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Spectrophotometric determination of methyldopa by oxidative coupling reactions using 2,4 -dinitrophenylhydrazine reagent

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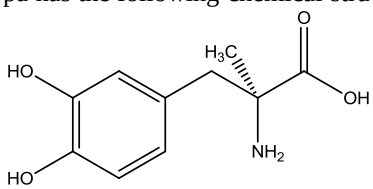
ABSTRACT

A spectrophotometric method was developed for the determination of methyldopa. The method is based on oxidative coupling reactions with 4,2-dinitrophenylhydrazine as a reagent and in the presence of the oxidizing agent potassium periodate in the acidic medium. The product exhibits maximum absorption at 428 nm, Beer's law-subjective concentration in the range of (1-30) µg/ml, and molar absorbance of 6589,908 liters. $mol^{-1}.cm^{-1}$. The relative standard deviation was 0.418 percent, the recovery rate was 99.60 percent, and the quantitative limit attained (LOQ) was 0.261 µg/ml. The limit of detection (LOD) was 0.078µg/ml. Following the procedures of continuous changes and molar ratio, the nature of the product created from the reaction was examined, and the ratio was 2:1. (drug compound: reagent) and the value of the stable rate of stability was 8.84 x 10^{12} liters ^{2}. mol^{-2}, which indicates the good stability of this product. This method was successfully applied to estimate methyldopa in tablet form.

Introduction

Methyldopa is one of the medications used to control blood pressure. Although its use has decreased as safer and more effective medications have come to light, it is still used to treat high blood pressure and pregnancy hypertension. The drug's side effects include drowsiness, stomach pain, pancreatitis, slow muscle movement, jaundice, hepatitis, and swelling in the feet[1,2]. Various techniques have been described for estimating dopa, including spectroscopic methods[3-14], chromatography[15,16], and voltage measurement. These techniques are effective in estimating dopa in people with decreased kidney function, depression, and mental disorders[17]. Due to the importance of this medicinal compound, a spectrophotometric method was developed for its determination.

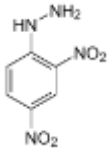
Methyldopa has the following chemical structure:



M.Wt= 211.215 g/mol

S-2-amino-3-(3,4-dihydroxyphenyl)-2-methyl-propanoic acid

As for the 4,2-dinitrophenylhydrazine reagent, it is a reddish-orange solid compound. It is substituted hydrazine. It is frequently used in the qualitative detection of the carbonyl group attached to ketones and aldehyde. It does not dissolve in water but dissolves in sulfuric acid [18]. The structural formula of the reagent is :



M.Wt= 198.14 g/mol

practical part:

Devices used:

Use of a UV-Visible spectrophotometer of the type Double_beam-Spectrophotometer Shimadzu UV-1800 PC.The solutions were heated using an Electro-mag water bath. As for the weight, it was using a sensitive scale, type ADAM.

Chemical solutions

Methyldopa (100 µg/mL): was prepared by dissolving 0.01 g of the pure substance in a volumetric vial of 100 mL of distilled water.

4,2 - Dinitrophenylhydrazine (100 µg/ml): Prepared by dissolving 0.01 g of the pure substance in a volumetric bottle of 100 ml of distilled water.

Potassium periodate (1 x 10^{-2} M): The solution was prepared by dissolving 0.23 g of the pure substance in a volumetric bottle of 100 ml of distilled water.

Hydrochloric acid (1.0 M): To make the solution, 8.5 ml of concentrated hydrochloric acid (11.7M) was diluted with 100 ml of distilled water in a volumetric bottle. Using the dilution law, solutions with lower concentrations were created from it.

Setting conditions:

It was observed that when a dilute aqueous solution of methyldopa (10 µg/ml) was mixed with the reagent 4,2-dinitrophenylhydrazine and using potassium periodate as an oxidizing agent in the acidic medium in a volumetric bottle (10 ml) a yellow product was obtained whose maximum absorption was measured. At a wavelength of 428 nm vs blank.

Study of the type of oxidizing agent

Various types of oxidizing agents were tested at a concentration of 1×10^{-3} molarity. Figure (1) shows that the best oxidizing agent is potassium periodate used and it was adopted in subsequent experiments.

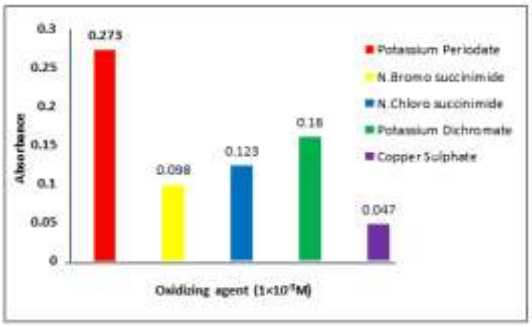


Fig. 1: Studying the effect of the type of oxidizing agent on the absorption of the reaction product

Study the effect of oxidizing agent concentration

This study was conducted to obtain the best concentration of potassium periodate to obtain the highest absorption, and the results recorded in Table (1) show the best concentration of potassium periodate is 1×10^{-2} which was relied upon in subsequent experiments.

Table 1: The concentration of the oxidizing agent on the absorption of the reaction product

Different of concentrations(M)	Absorbance
1×10^{-4}	0.187
5×10^{-4}	0.219
1×10^{-3}	0.273
5×10^{-3}	0.279
1×10^{-2}	0.287
5×10^{-2}	0.232

Study the volume of the oxidizing agent

This investigation was carried out to determine the ideal concentration of potassium periodate by adding increasing amounts (0.25-1.5) milliliters after the type and concentration of the oxidizing agent were fixed. The results are in Table (2).

Table 2: The size of the oxidizing agent on the absorption of the reaction product

X ml of KIO_4 (1×10^{-2} M)	0.25	**0.5**	0.75	1.0	1.25	1.5
Absorbance	0.189	**0.287**	0.178	0.125	0.098	0.066

Study the effect of acid type

This study was conducted to select the appropriate acid and to obtain high sensitivity. Figure (2) shows that the best acid is hydrochloric acid, so it was adopted in subsequent experiments.

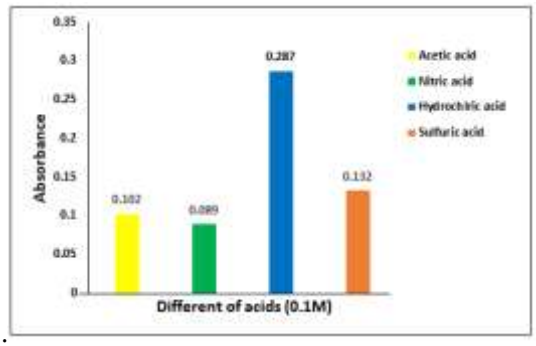


Fig. 2: Studying the effect of acid type on the absorption of the reaction product

Study the effect of acid concentration

After fixing the appropriate type of acid, this study was conducted to find out the appropriate concentration of hydrochloric acid, which gives the maximum absorption of the formed product, and Table (3) shows the best concentration of the acid that was relied upon in subsequent experiments.

Table 3: study of the acid concentration on the absorption of the reaction product

Concentration of HCl (M)	Absorbance
0.1	**0.288**
0.3	**0.294**
0.5	**0.301**
0.8	**0.278**
1.0	**0.245**

Study of the amount of hydrochloric acid

The effect of adding increasing amounts of hydrochloric acid at a concentration (0.5 M) on the intensity of absorption of methyldopa with 4,2-dinitrophenylhydrazine reagent at laboratory temperature (20 °C) was studied. The results were recorded in Table (4) and it was found that the optimal amount of Acid is 0.75 milliliters.

Table 4: Studying the volume of acid on the absorption of the reaction product

X ml of HCl (0.5M)	Absorbance
0.25	0.265
0.5	0.301
0.75	0.321
1.0	0.288
1.25	0.224

Study the effect of temperature

This study was conducted to find out the effect of different temperatures on the absorption of the formed product, and Figure (3) illustrates this

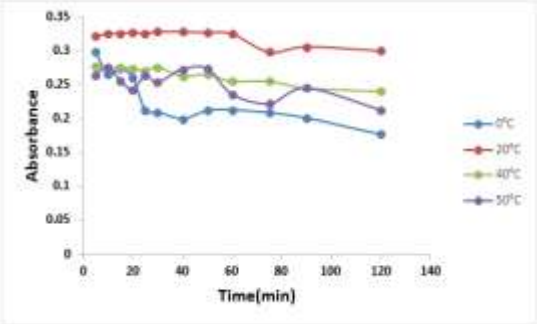


Fig. 3: Study of the effect of temperature on the absorption of the reaction product

Final absorption spectrum

The absorption spectrum was plotted with wavelengths ranging from 350-700 nm against the mock solution, and the complex showed the highest absorption at 428 nm, as shown in Figure (4).

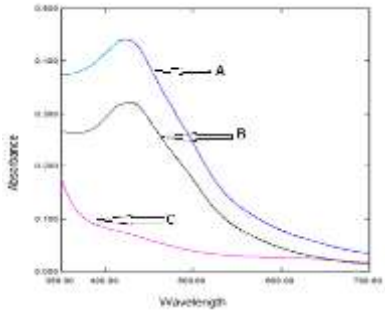


Fig. 4: The final absorption spectrum of the product (10 µg/mL
A: Methyldopa vs. distilled water. B: methyldopa vs blank C: sauerkraut vs. distilled water.

Standard curve for methyldopa

By following the previous optimal conditions, a standard curve was prepared for the determination of methyldopa in an aqueous solution. The concentration range of Beer's law was (1-30) µg/ml, and the molar absorbance of the product was 0.658 x 104 liters.mol-1.cm-1. The limit of detection was (LOD). 0.078 µg/ml, and the limit of quantification (LOQ) was 0.261 µg/ml. Figure (5) shows the standard curve for methyldopa

Study of Reagent quantity

This study was conducted to find out the effect of the amount of 4,2-dinitrophenylhydrazine reagent on the absorption of the formed product. At 428 nm, the absorbance of solutions and imitation solutions was compared. Since Table (5) indicates that 0.5 ml is the optimal volume for the reagent, this value was used in the following trials.

Table 5: study of the amount of reagent on the absorption of the product of the lag

X ml of 2,4- dinitro phenylhydrazine	0.25	0.5	0.75	1.0	1.25	1.5
Absorbance	0.267	0.322	0.278	0.219	0.187	0.122

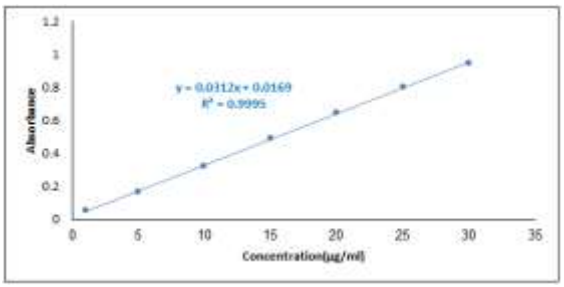


Fig. 5: Standard curve of the product

Method accuracy and compatibility

The accuracy and agreement of the method were calculated using five readings for three different concentrations of methyldopa, and the results in Table (6) show that the method is of high accuracy.

Table 6: Accuracy and compatibility of the method

Compound	Amount added (µg.ml^{-1})		Recovery* (%)	Average Recovery (%)	RSD* (%)
	Taken	Found			
Methyldopa	5	4.87	97.40	99.60	0.418
	15	15.16	101.06		0.322
	25	25.09	100.36		0.278

* Average of Five determinations

Studying the nature of the resulting product

To study the nature of the product formed from the reaction of methyldopa with 4,2-dinitrophenylhydrazine reagent, Jobe method and molar ratios method were followed

Continuous changes method (Job method)

Job's method[19] was applied to dilute solutions to find out the structural molar ratio. The total volume of the drug compound and the reagent was 1.5 ml with a concentration of 5 x 10^{-4} molar each with a final volume of 10 milliliters, and the results included in Figure (6) show that the ratio was 2:1 between methyldopa:2,4-Dinitrophenylhydrazine.

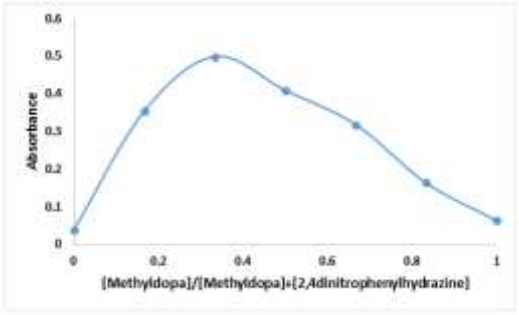


Fig. 6: Continuous variation Method

molar ratio method

To verify the validity of the results obtained from the continuous changes method, the molar ratio method[20] was resorted to, by adding increasing volumes (0-1.5) milliliters of 4.2-dinitrophenylhydrazine solution at a concentration of 5 x 10^{-4} molar. to a fixed volume (0.5 ml) of methyldopa solution at a concentration of 5 x 10^{-4} molar in a final volume of 10 ml. Figure (7) illustrates this.

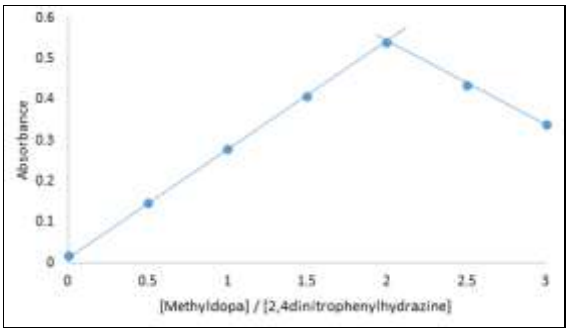


Fig. 7: The molar ratio method

Suggested chemical reaction

Methyldopa reacts with 2,4-dinitrophenylhydrazine reagent at a ratio of 2:1, leading to the formation of a yellow-colored product in the presence of potassium periodate as an oxidizing agent in an acidic medium. The following proposed mechanism shows this:

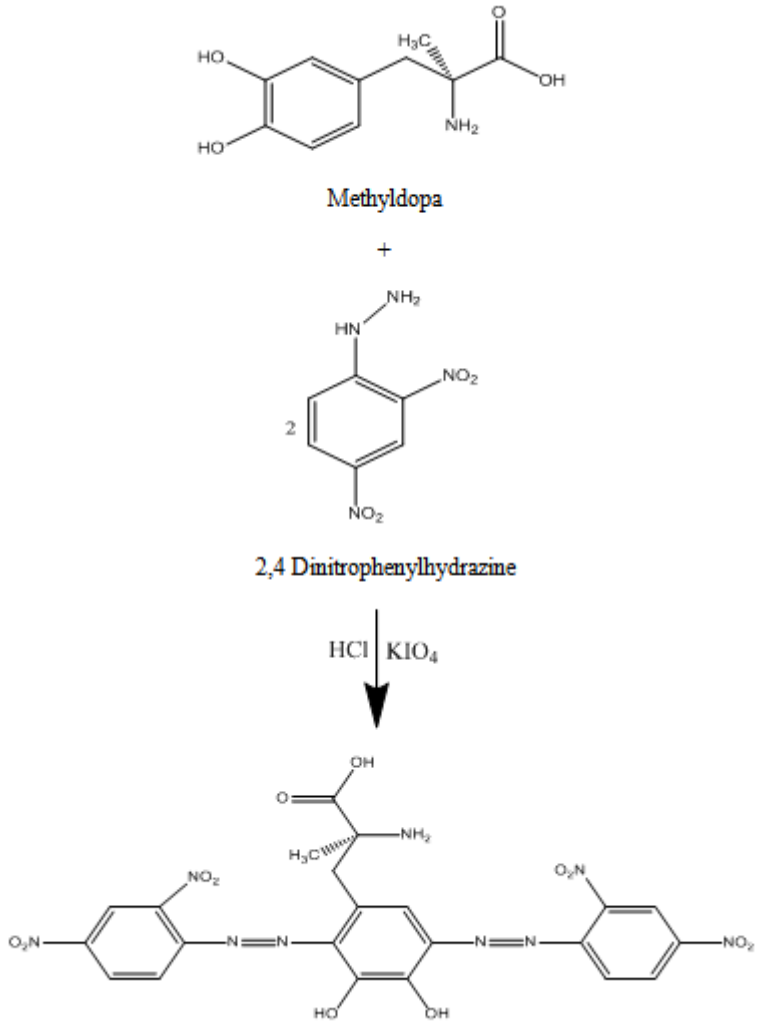


Fig. 8: The proposed reaction mechanism

Calculation of the* stability constant *of the resulting product

The stability constant of the product formed in a ratio of 2:1 between methyldopa and 4,2-dinitrophenylhydrazine was calculated and the results are shown in Table 7

Table 7: The stability constant of the output formed

Compound	Conc.(mol.l^{-1})	Absorbance		α	Average K_{st} (l^2.mol^{-2})
		As	Am		
Methyldopa	2.5×10^{-5}	0.059	0.163	0.638	8.84×10^{12}
	5.0×10^{-5}	0.219	0.322	0.319	
	7.5×10^{-5}	0.371	0.488	0.117	

Through the results, it was found that the stability constant is very high for the complex formed Application of the developed method to pharmaceutical reparations

Methyldopa Tablets Analysis. The concentration of methyldopa in the disc was found using the standard curve of methyldopa in its pure form, the results are listed in Table 8

Table 8: Determination of the drug compound in the pharmaceutical preparation

Pharmaceutical Preparation	Certified Value(mg)	Amount Present(µg/m)		Drug content found* µg/ml	Recovery (%)*	Average Recovery (%)
		Taken	Found			
Tablets	250	2.50	2.53	253.00	101.20	100.40
		5.00	4.97	248.50	99.40	
		10.00	10.06	251.50	100.60	

*Average of five determinations.

It can be concluded from the table that the proposed method for estimating methyldopa in its pharmaceutical preparation is of high accuracy and is in good agreement with the original content.

Evaluation of the results of the proposed method with the standard addition method

In order to prove the efficiency of the proposed spectroscopic method and its success in estimation, the standard addition method was applied to estimate methyldopa. It can be concluded from the results shown in Figure (9) and shown in Table (9) that the results obtained are in good agreement with the method. Good selectivity.

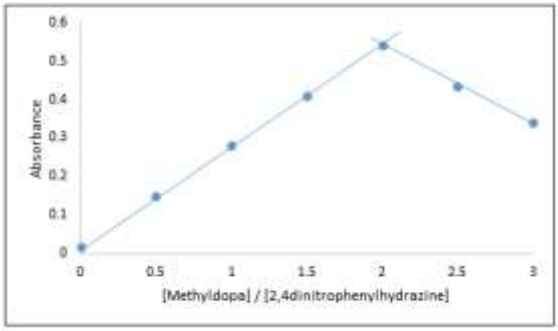


Fig. 9: Standard addition curve for the determination of methyldopa in tablets

Table 9: Determination of methyldopa by the standard and suggested methods of addition

Pharmaceutical Preparation	Certified Value(mg)	Amount Present (µg/ml)	Drug content found (mg)		Recovery (%) of standard Addition procedure
			Present method*	Standard Addition procedure	
Tablets	250	2.5	253.00	251.00	100.40
		5.0	248.50	250.50	100.20

Comparing the proposed method with another method

The present method of methyldopa was compared by oxidative coupling reaction using a 2,4-di nitrophenylhydrazine reagent with another spectrophotometric method, and the results of the comparison are included in Table 10.

Table 10: Comparison of the proposed method with other spectroscopic method

Analytical Parameters	Present method	Literature method [40]
Type of method	Oxidative coupling reaction	Oxidative coupling reaction
Reagent used	2,4-dinitro phenylhydrazine	3- amino pyridine
λ_{max} (nm)	428	476
Beer's law (µg/ml)	30-1	40-1
Molar absorptivity (l.mol^{-1}.cm^{-1})	0.658×10^4	0.230×10^4
Recovery (%)	99.6	100.4
RSD (%)	≤ 0.42	< 0.44

Conclusion

A rapid spectrophotometric method has been developed for the determination of microgram amounts of methyldopa using the 4,2-dinitrophenylhydrazine reagent at a wavelength of 428 nm based on the oxidative coupling reaction with good accuracy and agreement. The developed method is in good agreement with the standard addition method. The method was characterized by its simplicity and sensitivity, and it was carried out in an aqueous medium.

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التقدير الطيفي للمثيل دوبا بواسطة تفاعلات الاقتران التاكسدي باستخدام الكاشف 4,2- ثنائي نايترو فنيل هيدرازين

ضحى نوفل علي الغنام ، محمد سالم شيت العنزي

قسم الكيمياء ، كلية التربية للبنات ، جامعة الموصل ، الموصل ، العراق

الملخص

تم تطوير طريقة قياس الطيف الضوئي لتقدير المثيل دوبا . اذ تعتمد الطريقة على تفاعلات الاقتران التاكسدي مع 4,2 – ثنائي نايترو فنيل هيدرازين ككاشف وبوجود العامل المؤكسد بيرأيودات البوتاسيوم في الوسط الحامضي. ويظهر الناتج اقصى امتصاص عند الطول الموجي 428 نانوميتر والتراكيز التي تخضع لقانون بير هي من (1–30) مايكروغرام/مللتر وكانت الامتصاصية المولارية 6589.908 لتر.مول$^{-1}$.سم$^{-1}$ وبلغت قيمة حد الكشف (LOD) 0.078 مايكروغرام/مللتر وحد التقدير الكمي (LOQ) 0.261 مايكروغرام/مللتر وتراوح معدل نسبة الاسترجاع 99.60 % بانحراف قياسي نسبي ≤ 0.418 % . وتمت دراسة طبيعة الناتج المتكون من التفاعل بإتباع طريقتي التغيرات المستمرة والنسبة المولية وكانت النسبة 1:2 (المركب الدوائي: الكاشف) وبلغت قيمة معدل ثابت الاستقرار 8.84×10^{12} لتر^{2}. مول$^{-2}$ مما يدل على الاستقرارالجيد لهذا الناتج .وتم تطبيق هذه الطريقة بنجاح في تقدير المثيل دوبا بشكل أقراص دوائية .

الكلمات المفتاحية : اقتران تاكسدي ; قياس الطيف الضوئي ; 4,2 – ثنائي نايترو فنيل هيدرازين ; مثيل دوبا.