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Spectrophotometric Assay of Salbutamol Sulphate in Pharmaceutical Preparations by Coupling with Diazotized ρ-bromoaniline

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Abstract:

In this research, salbutamol sulphate (SAS) has been determined by a simple, rapid and sensitive spectrophotometric method. Salbutamol sulphate in this method is based on the coupling of SAS with diazotized ρ- bromoaniline reagent in alkaline medium of Triton X-100 (Tx) to form an orange azo dye which is stable and water-soluble. The azo dye is exhibiting maximum absorption at 441 nm. A 10 - 800 µg of SAS is obeyed of Beer's law in a final volume of 20 ml, i.e., 0.5- 40 ppm with ε, the molar absorptivity of 48558 $L.mol^{-1}.cm^{-1}$ and Sandell's sensitivity index of 0.01188 $\mu g.cm^{-2}$. This new method does not need solvent extraction or temperature control which is well applied to determine SAS in different types of pharmaceutical preparations.

Key words: Azo dye, diazotization coupling reaction, pharmaceuticals determination, spectrophotometric method, Triton X-100.

Introduction:

Salbutamol sulphate (SBS), SBS is chemically (RS) 2(hydroxymethyl)-4-{1-hydroxy-2-[(2-methyl-2-propanyl)-amino] ethyl} phenol sulfate (2:1) Fig. 1 (1,2).

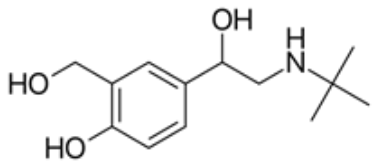

Figure 1. The chemical structure of salbutamol

Salbutamol is used in the treatment of asthma and chronic obstructive pulmonary disorder (COPD). It is 29 times more selective for beta2 receptors than beta1 receptors giving it higher specificity for pulmonary beta receptors versus beta1-adrenergic receptors located in the heart (3). It is also known as albuterol (4).

It's very slightly soluble or practically insoluble in ethanol (96 %) also in the methylene chloride but its soluble freely in water (5).

Many methods have been reported for the determination of SBS, these include HPLC, Reverse phase high performance liquid chromatography (4,6,7,8) , achiral supercritical fluid chromatography (9), ion pair liquid chromatography (10),atomic absorption spectrophotometric (11), Differential pulse voltammetric (12), titrimetric method (13), continuous/stopped flow injection method (14), spectrophotometric methods (1, 15, 16), first-order derivative spectroscopy (17) and Second Order Derivative method (18). The purpose of the present study is to develop a simple and sensitive method for the determination of salbutamol sulphate in pharmaceutical preparations using diazotization coupling reaction. The proposed method is based on a coupling reaction between salbutamol sulphate and ρ- bromoaniline reagent in alkaline medium of Triton X-100 (Tx) to form an orange azo dye which is stable, water-soluble and exhibits maximum absorption at 441 nm.

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Material and Methods

Apparatus

All measurements are performed using Spectro UV-VIS Double Beam UVD-3500, with 1-cm match quartz cells. The pH measurements are carried out using (HANNA pH-211) pH meter.

Reagents

All chemicals used are of the highest purity available.

Stock salbutamol sulphate solution, 1000 µg/mL.

It is prepared by dissolving 0.1 g of salbutamol sulphate (from the state company for drug industries and medical appliances- Ninevah, N.D.I) in distilled water and the volume is

completed to 100 ml with distilled water in a volumetric flask.

Working salbutamol sulphate solution, 100µg/mL.

Dilute salbutamol sulphate solutions are prepared by diluting the stock solution of salbutamol sulphate with the necessary volume of water.

Sodium hydroxide solution, 1N.

It is prepared by dilution of the concentrated volumetric (Fluka) solution with distilled water and then transferred to plastic bottle.

Triton X-100 solution (1%).

This solution is prepared by dissolving 1.0 g of Triton X-100, (BDH) in distilled water and the volume is completed to 100 ml in a volumetric flask.

Diazotized ρ-Bromoaniline reagent solution (DPBA), 15 mM.

A 0.2580 g of ρ-bromoaniline (Ridel-deHaën) is dissolved in about 75 ml of distilling water. The clear mixture is cooled to 0-5°C in an ice-bath after heating the solution containing 2 ml of concentrated HCl. Then 0.11 g of $NaNO_2$ is added and the mixture is stirred vigorously. The solutions made up to volume in 100 ml volumetric flask after 5 minutes with cold water. It is kept in a refrigerator in a brown bottle and is stable for at least 3 days.

Butadin tablets solution 100mg/L.

This solution is prepared by dissolving finally powdered 5 tablets of butadin drug (each tablet contains 2 mg salbutamol sulphate) in 10 mL ethanol with 20 mL distilled water, shake and warm the solution if necessary. Then the solution is Filtered into a 100 mL volumetric flask, wash the residue with distilled water and dilute to 1000 mL with distilled water to obtain 100 mg/L salbutamol sulphate.

Butadin syrup solution 100 mg/L.

The content of the container of butadin syrup was mixed (each 5 mL contains 2 mg salbutamol sulphate), 12.5 mL of the syrup diluted to 50 mL with distilled water to prepare 100 mg/L salbutamol sulphate solution.

Results and Discussion:

Study of the optimum reaction conditions

Optimum conditions of various parameters related to the orange dye formation have been studied .

Effect of base

The preliminary experiments have shown that the dye develops only completely in alkaline medium. Different volumes of bases (strong and weak) have been used, the results indicate that 0.3 mL of 1N NaOH gave highest intensity. Weak bases (Na_2CO_3, $NaHCO_3$ and NH_3) gave turbid solutions, 0.3 mL of 1N NaOH is recommended for the subsequent experiments.

Effect of diazotised ρ-bromoaniline concentration on absorbance

Effect of different concentration of DPBA (5 mM, 15 mM and 25 mM) with different amount of DPBA(0.1, 0.3,0.5,0.7,1 and 2 mL) have been studied .A 1 mL of 15 mM of DPBA gave the highest intensity therefore it has been recommended for the sequent experiments.

Effect of different acid on the diazotised ρ-bromoaniline

The effect of 1 N of acids (HCl, H_3PO_4, H_2SO_4 and CH_3COOH) in different ratio 1:8, 1:16 and 1:30 on the amount of DPBA have been studied. The results shows that 1 mL of 15 mM of DPBA using HCl (1:16) gave the highest intensity. Acetic acid gave turbid solutions. A 1 mL of 15 mM of DPBA using HCl (1:16) is recommended for the subsequent experiments.

Order of addition

The DPBA reagent used in the investigation has been added to the SAS in different orders to find which of the orders is optimum. The results are given in (Table 1).

Table 1. The order of addition

Reaction component	Order of addition	Absorbance	
		Sample	Blank
SAS+ DPBA +NaOH	I	0.188	0.045
SAS+NaOH+DPBA	II	0.158	0.052

Order I is recommended in the subsequent investigation for its relatively higher absorbance.

Effect of surfactants

Effects of different types and amounts of surfactants in various orders have been studied (cetyltrimethylammoniumbromide CTAB, sodium dodecyl sulphate SDS, TritonX-100, Tween80). As Shown in (Table 2), 5 mL of 1% TritonX-100 in order I are chosen in the subsequent experiments since they give the higher absorbance.

Table 2. Effect of surfactants on absorbance

Surfactants	Order of addition		Absorbance/ml of surfactant		
			1	3	5
CTAB	I	S	0.209	0.205	0.195
(1×10^{-3}) M		B	0.034	0.032	0.042
	II	S	0.185	0.278	0.196
		B	0.032	0.031	0.044
	III	S	0.166	0.165	0.280
		B	0.030	0.036	0.037
SDS	I	S	0.129	0.101	0.108
(1×10^{-3}) M		B	0.025	0.037	0.035
	II	S	0.142	0.127	0.130
		B	0.032	0.035	0.036
	III	S	0.198	0.242	0.181
		B	0.035	0.033	0.040
Triton X-100	I	S	0.396	0.455	0.551
(1%)		B	0.037	0.048	0.063
	II	S	0.394	0.476	0.534
		B	0.041	0.053	0.078
	III	S	0.183	0.201	0.286
		B	0.039	0.049	0.060
Tween 80	I	S	0.364	0.503	0.522
(1%)		B	0.058	0.106	0.106
	II	S	0.346	0.491	0.503
		B	0.079	0.108	0.132
	III	S	0.182	0.274	0.344
		B	0.064	0.091	0.106

Effect of time on color development

The effect of time on colour formed of the dye with different amount of SAS amount (50,100 and 200) µg has been investigated under the optimum experimental conditions described above. The results indicate that the colour formed within about one minute and remained stable for at least 1 hour.

Final absorbance spectra:

According to the recommended procedure when salbutamol sulphate in aqueous solution is reacted with diazotized p-bromoaniline reagent solution, an absorption peak is obtained showing intense orange dye absorption at 441nm as shown in Fig. 2.

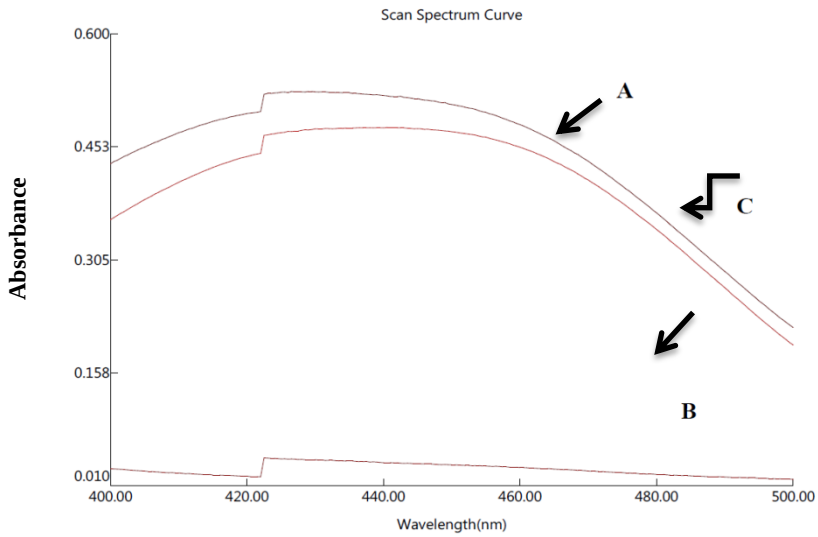


Figure 2. Absorption spectra of 100 µg of salbutamol sulphate /20 mL reacted as the recommended procedure and measured: (A) against blank, (B) against distilled water and (C) blank against distilled water.

Procedure and calibration grap

The effect of the SAS concentration is studied according to the optimum reaction conditions, by transferring aliquots of SAS solution into 20 ml volumetric flasks to cover the range 10-1000 of SAS. The 5 mL of Triton X-100 (1%), 1 mL of 15 mM diazotized ρ-bromoaniline and 0.3 mL of NaOH (1N) and the volume are completed to the mark with distilled water. The absorbance at 441 nm is measured for reaction mixtures against the reagent blank prepared in the same manner but without SAS.The resulting calibration graph obtained is shown in Fig. 3.

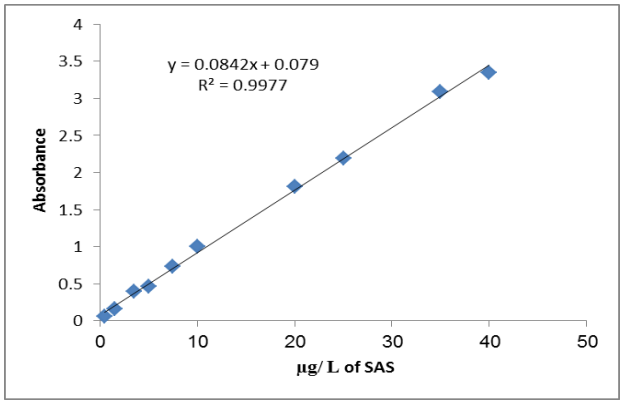
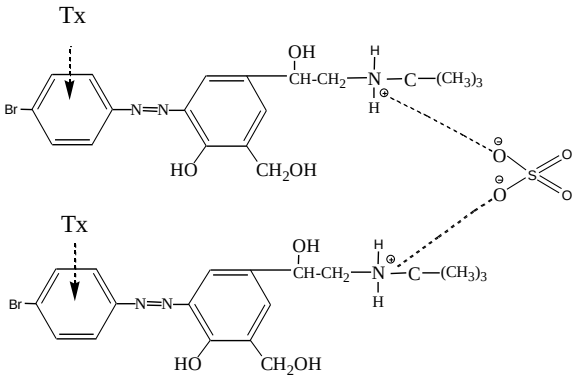


Figure 3. Calibration graph for SAS determination with diazotized ρ-bromoaniline reagent

The graph is a straight line passing through the origin. The absorbance is linear for 10 - 800 µg SAS/20 mL; i.e., 0.5- 40 µg/L. The molar absorptivity of the azo dye is calculated from the equation of calibration graph and found to be 48558 $L.mol^{-1}.cm^{-1}$ with Sandell's sensitivity index of 0.01188 $\mu g.cm^{-2}$. At higher concentration of SAS, a negative deviation is observed, Fig.3. The r^2 (coefficient of determination) is 0.9977, showing an excellent linearity.

The dye nature

Job's and mole-ratio methods (19) indicated that the dye has a composition of 1:2 SAS to diazotized ρ-bromoaniline reagent,i.e., 1:1salbutamol sulphate to diazotized ρ-bromoaniline. The proportion of Triton X-100 in the dye is determined by Job's and mole-ratio method which indicates that a molar ratio of diazotized ρ-bromoaniline to Triton X-100 is 1:2. The dye is thus, formulated as SAS $(DPBA)_2(Triton\ X\text{-}100)_4$. Therefore, the structure of the formed dye may be written as follows, scheme 1:



Scheme 1. Structure of orange azo dye

Stability constant of SAS- DPBA azo dye can be found by this equation (20):

$$K = \frac{C(1-\alpha)}{(\alpha C)(2\alpha C)^2} = \frac{1-\alpha}{4\,\alpha^3 C^2}$$

Knowing the value of α from the equation:

$$\alpha = \frac{A_m - As}{A_m}$$

Where Am is the absorbance of the solution containing excess amount of DPBA and As is the absorbance of the solution containing stoichiometric amounts of DPBA and C are essentially the same as the concentration of SAS. The conditional constant (K) can readily be calculated as shown in Table 3.

Table 3. Stability constant of SAS-DPBA azo dye

Ml of 3.55×10^{-4} M SAS/20 ml	Absorbance		α	K, M^{-2}
	A_s	A_m		
1	0.103	0.440	0.766	4.1312×10^8
2	0.246	0.890	0.724	1.4430×10^8

The stability constant value found shows that the azo dye is stable.

Effect of organic solvents

Different organic solvents (aceton, dimethyl sulfoxide, ethanol, formic acid, methanol and n-propanol) have been examined to evaluate their effects on the spectrum of the colored chelate formed by mixing 100 ppm of SAS solution with 5 ml of 1% Triton X-100, 1ml of 15mM DPBA and 0.3 ml of NaOH (1N) and diluted to the mark with different organic solvents in 20 ml volumetric flasks.

The result shows that there is no significant change in the spectral position of the solvents (with reference to water).

Effect of some pharmaceutical excipients on the determination of SAS

The efficiency and selectivity of the proposed method, a systematic study of excipients (e.g., glucose, dextrose gum acacia, lactose and sucrose) that usually present in dosage forms was performed. The results showed that there was no interference from additives or excipients up to 1000 µg in the present method as shown in Table 4 .

Table 4. Effect of some pharmaceutical excipients on the determination of SAS.

Excipients	Recovery* %			
	Amount added µg/ 100 µg SAS	100	300	500	1000
Glucose	102.50	96.93	103.07	94.13
Dextrose	99.29	103.55	102.37	102.36
Gum acacia	95.66	101.37	101.82	102.05
Lactose	97.21	98.60	98.60	100.56
Sucrose	100.47	98.80	99.29	100.70

* From three determinations

Application

The application of the method for the assay SAS in two drugs has been worked out. From the results, it can be shown that good agreement is found between amounts of SAS taken and those measured by the recommended procedure.

Results in Table 5 indicate that the proposed method can be successfully applied to the determination of SAS in pharmaceutical preparation.

Table 5. The results of application.

Type of drug	salbutamol sulphate present (µg/L)	Recovery*,%
Butadin Tablets	40	100.10
	200	99.22
	400	103.10
Butadin Syrup	40	100.20
	200	101.10
	400	101.10

* From three determinations.

Comparison of Method

Table 6 shows the comparison of the present method and literature method for the determination of SAS.

Table 6. The comparison of method

Analytical parameters	Present method	Literature method (21)
medium	alkaline	---
Temperature (C°)	Room temperature	---
λ_{max} (nm)	441	530
Medium of reaction	Aqueous	Non aqueous
Type of reaction	diazotisation	Oxidative coupling
Reagent	Diazotized ρ-bromoaniline	3-Methyl benzthiazoline-2-hydrazone
Beer's law rang (µg/L)	0.5- 40	0.5-15
Colour of the dye	Orang	Red
Application of the method	Determination of salbutamol sulphate in two drugs	Determination of salbutamol sulphate in two drugs

The results in Table (6) shows that the suggested method is simple, sensitive and the method has been applied to determine salbutamol sulphate in two drugs.

Conclusion:

The proposed method is simple, sensitive and does not need solvent extraction or temperature control which is well applied to determine SAS in different types of pharmaceutical preparations.

Conflicts of Interest: None.

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التقدير الطيفي لكبريتات السالبيوتامول في المستحضرات الصيدلانية بطريقة الازدواج مع بارا- بروموانيلين المؤزوت

تماضر عباس حمودي

قسم الكيمياء والكيمياء الحياتية، كلية طب نينوى، جامعة نينوى، نينوى، العراق.

الخلاصة:

طورت طريقة طيفية بسيطة و سريعة وحساسة لتقدير كميات نزرة من كبريتات السالبيوتامول . تعتمد هذه الطريقة على ازدواج كبريتات السالبيوتامول مع كاشف بارا- بروموانيلين المؤزوت في وسط قاعدي من ترايتون اكس 100 لتكوين صبغة آزوية ملونة ذائبة في الماء ومستقرة والتي تظهر اعلى امتصاص لها عند 441 نانوميتر. وكانت حدود قانون بير بحدود 10- 800 مايكروغرام من كبريتات السالبيوتامول في حجم نهائي 20 مل والذي يعادل 0.5-4 جزء لكل مليون بامتصاصية مولارية مقدارها48558 لتر.مول-1.سم-1 ودلالة ساندل 0.01188 مايكروغرام /سم2 . تمتاز هذه الطريقة لعدم احتياجها الى التحكم بدرجات الحرارة ولا تحتاج الى الاستخلاص بالمذيب وتم تطبيق الطريقة بنجاح لتقدير كبريتات السالبيوتامول في العديد مختلف المستحضرات الصيدلانية.

الكلمات المفتاحية: الصبغات الآزوية، تفاعلات الأزوتة والاقتران، تقدير المستحضرات الصيدلانية، الطرق الطيفية، ترايتون اكس 100.