Spectrophotometric determination of salbutamol sulphate in bulk and in pharmaceutical formulations

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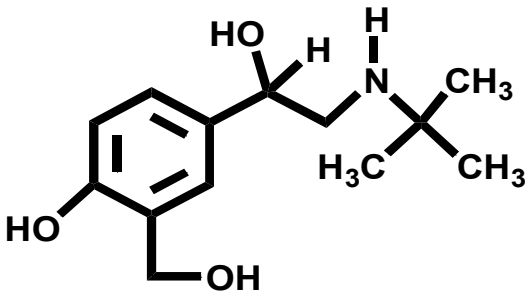
Abstract

A new simple and sensitive colorimetric method has been developed for determination of salbutamol in aqueous solutions. The method is based on the formation of a yellow colored azo dye by diazotization of 2,4-dichloroaniline, followed by a azo-coupling reaction between the resulting product and salbutamol. The maximum absorbance of azo dye at 447 nm. Beer's law was found to be obeyed in the concentration range of 2.5-20 μg/ml with range of molar absorptivity between(4000-7265) $Lmol^{-1}cm^{-1}$. The optimum reaction conditions and other analytical parameters were evaluated.

Keywords:-Determination**,** salbutamol, 2,4-dichloroaniline azo Coupling Reactions.

Introduction

Salbutamol (Ventolin) is a 2-sympathicomimetic drug, which was granted a marketing authorization in 1973. It is indicated for the treatment of reversible air way obstruction in bronchial asthma, chronic bronchitis and emphysema[1]. Salbutamol [1-(4-hydroxy-3-hydroxymethylphenyl)-2-tert-butylamino-ethanol] with formulae structural explain below is widely used as a bronchodilator in the treatment of respiratory diseases in humans [2, 3].



Salbutamol

Some different methods of analysis have been reported for the determination of SBS, including HPLC [4–6] and spectrophotometric[7-9],Flow-injection spectrophotometric [10], liquid chromatography(11) diazotization and coupling [12,13]. In current study describes assay sensitive colorimetric method for SBS in tablets and cyrop.. The method is based on the formation of a yellow colored azo dye by diazotizaiotn of 2,4-chloroaniline, followed by a azo -coupling reaction between the resulting product and alkali salbutamol sulphate solution. The proposed method have the advantages of being rapid and simple

Materials and Methods:

Equipment:

All spectral and absorbance measurements were by using a Computerize UV-Visible, ,shimadzu T60U Spectrophotometer, with 1cm matched quartz cell

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Chemicals and Reagents

All chemicals used were of analytical reagent grade and salbutamol sulphate standard material was provided from state company for Drug Industries and Medical appliance-(SDI) Samara -Iraq. of (99% purity) was obtained from (BDH)

1-A stander solution of 100 µg ml^{-1} salbutamol sulphate was freshly prepared by dissolving 0.01gm of salbutamol sulphate with distilled water to 100 ml

2-.2,4-dichloro aniline of (98.0% purity) was obtained from (Merck) a stander solution of 100 µg ml^{-1} was freshly prepared by dissolving 0.01gm of 2,4-dichloro aniline in 10ml absolute ethanol and then diluted with distilled water to 100 ml.

3-Sodium nitrite (99.8 purity) from(BDH) and stander solution of 1% was prepared.. **4-**Sodium Hydroxide of (98% purity) from (RDL), solution of 1M was prepared by dissolving 4 gm in 100 ml distilled water

5-1M HCl was prepared and used.

General procedure:

The 0.5 ml of Salbutamol standard solution 100µgml^{-1} and 0.5 ml of 1M Sodium Hydroxide solutions were added to 0.5 ml of of 2,4-dichloroaniline and 0.5 ml of 1% sodium nitrite and 0.5 ml of 1M HCl were mixed in and completed with distilled water to the mark in10 ml volumetric flask and shaked for 2 minutes, with shaking and cooling ice bath for 2 minute, after 5 minutes the yellow color is completely developed and the absorbance measurement was carried out at a wavelength at 447 nm, against a blank solution prepared in the same method but without Salbutamol.

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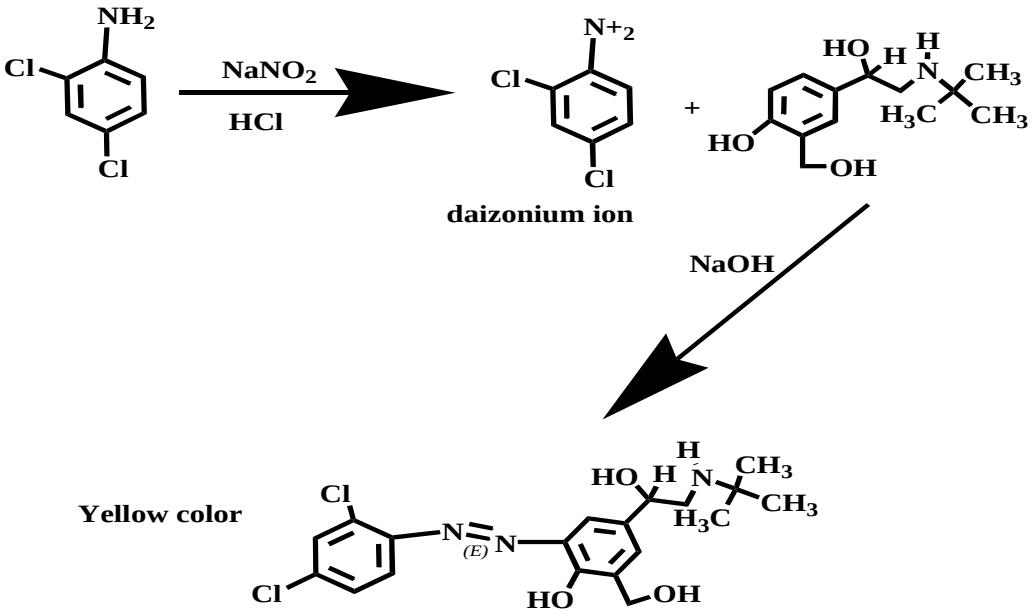
Procedure for Assay of Salbutamol Salphate in Pharmaceutical Preparations

Tablets: ten tablets were weighed and finely powdered. A weighed amount of the powder containing 200 mg of Salbutamol Salphate (equivalent to one tablet) was dissolved in 50 ml of volumetric flasks and diluted up to the mark to obtain 200 µg ml^{-1}.

Oral Solution 2 ml was taken from container containing 400 µg$ml^{-1}$of Salbutamol Salphate was transferred into 100 ml volumetric flasks and diluted up to the mark with distilled water. Working standard was prepared by suitable dilution and the recommended procedure was used for Salbutamol Salphate for its determination.

Reaction mechanism of the method :

Salbutamol forms colored products after coupling with an electrophilic of 2,4-dichloroaniline diazotization ion in the presence of alkaline medium The following mechanism has explain the reaction



4-(2-tert-Butylamino-1-hydroxy-ethyl)-2-(2,4-dichloro-phenylazo)-6-hydroxymethyl-phenol

Results and discussion:

The result of this investigation indicated that the reactions between Salbutamol with 2,4-dichloroaniline in the presence of sodium nitrite and hydrochloric acid yield highly soluble colored condensation products which can be utilized as a suitable assay procedures for Salbutamol These colored products have maximum absorption at 447 nm and. The blank at these wave lengths shows zero absorbance. (Figures 1,A). The influence of various reaction variables on the color development was tested to establish the most favorable conditions

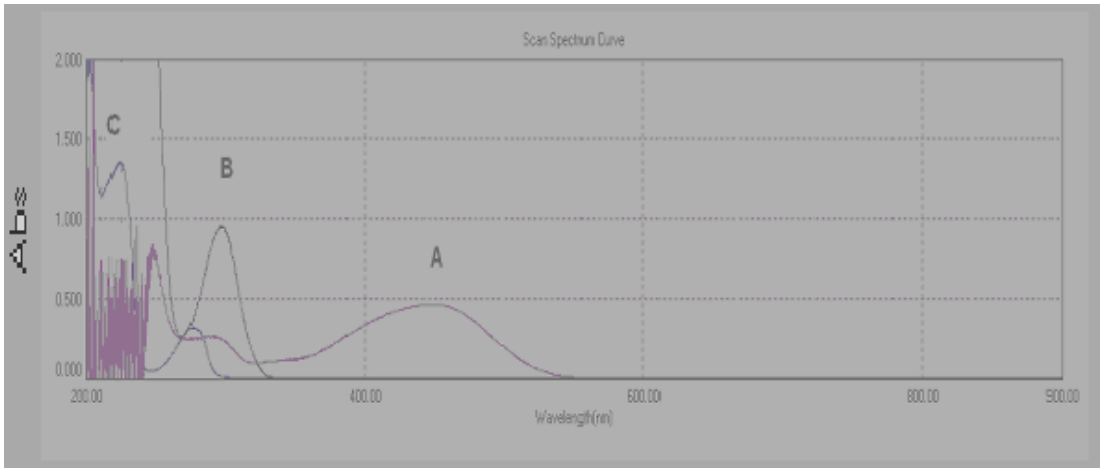


Figure 1: **(**A)Absorption spectrum of the azo dye formed ,salbutamol (10mg/ml) and 2,4-dichloroaniline**.,** (B)Absorption spectrum of: 2,4-dichloroaniline .**(**C) Absorption spectrum of salbutamol**.**

Development time and stability period :
The color intensity reached maximum after formation of azo dye of salbutamol. The color obtained was stable for at least 1day and this stability, period was sufficient to allow several measurements to be performed sequentially.

Effect of Base: It was found that the presence of a base led to increase the intensity of the produced product so;1M of NaOH was selected which was found that the best volume equal to 0.75 ml of this base give high sensitivity which selected in subsequent experiments Fig 2.

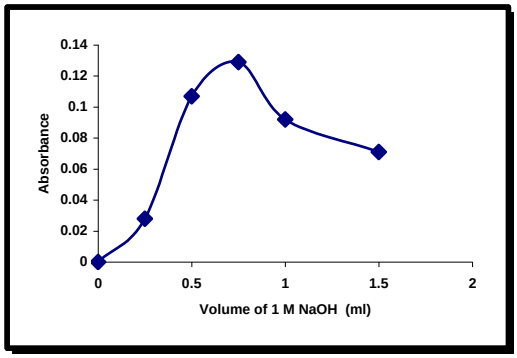


Fig 2: Effect of Sodium hydroxide volume

Effect of acid:
It was found experimentally that the colored products were formed stable by. using of the amount of 0.5 ml of (1M)hydrochloric acid and then used in determination of salbutamol Fig 3.

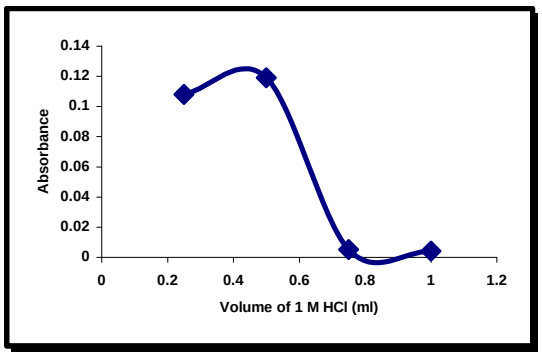


Fig 3 : Effect of hydrochloric acid volume

Effect of sodium nitrite
The optimum concentration of sodium nitrite solution that gave maximum absorption was found to be 0.5ml of 1% of sodium nitrite solution. Fig.4 explained these results.

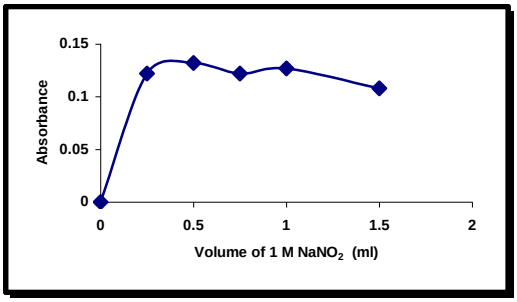


Fig 4: Effect of Sodium Nitrite volume

Effect of reagent concentration:
The effect of various concentrations of 2,4-dichloroaniline were investigated using the proposed procedure and adding 0.5-6 ml of 100µg ml^{-1} 2,4-dichloroaniline. It was found necessary for developing the colored products 5 ml of 100µg ml^{-1} 2,4-dichloroaniline solution in final volume of 10 ml. Fig. 5 explained these results.

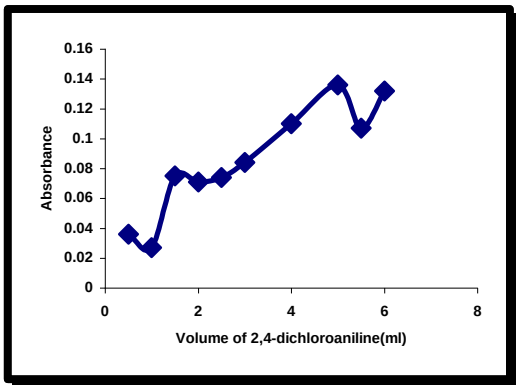


Fig.5: Effect of the volume of reagent .

Calibration curves :
Employing the conditions described in the procedure, a linear calibration graph for salbutamol is obtained Fig. 6, which shows that Beer's law is obeyed over the concentration range of **2.5-20** µg/ml with correlation coefficient of

0.9998 and The conditional molar absorptivity of the red product formed was found to be **4000-7265** L.mol^{-1}.cm^{-1}.

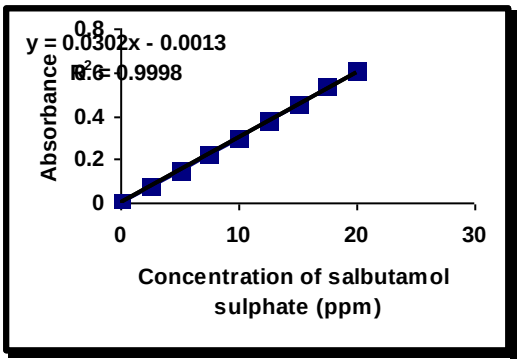


Fig.6 the calibration curve of salbutamol.

Order of addition of reagents :

To obtain the optimum results, the order of addition of reagent and other chemicals should be added to formation the azo dye are given by the following sequence volumes were taken in three procedures:

A- 5ml of 100µg ml^{-1} 2,4-dichloroaniline., 0.5ml 1% sodium nitrite and, 0.5 ml of 1M hydrochloric acid , 2ml of 100µg ml^{-1} salbutamol and0.75ml of sodium hydroxide

B- 5ml of 100µg ml^{-1} 2,4-dichloroaniline., 0.5 ml of 1M hydrochloric acid, 0.5ml 1% sodium nitrite and, 2ml of 100µg ml^{-1} salbutamol and 0.75ml of sodium hydroxide

C- 5ml of 100µg ml^{-1} 2,4-dichloroaniline., 0.5 ml of 1M hydrochloric acid, 0.5ml 1% sodium nitrite , 0.75ml of sodium hydroxide and 2ml of 100µg ml^{-1} salbutamol

The absorbance of three methods (A,B,C) were 0.196, 0.207, 0.039 respectively. Method(b) demonstrate the best of the order of addition of reagent and other chemicals which gave azo dye had high intensity and high absorbance at the wavelength.447nm.

Composition of the Formula structure :

The composition of the formula structure of azo dye were studied by the mole ratio Method(14,15). A break of 1:1 suggested the formation of salbutamol drug with 2,4-di chloroaniline Fig.7 explain the formula of azo dye.

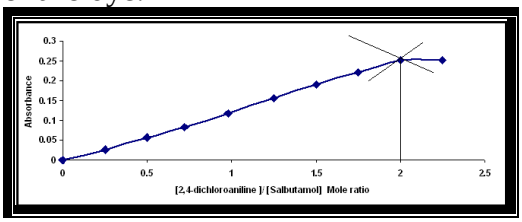


Fig 7: Mole ratio of the azo dye of salbutamol and 2,4-di chloroaniline.

The apparent stability constants were calculated by comparing the absorbance of solution containing a similar amounts of salbutamol with 2,4-dichloroaniline that of a solution containing a five-fold excess of reagent. The average conditional stability constants of the dye in water, under the described experimental conditions is 0.12×10^4

Effect of Organic solvents

The effete of organic solvents such us methanol ,ethanol ,ether and distill water were studied by using in the dilution and measuring the absorbance the absorbance were found 0.38,0.311,0.408 and 0.466 respectively. We used distill water because it had best absorbance for it is abundance.

Effect of Interferences

The effete of interference by common organic compounds was determined by measuring the absorbance of a dye solution containing 1 ml of $4x10^{-4}$M of glucose

and various amounts of other species such us p-phenylen diamine ,O-aminophenol,4-chloro nitro aniline , resosenol ,paracetamol ,salicylic acid, and starch .The results showed that 4-chloro nitro aniline major of the common compound does interfere The results are given in Table (1).

Table (1) Effects varies interference of organic compounds on the absorption.

Interference	Without addition	glucose	p-phenylen diamine	o-aminophenol	4-chloro nitro aniline	resosenol	paracetamol	salicylic acid	starch
Absorbance		0.144	0.136	0.192	0.214	0.062	0.166	0.092	0.196

Accuracy and precision :

To determine the accuracy and precision of the method, salbutamol is determined at three different concentrations. The results shown in Table 2 indicate that satisfactory precision and accuracy could be attained with the proposed method

Table 2. Accuracy and precision of the proposed method.

Concentration of Salbutamol µg ml^{-1}	* Recovery%	Average recovery%	%E Relative Standard error	Relative Standard Deviation* %
5	101.2	99.96	1.2	2.55
15	99.13		0.86	2.75
20	99.55		0.45	3.25

* **Average of five determinations**

Analytical application

Proposed method have been used of two types of drug containing salbutamol sulphate (tablet and **Oral**) and they gave good accuracy and precision, the proposed method was compared with brititish pharmocopeias standard method ,since F-test,T-test showed that three was no significant differences between the proposed methd and official method(16),the results obtained were tabulated in Table 3.

Table 3: Determination of salbutamol sulphate in pharmaceutical preparations by the proposed method and comparison with the British pharmacopoeia method

Procedure Applied	Pharmaceutical Formulation*	Recovery%	%E Relative Standard error	Relative Standard Deviation* %
Proposed method	Tablets [a] Ventorain	100.2	0.2	2.3
	Oral Solution [a]Salorain	99.87	0.125	4.3
British Pharmacopoeia method [17]	Tablets [a] Ventorain	100	……	…….
	Oral Solution [a]Salorain	100.15	……..	……..

* Mean of three determinations. a Marketed by S.D.I, Iraq.

Conclusions :

The present study demonstrates an excellent approach for the development of spectrophotometric method for determination of salbutamol sulphate high selectivity and excellent sensitivity for the oxidative coupling reaction of salbutamol sulphate are achieved with 2,4-dichloroaniline. the method was applied successfully on pharmaceutical samples.

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تقدير دواء كبريتات السالبيتيمول بطريقة طيفية في المادة النقية وفي المستحضرات الصيدلانية

مها كاظم سلمان *

*جامعة بغداد / كلية العلوم للبنات / قسم الكيمياء ، بغداد ، العراق .

الخلاصة

طُوّرتْ طريقة لونية جديدة بسيطة وحسّاسة لتعيين تركيز السالبيتمول في المادة النقية وفي بعض المستحضرات الصيدلانية إنّ تعتمد الطريقةَ على الحصول على لون لصبغة الازو الصفراء عند طول موجي 447 نانومتر والمتكونة مِنْ ازدواج السالبيتمول مع ملح الديازونيوم ل 2و4- ثنائي كلورو أنيلين, قانون بير وجد مطاعاً في مدى تركيزَ 2.5-20 ملغم/لتر مع ظهور مدى لقيم الامتصاصية المولارية بين(7265-4000) لتر/مول.سم.وقد قدرت الظروف المثلى للتفاعل وكذلك العوامل التحليلية الأخرى.