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Spectrophotometric Determination of Some Adrenergic Drugs Using H_2O_2 and Dichlorophenolindophenol

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Abstract

Based on the catalytic response between hydrogen peroxide and dichlorophenolindophenol, a spectrophotometric method was developed to estimate adrenaline hydrochloride (ADR), Salbutamol sulfate (sal), and dopamine hydrochloride (DOPA) in pharmaceutical preparations. A calculated value of the oxidizing agent (hydrogen peroxide) was added to the basic medium in the presence of dichlorophenolindophenol to form a blue color, which suffers from a bleaching process due to the oxidation process of the drugs mentioned above by the oxidizing agent at λmax 552 nm. The estimated amounts of adrenaline hydrochloride and Salbutamol sulfate were between 0.25 - 5 µg/ml and 0.5 - 5 µg/ml, respectively. The amount of dopamine hydrochloride was between 1 - 7 µg/ml with good sensitivity, as the molar absorbance was 3.8023×104, 8.3506×104and 2.7213×104, respectively. All other statistical values were thoroughly examined. Good agreement with certified value was observed.

Keywords: adrenaline hydrochloride, catalytic response, dopamine hydrochloride , salbutamol sulfate, Spectrophotometric.

Introduction

Adrenaline hydrochloride is 4-(1-hydroxy-2-(methylamino) ethyl) benzene-1,2-diol hydrochloride, Fig. 1. It's a hormone that the adrenal glands secrete that helps the sympathetic nervous system fight or flight[1]. In addition, it speeds up the body's response to stress or threat. It also increases blood flow to the muscles[2] releasing sugar into the bloodstream, along with a series of other effects that make the body alert and better able to fight off the attacker. It is used as a drug in acute asthma by relieving the contraction of bronchial smooth muscles by stimulating beta receptors. Therefore, it reduces the spasm of the pulmonary bronchial tubes and combats bronchospasm, anaphylaxis and anaphylactic shock: thus, preventing the risk of fluid accumulation in the arteries, and cases of cardiac arrest[3].

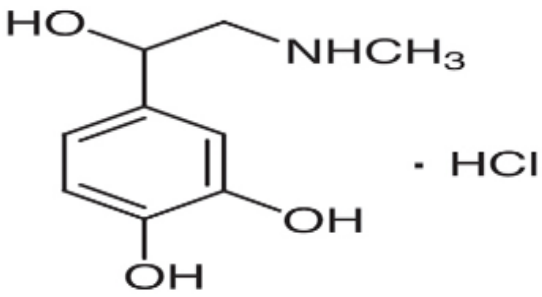

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Figure 1. The chemical formula of adrenaline hydrochloride

Salbutamol sulfate is bis(4-[2-(tert-butylamino)-1-hydroxyethyl]-2- (hydroxymethyl)phenol); sulfuric acid, Fig. 2, is a medication used to treat asthma and other respiratory conditions such as chronic obstructive pulmonary disease (COPD)[4]. It belongs to a class of drugs called bronchodilators, which work by relaxing the muscles in the airways and allowing more air to flow into the lungs. It is usually taken through an inhaler or nebulizer and can provide quick relief of symptoms such as wheezing, coughing, and shortness of breath. It may also be used to prevent exercise-induced asthma or other triggers that can cause breathing difficulties[5].

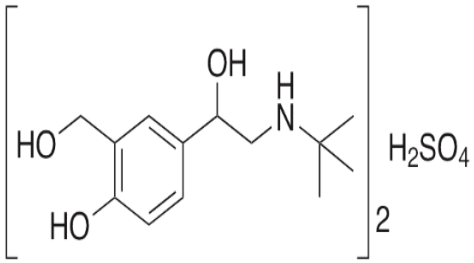

Figure 2. The chemical formula of Salbutamol sulfate

Dopamine hydrochloride, or 4-(2-aminoethyl) benzene-1,2-diol; hydrochloride , Fig. 3 ,is a chemical or hormone found in the human body. It can increase feelings of happiness and acts as a neurotransmitter, sending signals to the body and brain. Maintaining a proper balance of dopamine hydrochloride in the body is very important as it plays a role in controlling motor skills and emotional responses, which are essential for physical and mental health. Its effects can be found in the major areas of the brain, impacting mood, sleep, focus, learning, and memory. Low dopamine hydrochloride levels can lead to illnesses such as Parkinson's and depression, which can be caused by either a low production of dopamine hydrochloride in the body or a problem with the nerve receptors in the brain [6].

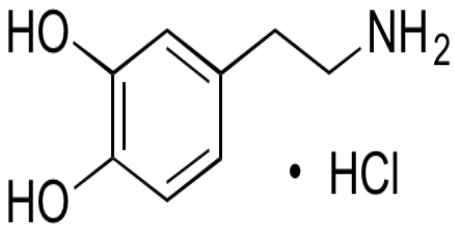

Figure 3. The chemical formula of dopamine hydrochloride

In the chemical literature, there are numerous techniques for measuring adrenaline hydrochloride, Salbutamol sulfate, and dopamine hydrochloride, including spectrophotometric [7-10], fluorometric[11,12], electric[13-15] and chromatographic methods[16-18]. The article talks about a straightforward spectrophotometric technique that can be used in water to measure the drugs mentioned previously.

Materials and Methods

Apparatus

Absorbance and spectral measurements were done using a PG Instruments-England T92+ UV spectrophotometer using a 1.0 cm thick quartz cell.

Reagents

Drug solutions were prepared by dissolving 0.01 g of these pure substances in a small amount of distilled water, then completing the volume in a 100ml volumetric flask up to the mark.

Hydrogen peroxide Solution 0.1 M

Prepared by dissolving 0.9406g of urea-H_2O_2 compound in 100 ml water, the other oxidizing agents were prepared at a concentration of 0.01 M by dissolving the appropriate amount in a volumetric flask of 50 ml capacity, then filling the volume up to the mark with distilled water. This ought to be freshly prepared each day. Bases stock solutions were prepared with a 1 M concentration, and the correct amount of each was dissolved in a 100 mL volumetric flask of distilled water for a perfect result and then diluted as needed. All surfactants were prepared at a concentration of 1% by dissolving the optimal amount in a suitable volume and as needed.

2,6-Dichloroindophenol Sodium 0.1%

It is prepared by dissolving 0.1g of 2,6-Dichloroindophenol sodium in 100ml distilled water**.**

Procedure

Aliquots of solutions containing 0.25–5 µg/ml adrenaline hydrochloride, 0.5-5 µg/ml Salbutamol sulfate, and 1–7 µg/ml dopamine hydrochloride, respectively, were added to three separate groups of 10 ml volumetric flasks, followed by the addition of 1 ml 0.1 M H_2O_2 and 1.25 ml of 1M NaOH to each group. This was allowed to stand for 10 minutes.

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Baghdad Science Journal

Then the optimal amount of dichlorophenolindophenol was added 40 µg/ml and supplemented with distilled water to the mark. The absorbance was measured versus blank at λ max 552 nm room temperature.

The Procedure of Pharmaceutical Preparations

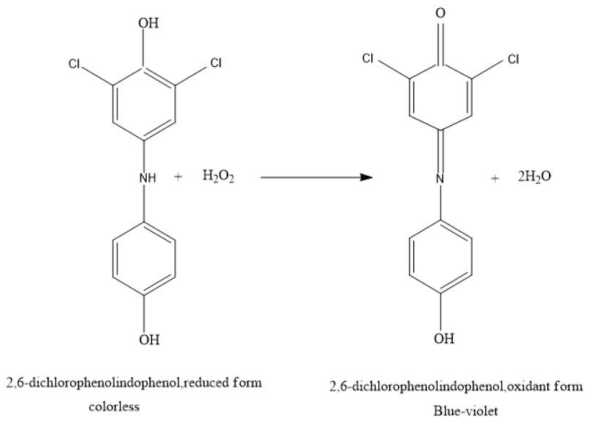
Adrenaline Hydrochloride Injections
The two adrenaline hydrochloride injections each contained 1.0 mg of adrenaline hydrochloride in 1.0 mL. They were diluted in 20 mL of distilled water to create the 100 g/mL solution, which was then withdrawn in various volumes as needed and administered under the method of action.

Butadin Tablets
Five butadin tablets, each containing 2 mg, were used in total. They were thoroughly ground before being dissolved in 100 ml of distilled water to produce 100 ppm. The suggested approach to doing the job was followed.

Dopamine Hydrochloride Injection Analysis
In a 200 ml volumetric flask, distilled water was used to dilute a pharmaceutical injection containing 200 mg/5 ml of dopamine hydrochloride to produce 1000 g/ml. Subsequent samples were then taken from it and handled according to the method.

Results and Discussion

It is known that a solution of 2,6-Dichlorophenolindophenol produces a blue colour with maximum absorption in the visible region between 500 and 600 nm in the presence of some oxidizing agents. This depends on the nature of the oxidizing agent used and the estimated material. As a result, after oxidizing drugs (adrenaline hydrochloride, salbutamol sulfate, and dopamine hydrochloride) with the same oxidizing agent, this property can be used to evaluate them. The proposed method is considered an indirect method based on quantitatively reducing or bleaching the colour of the 2,6-dichlorophenolindophenol due to the oxidation of the added pharmaceutical compounds[19,20] ,scheme1.



Scheme 1. The redox reaction between 2,6-Dichlorophenolindophenol

Optimum Conditions:
Because the estimation of the drugs under study depends on measuring the optimal amount of dye (2,6-dichlorophenolindophenol) according to Beer's law, the effect of the oxidizing agent, the medium, and stability time on dye formation must be investigated. All preliminary tests were performed in a 10 ml volumetric flask.

The Effect of Oxidant Amount
Suitable oxidizing agents were chosen to obtain a stable color for the dye. The preliminary study found that hydrogen peroxide is the best. The reason is that the resulting dye was stable, so it was studying the effect of the amount of oxidizing agent 0.1 M on the color of (5 µg/ml) dichlorophenolindophenol in the presence of 1ml of each HCl and NaOH (1M). Fig. 4 shows that 1ml of 0.1 M H_2O_2 was the best volume in the basic medium and was used in the subsequent study.

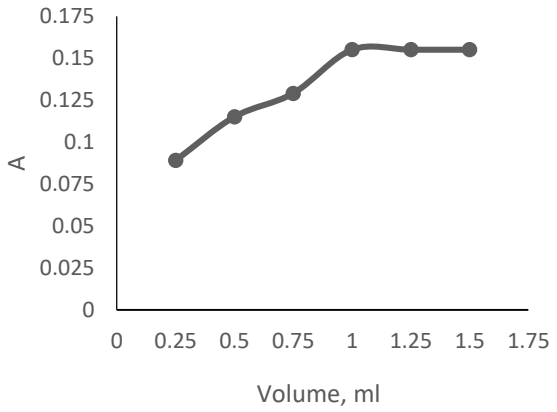


Figure 4. The effect of oxidant amount.

The Effect of Type Base
To find the best base that increases the intensity of the colour of the dye, some bases were added in a

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volume of 1 ml with a concentration of 1 M., Fig. 5. This shows that sodium hydroxide gave the highest intensity and was used in the subsequent study.

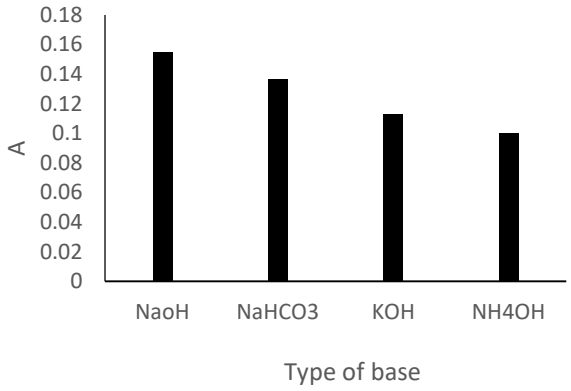

Figure 5. The effect of type base.

The Effect of the Base Volume Used

Since the reaction takes place in an alkaline medium, taking different volumes of the base is appropriate. Figure 6 shows that 1.25 is the most suitable. The pH of the final solution was measured, and its value was 10. Then several buffer solutions with the same pH10 were used, which found that using the base sodium hydroxide was better, so it was used in subsequent studies.

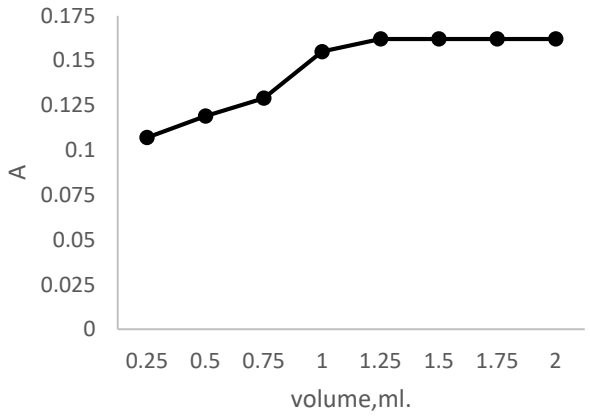

Figure 6. The impact of base volume

The Effect of Time

To find the best stability time for colour development of the dye (5 µg/ml) after adding the optimal amount of oxidizer (1.0 ml) and 1.25ml of 1M NaOH, the absorbance was measured at contrasting times, and the graph shows that the maximum absorbance was reached after 10 minutes and remained stable for four hours, Fig.7. So, an increasing amount of 2,6-dichlorophenolindophenol was added to obtain the highest value that obeys Beer's law for use in determining the drugs under study. Figs. 8 and 9 show the peak and that 40 µg/mL is the highest.

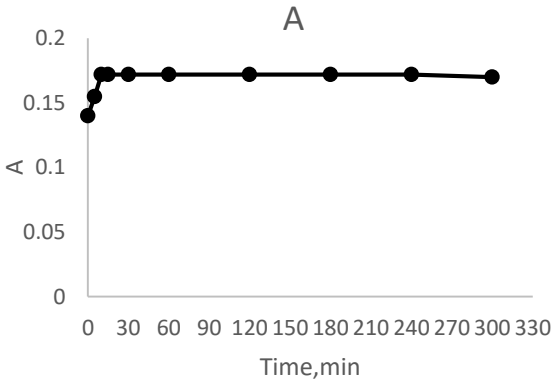

Figure 7. The effect of time.

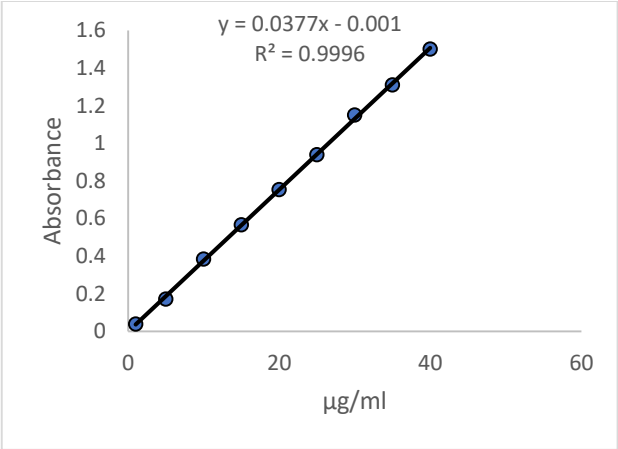

Figure 8. The calibration graph of 2,6-dichlorophenolindophenol

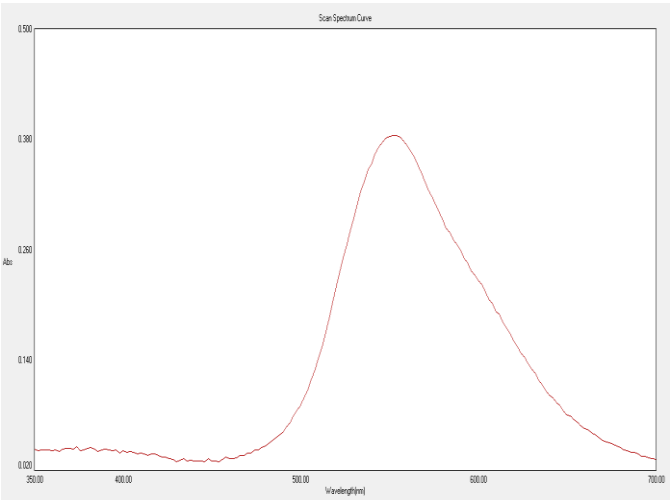

Figure 9. The peak of 10 µg/ml of 2,6-dichlorophenolindophenol Vs blank

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Baghdad Science Journal

The Effect of Oxidation Time on the Drugs

It is expected that when adding the oxidizing agent in the basic medium to adrenaline hydrochloride, salbutamol sulfate, and dopamine hydrochloride (2µg/ml), an oxidation process will occur, and the remaining oxidizing agent works to show the colour of the dye quantitatively. Therefore, different times for the oxidation process were studied. Fig. 10 indicates that after 10 minutes, the oxidation process was completed, as is evident from ΔA values.

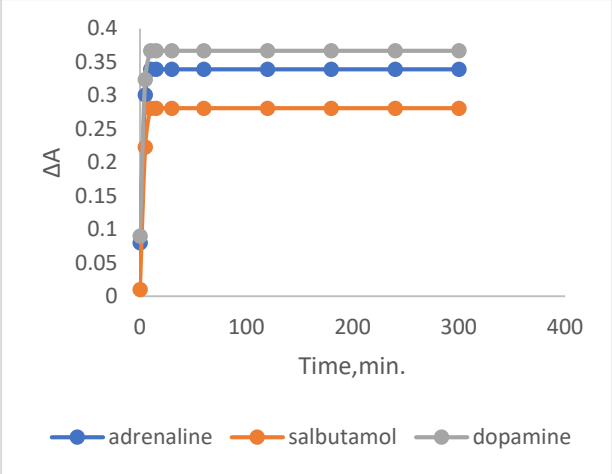

Figure 10. The effect of oxidation time on the drugs

Effect of Surfactant

Both negative and positive surfactants were used in this study and the result was negative, so they were not used.

Calibration Plot

After following the optimal conditions above, it was found that each of the adrenaline hydrochloride, salbutamol sulfate, and dopamine hydrochloride can be estimated Fig. 11. Table 1 shows the limits of the estimate in addition to the values of LOD, LOQ and the rest of the other statistical values.

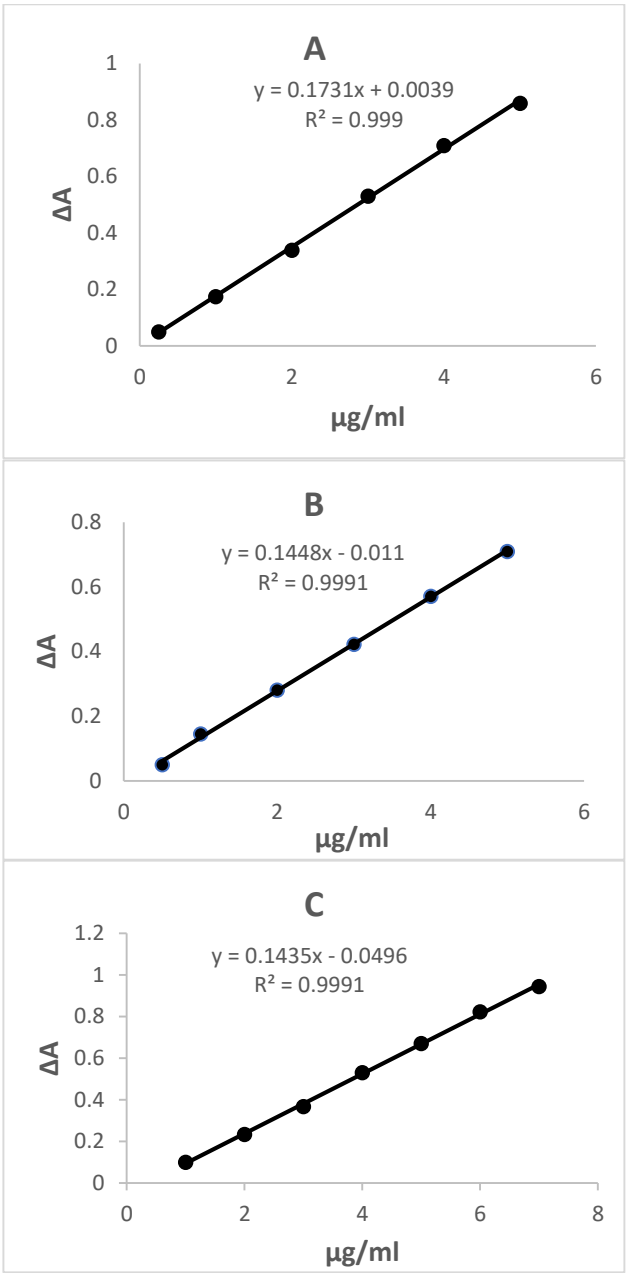

Figure 11. Calibration plot of adrenaline hydrochloride (A), salbutamol sulfate (B) and dopamine hydrochloride(C)

Table 1. Analytical and statistical values of the drugs under study

Parameters	Adrenaline hydrochloride HCl	salbutamol sulfate	Dopamine hydrochloride hydrochloride
Linearity range(µg/ml)	0.25-5	0.5-5	1-7
Molar absorptivity (L.mol^{-1}. cm^{-1})	3.8023×10^4	8.3506×10^4	2.7213×10^4
Intercept	0.0039	-0.011	-0.0496
Slope	0.1731	0.1448	0.1435
R^2	0.9990	0.9991	0.9991
LOD* (µg/ml)	0.0421	0.0393	0.0715
LOQ* (µg/ml)	0.1390	0.1297	0.2360

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P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

Method accuracy and Precision

The method was verified by evaluating the accuracy and precision of the method by calculating the relative standard deviation (RSD) of the three different concentrations. The results presented in Table 2 show that the method has good precision, as the recovery rate reaches 99.69-101.83 and the good agreement (RSD) is less than 3%.

Table 2. Accuracy and precision of the method

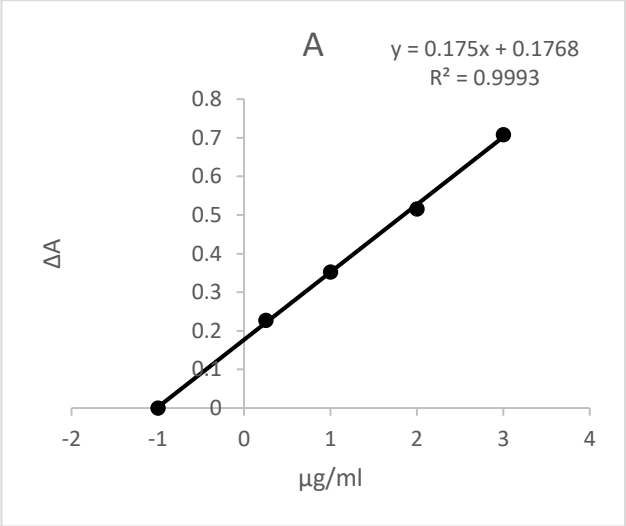
Drug	Amount taken µg/ml	Recovery%	Average recovery %	RSD	Average RSD%
Adrenaline hydrochloride HCl	2	98.45	100.78	2.11	1.88
	3	103.11		1.66	
Salbutamol sulfate s	2	103.35	101.83	2.78	2.31
	3	100.32		1.85	
Dopamine hydrochloride HCl	3	98.16	99.69	1.94	2.2
	5	101.22		2.46	

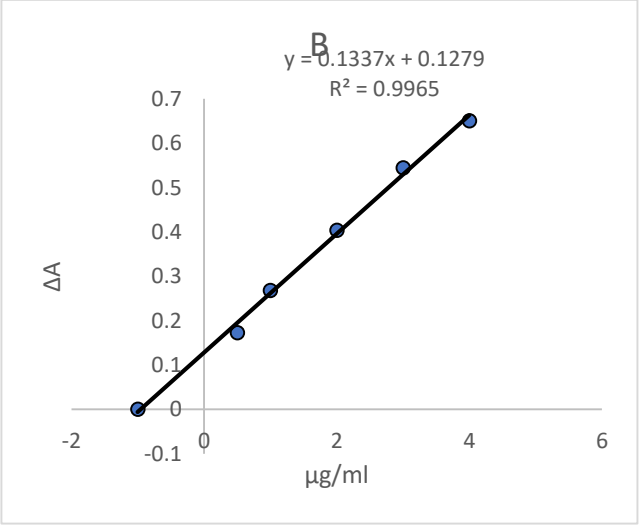
Analytical Application

The standard addition methods were applied to pharmaceutical preparations of adrenaline hydrochloride, Salbutamol sulfate, and dopamine hydrochloride as there were no requirements for the three standard methods adopted in the British Pharmacopoeia to demonstrate the lack of spectral overlap in the proposed method. Figs. 12 and Table 3,4 show that the results obtained for adrenaline hydrochloride and dopamine hydrochloride agree well with the original levels of these drugs. This means that the process has a satisfactory selectivity.

Table 3. The analytical application of the method

Pharmaceutical preparation	Certified value	Amount present (µg/ml)	Recovery %
Adrenaline hydrochloride ampoule Egypt	1 mg	2	101.375
		4	96.925
Butadin tablets Iraq	2 mg	2	102.500
		4	98.750
Dopan ampoule Mumbai	200 mg/5ml	4	102.600
		6	98.550





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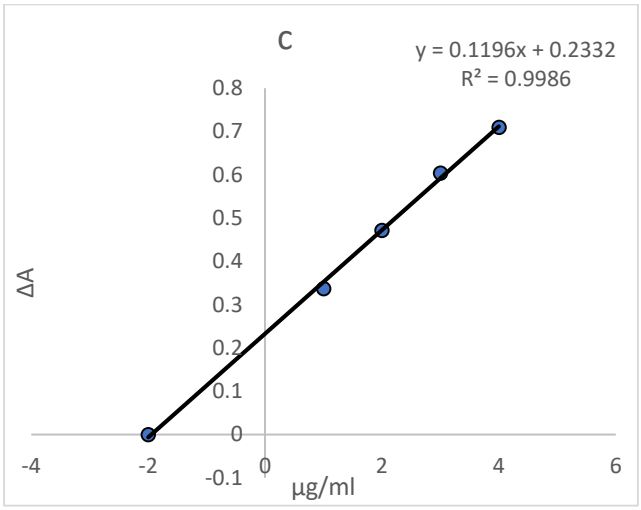

Figure 12. Standard addition method of adrenaline hydrochloride(A), Salbutamol sulfate (B), and dopamine hydrochloride(C).

Table 4. Standard addition method of adrenaline hydrochloride, Salbutamol sulfate, and dopamine hydrochloride summary

Pharmaceuticals	Certificate value	Amount present (µg/ml)	Drug content (µg/ml)	Recovery %
Adrenaline hydrochloride ampoule	1mg/1ml	1	1.010	101.02
Butadin tablets	2 mg	1	0.9566	99.566
Dopan ampoule	200 mg/5ml	2	1.949	97.491

Conclusion

The current method implements the evaluation of the above-mentioned three drugs with high accuracy and efficiency in the water with short steps in addition to the successful application of the method to a pharmaceutical preparation. It is also possible to estimate many drugs with a condition and compounds that can suffer from the oxidation process, and the reason is that it depends on the standard curve.

Acknowledgment

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Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Authors sign on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee at Mosul University.
- Ethics statement:
  No animal studies are present in the manuscript.
  No human studies are present in the manuscript.
  No potentially identified images or data are present in the manuscript.

Authors' Contribution Statement

Each of the individuals listed in the study made an equal contribution to the work and writing.

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التقدير الطيفي لبعض الأدوية الأدرينالية باستخدام H2O2 وثنائي كلوروفينولليندوفينول

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الخلاصة

بناءً على الاستجابة التحفيزية بين بيروكسيد الهيدروجين وثنائي كلورو فينول اندوفينول، تم تطوير طريقة قياس طيفية لتقدير هيدروكلوريد الأدرينالين (ADR) وكبريتات السالبوتامول (sal) وهيدروكلوريد الدوبامين (DOPA) في المستحضرات الصيدلانية.اذ تمت إضافة كمية محسوبة من العامل المؤكسد (بيروكسيد الهيدروجين) في الوسط القاعدي وبوجود ثنائي كلورو فينول اندوفينول لتكوين صبغة ذات لون أزرق، والتي تعاني من عملية القصر نتيجة لعملية أكسدة الأدوية المذكورة في أعلاه بواسطة العامل المؤكسد (بيروكسيد الهيدروجين) عند طول موجي مقداره 552 نانوميتر. كانت الكميات المقدرة من هيدروكلوريد الأدرينالين وكبريتات السالبوتامول بين 0.25 - 5 مايكروغرام / مل و0.5 - 5 مايكروغرام / مل على التوالي. كانت كمية الدوابامين هيدروكلوريد ما بين 1 - 7 مايكروغرام / مل مع حساسية جيدة حيث اذ أن قيمة الامتصاص المولاري كانت 3.8023×10^4 و8.3506×10^4 و2.7213×10^4 على التوالي. تم فحص جميع القيم الإحصائية الأخرى بدقة وتوافق جيدين ومقارنتها مع القيمة الحقيقية.

الكلمات المفتاحية: ادرينالين هيدروكلوريد،الاستجابة التحفيزية، القياس الطيفي، دوبامين هيدروكلوريد، كبريتات السالبيوتامول.