DEVELOPED SPECTOPHOTOMETRIC DETERMINATION OF SALBUTAMOL SULPHATE IN PHARMACEUTICAL SAMPLES BY COUPLING WITH O-NITROANILINE

Hind Hadi

Department of Chemistry, College of Science, University of Baghdad. Baghdad-Iraq.

Abstract

A simple, rapid and sensitive spectrophotmetic method for trace determination of salbutamol sulphate (SBS) in aqueous solution and in pharmaceutical preparations is described .The method is based on the coupling reaction of the intended compound with diazotized o-nitroaniline (DONA) in alkaline medium to form an intense yellow-orange, water soluble dye that is stable and shows maximum absorption at 448 nm .A working curve of absorbance versus concentration indicates that Beer's low is obeyed over the concentration range of 50-1000 μg of SBS in a final volume of 25-ml (i.e. 2-40 ppm), with a molar absorpativity of 1.58×10^4 $L.mol^{-1}.cm^{-1}$,a sandell's sensitivity of 0.0365 $\mu g.cm^{-2}$,a relative error of (-1.156) – 2.874 % and a relative standard deviation of 0.735-1.992 % depending on the concentration of SBS. The optimum conditions and stability of the colored product have been investigated and the method was applied successfully to the determination of SBS in dosage forms.

Keywords*:* Salbutamol sulphate, diazotization, spectophotometry.

الخلاصة

يتضمن البحث وصف طريقة طيفية بسيطة و سريعة وحساسة للتقدير الكمي للمقادير الضئيلة من مستحضركبريتات السلبيوتامول في المحاليل المائية باستخدام الطريقة الطيفية . تعتمد الطريقة على تفاعل ازدواج المستحضر المذكور مع كاشف اورثونايتروانلين المؤزوت في وسط قاعدي حيث يتكون ناتج اصفر– برتقالي مستقر وذائب في الماء اعطى اعلى امتصاص عند طول موجي 448 نانومتر .يشير الرسم البياني للامتصاص مقابل التركيزبان قانون بير ينطبق ضمن مدى التركيز 50– 1000 مايكروغرام من كبريتات السلبيوتامول في حجم نهائي 25 مل (اي ما يكافئ 2–40 جزء بالمليون) وكانت قيمة الامتصاصية المولارية مساوية الى 1.58×10^4 لتر.مول$^{-1}$.سم$^{-1}$ وقيمة حساسية ساندل 0.0365 مايكروغرام .سم$^{-2}$ مع خطأ نسبي (1.156–) 2.874– % وانحراف قياسي نسبي 0.735-1.992% أعتمادا على مستوى التركيز المراد تقديره.تمت دراسة الظروف المثلى للتفاعل وتطبيق الطريقة على المستخضرات الصيدلانية الحاوية على كبريتات السلبيوتامول .

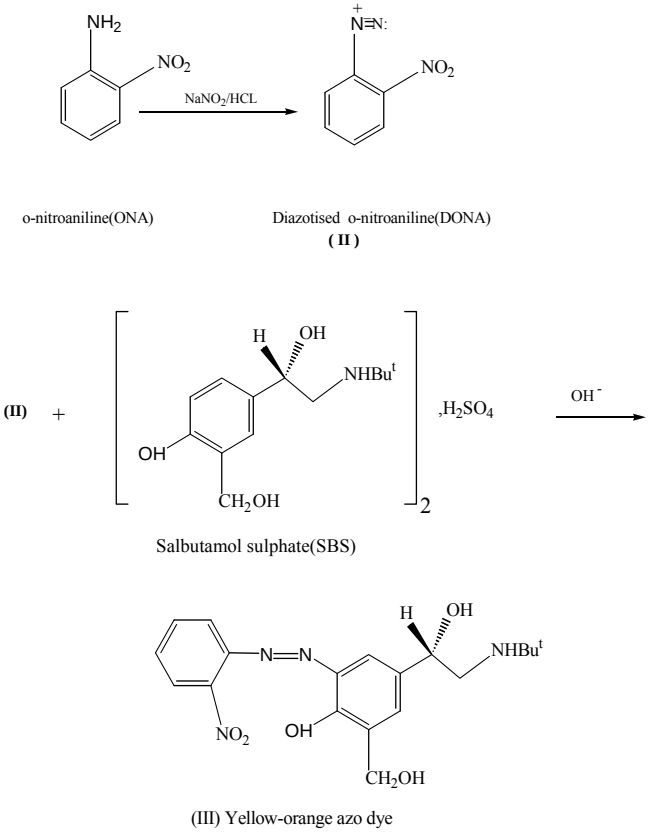
Introduction

Salbutamol sulphate (SBS) is one of a series of selective stimulants of the β_2 receptors which are presents in the bronchioles of lungs of human body, so it acts as a bronchodilator, and its cardiovascular effects are less than its bronchodilator actions [1]. Various methods have been reported for the determination of SBS, these include spectrophotometric [2], high performance liquid chromatography [3-5], liquid chromatography-tandem mass spectrometry [6], gas chromatography mass spectrometry [7], flow injection analysis[8],and polarographic[9]. Various methods based on diazotization and coupling reactions have been developed for the determination of drugs such as diazotized aminoantipyrine [10],sulphonamide [11],acetaminophen and dopamine [12].

The purpose of the present investigation is to develop a simple and sensitive method for the determination of SBS in pharmaceutical preparations using diazotization coupling reaction. The proposed method is based on a coupling reaction between SBS and DONA in alkaline medium to form an intense yellow-orange color product which shows an absorption maximum at 448 nm.

Reaction mechanism of the method

Salbutamol sulphate forms a yellow-orange colored product (λ_{max} 448nm) with DONA in alkaline medium .Due to the phenolic nature of the drug, it can readily be coupled with DONA according to scheme 1[13].



Scheme (1): The proposed mechanism of the reaction between SBS and DONA

Experimental:

Apparatus

All spectra and absorbance measurements were carried out on a Shimadzu UV-visible 260 digital double beam recording spectrophotometer using 1-cm silica cells.

Reagents:

All chemicals used were of analytical reagent grade and pure salbutamol sulphate drug sample was kindly provided from state company for Drug Industries and Medical Appliance,SDI,Samara.Iraq .Dosage forms were obtained from commercial sources.

Salbutamol sulphate stock solution (1000 µg ml^{-1})

A 0.1000 gm amount of pure SBS was dissolved in distilled water and the solution was made up to volume of 100 ml in volumetric flask with the same solvent .To obtain SBS working solution (500 µg ml^{-1}) a 50 ml volume of the stock solution was transferred into a 100 ml volumetric flask and made up to the mark with distilled water.

Diazotized o-nitroaniline solution (3×10^{-3}M)

Prepared daily by dissolving 0.0414 gm of o-nitroaniline (ONA) in 5 ml ethanol, 20 ml distilled water and 3 ml of 0.8M hydrochloric acid in a 100-ml volumetric flask. Cool the mixture to 0-5°C for 5 min using an ice-bath, add 0.0207 gm amount of sodium nitrite and stir the mixture. After 5min the volume is made up to the mark with addition of cooled distilled water. More dilute solutions were prepared by suitable dilution with distilled water.

Hydrochloric acid solution (0.8M)

Prepared by dilution of concentration hydrochloric acid and standardized against sodium carbonate.

Sodium hydroxide solution (0.1M)

A 0.4000gm of sodium hydroxide was dissolved in distilled water and made up to the 100 ml volumetric flask with the same solvent.

Procedure of pure drug

An aliquot of sample containing 50-1000 µg of SBS was transferred into a series of 25 ml standard flasks to cover the range of 2-40 µg. ml^{-1}.A volume of 3ml of 3×10^{-3}M DONA solution and 1ml of 0.1M sodium hydroxide solution were added . The contents of flasks were diluted to the mark with distilled water ,mixed well and left for 20 min. The absorbance was measured at 448 nm (at room temperature 20°C). The color of the formed dye is stable for more than 3hr.

For optimization of conditions and in all subsequent experiments, a solution of 500 µg was used and the final volume was 25 ml (i.e.20 µg ml^{-1}).

Analysis of commercial dosage forms:

Fifty to 100 tablets were accurately weighed and powdered .An amount to tablets equivalent to 100 mg of the pure drug, was dissolved in distilled water and transferred into a 100 ml calibrated flask and completed to the mark with the same solvent. The flask with its contents was shaked well and filtered. Samples of 20 and 30 ppm of SBS were taken and the measurements were carried out as described earlier under general procedure.

Stoichiometry of the reaction

The stoichiometry of the reaction between each SBS and DONA was investigated under the recommended optimum conditions and applying Job's method [14].Volumes of 0.5-4.5ml of 4.34×10^{-4} M portions of DONA were coupled with corresponding 4.34×10^{-4} M SBS solution to give a total volume of 5ml .The results obtained (Fig.1) showed that a 1:1 (SBS: DONA)product was formed between SBS and DONA. The apparent stability constant was calculated by comparing the absorbance of solution containing stoichiometric amounts of SBS and DONA with that of a solution containing a five-fold excess of DONA reagent. The stability constant of the product in water under the described experimental conditions was 2.4299×10^{4} $L.mol^{-1}$.

Results and discussion

Absorption spectra

When a very diluted aqueous solution of SBS was mixed with DONA reagent in alkaline medium , an intense yellow-orange azo dye formed immediately , which became stable after 20 min .The yellow-orange product has a maximum absorption at 448 nm .Fig.2 shows the spectra of the product formed and of the reagent blank, the maximum absorption at 448 nm was used in all subsequent experiments.

Study of the optimum reaction conditions

The effects of various parameters on the absorption intensity of the formed product were studied and the reaction conditions were optimized.

Effect of diazotized reagent concentration

When various concentrations of DONA solution were added to a fixed concentration of SBS ,3ml of 3×10^{-3} M DONA solution was sufficient to develop the color to its full intensity and give minimum blank value ;above 3ml the absorbance of blank value increased ,causing a decrease in the absorbance of sample .Therefore ,3ml of 3×10^{-3} M DONA solution was found optimum and was used in all subsequent experiments .

Effect of alkaline solution

Preliminary results indicated that the presence of an alkaline in the reaction mixture is essential for developing a more intense yellow-orange color .In this respect, sodium hydroxide, potassium hydroxide, sodium acetate, ammonium hydroxide and sodium carbonate were examined. It was found that the best results were obtained with sodium hydroxide, therefore, sodium hydroxide was chosen and 1ml of 0.1M solution was added as optimum after the diazotized reagent as it gives a high sensitivity and minimum blank value.

Effect of order of addition

Different orders of addition of reagents were experimented and it was found that the order of addition of reagents cited under general procedure was optimum and was used in all subsequent experiments.

Effect of reaction time

In spite of the rapid color development (formed immediately) the color intensity reached a maximum after SBS solution had been reacted with DONA and sodium hydroxide for 20 min, therefore 20 min development time was selected as optimum in the general procedure .The color obtained was stable for 3hr.

Accuracy and precision

To determine the accuracy and precision of the method, SBS was determined at three different concentration .The overall relative standard deviations and recoveries were summarized in Table (1).Small relative standard deviation (less than 1.992) and a good recovery (98.844-102.874) indicated high precision and accuracy of the proposed method.

Working curve

Under the recommended conditions described above and mentioned in the general assay procedure, a linear calibration graph (Fig.3) for SBS was obtained, which shows that Beer's law obeyed over the concentration range of 2-40 ppm with a correlation coefficient of 0.9992. The conditional molar absorpativity of the

product formed with SBS was found to be 1.5802×10^4 $L.mol^{-1}.cm^{-1}$ with reference to the SBS and sandell's sensitivity was 0.0365 $\mu g.cm^{-2}$ [Table2] .

Analytical Applications

The suggested method was applied to the quantitative determination of SBS in pharmaceutical formulations. Two types of tablets containing SBS have been analyzed and they gave a good accuracy and precision as shown in Table (3).

The proposed method was compared successfully with the British pharmacopeia's standard method, since F-test and T-test showed that there was no significant differences between the proposed and official methods [15] as shown in Table(4).

Conclusion

A simple, rapid and sensitive spectrophotometric method has been developed for the determination of trace amount of SBS in aqueous solution based on diazotization coupling reaction with DONA reagent in the presence of sodium hydroxide .

The proposed method does not require temperature control or solvent extraction step [Table 5], the method was applied successfully to pharmaceutical tablets containing SBS.

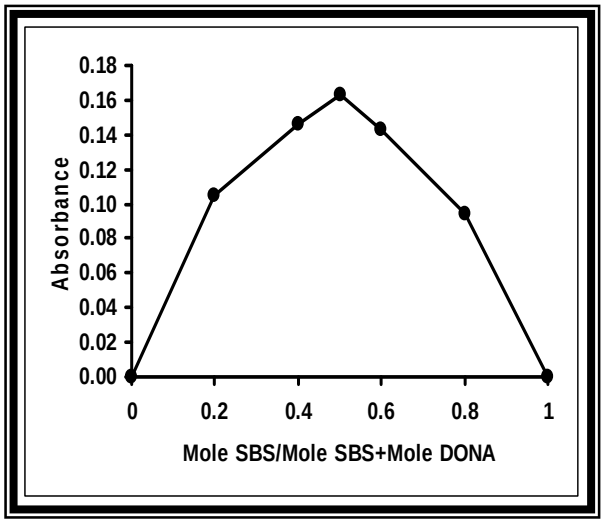


Figure (1): Job's method for the complex produced

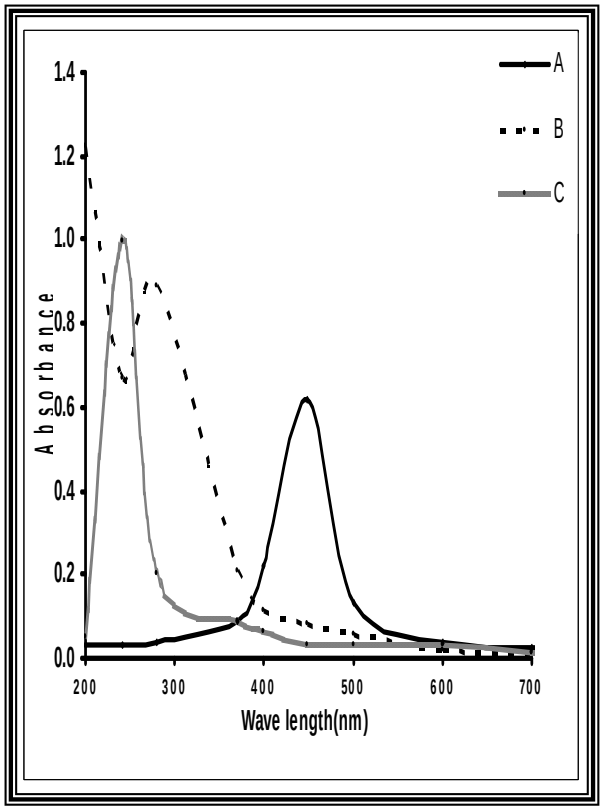


Figure (2): Absorption spectra of A (20 µg ml^{-1}) of SBS treated as described under procedure and measured against blank , B the reagent blank and C (20 µg ml^{-1}) of SBS measured against distilled water.

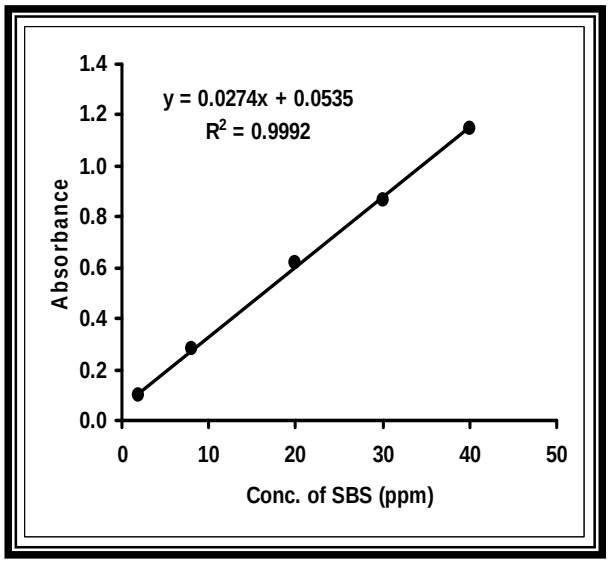


Figure (3): Calibration curve of Salbutamol sulphate

Table (1): Accuracy and precision of the proposed method.

Amount of SBS ($\mu g.ml^{-1}$)		Recovery %*	R.S.D %*
Present	Found		
8.000	8.229	102.874	0.735
30.000	29.653	98.844	1.992
40.000	40.018	100.046	1.831

* Average of four determinations.

Table (2): Analytical data obtained from proposed method.

PARAMETER	VALUE
Beer's Low limits($\mu g.ml^{-1}$)	2-40
Molar absorbativity ($lit.mole^{-1}.cm^{-1}$)	1.5802×10^{4}
Sandell's sensitivity($\mu g.cm^{-2}$)	0.03649
Slope(b)	0.0274
Intercept(a)	0.0535
Correlation coefficient (R^2)	0.9992
λ_{max} (nm)	448
R.S.D (%)	≤1.9915
Limit of detection ($\mu g.ml^{-1}$)	0.3656

Table (3): Application of the proposed method for the determination of SBS in pharmaceutical preparations.

SBS Sample	SBS ($\mu g.ml^{-1}$)		R.S.D %*	Rec. %*	Average %Rec.
	Taken	Found			
Butadin tablets (SDI)	20.000	20.323	0.772	101.613	101.326
	30.000	30.312	0.439	101.039	
Butadin tablets (Dijla)	20.000	20.387	1.236	101.936	100.564
	30.000	29.758	1.143	99.192	

* Average of five determinations.

Table (4): Comparison of the proposed method with standard method to determination of SBS in pharmaceutical injections.

SBS Sample	Recovery%*	
	Proposed method	Standard method**
Pure salbutamol sulphate	100.00	100.00
Butadin tablets (SDI)	101.33	101.15
Butadin tablets (Dijla)	100.56	98.55

* Average of five determinations.

* * British Pharmacopoeia standard method[15].

Table (5):Comparison of the proposed method with some of the visible spectrophotometric methods for the determination of SBS

Reagent(s)used	Wave length (nm)	Limits $\mu g.ml^{-1}$	Remarks	Ref.
Chloramine-T N,N-Dimethyl-p-phenylenediamine	620	10-40	Extraction with butan-2-ol	16
CeriumIV/3-Methylbenzothaiazolin2-onehydrazone	530	Up to 15	Extraction ,expensive reagent	17
Ferricyanide 4-aminophenazone	505	25-175	Heating ,waiting for 30 min	18
Diazotized o-nitroaniline	448	2-40	Reaction carried out at room temperature(25 ºC)	The present work

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