqueous environment. Salbutamol sulphate, a weak base (secondary amine), contributes marginally to the overall pH. The variation in salbutamol concentration, ranging from 2 to 12 µg mL^{-1},, elicited only marginal alterations in pH: lower concentrations (2–4 µg mL^{-1},) exhibited a modest increase in pH (~4.45–4.5), while higher concentrations (12 µg mL^{-1},) prompted a slight decrease (~4.35–4.4). This minimal effect is attributable to the fact that the acidic constituents of the compound overshadow the weak basicity of Salbutamol. Consequently, the compound maintains a consistently acidic environment, which also explains why the UV absorbance at 426 nm remains largely associated with chemical interactions within the compound rather than the Salbutamol molecule itself show in Figure 5(C).

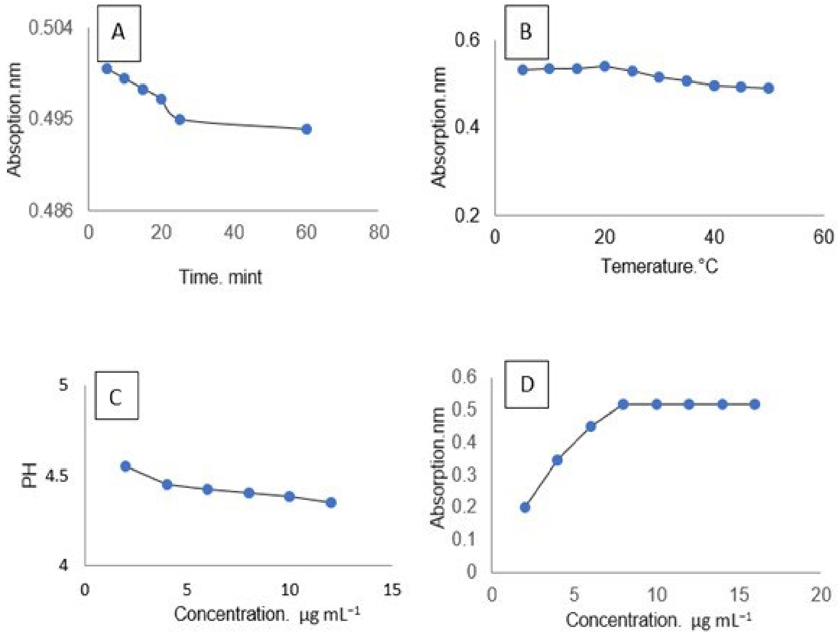

Figure 5 . (A) Effect of the time of H_3NSO_3 addition on the absorbance of the product, (B) Effect of temperature on the absorbance of the yellow azo dye formed from the reaction of p-methoxy aniline with SBS ,(C) Effect of PH on compound , ,(C) Effect of ($NaNO_2$) concentration on the absorbance of the colored product

Effect of solvent

The solvent utilized in the compound's preparation exhibited a discernible influence on the stability and spectral behavior of the resulting solution. The completion of the final volume with ethanol yielded a transparent and homogeneous compound, producing a smooth and well-defined UV absorption peak. Conversely, the addition of water to the volume consistently resulted in turbidity and visible precipitation, thereby hindering light transmission and yielding unstable or noisy absorbance readings. These findings suggest that ethanol functioned as an effective co-solvent, thereby enhancing the solubility and stability of the reaction components. In contrast, water alone proved to be inadequate in maintaining the compound in a clear and stable form that was suitable for UV analysis.

Final Absorbance Spectrum

Under the optimized experimental conditions, the reaction of salbutamol sulfate in aqueous solution with diazotized p-Methoxy aniline led to the formation of a stable yellow azo dye. The resulting product exhibited a sharp and well-defined absorption peak at λmax = 426 nm, clearly indicating the successful formation of the extended conjugated azo chromophore. This spectral feature confirms efficient coupling between the aromatic moieties and demonstrates the stability and reproducibility of the dye under the established conditions (Figure 6).

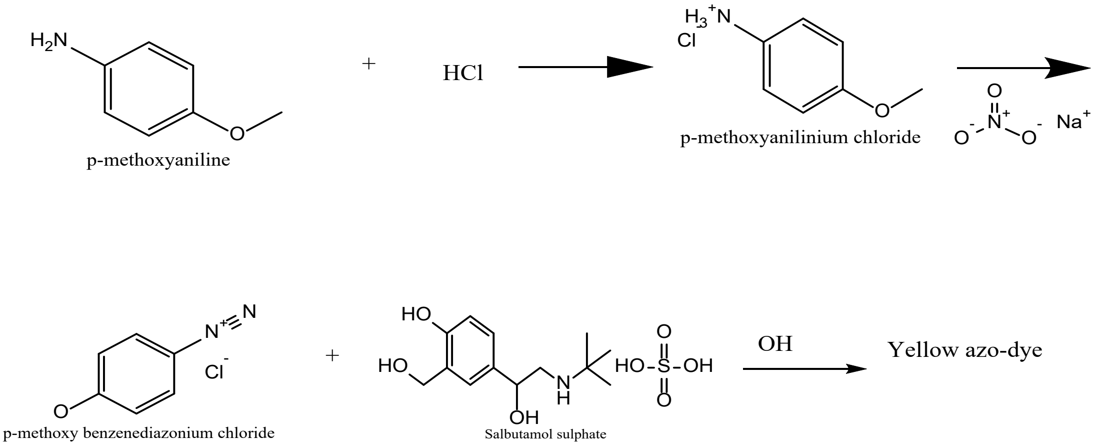

Figure 6. Synthesis of yellow azo-dye derivative

Procedure and calibration carve

Under the optimized experimental conditions, a calibration curve was established for SBS. Standard solutions covering the concentration range of 2-12 µg mL^{-1} were prepared and reacted with diazotized p-methoxy aniline in the presence of sodium hydroxide. Then, the solutions were diluted with ethanol to the mark. The solutions were stabilized in an ice bath, and their absorbance was recorded at 426 nm against a reagent blank. A linear relationship was obtained between absorbance and SBS concentration . The correlation coefficient (R^2) was found to be 0.9957, indicating excellent linearity. The limit of detection (LOD) calculated based on $LOD = 3.3\sigma/b$ (Eq. 1) was (0.0096) µgmL^{-1}. These results confirm the reliability of the proposed method for the quantitative determination of SBS (Figure 7).

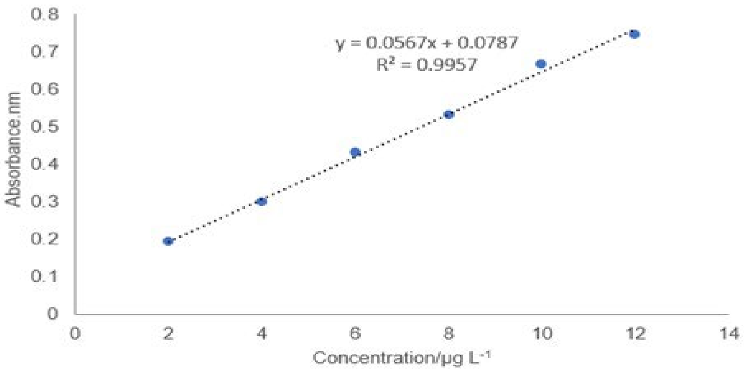

Figure 7. Calibration curve of SBS evaluation with diazotized ρ-Methoxy aniline reagent

The absorbance at 0 µg/mL (blank) was found to be approximately zero, which is consistent with the expected baseline response of the developed method.

The calibration range (2–12 µg/mL) was carefully selected based on the method's linearity, sensitivity, and reproducibility, where reliable analytical performance was achieved.

At concentrations below 2 µg/mL, the absorbance values approach the method's limit of detection (LOD) and limit of quantification (LOQ), which may lead to reduced accuracy and precision. Therefore, this range was not included in the calibration curve. This point has now been clarified in the revised manuscript.

Job plot method

The stoichiometric ratio of the interaction between SBS and p-methoxy aniline was determined using Job's method of continuous variation. Absorbance was measured at 426nm while varying the SBS volume, keeping the total volume constant. As shown in Figure 4, the maximum absorbance was reached at 2.5 mL of the drug, indicating that the most stable complex formed at this ratio. These results suggest a stoichiometric ratio between SBS and p-Methoxy aniline. The symmetry of the plot supports the formation of a well-defined complex under these conditions, which is consistent with the behavior typically observed in charge-transfer complex studies see (Figure 8).

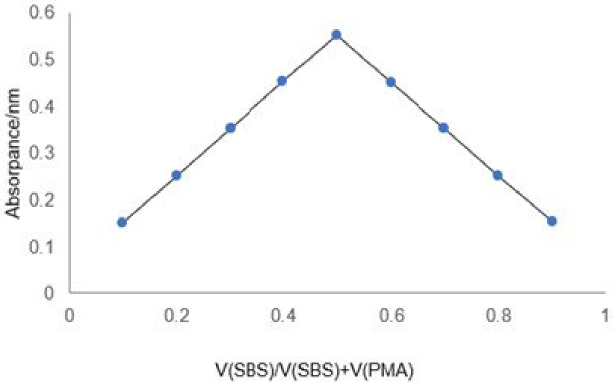

Figure 8. Job's plot (Continuous variation method) for the interaction between SBS and p-Methoxy aniline.

To achieve maximum absorbance and stability of the formed colored product, all experimental parameters affecting the proposed spectrophotometric method were carefully optimized, including reagent concentration, reaction time, pH, and wavelength.

The method exhibited good linearity over the studied concentration range, with a high correlation coefficient indicating excellent agreement between absorbance and concentration. The method's precision was evaluated through replicate measurements, which showed low relative standard deviation values and confirmed the method's reproducibility.

Additionally, the method's sensitivity was evaluated by determining the detection and quantification limits, demonstrating its suitability for analyzing salbutamol sulfate at low concentrations.

These results confirm that the proposed method is reliable and reproducible for routine analytical applications.

Comparison with reported method

The comparison of current method with literature methods to determine of SBS (Table 2).Although the literatures revealed different techniques which have been reported for determination of SBS, there are some drawbacks observed (Table 1) . Unfortunately, the separation techniques provide a quick PC controlled and highly sensitive determination of many chemicals and pharmaceutical products; they still have many drawbacks such as high cost, need for large amounts of organic solvents and high operating skills [30]. Moreover, electrochemical approaches may also be carried out very quickly, and the results are available as electrical signals. These techniques displayed some errors in analysis and require high environmental protection in order to minimize the toxicity. However, spectrophotometry is very simple, fast and provides promising results as well. And the comparative evaluation demonstrated that the analytical method developed in this study possesses several strengths that render it more efficient and sensitive than the previously published method [31], even though some general performance indicators appear comparable. The proposed method exhibited significantly higher spectral sensitivity, as evidenced by its markedly greater molar absorptivity value (3.27×10^4 L mol^{-1} cm^{-1}), which clearly surpasses that of the literature method and reflects an enhanced ability to detect lower analyte concentrations. In addition, our method achieved a substantially lower detection limit (0.0096 µg mL^{-1}) and a much lower standard deviation (0.0017), confirming its superior precision and repeatability. The high recovery percentage (98.75%) and reduced %RSD further demonstrate the reliability and robustness of the proposed procedure.

Table 1. Comparative analytical results of salbutamol determination using the proposed method and other previously reported methods

Method of analysis	Principle	Linear concentration range	Limitation
Voltammetry	This method was based on the direct Electrochemical determination of SAL using an iron titanate nano powder-modified carbon paste electrode.	0.2–25 nmol L^{-1}	This electrochemical method may display some errors in analysis, requiring environmental protection to minimize the toxicity[31].
High performance liquid chromatography coupled with mass spectrometry	The separation was carried out using C18 column, 0.1% formic acid in water and 0.1% formic acid in methanol (20 : 80 v/v) using isocratic mode at 30 °C. under typical mass setting Capillary voltage 3.5, cone voltage 30, temperature source 100 °C and de solvation temperature 450 °C.	1-50 µg mL^{-1}	Very expensive method[32]
Flow-injection spectrophotometry	The methods based on an oxidative-coupling reaction of 2,4-dinitro phenyl hydrazine with sodium periodate, where an electrophilic intermediate diazonium salt of the reagent is produced, which coupled with SAL. The determination at 525 nm.	1–24 µg mL^{-1}	Consuming large quantity of reagents[33]
Spectrophotometry	Azo-coupling reaction of salbutamol sulfate (SBS) coupled with p-Methoxy aniline. The resulting yellow color product was measured at maximum absorption of 426 nm.	2–12 µg mL^{-1}	---

Table 2. The comparison of current method with literature methods to determine of SBS

Parameters	Method	Literature method
Maximum wavelength (nm)	426	499
Color	Yellow	Red
Regression equation (y)	Y=0.0567x+0.0787	Y=0.0383x-0.0185
Concentration range of SBS (µg mL^{-1})	2–12	2-32
R^2(Correlation Coefficient)	0.9957	0.9995
SD(µg mL^{-1})	0.0017	0.03
Molar Absorptivity (L $mol^{-1}.cm^{-1}$)	3.269889×10^4	9.165×10^3
Sandalls' sensitivity (µg cm^{-2})	1.76×10^{-2}	0.026
Percent Relative Error (%Erel)	-1.25%	---
Slope (µg mL^{-1})	0.0567	0.0383
Intercept (C)	0.080133	−0.1667
%RSD	0.32%	0.98%
Percent Recovery (%Re)	98.75%	98.8
LOD (µg mL^{-1})	0.0096	0.0317

Accuracy and precision

The accuracy of the suggested spectrophotometric method was evaluated by calculating the % mean recoveries of SBS solutions in the range of(2,4,8) µg mL^{-1}. The outcome result was 98.75 revealing high accuracy of the suggested method (Table 3). Moreover, to study the precision of the suggested method, inter-day precision was applied by determining three different concentrations of SBS in pure form through replicating the analysis for a period of 5 successive days. The evaluated % RSD ranged from 0.14 to 0.32 % for inter-day, revealing good precision of the proposed method towards the determination of SBS (Table3) .

Table 3. parameters values of accuracy and precision, data obtained for the evaluation of proposed method precision using inter-day assay.

Sample	SBS determination using p-Methoxy aniline		
	Taken, µg mL^{-1}	**Found, µg mL^{-1}**	**% Recovery**
1	2	1.989	99.47
2	4	4	100.0
3	8	7.9	98.75

Sample	SBS determination using p-Methoxy aniline			
	Taken, µg mL^{-1}	**Found, µg mL^{-1}**	**%Recovery**	**%RSD**
1	2	1.978	98.9	0.144
2	4	4.07	101.75	0.391
3	8	7.9	98.75	0.320

4. Conclusions

A novel spectrophotometric method was developed and validated for the determination of (SBS) in pharmaceutical formulations. This method was characterized by its simplicity, rapidity, and reliability. The proposed method is predicated on an azo coupling reaction between salbutamol sulfate and p-methoxy aniline reagent, resulting in the formation of a stable and intensely colored azo dye with a well-defined maximum absorbance at the selected wavelength.

Notably, this study signifies the inaugural instance of p-methoxy aniline's utilization as a coupling reagent in the spectrophotometric determination of salbutamol sulfate. This contribution underscores the innovation and originality of the proposed analytical approach. The method demonstrated adequate linearity over the concentration range of 2–12 μg mL^{-1}, exhibiting a high correlation coefficient, thereby confirming satisfactory compliance with Beer–Lambert's law.

The analytical performance of the developed method demonstrated high sensitivity, accuracy, and precision, as evidenced by low limits of detection and quantification, acceptable recovery values, and low relative standard deviation (RSD%). Moreover, the method exhibited remarkable selectivity, with no substantial interference from prevalent excipients, co-formulated substances, or potential interfering compounds found in pharmaceutical dosage forms. The primary objective of this study was to develop and validate a sensitive spectrophotometric method under controlled laboratory conditions using standard solutions. This ensured accuracy, reproducibility, and proper evaluation of analytical performance.

The implementation of this method in actual water samples may introduce matrix effects that could potentially influence the measurements. Consequently, separate optimization may be necessary to ensure accuracy and precision.

This aspect is regarded as a significant direction for future research and will be addressed in subsequent studies.

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Conflict of interest

The authors declare no conflict of interest

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