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Spectrophotometric Determination of Salbutamol Sulphate by Coupling with Diazotized 5-Amino-2-chlorobenzotrifluoride – Application to Pharmaceutical Preparations

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ABSTRACT

A simple, rapid and sensitive spectrophotometic method for the estimation of trace amounts of salbutamol sulphate (SBS) in pharmaceutical preparations has been proposed .The method is based on the coupling reaction of the intended compound with diazotized 5-amino-2-chlorobenzotrifluoride in alkaline medium and in the presence of Triton X-100 to form a yellow-orange dye that shows maximum absorption at 467 nm. Beer's law is obeyed over the range 5 – 300 µg / 10 ml (i.e.,0.5-30 ppm) with a molar absorptivity of 4.20×10^4 $l.mol^{-1}.cm^{-1}$ and Sandell's sensitivity index of 0.0139 $\mu g.cm^{-2}$, a relative error of +1.08 to +3.46 % and a relative standard deviation of ±0.76 to ±3.50 %, depending on the concentration. The method has been applied to estimate salbutamol sulphate in syrup, tablet and ventolin.

Keywords: Salbutamol sulphate, diazotized 5-amino-2-chlorobenzotrifluoride, diazo-coupling, Triton X-100, spectrophotometry

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التقدير الطيفي لكبريتات السالبيوتامول بالاقتران مع الكاشف المؤزوت 5-امينو-2- كلوروبنزو-ثلاثي فلوريد- تطبيقات في مستحضرات صيدلانية

الملخص

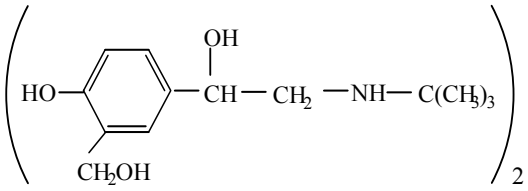
اقترحت طريقة طيفية بسيطة وسريعة وحساسة لتقدير كميات متناهية في الـصغر مـن كبريتـات الـسالبيوتامول فـي المستحضرات الصيدلانية. تعتمد الطريقة على اقتران المادة المراد تقديرها مع الكاشف 5-امينو-2-كلوروبنزوثلاثي الفلوريـد المؤزوت بوجود Triton X-100 في الوسط القاعدي لتكوين صبغة آزوية ذات لون برتقالي مصفر تكون ذائبة فـي المـاء ومستقرة والتي تعطي أقصى امتصاص عند الطول الموجي 467 نانوميتر. كانت حدود قانون بير في مدى التراكيز من 5 إلى 300 مايكروغرام/ 10مل (أي من0.5-30 جزء/مليون). وكانت الامتصاصية المولارية 4.20×10^4 لتر. مـول$^{-1}$ سـم$^{-1}$، ودلالة ساندل للحساسية 0.0139 مايكروغرام / سم^2، وتراوح الخطأ النسبي بين 1.08+ إلى 3.46+% والانحـراف القياسـي النسبي في مدى 0.76± إلى 3.50 % ± اعتماداً على التركيز. تم تطبيق الطريقة بنجاح لتقدير كبريتات الـسالبيوتامول فـي الشراب والأقراص والفنتولين.

الكلمات الدالة: كبريتات السالبيوتامول، كاشف5-امينو-2-كلوروبنزوثلاثي الفلوريد المؤزدت، الاقتران الازوي، TritonX-100 تقنية مطيافية.

INTRODUCTION

Salbutamol sulphate is the racemic form of salbutamol, it is an important drug, indicated for the relief of severe bronchial spasm associated with asthma (Fattah *et al*., 1998). Salbutamol

sulphate is [di[(RS)-2-(1,1-dimethyl) ethylamino-1-[4-hydroxy-3-(hydroxymethyl)phenyl] ethanol] sulphate] a white or almost white, crystalline powder, freely soluble in water, slightly soluble in alcohol and in ether, very slightly soluble in methylene chloride and has the following structure (British Pharmacopoeia, 2007).



M.wt = 576.7g/mol

Salbutamol sulphate

The assay of Salbutamol sulphate officially listed in British Pharmacopoeia describes a potentiometric titration procedure (British Pharmacopoeia, 2007).

Several analytical methods for the determination of salbutamol were developed and reported such as TLC (Dave *et al*., 2011), GC and GC-MS (Caban *et al*., 2011; Wang *et al*., 2010), cyclic voltammetry (Ganjali *et al*., 2005), adsorptive stripping voltammetry on a carbon paste electrode (Attaran *et al*., 2012), Capillary electrophoresis coupled with electrochemilumencesis (Bao *et al*., 2012), flow injection method (Al-Abachi and Subhi, 2013), HPLC and RP-HPLC (Mukesh and Ranjit, 2011; Pai *et al*., 2009 ; Ghulam *et al*., 2009 ; Li *et al*., 2010), HPLC-chemiluminescence (Zhang *et al*., 2011), solid phase extraction (SPE)-HPLC (Liu and Wang , 2011; Yan *et al*., 2012) , and fluorescence (Tang *et al*., 2010).

Various spectrophotometric procedures have been reported for the determination of salbutamol sulphate as a pure and in dosage form using different reagents such as diazotized sulphanilic acid (Othman and Zakaria, 2004), diazotized p-nitroaniline (Othman and Hamdoun, 2005), Folinciocalteu (Basavaiah and Prameela, 2003), iron(III) with ferricyanide (Kanakapura and Huiikal, 2003), hydroxyl ammonium chloride in alkaline medium (Manasa, 2013), p-phenylenediamine in presence of sodium meta periodate (Al-Hafith, 2005), 2,6-dichloroquinonechlorimide and 7,7,8,8-tetracyanoquindimethan (Mohamed *et al*., 2002), and sodium hydroxide (Eswarudu *et al*., 2012). The present work describes a spectrophotometric method for the determination of salbutamol sulphate . The method is based on coupling the salbutamol sulphate with diazotized 5-amino-2-chlorobenzotrifluoride to form a stable and soluble azo dye product .

THE EXPEREMENTAL

Apparatus:

The spectrophotometric measurements were carried out on Jasco V-630 using 1cm glass cells.

Reagents

All chemicals used are of the highest purity available.

Salbutamol sulphate solution, 100 μg. ml^{-1}.

A 0.0100g amount of salbutamol sulphate was dissolved in distilled water, then the volume was completed to 100 ml in a volumetric flask with distilled water.

Diazotized 5-amino-2-chlorobenzotrifluoride solution 5x10^{-3} M.

This solution was prepared daily by dissolving 0.0978 g of 5-amino-2-chlorobenzotrifluoride in 10 ml of ethanol then a 3.0 ml of concentrated HCl was added, followed by dilution to 80 ml with distilled water, then the solution was transferred into a 100-ml volumetric flask and cooled to (0 - 5) ºC in an ice-bath, a 0.0345 g of sodium nitrite was added then stirred vigorously, after 5 minutes the solution was made up to 100 ml with cooled distilled water and stored in a dark bottle.

Sodium hydroxide solution, 2 M.

This solution was prepared by the dilution of the concentrated volumetric solution (1Ampoule, Fluka) to 500 ml with distilled water and then transferred to a plastic bottle.

Triton X-100, (1%).

A 1.0 g of Triton X-100 was dissolved in 100 ml of distilled water.

Interferences solutions, 1000 μg.ml^{-1}.

These solutions were prepared by dissolving 0.1 g of each of them in 100 ml of distilled water.

Butadine syrup solution, 100 μg .ml^{-1}.

This solution was prepared by diluting 25 ml of butadine syrup (2 mg salbutamol sulphate per 5 ml) to 100 ml with distilled water in a volumetric flask.

Butadin a tablets solution, 100 μg .ml^{-1}.

Finely 5 powdered tablets of butadine drug (each tablet contains 2 mg salbutamol sulphate) were dissolved in 80 ml of distilled water, and the solution was shaked and warmed. The solution was filtered into a 100-ml volumetric flask, the residue was washed with distilled water and diluted to volume with distilled water to obtain 100 mg/l salbutamol sulphate.

Solution, 100 μg .ml^{-1}.Salb. Vent.

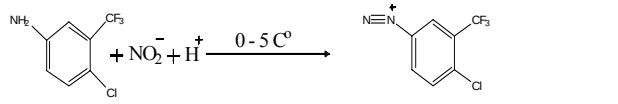
A 2 ml of salb. vent. (5mg salbutamol sulphate per 1ml) diluted to 100 ml with distilled water in a volumetric flask to obtain 100 μg.ml^{-1} salbutamol sulphate solution.

RESULTS AND DISCUSSION

Principles of the method

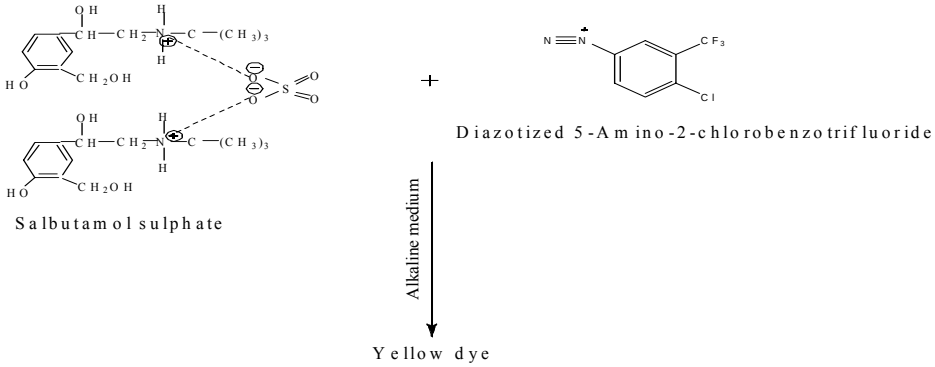
The method included the following steps:

- Preparation of diazotized 5-amino-2-chlorobenzotrifluoride.



5-Amino-2-chlorobenzotrifluoride Diazotized 5-Amino-2-chlorobenzotrifluoride

- Coupling of salbutamol sulphate with diazotized 5-amino-2-chlorobenzotrifluoride to form a yellow dye in a basic medium.



Optimum reaction conditions

The effect of different factors on the formation of the colored dye is investigated and the reaction conditions have been optimized.

For the subsequent experiments, 100 μg of salbutamol sulphate is taken in 10 ml as a final volume and absorbance measurements are achieved directly after dilution with distilled water at 451.5 nm.

Selection of diazotized reagent

Some diazotized reagents (1 ml of $5x10^{-3}$ M) have been selected for optimum conditions. The results in Table (1) show that 5-amino-2-chlorobenzotrifluoride gives the highest intensity with high value of color contrast, therefore, it has been selected for the subsequent experiments.

Table 1: Selection of diazotized reagent

1 ml of ($5x10^{-3}$M) diazotized reagent	λ_{max}(nm)	$\Delta\lambda_{max}$(nm)*	Absorbance
5-Amino-2-chlorobenzotriflouride	451.5	141.5	0.3068
3-Aminobenzotriflouride	437	138	0.2023

- $\Delta\lambda_{max,} = \lambda_{max}S - \lambda_{max}B$ where S = Sample, B = Blank

Optimum amount of diazotized 5-amino-2-chlorobenzotriflouride reagent

The effect of different amounts (1.0 – 6.0 ml) of 5-amino-2-chlorobenzotriflouride ($5x10^{-3}$ M) reagent on the absorbance of solutions containing different amounts of salbutamol sulphate (50-300 µg/10ml) has been studied; the results in Table (2) show that 4 ml of diazotized 5-amino-2-chlorobenzotriflouride reagent gives highest absorbance and with best determination coefficient (R^2 = 0.999), therefore this volume of diazotized reagent was selected for the subsequent experiments.

Table 2: The optimum amount of diazotized reagent

ml of (5×10^{-3}M) diazotized reagent solution	Absorbance / µg of salbutamol sulphate				R^2
	50	100	200	300	
1.0	0.1701	0.3068	0.5642	0.6468	0.947
2.0	0.1963	0.4054	0.6835	1.0793	0.994
3.0	0.2121	0.3984	0.7602	1.3131	0.988
4.0	0.2272	0.4596	0.9413	1.3872	0.999
5.0	0.2200	0.4272	0.9258	1.3430	0.998

Effect of pH

Salbutamol sulphate undergoes complete diazo-coupling reaction in alkaline medium (Othman and Zakaria , 2004), so that several bases have been tested Table (3) for optimum conditions (1 ml of 2 M of each base was added).

Table 3: Selection of base

1ml of Base (2 M)	$\lambda_{max(nm)}$	$\Delta\lambda_{max(nm)}$	Absorbance		Final PH
			Sample Vs. Blank	Blank Vs.DW.	
NaOH	452	143	0.4775	0.0746	12.01
KOH	361.5	46	0.4724	0.1877	12.23
$NaHCO_3$	Turbid				
Na_2CO_3	Turbid				

The experimental data in Table (3) showed that the reaction needs a strong alkaline medium and NaOH gives a highest sensitivity with best color contrast, therefore it has been fixed for the subsequent experiments.

The optimum amount of sodium hydroxide

The results in Table (4) indicate that 1ml of NaOH (2 M) gives the highest intensity of the colored dye and the value of determination coefficient (0.999), therefore this volume has been recommended for the subsequent experiments.

Table 4: The optimum volume of sodium hydroxide

ml of NaOH (2M)	Absorbance / µg of salbutamol sulphate				Determination coefficient (R^2)
	50	100	200	300	
0.5	0.0338	0.0610	0.0882	0.2092	0.903
0.8	0.2203	0.4821	1.0587	1.5800	0.999
1.0	0.2384	0.5056	1.0518	1.5946	0.999
2.0	0.2342	0.5001	0.9011	1.1977	0.987
3.0	0.2415	0.3838	0.8325	1.0311	0.974

Effect of surfactant

The effect of several types of surfactants with different orders of addition on color intensity and color contrast of the dye has been investigated. (Table 5 and Fig. 1).

Table 5: The effect of surfactant on dye absorbance

Order of addition	Absorbance/ 1 ml of surfactant solution					
	CTAB ($1x10^{-3}$M)		SDS (1%)		Triton X-100 (1%)	
	Abs.	$\lambda_{max.(nm)}$	Abs.	$\lambda_{max.(nm)}$	Abs.	$\lambda_{max.(nm)}$
I	0.4563	440.5	0.5244	452	0.7033	466.5
II	0.4895	441	0.6022	451.5	0.7090	467
III	0.5016	438	0.5138	452.5	0.6045	467

Note: Absorbance = 0.5259 without surfactant and $\lambda_{max.}$ = 451.5 nm

I. Salbutamol sulphate (S) + surfactant (C) + Reagent (R) + NaOH (B)

II. S + R + C + B

III. S + R + B + C

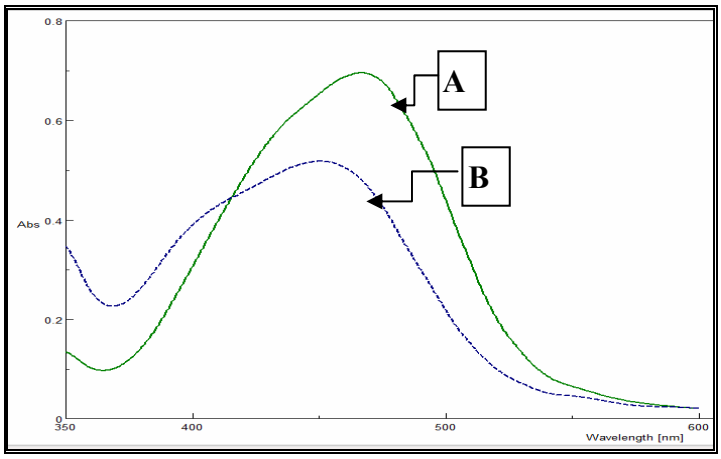


Fig. 1: The effect of Triton X-100 on absorbance (order II)
A-Sample with Triton X-10
B- Sample without Triton X-100

The results in Table (5) and (Fig. 1) indicate that the addition of Triton X-100 in order (II) increases the intensity of the formed dye and color contrast from 141.5 nm To 158 nm, therefore it has been recommended in subsequent experiments.

The optimum amount of Triton X-100

From the results in Table (6), it was found that 1 ml of (1%) TritonX-100 solution was adequate for the maximum absorbance, therefore it has been used in the subsequent experiments.

Table 6: The effect of TritonX-100 amount on absorbance

ml of (1%) TritonX-100	0.5	1.0	2.0	3.0
Absorbance	0.6756	0.6913	0.6715	0.6710

Effect of time and amount of salbutamol sulphate on absorbance

A study of the time effect on color development showed that the color formed immediately and remained stable for at least 90 minutes, Table (7).

Table 7: Stability of azo dye

Time/min.	Absorbance/µg of Salbutamol sulphate present		
	50	100	200
After dilution	0.3678	0.7061	1.3129
10	0.3652	0.6903	1.2981
20	0.3636	0.6933	1.2939
30	0.3670	0.6964	1.2940
40	0.3727	0.6916	1.2958
50	0. 3721	0.6946	1.2906
60	0. 3752	0.6949	1.2969
90	0. 3754	0.6962	1.2952
120	0.3904	0.6821	1.2390
Over night	0.1130	0.4103	0.9259

Final Absorption Spectra

Absorption spectra of the colored dye formed from treatment salbutamol sulphate with diazotized 5-amino-2-chlorobenzotriflouride reagent in basic medium, in the presence of TritonX-100, according to the above recommended procedure, showed that the maximum absorption is obtained at 467 nm (Fig. 2).

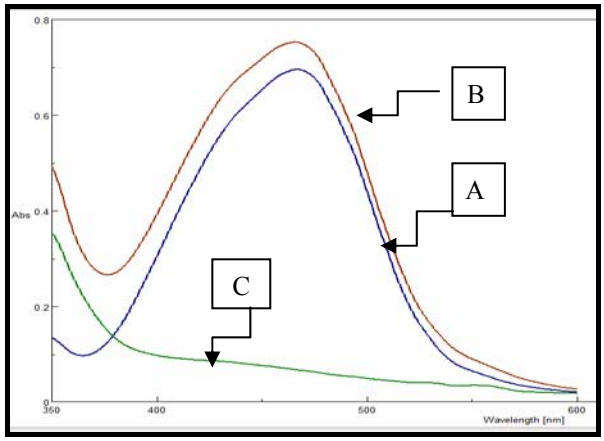


Fig. 2: Absorption spectra of 100 µg salbutamol sulphate/10 ml treated according to the recommended procedure and measured against (A) blank, (B) distilled water and (C) blank measured against distilled water.

Procedure and calibration graph

To a series of 10-ml volumetric flasks, increasing volumes of aqueous solution containing 5–300 μg salbutamol sulphate are transferred, 4 ml diazotized 5-amino-2-chlorobenzotrifluoride ($5x10^{-3}$ M) followed by the addition at 1 ml of (1%) Triton X-100 and 1 ml NaOH (2 M) then the volumes were completed to the mark with distilled water. The absorbance for each flask was measured directly after dilution at 467 nm against blank. The calibration graph is linear over the range 0.5 – 30 $\mu g.ml^{-1}$ and higher concentrations show negative deviation from Beer's law (Fig. 3). The apparent molar absorptivity referred to salbutamol sulphate, has been found to be 4.2×10^4l. $ol^{-1}.cm^{-1}$.

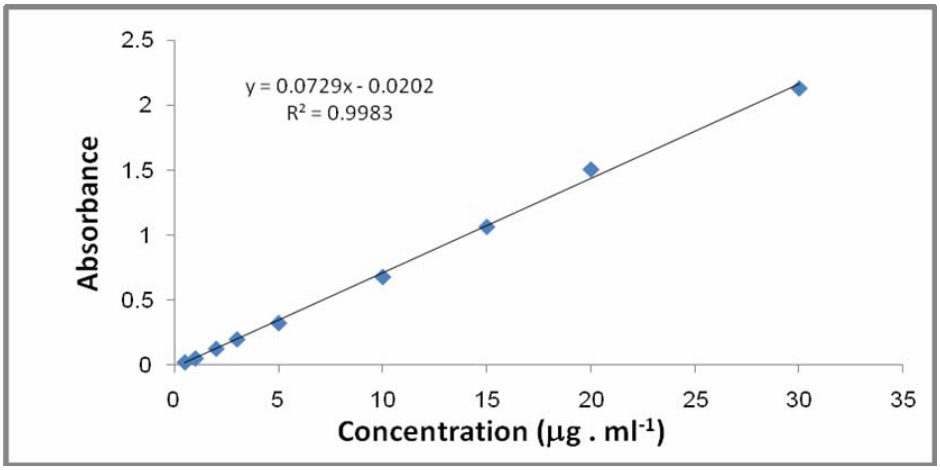


Fig. 3: Calibration graph for salbutamol sulphate determination using the proposed method

Nature of the dye

Continuous variations (Job's method) and mole – ratio methods (Delevie, 1997) indicate that the dye has a composition of 1:1 salbutamol sulphate [SBS] to diazotized 5-amino-2-chlorobenzotriflouride [Diaz.] reagent (Fig. 4 and 5).

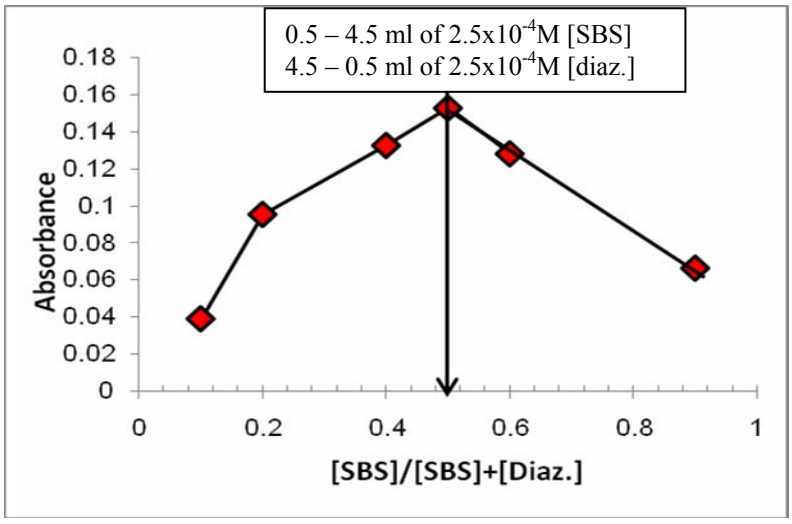


Fig. 4: Job`s method plot

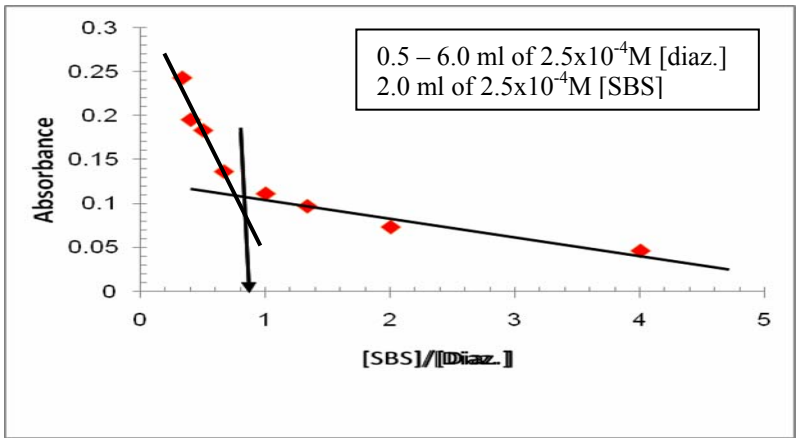
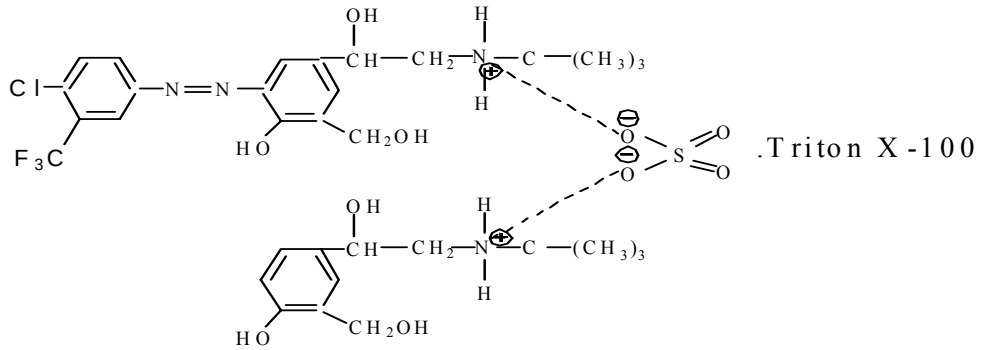


Fig. 5: Mole ratio method plot

Hence the dye may have the following structure.



Interference

The extent of interferences by some excipients which often accompany pharmaceutical preparations as studied by measuring the absorbance of solutions containing 100 µg ml^{-1} of salbutamol sulphate and various amounts (50,100 and1000) of exaplents in a final volume of 10 ml. It was found that the studied excipients do not interfere in the determination of salbutamol sulphate in its dosage forms. Typical results are given in Table (8).

Table 8: Effect of foreign compounds on the determination of 100 µg salbutamol sulphate

Foreign compound	Recovery (%) of 100 µg salbutamol sulphate per µg foreign compound added		
	100	500	1000
Glucose	98.36	99.86	97.21
Arabic Gum	102.52	100.53	101.46
Lactose	96.29	95.55	98.04
Starch	101.19	97.95	101.97

Application of the method

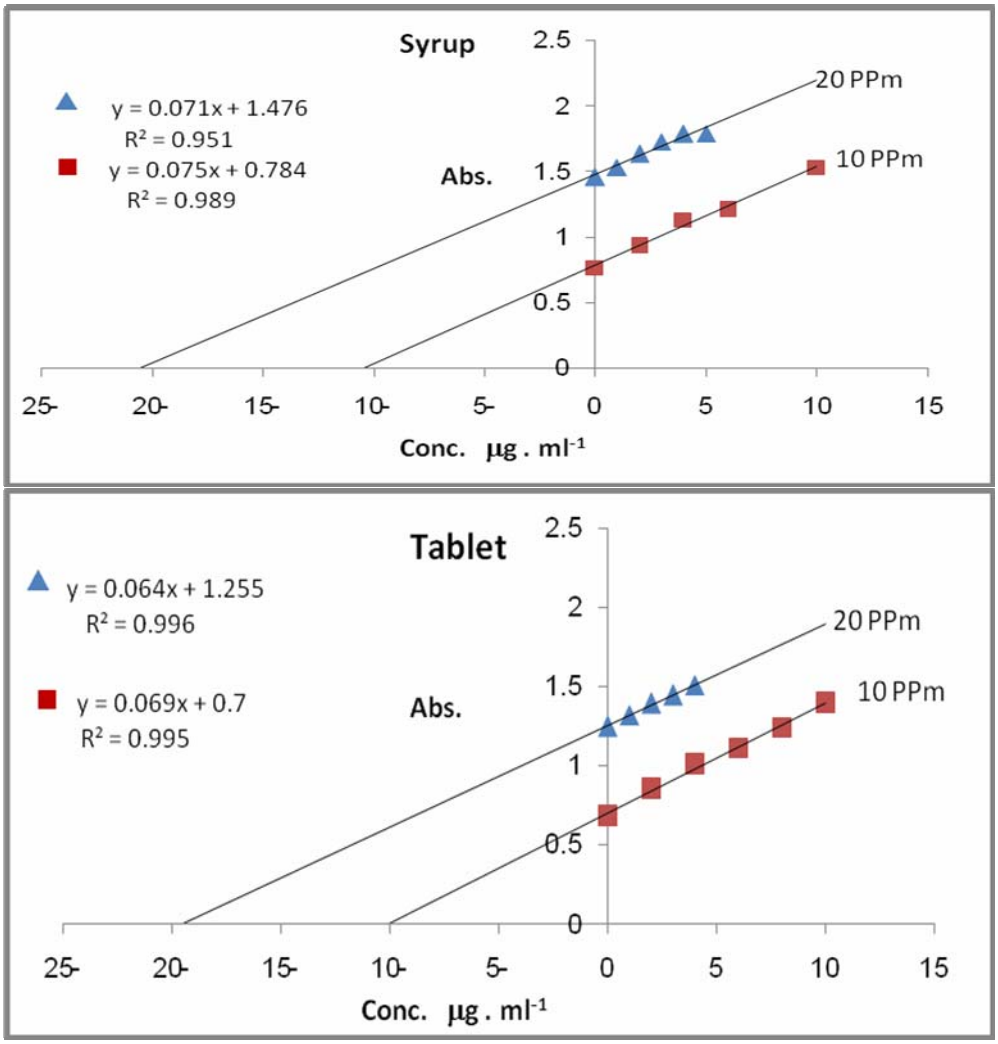
The proposed method was applied to determine salbutamol sulphate in its pharmaceutical preparations (Butadin syrup, tablet and Salbu. Vent.). The results shown in Table (9) indicated that a good recovery and the RSD% was better than ±3.50%.

Table 9: Analytical applications of the proposed method

Pharmaceutical preparation	Amount taken (µg)	Amount measured (µg)	Recovery,(%*)	RSD,(%*)
Butadin syrup 2 mg salbutamol sulphate/ 5 ml (S.D.I Iraq)	50	51.72	103.44	3.17±
	100	101.08	101.08	0.76±
Butadin tablet 2 mg salbutamol sulphate/ tablet (S.D.I Iraq)	50	51.69	103.38	1.73±
	100	102.16	102.16	2.47±
Salbu. Vent. 5 mg salbutamol sulphate/ 1 ml (Diamond pharma-syria)	50	50.90	101.80	3.50±
	100	101.63	101.63	2.35±

*Average of five determinations

The validity of the method was confirmed by applying the standard addition procedure (Al-Abachi, and Al-Ghabsha, 1986) (Fig. 6).



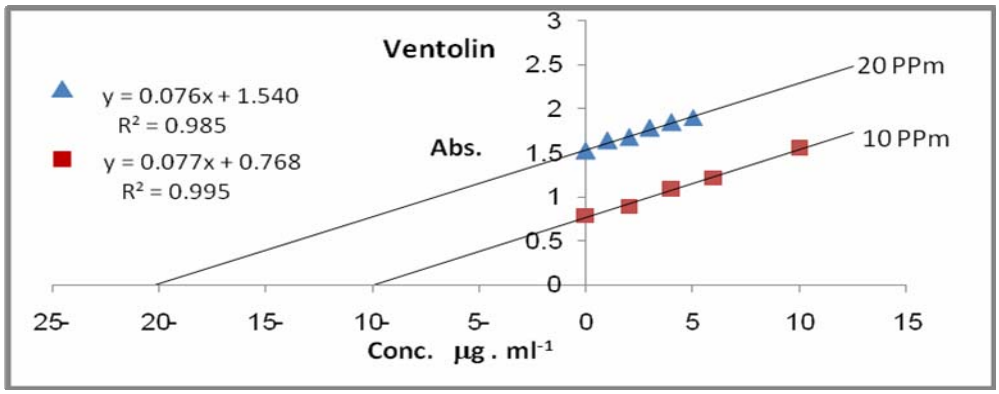


Fig. 6: Standard addition plot for the recoveries of 10,20 µg of salbutamol sulphate in butadin syrup, tablet and ventolin respectively.

The recoveries calculated by using the equations of the linearity in (Fig. 6) and the results obtained are in agreement with the certified value Table (10).

Table 10: The results of standard addition method

Pharmacetical preparation	Amount taken ($\mu g.ml^{-1}$)	Amount measured ($\mu g.ml^{-1}$)	Recovery, %
Butadin syrup 2 mg salbutamol sulphate/ 5 ml (S.D.I Iraq)	10	10.45	104.50
	20	20.78	103.90
Butadin tablet 2 mg salbutamol sulphate/ tablet (S.D.I Iraq)	10	10.14	101.40
	20	19.61	98.05
Salbu. Vent. 5 mg salbutamol sulphate/ 1 ml (Diamond pharma-syria)	10	9.97	99.70
	20	20.26	101.30

Comparison of the methods

Table (11) shows the comparison between some of analytical variables obtained from the present method with other spectrophotometric methods.

Table 11: Comparison of the methods

Parameter	Present method	Othman and Zakaria , 2004	Othman and Hamdoun, 2005	Al-Hafith (2005)
Reagent	Diazotized 5-amino-2-chlorobenzotriflour-ide	Diazotized sulphanilic acid	Diazotized p-nitroaniline	p-Phenylenedi-amine in presence of meta per iodate
pH	12.01	-	12.2	9
Temperature (C^o)	R.T.	R.T.	R.T.	R.T.
Development time (min.)	After dilution	10	After dilution	50
λ_{max} (nm)	467	445	488	552
Beer's law range (ppm)	0.5 - 30	0.8 – 4.0	0.4 – 4.8	0.8 – 40
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	4.15×10^4	3.53×10^4	3.13×10^4	2.30×10^4
Colour of the dye	Yellow - orange	Yellow	Orange	Violet
Application of the method	Syrup, tablet and ventoline	Syrup and tablet	Syrup and tablet	Syrup, tablet and ventoline

CONCLUSION

The advantage of the proposed method compared to the reference methods was a higher sensitivity than the refered methods which were linear from 0.5-30 $\mu g.ml^{-1}$.Moreover, the proposed method could be applied successfully to the determination of the salbutamol sulphate in a pure form as well as in its dosage forms.

REFERENCES

Al-Abachi, M.Q.; Al-Ghabsha, T.S. (1986). "Fundamentals of Analytical Chemistry". Mosul University Press, p.197 (in Arabic).

Al-Abachi, M.Q.; Subhi, S. (2013). Flow injection- spectrophotometric determination of salbutamol sulphate and pyridoxine hydrochloride using 2,4- dinitrophenylhydrazine. *Iraqi J. Sci.*, **54**(1), 6-16.

Al-Hafith, H.A. (2005). Development of spectrophotometric methods for the determination of some phenolic compounds and catecholamines in pharmaceutical preparations. M.Sc. Thesis, Mosul University, 45 p.

Attaran, M.A.; Javanbakht, M.; Fathollahi, F.; Enhessari, M. (2012). Determination of salbutamol in pharmaceutical and serum samples by adsorptive stripping voltammetry on a carbon paste electrode modified by iron titanatenano powders. *Electroanal.*, **24** (10), 2013-2020.

Bao, Y.; Yang, F.; Yang, X. (2012). Capillary electrophoresis coupled with electrochemiluminescence for the facile separation and determination of salbutamol and clenbuterol in urine. *Electroanal.*, **24**(7),1597-1603.

Basavaiah, K.; Prameela, H.C. (2003). Spectrophotometric determination of salbutamol sulfate and pyrantel pamoate in bulk drugs and pharmaceuticals. *Chem. Anal.*, **48**(2), 327-334.

British Pharmacopoeia on CD-ROM, (2007). Copyright by System Simulation Ltd. The Stationery Office Ltd., London.

Caban, M.; Stepnowski, P.; Kwiatkowski, M.; Migowska, N. (2011). Determination of b-blockers and b-agonists using gas chromatography and gas chromatography-mass spectrometry - A comparative study of the derivatization step. *J. Chromatogr. A.* **1218**(44), 8110-8122.

Dave, H.N.; Mashru, R.C.; Patel, A.K. (2011). Thin Layer chromatography method for the determination of ternary mixture containing salbutamol sulphate, bromhexine hydrochloride and etofylline. *J. Pharma. Sci. Res.*, **2**(3), 143-148 .

Delevie, R. (1997)." Principles of Quantitative Chemical Analysis". McGraw-Hill, International Edn., Singapore, p.498.

Eswarudu, M.M.; Sushma, M.; Sushmitha, M.; Yamini, K. (2012). Validated spectrophotmetric method for the determination of salbutamol sulphate in bulk and pharmaceutical dosage forms. *Int. Res. J. Pharma.*, **3**(4), 423-425.

Fattah, E.; Grant, D.; Gabr, K. ; Meshali, M. (1998). Physical characteristics and release behaviour of salbutamol sulphate beads prepared with different ionic poly–saccharides. *Drug Devel. Indust. Pharma.*, **24**(6), 541 – 547.

Ganjali, M.R.; Norouzi, P.; Ghorbani, M.; Sepehri C.R. (2005). A fourier transform cyclic voltammetric technique for monitoring ultratrace amounts of salbutamol at gold ultra microelectrode in flowing solutions *Talanta*, **66**(5), 1225-1233.

Ghulam, M.; Mahmood, A.; Muhammad, A.; Muhammad, W. (2009). A new reverse phase HPLC method with fluorescent detection for the determination of salbutamol sulphate in human plasma. *Bull. Chem. Soc. Ethiopia*, **23**(1), 1-6.

Kanakapura, I.; Huiikal, C. (2003). Spectrophotometric determination of salbutamol sulphate and acyclovir using iron(III) and ferricyanide., *Sci. Asia*, **29**,141-146.

Li, C.; Wu, Y.L.; Yang, T.; Zhang, Y. (2010). Simultaneous determination of clenbuterol, salbutamol and ractopamine in milk by reversed-phase liquid chromatography tandem mass spectrometry with isotope dilution. *J. Chromatogr. A* , **1217**(50), 7873-7877 .

Liu, C.; Wang, L. (2011). Research on determination of clenbuterol and salbutamol in pork by SPE-HPLC. *Inter. Conf. New Technol. Agr.*, 1024-1026.

Manasa, A. (2013). Spectrophotometric determination of salbutamol sulphate in bulk form and in various dosage forms. *The Experiment*, **7**(4), 445-449.

Mohamed, G.G.; Khalil, S.M.; Zayed, M.A.; El-Shall, M.A. (2002). 2,6-Dichloroquinone chlorimide and 7,7,8,8- tetracyanoquinodimethane reagents for the spectrophotometric determination of salbutamol in pure and dosage forms. *J. Pharma. Biomed. Anal.*, **28**(6), 1127-1133.

Mukesh, M.; Ranjit, S. (2011). Development and validation of a stability- indicating HPLC method for the simultaneous determination of salbutamol sulphate and theophylline in pharmaceutical dosage forms. *J. Anal. Bioanal. Tech.*, **2**(1),1-5.

Othman, N.S.; Hamdoun, E.A. (2005). Diazotized p-nitroaniline reagent for the determination of trace amount of salbutamol sulphate in aqueous solution-application to pharmaceutical preparation. *Raf. J. Sci*., **16**,60-67.

Othman, N.S.; Zakaria, R.Z. (2004). Use of diazotized sulphanilic acid reagent in the spectrophotometric determination of salbutamol sulphate-application to pharmaceutical preparation. *J. Edu. Sci*., **16**, 27-37.

Pai, P.N.; Rao, G.; Murthy, M.; Agarwal, A. (2009). Simultaneous determination of salbutamol sulphate and bromhexine hydrochloride in tablets by reverse phase liquid chromatography. *Indian J. Pharma. Sci.*, **71**(1), 53-55.

Tang, J.; Liu, Z.; Kang, J.; Zhang, Y. (2010). Determination of salbutamol using R-phycoerythrin immobilized on egg shell membrane surface as a fluorescence probe. *Anal. Bioanal. Chem.*, **397**(7), 3015- 3022.

Wang, L.; Li, Yuan-Q.; Zhou, Yun-K.; Yang, Y. (2010). Determination of four β2-agonists in meat, liver and kidney by GC-Mass with dualinternal standards. *Chromatographia* ,**71**(7), 737-739.

Yan, H.; Wang, R.; Han, Y.; Liu, S. (2012). Screening, recognition and quantization of salbutamol residues in ham sausages by molecularly imprinted solid phase extraction coupled with high-performance liquid chromatography-ultraviolet detection. *J. Chromatogr. B*, **900,** 18-23.

Zhang, Y.; Zhang, Z.; Sun, Y.; Wei, Y. (2011). Development of an analytical method for the determination of β_2-agonist residues in animal issues by high-performance liquid chromatography with on-line electrogenerated $[Cu(hio_6)_2]^{5-}$luminol chemiluminescence detection. *J. Agr. Food Chem.*, **55**(13), 4949-4956.