Article

Spectral Estimation Of Methyldopa By The Oxidative Coupling Method Using A Benzidine Reagent

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Abstract

The study included the development of a spectrophotometric method for assessing methyldopa (MDP) in pharmaceutical and pure forms. Based on an oxidative coupling reaction, this process entails oxidizing the medication using sodium periodate ($NaIO_4$) and combining it with a benzidine reagent in a diluted base medium. The outcome is a water-soluble, orange-colored compound with maximal absorption at 488 nm in wavelength. The concentration range of methyldopa that aligns with the beer law is 1.25–25 µg/mL , the molar absorption coefficient value is 5597.198 L. $mol^{-1}.cm^{-1}$,and the sandell's significance value, is 0.038 µg. cm^{-2} indicating the sensitivity of the method. The low relative standard deviation of 1.73% indicates that the approach exhibits good precision, the method's detection limit is found to be 0.26 µg/ml, indicating that it can accurately identify methyldopa at such low concentrations. The established technique has been effectively used to examine the presence of methyldopa in tablets. The method's accuracy in identifying the methyldopa content of real-world samples is demonstrated by the reported 100.33% recovery rate for methyldopa in these samples.

Key words: Methyldopa, benzidine, oxidative coupling, sodium periodate, spectrophotometric.

Introduction

Catechol amines are one of the groups of neurotransmitters called monoamines, which contain the nucleus of the catechol group represented by the benzene ring, and there are also two adjacent hydroxyl groups, in addition, a side chain of ethylamine with a single amine group, and there may be additional compensators. The tissue sources of catecholamines in the body. of the organism are mainly dependent on the presence of the enzyme(TH), which is significantly present in the dopamine-and norepinephrine-generating neurons of the central nervous system [1], the fibroblasts of the adrenal medulla and nerve nodes in the periphery that act as neurotransmitters for the processing of voluntary and involuntary information, which are produced mainly in the brain stem. Measuring the levels of catecholamine's and their receptors in biological fluids such as

urine, plasma, and serum is very important in clinical diagnosis [2], and catecholamine's are widely used in the treatment of high blood pressure, Parkinson's disease, and the treatment of bronchial asthma [3].

Methyldopa which is a derivative of catecholamine is widely used as an antihypertensive agent and is an agonist of Alpha-2 receptors with a central effect, which reduces sympathetic tone and results in a decrease in blood pressure; methyldopa is considered a primary medicine because it works mainly due to its metabolism in the central nervous system of methyl norepinephrine [4, 5] and it works by relaxing blood vessels so that blood flows more easily through the body. It is one of the preferred antihypertensive drugs during pregnancy; especially in complicated pregnancies and failures [6]. Methyldopa inhibits the enzyme aromatic amino acid decarboxylase DOPA decarboxylase and thus prevents the conversion of L-DOPA to dopamine, which is a precursor for the synthesis of epinephrine and norepinephrine [7].

The medical significance of methyldopa where the drug acts as an agonist via or through its active receptor α-methyl norepinephrine, where methyldopa is transported through the blood-brain barrier and the carboxyl group in it is extracted by (AADC) in the brain to α-Methyl dopamine and then the hydroxyl group is introduced stereo specifically into α-Methyl norepinephrine 1R- 2S-this stereoisomer acts as a blood pressure lowering agent, meaning that methyldopa works by stimulating alpha receptors in the brain, which leads to stimulation of the brain to send nerve signals to blood vessels, which helps them expand and as a result, blood pressure decreases [8].

Methyldopa is an organic compound characterized as a white or colourless crystalline powder, slightly soluble in alcohol, practically insoluble in ether, moderately soluble in water, and freely soluble in mineral acids [9].

Methyldopa, is chemically known as 3-(3,4-dihydroxyphenyl)-2-Methyl-L-alanine. Its structural formula appears as in the figure below [9]:

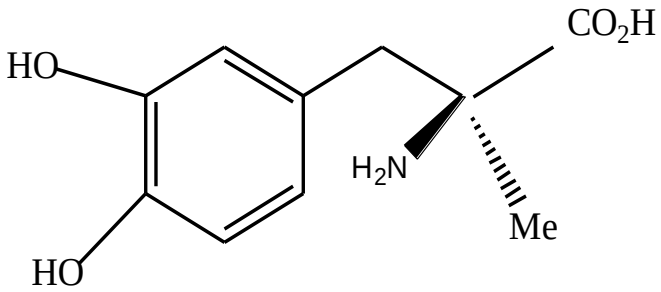


Figure 1. Structural formula of methyldopa

The molecular formula of the methyldopa is $C_{10}H_{13}NO_4$, and the molar mass is 211. 215 g / mole.

Various analytical methods have been used to analyses methyldopa in pharmaceutical preparations or biological samples; including the spectroscopic method; the methyldopa used in the treatment is a left-handed photo cycling (L-methyldopa) [10].

A range of analytical techniques have been used to assess the drug due to its medical significance in both the pharmaceutical industry and the medical field. These techniques include spectroscopic methods [11 –20], chromatographic procedures [21–24], flow injection methods [25–28], Electrochemical methods [29–33]. This study quantified this widely used medication using the oxidative coupling reaction, a novel, straightforward, and sensitive spectroscopic method. In order to obtain the best results required to develop methods with high sensitivity and accuracy, the study looked at the optimal circumstances for the chemical and physical variables that determine the course of the reaction.

Experimental section

Materials and Methods

Analytical materials and chemical reagents of high purity were used in this research. Methyldopa (99.8%, Factory of Samara Pharmaceuticals), benzidine (98%, BDH), sodium hydroxide(98.8%, BDH), sodium per iodate (99%, BDH).

Instrumentation

The following instruments were used for all spectrum measurements: a Shimadzu UV/vis 160 spectrophotometer (Japan),a water bath, a pH meter (WTW 720, Germany), a magnetic stirrer (BIOSAN MSH 300, Germany), and a sensitive balance Precisa (XR-205gm, Sweden).

Solution preparation

A standard methyldopa solution of 4.7345×10^{-3} M (1000 ppm) was prepared by dissolving 0.1000 g of pure methyldopa powder with distilled water in a 100 ml volumetric flask, and stored in an opaque bottle away from light. Dilution solutions were prepared by diluting with distilled water.

A 0.001 M benzidine reagent solution was prepared by dissolving 0.0184 g (M.w.t. = 184.24 g/mole) of the reagent in 10 ml of ethanol, then completing the volume to the mark in a 100 ml volumetric flask.

A 0.01 M sodium periodate solution was prepared by dissolving 0.230 g (M. wt = 230 g/mole) of oxidizing agent in an amount of distilled water and completing the volume to 100 ml with distilled water.

A 0.01 M NaOH solution was prepared by dissolving 0.04 g of sodium hydroxide in a quantity of water and completing the volume with distilled water to the 100 ml mark.

An HCl solution with an approximate concentration of 0.01 M was prepared by adding 0.085 ml of concentrated acid (11.8 M) to distilled water in a 100 ml volumetric flask and then the volume was completed to the mark.

Methyl dopa (250 mg), production of general company of pharmaceutical industry and medical supplies - Samarra - Iraq, each tablet containing 250 mg of MDP and the solution is prepared as follows:

Ten tablets, weighing 3.40 g, are well ground into a fine powder, then a weight of 0.0136 g of this powder is dissolved in an amount of distilled water, and the resulting solution is then filtered using paper filtration (604, RUNFILTER, Q240 mm), the volume is completed with distilled water in a volumetric flask of 100 ml , yielding the concentration of 100 µg/mL.

preliminary examinations

A simple and sensitive spectrophotometric method for the determination of methyldopa was developed by studying the optimal conditions for the oxidative coupling reaction. This method was observed to produce an orange-colored compound when a methyldopa solution was mixed with a benzidine solution in the presence of a sodium periodate solution in an acidic medium, with maximum absorption at 488 nm. In contrast, the blank solution did not show any absorption at the wavelength above, Figure 2.

Figure 2. Right, methydopa solution 100 µg/mL(orange color);
Left, blank solution (less color)

Results and discussion

Study of the optimum reaction conditions

In the following experiments, the effect of different conditions on the optical properties of the product formed by coupling methyldopa with the benzidine reagent was studied by taking 1 ml of sodium periodate solution (0.01 M), 1.5 ml of benzidine reagent solution (0.001 M), and 3 ml of methyl dopa (100 µg /ml) in a volumetric flask (20 ml), and the

absorbance of the resulting solutions was measured at 488 nm compared to the blank solution, and then the optimal conditions for the reaction were tested as follows:

The oxidizing agent's selection

A number of oxidizing agents, including sodium periodate, N-bromosuccinimide, potassium ferricyanide, ferric chloride, ammonium ceric sulphate dehydrate, and sodium nitroprusside were tested in order to determine which one would work best for creating the colored product. 1.0 ml of each of the aforementioned agents (0.01 M) was added to a series of volumetric bottles that contained 3.0 ml of methyldopa (100 µg /ml) in the presence of 1.5 ml of benzidine (0.001 M) and 1.0 ml of sodium hydroxide (0.01 M). As demonstrated by the data in Table 1, sodium periodate is the most efficient oxidizing agent and produces the largest absorption of the colored product at 556 nm when compared to the other oxidizing agents. For this reason, it was utilized in the subsequent studies.

Table 1. Choosing the best oxidizing agent to create the colored product

Oxidizing agent (0.01M)	λ_{max} (nm)	Absorbance
Sodium periodate	**488**	**0.485**
N- Bromosuccinimide	440	0.143
potassium ferricyanide	438	0.086
Ferric chloride	454	0.353
ammonium ceric sulphate dihydrate	425	0.079
Sodium nitroprusside	450	0.295

Choosing the coupling agent

The best reagent for producing the highest color intensity was determined by examining several coupling reagent types (0.001 M), including benizdine, N-(Naphthyl) ethylene diamine dihydrochloride, P-bromoaniline, 2,4-dinitro phenylhydrazine, sulphanilamide, and 4-amino-2-naphthol-4-sulphanilic acid. As demonstrated by the data in Table 2, the results indicated that benzidine produced the highest colour intensity and good colour contrast when compared to other reagents. Thus, in the trials that followed, this reagent was selected.

Table 2. Choosing the coupling reagent to create the colored

Coupling agent (0.001M)	λ_{max} (nm)	Absorbance
Benzidine	**488**	**0.485**
N-(Naphthyl) ethylene diamine dihydrochloride	446	0.174
P-bromoaniline	398	0.391
2,4-dinitro phenylhydrazine	351	0.067
Sulphanilamide	405	0.196
4-Amino-2-naphthol-4-sulphanilic acid	429	0.383

Influence of base type and investigate base volume effect

Experimental results showed that the reaction occurs exclusively in basic media. In order to determine the base type that provides the greatest absorption of the final product, 1.0 ml of different concentrations of strong and weak bases ranging from (0.01 – 1.0) M were taken to study the effect of base type.

Table 3. Base type influence on colored product absorption

1.0 ml of base	**Absorbance**			
	0.01 M	**0.1 M**	**0.5 M**	**1.0 M**
NaOH	**0.485**	0.462	0.354	0.167
KOH	0.387	0.348	0.253	0.234
NH_4OH	0.365	0.289	0.278	0.209

Table 3 showed that using 0.01 M sodium hydroxide produced the highest absorption, which is why it was used in the studies that followed.

A study was carried out to identify the perfect base volume that yields the maximum absorption of the final product, after the type of base and optimal concentration were determined. As a consequence, 2.0 mL is the optimal base volume, as indicated by the data in Table (4), it was utilized in the studies that followed.

Table 4. Base volume influence on colored product absorption

ml of 0.01M NaOH	Absorbance
0.0	0.034
0.5	0.205
1.0	0.485
1.5	0.516
2.0	**0.589**
2.5	0.467
3.0	0.442
3.5	0.373

Influence of concentration of benzidine solution

Initial solutions (stock solution) of the benzidine coupling reagent were prepared at different concentrations, and their effect on the absorption of the colored product was studied by taking a fixed volume of 1.5 mL of the prepared concentrations of the coupling reagent. The results obtained in Table (5) indicate that the concentration 3×10^{-3} M of benzidine solution is the most appropriate for estimation as it produced the maximum absorption of the product and was used in subsequent experiments.

Table 5. Influence of benzidine solution concentration

Molarity of benzidine	**Absorbance**
1×10^{-3}	0.139
2×10^{-3}	0.254
3×10^{-3}	**0.589**
4×10^{-3}	0.520
5×10^{-2}	0.478

A number of experiments were also conducted to find the ideal benzidine volume (0.5 - 4.5 mL) at a concentration of 3×10^{-3} M. Methyldopa assaying requires a volume of 2.5 mL since it produced the maximum absorption of the product, as Table (6) demonstrates.

Table 6. Influence of benzidine solution volume on the absorbance

Volume (mL) of 3×10^{-3} M benzidine	**Absorbance**
0.5	0.143
1.0	0.282
1.5	0.541
2.0	0.557
2.5	**0.598**
3.0	0.554
3.5	0.527
4.0	0.486
4.5	0.372

Influence of concentration of sodium periodate solution

The influence of increasing volumes (0.5 - 4.5 ml) of sodium periodate solution (1 x 10^{-2} M) on the absorption of the final product was tested, and from the data presented in Table (7) it can be concluded that the optimal volume is 2.0 ml. It was used in further studies because it gave the highest absorption.

Table 7. Influence of the sodium periodate solution volume on the absorbance

Volume (mL) of 1×10^{-2} M $NaIO_4$	**Absorbance**
0.5	0.447
1.0	0.579
1.5	0.599
2.0	**0.628**
2.5	0.561
3.0	0.537
3.5	0.486
4.0	0.439
4.5	0.412

Temperature's influence on the formation time of the final product and its stability

Table 8 displays the effects of varying temperatures (10 to 60°C) on the final product's sensitivity and stability . After 15 minutes of dilution and more than 60 minutes of stabilization, room temperature was found to be the optimal temperature for the coupling of methyldopa with benzidine in the presence of sodium periodate in a basic medium of sodium hydroxide. Because of this, absorbance was measured in the subsequent experiments after 15 minutes from the proper dilution.

Table 8. The impact of temperature on the duration of colour product development and its stability

Temp. (^{0}C)	Absorbance / min standing time									
	5	**10**	**15**	**20**	**25**	**30**	**40**	**50**	**60**	**70**
10	0.176	0.201	0.256	0.266	0.297	0.270	0.264	0.261	0.249	0.228
15	0.363	0.427	0.487	0.498	0.494	0.489	0.475	0.453	0.432	0.387
25	**0.545**	**0.597**	**0.628**	**0.629**	**0.631**	**0.628**	**0.628**	**0.629**	**0.630**	**0.629**
35	0.421	0.438	0.456	0.473	0.495	0.511	0.485	0.467	0.449	0.439
50	0.328	0.322	0.319	0.307	0.304	0.301	0.278	0.253	0.236	0.215
60	0.301	0.327	0.346	0.335	0.318	0.307	0.289	0.267	0.243	0.229

Sequence addition's influence

The sequences indicated in Table (9) were run in order to determine the optimal order for adding the reactants. The subsequent experiments used the following arrangement (IV), which was determined by the recorded results to be the most effective in forming a colored product with maximum absorption: methyldopa solution (D) + sodium periodate (O) + benzidine (R) + sodium hydroxide (B).

Table 9. Sequence addition's influence

Order number	Reaction Components	Absorbance
I	D + R + B + O	0.484
II	R + B + D + O	0.471
III	D + R + O + B	0.509
IV	**D + O + R + B**	**0.628**
V	B + O + R + D	0.426
VI	R + O + B + D	0.353
VII	D + O + B + R	0.431

Influence of a solvent

The influence of various solvents on the absorption of the colourful reaction product arising from the reaction of methyldopa and benzidine was investigated in sodium hydroxide medium containing potassium periodate as an oxidizing agent. In this study, 20 ml volumetric flask were employed. Following the completion of the additions under ideal circumstances, several organic solvents were added to the appropriate volume. Each solution's absorption spectra were then measured against a blank solution. The outcomes are displayed in Table 10.

Table 10. Influence of a solvent

Solvent	λ_{max}. nm	Absorbance
Ethanol	468	0.582
Acetone	397	0.157
Dimethyl sulphoxide	452	0.321
Water	**488**	0.628
n-propanol	495	0.045

The data displayed in Table 10 demonstrate that water is an inexpensive, readily available, and effective medium for the reaction. It also exhibits the maximum absorption at a wavelength of 488 nm.

Final spectrum of absorption

After achieving the best circumstances: 2.5 mL of 0.001 M benzidine reagent solution, 3.0 mL of 100 µg/ml methyldopa solution, 2.0 mL of 0.01 M sodium periodate as an oxidizing agent solution and 2.0 mL of 0.01 M sodium hydroxide solution (pH 9.3) at room temperature, it was left for 15 minutes to complete the reaction, then add enough distilled water to a 20 mL volumetric flask to fill it to the proper level. After diluting the solution and allowing it to sit for a further 15 minutes to complete the reaction, the orange product's final absorption spectrum was measured against of the blank solution.

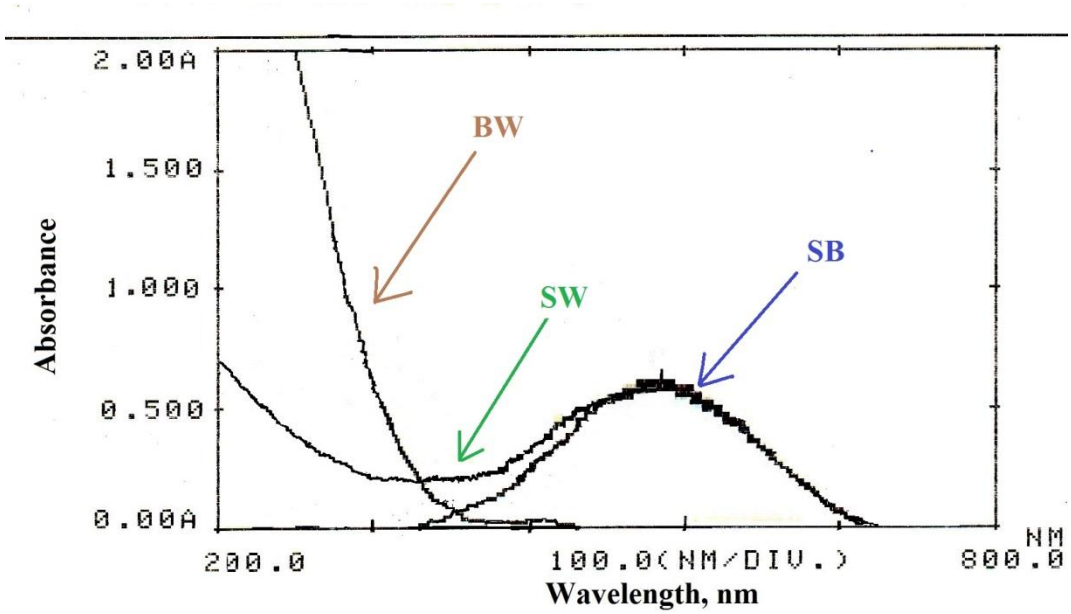


Figure 3. Methyldopa absorption spectra processed using the suggested method: (SB) Sample compared to blank solution , (SW) Sample compared to Distilled water , and (BW) blank compared to Distilled water

Standard curve for the computation of methyldopa

Following the establishment of the best conditions for methyldopa quantitation, the following standard curve was created:

Increasing amounts (0.25 - 5.5 mL) of 100 µg/mL methyldopa solution (1.25 - 27.5 µg/mL), 2.0 M of 0.01 M sodium periodate solution, 2.5 mL of 0.001 M benzidine solution, and 2.0 mL of 0.01 M sodium hydroxide solution (pH 9) were added to a set of 20 mL volumetric flasks. After the colored product was diluted to the appropriate level with distilled water and left for 15 minutes, its absorbance was measured at a wavelength of 488 nm in comparison to the blank solution. The standard curve for methyldopa determination is displayed in Figure 4, where Beer's law is applicable across a wide range from (1.25 – 25 µg/mL), and it deviates negatively from the expected upper limits. The standard curve's correlation coefficient value was more than 0.9951, indicating statistically that its linear specifications are excellent. After computation, the molar absorbance was discovered to be 5597.198 L. $mol^{-1}.cm^{-1}$. The method's great sensitivity is indicated by the sandell's significance value of 0.038 $\mu g.cm^{-2}$.

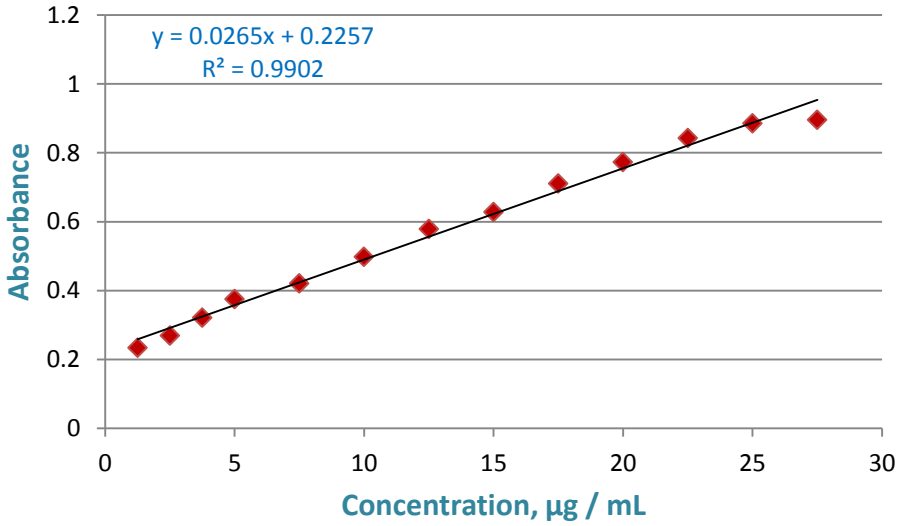


Figure 4. Standard curve for measuring methyldopa

The limits of detection (LOD) of methyldopa were calculated by the developed method following the steps described below:

Ten solutions were prepared from the blank solution (according to the conditions of the standard curve) and their absorbance was measured at 488 nm, then their arithmetic average X_B was found through the following mathematical relationship:

$$\overline{X}B = \frac{\Sigma Xi}{n}$$

∑ Xi = total reads, n = number of reads

The standard deviation σ_B is found by the following formula:

$$\sigma_B = \sqrt{\frac{\sum\left(Xi - \overline{X}\right)^2}{n-1}}$$

Then, the LOD detection limits were calculated by applying the following mathematical relationship:

$$C_{LOD} = \frac{3\sigma_B}{S}$$

Where S = slope of the standard curve.

Table 11. computations to determine the suggested method's detection and quantification limits

Absorbance of blank (xi)	$\left(Xi - \overline{X}\right)$	$\left(Xi - \overline{X}\right)^2$
0.058	0.0021	4.41×10^{-6}
0.057	0.0011	1.21×10^{-6}
0.055	- 0.0009	0.81×10^{-6}
0.058	0.0021	4.41×10^{-6}
0.056	0.0001	0.01×10^{-6}
0.056	0.0001	0.01×10^{-6}
0.053	- 0.0029	8.41×10^{-6}
0.051	- 0.0049	24.01×10^{-6}
0.058	0.0021	4.41×10^{-6}
0.056	0.0001	0.01×10^{-6}
∑ Xi = 0.559		**∑= 47.7 × 10^{-6}**

$$\overline{X}_B = \frac{\sum Xi}{n} = \frac{0.559}{10} = 0.0559 \quad ; \quad \sigma_B = \sqrt{\frac{47.7\times10^{-6}}{10-1}}$$

$$C_{LOD} = \frac{3x0.0023}{0.0265} = 0.26ug/mL$$ **0.26 µg / m**

Similarly, the limit of quantification (LOQ) was determined by utilizing the following relationship:

$$C_{LOQ} = \frac{10\sigma_B}{S}$$

$$C_{LOQ} = \frac{10X\,0.0023}{0.0265} = 0.8679\ \mu g / mL$$

The method's accuracy and compatibility

The accuracy and compatibility of the method were assessed under the ideal circumstances outlined in the previously mentioned procedure. The recovery percentage and the relative standard deviation were calculated to assess the method's accuracy and compatibility for four different concentrations of methyldopa: 2.5, 5, 15, and 25 µg/mL. The results obtained are summarized in Table (12), which demonstrates that the method has good accuracy (recovery rate 100.75%) and good agreement (RSD less than 3.5%), as shown in the following laws:

$$\text{Rec.} = \frac{X_i}{u} \times 100$$

whereas:

Xi = analytical value.

u = true value.

To determine the relative standard deviation as a percentage, the following law is utilized:

$$\text{RSD} = \frac{S}{\overline{X}} \times 100$$

whereas:

S = standard deviation

$\overline{X}$ = rate of reads

Table 12. Accuracy and compatibility of the method for the determination of methyldopa

Concentration of methyldopa (µg / ml)		**Recovery*(%)**	**RSD* (%)**
Present	**Found**		
2.5	2.47	98.80	3.21
5	5.08	101.60	2.59
15	15.11	100.73	2.18
25	24.79	99.16	1.73

***Average of six determinations**

Calculate the compositional proportion of the colored product and its stability constant in solutions using the molar ratio and continuous variation methods.

In the basic medium of sodium hydroxide solution (pH9), the molar compositional ratio of the colored product generated between methyldopa and benzidine in the presence of sodium periodate was examined using the molar ratio and the continuous variation(Job's method) methods. As observed in Figure (5), the Job's method is used to calculate the ratio of methyldopa to benzidine. The absorbance was calculated for solutions containing 0.1-0.9 ml of methyldopa and 0.9-0.1 ml of benzidine at a concentration of (4.73×10^{-4}) for each. The coupling ratio of the methyldopa with benzidine was found to be 1:1 when the absorbance was measured at a wavelength of 488 nm using the approved method against the blank solution.

This ratio was verified using the molar ratio method. By using this procedure, the volume of 2 ml of methyldopa (4.73×10^{-4}) M was fixed, and the volume of the reagent was adjusted to (0.3 – 5.5) ml with a concentration of (3.56×10^{-4}) M. As seen in Figure 6, the final product is a 1:1 molar ratio coupling of methyldopa and benzidine.

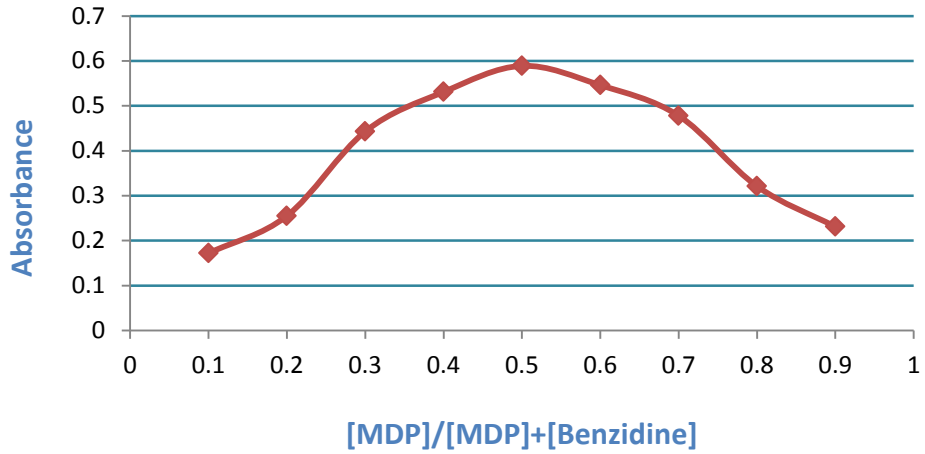


Figure 5. Job's method diagram showing the chemical coupling of methyldopa – benzidine reagent

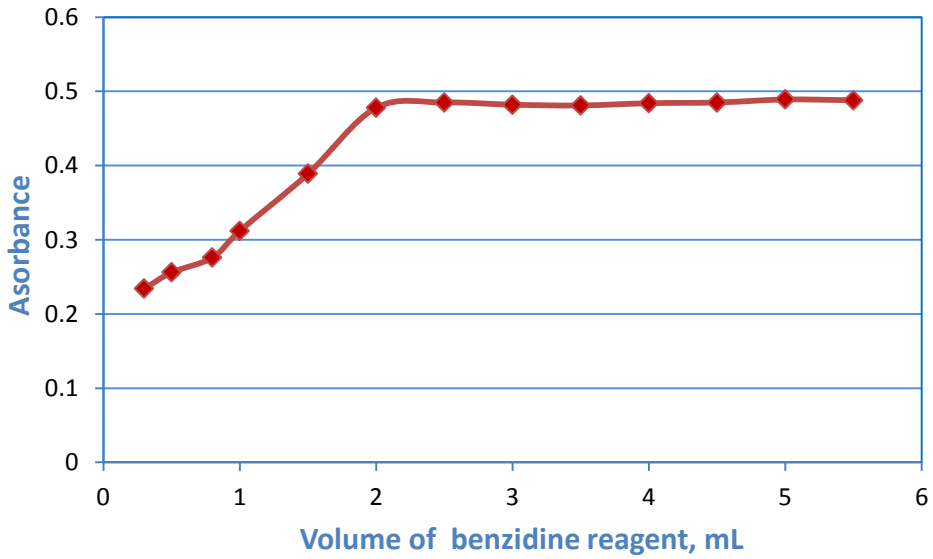


Figure 6. Molar- ratio diagram showing the chemical coupling of methyldopa – benzidine reagent

To determine the stability constant for the MDP reaction product with benzidine reagent, the molar ratio method's results are employed is one way to express the reaction (126,127).

$$\underset{\alpha c}{M} + \underset{\alpha c}{L} \longrightarrow \underset{(1-\alpha)\,c}{ML}$$

The stability constant can be written as follows:

$$K=\frac{[ML]}{[M][L]} = \frac{[C\,(1-\alpha)]}{[\alpha\,C][\alpha\,C]}$$

If it is:

α = is the degree of dissociation.

C = is the concentration of the coupling product.

The aforementioned equation can be written as follows:

$$K =\frac{[C\,(1-\alpha)]}{\alpha^2 C^2} \longrightarrow K = \frac{1-\alpha}{\alpha^2 C}$$

The values of α can be determined from the following equation:

$$\alpha = \frac{Am - As}{Am}$$

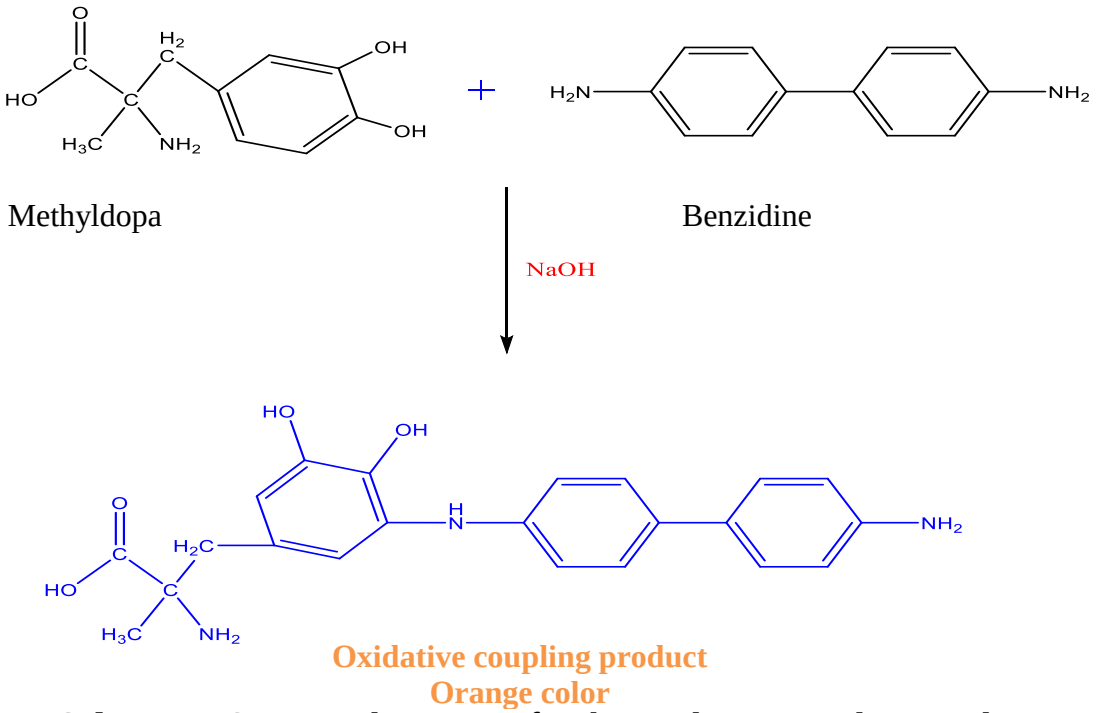
whereas:

Am = is the highest absorption value.

As = absorption value at the equivalency point (with the ratio of product 1: 1).

The stability constant is calculated for the value of AS (0.488) when the volume of both the drug and reagent is 2.0 ml of 4.73×10^{-4} M, whereas the value of Am (0.478) is obtained at a volume of 5.5 ml for the excess reagent. The colourful product MDP-benzidine has a stability constant value of 4.93×10^{6} L/mole under optimal experimental circumstances, showing good stability.

An oxidative coupling product is produced when MDP reacts with the benzidine reagent in the presence of sodium periodate, as indicated in the suggested equation in Scheme 1.



Scheme 1. Suggested equation for the oxidative coupling product

Examination of the influence of excipients

The influence of various amounts of foreign compounds was investigated in order to ensure the method's selectivity and its application in routine analyses on various samples, particularly pharmaceutical preparations. This was achieved by adding an increase of each of these substances separately to 100 µg / 20 mL of methyldopa while adhering to the ideal conditions for estimation. The outcomes were listed in Table (13).

Table 13. Influence of excipients on the determination of methyldopa

Excipient	**Recovery (%) of 100µg Methyldopa per µg foreign compound added**				
	100	**200**	**300**	**400**	**500**
Glucose	99.3	97.87	98.7	101.28	100.32
Lactose	97.8	96.05	99.38	99.7	100.12
Sucrose	100.9	100.4	101.5	99.8	100.18
Acacia	98.72	99.37	98.42	99.60	101.20
Starch	100.40	99.81	100.31	99.87	99.88
Sodium bicarbonate	98.36	99.87	97.78	101.05	99.67

As can be seen from the above-mentioned table that there was no interference with the substances that were anticipated to be present in pharmaceutical preparations together with the drug. Implementation of the proposed method for evaluating methyldopa in pharmaceutical formulations by analyzing methyldopa tablets by the direct method.

Using a 20 mL volumetric flask, various volumes (0.25, 0.5, and 0.75 mL) of the pharmaceutical formulation solution (100 µg/mL) are transferred in this procedure. Next, the concentrations that result (1.25, 2.5, 3.75 and 5.0 µg/mL) are handled in the same way as in the calibration curve design. Six readings are averaged for each concentration after the absorbance is measured at 488 nm. Following computations, the recovery and RSD are displayed in the table (14).

Table 14. Results of the direct method for determining the amount of methyldopa in tablet solution

Pharmaceutical preparation	**Certified value (mg)**	**Amount present (µg/ ml)**	**Recovery (%)**	**RE%**	**Drug content found (mg)**
Methyldopa tablets	250	1.25	100.41	2.37	251.025
		2.5	99.21	1.19	248.025
		3.75	101.09	3.06	252.725
		5	100.60	1.49	251.500

It can be concluded from the results recorded in Table 14 that the average recovery percentage for the analysis of methyldopa in tablets solution was 100.33% ,which indicates that the method has good efficiency and accuracy in estimation.

The proposed method was compared with the method used in the literature as shown in Table 15.

Table 15. The comparison of the suggested method with the literature's method

Analytical parameter	Literature method[34]	Present method
Reagent	chloro-7-nitrobenzo-2-oxa-1, 3--4 diazole	Binzidine
Beers law range µg.ml^{-1}	1.6 – 17.6	1.25 - 25
Solvent	Water	Water
Molar absorptivity (l.mol^{-1}.cm^{-1})	1.9337×10^{4}	5597.198
λ_{max} (nm)	470	488
Average recovery(%)	100.11	100.07
RSD(%)	0.033	1.37
Temperature	RT	RT
LOD	5.536×10^{-3}	0.2600
LOQ	-------	0.8679
Colour of the dye	Brown	0range
Sandel Index µg.cm^{-2}	----	0.038
Pharmaceutical Preparation	Tablet	Tablet

Conclusions

In conclusion, The analytical process's outcomes validated that the suggested approach is simple, quick, appropriate, and highly sensitive for determining methyldopa (MDP) in pure and pharmaceutical formulations.

According to the study's findings, the absorption of the MDP concentrations under investigation (1.25–27.5 µg/mL) follows Beer's law, with a correlation coefficient of 0.9902. The molar absorbance and sandell's significance values were, respectively, 5597.189 L. mol^{-1}.cm^{-1}and 0.038 µg. cm^{-2}. Furthermore, it was found that, depending on the concentration under investigation, the relative standard deviation (RSD) value varied from 1.73 to 3.21%.

Unlike most methyldopa assay methods, which call for expensive equipment's or meticulously regulated procedures, the present method satisfies all the primary

requirements for routine analysis since it is reliable, does not call for particular working circumstances, such as the use of organic solvents or controlling of temperature. Because the final product is stable, soluble in water, and yields a bright orange colour, making this process straightforward to employ.

It is important to note that the use of benzidine as a novel coupling reagent with the medication employing an oxidizing agent and the creation of a colored product absorbed at wavelength 488 nm distinguish this study from earlier research. This reagent has never been used in conjunction with this medication as a coupling reagent. When applied to solid medications, this technology produced positive outcomes.

References

[1] Lelou , E. Corlu , A., Nesseler , N., Rauch , C., Mallédant ,Y., Seguin , Ph. & Aninat, C. (2022). The Role of Catecholamines in Pathophysiological Liver Processes. Cells 11.6,1021.

[2] Brady, S., Siegel, G., Albers, R. W. & Price., D. L. (2011). Basic Neurochemistry: Principles of Molecular, Cellular and Medical Neurobiology. 8th Edition.

[3] Goldstein, D. S. (2010). Catecholamines 101. Clinical Autonomic Research, 20(6), 331-352.

[4] Hashem, E. Y., & Youssef A. K. (2013). Spectrophotometric determination of norepinephrine with sodium iodate and determination of its acidity constants. Journal of Applied Spectroscopy, 80, 258-264.

[5] Easterling, T., Mundle, S., Bracken, H., Parvekar, S., Mool, S., Magee. L.A., von Dadelszen, P., Shochet, T.& Winikoff, B. (2019). Oral antihypertensive regimens (nifedipine retard, labetalol, and methyldopa) for management of severe hypertension in pregnancy: an open-label, randomised controlled trial. The Lancet, 394(10203), 1011-1021.

[6] Fouladgar, M. & Karimi-Maleh, H. (2012). Ionic liquid / multiwall carbon nanotubes paste electrode for square wave voltammetric determination of methyldopa. Ionics, 19(8), 1163 - 1170.

[7] Shihab, A. I. & Al-Sabha, Th. N.(2020). Application of cloud point method for spectrophotometric determination of Salbutamol sulphate and Methyldopa. Pak. J. Anal. Environ. Chem, 21(1), 10-18.

[8] El-sadek, Sh. E. El-sadek, A. M. & Ahmed, A.K. (2021). Labetalol versus Alpha Methyldopa for Control of Pregnancy Induced Hypertension. Al-Azhar International Medical Journal, 2(6), 23-28.

[9] British Pharmacopeia, (2009). 6th Ed., by system simulation ltd., thestationary office, London, in CD-ROM".

[10] Rezk, M., Emarh, M., Masood, A., Dawood, R., El-Shamy, E., Gamal, A.& Badr , H. (2020). Methyldopa versus labetalol or no medication for treatment of mild and moderate chronic hypertension during pregnancy: a randomized clinical trial." Hypertension in Pregnancy, 39(4) , 393-398.

[11] Fathi, S.A., Othman, N.S. & AL-Taee, A.Th. (2023). Indirect Spectrophotometric Method for Determination of Methyldopa in Pure and Pharmaceutical Formulation. Biomedicine and Chemical Sciences, 2(2).

[12] Fadhel, S.R. & Kaddouri, R.M. (2023). A New Visible Spectrophotometric Approach for Determination of Methyldopa in Pharmaceuticals. Baghdad Science Journal, DOI:10.21123/bsj.2023.8558.

[13] Alaallah, N.J., Dhahir, S.A. & Ali, H.H. (2020). Spectrophotometric evaluation of methyldopa in pure and pharmaceutical formulation using Ecological-friendly Method. Materials Science and Engineering, 1- 10, doi:10.1088/1757-899X/871/1/012033.

[14] Shakkor S.J., Mohammed, N. & Shakor, S.R. (2022). Spectrophotometric Method for Determination of Methyldopa in Bure and Pharmaceutical Formulation Based on Oxidative Coupling Reaction. Chemical Methodologies, 6(11), 851-860.

[15] Ghaib Allah, N. M., Ahmed, A. M. K. & Tapabashi, N.O. . (2022). Spectrophotometric Determination of Methyldopa in Pure and Pharmaceutical Preparations by the Oxidative Coupling Reaction with 1, 5-Diaminonaphthalene in the Presence of Ammonium Ceric (IV) Nitrate. Kirkuk Univ. J. Sci. Stud, 17(4), 41-48.

[16] Humeidy, I.T., Salman, S.A. & Hashim, K.K. (2020). SPECTROPHOTOMETRIC DETERMINATION OF METHYLDOPA WITH 2, 6-DIAMINOPYRIDINE REAGENT USING OXIDATIVE COUPLING REACTION. Journal of Engineering Science and Technology,15(3), 1824- 1839

[17] Abood, N.K., Hassan, M. J.M. & AL-Da,amy, M.A. (2019). Spectrophotometric Determination Methyldopa and Salbutamol by Oxidative Coupling, Cloud Point and Flow Injection in Pharmaceutical Formulations. International Journal of Drug Delivery Technology, 9(2), 182-192

[18] AL-ghanam, D. N. A., & AL-Enizzi, M. S. S. (2022). Spectrophotometric determination of methyldopa by oxidative coupling reactions using 2, 4-dinitrophenylhydrazine reagent. Tikrit Journal of Pure Science, 27(5), 16-22.

[19] Al Samarrai ,K.F., Al Samarrai, E. Th .A. & Al Samarrai, B. A. (2019). Spectrophotometric determination of methyldopa in pharmaceutical preparation via ion pair formation. International Journal of Research in Pharmaceutical Sciences, 10(2).

[20] Salman, S.D. & Khalaf, H. S. (2024). Spectrophotometric Estimation of Methyldopa Drug in pure and pharmaceutical formulations. Egyptian Journal of Chemistry, Doi: 10.21608/EJCHEM.2024.248561.8873.

[21] Gonçalves, M.S., Armstrong, D.W., Cabral, L.M., Pinto, E. C. & Sousa, V.P. (2021). Development and validation of a fast HPLC method for methyldopa enantiomers using superficially porous particle based macrocyclic glycopeptide stationary phase. Microchemical Journal, 164, 105957.

[22] Emara, S., Masujima ,T., Zarad,W., Kamal,M., Fouad, M. & El-Bagary ,R. (2015). An Eco-Friendly Direct Injection HPLC Method for Methyldopa Determination in Serum by Mixed-Mode Chromatography Using a Single Protein-Coated Column. J Chromatogr Sci, 53(8),1353-1360.

[23] Jin-qi, Z.H.E.N.G. , Ju-zhen, J.I.N, Liang-ping, C.H.E.N. & Hui-lin, L.I. (2010). HPLC determination of content of methyldopa and its related substances. Chinese Journal of Pharmaceutical Analysis, 30(8),1440 – 1444(5).

[24] Kharnoob, H.H. (2015). Developments of HPLC Method for Determination of MethylDopa. International Journal of Scientific Research in Science, Engineering and Technology, (1)6, 22-28.

[25] Al-Abachi,M.Q., Al-Uzri, W.A. & Abed, S.S. (2013). Batch and Flow-Injection Spectrophotometric Determination of Methyldopa Using Metochlopramide as diazotized Chromogenic Reagent. Iraqi National Journal of Chemistry, 49, 12–24.

[26] Al-Abachi, M.Q., Sinan, R. & Haddi, H. (2009). Spectrophotometric Determination of Methyldopa and Dopamine Hydrochloride in Pharmaceutical Preparations Using Flow Injection Analysis. National Journal of Chemistry, 36, 597- 604.

[27] Turkey , N.S. & Mezaal, E.N. (2020). Continuous Flow Injection Analysis,Turbidmetric and Photometric Determination of Methyldopa Using a New Long Distance Chasing Photometer (NAG-ADF-300-2). Indian Journal of Forensic Medicine & Toxicology, 14(4), DOI: https://doi.org/10.37506/ijfmt.v14i4.

[28] Tubino, M., Junior, A.R. & Vila, M. M. D. C (2004). A Simple Flow Injection Spectrophotometric Method for the Determination of -Methyldopa in Pharmaceutical Preparations. J. Flow Injection Anal., 21(2), 132 – 136.

[29] Sanati, A.L. & Faridbod, F. (2017). Electrochemical Determination of Methyldopa by Graphene Quantum Dot / 1-butyl-3-methylimidazolium hexafluoro phosphate Nanocomposite Electrode. International Journal of Electrochemical Science, 12(9), 7997-8005.

[30] Halakoei, H., Ghalkhani, M., Sobhani-Nasab, A. & Rahimi-Nasrabadi, M. (2021). An efficient electrochemical sensor based on CeVO4-CuWO4 nanocomposite for methyldopa. Mater Res Exp 8:085001

[31] Upan, J., Themsirimongkon, S., Saipanya, S. & Jakmunee, J. (2019). Gold Nanoparticles Decorated on Carbon NanotubeModified Screen-printed Electrode for Flow Injection Amperometric Determination of Methyldopa. Chiang Mai J. Sci., 46(3) , 537-546.

[32] Imani, R., Shabani-Nooshabadi, M. & Neda Ziaie, N. (2022). Fabrication of a sensitive sensor for electrochemical detection of diltiazem in presence of methyldopa. Chemosphere, 297, 134170.

[33] Erdogdu, G., Yagcl, S.Z. & Savan, E.K. (2019). Investigation of the Voltammetric Behavior of Methyldopa at a Poly (p-Aminobenzene Sulfonic Acid) Modified Senso. Turk J Pharm Sci, 16(4),450-456.

[34] Rashid Q.N., Bakir M.H., Baban Sh.O. 2016, Spectrophotometric determination of Methyldopa in pure form and in the pharmaceutical preparations. Tikrit Journal of Pharmaceutical Sciences, 11(1): 67-77