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Indirect Spectrophotometric Determination of Antidepressant Drugs by Oxidation Using Ponceau 4R Dye

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Abstract

A simple and sensitive indirect spectrophotometric method is developed for the assay of Adrenaline, Dopamine hydrochloride, and Methyldopa in their pure and pharmaceutical preparations. The method is based on the principle of oxidation of the above drugs with a known amount of the oxidizing agent, N-bromosuccinimide (NBS), in hydrochloric acid. The unreacted oxidant bleaches Ponceau 4R dye, then the absorbance of the remaining dye (ΔA) is measured at 507 nm. The method obeyed Beer's law within the concentration range of 3.5-10, 4-15, and 5-20 µg/mL, with molar absorptivity values of 8.01×10^4, 5.32×10^4, and 4.93×10^4 L. $mol^{-1}cm^{-1}$ for Adrenaline, Dopamine hydrochloride and Methyldopa, respectively. The recovery ranged between 99.15 to 100.61%, and the relative standard deviation (RSD) was less than 1.42 % for all drug compounds. The method was applied successfully to pharmaceutical preparations for the studied medicinal compounds from different origins, as its results were in good agreement with the original content of the drugs in their pharmaceutical formulations and with the standard addition procedure, which proved that there was no interference by the excipients.

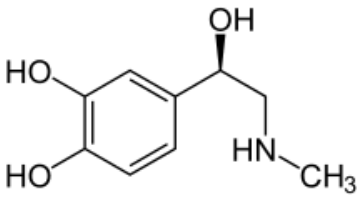
Keywords: Antidepressant drugs, Ponceau 4R, NBS, Spectrophotometry

Introduction

Adrenaline or epinephrine, (3,4-Dihydroxy-α-[2-(methyl amino) ethyl] benzyl alcohol) (I) belongs to a class of neurotransmitters called catecholamines and is a hormone released by the adrenal gland in situations of severe stress or hypoglycemia [1]. Asthma, heart attack, and high blood pressure symptoms are all treated with it as medicine [2]. It is also suggested as an emergency treatment for cardiopulmonary resuscitation and emphysema. It is used as a medication to treat the symptoms of high blood pressure, asthma, and heart attacks. [2]. It is also recommended as an emergency medication for emphysema and cardiopulmonary resuscitation [3]. Methyldopa [3-(3,4-dihydroxyphenyl)-2- L-alanine] (II) is one of the most significant and popular medications used to treat high blood pressure, particularly during pregnancy [4], and this effect is mainly due to its effect on the central nervous system [5]. Methyldopa can increase the human heart rate and constrict blood vessels in the body [6]. High doses of Methyldopa increase the risk of headache, drowsiness, weakness, liver problems, red blood cell damage, and some allergic side effects [7].

Dopamine [4-(2-Aminoethyl)benzene-1,2-diol hydrochloride] (III) is one of the neurotransmitters in the human brain's central nervous system and is crucial to the system's activities (renal, hormonal, and cardiovascular). Disordered hormone secretion causes

illnesses including Alzheimer's and Parkinson's disease [8]. In addition to its main role in improving mood, movement, perception, and breathing, it participates in regulating blood pressure [9].



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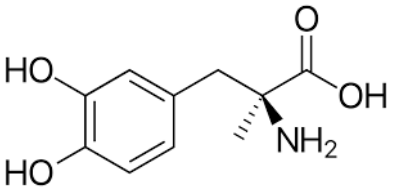

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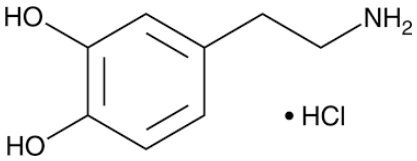
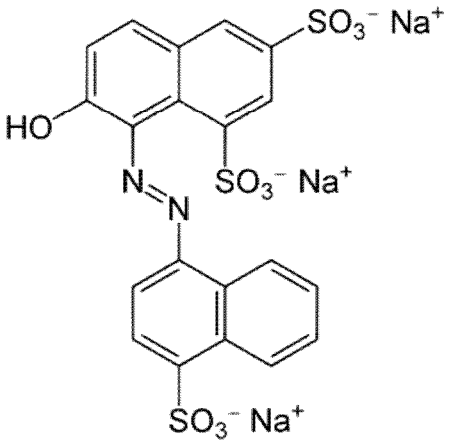


(III)

For the determination of adrenaline, methyldopa, and dopamine hydrochloride in their pure and pharmaceutical forms, many analytical techniques have been reported. These techniques include fluorometry [10-12], voltammetry [13-18], chromatography [19-22], flow injection [23], and chemiluminescence [24].

In the literature, a number of spectrophotometric methods for the direct and indirect determination of catecholamine medicines were described. These methods depended on using different reagents, such as 5-benzimino-1,3,4-thiodiazole-2-thione [25], ferric ion [26] , and chloranil [27] used for the determination of Adrenaline. Eriochrom black T [28], neocuproine [29], 2-aminopyridine [30], ferrous ion [31], and 3-methyl-2-benzothiazolinone hydrazone hydrochloride monohydrate (MBTH) [32] were used for determination of Methyldopa. 2,4-Dinitrophenylhydrazine (DNP) [33], and potassium permanganate [34] were used for the determination of Dopamine. Thionine–bromate [35,36] and Ag^{+}–tetramethylbenzidine [37] were used for the determination of catechol amine drugs.

Ponceau 4R dye, chemical name is 1-(4-sulfo-1-napthylazo)-2-napthol-6,8-disul-fonic acid, trisodium salt [38] (IV), was decolorized in the presence of an oxidizing agent [39], and through the scientific literature, it was noted that this dye was not used in the analysis of pharmaceutical compounds.



(IV)

The objective of this work was to develop an indirect spectrophotometric method for the determination of Adrenaline, Methyldopa, and Dopamine hydrochloride using Ponceau 4R dye in the presence of the oxidizing agent NBS, and application to their pharmaceutical preparations. The devolped method was characterized by simplicity, sensitivity, accuracy, and good precision.

Materials and Methods

Chemicals and Reagents

High purity chemicals and reagents were used and supplied by Fluka and BDH Chemicals Ltd. (England). The drugs used were supplied by the state company for drugs industry and medical appliances. A concentration of 500 µg/mL Ponceau 4R dye solution was prepared by dissolving 0.05 g of the dye in distilled water. Standard solutions of each of NBS, periodate, potassium iodate, potassium dichromate, chloramine-T and cerium IV sulphate oxidizing reagents, at a concentration of 5×10^{-3} M, were prepared in distilled water. A phthalate and citrate buffer solutions of pH 2.5 and 3 were prepared according to the reference [40]. A stock solution of Adrenaline, Dopamine hydrochloride and Methyldopa of 100 µg/mL of pure forms was prepared in distilled water and kept in the refrigerator

Instrumentation

Spectral measurements and absorption spectra were drawn using a Shimadzu UV-1800 PC UV-Visible spectroph-otometer using quartz cells with a thickness of 1 cm, and the substances were weighed using a KERN ABS-Germany sensitive balance. The solutions were prepared using an Ultrasonic Shaker Cleaner Power Sonic 405. A water bath of type BS-11 from Lab Companion-Korea and pH-meter with a combined glass electrode type Philips PW (9421) was used for heating and maintaining pH of the solutions, respectively.

Assay Procedure for pure drugs

An aliquot of the solution containing 3.5-10 µg/mL Adrenaline, 4-15 µg/mL Dopamine hydrochloride, and 5-20 µg/mL. Methyldopa and were transferred to series sets of 10 mL volumetric flasks separately, followed by the addition of 1 mL of the NBS and 1.5, 2, and 1.5 mL of buffered phthalate solution (pH 2.5) were added to the above drugs, respectively. The solutions were left for 10 min in a water bath adjusted at 30 °C to complete the oxidation process, then a 100 µg/mL solution of ponceau 4R dye was added to each set, and the volume was completed to the mark with distilled water and left for another 10 min in the same temperature. Then the absorbance was measured at 507 nm against their reagent blank.

Assay Procedure of Drugs Formulations

Adrenaline injection

The contents of five injections of Adrenaline were mixed, each injection containing 1 mg/mL of Adrenaline, and the mixture was diluted to 50 mL with distilled water to obtain a solution with a concentration of 100 µg/mL. Different volumes of the solution were taken to obtain concentrations (4, 5, and 8 µg/mL) in a final volume of 10 mL, and were treated according to the recommended procedure, and the concentration of adrenaline in the injection was found using the standard curve of the drug compound in its pure form.

Dopamine Hydrochloride Injection

Three injections of Dopamine hydrochloride were mixed, each injection containing 200 mg/5 mL, then 2.0 mL of the mixture was diluted to 100 mL with distilled water to obtain a solution with a concentration of 800 µg/mL, further dilution was made to obtain a solution with a concentration of 100 µg/mL. Different volumes of the solution were taken to obtain concentrations (9, 7, and 5 µg/mL) in a final volume of 10 mL, and it was treated according to recommended procedure, and the concentration of Dopamine was found in the injection using the standard curve of the drug compound in its pure form.

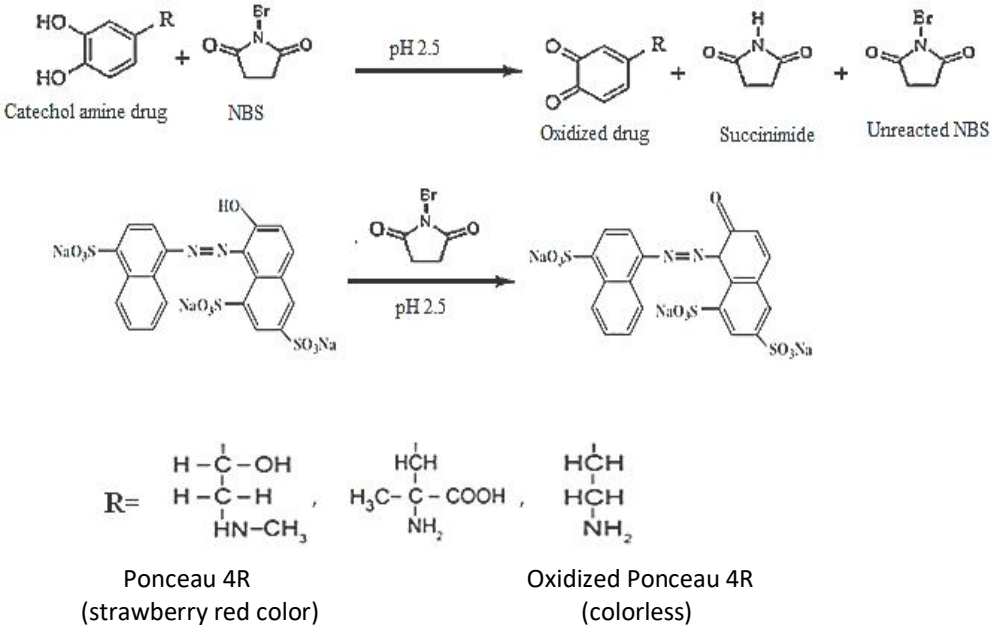
Methyldopa Tablet

Accurately weighed the content of 10 tablets of each pharmaceutical preparation of Methyldopa, crushed and mixed well, then weighed the powder equivalent to one tablet (250 mg Methyldopa) and dissolved in a beaker with 20 mL distilled water and heated in a water bath for 5 min, and left the solution to cool, then filtered the solution and transferred to a 100 mL volumetric flask. The volume was completed to the mark with distilled water to obtain a solution with a concentration of 2500 µg/mL. Then the flask was placed in the ultrasonic shaker for 10 min to complete the dissolution and homogenization of the solution. A solution with a concentration of 100 µg/mL was prepared from it, and different volumes were taken from it to obtain concentrations (4, 10, 16, and 24 µg/mL) in a final volume of 10 mL, and were treated according to the recommended procedure. The concentration of Methyldopa in the tablet of each medicinal preparation was found using the calibration curve of the pure form.

Results and Discussion

Preliminary Investigation

Ponceau 4R is a strawberry red color having maximum absorbance at 507 nm in the aqueous medium. It has been observed that the color of this dye was bleached in the presence of an oxidizing agent, such as NBS. Based on this phenomenon, an indirect spectrophotometric method has been developed for the determination of some oxidizable drug compounds including Adrenaline, Dopamine hydrochloride, and Methyldopa. It was noted that the ponceau 4R dye absorption was reduced by using an acidic medium. However, when using a buffer solution of pH 2.5, an increase in dye absorption at 507 nm was observed. However; the present method depended on the oxidation of the above drugs by a fixed amount of NBS in the medium of a pH 2.5 buffered solution and the unreacted NBS was reacted with a known amount of dye as shown in Scheme 1.



Scheme 1: **Proposed mechanism for the oxidation of drugs and dye**

Study of Optimal Reaction Conditions

Subsequent experiments were carried out using 10 mL volumetric flasks containing 5, 7, and 10 µg/mL solutions of Adrenaline, Dopamine hydrochloride, and Methyldopa, respectively, and measured the absorbance at 507 nm.

Selection of Ponceau 4R Amount

To determine the optimal amount of ponceau 4R dye that can be used in the determination of the studied pharmacological compounds that follow Beer's law, increasing volumes (mL) of the dye solution with a concentration of 500 µg/mL were added to volumetric flasks of 10 mL containing 1.0 mL of phthalate buffer solution of pH 2.5 and the volume was completed to the mark with distilled water and the absorbance was measured at 507 nm. It was found that the range of linear concentration of the dye was from 1.0-100 µg/mL, and accordingly, the concentration was selected at 100 µg/mL in subsequent studies.

Selection of oxidizing agent for dye bleaching

Different oxidizing agents such as NBS, sodium periodate, potassium iodate, potassium dichromate, chloramine-T, and cerium IV sulphate, at a concentration of 5×10^{-3} M and a volume of 0.5 mL for each, on the bleaching of 100 µg/mL ponceau 4R dye were studied in the presence of 1 mL phthalate buffer solution of pH 2.5. The solutions were left for 5 min, and then the absorbance of the solutions was measured at 507 nm, as shown in Fig. 1 NBS was the best as it gave less absorbance and therefore it was adopted in subsequent studies. However; the concentration and volume of NBS were studied and found that 1 mL of 5×10^{-3} M was sufficient for bleaching of the dye (Fig. 2 and 3).

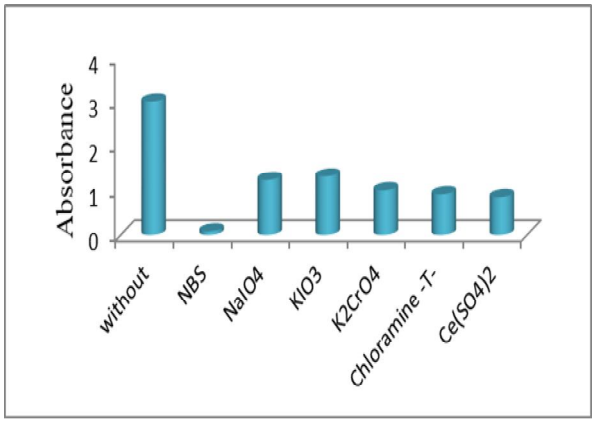


Figure 1. **Effect of oxidizing agents on the bleaching of dye**

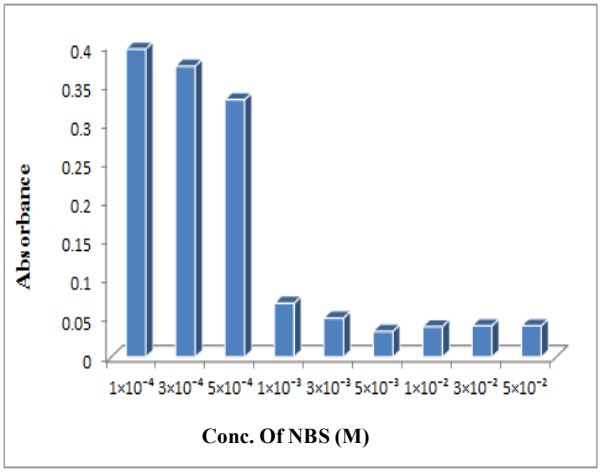


Figure 2. **Effect of NBS concentration on the bleaching of ponceau 4R dye**

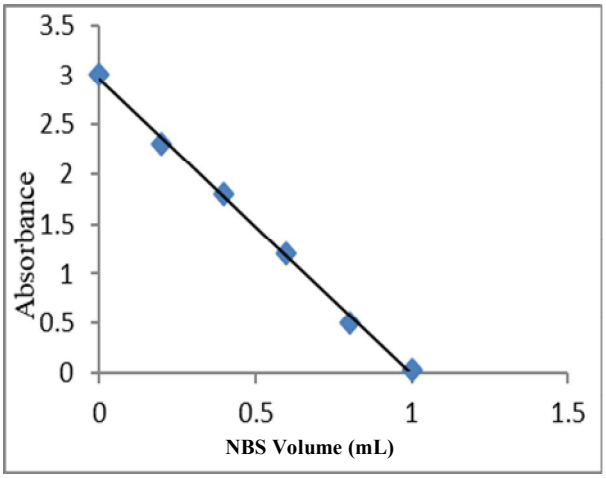


Figure 3. **Effect of NBS volume on the bleaching of ponceau 4R dye**

Selection of buffer solution and volume

The preliminary experimental results that were showed that the oxidation of the ponceau 4R and the studied drug compounds by NBS gives good results using an acidic buffer solution instead of using acid at room temperature. Two types of acidic buffer solutions, phthalate and citrate of pH 2.5 and 3 were prepared and a fixed amount of 1 mL of these buffers was added individually to volumetric flasks containing microgram quantities of drug compounds separately followed by the addition of 1 mL of 5×10^{-3} M NBS. Waiting 5 min at room temperature and then 100 µg/mL ponceau 4R dye was added. The absorbance was then measured at 507 nm after 5 min. The results shown in Table 1 indicated that the buffered phthalate solution of pH 2.5 was the most appropriate which gave the highest absorption. The volume of phthalate buffer solution has been also studied and found that 1 mL was the optimum for Adrenaline and Methyldopa, and 2 mL for Dopamine.

Table 1. **Effect of buffer solution on the absorbance of Adrenaline, Dopamine and Methyldopa.**

Drug	Absorbance*			
	Without buffer	Phthalate buffer pH 2.5	Phthalate buffer pH 3	Citrate buffer pH 3
Adrenaline (5 µg/mL)	0.399	0.769	0.669	0.678
Dopamine HCl (7 µg/mL)	0.228	0.345	0.333	0.315
Methyldopa (10 µg/mL)	0.459	0.656	0.623	0.626

*1.0 mL of buffer solution added

Effect of temperature and time on the oxidation reaction of drugs and a dye

The effect of different temperatures (23, 30, and 40 °C) and time on the oxidation reaction of the studied drug compounds, at the optimal conditions as proven in previous experiments, has been studied with a time period of 10 min and on the stability of the dye after 10 min of dilution for each drug compound. The absorbance of the dye was measured after 5 min intervals at 507 nm. The results indicated in Fig. 4, a, b, and c showed that the temperature of 30 °C was optimal for the determination of drug compounds, as a higher absorption was obtained 10 min after the start of the reaction for all drugs, with stability of not less than two hours, so they were adopted in the subsequent study. While it was found that higher temperature decreases the absorbance.

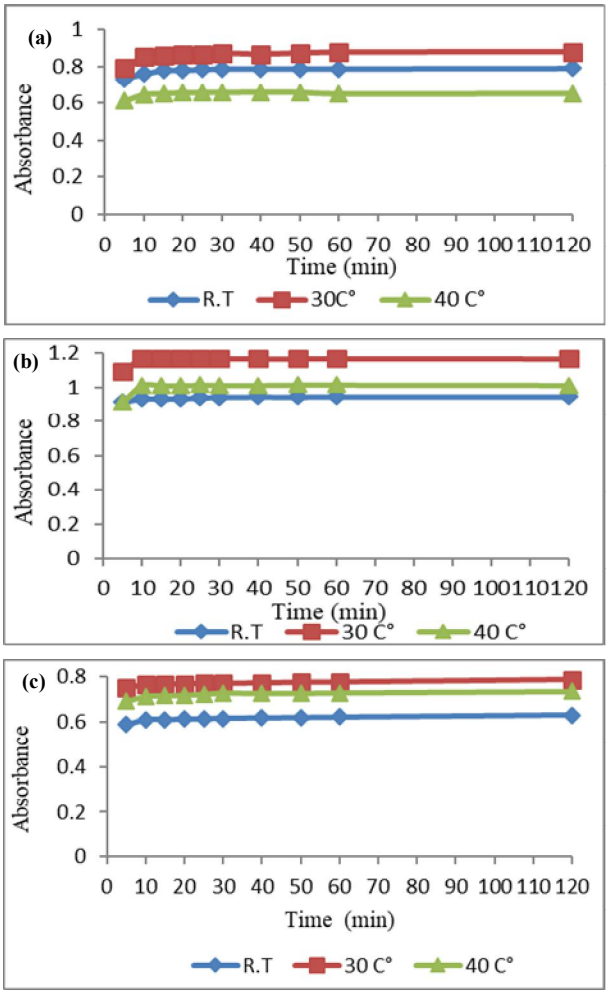


Figure 4. **Effect of temperature and time on the absorbance and stability of the oxidization of Adrenaline (a), Dopamin (b) and (c) Methyldopa**

Addition sequence effect

The results shown in Table 2 indicate that sequence II was appropriate in the determination of drugs and that any change in the addition sequence negatively affects absorption.

Table 2. **Effect of sequence addition.**

No.	Reaction sequence	Absorbance		
		Adrenaline	*Dopamine.HCl*	*Methyldopa*
I	D+B+NBS+P	0.623	0.661	0.890
II	D+NBS+B+P	0.877	0.770	1.171
III	D+NBS+P+B	0.447	0.613	0.542
IV	P+B+D+NBS	0.201	0.184	0.058
V	NBS+D+B+P	0.375	0.233	0.439

Where D= drug, NBS= N-bromosuccinamide, P= ponceau 4R and B= phthalate buffer (pH 2.5)

Final absorption spectra

Fig. 5 shows the final absorption spectra of the studied drugs after fixing the optimum conditions for the reaction, where the maximum absorption of the ponceau 4R was at the wavelength of 507 nm, whereas the blank solution gave no absorbance at this wavelength.

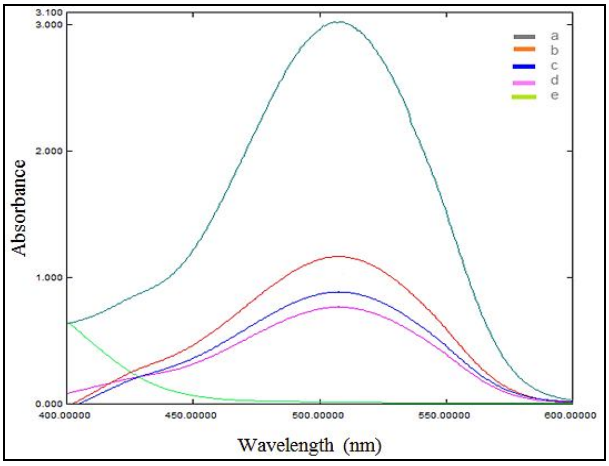


Figure 5. **Absorption spectra: (a) Ponceau 4R (100 µg/mL) in the presence of phthalate buffer solution (pH 2.5), (b, c, d) Ponceau 4R (100 µg/mL) in the presence of 10,5,7 µg/mL Methyldopa, Adrenaline, and Dopamine respectively, measured under the optimum conditions, (e) Blank solution**

Linearity and Quantification

Standard calibration graphs for Adrenaline, Dopamine HCl, and Methyldopa have been plotted by concentration versus absorbance under the optimal conditions (Fig. 6). The method follows Beer's law within the linear ranges shown (Table 3) for the studied drugs and there was a negative deviation from Beer's law after the estimated upper limits, as the square values of the correlation coefficient were ≥ 0.9977 for all drugs, which indicates that the calibration curves have excellent linear characteristics. The molar absorptivity values are 8.01×10^4, $5.32\times10^{4,}$ and 4.93×10^4 $L.mol^{-1}.cm^{-1}$ for the above drugs, respectively, indicating the method is sensitive. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated by the following 1 and 2 equations:

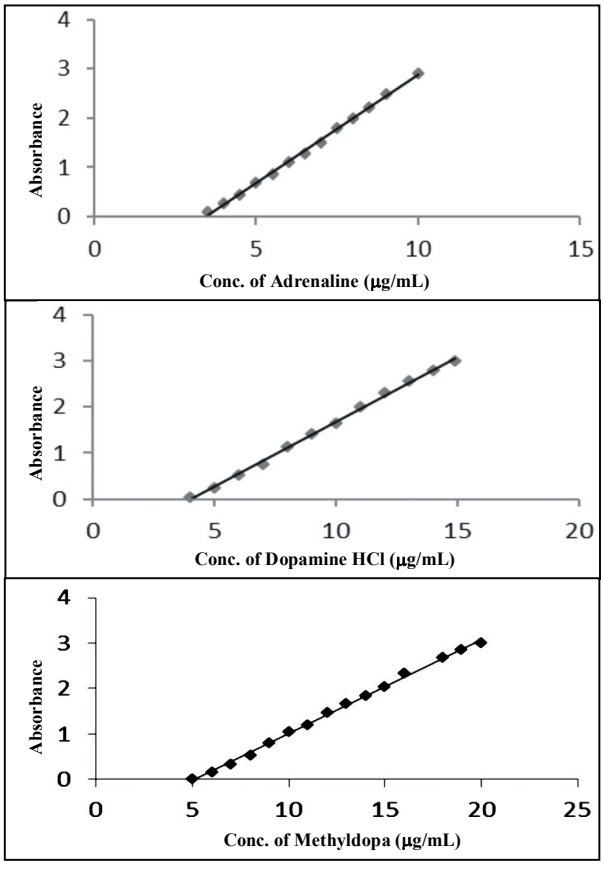


Figure 6. **Calibration graphs of Adrenaline, Dopamine HCl and Methyldopa**

$$LOD = 3.3\sigma/s \quad (1)$$
$$LOQ = 10\ \sigma/s \quad (2)$$

Where σ= The standard deviation of the absorption of the lowest concentration on the calibration graph, and s= slope of the calibration curve.

The results, cited in Table 3 are below the lower limit of the calibration curve. The results of accuracy (average recovery %) and the relative standard deviation (RSD) indicated the method was accurate and precise (Table 3).

Table 3. **Statistical data of the quantitative parameters for determination of Adrenaline, Dopamine.HCl and Methyldopa.**

Parameter	Adrenaline	Dopamin. HCl	Methyldopa
Linearity range (µg/ml)	3.5-10	4-15	5-20
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	8.01×10^4	5.32×10^4	4.93×10^4
Sandell Sensitivity ($\mu g/cm^2$)	2.28	3.56	4.83
Average recovery * %	100.61	99.61	99.15
LOD (µg/ml)	0.121	0.234	0.118
LOQ (µg/ml)	0.372	0.712	0.357
RSD*	≤ 0.46	≤1.42	≤ 0.96
Intercept	-1.5013	-1.1278	-1.0645
Slope	0.4377	0.2809	0.207
Correlation coefficient (R^2)	0.9977	0.9982	0.9978

*Average of six determinations

Validity and Application

To prove the efficiency of the proposed method and its success and free from the interference of drug additives in estimating the drugs in their pharmaceutical preparations, the standard addition procedure was applied to pharmaceutical preparations for each Adrenaline (ampoule), Dopamine HCl (ampoule) and Methyldopa (tablet) were produced from different origins (Table 4), with the addition of intercept value to each absorbance for each drug. As it can be inferred from Fig. 7 and Table 5, the method was free from interferences of excipients. However; the accuracy of the method was checked by applying the following equation 3 of the student t-test:

Table 4. Pharmaceutical formulations and their origin.

Pharmaceutical preparation	Declared composition	Company
	Adrenaline	
Adrenaline ampoules	Per ampoule: 1 mg Adrenaline	OSEL-Turkey
Adrenaline ampoules	Per ampoule: 1 mg Adrenaline	LINCOLN Pharmaceuticals India
	Dopamine HCl	
Dopadren ampoules	Perampoule: 200 mg Dopamine HCl	Vem Pharmaceutical – Turkey
Dopamine hydrochloride ampoules	200 mg Dopamine HCl	Hospira, INC, LAKE FOREST, USA
	Methyldopa	
Aldosam (10 tablets)	Per tablet: 250 mg Methyldopa	SDI- Iraq
Aldomac (10 tablets)	Per tablet: 250 mg Methyldopa	CAIRO- EGYPT

Table 5. **Standard addition method for the determination of the studied drugs.**

Pharmaceutical preparation	Certified value (mg)	Amount present (µg/mL)	Recovery (%)	Drug content found (mg)
		Adrenaline		
Adrenaline ampoule Turkey	1mg	1.01	101.25	4
		1.00	100.60	5
Adrenaline ampoule India	1 mg	1.01	101.00	4
		1.01	101.00	5
		Dopamine HCl		
Dopamine ampoule Turkey	200 mg	5	98.80	197.60
		7	99.28	198.56
Dopamine ampoule USA	200 mg	5	99.60	199.20
		7	99.85	199.70
		Methyldopa		
Aldosam tablets Iraq	250 mg	8	100.12	250.30
		10	99.80	249.50
Methyldopa tablets Egypt	250 mg	6	96.83	242.07

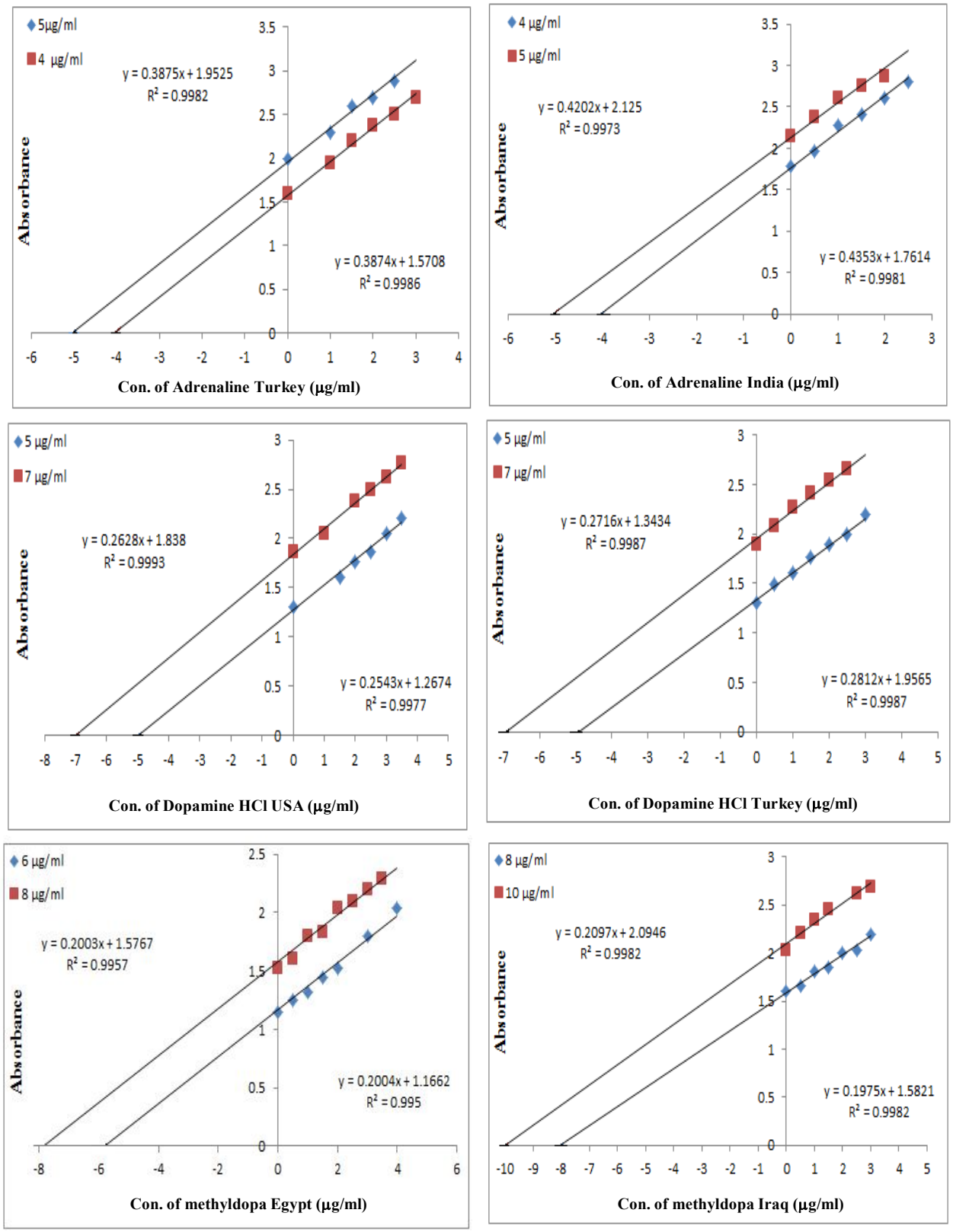


Figure 7. **Effect of excipient interferences by the standard addition procedure**

Comparison of the Developed Method with Other Methods

The developed method for the determination of Adrenaline, Methyldopa, and Dopamine hydrochloride has been compared with direct and indirect spectrophotometric methods described in the literature. Table 7 shows that the proposed method has high sensitivity and low detection and quantification limits, in addition to that it does not require temperature control, but it is consistent in terms of accuracy, compatibility, and analysis of medicinal compounds in its pharmaceutical preparations compared to other methods.

Table 6. **Estimation of the studied drugs in their pharmaceutical preparations.**

Pharmaceutical preparation	Certified value	Amount present (µg/mL)	Drug content found* (µg/mL)	Recovery* (%)	Average recovery (%)	Average recovery (mg)	t-test
Adrenaline							
Adrenaline ampoules Turkey	1 mg	4	4.04	101.00	100.04	1.00	0.89
		5	4.95	99.00			
		8	8.01	100.12			
Adrenaline ampoules India	1 mg	4	4.02	100.50	99.09	0.99	1.06
		5	4.92	98.40			
		8	7.87	98.37			
Dopamine HCl							
Dopamine ampoules Turkey	200 mg	5	5.12	102.40	99.37	198.74	0.74
		7	6.71	95.85			
		9	8.99	99.88			
Dopamine ampoules USA	200 mg	5	5.08	101.60	98.95	197.90	1.19
		7	6.70	95.71			
		9	8.96	99.55			
Methyldopa							
Aldosam tablets Iraq	250 mg	8	7.67	95.87	98.03	245.07	2.39
		9	8.95	99.44			
		10	9.88	98.80			
Methyldopa tablets Egypt	250 mg	6	5.71	95.16	96.87	242.17	0.74
		7	6.71	95.85			
		8	7.97	99.62			

Table 7. **Comparison of the proposed method with other methods.**

Analytical parameters	Present method			Literature method [41]			Literature method [42]
	Ad	***Md***	***Dm***	***Ad***	***Md***	***Dm***	***Md***
λmax (nm)		531			510		530
Reagent		Ponceau 4R			Calcon		EBT
Method		Oxidation-bleaching			Oxidation-bleaching		Oxidation-bleaching
Linearity (µg/mL)	3.5-10	5-20	4-15	1.0-36.0	2.0-40.0	0.5-16.0	0.1-9
Molar absorptivity L/mol.cm	8.01×10^4	4.93×10^4	5.32×10^4	1.10×10^4	0.32×10^4	0.43×10^4	4.60×10^4
LOD (µg/mL)	0.121	0.234	0.118	0.053	0.234	0.141	0.066
LOQ (µg/mL)	0.372	0.712	0.357	0.176	0.781	0.468	0.192
Temp. (°C)		30			30		28
RSD	0.46	0.96	1.42	0.35	0.85	0.38	≤ 1.0
Applications	Injection	Tablet	Injection	Injection	Tablet	Injection	Tablet

Ad= Adrenaline Md= Methyldopa Dm= Dopamine

Conclusion

Ponceau 4R dye has been used for indirect spectrophotometric determination of Adrenaline, Methyldopa, and Dopamine hydrochloride in their pure forms and pharmaceutical preparations. The method depended on the oxidation of the above drugs by measuring the amount of NBS, and the residue oxidized the dye in an acidic medium. The remaining dye was measured at 531 nm. The method is sensitive, accurate, and precise. The method was successfully applied to pharmaceutical preparations produced from different origins. The standard addition procedure was applied and proved that there was no interference by the excipients.

Conflict of Interest

The authors declare no conflict of interest.

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