

## USING PARACETAMOL HYDROLYSIS IN THE SPECTROPHOTOMETRIC DETERMINATION OF DOPAMINE HYDROCHLORIDE AND METHYLDOPA BY OXIDATIVE COUPLING REACTIONS

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**Abstract:** A simple and accurate spectrophotometric method was developed for the determination of dopamine hydrochloride and methyl dopa. This method depends on the oxidative coupling reaction of these compounds for paracetamol hydrolysis. It utilizes a potassium bromide-bromate mixture as an oxidative agent in a basic solution and CPC as a surfactant. A violet and blue color is formed for both dopamine hydrochloride and methyl dopa, with maximum absorption at wavelengths of 577 and 596 nm, respectively. The method followed Beer's law with a range of (1.0-17.5) and (3.0-25.0)  $\mu\text{g/mL}$ , and their molar absorptivity was ( $9.33 \times 10^3$ ) and ( $9.56 \times 10^3$ )  $\text{L/mol.cm}$  for the above compounds, respectively. The recovery of the method was 100.65% and 102.30%, while the relative standard deviation was 0.70% and 0.61% for the studied compounds. The LOD values of dopamine hydrochloride and methyl dopa were (0.1631) and (0.1056)  $\mu\text{g/mL}$ , and the LOQ values were (0.5436) and (0.3521)  $\mu\text{g/mL}$ , in that order. Pharmaceutical preparations of the two drug compounds were well applied using the method.

**Keywords:** spectrophotometry, oxidative coupling, dopamine hydrochloride, methyl dopa, paracetamol.

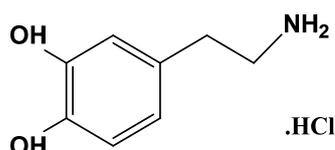
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### Introduction

Dopamine hydrochloride is an off-white crystalline powder that is soluble in water and most alcoholic solvents but insoluble in chloroform and ether. It is a catechol derivative (catecholamine). It has the structural formula shown in Fig. (1A) [1, 2]. Dopamine is an important drug that stimulates the heart when the blood pressure is low (antihypertensive) and the pulse slows down [3], in addition to being a hormone secreted from the adrenal gland and involved in controlling mood, movement, and cognition, controlling balance in the kidneys, and regulating immune cells [4]. Therefore, its deficiency leads to certain diseases,

including Parkinson's disease [5].

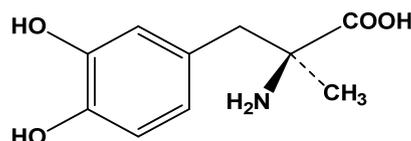
Methyl dopa is a white-colored powder that dissolves in water and is easily dissolved in alcohol. It is a derivative of catechol (catecholamine) and has the structural formula shown in Fig. (1B) [2]. Methyl dopa is one of the drugs used to lower blood pressure (an antihypertensive agent), as it works to dilate blood vessels to facilitate blood flow through them [6]. Its use has decreased after the discovery of safer and more effective drugs, but it is still used to treat high blood pressure and gestational hypertension [7, 8].



Dopamine hydrochloride ( $\text{C}_8\text{H}_{11}\text{NO}_2 \cdot \text{HCl}$ )  
4-(2-Aminoethyl)benzene-1,2-diol hydrochloride

Molar mass = 189.64 g/mol

(A)



Methyl dopa ( $\text{C}_{10}\text{H}_{13}\text{NO}_4$ )  
S-2-amino-3-(3,4-dihydroxyphenyl)-2-methyl-propanoic acid

Molar mass = 211.215 g/mol

(B)

**Fig. 1.** Chemical formula of dopamine hydrochloride and methyl dopa

Various analytical methods have been used for the determination of dopamine hydrochloride and methyl dopa, including spectrophotometric

methods [9-18], chromatographic methods [19-21], electric methods [22, 23], and flow injection methods [24, 25].

### Experimental part

**Apparatus.** A Shimadzu UV-1900i double-beam spectrophotometer was used, using 1 cm quartz cells. Weighing was done using a sensitive balance (ae ADAM). Heating was carried out using an (elektro-mag) water bath, and the acidity of the solutions was measured using a pH meter

(EUTECH instruments).

**The Chemicals and Reagents.** The chemicals and analytical reagents used were all of high purity. Detailed information is given in Table 1.

**Table 1.** Preparing the chemical compounds involved in the experiments

Chemicals	Cons.	Preparation	Final dilution with water
<b>Dopamine hydrochloride</b>	100 µg/mL	The solution was prepared by dissolving 0.0100 g of the pure substance in 2 mL of absolute ethanol.	100 ml
<b>Methyl dopa</b>	100 µg/mL	The solution was prepared by dissolving 0.0100 g of the pure substance in 2 mL of absolute ethanol.	100 ml
<b>Paracetamol solution</b>	0.5%	The solution was prepared by dissolving 0.5000 g in 20 mL of ethanol.	100 ml
<b>Potassium bromide-bromate mixture solution</b>	0.01 - 0.001 M	The oxidizing agent, a mixture of potassium bromide and bromate, was prepared by dissolving 0.1190 - 0.0167 g of the substance.	100 ml
<b>Sodium hydroxide solution</b>	1 M	The solution was prepared by dissolving 4 g of the base and was used for methyl dopa. A solution of 0.1 M concentration was prepared and used for dopamine hydrochloride.	100 ml
<b>Surfactant solution</b>	0.1%	Solutions were prepared by dissolving 0.100 g of the substances in hot distilled water.	100 ml

**The procedure of paracetamol hydrolysis.** In a 250 mL round-bottom flask that has a condenser attached, 100 mL of paracetamol (0.5%) was added, followed by 25 mL of concentrated hydrochloric acid (12.06 M), refluxed for 45 min, and then moved to a 250 mL volumetric flask, and distilled water was used to

complete to the mark required [26].

**Standard Paracetamol Solution (50 µg/mL):** A 50 µg/mL solution was prepared by neutralizing 2.5 mL of paracetamol hydrolysis with 20% sodium carbonate and finishing the volume with distilled water in a 100 mL volumetric flask.

### Result and Discussion

**Preliminary study and install optimum conditions.** After the conversion of paracetamol to p-aminophenol by hydrolysis, 1.5 mL of p-aminophenol was oxidized by a mixture of potassium bromide-bromate, and 10 µg/mL of dopamine hydrochloride and 10 µg/mL of methyl dopa were added separately in sodium hydroxide medium using a 10 mL volumetric

flask. Absorbance measurements were performed at a wavelength of 561.50 nm for dopamine hydrochloride and 575 nm for methyl dopa, and various conditions were studied to obtain results with good stability.

**The effect of different types of oxidizing agents.** Different types of oxidizing agents were used, and their effect on the intensity of the

absorption spectrum of the colored products was studied to select the best oxidizing agent that leads to the oxidation of the reagent. Table 2 shows that

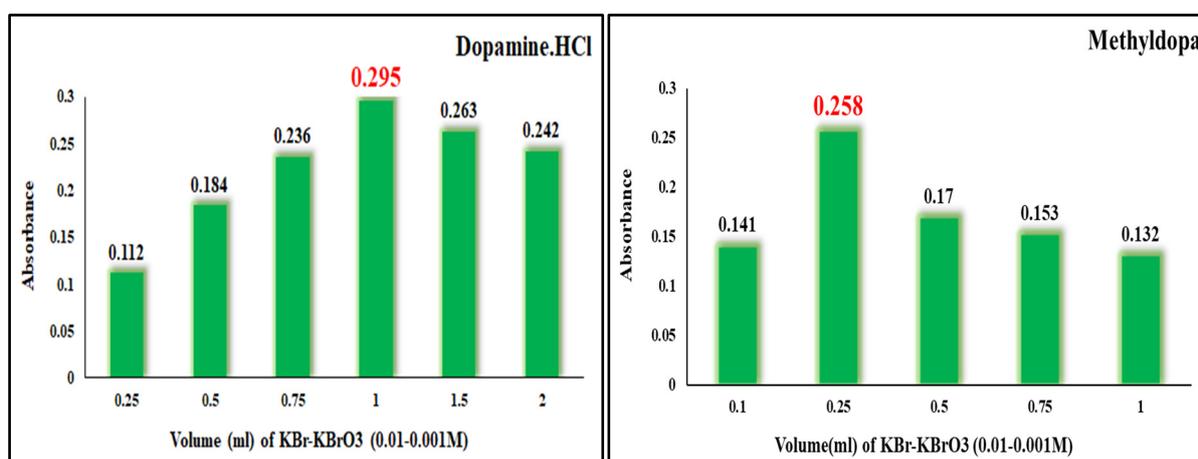
the mixture of potassium bromide and bromate is the best.

**Table 2.** Effect of different types of oxidizing agents

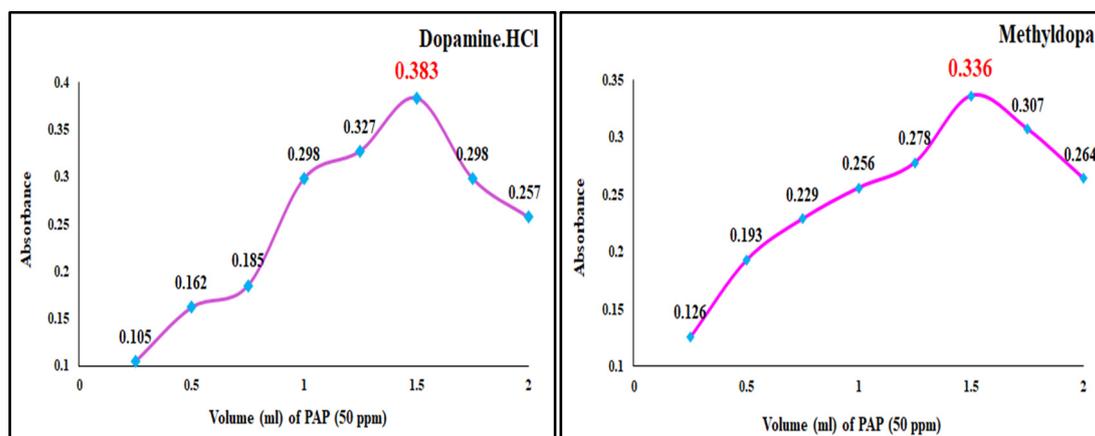
Type of oxidizing agent ( $1 \times 10^{-2}M$ ) (1mL)	Dopamine.HCl		Methyldopa	
	$\lambda_{max}$ (nm)	Absorbance	$\lambda_{max}$ (nm)	Absorbance
Potassium Iodate ( $KIO_3$ )	565	0.174	570	0.108
Sodium Periodate ( $NaIO_4$ )	435	0.295	434.50	0.087
N-Chlorosuccinimide (NCS)	308	0.295	491.50	0.034
Ferric Chloride ( $FeCl_3$ )	305	-0.346	301	0.777
Potassium Dichromate ( $K_2Cr_2O_7$ )	563.50	0.155	568	0.125
Copper Sulfate pentahydrate ( $CuSO_4 \cdot 5H_2O$ )	558	0.162	297	0.461
Potassium Bromide-Potassium Bromate ( $KBr - KBrO_3$ ) (0.01 – 0.001M)	561.50	0.296	575	0.132

The effect of increasing amounts of the oxidizing agent  $KBr-KBrO_3$ . Different amounts of the oxidizing agent (0.1-2.0 mL) were studied.

Fig. 2 shows that the best amount leading to oxidation of the reagent is 1 mL for dopamine hydrochloride and 0.25 mL for methyldopa.



**Fig. 2.** Effect of increasing amounts of  $KBr-KBrO_3$



**Fig. 3.** Effect of increasing amounts of paracetamol hydrolysis reagent

The effect of increasing amounts of paracetamol hydrolysis reagent (50 ppm). Increasing amounts (0.25-2.0 mL) of the reagent

p-aminophenol produced from the paracetamol hydrolysis were studied for their effect on the absorption of complexes, and absorbance

measurements were performed against blank solutions at 561.50 nm for dopamine hydrochloride and 575 nm for methyl dopa. Fig. 3 shows that the optimal amount of p-aminophenol reagent for both dopamine hydrochloride and methyl dopa is 1.5 mL.

**The effect of different types of bases.** The effect of different types of bases has been studied to see which one gives the highest absorption of the product formed. Fig. 4 shows that the best base for dopamine hydrochloride and methyl dopa is sodium hydroxide.

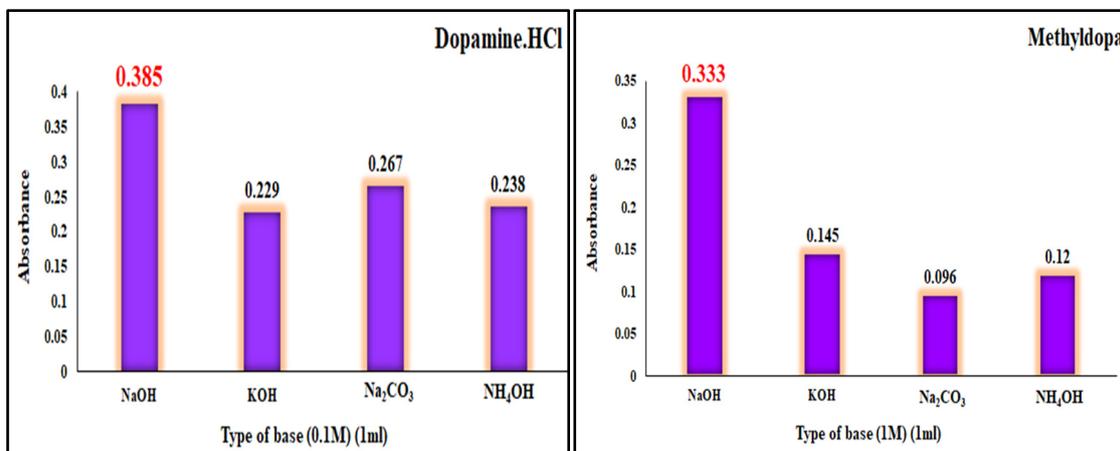


Fig. 4. Effect of different types of bases

**The effect of different concentrations of sodium hydroxide base.** Several concentrations of sodium hydroxide base were taken, and their effect on the absorption intensity was studied. Table 3 shows that the best concentration for dopamine hydrochloride is 0.1 M and 1 M for methyl dopa.

Table 3. Effect of different concentrations of sodium hydroxide

Concentration (M) of NaOH (1ml)	Absorbance	
	Dopamine. HCl	Methyl dopa
0.01	0.186	-----
0.1	0.382	0.147
0.3	0.287	0.201
0.5	0.261	0.230
0.75	0.194	0.254
1.0	0.089	0.335
1.5	-----	0.232
2.0	-----	0.226

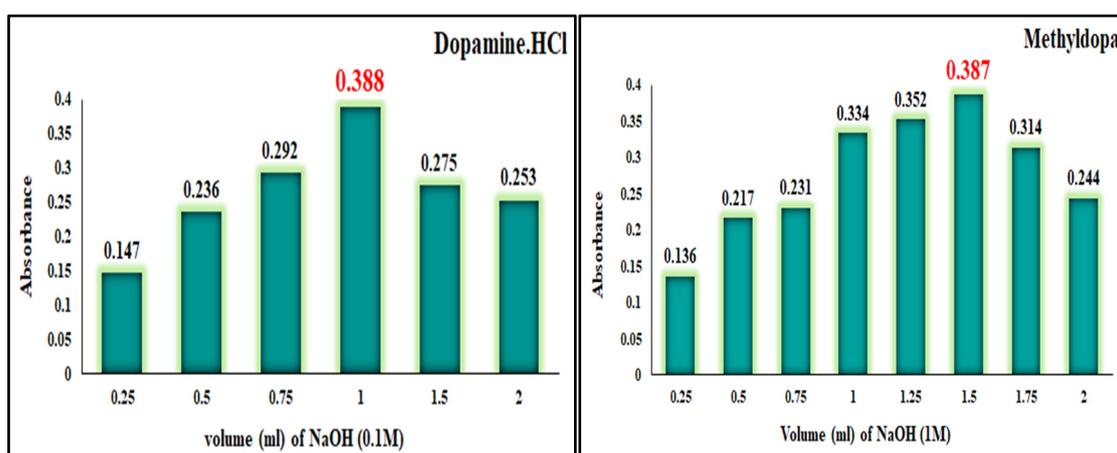


Fig. 5. Effect of increasing amounts of sodium hydroxide

**The effect of increasing amounts of sodium hydroxide base.** The effect of the amount of base added to the reaction (0.25-2.0 mL) was studied to obtain the highest absorption of the formed product. Fig. 5 shows that the best amount of dopamine hydrochloride is 1 mL and the best amount of methylidopa is 1.5 mL.

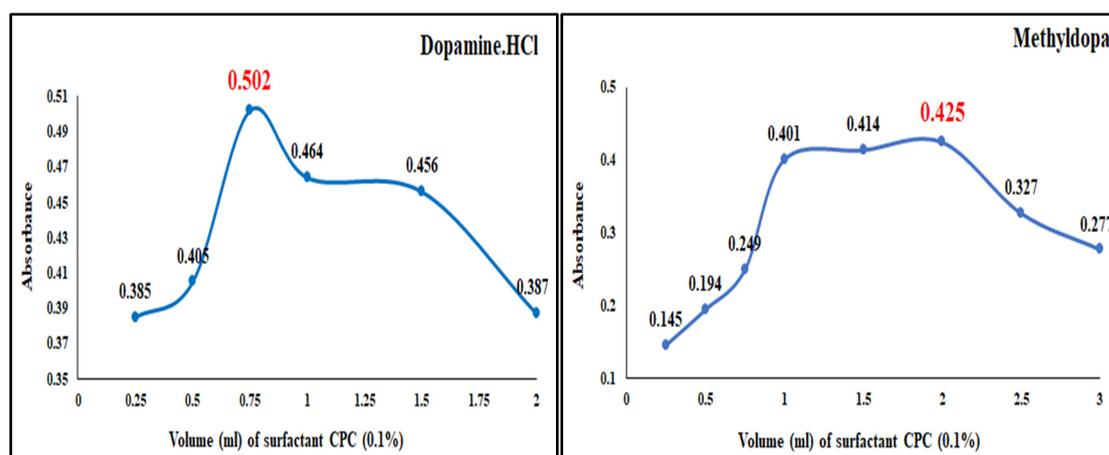
**The effect of surfactants (0.1%).** Use several surfactants (positive, negative, and neutral) and observe their effect on the color intensity and absorbance of the product formed. Table 4 shows that the best surfactant is CPC for both dopamine hydrochloride and methylidopa.

**Table 4.** Effect of surfactants

Surfactant 0.1% (1ml)	Dopamine.HCl		Methylidopa	
	$\lambda_{max}$ (nm)	Absorbance	$\lambda_{max}$ (nm)	Absorbance
Without	561.50	0.390	575	0.385
CPC	577	0.467	596	0.403
SDS	564.50	0.310	582	0.214
Cetavlon	570	0.418	581	0.283
Triton X-100	564.50	0.319	578	0.267

**The effect of increasing amounts of the surfactant CPC (0.1%).** Increasing amounts of CPC surfactant (0.25-3.0 mL) were taken. Fig. 6

shows that the optimal amount of CPC for dopamine hydrochloride was 0.75 mL and for methylidopa was 2 mL.



**Fig. 6.** Effect of increasing amounts of CPC surfactant

**The effect of oxidation time.** The oxidation time of dopamine hydrochloride and methylidopa was determined by adding 1.5 mL of the paracetamol hydrolysis reagent (50  $\mu\text{g}/\text{mL}$ ), then adding the oxidizing agent potassium bromide-bromate mixture, and leaving it for different

periods of 1-15 minutes, and then the other substances were added. After that, the absorption was measured. Table 5 shows the oxidation time of dopamine hydrochloride as 1 minute and methylidopa as 3 minutes.

**Table 5.** Effect of oxidation time

Time (min)	Absorbance	
	Dopamine.HCl	Methylidopa
1	0.524	0.396
3	0.513	0.446
5	0.502	0.421
7	0.421	0.433
10	0.377	0.388
15	0.373	0.357

**The effect of temperature and stability time.** The effect of different temperatures (20, 40, and 50°C) on the absorption intensity and stability of the formed product was studied separately for dopamine hydrochloride and methyldopa using the optimum conditions that were previously

established. Fig. 7 shows that the best temperature is the laboratory temperature (20°C), as the Development time for both was 10 minutes, and the stability period for them lasted for more than 60 minutes.

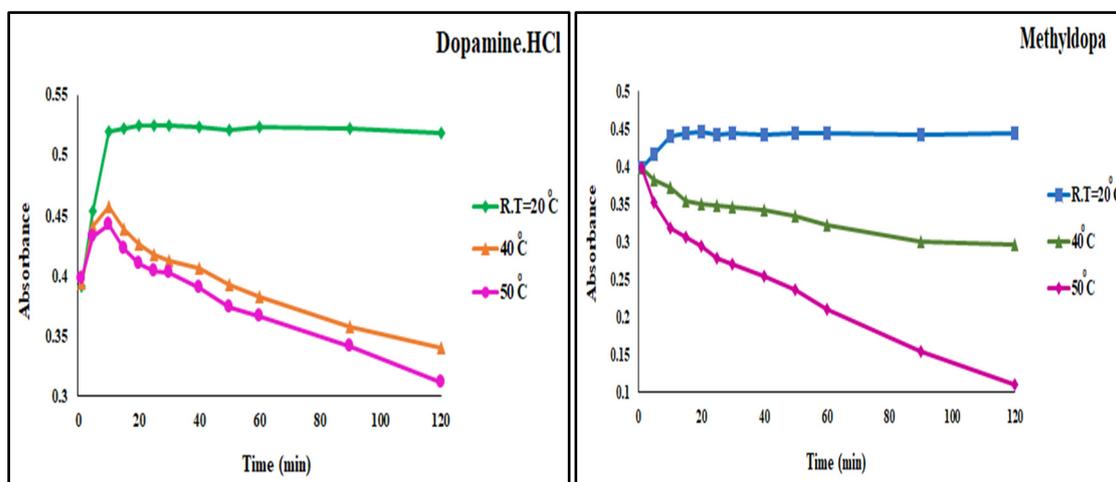


Fig. 7. Effect of temperature and stability time

Table 6 shows a synopsis of the optimized conditions obtained regarding the determination of

dopamine hydrochloride and methyldopa with paracetamol hydrolysis reagent.

Table 6. Summary of optimized conditions

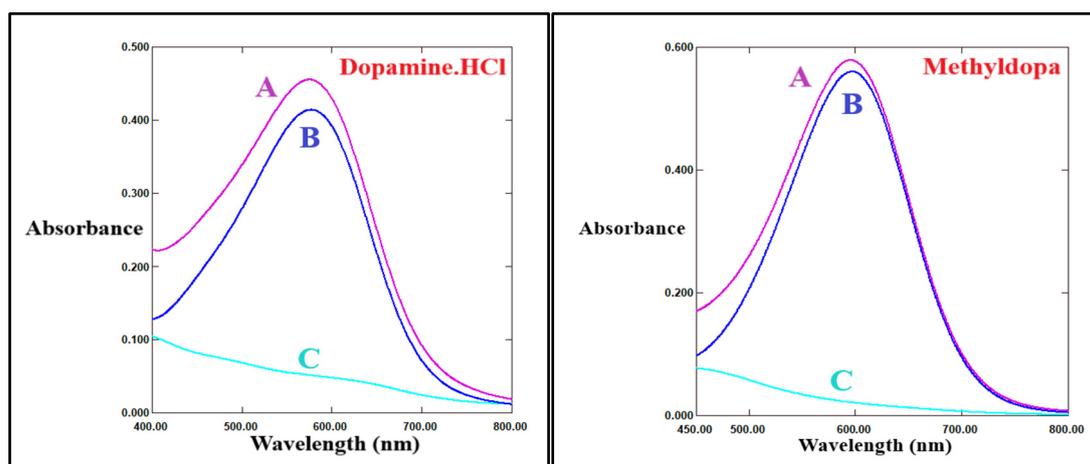
Parameters	Experimental Conditions			
	Dopamine.HCl		Methyldopa	
	Concentration	Volume (ml)	Concentration	Volume (ml)
Paracetamol hydrolysis	50 µg/ml	1.5	50 µg/ml	1.5
KBr-KBrO <sub>3</sub>	0.01-0.001 M	1	0.01-0.001 M	0.25
NaOH	0.1 M	1	1 M	1.5
CPC	0.1%	0.75	0.1%	2
Color	Violet		Blue	
λ <sub>max</sub>	577 nm		596 nm	
Oxidation Time	1 min		3 min	
Temperature	R.T=20 °C		R.T=20 °C	
Development Time	10 min		10 min	
Stability Period	> 100 min		> 100 min	

**Final absorption spectrum.** After the optimal conditions were established, the absorption spectra of dopamine hydrochloride at a wavelength of 577 nm and methyldopa at a wavelength of 596 nm, as shown in Fig. 8.

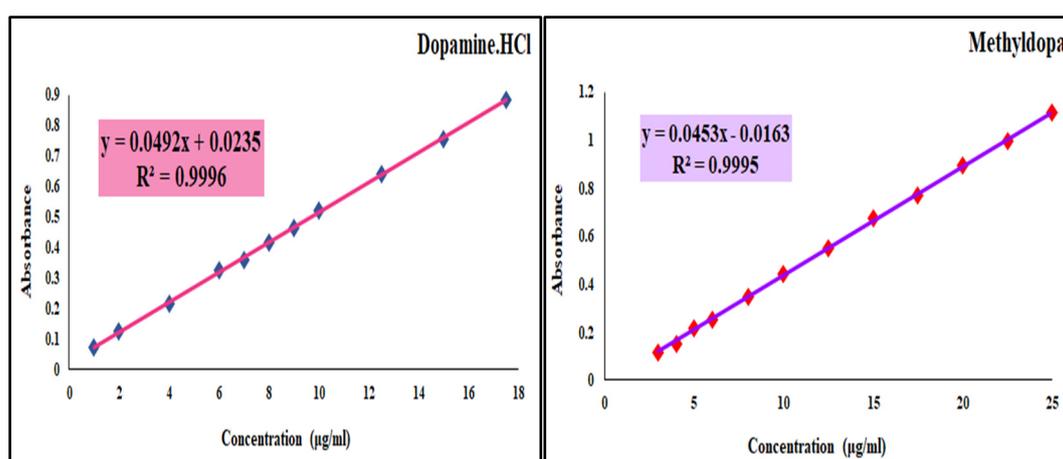
**Procedure method and standard curves of dopamine hydrochloride and methyldopa.** Following the optimal conditions found in Table 5, it was possible to prepare standard curves for both dopamine hydrochloride and methyldopa.

Increasing amounts of the drug compounds were added to a set of volumetric flasks after adding the optimal amounts of the substances studied, and then the absorbance was measured after 10 minutes at a wavelength of 577 nm for dopamine hydrochloride and 596 nm for methyldopa. The molar absorbance of dopamine hydrochloride was  $9.33 \times 10^3$  L/mol.cm, and for methyldopa it was  $9.56 \times 10^3$  L/mol.cm. Fig. 9 shows that the method

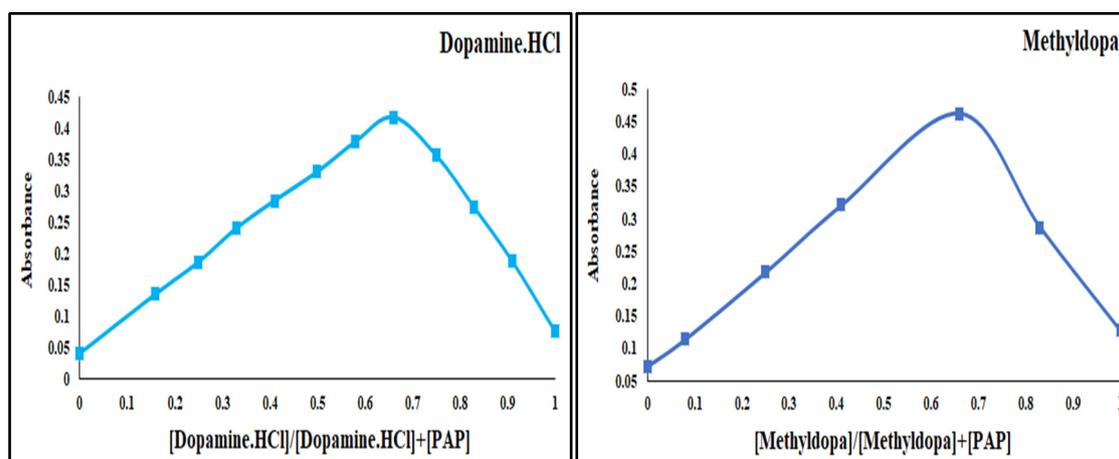
follows Beer's law, including the range (1.0-17.5)  $\mu\text{g/mL}$  for methyl dopa.  $\mu\text{g/mL}$  for dopamine hydrochloride and (3.0-25.0)



**Fig. 8.** Final absorption spectrum of dopamine hydrochloride and methyl dopa. (A) Absorption spectra of dopamine hydrochloride (8  $\mu\text{g/mL}$ ) and methyl dopa (12.5  $\mu\text{g/mL}$ ) versus distilled water. (B) Absorption spectra of dopamine hydrochloride (8  $\mu\text{g/mL}$ ) and methyl dopa (12.5  $\mu\text{g/mL}$ ) versus their blank solution. (C) Absorption spectra of their blank solution versus distilled water



**Fig. 9.** Standard curves for the drug compounds



**Fig. 10.** Continuous changes for dopamine hydrochloride and methyl dopa

**The effect of the nature of the products formed.** The continuous changes method (Job's

method) and the mole ratio [27] were employed to calculate the compositional ratio between the drug

compound dopamine hydrochloride or methyldopa and the reagent paracetamol hydrolysis by using dilute solutions of the reagent and the two drug compounds at a concentration of

$3.3 \times 10^{-4}$  M. The results obtained in Fig. (10) and Fig. 11 confirm that the ratio between them is 2:1 (drug compound:reagent) using both methods.

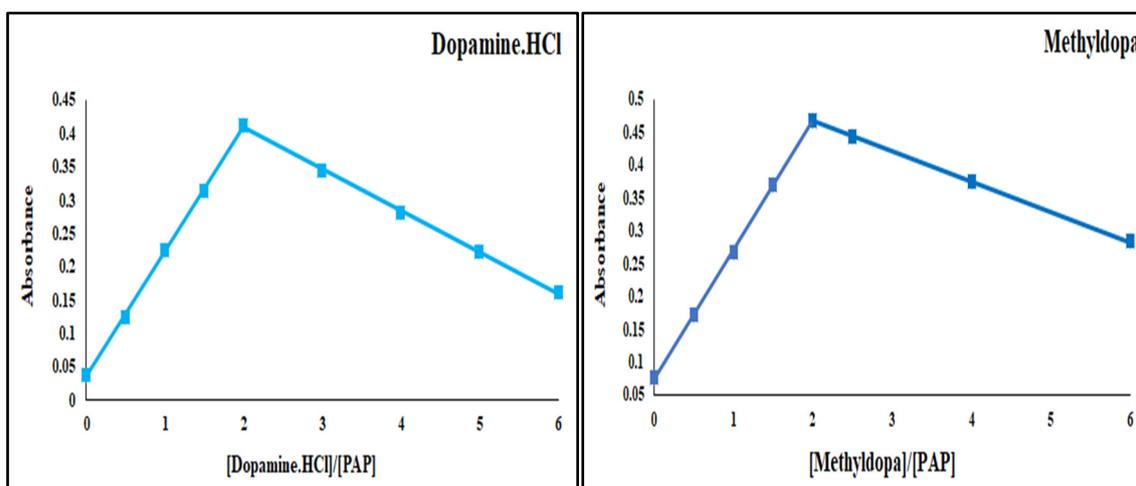


Fig. 11. Molar ratio of dopamine hydrochloride and methyldopa

The stability constant of the products formed in a 2:1 ratio of dopamine hydrochloride and

methyldopa was calculated separately by applying the following law:

$$K_{st} = 1 - \alpha / 4\alpha^3 c^2$$

The stability constant of dopamine hydrochloride was  $6.568 \times 10^{11}$  L<sup>2</sup>/mol<sup>2</sup>, and that of methyldopa

was  $1.319 \times 10^{11}$  L<sup>2</sup>/mol<sup>2</sup>, indicating the high stability of the formed products.

Table 7. shows the stability constant of the products formed

Concentration (mol/l)	Absorbance		$\alpha$	$K_{st}(l^2/mol^2)$	Average $K_{st}(l^2/mol^2)$
	As	Am			
<b>Dopamine.HCl</b>					
$1.65 \times 10^{-5}$	0.1812	0.2032	0.10826	$6.4527 \times 10^{11}$	$6.568 \times 10^{11}$
$3.3 \times 10^{-5}$	0.2682	0.3740	0.28288	727205	
$6.6 \times 10^{-5}$	0.4018	0.5146	0.219199	425475	
<b>Methyldopa</b>					
$1.65 \times 10^{-5}$	0.2514	0.3128	0.19629	$9.7581 \times 10^{10}$	$1.319 \times 10^{11}$
$3.3 \times 10^{-5}$	0.3334	0.4248	0.21516	$1.8088 \times 10^{10}$	
$6.6 \times 10^{-5}$	0.4658	0.5444	0.14437	$1.6316 \times 10^{10}$	

**Suggested chemical reaction.** After studying the nature of the products formed through the Job's and molar ratio methods, it was found that the correlation ratio between the paracetamol hydrolysis reagent with dopamine hydrochloride and methyldopa is 2:1 (drug compound:reagent), and it is expected that the reaction proceeds according to the mechanism in Fig. 12.

**Application of the suggested method to pharmaceutical preparations.** The method was applied to the determination of dopamine hydrochloride and methyldopa in their

pharmaceutical preparations in the form of injections and tablets from different origins, as shown in Table 8.

Dopamine hydrochloride injection analysis (200 mg/5 mL): the dopamine injection was analyzed by withdrawing 5 mL of the drug solution and placing it in a 100 mL volumetric flask so that the final concentration was 2000  $\mu$ g/mL, and from this concentration, 100  $\mu$ g/mL was prepared, and then the concentrations of 4, 8, and 10  $\mu$ g/mL of dopamine hydrochloride were taken from it. Table 8 shows the results obtained.

Methyldopa tablets analysis (250 mg): The methyldopa tablets were analyzed by weighing 10 tablets of the pharmaceutical preparation and grinding them well, and approximately the weight of one tablet was taken and dissolved in distilled

water in a volume of 100 mL to obtain a concentration of 2500 µg/mL. Then, 100 µg/mL was prepared, and then concentrations of 5, 8, and 15 µg/mL of methyldopa were taken. Table 8 shows the results obtained.

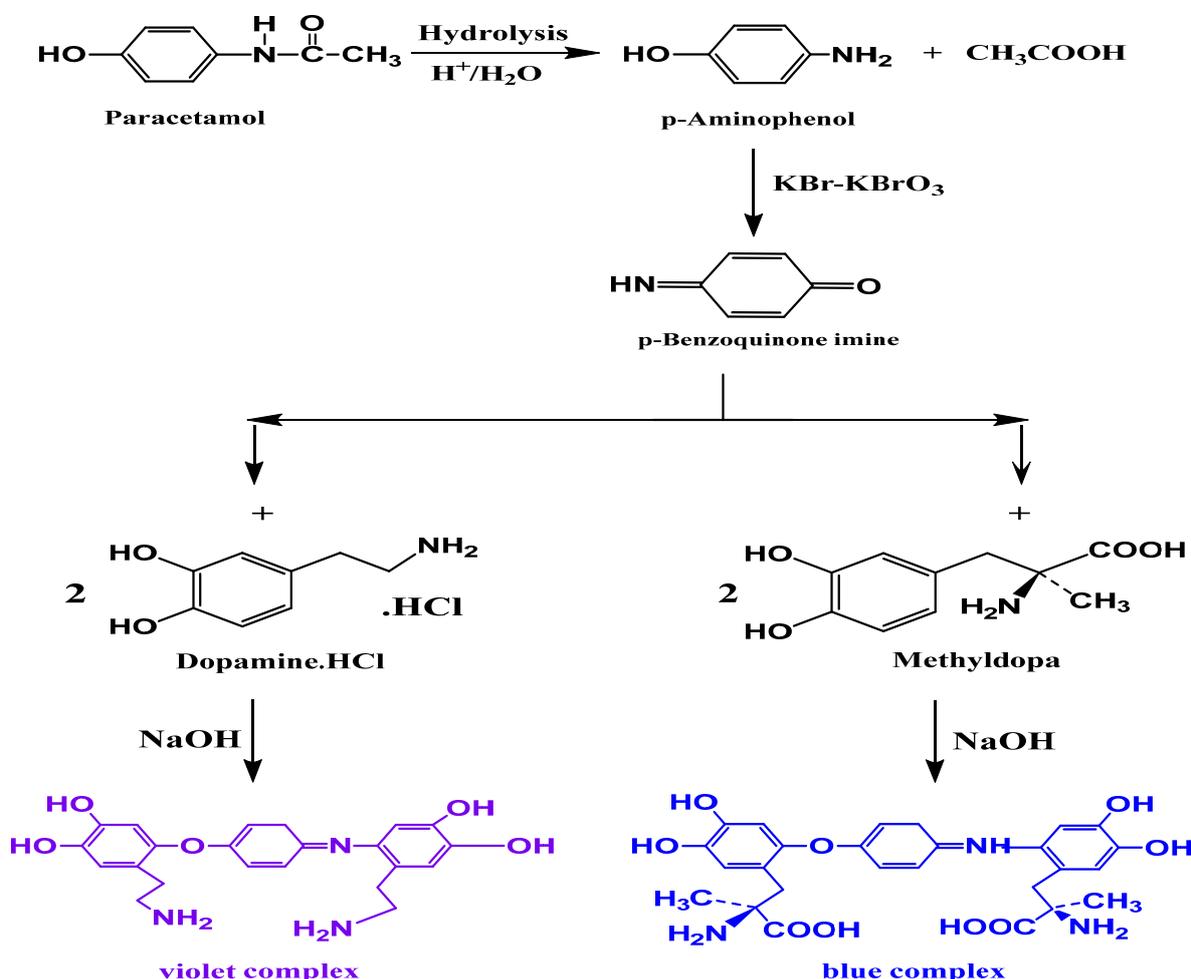


Fig. 12. Suggested interaction mechanism of dopamine hydrochloride and methyldopa

Table 8. Determination of drug compounds in pharmaceutical preparations

Pharmaceutical preparation	Validated value	Amount present (µg/mL)		Drug content found(mg)	Recovery %	RSD%
		Taken	Found			
Dopamine.HCl						
Oterop Injection	200mg / 5mL	4.0	3.87	193.50	96.75	1.20
		8.0	7.93	198.24	99.12	0.43
		10.0	10.14	202.80	101.40	0.36
Methyldopa						
SAFA Tablet	250mg	5.0	5.05	252.50	101.00	0.71
		8.0	8.03	250.92	100.37	0.32
		15.0	15.22	253.65	101.46	0.23

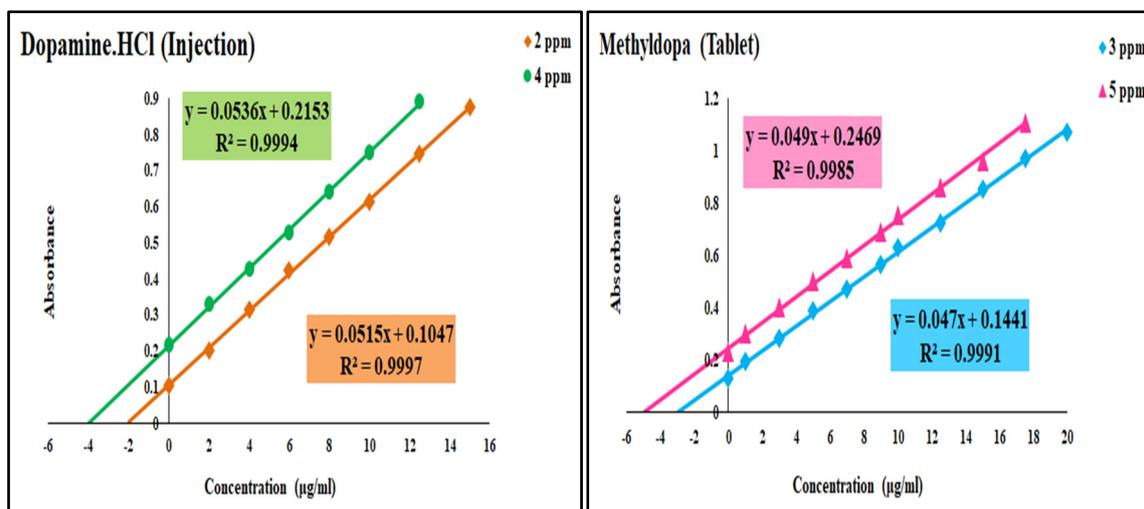
**Application of the standard addition method to pharmaceutical preparations.** To prove the efficiency and success of the suggested method, the standard addition method was applied to the

pharmaceutical preparation in the form of an injection of dopamine hydrochloride at a concentration of 2 and 4 µg/mL and in the form of

tablets of methyl dopa at a concentration of 3 and 5  $\mu\text{g/mL}$ , as shown in Fig. 13.

**Comparing the suggested method with another method.** The suggested way for dopamine hydrochloride and methyl dopa using

paracetamol hydrolysis reagent was compared with another spectrophotometric method. Table 9 shows that the suggested method has a higher sensitivity, a higher estimation range, and a higher wavelength.



**Fig. 13.** The standard addition to the pharmaceutical preparation

**Table 9.** Comparing the suggested method with another method

Analytical parameters	Present method		Literature method[28]	
	Dopamine.HCl	Methyl dopa	Dopamine.HCl	Methyl dopa
Type of reaction	oxidative coupling	oxidative coupling	Diazo-coupling	Diazo-coupling
Reagent used	Paracetamol hydrolysis	Paracetamol hydrolysis	Sulphanillic acid	Sulphanillic acid
$\lambda_{\text{max}}$ (nm)	577	596	475	507
Color of product	Violet	Blue	Orange-red	Orange-red
Medium of reaction	Basic medium	Basic medium	Basic medium	Basic medium
Beer's law ( $\mu\text{g/ml}$ )	1.0-17.5	3.0-25.0	0.6-15.0	0.5-17.0
Molar absorptivity ( $\text{l/mol.cm}$ )	$9.33 \times 10^3$	$9.56 \times 10^3$	$9.27 \times 10^3$	$9.47 \times 10^3$
Recovery %	100.65	102.30	99.83	99.83
RSD %	0.70	0.61	0.063	0.826
Application	Injection	Tablet	Injection	Tablet

## Conclusion

A rapid and simple, straightforward spectrophotometric method was developed for the determination of dopamine hydrochloride and methyl dopa by oxidative coupling between the reagent paracetamol hydrolysis and the drug compounds in the presence of the oxidizing agent  $\text{KBr-KBrO}_3$  in a basic medium with CPC as a surfactant. The absorption intensity was measured

at wavelengths of 577 nm for dopamine hydrochloride and 596 nm for methyl dopa. The method was successfully applied to the pharmaceutical preparation in the form of an injection for dopamine hydrochloride and the form of tablets for methyl dopa, and the results were of good accuracy and precision.

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