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p-Aminobenzoic Acid as a New Reagent in Spectrophotometric Determination of Salbutamol Sulphate Via Oxidative Coupling Reaction

Nabeel S. Othman Ali M. Asaad

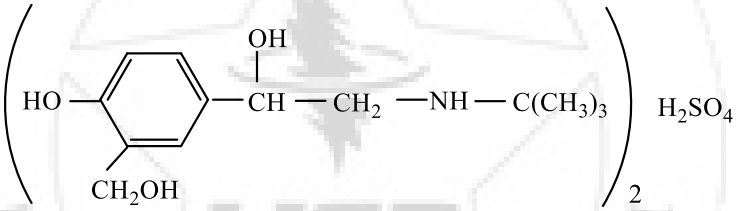
Department of Chem., College of Science, Mosul University, Mosul, Iraq

Abstract: ***A simple spectrophotmetic method for the determination of salbutamol sulphate(SBS) in aqueous solution has been suggested. The method is based on the oxidative coupling reaction of SBS with p-aminobenzoic acid(PABA) in an alkaline medium (pH=11.96) in presence of N-chlorosuccinimide (NCS) as oxidizing agent to produce an intense blue colored, water soluble and stable product, which showed maximum absorption at 622 nm. Beer's law is obeyed over the range 2.0- 25.0 µg.ml^{-1} of SBS and limit of detection (LOD) of 0.460 µg.ml^{-1}, limit of quantitation(LOQ) of 1.536 µg.ml^{-1}, a molar absorptivity of 1.828×10^{4} l.mol^{-1}.cm^{-1}. and Sandell's sensitivity index of 0.0315 µg.cm^{-2}. The method has been successfully applied to the determination of SBS in pharmaceutical preparations.***

Keywords: spectrophotometry, salbutamol sulphate, p-aminobenzoic acid, oxidative coupling reaction, N-chlorosuccinimide

1. Introduction

SBS is almost white crystalline powder, freely soluble in water, slightly soluble in alcohol and in ether, very slightly soluble in methylene chloride and has the following structure and chemical name [1].



Bis[(1RS)-2-[(1,1-dimethylethyl)amino]-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanol] sulphate
M.wt. = 576.7 g/mol

SBS is a β2 adrenergic receptor agonist, primarily used in the treatment of bronchial asthma and other forms of allergic airways disease. The drug is also used in obstetrics for the prevention of premature labour and as a nasal decongestant[2,3]. The standard method of SBS determination is non-aqueous titration[1]. Other methods have been mentioned in the literature for the determination of salbutamol such as titrimetric [4], fluorescence probe[5],HPLC[6-8]LC-tandem mass[9], GC-Mass[10],SPE-UPLC[11], fluorospectrophotometry[12],votametric[13-15] and spectrophotom-etric methods[16-25].

The objective of the investigation reported in this paper is to evaluate simple spectrophotometric method for the determination of SBS. The method is based on the oxidative coupling reaction of SBS with PABA reagent in alkaline medium in the presence of NCS as oxidizing agent to produce an a stable and soluble blue product.

2. Experimental

2.1 Apparatus

The spectrophotometric measurements were carried out on JASCO V-630 and SHIMADZU UV-1650 PC using 1cm glass cells, the pH measurements were performed on HANNA pH 211 pH meter and the heating operations were carried out by using MEMMERT water-bath.

2.2 Reagents

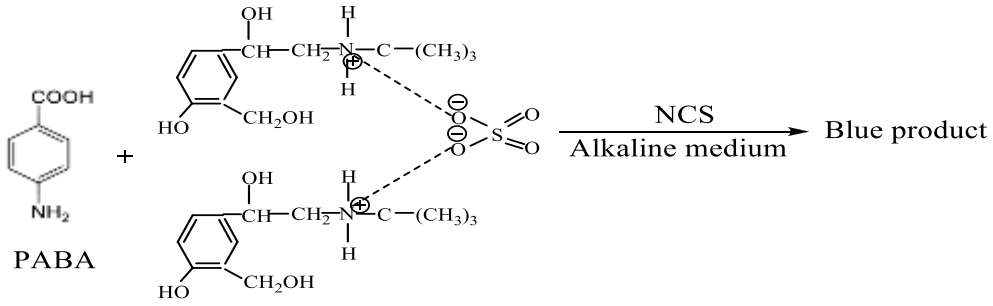
All chemical used are of the highest purity available. Working SBS solution(200 µg.ml^{-1}), PABA solution(0.02M), NCS solution (0.01M), sodium hydroxide solution (0.2M) were prepared by dissolving an a appropriate weight in distilled water in a volumetric flask.

2.3 Pharmaceutical preparations

Butadin syrup solution, 200 µg.ml^{-1}
This solution was prepared by diluting 50 ml of butadin syrup (2mg SBS per 5ml) to 100 ml with distilled water in a volumetric flask.

Butadin tablets solution, 200 µg.ml^{-1}
Dissolve finally powdered 10 tablets of butadin drug (each tablet contains 2 mg SBS) in 80 ml distilled water, shake and warm the solution. Filter the solution into a 100 ml volumetric flask, wash the residue with distilled water and dilute to volume with distilled water to obtain 200 µg.ml^{-1} SBS.

Salbutamol vent. 200 µg.ml^{-1}. Salbu. Vent. solution, 200 µg.ml^{-1}.
A 4 ml of salbu. vent. (5mg SBS per 1ml) diluted to 100 ml with distilled water in a volumetric flask to obtain 200 µg.ml^{-1} SBS solution.



Salbutamol sulphate

3. Results and discussion

For the subsequent experiments, 200 µg of SBS was taken and the final volumes were diluted to 10 ml with distilled water.

3.1 Principle of the method

When addition of PABA to SBS and then NCS was added in alkaline medium, an a blue product formation which showed maximum absorption at 622 nm.

3.2 Study of the optimum reaction conditions

The various parameters related to the colored product formation have been studied and optimum conditions have been selected.

3.2.1 Selection of reagent
Several aromatic reagents (1ml of 0.02M) have been tested for optimum conditions. The results in Table 1 display that PABA give the highest intensity; therefore, it has been selected for this work.

Table 1: Selection of coupling reagent

Reagent	$\lambda_{max.}$ (nm)	Absorbance
p-Aminobenzoic acid	618.5	0.3042
3,4-Diaminobenzoic acid	430	0.1154
3,5-Diaminobenzoic acid	No colour contrast *	
1,5-Diaminonaphthaline	Turbid	

*color contrast:$\Delta\lambda_{max} = \lambda_{maxS}-\lambda_{maxB}$ when S=The colored product, B=Blank

3.2.2 Selection of oxidizing agent
The effect of different oxidizing agents (1ml of 0.01M) on the absorbance of the colored product was studied. The obtained results indicated that the best oxidizing agent can be used for colored product formation with high intensity was NCS, therefore it has been selected for the subsequence experiments.

3.2.3 The optimum amount of PABA
The effect of the amount of the PABA on the absorbance of the colored product formed, has been investigated, the results showed that 2.5ml of PABA gave the highest intensity, thus it fixed in the subsequent experiments.

3.2.4 The optimum amount of NCS
The experimental results showed that 2ml of NCS gives highest intensity of absorbance, so th that this volume selected for the subsequent experiments.

3.2.5 Effect of base
Various bases (strong and weak) have been tested (1 ml of 0.2M of each base was added) and their results in Table 2 indicated that NaOH gave the highest intensity of the colored product, this meaning that the colored product needed a strong alkaline medium.

Table 2: The effect of base on absorbance

Base (1ml of 0.2M) soln.	λ_{max}^{S} (nm)	Absorbance	
		Sample VS. blank	Blank VS. D.W.*
NaOH	619.5	0.4017	0.0540
KOH	614.5	0.4008	0.0634
Na_2CO_3	628	0.3880	0.0524
$NaHCO_3$	634	0.1868	0.0511
Without	No color contrast		

Also the amount of NaOH has been studied, the results indicated that 0.5 mL of NaOH was the optimum volume due to the highest intensity for the colored product ,therefore it has been recommended for the subsequent experiments.

3.2.6 Effect of time and temperature on the stability of the colored product
The effect of time and temperature [20(R.T.) to 65C^{o} , with 20 minute heating in water-bath] on stability of the colored product has been studied, the results indicated that 45C^{o} considered as a suitable temperature because it showed high intensity and stability of the colored product, also the time of heating has been studied ,20 minutes was sufficient time to produced the colored product with high intensity, therefore 45C^{o} and 20 minutes have been fixed in the subsequent experiments.

4. Final Absorption Spectra

When SBS in aqueous solution was treated with PABA solution according to the recommended procedure, an absorption peak was obtained showing an intense absorption at 622nm, characteristic of the blue product which was used in all subsequent studies in this investigation (Fig. 1).

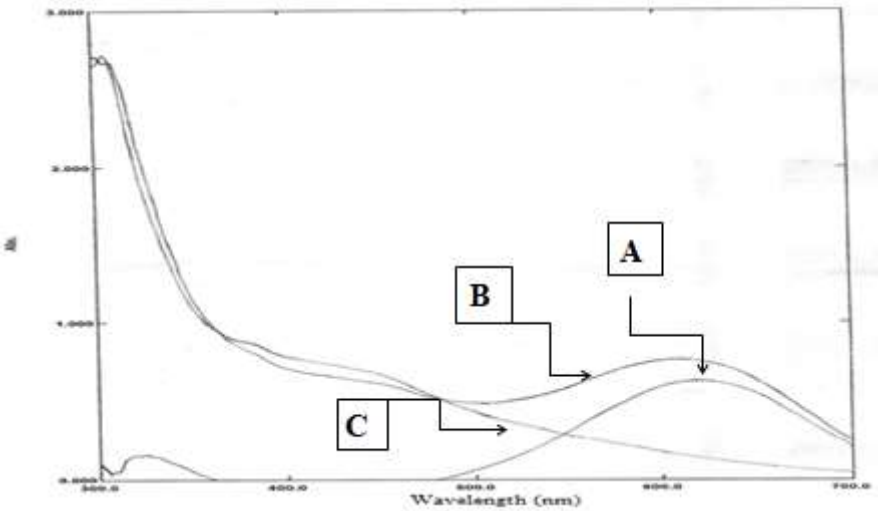


Figure 1: Absorption spectra of 200µg SBS/10 ml treated according to the recommended procedure and measured against: blank(A), distilled water(B) and (C) blank measured against distilled water

General procedure and calibration graph

To a series of 10 ml volumetric flasks transferred 20 - 250 µg of SBS, then 2.5 ml of PABA (0.02 M), 2.0 ml of NCS (0.01 M) and 0.5 ml of sodium hydroxide solution (0.2 M) have been added. The volumes were completed to the mark with distilled water. The reaction mixtures were heated in water bath (20 minute) at 45 C°. The absorbance was measured at 622nm against the reagent blank. The calibration graph as shown in Fig. 2 was linear over the range of 2 - 25 µg of SBS.ml^{-1}.

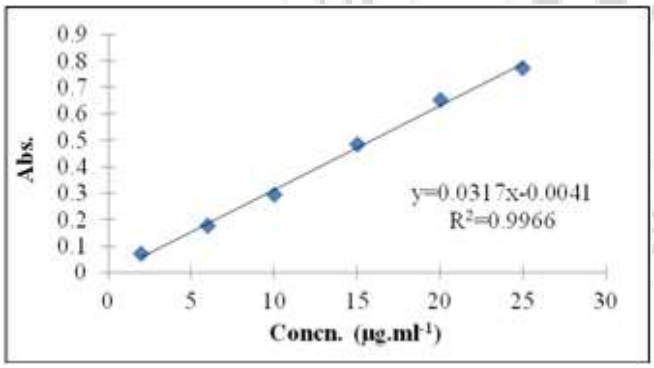


Figure 2: Calibration graph for SBS determination using the proposed method

The characteristic and the results of statistical analysis of the experimental data were summarized in Table 3

Table 3: Optical and regression characteristics of the suggest method.

Parameter	Value
Beer's law limite(µg.ml^{-1})	2-25
λ_{max} (nm)	622
Molar absorptivity (l.mol^{-1}.cm^{-1})	1.828×10^4
Linear regression equation	Y = ax* +b
Slope (a)	0.0317
Intercept (b)	-0.0041
Determination coefficient (R^2)	0.9966
Limit of Detection (LOD)	0.460
Limit of Quantitative (LOQ)	1.536
Relative Standard Deviation(RSD)	not more than ±3.34%

* = concentration

5. Nature of the dye

The result of appling Job`s and Mole-ratio methods [26] indicate that the colored product has a compostion of 1:1SBS to PABA. (Fig.3 and 4).

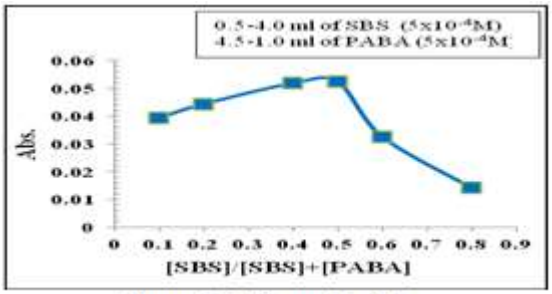


Figure 3: Job`s method plot

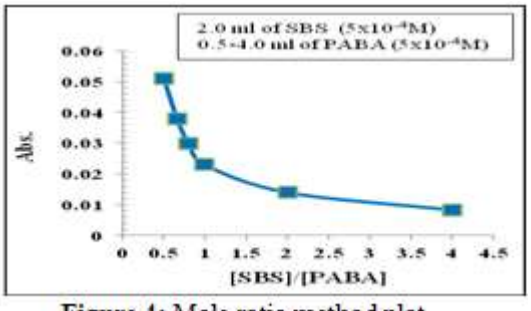
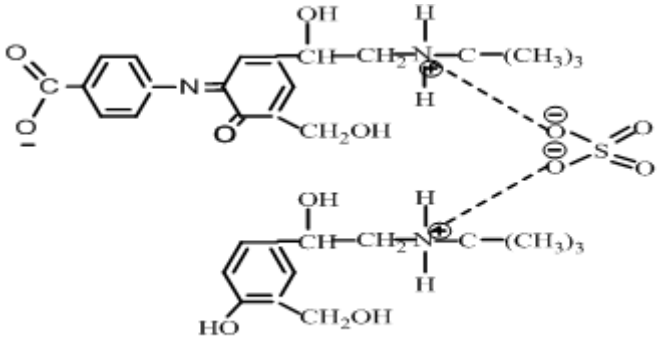


Figure 4: Mole-ratio method plot

Hence the color product may have the following suggested structure:



Blue product

6. Application of the method

The proposed method was applied to determine SBS in its pharmaceutical preparations (Butadin syrup, tablet and Salbu. Vent.).The results in Table 4 indicated that a good recovery and the RSD% not more than ±3.34% were obtained.

Table 4: Analytical applications of the proposed method

Phar Pharmaceutical preparations maceutical preparation	Amount taken (µg)	Recovery (%*)	RSD (%*)
Butadin syrup, 2 mg SBS/5 ml (S.D.I Iraq)	100	103.32	1.77±
	200	100.6	0.89±
Butadin tablet, 2 mg SBS/tablet (S.D.I Iraq)	100	100.90	1.68±
	200	97.04	0.87±
Salbu. Vent., 5 mg SBS/1 ml (Diamond pharma-Syria)	100	101.47	2.15±
	200	102.14	3.34±

* Average of five determinations.

7. Conclusion

The proposed method is simple , sensitive and has a wide application for the determination of SBS in various pharmaceutical preparations.

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