Spectrophotometric method for Assay of Salbutamol in Pharmaceutical Formulations

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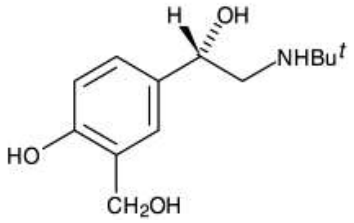
Abstract

A simple and sensitive spectrophotometric method is proposed for the determination of salbutamol. The method based on coupling of 4-aminoantipyrine (4-AAP) with salbutamol to give a new ligand that reacts with copperII to give intensely red colored chelate at 60°C. The colored product is quantified spectrophotometrically at 500nm. The optimization of the experimental conditions is described. The method has been used for the determination of 0.5–30.0 μgml^{-1} salbutamol. The accuracy of the method is achieved by the values of recovery (100 ± 0.05%) and the precision is supported by relative standard deviation (1.67%) values. The sensitivity of the method is indicated by molar absorptivity of 10106 l.mol^{-1}.cm^{-1}. The results of the method is compared with the official method. The interference of common drug additives was also studied. The proposed method is applied successfully to the determination of salbutamol in pharmaceutical preparations.

Introduction

Salbutamol,[1-(4-hydroxy-3-hydroxymethylphenyl)-2-(t-butylamino) ethanol] (I), also known as albuterol, is a β_2 adrenergic receptor agonist, primarily used in the treatment of bronchial asthma and other forms of allergic airways disease. The drug is also used in obstetrics for the prevention of premature labour and as a nasal decongestant[1,2].



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Among the techniques reported for the determination salbutamol in pharmaceuticals, methods using high-performance liquid chromatography (HPLC) [٣-٩] are tedious, time consuming, and require special and expensive apparatus. Besides, all the reported HPLC methods are relatively insensitive. Another chromatographic method, thin-layer chromatography [١٠], although simpler than HPLC methods, is also less sensitive with the linear range being 20–580 μg ml^{-1}. Non-chromatographic methods such as derivative ultra-violet spectrophotometry[١١], derivative difference spectrophotometry[12], capillary electrophoresis [13] and a.c. oscillopolarography [14] are also relatively complicated in terms of assay procedure or equipment required for analysis. The most widely used technique for the assay of salbutamol in pharmaceuticals has been visible spectrophotometry; procedures based on such varied reactions as redox [15,16], reduction followed by chelation [17], oxidative coupling [18,19], diazotization and coupling [20,21], nitrosation [22], nitration [23], nitration followed by Meisenheimer complex formation [24], and charge-transfer complex formation [25] are found in literature. However, many of these procedures suffer from one or the other disadvantage such as poor sensitivity, a heating or extraction step, critical working conditions, or the use of organic solvents; and hence are unsatisfactory for routine analysis. The only visual titrimetric method [26] reported employs *N*-bromosuccinimide as the oxidimetric titrant; this compound is unstable in solution and requires daily standardisation [27]. Recently Issa et al. [28] have reported a conductimetric titration method using phosphotungstic and phosphomolybdic acids as titrants. Even these procedures are time-consuming and poorly sensitive. The present research aims chiefly to developing a sensitive, simple spectrophotometric method for the determination of salbutamol based on its coupling with 4-aminoantipyrine (4-AAP) to give a new ligand that reacts with copperII to give intensely red colored chelate at 60°C.

Experimental

Apparatus

All absorption measurements were made on double-beam spectrophotometer Shimadzu (UV-160A) and matched 1-cm optical silica cells. The pH of the solutions were measured by HANNA (pH_{211}) Microprocessor pH meter. Heating of solutions is carried out on a water bath of frost instruments, LTD.

Reagents

All reagents used were of analytical grade and obtained from Fluka and BDH companies.

Copper sulphate ($CuSO_4.5H_2O$) solution (0.1%) was prepared by dissolving 0.1 g of $CuSO_4.5H_2O$ with distilled water and diluted to 100 ml in calibrated flask.

4-Aminoantipyrine(4-AAP)*(1%)* was prepared by dissolving 1g of 4-AAP in small amount of ethanol and diluted to the mark in a 100ml-volumetric flask with distilled water.

Sodium hydroxide (0.05M) was prepared by dissolving 0.2 g of sodium hydroxide in distilled water and diluted to 100 ml in calibrated flask.

Standard solution of salbutamol(1000ppm) was prepared by dissolving 0.1g of pure salbutamol, provided from Sammara drug industries(SDI), in distilled water and diluted to the mark in 100ml-volumetric flask with distilled water and stored in amber coloured bottle and kept in refrigerator. The solution were diluted as needed.

Recommended procedure

Aliquots containing 0.5–30 μgml^{-1} of salbutamol in final dilution, were transferred into a series of 10 ml volumetric flasks, followed by 1.5 ml of 1% 4-AAP, 0.75 ml of 0.1% copper sulphate and 0.1 ml of 0.05 M sodium hydroxide to have pH 7.5. The red color mixture was placed in water bath adjusted at 60°C for 70 min. cooled and completed to 10ml with distilled water, the absorbance values were measured at 500 nm against the blank solution. The calibration curve was obtained applying the same procedure using standard drug solutions.

Analysis of tablets

Weighed and finely powdered 10 tablets (each containing 2 mg salbutamol). An amount of powder equivalent to one tablet was weighed accurately and transferred into a 50 ml beaker. The powder was completely dissolved in distilled water and the solution was filtered through a Whatmann 41 filter paper. The filtrate was made up to 50 ml with distilled water in a volumetric flask to obtain 40 µgml^{-1} salbutamol in a final dilution. An a liquid of drug solution was analyzed as described in general procedure.

Result and discussion

Optimum conditions affecting the reaction of salbutamol with copper sulphate and 4-AAP were studied carefully.

Absorption spectrum

Salbutamol reacts with 4-AAP and copper sulphate in the presence of sodium hydroxide when heated for ٧٠ min at ٦0°C to give a red colored product, as shown in Figure 1, the absorption spectrum of which under optimum conditions shows a maximum at 500 nm, whereas the reagent blank gave maximum absorption at 300 nm.

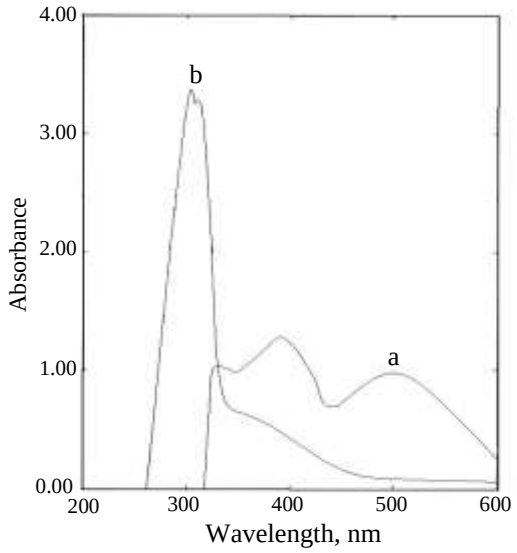


Fig.1. Absorption spectra of
(a) salbutamol (25 µgml^{-1}) – 4-AAP-Cu system and its reagent blank
(b), in a total volume of 10 ml.

Effect of pH

The effect of pH on the absorbance of the mixture solution was studied by addition of sodium hydroxide. As shown in Figure 2, it was found that the chelating complex gave highest absorbance at pH 7.5 with 0.1 ml of 0.05 M NaOH at λ_{max} 500nm. Other bases such as sodium carbonate, ammonia and potassium hydroxide were also investigated but gave less sensitivity than sodium hydroxide. Therefore; the upper fixed amount of NaOH was used in all subsequent experiments.

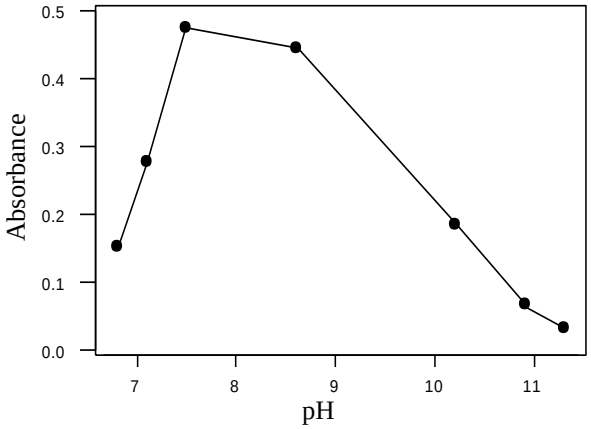


Figure 2: Effect of pH on the absorbance of 25 µgml^{-1} salbutamol

Effect of 4-AAP amount

When various concentrations of 4-AAP solution were added to fixed amount of the drug solution, 1.5 ml of 1% solution was found enough to develop the colour to its full intensity (Figure 3), with a minimum blank value and was considered to be optimum.

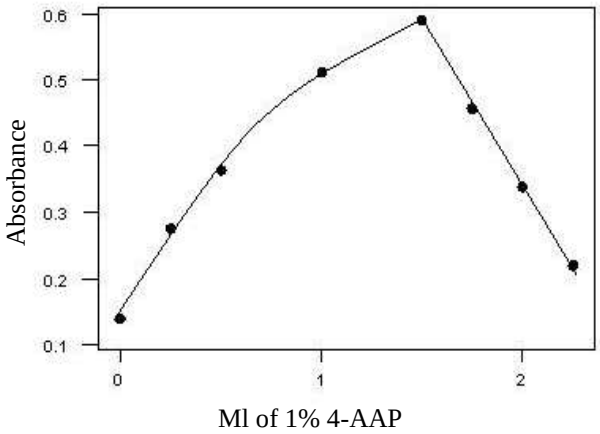


Figure 3: Effect of 4-AAP concentration on the absorbance of 25 µgml^{-1} salbutamol

Effect of $CuSO_4.5H_2O$ concentration

The chelating complex formation reached its maximum when 0.75 ml of 0.1% of $CuSO_4.5H_2O$ solution were added to a mixture of salbutamol, 4-AAP and sodium hydroxide (Figure 4), therefore, this amount was used in the procedure since it gives high sensitivity and minimum blank value.

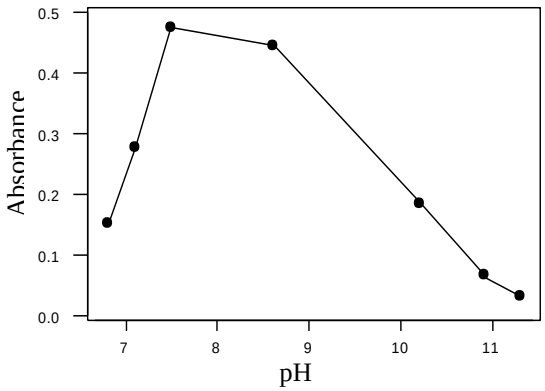


Figure 4: Effect of $CuSO_4.5H_2O$ concentration on the absorbance of 25 µg/ml salbutamol In the presence of 4-AAP

Effect of temperature and reaction time

The reaction time was determined by following the colour development at room temperature and at different temperatures in thermostatically controlled water-bath. The absorbance was measured at 5 min intervals against reagent blank treated similarly. As shown in Figure 5, it was observed that formation of coloured complex for salbutamol was achieved maximum after 70 min at 60°C and stable for at least 1 hour.

Effect of order of addition

To obtain optimum results the order of addition of reagents should be followed as given under the general procedure, otherwise a loss in colour intensity was observed.

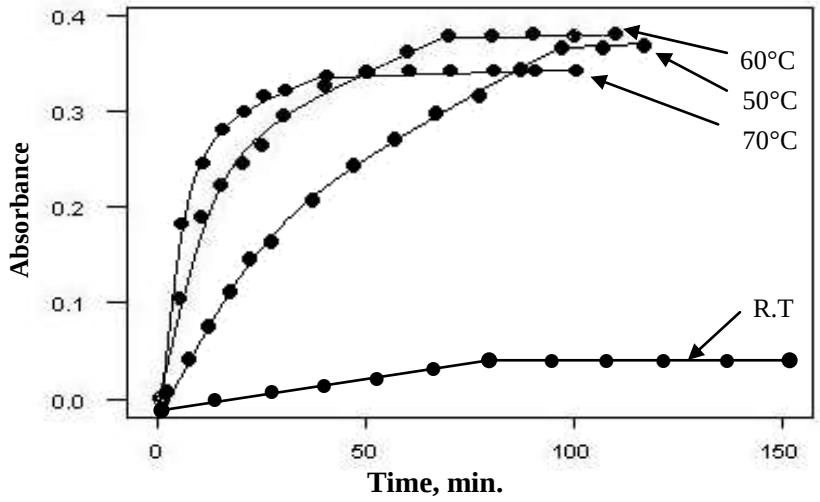


Figure 5 Effect of temperature and developing time on the absorbance of 10 µg/ml salbutamol.

Quantification

In order to investigate the range in which the coloured complex adhere to Beer's law, the absorbance of the complex was measured at λ_{max} value after developing the color by following the suggested procedure for a series of solutions containing increasing amounts of salbutamol drug (Figure 2). The Beer's law limits, molar absorptivity and Sandell's sensitivity values were evaluated and are given in Table 1, which are indicated that the method is sensitive. The linearity was represented by the regression equation and the corresponding correlation coefficient for the salbutamol determined by the proposed method represents excellent linearity. The relative standard deviation (RSD) and accuracy (average recovery %) for the analysis of six replicates of each three different concentrations of salbutamol (5, 15 and 25 µg/ml) indicated that the method is precise and accurate. LOQ is determined by taking the ratio of standard deviation of the blank with respect to water and the slope of calibration curve multiplied by a factor of 10. This means that LOQ is approximately 3.3 times LOD. Naturally, the LOQ slightly crosses the lower limit of Beer's law range. However, LOD is well below the lower limit of Beer's law range[30] .

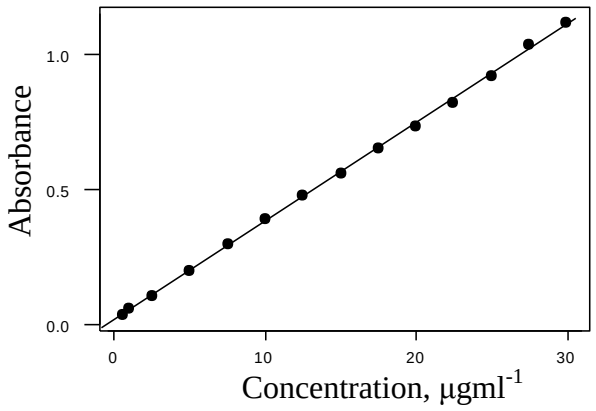


Figure 2. Calibration graph of salbutamol

Table 1. Summary of optical characteristics and statistical data for the proposed method

Parameter	Values of method
Beer's law limits (µg/ml)	0.5-30.0
Molar absorptivity ($l.mol^{-1}. cm^{-1}\times10^4$)	1.0106
Limit of detection (µg/ml)	0.1251
Limit of quantitation (µg/ml)	0.3812
Correlation coefficient	0.9990
Regression equation (*Y*)*	
Slope, *a*	0.0422
Intercept, *b*	0.0165
RSD**	1.67
Average recovery %	100 ± 0.05

* $Y = aX + b$, where X is the concentration of salbutamol in µg/ml.
** Average of six determinations.

Interference

The extent of interferences by some excipients which often accompanied pharmaceutical preparations were studied by measuring the absorbance of solutions containing 10 µg/ml of salbutamol and various amounts of diverse species in a final volume of 10 ml. It was found that the studied excipients do not interfere in the determination of salbutamol in its dosage forms. Starch maize and Vitamin C showed an interference effect when present in a large excess. An error of 5.0% in the absorbance readings was considered tolerable. Typical results are given in Table 2.

Table 2: Effect of excipients for assay of salbutamol

	Recovery %* of 10µg/ml of salbutamol per µg/ml excipients added in			
Excipients	**25**	**50**	**100**	**250**
Glucose	102.5	103.5	102.0	103.0
Lactose	104.4	104.5	102.2	103.3
Starch maize	97.7	99.2	82.5	62.5
Acacia	104.2	104.5	103.5	101.5
Talc	97.5	101.5	95.5	82.5
Sodium chloride	102.5	103.5	101.5	100.2
Glycerin	98.5	96.5	96.25	95.7
Vitamin C	96.55	91.0	37.5	24.5

* Average for three determinations

Analytical applications

The proposed method was successfully applied to determine salbutamol in its pharmaceutical preparations. The obtained results were compared statistically by a Student's *t*-test for accuracy and a variance ratio *F*-test for precision with the official method[29] (depending on potentiometric titration for pure drug using 0.1 M perchloric acid) at the 95% confidence level with five degrees of freedom, as cited in Table 3. The results showed that the *t*-test and *F*-test were less than the theoretical value(t=2.776, F=6.39), indicating that there was no significant difference between the proposed method and official method. Moreover, the proposed methods are compared favorably with other reported methods[15,17,20] as shown in Table 4.

Table 3. Assay of salbutamol in pharmaceutical preparations using the proposed method and comparison with the official method.

		Recovery % ± RSD[a]	
Preparation[b]	Nominal Value	Method	Official method[c]
Butadin (tablet)	2mg/Tab	100.30 ± 0.82 t =0.98 F =1.81	99.85±0.85
Butadin (syrup)	2mg/5ml	97.85 ±1.47 t =1.26 F=3.26	99.85±0.85

[a] Average of three determinations.
[b] Marketed by S.D.I Iraq
[c] Official method was applied for determination of pure drug.

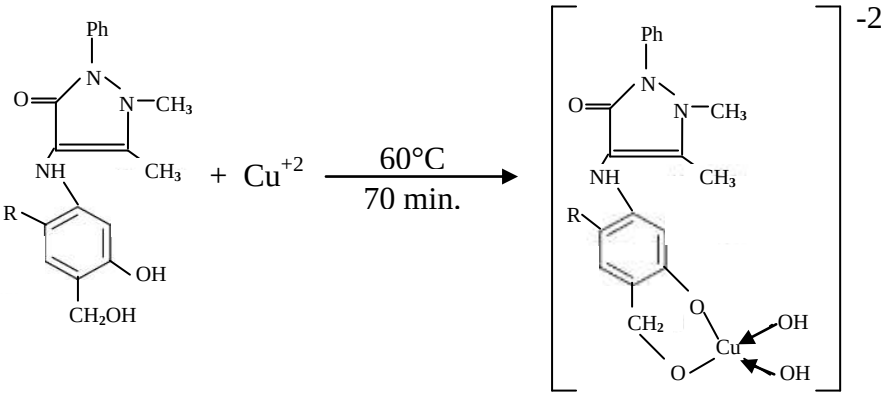
Table 4: Comparison of results for the determination of salbutamol by the proposed method and the reported methods

Reagent used	λ_{max} (nm)	Beer's law limit (µg/ml)	Molar absorptivity (L.mol-1cm-1)	Application	Remarks
Cerium(IV)–MBTH[a] [17]	530	0-15	2.4×10^4	---	Involves extraction and an expensive reagent
BrO_3^-–Br^- /methyl orange [20]	510	0.5-5	7.17×10^4	Tablet	Involves several reagents a critical conditions
F–C reagent[b] [15]	750	1-15	---	Tablet, urine	Uses on-line solid phase extraction and flow-injection
4-AAP-Cu	500	0.5-30	1.01×10^4	Tablet, syrup	Proposed method

[a]MBTH, 3-methylbenzothiazolin-2-one hydrozone.
[b] F–C reagent, Folin–Ciocalteu reagent.

Nature of complex

By applying the molar ratio method, it was found that salbutamol form a dye-coupled product with 4-AAP in the ratio 1 : 1 Cu : dye products. However: the solid products of the same reaction but with catecholamines are separated and characterized using different tools like elemental analysis, IR, magnetic, UV-Vis and thermal analysis [31]. The reaction may proceed as given in Scheme 1:



R= $CHOHCH_2NHBu^t$

Scheme 1

Conclusion

The proposed method is simple, fairly sensitive and economical when compared to already reported methods especially those based on non-aqueous medium and expensive technique like HPLC and do not require any pretreatment of the drugs or extraction procedure and has a good accuracy and precision. The method is important to the assay of pharmaceutical samples of salbutamol (tablet and syrup), and the results suggested that there is no interference with which are present in commercial dosage forms.

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طريقة طيفية لتقدير السالبيوتامول في المستحضرات الصيدلانية

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الخلاصة

تم وصف طريقة طيفية بسيطة وحساسة لتقدير عقار السالبيوتامول. تعتمد الطريقة على اقتران ٤–امينوانتي بايرين مع السالبيوتامول لتكوين ليكاند يتفاعل مع النحاس الثنائي ليعطي معقد مخلبي أحمر اللون عند درجة حرارة ٦٠ °م. تم تقدير الناتج الملون طيفيا عند ٥٠٠ نانوميتر. تم دراسة الظروف العملية المثالية. لقد أمكن تطبيق قانون بير بحدود ٠,٥– ٣٠ مايكروغرام/ مللتر من السالبيوتامول. لقد كانت دقة الطريقة بدلالة نسبة الاسترجاع (100 ± 0.05%) وبانحراف قياسي نسبي أقل من (1.67 %) . كما وجدت الحساسية بدلالة الامتصاصية المولارية ١٠١٠٦ لتر/مول.سم. تم مقارنة الطريقة المقترحة مع الطريقة القياسية في الدستور البريطاني للأدوية. كذلك تم دراسة تأثير المتداخلات من المضافات على المركبات الدوائية. تم تطبيق الطريقة المقترحة بنجاح في تقدير السالبيوتامول في مستحضراته الصيدلانية.