DEVELOPED SPECTOPHOTOMETRIC DETERMINATION OF SALBUTAMOL SULPHATE IN PHARMACEUTICAL SAMPLES BY COUPLING WITH DIAZOTIZED 4-AMINOACETOPHENONE

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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 4-aminoacetophenone (DAAPH) in alkaline medium to form an intense yellow-orange, water soluble dye that is stable and shows maximum absorption at 463 nm .A graph of absorbance versus concentration indicates that Beer's law is obeyed over the concentration range of 12.5-750.0 μg of SBS in a final volume of 25-mL (i.e. 0.5-30.0 ppm), with a molar absorbtivity 2.722×10^4 $L.mol^{-1}.cm^{-1}$,a sandell's sensitivity of 0.0212 $\mu g.cm^{-2}$, a relative error of (–1.314) –0.763 % and a relative standard deviation of 0.216-1.131 % 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.

Key words: Salbutamol sulphate, diazotization, spectophotometry.

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 chromato-graphy-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 aminoantipyrin [10], sulphonamide [11] and dopamine [12].

Aim of Research

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 DAAPH in alkaline medium to form an intense yellow-orange color product which shows an absorption maximum at 463 nm.

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 sulbutamol 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 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 4-aminoacetophenone solution (3×10^{-3}M)

Prepared daily by dissolving 0.0405 gm of AAPH in 5 mL ethanol, 20 mL distilled water and 2 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 12.5-750.0 µg of SBS was transferred into a series of 25 mL standard flasks to cover the range of 0.5-30 µg. mL^{-1}. A volume of 3 mL of 3×10^{-3}M DAAPH 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 463 nm (at room temperature 20°C). The color of the formed dye is stable for more than 5hr.

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. A samples of 375 and 500 µg of SBS in a final volume of 25 mL were taken and the measurements were carried out as described earlier under general procedure.

Results and discussion

Absorption spectra

When a very diluted aqueous solution of SBS was mixed with DAAPH 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 463 nm .Fig.(1) shows the spectra of the product formed and the reagent blank, the maximum absorption at 463 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 DAAPH solution were added to a fixed concentration of SBS, 3mL of 3×10^{-3} M DAAPH solution was sufficient to develop the color to its full intensity and give minimum blank value; above 3 mL the absorbance of blank value increased ,causing a decrease in the absorbance of sample .Therefore ,3 mL of 3×10^{-3} M DAAPH solution was used for all experiments.

Effect of alkaline solution

Preliminary results indicated that the presence of base 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 after the diazotized reagent, to produce the optimum absorbance (minimum blank and high color stability).

Effect of order of addition

Different orders of addition of reagents were examined and it was found that the order of addition of reagents cited under general procedure 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 DAAPH 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 5hr.

Accuracy and precision

To determine the accuracy and precision of the method, SBS was determined at three different concentrations .The results are shown in Table (1); indicate that a satisfactory precision and accuracy could obtained with the proposed method.

Calibration graph

Under the recommended conditions described above and mentioned in the general assay procedure, a linear calibration graph Fig.(2) for SBS was obtained, which shows that Beer's law obeyed over the concentration range of 12.5-750.0 µg/25 mL or 0.5-30 ppm with a correlation coefficient of 0.9992. The conditional molar absorptive of the product formed with SBS was found to be 2.722×10^4 $L.mol^{-1}.cm^{-1}$ with reference to the SBS and sandell's sensitivity was $0.0212 \mu g.cm^{-2}$ Table (2).

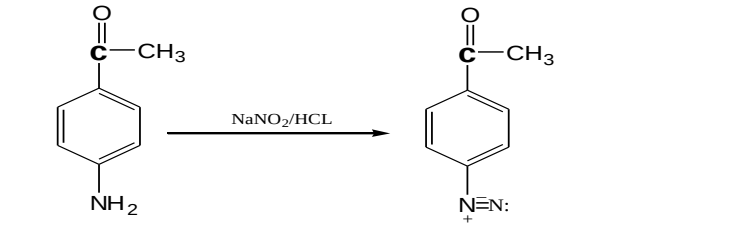
Analytical Applications

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 [13] as shown in Table (4).

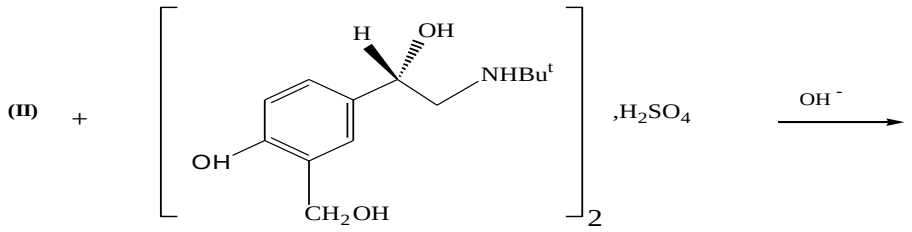
Structure of the product

The stoichiometry of the reaction between each SBS and DAAPH was investigated under the recommended optimum conditions and applying Job's method[14]. The obtained results in Fig.(3) showed that a 1:1 (SBS:DAAPH) product was formed between drug and diazotized reagent at 463 nm, and due to the phenolic nature of the drug, it can readily be coupled with DAAPH according to scheme (1) [15].

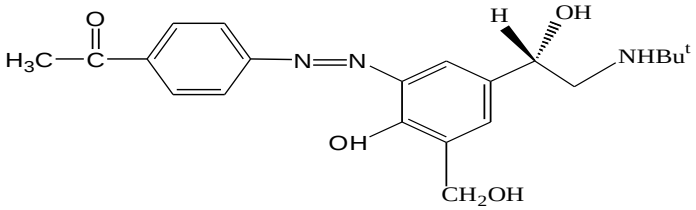


4-aminoacetophenone(AAPH) (I)

Diazotised 4-aminoacetophenone(DAAPH) (II)



Salbutamol sulphate(SBS)



(III) Yellow-orange azo dye

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

The apparent stability constant was calculated by comparing the absorbance of solution containing stoichiometric amount of SBS and DAAPH with that of a solution containing a five-fold excess of DAAPH reagent. The stability constant of the product in water under the described experimental conditions was 3.872×10^5 L.mol^{-1}.

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 DAAPH 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. A stable-soluble yellow-orange color azo dye was formed which can be measured at 463 nm.

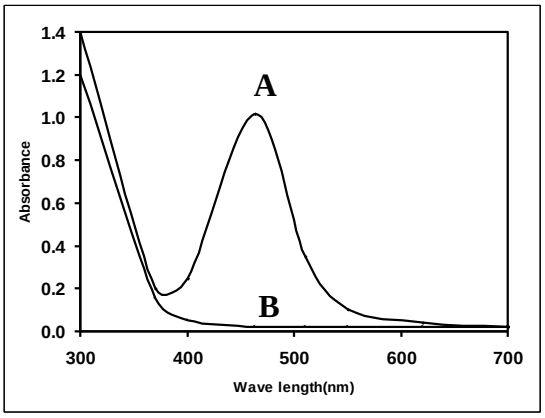


Fig. (1) : Absorption spectra of A (20 µg mL^{-1}) of SBS treated as described under procedure and measured against blank and B the reagent blank measured against distilled water.

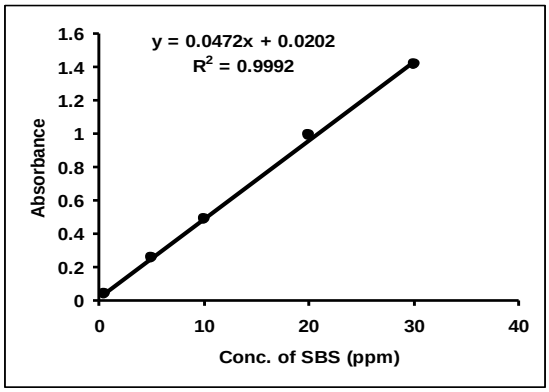


Fig.(2) : Calibration graph of Salbutamol sulphate.

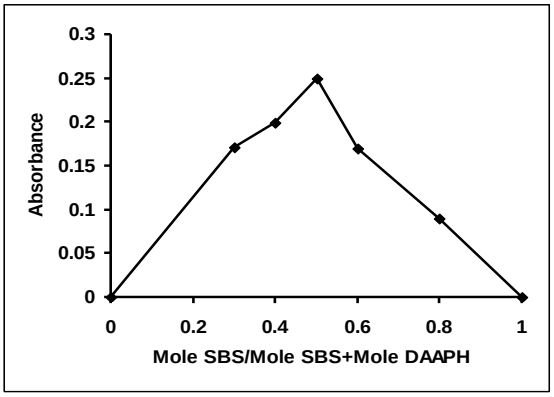


Fig.(3) : Job's method for Salbutamol sulphate.

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

Amount of SBS (µg.mL^{-1})		Recovery %*	R.S.D %*
Presen	Found		
5.00	5.038	100.763	0.216
10.00	9.869	98.686	0.545
30.00	29.636	98.785	1.131

Table (2)
Analytical data obtained from proposed method.

Parameter	Value
Beer's Law limits(µg.mL^{-1})	0.5-30.0
Molar absorpativity (lit.mole^{-1}. cm^{-1})	2.722×10^4
Sandell's sensitivity (µg.cm^{-2})	0.0212
Slope(b)	0.0472
Intercept(a)	0.0202
Correlation coefficient (R^2)	0.9992
λ_{max} (nm)	463
R.S.D (%)	<1.131
Limit of detection (µg.mL^{-1})	0.414

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

SBS Sample	SBS (μg.mL^{-1})		R.S.D %*	Rec. %*	Average %Rec.
	Taken	Found			
Butadin tablets (SDI)	15.000	15.447	0.545	103.311	101.855
	20.000	20.079	0.962	100.398	
Butadin tablets (Dijla)	15.000	15.119	0.837	100.745	99.775
	20.000	19.761	1.369	98.805	

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

SBS Sample	Recovery%*	
	Proposed method	Standard method**
Pure salbutamol sulphate	100.00	100.00
Butadin tablets (SDI)	101.86	101.15
Butadin tablets (Dijla)	99.78	98.55

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 μg.mL^{-1}	Molar absorpitivity L.mol^{-1}.cm^{-1}	Remarks	Ref.
Folin-Ciocalteau agent	750	1-15	2.33×10^4	On-line solid phase extraction and flow injection	16
CeriumIV/3-Methylbenzothaiazolin2-nehydrazone	530	Up to 15	2.4×10^4	Extraction ,expensive reagent	17
Ferricyanide 4-aminophenazone	505	25-175	Not reported	Heating ,waiting for 30 min	18
Diazotized 4-minoacetophenone	463	0.5-30.0	2.72×10^4	Reaction carried out room temperature (25ºC)	The present work

الخلاصة

يتضمن البحث تطوير 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 4–امينواسيتوفينون المؤزوت في وسط قاعدي حيث يتكون ناتج اصفر–برتقالي مستقر وذائب في الماء اعطى اعلى امتصاص عند طول موجي 463 نانومتر .يشير الرسم البياني للامتصاص مقابل التركيزبان قانون بير ينطبق ضمن مدى التركيز 12.5–750.0 مايكروغرام من كبريتات السلبيوتامول في حجم نهائي 25 مل (اي ما يكافئ 0.5–30.0جزء بالمليون) وكانت قيمة الامتصاصية المولارية مساوية الى 2.722 ×10^4 لتر.مول$^{-1}$.سم$^{-1}$ وقيمة حساسية ساندل 0.0212 مايكروغرام .سم$^{-2}$ مع خطأ نسبي (– 1.314)– 0.763 % وانحراف قياسي نسبي 0.216-1.131% أعتمادا على مستوى التركيز المراد تقديره.تمت دراسة الظروف المثلى للتفاعل وتطبيق الطريقة على المستحضرات الصيدلانية الحاوية على كبريتات السلبيوتامول.

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