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Spectrophotometric Determination of Salbutamol Sulphate and Isoxsuprine Hydrochloride in Pharmaceutical Formulations

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Abstract:

A simple, sensitive and accurate spectrophotometric method has been developed for the determination of salbutamol sulphate (SAB) and isoxsuprine hydrochloride (ISX) in pure and pharmaceutical dosage. The method involved oxidation of (SAB) and (ISX) with a known excess of N-bromosuccinamid in acidic medium, and subsequent occupation of unreacted oxidant in decolorization of Evans blue dye (EB). This, in the presence of SAB or ISX was rectilinear over the ranges 1.0-12.0, 1.0-11.0 µg/mL, with molar absorptivity 4.21×10^4 and 2.58×10^4 $l.mol^{-1}.cm^{-1}$ respectively. The developed method had been successfully applied for the determination of the studied drugs in their pharmaceutical dosage resulting in a good agreement with certified value and standard addition procedure.

Keywords: Asoxsuprine hydrochloride, Evans blue dye, N-Bromosuccinamid, Salbutamol sulphate, Spectrophotometric.

Introduction:

Salbutamol is RS-[4-[2-(tert-butyl amino)-1-hydroxyethyl]-2-(hydroxymethyl) phenol] sulphate, Fig.1. Salbutamol is a short-acting β2 adrenergic receptor agonist, which works by causing relaxation of airway smooth muscle. It is used to treat asthma[1], including asthma attacks and exercise-induced bronchoconstriction, and chronic obstructive pulmonary disease [2].

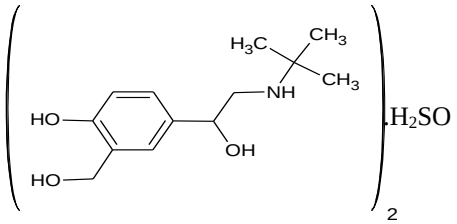


Figure 1. Chemical structure of salbutamol

Isoxsuprine (ISX); p-hydroxy-N-(1-methyl2-phenoxyethyl) norephedrine hydrochloride, Fig. 2, is a vasodilator that produces the effects of β-adrenoceptor stimulation and α-adrenoceptor antagonism; the former effect is the more predominant. It is used in the treatment of cerebral and peripheral vascular diseases [3-4]. It has a direct relaxant effect on the smooth muscular tissue of the blood vessels and uteruses also; it is used to arrest premature labor[5].

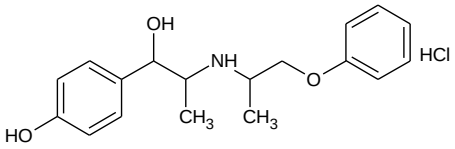


Figure 2. Chemical structure of isoxuprine

Various analytical methods and techniques have been used to determine the above salbutamol, and isoxsuprine, in pure form and pharmaceutical dosage including spectrophotometric [6-9], electrochemical techniques[10-14] and HPLC methods [15-18]. The aim of the present work is to develop a very simple, rapid, accurate, precise, sensitive and less time consuming quantitative determination of salbutamol and isoxsuprine in pure form and pharmaceutical dosage.

Experimental:

Instrument

All the absorption spectral measurements were employing UV-1650 PC UV-Visible

spectrophotometer equipped with 1.0 cm matched quartz cells.

Reagents and Chemicals

Salbutamol sulphate and isoxsuprine hydrochloride are from the State Company for Drug Industries and Medical Appliances. Solutions of SAB and ISX were prepared in a concentration of 50µg/mL by dissolving 0.01 g of each drug in 200 ml distilled water in volumetric flasks. Evans blue dye (100µg/mL) was prepared by dissolving 0.025 g in distilled water in a 250 mL volumetric flask. N-Bromosuccinimide (NBS) was prepared of 200 µg/mL by dissolving 0.02 g in 100 mL distilled water. Hydrochloric acid was prepared in a concentration of 2 M by diluting 50 mL of conc. HCl (10 M) with distilled water in a 250 ml volumetric flask.

General Procedure

Into two series of volumetric flasks (10 mL) aliquots of solutions containing 1-9 and 1-10 µg/mL of SAB and ISX respectively, were added separately, followed by addition of 1 mL of 2M HCl and 2 mL of 200 µg/mL NBS to each flask. The solutions were gently shaken and left for 10 min at room temperature for oxidation. 2.5 mL of 100µg/mL EB were added to the solutions. The flasks were diluted to the mark with distilled water, mixed well and measure the absorbance at 600 nm after 5 min at room temperature versus reagent blank

Procedure for the Pharmaceutical Preparations

Analysis of Tablet

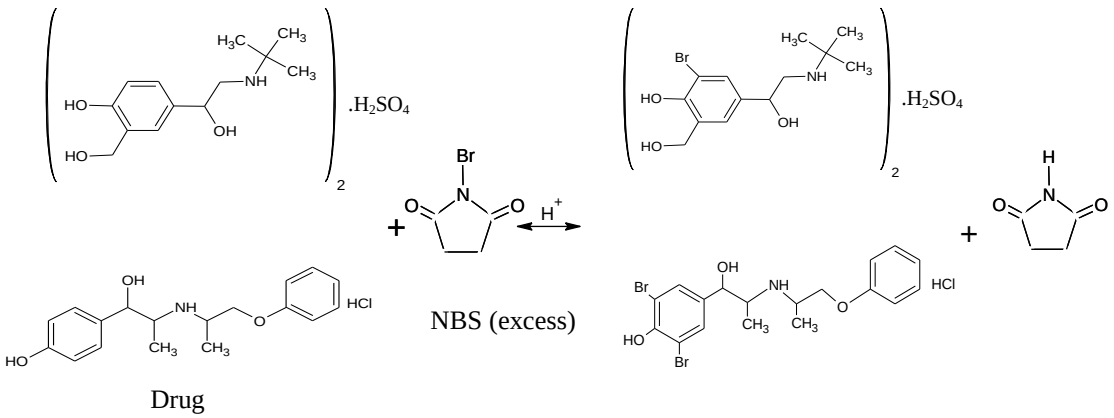
Ten tablets were weighed and pulverized into a fine powder from each of butadin (each tablet containing 2 mg SAB) and dulivan (each tablet containing 10 mg ISX). An accurate weight, equivalent to 10 mg of pure drug, was dissolved in 100 mL distilled water, and then filtered after spraying in the ultrasonic device for 5 minutes to obtain a solution with a concentration of 100 µg/mL. A suitable volume was diluted with distilled water and followed the recommended procedure.

Syrup

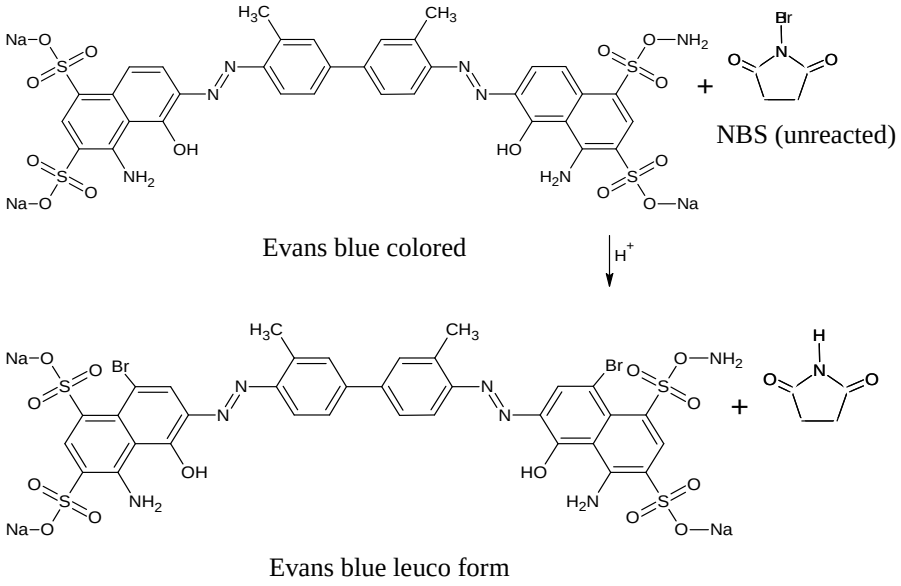
25 mL of butadin syrup of 2mg/ 5mL was transferred into a100 mL volumetric flask and diluted with distilled water to obtain 100 µg/mL of SAB. Aliquots of this solution were treated as described under the recommended proceedings.

Results and Discussion:

This suggested method is the indirect determination of phenolic drugs SAB and ISX involves the oxidation of these drugs by NBS in acidic medium, scheme 1 and the residual oxidant bleaches the blue color of EB dye[19], scheme 2. When the known volume of NBS is added to an increasing amount of drug, there was a decrease in the concentration of oxidant and increasing the absorbance of EV dye, Scheme1 and Fig. 3.



Scheme1. Possible reaction pathways between the cited drugs and NBS



Scheme2. Possible reaction between the dye and NBS.

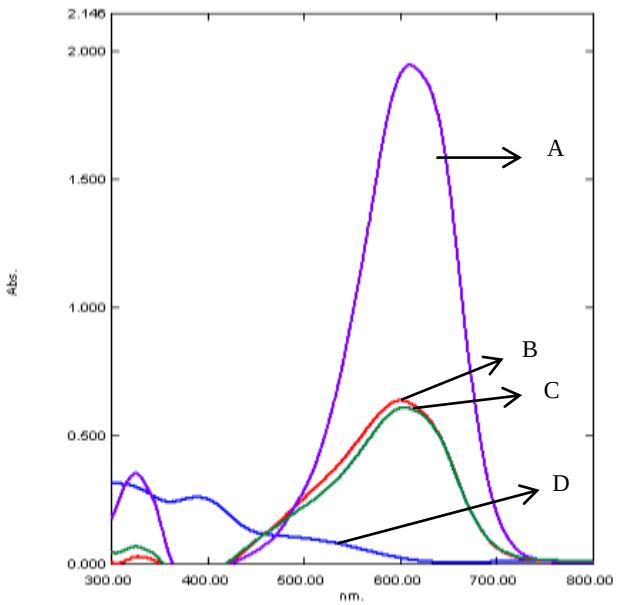


Figure 3. Absorption spectrum of 25 µg/mL EB dye against in acidic medium(A), in determination of SAB or ISX 8 µg/mL in presence of NBS(B andC), reagent blank agents distilled water (D).

Optimization of Experimental Variables

Optimum Amount of Evans Blue Dye

The preliminary experiments were performed to optimize the useful and optimum concentration of EB dye that can be determined spectrophotometrically. The results indicated that 250 µg/mL from EB dye was found to be a useful agent for reaction, Fig. 4.

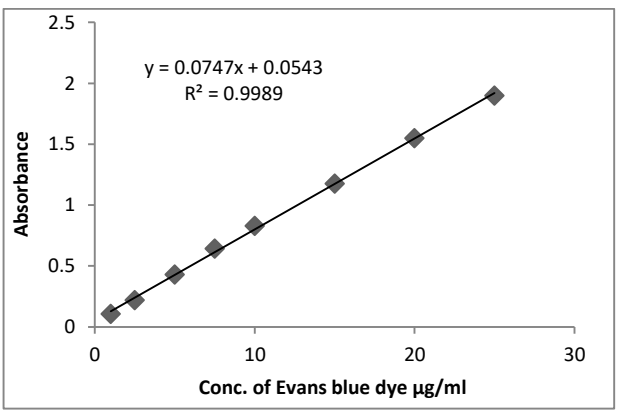


Figure 4. The calibration curve of EB dye

The Effect of Oxidant Reagents

N-bromosuccinamid was found to be a useful oxidizing agent, other oxidizing agents (chloramine-T and bromate-bromide) have also been tested, but none offered real advantages over N-bromosuccinamid, Fig 5.

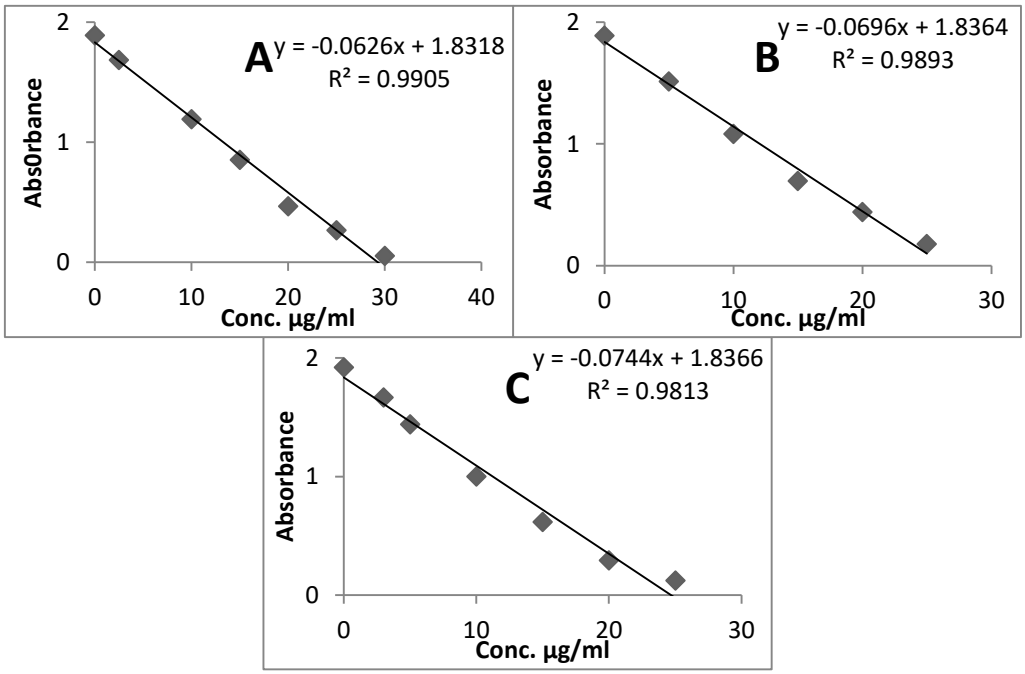


Figure 5. the calibration carves for selecting the amount of oxidizing agent in 25 µg/mL Evans blue dye in acidic medium, A: N-bromosuccinamid, B: chloramine-T, C: bromate-bromide.

Figure 5 shows that 1.5 mL of 200 µg/mL NBS solution was enough to obtain maximum bleaching of the color of EB dye therefore it was recommended in the subsequent experiments.

The Effect of Acid

Experimental results show that the oxidation of EB dye and the studied drugs by NBS take place in an acidic medium. Therefore, the effect of different amounts from various acids (2 M) have been tested, using 8 µg/mL of each drug, to obtain high sensitivity. It was found that HCl is the best acid for the system (Table 1). In addition, 1 mL of 2M HCl was selected as optimum amount for two drugs as shown Fig. 6.

Table 1. Effect of acid

Drug (8 µg/mL)	**Absorbance**				
	HCl	**H_2SO_4**	**CH_3COOH**	**HNO_3**	**H_3PO_4**
salbutamol	0.530	0.441	0.298	0.339	0.348
Isoxsuprine	0.541	0.450	0.307	0.357	0.360

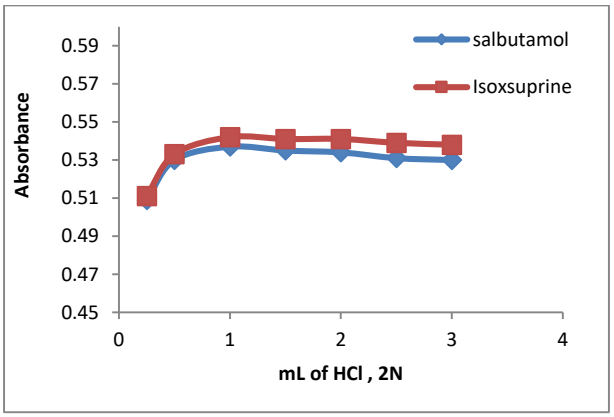


Figure 6. The effect of HCl amount

The Effect of Time on Oxidation

The effect of oxidation time of SAB and ISX drugs was studied by adding 1.5 mL of 200 µg/mL NBS to 8 µg/mL for each drug in the presence of 1mL of 2N HCl. The solutions were shaken and left at room temperature for different periods. Then 2.5 mL of 100 µg/mL EB were added to each drug and the solutions were agitated and diluted to 10 ml in volumetric flasks. The absorbance of the residual EB was measured after 5 min standing time at 600 nm against blank solution. The results obtained in Table 2 indicated that 10 min is sufficient for the oxidation of drugs and the absorbance remain constant for one hours.

Table2. The effect time on the oxidation of drugs and EB

Standing time before adding EB & dilution (min)	Absorbance/ standing time (min.)							
	5	10	15	20	30	40	50	60
	salbutamol							
After addition	0.510	0.512	0.511	0.508	0.507	0.506	0.506	0.504
5	0.531	0.532	0.530	0.530	0.528	0.527	0.526	0.528
10	0.538	0.537	0.536	0.536	0.535	0.534	0.534	0.531
15	0.536	0.536	0.534	0.534	0.533	0.533	0.532	0.530
20	0.535	0.536	0.535	0.533	0.532	0.532	0.530	0.530
	Isoxsuprine							
After addition	0.520	0.522	0.521	0.521	0.520	0.520	0.519	0.517
5	0.540	0.538	0.538	0.537	0.537	0.535	0.536	0.535
10	0.544	0.544	0.543	0.542	0.540	0.539	0.538	0.537
15	0.541	0.542	0.542	0.543	0.541	0.540	0.534	0.536
20	0.546	0.543	0.542	0.540	0.541	0.540	0.538	0.534

Calibration Curves

Under the described experimental conditions, standard calibration curves for, salbutamol and Isoxsuprine drugs with EB dye were constructed by plotting absorbance against concentration (Fig.7). Beer's law limits, molar absorptivity values and Sandell Sensitivity[20] were evaluated and given in Table.3, as well as the limit of detection (LOD) and limit of quantitation (LOQ) [21] were calculated according to the following equations:

$LOD = 3\sigma C_{low}/\bar{X}$, $LOQ = 10\sigma C_{low}/\bar{X}$

Where (σ) is the standard deviation of absorbance of low concentration and $\bar{X}$ is the average absorbance of low concentration.

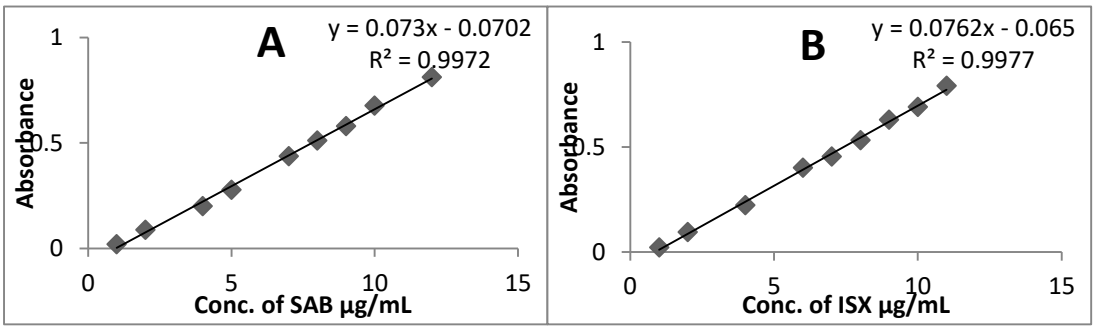


Figure 7. Calibration graphs of Salbutamol (A) and Isoxsuprine (B).

Table 3.The analytical and statistical values for the determination of SAB and ISX

Parameter	salbutamol	Isoxsuprine
Linearity range (µg/mL)	1-12	1-11
Intercept	-0.07018	-0.065
Slope	0.072976	0.076242
Correlation coefficient (R^2)	0.9972	0.9977
Molar absorptivity ($l.mol^{-1} cm^{-1}$)	4.21×10^4	2.58×10^4
Sandell Sensitivity (g/cm^2)	0.0137	0.0131
LOD*(µg/mL)	0.3122	0.2137
LOQ*(µg/mL)	1.0407	0.7124

* average of five determinations

Accuracy and Precision

The accuracy and precision was evaluated by calculating the recovery ratios and the relative standard deviation of three different concentrations of each drug compound. The results in Table 4, indicate the method is precise accurate.

Table 4. The accuracy and precision for the determination of study drugs.

Drug	Conc of drug (µg/ml)		Recovery* (%)	Average recovery (%)	RSD* (%)
	Taken	Found			
salbutamol	2	1.93	96.5	98.1	4.28
	4	3.93	98.3		2.65
	8	7.96	99.5		2.14
Isoxsuprine	2	2.06	103.0	100.8	4.09
	4	3.98	99.5		2.13
	8	8.01	100.1		1.08

Analytical Applications

The suggested method was tested for the determination salbutamol and isoxuprine in some of their pharmaceutical dosage. The concentration of drugs was calculated by direct measurement on appropriate standard calibration curve (Table 5). The similar results were obtained by applying the standard addition technique Fig. 8, indicating that suggested method is free from interferences.

Table 5. Determination of study drugs in pharmaceutical dosage.

Pharmaceutical formulation	Certified value	Amount present (µg.ml^{-1})	Found (mg)	Recovery* (%)
		salbutamol		
Butadin tablets	2 mg	1	0.98	98.0
S.D.I -Iraq		4	3.89	97.3
		8	7.9	98.8
Butadin surp	2mg/5ml	1	0.99	99.0
S.D.I -Iraq		4	3.91	97.8
		8	7.92	99.8
		Isoxsuprine		
Dulivan tablets	10 mg	1	0.97	97.0
ASIA-Syria		4	3.97	99.3
		8	7.94	99.3
Duvilane tablets	10 mg	1	0.99	99.0
Aleppo-Syria		4	3.95	98.8
		8	8.01	100.1

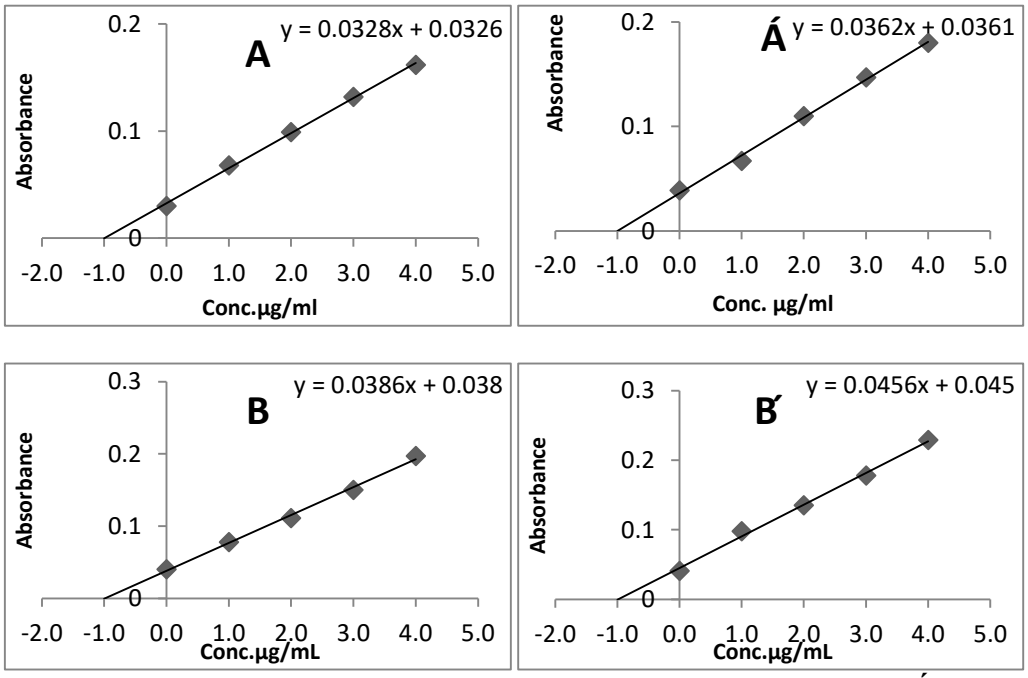


Figure 8. Plots of standard addition technique for determination of salbutamol (A, Á) and Isoxsuprine (B, B́)

Table 6. Assay of study drugs in pharmaceutical preparation using standard addition method

Pharmaceutical formulation	Certified value (mg)	Recovery* (%)	Drug content found (mg)	
			Present method	Standard addition
salbutamol				
Butadin tablets S.D.I-Iraq	2mg	99.4	1.960	1.988
Butadin syrup S.D.I-Iraq	2 mg/5 mL	99.7	1.98	1.994
Isoxsuprine				
Dulivan tablets ASIA-Syria	10 mg	98.44	9.70	9.844
Duvilane tablets Aleppo-Syria	10 mg	98.68	9.90	9.868

*Average of three determinations.

Conclusion:

The suggested method described the successful development of simple, accurate and sensitive spectrophotometric method for the determination of salbutamol and isoxuprine using N-bromosuccinamid as oxidant agent of the two drugs. The unreacted N-bromosuccinamid bleached the Evans blue dye. The method has been applied successfully to determine salbutamol and isoxuprine in various pharmaceutical formulations.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Mosul.

Authors' contributions statement:

Z. Z. Al. carried out the design, acquisition of data, analysis, interpretation, and participated in the drafted the manuscript. N. N. H. helped in interpretation and analysis. E. S. S. contributed to manuscript conceptualization, and interpretation. All authors read, revision and proofreading the final manuscript

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التقدير الطيفي لكبريتات السالبيوتامول والايزوكسوبرين هيدروكلوريد في المستحضرات الصيدلانية

زينة زهير العبدلي **نغم ناظم حبيب** **الهام سعد الله صالح**

قسم الكيمياء ,كلية التربية للعلوم الصرفة, جامعة الموصل, موصل, العراق

الخلاصة:

امكن تطوير طريقة يسيرة وحساسة لتقدير كبريتات السالبيوتامول وايزوكسوبرين هيدروكلوريد بشكلهما النقي وفي مستحضراتهما الصيدلانية, استندت الطريقة على مبدأ اكسدة المركبين الدوائيين بزيادة محسوبة من العامل المؤكسد N- بروموسكسينميد في وسط حامض الهيدروكلوريك وادخال غير المتفاعل من العامل المؤكسد في تفاعل اكسدة صبغة ايفانز الزرقاء المضافة بكمية ثابتة مؤديا الى قصر لونها الازرق وقياس المتبقي من الصبغة عند الطول الموجي 600 نانوميتر, اذ وجد ان امتصاص الصبغه المتبقية يزداد خطيا مع زيادة تركيز المركبين الدوائيين ضمن مدى التراكيز 1-12 و 1-11 مايكروغرام/مللتر بامتصاصية مولارية 4.21×10^4 و 2.58×10^4 لتر .مول$^{-1}$.سم$^{-1}$ لكل من كبريتات السالبيوتامول وايزوكسوبرين هيدروكلوريد على التوالي. طبقت الطريقة بنجاح على المستحضرات الصيدلانية للمركبين الدوائيين وكانت نتائجها متوافقة مع نتائج طريقة الاضافة القياسية مما يدل على ان الطريقة ذات دقة وصلاحية تطبيق تحليلي جيدة.

الكلمات المفتاحية: الايزوكسوبرين هيدروكلوريد, صبغة ايفانز الزرقاء، كبريتات السالبيوتامول ,N - بروموسكسينميد, التقدير الطيفي.