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Diazotised *p*-Nitroaniline Reagent for the Determination of Trace Amounts of Salbutamol Sulphate in Aqueous Solution– Application to Pharmaceutical Preparations

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ABSTRACT

A simple, rapid and sensitive spectrophotometric method for the determination of micro amounts of salbutamol sulphate in aqueous solution has been developed. The method is based on the coupling of salbutamol sulphate with diazotised *p*-nitroaniline in basic medium to form a coloured mono azo dye which is stable, water- soluble and exhibits maximum absorption at 488 nm. Beer's law is obeyed over the range 10–120 μg of salbutamol sulphate in a final volume of 25 ml, i.e., 0.4–4.8 ppm with a molar absorptivity of 3.13×10^4 $l.mol.^{-1}cm^{-1}$, a relative error of +1.80 to –3.40% and a relative standard deviation of $\pm$5.70 to $\pm$1.65%, depending on the concentration level. The proposed method does not require temperature control or solvent extraction and the method has been successfully applied to the determination of salbutamol sulphate in different types of pharmaceutical preparations, tablets and syrup.

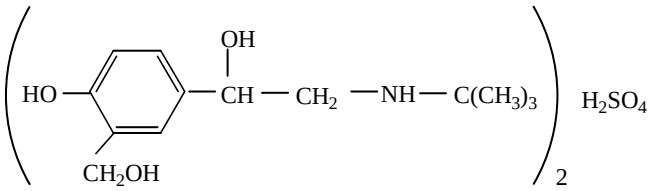
العامل المؤزوت بارا-نايتروانلين في تقدير كميات متناهية في الصغر من كبريتات السالبيوتامول في المحلول المائي- تطبيقات في مستحضرات دوائية

الملخص

يتضمن البحث طريقة طيفية بسيطة، سريعة وحساسة لتقدير كميات متناهية في الصغر من كبريتات السالبيوتامول في الوسط المائي. تعتمد الطريقة على اقتران العامل المؤزوت بارا-نايتروانيلين مع كبريتات السالبيوتامول في وسط قاعدي لتكوين صبغة آزوية أحادية ملونة، كانت الصبغة مستقرة وذائبة في الماء وأعطت أعلى امتصاص عند 488 نانوميتر. كانت حدود قانون بير في مدى التركيز 10 – 120 مايكروغرام كبريتات سالبيوتامول في حجم نهائي 25 مل (0.4 – 4.8 جزء/مليون) وكانت قيمة معامل الامتصاص المولاري 3.13×10^4 لتر مول$^{-1}$ سم$^{-1}$، الخطأ النسبي بين + 1.80و -3.40% والانحراف القياسي بين $\pm$ 5.70 و$\pm$ 1.65% اعتماداً على مستوى التركيز. لا تحتاج الطريقة المقترحة إلى سيطرة على درجة الحرارة أو الاستخلاص وتم تطبيق الطريقة بنجاح في تقدير كبريتات السالبيوتامول في نماذج مختلفة من المستحضرات الدوائية، الحبوب والشراب.

INTRODUCTION

Salbutamol is (RS)-1-(γ-hydroxy-3-hydroxymethyl) phenyl-2-(tert-butylamine) ethanol (albuterol) which has the chemical formula $C_{13}H_{21}NO_3$. Salbutamol sulphate (Albuterol sulphate; salbutamol hemsulphate) has the chemical formula $(C_{13}H_{21}NO_3)_2$ H_2SO_4 (James, 1996) , and it has the following structure:



Salbutamol is a direct acting sympathoimetric agent with a selective action on β_2-adrenergic receptors. It is used for the treatment of both acute and chronic asthma (Fattah *et al.,* 1998). Various methods have been reported for the determination of salbutamol such as:

Gas chromatography – mass spectrometry (GC-MS) method has been used for the determination of several beta (2)-against compounds in bovine retina (Hernandez, 2000).

A simple, selective and accurate high performance liquid chromatographic method for the determination of cephalexine, sefolaxime, and salbutamol sulphate has been developed. The suggested method uses ODS column with methanol-phosphate buffer (pH7) 6:4, as a mobile phase (Shalaby, 1998).

Sodium cobaltinitrite $Na_3Co(NO_2)_6$ has been used as a reagent for determining some phenolic drugs, the reaction occurs in aqueous acetic solution to form yellow complexes which extracted into $CHCl_3$ and measured colorimetrically (Wahbi *et al*., 1978).

A colorimetric method for salbutamol sulphate in tablet has been proposed, the method is based on treating salbutamol sulphate with (0.04%) 3,5-dichloro-*p*-benzoquinone-chlorimine reagent at pH 8.2 (borate buffer solution) and the absorbance is measured at 610 nm (Shinghal and Naik, 1981).

Another colorimetric method based on the nitration of the salbutamol sulphate followed by the subsequent formation of meisenheimer complex with a nucleophilic reagent in alkaline medium (Bakry *et al*., 1995).

A flow injection method has been used for determination of some phenolic drugs; based on the reaction of the phenolic drugs with 4-aminoantipyrine and potassium hexacyanoferrate (III). (El-Gendy, 2000).

An improved extractive spectrophotometric method for determination of salbutamol sulphate based on its oxidative coupling with 3-methylbenzthiazolinove-2-hydrazone in the presence of ceric ammonium sulphate as an oxidizing agent (Gecta and Baggi, 1989).

In this study, the reaction between salbutamol sulphate with diazotised *p*-nitroaniline in alkaline medium has been adopted for a spectrophotometric method. The proposed method has the advantages of simplicity, rapidity, sensitivity and stability of the colour formed.

EXPERIMENTAL

Apparatus

All measurements are performed using Shimadzu UV-Visible Recording Spectrophotometer UV-160, with 1-cm matched silica cells.

The pH measurements are performed on Philips PW 9420 pH meter with a combined glass electrode.

Reagents

All chemicals used are of highest purity available.

Working salbutamol sulphate solution, 100μg / ml. A 0.01 g of salbutamol sulphate is dissolved in 20 ml distilled water with stirring and the volume is completed with distilled water to 100ml in a volumetric flask.

Sodium nitrite solution, 1%. This solution is prepared by dissolving 1g of sodium nitrite in 100 ml distilled water.

Hydrochloric acid solution, 1N. This solution is prepared by appropriate dilution of the concentrated acid with distilled water.

Diazotised *p*-nitroaniline reagent solution, 5 mM. A 0.1727g of a *p*-nitroaniline (Fluka) is dissolved in about 40ml distilled water. Then 20 ml of 1M HCl is added and the solution is heated, the clear mixture is then transferred to a 250 ml volumetric flask and is cooled to 0-5^{O}C in an ice – bath. A 8.65 ml of 1% $NaNO_2$ is added and the mixture is stirred vigorously. After 5 minutes the solution is made up to volume in 250- ml volumetric flask with cold water. The solution is kept in a brown bottle in a refrigerator and is stable for at least 3 days.

Sodium hydroxide solution, 1N. This solution is prepared by appropriate dilution of the concentrated volumetric (Fluka) solution with distilled water and then transferred to plastic bottle.

Butadin tablets solution 100mg/l. Dissolve 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. Filter the solution into a 100-ml volumetric falsk, wash the residue with distilled water and dilute to volume with distilled water to obtain 100 mg/l salbutamol sulphate.

Butadin syrup solution 100mg/l.

The content of the container of butadin syrup was mixed (each 5ml contains 2 mg salbutamol sulphate), 6.25 ml of the syrup diluted to 25 ml with distille water to prepare 100 mg/l salbutamol sulphate solution.

RESULTS AND DISCUSSION

Study of the optimum reaction conditions

The various parameters related to the orange azo dye formation have been studied and optimum conditions have been selected.

Effect of base

The preliminary experiments have shown that the dye develops only completely in alkaline medium. Different amounts of bases (strong and weak) have been used, the results indicate that 4 ml of 1N NaOH gave highest intensity with a good colour contrast ($\simeq$ 110nm).Weak bases (Na_2CO_3 and $NaHCO_3$) gave turbid solutions, 4 ml of 1N NaOH is recommended for the subsequent experiments.

Effect of surfactants

The effect of different types and amounts of surfactants (cetylpyridinium chloride, sodium dodecyl sulphate and triton-x-100) on the colour intensity of the orange dye and colour contrast have been examined and the results show that there is no useful effect.

Order of reagents addition

Table (1) shows three order of addition.

Table 1. The order of addition.

Type	The order	A	Δλ, nm
I	Sample + diazotized reagent + base	0.206	110
II	Sample + base + diazotized reagent	*-	-
III	Diazotised reagent+ sample + base	0.202	107

*Turbid solution

From the results in Table (1), order I is recommended for the subsequent experiments.

Effect of diazotised *p*-nitroaniline reagent amount

The effect of the amount of the diazotised *p*-nitroaniline on the maximum absorbance of the dye formed has been investigated (Table 2).

Table 2. Effect of *p*-nitroaniline diazotised amount on the absorbance.

Ml of diazotised *p*-nitroaniline	Absorbance / μg salbutamol present as sulphate							
	10	20	40	60	80	100	120	r
0.5	0.023	0.047	0.063	0.124	0.137	0.172	0.219	0.991345
1.0	0.021	0.037	0.082	0.137	0.186	0.200	0.253	0.994201
2.0	0.033	0.042	0.081	0.151	0.177	0.222	0.299	0.991754
3.0	0.042	0.060	0.090	0.139	0.186	0.260	0.287	0.993026

The results show that 1 ml of diazotised *p*-nitroaniline (5mM) reagent solution gave the highest value of a correlation coefficient (r =0.9942) over a range of salbutamol sulphate concentration 10–120 μg/25 ml solution, therefore 1 ml is recommended for the subsequent experiments.

Effect of time on colour development

Table (3) shows the effect of time on colour development of the dye obtained from three different concentrations of determined.

Table 3: Effect of time on colour development.

μg salbutamol sulphate / 25 ml	Absorbance / minute						
	0	10	20	30	40	50	60
20	0.056	0.058	0.059	0.059	0.059	0.058	0.059
60	0.140	0.142	0.142	0.144	0.144	0.144	0.143
100	0.214	0.216	0.216	0.216	0.216	0.216	0.216

The results above indicate that the colour formed within about one minute and remained stable for at least 1 hour.

Procedure and calibration graph

To a series of 25- ml volumetric flasks transferred 10 – 160 μg (0.4–6.4 ppm) of salbutamol sulphate, 1 ml diazotised *p*-nitroaniline (5 mM) and 4 ml of 1N NaOH were added, then the volumes were made to the mark with distilled water. The absorbances were read against a reagent blank prepared in the same manner but without salbutamol sulphate at 488 nm using 1-cm cells. The colour is formed immedietly and is stable for at least 1 hour. The calibration graph is linear over the range 0.4 – 4.8 ppm, and higher concentrations show negative deviation (Fig. 1). The molar absorptivity, calculated in the region of least photometric error and at the wavelength of maximum absorption, is found to be 3.13×10^4 $l.mol^{-1}.cm^{-1}$.

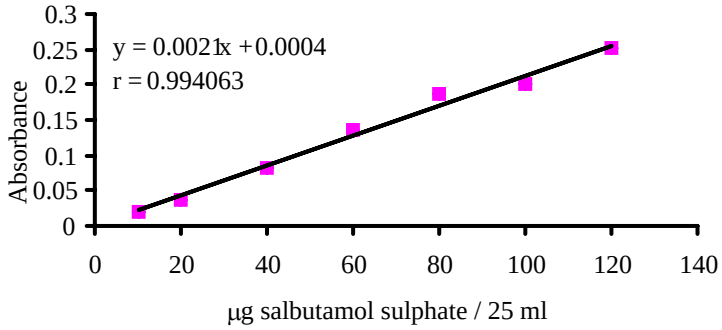


Fig. 1: Calibration graph for salbutamol sulphate determination using diazotised *p*-nitroaniline reagent

Absorption spectra

When salbutamol sulphate in aqueous solution is treated with diazotised *p*-nitroaniline reagent solution, according to the recommended procedure, an absorption peak is obtained showing intense orange dye absorption at 488 nm , the reagent blank showed almost nill absorption at this wavelength of maximum absorption (Fig. 2).

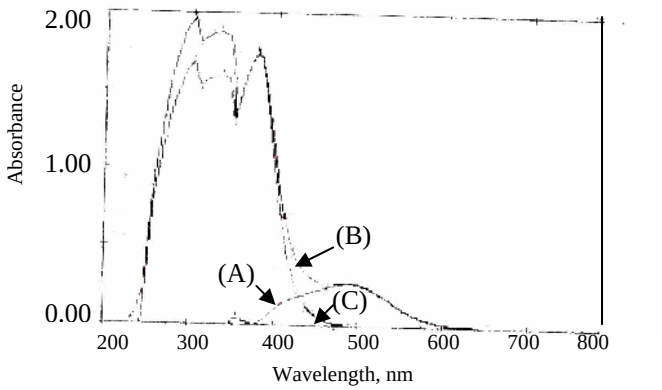


Fig. 2: Absorption spectra of 100 g salbutamol sulphate / 25 ml treated according to the recommended procedure and measured against (A) blank, (B) distilled water and(C) blank measured against distilled water.

Application

The application of the method for the assay of salbutamol sulphate in two drugs has been worked out. From the results, it can be shown that good agreement has occurred between amount of salbutamol sulphate taken and those measured by the recommended procedure. The recovery has further been confirmed with the dizotised sulphanilic acid method (Table4).

Table 4: The results of application.

Drug	%Recovery*	
	Present method	dizotised sulphanilic acid method**
Butadin tablet	98.2	97.8
Butadin syrup	97.4	97.3

*Average of three determinations

** R.A. Zakaria"M.Sc.Thesis"Mosul University,(2003),p 49.

Accuracy and precision

To check the accuracy and precision of the method, three different concentrations of salbutamol sulphate are determined. The results shown in Table 4 indicate that the method is satisfactory.

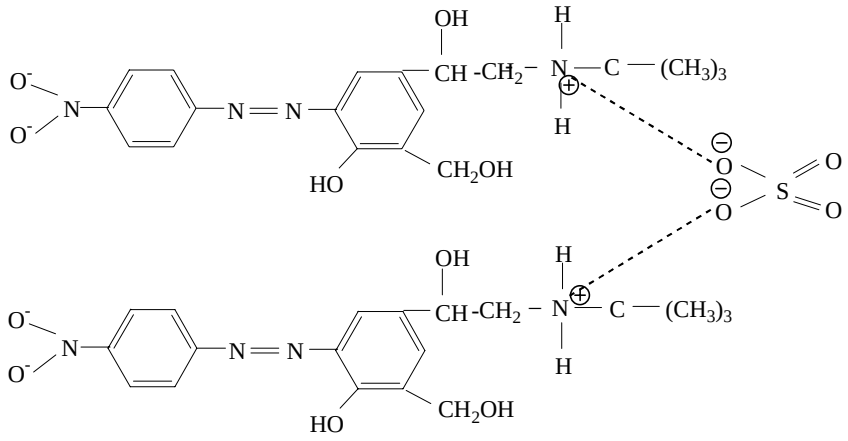
Table 5. Accuracy and precision of the method.

μg salbutamol sulphate	Relative error, %*	Relative standard deviation, %*
20	-3.40	±5.70
80	-1.30	±1.65
100	+1.80	±1.75

*Average of five determinations

Nature of the dye

Job's and mole–ratio methods indicated that the dye has a composition of 1:2 salbutamol sulphate to diazotised *p*-nitroaniline reagent, i.e., 1:1 salbutamol to diazotised *p*-nitroaniline. Hence the dye may have the following suggested structure:



Orange azo dye

Comparison of the methods

Table 6 shows comparison between some of analytical variables of the suggested method with another spectrophotometric method.

Table 6. Comparison of methods.

Analytical parameters	Present method	Literature method*
pH	12.2	…
Temperature (C^o)	Room temperature	…
λ_{max} (nm)	488	530
Medium of reaction	Aqueous	Non queous
Type of reaction	Diazotisation	Oxidative coupling
Reagent	Diazotised *p*-Nitroaniline	3-Methyl benzthiazoline-2-hydrazone
Beer's law range (ppm)	0.4 – 4.8	0.5 – 15
Colour of the dye	Orange	Red
Application of the method	Determination of salbutamol sulphate in two drugs	Determination of salbutamol sulphate in two drugs

* N. Gecta, and T. Baggi (1989), Microchem J., 39(2), 137 – 144.

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

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