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Application of Cloud Point Method for Spectrophotometric Determination of Salbutamol Sulphate and Methyldopa

Intisar A. Shihab[1] and Theia'a N. Al-Sabha[2]*

[1]*Department of Chemistry, College of Education for Girls, University of Mosul, Mosul, Iraq.*
[2]*Department of Chemistry, College of Education for Pure Sciences, University of Mosul, Mosul, Iraq.*
**Corresponding Author Email: dr_theiaa@yahoo.co.uk*
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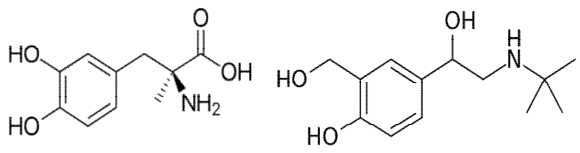
Abstract

A simple and efficient cloud point spectrophotometric method has been used for the determination of salbutamol sulphate and methyldopa both in pure and pharmaceutical preparations. The procedure was based on the ion association formation with eosin Y. The extraction of ion association, drown to Triton X-114 micelles, was measured spectrophotometrically. The phase separation was studied and optimized. Beer's law was rectilinear over the concentration ranges of 0.1-20 and 0.3-10 µg/mL with molar absorptivity $4x10^4$ and $5.7x10^4$ $L.mol^{-1}$ cm^{-1} and average recovery 98.21% and 101.27% for the above drugs, respectively. The method was applied successfully for the determination of salbutamol sulphate and methyldopa in pharmaceuticals.

Keywords: Cloud point, Ion association, Eosin Y, Salbutamol, Methyldopa.

Introduction

Methyldopa [Lα-Methyl-3,4-dihydroxyphenyla-lanine; $C_{10}H_{13}NO_4$] [I] is an aromatic-amino acid decarboxylase inhibitor in animals and in man [1]. It is a medication that has been used to treat high blood pressure since the 1960s. While there is some belief that it reduces blood pressure [2]. Salbutamol, [1-(4-hydroxy-3-hydroxymethylp-henyl)-2-(t-butylamino) ethanol] (II), marketed as Ventolin, is usually considered the drug of choice as relief medication for symptoms of bronchospasm. It is an agonist of β2 receptors which are present in the bronchioles of lungs of the human body. Athletes using β2-agonists, usually inhale them prophylaxis prior to competition or training [3].



For the determination of salbutamol sulphate and methyldopa, different analytical techniques have been proposed, like spectrophotometric [4-15], chromatographic [16-19], voltammetric [20-21], potentiometric [22], flow injection [23] and kinetic [24] methods. Our determination of salbutamol sulphate and methyldopa spectrophotometrically is actually new, simple, and sensitive. The method has been applied for the determination of the pharmaceutical formulations of both drugs. The work has aimed at developing a new spectrophotometric method with cloud point extraction (CPE) preconcentration for Salbutamol and Methyldopa by using of eosin Y as a reagent. In the CPE method, surfactant Triton X-114 was used as the extractant solution which is used for promoting phase separation [25].

Materials and Methods

Visible spectrophotometer (T92 UV) equipped with a 1.0-cm glass cell and RLO 60P pH-meter with a combined glass electrode has been used. A centrifuge was used for separation

(laboratory centrifuge-INDIA). Statistically, Excel 2010 software has been used.

Reagents

In this research, the chemical materials used were from Fluka and BDH companies. eosin Y concentrations of 1% and 2 % were prepared by diluting 1 and 2 g in distilled water respectively, using calibrated flasks of volume 100 mL. The acetate and citrate buffer solutions of pH 3.9 and 4 were adjusted by pH meter. Salbutamol sulphate and methyldopa of 100 µg/mL were prepared by diluting of 0.01 g of each pure drug in 100 mL distilled water separately. Triton X-100 of 1% was prepared by dissolving 1 g of surfactant in 100 mL distilled water in volumetric flask.

General cloud point extraction procedure for drugs

Into two sets of 10 mL volumetric flasks, volumes of both salbutamol and methyldopa within concentrations 0.1-20 and 0.3-10 µg/mL respectively, were added separately. Then, followed by the addition of 1 mL of citrate buffer or acetate buffer solution, 1 mL of 1%Triton X-114 followed by addition of 2 mL of 1% eosin Y for salbutamol or methyldopa, respectively. Then, the volume was completed to the mark with distilled water. The solutions were placed in a water bath adjusted at 60°C or 50°C for above drugs respectively. The turbid contents of the flasks were transferred into a 10 mL centrifuging tubes. Surfactant-rich phase were accomplished by centrifugation for 10 min at 3500 rpm. After cooling in an ice bath for 5 min the rich layer became sticky, and the aqueous phases were decanted. Ethanol was added to surfactant-rich phase and the volume was completed to 10 ml by distilled water. The absorbance was measured at 558 nm and 564 nm for salbutamol and methyldopa, respectively.

Results and Discussion

The preliminary investigation was found that salbutamol and methyldopa reacted with Eosin Y in acidic medium, a reddish-orange ion-pair complexes with λ_{max} at 558 nm and 564 nm respectively (Fig. 1) as a result of the reaction. Which attributed to the electrostatic interaction between the most basic center in the drug's molecules (hydroxyl groups) and the carboxylate anion of the dye formed complexes.

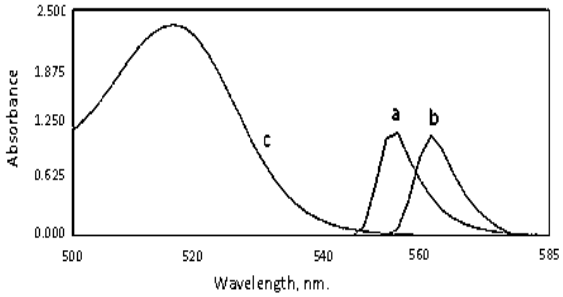


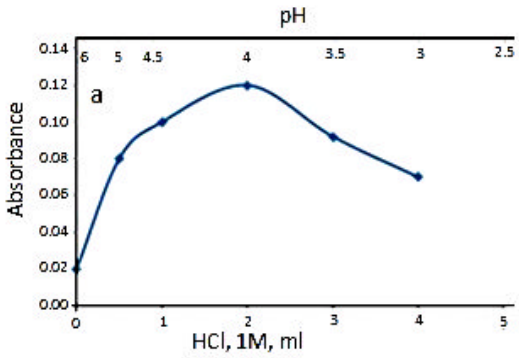
Figure 1. **Absorption spectra of 3 µg/mL methyldopa (a) and 10 µg/mL salbutamol (b) with Eosin against reagent blank (c) under optimum conditions**

Optimization of reaction conditions

High sensitivity was achieved via different parameters influences such as pH, reagent concentration, temperature and developing time.

Effect of pH and buffer solution

The effect of pH for the reaction of salbutamol sulphate and methyldopa was studied with eosin Y in the acidic medium by adding increasing amounts of hydrochloric acid with a concentration of 1 M. It has been found that the maximum absorption was reached at pH4 by adding 2 mL HCl for both drugs (Fig. 2). Different types of buffers such as acetate, phthalate, glycine and citrate buffers, with the same values of pH 4 have been studied. It was observed that maximum absorption on using citrate buffer for salbutamol, and acetate buffer for methyldopa with quantities of 1 and 2 mL, respectively (Fig. 3).



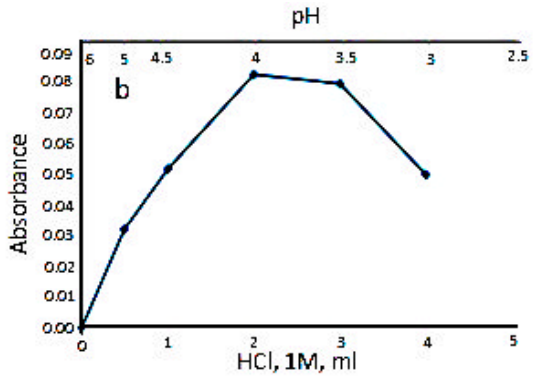


Figure 2. **Effect of pH on the absorbance of 3µg/mL methyldopa (a) and 3µg/mL salbutamol (b) in the presence of 1 mL of 1% eosin Y and HCl.**

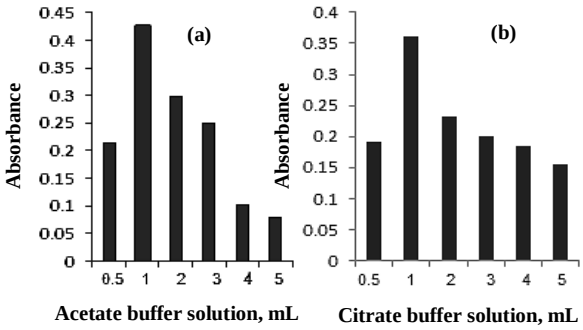


Figure 3. **Effect of buffer solution on the absorption of (a) methyldopa and (b) salbutamol**

The effect of eosin Y concentration

Different concentrations of 2 mL eosin Y have been studied. It was found that 1% and 2% concentrations gave maximum absorbance for ion-pair complexes of salbutamol and methyldopa, respectively. However; different quantities of these concentrations have been added to the solutions and found that, 2 mL for both drugs gave maximum absorbance respectively as seen in Fig. 4.

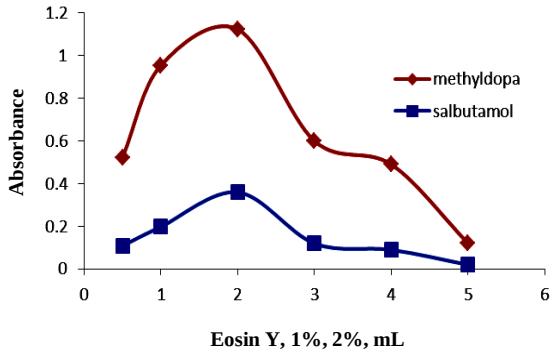


Figure 4. **Effect of 1% and 2% eosin Y volume on the absorbance of salbutamol and methyldopa, respectively**

Effect of triton X-114 surfactant concentration

The effect of Triton X-114 concentration on the efficiency of extraction has been studied. The extraction efficiency increases with increasing the concentration of the surfactant and gave maximum sensitivity at concentration 1% v/v for both drugs (Fig. 5). However; it was found that 2 mL and 1 mL of surfactant gave maximum absorbance for salbutamol and methyldopa, respectively, which are recommended in the next experiments (Fig. 6).

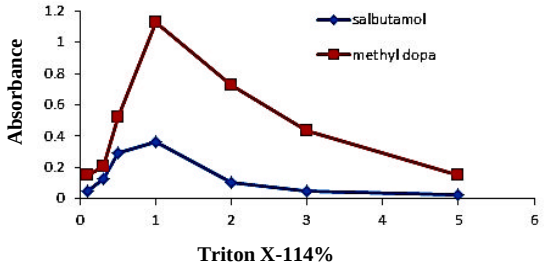


Figure 5. **Effect of Triton X-114% on the absorbance of salbutamol and methyldopa**

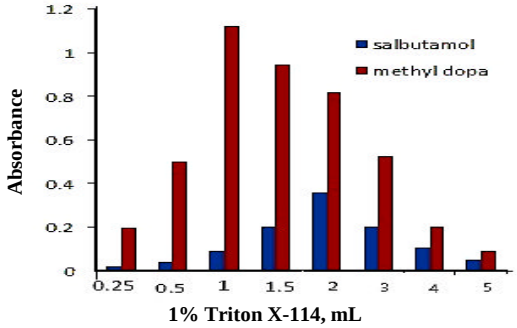


Figure 6. **Effect of 1% Triton X-114 volume on the absorbance of salbutamol and methyldopa**

Effect of the centrifugation time

The effect of the centrifugation time on extraction efficiency was studied within a range of 1-30 min. It has been found that the separation is not completed until 10 min at 3500 rpm which was selected in this procedure (Fig. 7).

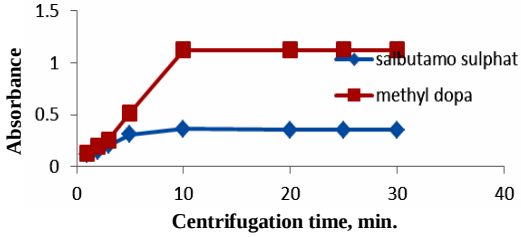


Figure 7. **Effect of centrifugation time**

The effect of time and temperature

Equilibration temperature and optimal incubation time are necessary to achieve complete extraction and easy phase separation. The effect of temperature, ranging from room temperature up to 70ºC, has been studied. An optimum of 50ºC and 60ºC were selected for salbutamol and methyldopa, respectively. The extraction efficiency upon equilibration time was studied within a range of 5-90 min. It was found that complexes were formed within 20 min, which is selected as the best, and remained stable for 50 min and 60 min for above drugs respectively, (Fig. 8a & 8b).

Composition and stability constant of the ion-pair complexes

The composition of the ion-pair was studied by Job's of continuous variation [26] and slop ratio methods [27] using equimolar solutions of 1×10^{-3} M of each drug and eosin Y. The results shown in Fig. 9 indicated that the ion-pair complexes were formed in the ratio of 1:1. The apparent stability constant was estimated by comparing the absorbance of a solution containing stoichiometric amounts of the drug and eosin Y (As) to one containing an optimum amount of eosin Y reagent (Am). The average conditional stability constant of the complexes was calculated, according to the 1:1 ratio, by the following equations:

$$Kc = 1-\alpha / \alpha^2 C \qquad \alpha = Am-As/Am$$

Where Kc is the stability constant ($l.mol^{-1}$), α is dissociation degree and C the concentration of the complex which is equal to the concentration of the drug. The average stability constants for three different concentrations were found 7.4×10^6 and 1.6×10^6 l. mol^{-1} for salbutamol and methyldopa respectively indicating the good stabilities.

Mechanism

The ion-pair complexes were formed via electrostatic interaction between the amino group present in drug molecule and carboxylate anion of eosin Y in an acidic medium which increasing the electron delocalization of eosin Y and a red shift of the dye about 40-50 nm was occurred, (Fig. 1). Applying slope ratio and Job's methods (Fig. 9), it was found that, the reaction proceeds in the ratio of 1:1 of drug to eosin Y for both drugs, as seen in their chemical structures, they have one basic center, the proposed mechanism of the reaction pathway is shown in Scheme 1.

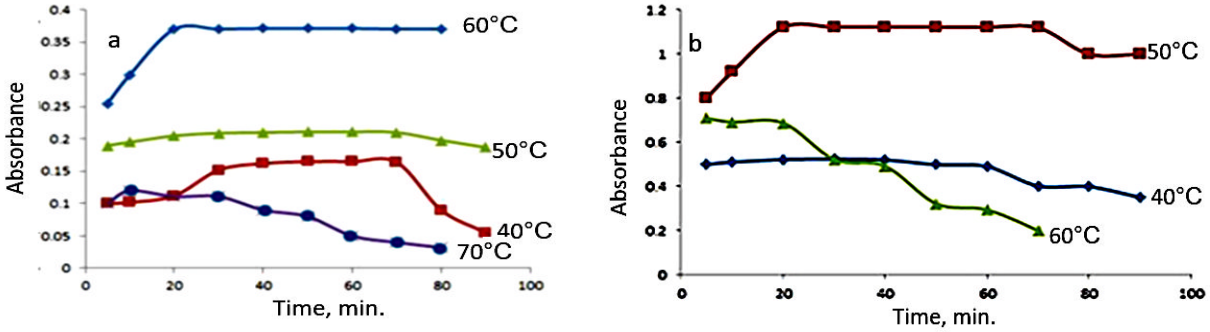
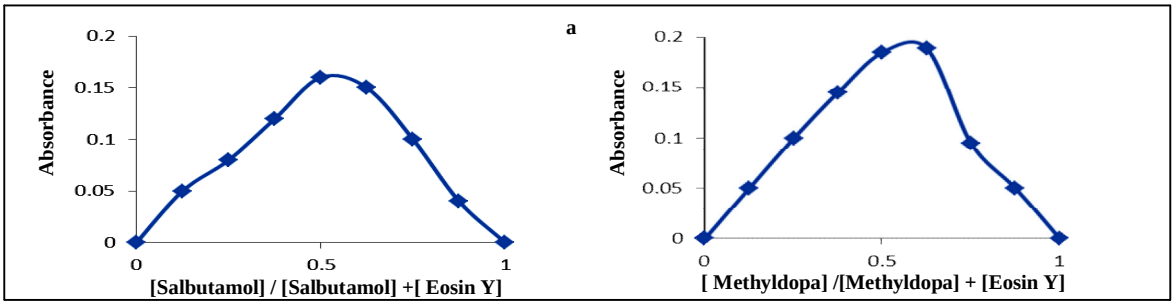


Figure 8. **ffect of equilibration temperature and time on the absorption of 3 µg/mL for each of salbutamol (a) and methyldopa (b) with eosin Y**



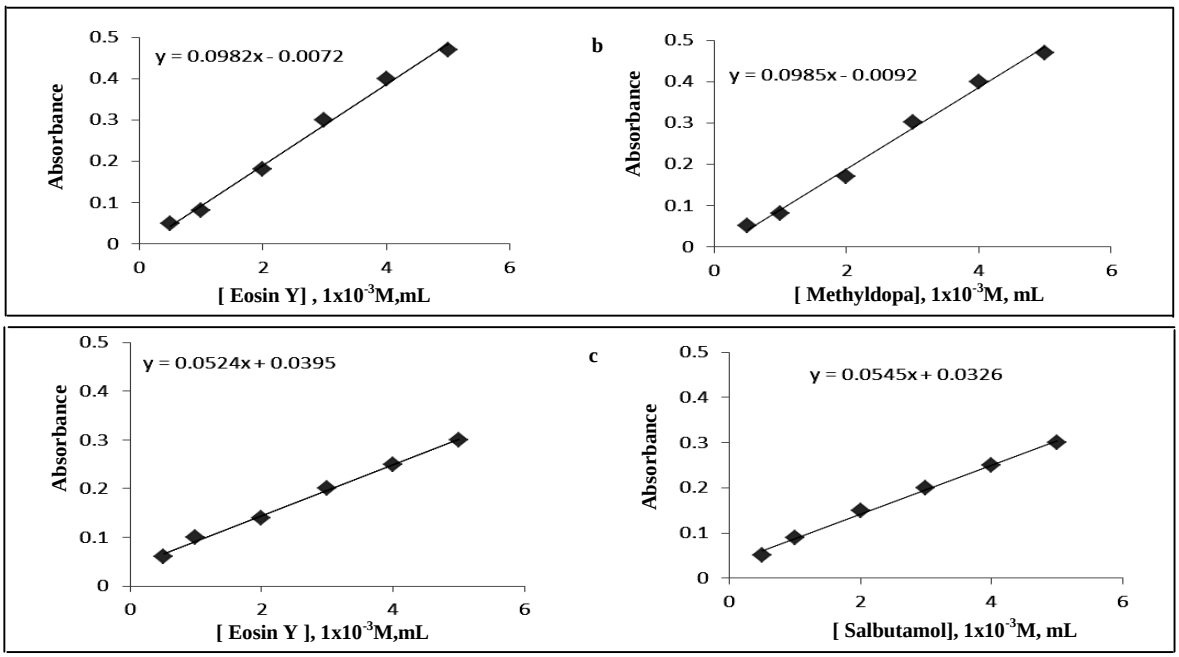
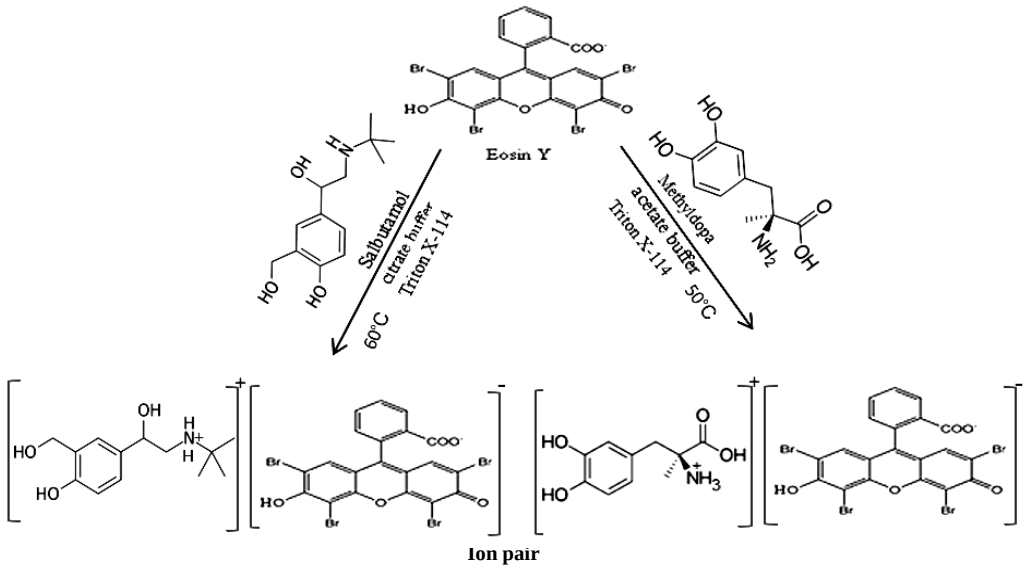


Figure 9. **Job's method for salbutamol and methyldopa (a) and slope ratio method for methyldopa (b) and salbutamol (c) ion pair complexes with eosin Y**



Scheme 1. **Suggested mechanisms of the reaction between salbutamol and methyldopa with eosin Y.**

Linearity and range

Linear relationships actually found between the absorbance and the concentration of the drugs in the ranges 0.1-20 and 0.3-10 µg/mL with molar absorptivity values $4x10^4$ and $5.79x10^4$ $L.mol^{-1}.cm^{-1}$ for salbutamol and methyldopa respectively, indicating the method is sensitive. The linearity was represented by the regression equation and the corresponding correlation coefficient for the studied drug by the proposed method represents excellent linearity. Five replicates of each three different concentrations for each drug were determined. The relative standard deviation and accuracy indicated that the method is precise and accurate. Limit of detection (LOD)

and limit of quantitation (LOQ) were then calculated. However; the analytical parameters for the proposed method are shown in Table 1.

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

Parameter	Salbutamol	Methyldopa
λ_{max} (nm)	558	564
Linear range (µg/mL)	0.1-20	0.3-10
Molar absorptivity ($L.mol^{-1}. cm^{-1}$)	$4x10^4$	$5.79x10^4$
LOD (µg/mL)	0.041	0.0165
LOQ (µg/mL)	0.139	0.055
Average recovery* (%)	98.21	101.27
Regression equation (Y)**		
Slope, a	0.0962	0.2435
Intercept, b	0.0749	0.336
RSD*	≤1.6	≤ 0.3

***Average for five determinations**
****Y=aX+b, where X is the concentration of salbutamol and methyldopa in µg/mL**

Specificity

The specificity of the method was investigated by the observation of any interference encountered from the common excipients of the pharmaceutical formulations by the measurement of the absorbance of solutions containing 3 µg/mL for each of drugs, and various amounts of different additives, up to 200 µg/mL for salbutamol and methyldopa respectively, in a final volume of 10 mL. It was found that the studied excipients did not interfere seriously (Table 2).

Analytical applications

The proposed method was successfully applied to determine salbutamol sulphate (tablet, spray, and syrup) and methyldopa tablet in their pharmaceutical formulations, using three different concentrations for each formulation. The average recovery (%) was in the range 99.84-102.60 % for salbutamol sulphate and 98.95-100.50 % for methyldopa indicating that the method is accurate (Table 3). The obtained results of methyldopa tablet and salbutamol sulphate tablet were compared statistically by a Student's t-test for accuracy and a variance ratio F-test for precision with the official method procedure [28] at the 95% confidence level with four degrees of freedom. The results showed that the experimental t-test = 1.272, 1.350 and F-test = 4.38, 4.303, for above drugs respectively, were less than the theoretical value (t=4.303, F=9.28), indicating that there was no significant difference between the proposed method and official method.

Comparison of the present method with other spectrophotometric methods

The present spectrophotometric method has been compared with other spectrophotometric methods for determination of salbutamol and methyldopa. These methods are depended on the reduction of gold to gold nanoparticles in the presence of SDS surfactant for determination of salbutamol [29], 2,6-dichloroquinone-4-chlorimide (DCQ) in the medium of acetate buffer as coupling reagent for determination of methyldopa [30], and application of cloud point extraction in oxidative coupling reaction for determination of methyldopa and salbutamol using thiosemicarbazide and 4-nitrophenyl hydrazine respectively [15]. As seen in Table 4, the suggested method is more sensitive, accurate and precise than other methods.

Table 2. **Effect of excipients for assay of salbutamol and methyldopa.**

Excipient	Recovery% of 3 µg/mL methyldopa per µg/mL excipient				Recovery % of 3 µg/mL salbutamol per µg/mL excipient			
	20	***50***	***100***	***200***	***20***	***50***	***100***	***200***
Glucose	104.55	104.46	100.44	110.8	100.79	100.0	100.0	100.50
Lactose	104.46	100.02	116.07	108.99	99.69	100.20	99.39	96.60
Starch	100.08	100.35	107.14	107.2	101.30	100.69	10.0	90.09
Arginine	98.03	100.80	101.60	97.75	100.02	100.39	99.69	98.69
NaCl	104.64	104.73	100.0	108.92	99.90	100.0	99.39	91.39
Acacia	100.44	100.26	100.0	106.4	100.0	100.0	102.3	94.70

Table 3. **Assay of salbutamol sulphate and methyldopa in pharmaceutical preparations.**

Pharmaceutical formulation	Drug amount present (µg/mL)	Recovery* (%)	Average drug content found (mg)	Certified value (mg)
Salbutamol sulphate tablet [a]	5	104.00	2.031	2.0
	10	102.00		
	15	98.66		
Butadin[a]	5	102.80	2.032	2.0
	10	103.40		
	15	98.60		
Aldosam [a]	0.8	100.00	250.350	250
	3	103.33		
	10	97.10		
Salbu Vent[b]	5	101.00	0.502%	0.5%
	10	102.00		
	15	98.66		
Alfamet [c]	0.8	103.75	258.25	250
	3	103.33		
	10	97.20		

*Average of four determinations
[a]SDI, [b] Diamond pharma-Damascus, [c]Turkey-Cyprus

Table 4. **Comparison of the present method with other methods.**

Analytical Parameters	Present method		Reported methods			
	Salbutamol	***Methyldopa***	***Salbutamol [29]***	***Methyldopa [30]***	***Salbutamol [15]***	***Methyldopa [15]***
λ_{max}	558	564	530	400	535	470
Linearity (µg/mL)	0.1-20	0.3-10	5.0-18.0	4–20	0.25-6	0.25-6
Development (time, min)	15	15	20	60	50	50
Recovery (%)	98.21	101.27	97.20	101.7	99.98	99.57
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	$4X10^4$	$5.79X10^4$	1.46×10^4	6.42×10^3	4.83×10^4	5.10×10^4
LOD (µg/mL)	0.041	0.0165	1.625	1.1	0.029	0.024
LOQ (µg/mL)	0.139	0.055	4.924	3.21	0.098	0.079
RSD (%)	≤ 1.55	≤ 0.23	1.216	0.84	≤0.23	≤0.18

Conclusion

For the determination of salbutamol sulphate and methyldopa, a simple, accurate and precise spectrophotometric method was developed. The method depended on the formation of ion-pair complexes between eosin Y dye and the drugs followed by cloud point extraction with Triton X-114 surfactant. Statistics indicated the high reproducibility and accuracy of the suggested method. Analysis of samples showed that there is no interference from common additives and auxiliary substances. The advantage of the method is less time-consuming and requiring no variety of elaborative treatments and tedious extraction procedures as well as the capability of successful application of pharmaceutical preparations**.**

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