Spectrophotometric determination of Salbutamol sulphate by coupling with diazotized 2,4-dinitroaniline

M. S. Saleem S. M. AL-Mtwaiti S. T. AL-Ramadhani
Department of Chemistry / College of Education
University of Mosul

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الخلاصة

تضمن البحث تطوير طريقة طيفية سريعة وملائمة وحساسة لتقدير كميات مايكروغرامية من كبريتات السالبيوتامول بهيئتها النقية وفي مستحضراتها الصيدلانية. تعتمد الطريقة على تفاعل اقتران المركب الدوائي مع الكاشف 4,2- ثنائي نايتروانيلين المؤزوت في الوسط القاعدي ليعطي صبغة آزوية زرقاء وذائبة في الماء ومستقرة ولها أقصى امتصاص عند 558 نانوميتر. اتبعت الطريقة قانون بير ضمن مدى 0.2- 6 مايكروغرام/مللتر و بامتصاصية مولارية 9.33× 10^4لتر.مول$^{-1}$.سم$^{-1}$ ودلالة ساندل 0.0061 غرام/سم^2. وبلغ حد الكشف 0.0089 مايكروغرام/مللتر فيما بلغ حد التقدير الكمي 0.029 مايكروغرام/مللتر. أظهرت الطريقة دقة وتوافق عاليين إذ بلغت الاسترجاعية 99.75% وانحراف قياسي نسبي (RSD) اقل من 2.2%. وأمكن تطبيق الطريقة المقترحة بنجاح في تقدير كبريتات السالبيوتامول في مستحضراته الصيدلانية من دون تداخل مواد السوًاغ. كما تم مقارنة الطريقة المقترحة بنجاح مع الطريقة المعتمدة في الدستور البريطاني.

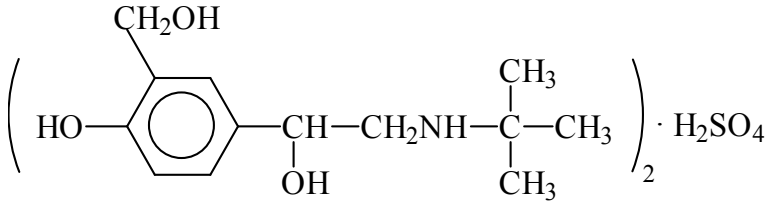
ABSTRACT

A quick, convenient and sensitive method has been developed for the determination of microgram amounts of salbutamol sulphate in its pure form and pharmaceutical preparations. The method is based on the coupling reaction of the drug with diazotized 2,4 - dinitroaniline reagent in an alkaline medium to produce an intense blue coloured Water soluble and stable azo dye which exhibits a maximum absorption at 558 nm. Beer's law was obeyed over the concentration range 0.2 - 6 µg/ml with a molar absorptivity of 9.33×10^4 L.mol^{-1}.cm^{-1} and Sandell's sensitivity index of 0.0061 g/cm^2. The limit of detection is 0.0089 µg/ml while the

limit of quantitation is 0.029 µg/ml. The method shows high accuracy (average recovery 99.75%) and precision (relative standard division (RSD) is less than 2.2%.The suggested procedure was applied for determination of salbutamol sulphate without any interference from common pharmaceutical excipients. The proposed method is successfully compared with the official method.

Introduction

Salbutamol is *RS*-[4-[2-(*tert*-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl)phenol] sulphate (Scheme1). Salbutamol belongs to the family of medicines known as adrenergic bronchodilators. Adrenergic bronchodilators are medicines that are breathed in through the mouth to open up the bronchial tubes (air passages) in the lungs. The relieve cough, wheezing, shotness of breath, and troubled breathing by increasing the flow of air through the bronchial tubes[1].



M.Wt 576.7g/mole

Scheme 1: Salbutaml sulphate structure

Salbutamol (SBS) is used to treat or prevent bronchospam in patient with asthma, bronchitis emphysema and other lung diseases. This medicine is also used to prevent wheezing cased by exercise–induced bronchospam[2].

Some of different methods of analysis have been reported for determination of SBS including HPLC[3-5], UV–spectrophotometry[6,7], redox[8-10], reduction followed by chelation[11], oxidative coupling[12-13] and charge transfer complex formation[14]. Some of those methods are less sensitive complicated in term of assay procedure, equipment required for analysis and need extensive sample preparation prior to the measurement step.

This paper describes an assay method for SBS determination in tablet and syrup. The method based on coupling of the drug with diazotized 2,4-dinitroaniline to form stable azo dye product.

Experimental

Apparatus

A Shimadzu model 1650 computerized spectrophotometer provided with 1 cm match quartz cell used for all absorbance measurements.

Reagents

All chemicals used were of analytical grade and obtained from Fluka and BHD companies.

Salbutamol sulphate was provided from the State Company for Drug Industries and Medical Applicances, Sammara –Iraq (SDI).

Salbutamol sulphate standard solution (100 μg/ml): is prepared by dissolving 0.01 g of pure salbutamol sulphate in100 ml distilled water.

2,4-dinitroaniline reagent solution(0.05%): this solution is prepared by dissolving 0.025 g of 2,4-dinitroaniline in 25 ml of acetonitrile and diluted to the mark in a 50 ml volumetric flask with distilled water.

Hydrochloric acid solution (1N): is prepared by diluting 8.5 ml of concentrated acid to the mark in a100 ml volumetric flask with distilled water.

Sodium nitrite solution(1%): is prepared by dissolving 1 g of $NaNO_2$ in distilled water and diluted to the mark in a 100 ml volumetric flask with distilled water.

Sulphamic acid solution(2%): is prepared by dissolving 2 g of sulphamic acid in distilled water and diluted to the mark in a 100 ml volumetric flask with distilled water.

Sodium hydroxide solution (1N): is prepared by dissolving 4 g of sodium hydroxide in distilled water and diluted to the mark in a 100 ml volumetric flask with distilled water.

Interference solution (1000 μg/ml): 0.1 g of each foreign compound is dissolved and completed to 100 ml with distilled water.

Surfactant solution(0.1%):0.1 g of each surfactant is dissolved and completed to 100 ml with distilled water.

Recommended produce and calibration graph

Employing the establish optimum condition, the calibration graph was constructed as follow.

To a series of 25 ml volumetric flask transfer 2 ml of 0.05 % of 2,4-dinitroaniline, 1ml of 1N HCl and 0.75 ml of 1 % of $NaNO_2$. The reaction mixture was shaked and left for 5 minutes then followed by the addition of 0.5 ml of 2% sulphamic acid with shaking and leaving for 5 minutes. After that an increasing amounts of salbutamol sulphate covering (5-150) μg are added followed by addition of 3 ml of 0.1% CTAB solution and 1.5 ml of 1N of NaOH. The volumes are made up to the marks with distilled water and left for 10 minutes and the absorbance of the coloured azo dye was measured versus reagent blank at 558nm.

Assay procedures for salbutamol sulphate in its pharmaceutical preparations:

1- Tablet:

Twenty tablets each contain 2 mg of salbutamol sulphate were weighed and finally powdered. An accurate quantity of powder equivalent to 20 mg was dissolved in distilled water and transferred to a 100 ml volumetric flask and the solution was made up to the volume with same solvent, mixed well and filtered.

A solution of 100 µg/ml was prepared by diluting 50 ml of filtrate to 100 ml with distilled water. Aliquots were treated as described under the recommended procedure for determination of salbutamol sulphate.

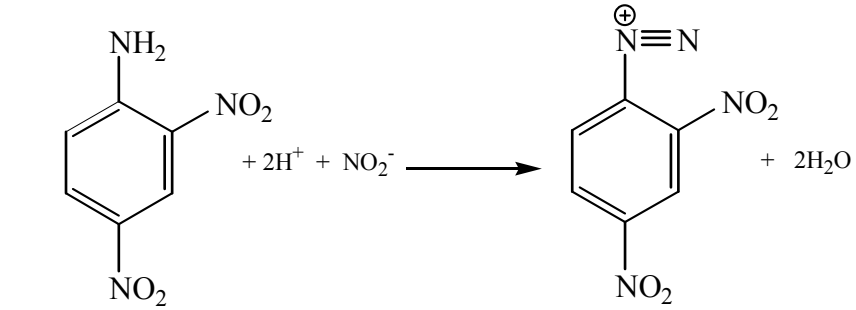
2- Syrup:

25 ml of butadin syrup of 40mg / 100ml was transferred into 100ml volumetric flask and diluted to the mark with distilled water to obtain 100µg/ml of salbutamol sulphate. Aliquots of this solution were treated as described under the recommended procedure for determination of salbutamol sulphate.

Result and Discussion

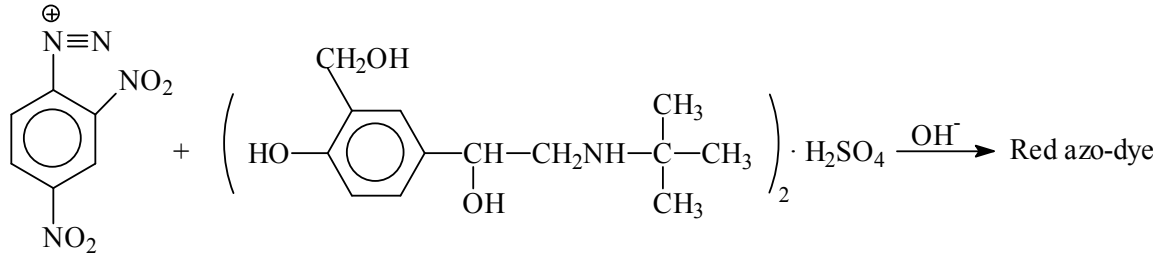
Principle of the colour reaction

2,4-Dinitroaniline has been reacted recently with excess nitrite in acidic medium to form the corresponding diazonium salt as follows.



2,4-Dinitroaniline Diazonium salt

Diazotized 2,4-dinitroaniline is then coupled with salbutamol sulphate in basic medium to form Red azo dye.



Study of the reaction condition

The various parameters affecting the related colour intensity of the formed azo dye have been studied in order to select the optimum conditions.

Choice of diazotized reagent amount

Diazotized 2,4-dinitroaniline has been selected in this study for the following reasons,(i) strongest diazotized electrophile especially when compared with diazotized p- nitroaniline due to the presence of two strong electron withdrawing nitro group. (ii) Strong colour contrast observed in its azo dye production due to the presence of two resonating nitro group.

The effect of the amount of produced diazotized 2,4-dinitroaniline on the absorbance of the resulting azo dye has been next investigated.The experimental results in table1 have been shown that 2 ml of 0.05% of 2,4-dinitroaniline is the optimum value due to its high colour intensity.

Table 1: The effect of 2,4-dinitroaniline amount in azo dye colour intensity

Volume of 0.05% of reagent (ml)	0.5	1.0	1.5	2.0	2.5	3.0
Absorbance	0.241	0.248	0.350	0.353	0.351	0.350

Effect of acid on diazotization formation

The diazotization of 2,4-dinitraniline was carried out in different amount of different acids (HCl, H_2SO_4, HNO_3, CH_3COOH) to evaluate the best acid with its optimum amount that can used for diazotization. The experimental data in table 2 revealed that 1 ml of 1N hydrochloric acid is to be the most suitable of the commonly known acids.

Table 2: The effect of different acid on azo dye colour

1N acid solution used	Absorbance / volume of acid used (ml)				
	0.25	0.5	1.0	1.5	2.0
HCl	0.321	0.339	0.340	0.336	0.336
H_2SO_4	0.302	0.305	0.313	0.328	0.326
HNO_3	0.308	0.336	0.328	0.334	0.339
CH_3COOH	0.115	0.237	0.235	0.238	0.241

Effect of nitrite amount and time

The produced coloured azo dye was reached its maximum colour intensity on using 0.75 ml of 1% (w/v) of nitrite solution within 5 minutes as a standing time (Table 3).

Table 3: Effect of 1% nitrite solution and time on azo dye colour

Time (min)	Absorbance / volume of 1% nitrite solution (ml)				
	0.25	0.5	0.75	1.0	1.25
3	0.315	0.320	0.322	0.328	0.327
5	0.347	0.357	0.359	0.355	0.352
7	0.349	0.354	0.355	0.348	0.346
10	0.349	0.356	0.351	0.350	0.348

Effect of sulphamic acid amount and time

The presence of unreacting nitrous acid was undesirable in diazotization reaction due to the probability of nitrosation reaction to take place, therefore it should be removed by sulphamic acid which fastly react with the excess nitrous acid. Table 4 showed that 0.5 ml of 2% sulphamic acid within 5 minutes as standing time was considered to be an optimum value.

Table 4: Effect of 2% sulphamic acid solution and time on the produced azo dye

Minute standing time (min)	Absorbance / volume of 2% sulphamic acid solution (ml)				
	0.25	0.5	0.75	1.0	1.25
3	0.346	0.350	0.350	0.348	0.348
5	0.347	0.352	0.345	0.350	0.351
7	0.342	0.345	0.349	0.351	0.346
10	0.342	0.340	0.348	0.350	0.348

*Absorbance without sulphamic acid =0.342

Effect of base type and its amount

It was found that the coupling reagent of 2,4-dinitroaniline with salbutamol sulphate occurs in basic medium, therefore several bases including NaOH, KOH, Na_2CO_3, $NaHCO_3$ have been examined at different amounts of these bases. The results cited in table 5 showed that 1.5 ml of 1N NaOH was selected to be optimum because it gave highest colour intensity of the azo dye formed.

Table 5: The effect of different base on azo dye colour

Base (1N)	Absorbance / volume of acid used (ml)				
	1.0	1.5	2.0	2.5	3.0
NaOH	0.102	0.358	0.355	0.352	0.351
KOH	0.122	0.351	0.350	0.348	0.347
Na_2CO_3	0.080	0.091	0.115	0.123	0.127
$NaHCO_3$	0.060	0.098	0.127	0.133	0.138

Effect of surfactant

The addition of surfactant to the reaction mixture may frequently leads to an increase in the colour intensity and my also leads to a shift in the wavelength, therefore the effect of various surfactant including cationic, anionic and non –ionic surfactants on the formation of azo dye are investigated in two different orders. The cationic cetyltrimethyl ammounium bromide (CTAB) was the best surfactant used owing to the highest colour intensity and the red bathochromic shift of the absorption maxima wavelength of the produce dye and also the colour change from red to blue colour. Further studies showed that 3 ml of 0.1% CTAB was selected as an optimum amount for subsequent work (Table 6&7).

Table 6: The effect of different surfactant on azo dye colour

1ml of 0.1% surfactant solution used	Absorbance / order of addition		
	I*	II*	λ_{max}
Without	0.356	0.356	521
CTAB	0.390	0.391	558
SDS	0.349	0.351	521
Tween-20	0.338	0.351	525
Triton-80	0.335	0.348	522

* Reagent (R) +Nitrite (N)+ Sulphamic acid(A) + Salbutamol sulphate(D) +surfactant (S) +Base (B)
** R+N+A+D+B+S

Table 7: Effect of CTAB amount on colour intensity of the azo dye

Volume of 0.1% of CTAB (ml)	1.0	2.0	3.0	4.0	5.0
Absorbance	0.392	0.432	0.454	0.452	0.451

Effect of reaction time and temperature

The reaction time was determined by following the formation of dye colour at different time intervals at different temperatures ranging from 0 to 50 C. The produced dye formed after 10 minutes and remain stable for at least 3 hour at temperature up to 40 ˚C therefore,10 minutes as a reaction time at a room temperature was chosen as optimum in the subsequent studies.

Absorption spectra

A blue azo dye formed when diazotized 2,4-dinitroaniline was coupled with salbutamol sulphate in basic medium in presence of CTAB surfactant under the established optimum amounts, the formed azo dye shows an absorption maxima at 558 nm while the coloured reagent blank shows no absorbance at this wavelength (Figure 1).

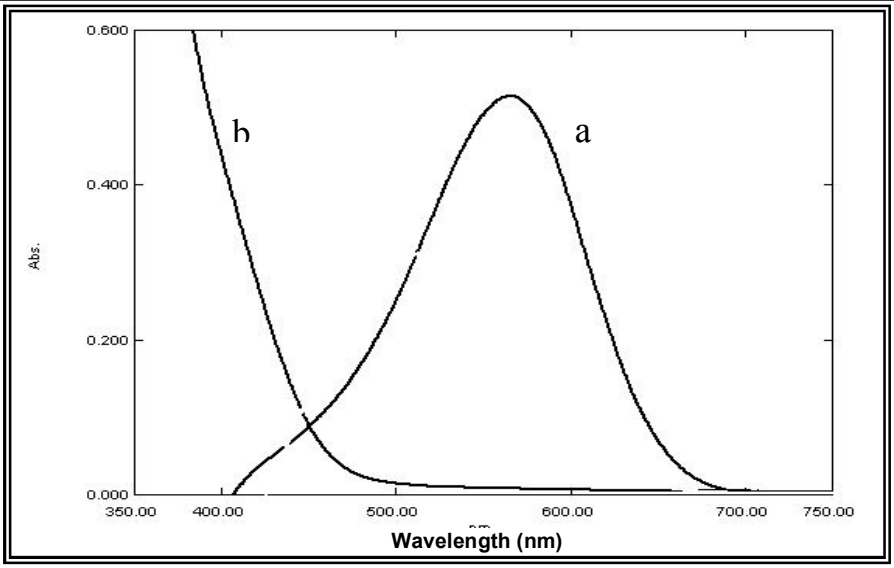


Figure 1: Absorption spectra of (a) 62.5 µg salbutamol sulphate against reagent blank, (b) reagent blank versus distilled water Quantitation

A plot of absorbance versus determination concentration shows that Beer's law is obeyed over the range 0.2-6 µg/ml from salbutamol sulphate after which a negative deviation from the straight line was observed (Figure 2), the average molar absorptivity is calculated as 9.33×10^{4} $L.mol^{-1}.cm^{-1}$. and Sandell's sensitivity is 0.0061 $g/cm^{2.}$ The limit of detection is 0.0089 µg/ml and limit of quantitation is 0.029 µg/ml.

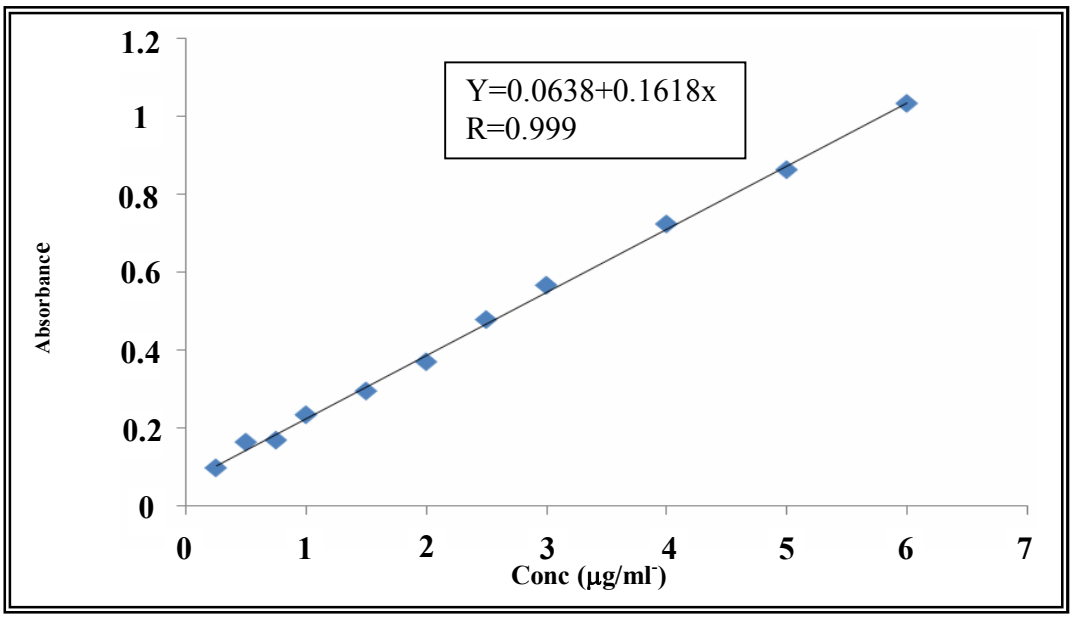


Figure 2: Calibration graph for determinationof salbutamol sulphate

Accuracy and Precision

To evaluate the accuracy and precision of the method, a pure salbutamol sulphate was analyzed at four different concentration by seven replicates. The results shown in table 8 indicate that a satisfactory accuracy and precision could be attended by using the proposed method.

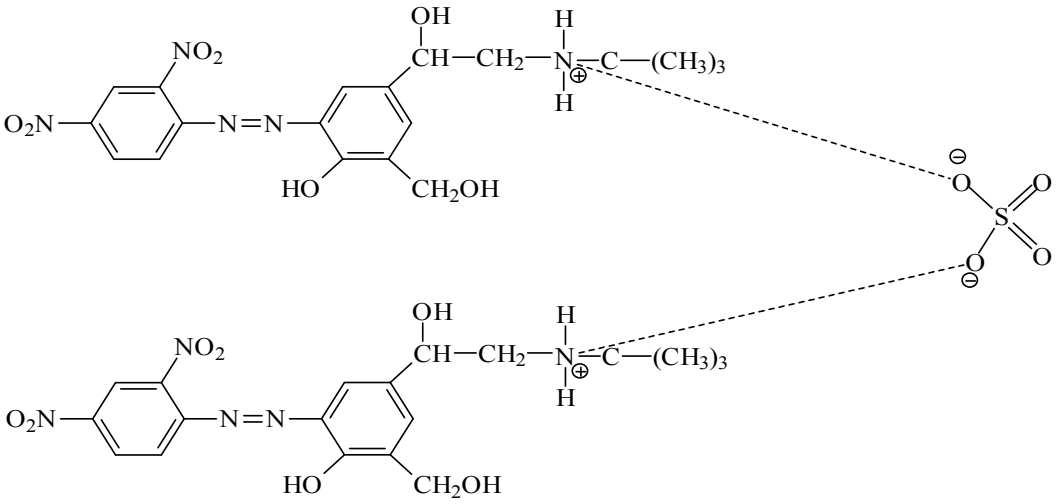
Table 8: Accuracy and Precision of proposed method

Concentration of salbutamol sulphate (µg/ml)		Recovery (%)	Average recovery* (%)	RSD* (%)
Present	Found			
1	0.98	98	99.75	1.47
2	2.05	102.5		2.15
4	3.95	98.75		1.38

* Average for four determinations

Nature of the dye

Applying Job's method of continuous variation and mole ratio method [15] showed that the formed dye has the composition of 1:2, salbutamol: diazotized 2,4-dinitroaniline (Figure 3), therefore; the formed dye may be written as follows:



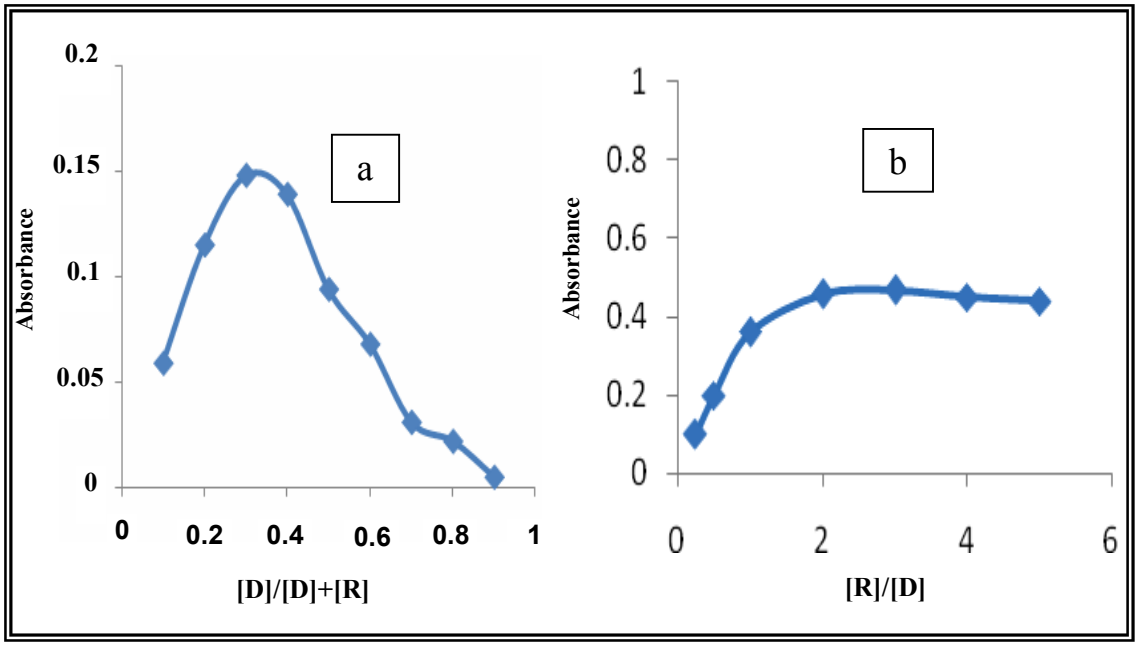


Figure 3: Job's plot (a) and mole-ratio plot (b) for diazotized 2,4- dinitroaniline coupled with slbutamol sulphate.

The apparent conditional stability constant of the produced azo dye described under the recommended procedure has been estimated to be 2.92×10^{9} $L^{2.}$ $mol^{-2.}$ The formed dye may be written as follows:

Interferences

In order to assess the possible application of the present proposed method, the effect of some foreign compounds which often accompany this drug in pharmaceutical product were studied by adding different amounts of excipients to 100 µg of salbutamol sulphate. It was found that non excipients can introduce significant interference.

Analytical application

Appling the present method for the assay of two salbutamol sulphate drugs(tablet and syrup).The results shown in Table 9 give a reproducible and accurate result.

Table 9: Determination of salbutamol sulphate in pharmaceutical preparations

Pharmaceutical Preparations	Certified Value	Amount Present (µg/ml)	Recovery (%)	Drug content Found* mg
Butadine tablets (SDI)	2.0 (mg)	1.0	102.25	2.045
		2.0	102.8	2.056
		4.0	100.6	2.012
Butadine syrup (SDI)	0.4 (mg/ml)	1.0	103.15	0.4126
		2.0	100.8	0.4032
		4.0	101.05	0.4042

* Average for five determinations

The validation of the present proposed method was confirmed by applying official standard method[16]. Table 10 shows the assay of salbutamol sulphate in tablet and syrup formulations by the proposed and official standard method procedure.

The results were also compared statistically by student t-test and variance ratio F-test with those obtained by British pharmacopeia[16] at 95% confidence level with five degrees of freedom, as cited in Table 10. The result showed that the t- test and F-test were less than the theoretical value (t= 2.57, F= 5.05)[15], indicating that there was no significant difference between the proposed method and official method.

Table 10: The results of t-test and F-test analysis

Pharmaceutical Preparation	Recovery (%)*		t-test	F-test
	Present Method	Standard Method		
Tablet	101.88	102.57	0.832	2.46
Syrup	101.66	101.65	0.953	3.62

*Average for five determinations

Conclusion

The proposed method have advantages of being, simple and free from interferences of the common excipiets. The results obtained were closely comparable of those of a reported method and recovery test were also found to be satisfactory.

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