Article

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New Sensitive Spectrophotometric Method for Methyldopa Determination in Different Pharmaceutical Samples

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Abstract

Methyldopa (M. dopa) drug concentration in pharmaceutical and pure forms is determined using a simple spectrophotometric method. In this work, the medication is first oxidized using an excess of N-bromosuccinimide (NBS), and then it is reduced with 3,3-diaminobenzidine (DAB). The maximum absorption wavelength of the compound (λmax = 513 nm). Beer's law is obeyed in the concentration range of 0.5 – 10 µg mL^{-1} (R^2 = 0.9988) for determination of M. dopa. The conditions for compound formation were studied and optimized to obtain the highest absorbance available. the detection limits (LOD) determined and was 0.171 µg. mL^{-1}. The limits of quantities (LOQ) study and was 0.571 µg. mL^{-1}, Sandell sensitivity (S) was 0.008μ g cm $^{-2}$ with a molar absorptivity of 25.87×10^3 L mol^{-1} cm^{-1} and (recovery% 100.780 to 114.620). Common excipients did not appear to cause any interference in the formulations. The findings demonstrate an easy-to-use, quick, accurate, and accurate method for determining the amount of methyldopa in pharmaceutical items. The suggested technique was effectively

used to identify the medication in their pharmaceutical compositions.

Keywords: Spectrophotometry, Methyl dopa (M. dopa), N-Bromosuccinimide (NBS), 3,3Diaminobenzidine (DAB).

1. Introduction

Methyldopa (M. dopa) is a derivative of catecholamine and is highly recommended for managing hypertensive conditions. It is widely favored as an antihypertensive medication during pregnancy, especially in cases of complicated pregnancies and renal issues. Its effectiveness lies in its ability to maintain renal blood flow without impacting uterine and placental circulations [1,2] chemically, Figure 1 illustrates 3-hydroxy-a-methyl-L-tyrosine sesquihydrate, known as Methyldopa. Its main antihypertensive action occurs through its interaction with alpha-adrenoreceptors in the lower brain stem, where it tricks these receptors into preferring its metabolite, methyl-norepinephrine, over norepinephrine. As a result, neurotransmitter stimulation decreases, leading to reduced nervous sympathetic activity and consequently lowering blood pressure [3]. Methyldopa is available as colorless or nearly colorless crystals or as a white to yellowish-white fine powder; It's almost impeccably tasteless [4]. The pH of its saturated aqueous solution is around 5.0 when it is in its sesquihydrate state. Water, ethanol, and isopropanol are soluble in methyldopa [5]. When used alongside a diuretic, Methyldopa proves to be a potent antihypertensive medication. It functions as a prodrug, undergoing metabolism to produce an active metabolite known as α-methylnorepinephrine. This metabolite acts as an agonist for α2-adrenergic receptors in the brainstem, which in turn reduces the transmission of vasoconstrictor signals to the peripheral sympathetic nervous system, thereby contributing to its antihypertensive effects [6]. Various techniques have been suggested for quantifying methyldopa in pharmaceutical products. These techniques include flow injection analysis (FIA) [17,18], kinetic measurements [19,20], anodic voltammetry [21], chemiluminescence [22,23], differential pulse polarography [8], titrimetric assays [9], UV spectrophotometry [10], visible spectrophotometry [11–16],

and high-performance liquid chromatography (HPLC) with UV detection [7, 8]. This work presents a spectrophotometric method that is easy to use, sensitive, fast, specific, and affordable for determining the amount of M. dopa present in pharmaceutical dosage formulations and bulk materials.

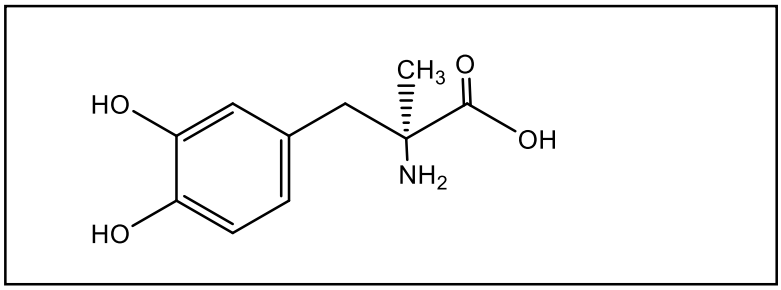

Figure 1: Structure of Methyl dopa.

2. Materials and Methods

2.1 Apparatus

The devices employed in this study included the "Biochrome Libra S60" double-beam spectrophotometer for absorbance measurements, the "Oakton 2100 Series pH/mV/Ion/0C/0F" Meter for pH value measurements, and the "Ohaus PA214 Pioneer Analytical Balance" for sample weighing. The wavelength maximum was determined using the "Shimadzu UV-1700" spectrophotometer. HPLC, along with ethanol and water as solvents, were utilized as techniques to characterize the reagent and Methyldopa compound in this investigation.

2.2 Chemicals

All chemicals utilized in this study were of analytical grade and were employed without additional purification. N-bromosuccinimide (NBS) with a purity of 99.9% was obtained from London, UK, while 3,3 Diaminobenzidine (DAB) with a purity of at least 98% was sourced from the German company Sigma-Chemicals. Methyldopa is a medicinal medicine that was purchased from the Samara, Iraqi market. It has a purity of 98%. Ethanol and distilled water were utilized to produce the solutions used in this study.

2.3 The Methyldopa Pharmaceutical Preparations [24]

Samarra Pharmaceuticals Corporation (S D I-IRAQ) provided the 250 mg tablets for each of the five samples under study, which comprised the methyldopa-containing pharmaceutical formulation: methyldopa, Aldosam, Aldomet, methyldopa Safa, and methyldopa Moroccan. To prepare the tablet solution, 10 tablets weighing 4.38 grams were ground, and 0.0526 grams of this mixture were dissolved in a small amount of hot water in a 100 mL volumetric flask. The solution was filtered and then repeatedly washed with hot distilled water to get rid of any leftover material. A solution with a concentration of 1000 µg/mL was then obtained by adjusting the filter volume with distilled water. A 100 mL volumetric flask was then filled with 30 mL of this solution, which was then diluted with distilled water to achieve a concentration of 300 µg/mL.

2.4 Preparation of reagent

While DAB was synthesized by dissolving it in 100% ethanol, NBS was prepared by dissolving it in water at a low temperature while stirring continuously.

2.5 The Preparation of the solutions for standard stocks.

A solution of M. dopa drug at a concentration of 100 mg/L was prepared by dissolving 0.01 grams of M. dopa in 100 mL of distilled water to create a stock solution. Working solutions were then prepared through additional dilution. The M. dopa standard used was procured from Samara, Iraq. A solution of NBS at a concentration of 1×10-2 mol/L was prepared by dissolving 0.0889 grams of the reagent in 50 mL of water.

A solution of DAB at a concentration of 1×10-3 mol/L was prepared by dissolving 0.0107 grams of the reagent in 50 mL of ethanol to create a stock solution. Further dilutions of the reagent were made as needed.

2.6 Interference solutions

All of the following interference samples were synthesized at a concentration of 100 mg/L: " Ascorbic acid, glucose, starch, $ZnCl_2$, $MgCl_2$, $CaCl_2.2H_2O$, and $AlCl_3.6H_2O$" in precise quantities (0.01, 0.01, 0.01, 0.0208, 0.0391, 0.367, and 0.0894 g), respectively,

were dissolved in distilled water that had been pH-corrected. The volume was then completed to 100 mL to obtain Ascorbic acid, Glucose, Starch, Zn^{+2}, Mg^{+2}, Ca^{+2}, and Al^{+3} ions as interference sources. Each interference ion was tested at two concentrations: 5 mg/L for the low-concentration test and 50 mg/L for the high-concentration test.

3. Results and Discussion

N-bromosuccinimide (NBS) and 3,3-diaminobenzidine (DAB), as well as the compounds containing them, were found to have the greatest absorbance wavelength. The Shimadzu UV-1700 spectrophotometer was employed to assess the absorbance maximum of NBS and DAB in conjunction with M. dopa medication across different media, aiming to enhance sensitivity. According to the findings, an acidic medium had the best spectra. According to Figure 2, In this study, the M. dopa compound exhibited its highest absorbance at 513 nm, while the reagent showed its peak absorbance at 281 nm. Thus, the determined maximum wavelength (λ) for this investigation was 513 nm.

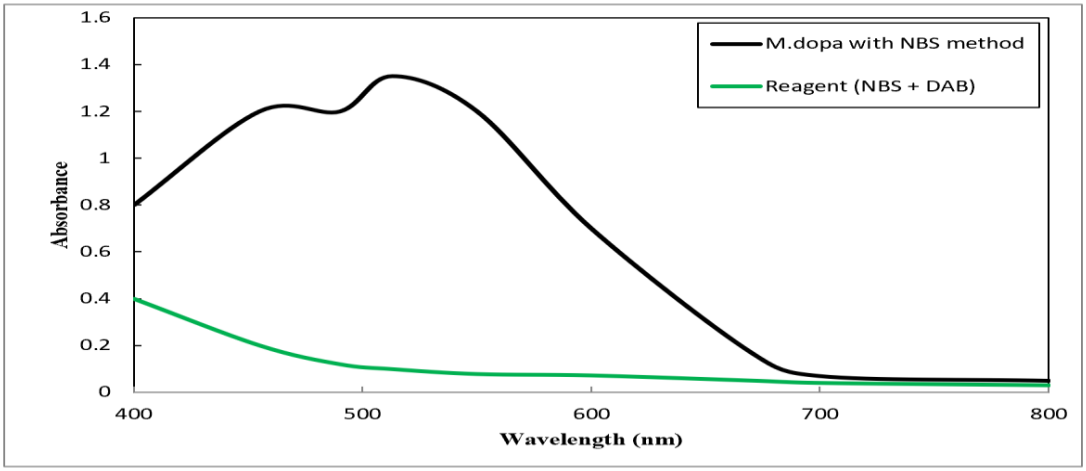

Figure 2: The absorption spectrum of M. dopa compound in acidic medium.

3.1 Proposed Mechanism of reaction of Methyl dopa with a Mixture of NBS and 3,3 DAB

The synthesis of the M. dopa medicinal molecule utilizing N-bromosuccinimide (NBS) and 3,3 Diaminobenzidine (DAB)[25] was the main focus of the spectrophotometric approach suggested in this investigation, as shown in Figure 3. This

mixture produced a magenta color with a 513 nm peak absorption. To enhance result precision, extensive research was conducted to optimize the conditions for this reaction using two spectroscopic techniques.

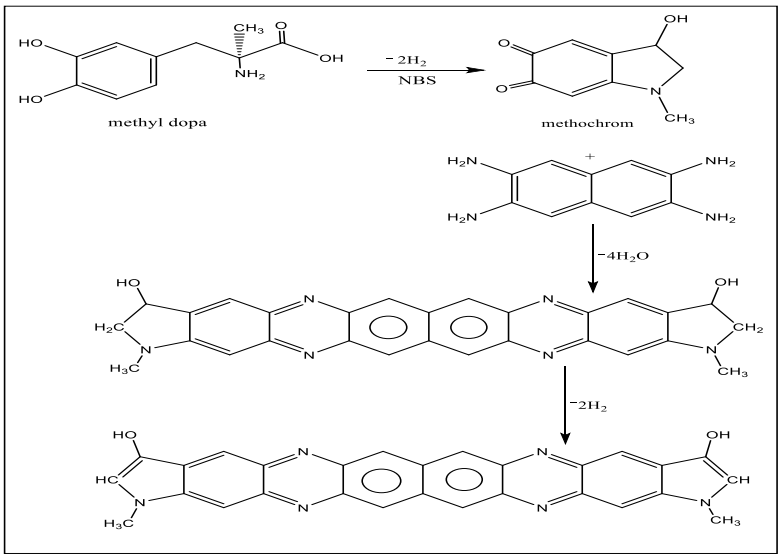

Figure 3: the proposed mechanism for the interaction between the M. dopa drug and the combination of N-bromosuccinimide (NBS) and 3,3 Diaminobenzidine (DAB) reagents.

3.2 Optimum conditions

3.2.1The Effect of pH on the M. dopa Reaction

The experiment involved using the same concentrations and volumes for each component, including M. dopa (10 mg/L, 1 mL), NBS ($1x10^{-2}$ mol/L, 0.15 mL), and DAB (1×10^{-3} mol/L, 0.15 mL). NaOH, HCl, and a pH meter is used to adjust the medium's pH from 3 to 7, which is the ideal pH for the chemical. As demonstrated in Figure 4, pH=5 is the value that is chosen as the ideal pH for the tests because it is obviously favorable to the compound's formation.

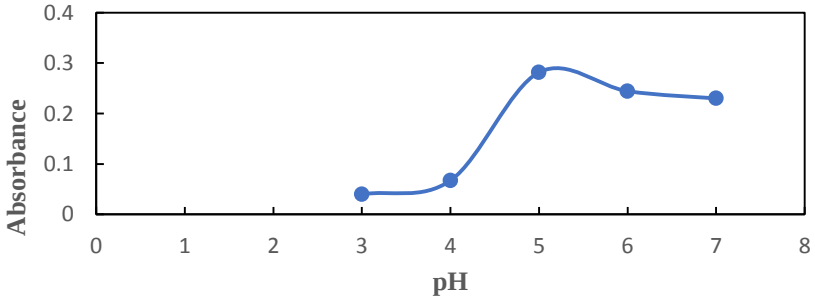

Figure 4: The influence of the acidity function on the oxidation-reduction reaction of M. dopa

3.2.2 The influence of the buffer solutions on the reaction involving M. dopa.

This experiment was conducted with constant concentrations and volumes of M. dopa, NBS, and DAB, set at (10 mg/L, 1 mL; 1×10^{-2} mol/L, 0.15 mL; 1×10^{-3} mol/L, 0.15 mL) respectively, at pH=5. The purpose was to investigate the impact of buffer solutions on absorbance values. Figure 5 displays the outcomes, indicating that the solution without a buffer at pH=5 for M. dopa exhibited the highest absorption value, thus it was selected for use in this study. Four types of solutions were analyzed, which include:

- Solution Type 1 involves using distilled water at pH=5 without a buffer solution.
- Solution Type 2 involves incorporating an acetate buffer (CH3COOH + CH3COONa) derived from Buffer Solution Type 1.
- Solution Type 3 involves utilizing a citrate buffer (sodium citrate + Citric acid) derived from Buffer Solution Type 2.

  Solution Type 4 involves incorporating a phosphate citrate buffer (Na_2HPO_4 + Citric acid) derived from Buffer Solution Type 3.

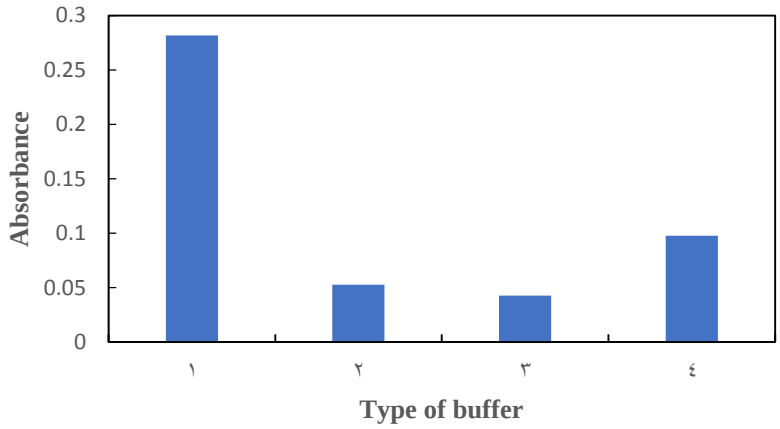

Figure 5: The impact of the buffer solution on the compound reaction of M. dopa.

3.2.3 The influence of N-bromosuccinimide (NBS) concentration on the reaction wit h M. dopa.

Several solutions ($1x10^{-2}$, $5x10^{-2}$, and $1x10^{-1}$) mol/L are prepared to demonstrate the impact of the reagent's concentration. The absorbance rapidly decreased without any precipitate in the solution, as demonstrated by Figure 6 results of the

reagent concentrations. At pH = 5, each reactant was evaluated with M. dopa (10 mg L^{-1}, 1 mL), NBS (0.15 mL), and DAB (1×10^{-3} mol. L^{-1}, 0.15 mL) at a constant concentration and volume.

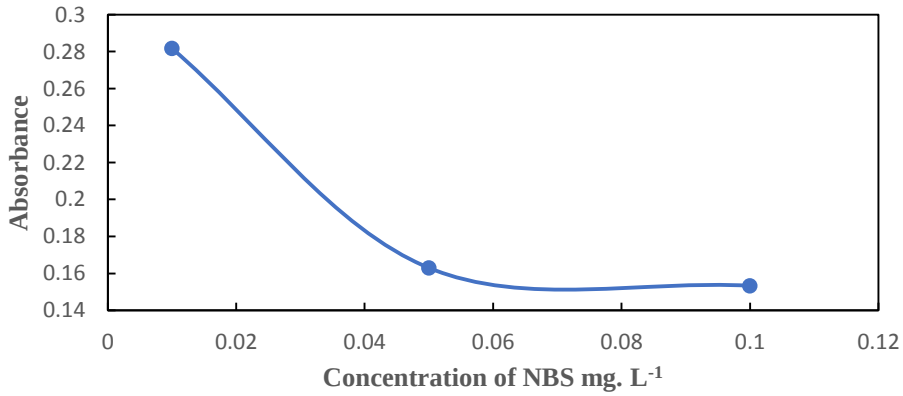

Figure 6: The influence of NBS concentration on M. dopa reaction.

3.3.4 The effect of varying concentrations of the DAB reagent on the oxidation-reduction reaction of M. dopa.

A range of solutions with varying concentrations of the DAB reagent (1×10^{-4}, 5×10^{-4}, 1×10^{-3} mol/L) were prepared, With a pH of 5, the experiment maintained a consistent concentration and volume of M. dopa (10 mg/L, 1 mL) and NBS (1×10^{-2} mol/L, 0.15 mL). These preparations aimed to investigate the impact of reagent concentration. According to Figure 7, it was determined that the ideal concentration for DAB is 1×10-3 mol/L. The results indicated a rise in absorbance without any noticeable precipitation in the solution.

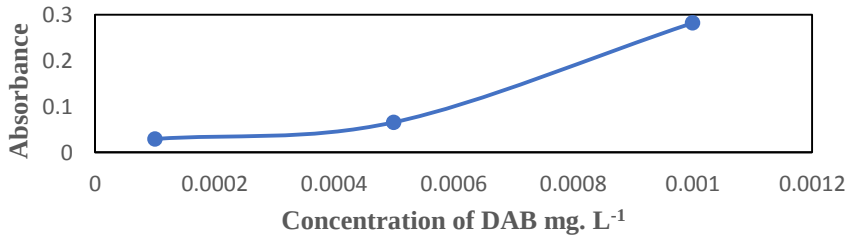

Figure 7: The impact of varying concentrations of DAB on the oxidation-reduction reaction of M. dopa.

3.2.5 Influence of NBS Volume on Oxidation-Reduction Reaction of M. dopa

To investigate the impact of the optimal NBS volume, solutions containing varying NBS (1 x 10^{-2} mol. L^{-1}) volumes (0.05, 0.1, 0.15, 0.2, and 0.25) mL are prepared. The concentration and volume of M. dopa (10 mg. L^{-1}, 1 mL) and DAB (1×10^{-3} mol. L^{-1}, 0.15 mL) are kept constant, and the solution's acidity is 5. It was discovered that 0.05 was the reagent volume that provided the highest absorbance in solution with no precipitation. according to Figure 8.

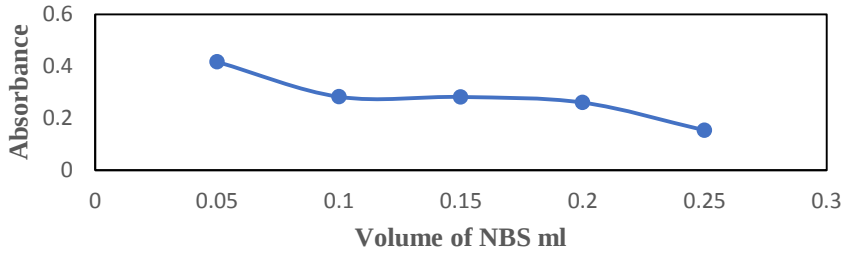

Figure 8: The M. dopa Reaction and effect of (NBS) volume.

3.2.6 The influence of varying DAB volumes on the oxidation-reduction reaction of M. dopa.

To evaluate the effect of the reagent volume, a range of amounts, including 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, and 0.40 mL for DAB, were employed to create solutions. The pH of pH=5 is the reaction pH, while M. dopa (10 mg L^{-1}, 1 mL) and NBS (1 x10^{-2} mol. L^{-1}, 0.05 mL) have constant concentrations and volumes, respectively. Figure 9 illustrates the volume of reagent that produced the highest absorbance in solution with no precipitation, indicating that the optimal DAB volume was 0.30 ml.

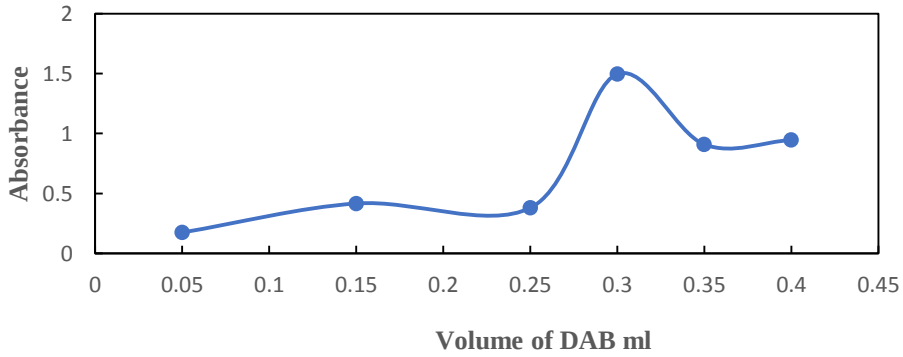

Figure 9: The oxidation-reduction reaction of M. dopa and the effect of DAB volume

3.2.7 The Effect Drug Volumes on Reaction

The investigation is focused on understanding how varying volumes of M. dopa medication, ranging from 1 to 3 mL, affect the reaction. This study was conducted at pH=5, maintaining a constant concentration of M. dopa (10 mg/L), along with constant volumes and concentrations of NBS (1×10^{-2} mol/L, 0.05 mL) and DAB (1×10^{-3} mol/L, 0.30 mL) respectively, as depicted in Figure 10. Results from this experiment indicate that the optimal medication dosage is 1 mL, as it produces the highest absorbance in the solution without any precipitation; however, precipitation begins to occur after this volume.

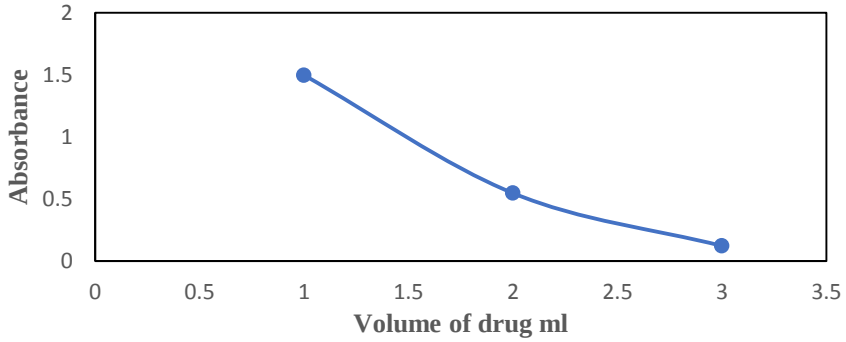

Figure 10: The effect of drug Volume on the Reaction.

3.2.8 The oxidation-reduction reaction of M. dopa and the effect of time

To determine the optimal absorbance measurement time under ideal conditions, It was investigated how time affected the stability and synthesis of the M. dopa molecule. The compound's absorbance was tested at many intervals: immediately following the procedure, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 100, 140, and 170 minutes later. The results showed that the compound had formed and had remained almost stable for 170 minutes. Based on the findings, it was appropriate to evaluate the compound's absorbance right after preparation, as depicted in Figure 11.

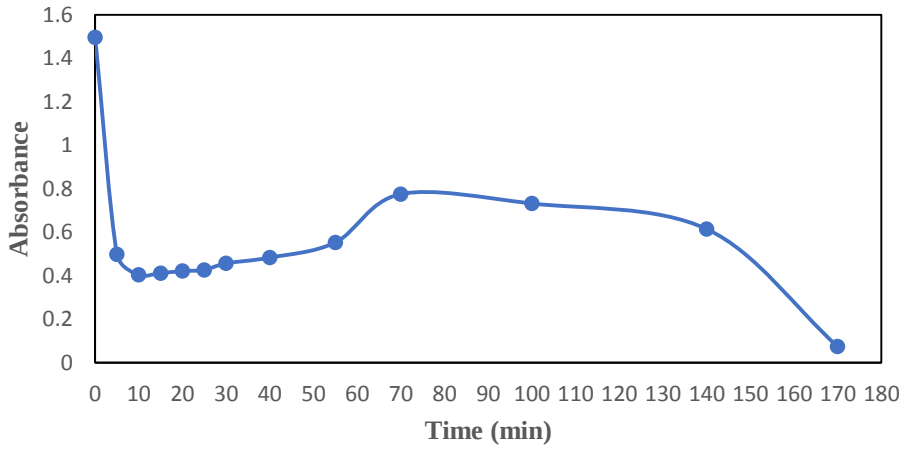

Figure 11: The effect of time on the M. dopa reaction.

3.2.9 Calibration Curve of for M. dopa Compound

The calibration curve of M. dopa can be determined under ideal circumstances by making a series of M. dopa solutions with a pH of 5 (distilled water) and a range of 0.05 to 50 mg L-1. The experiment kept a steady volume of 1 mL and maintained consistent concentrations and volumes for NBS (1x10-2 mol/L, 0.05 mL) and DAB (1×10-3 mol/L, 0.30 mL) accordingly. The absorbance of the compound is assessed at 513 nm, and as depicted in Figure 12, Beer's law is observed within the range of 0.5–10 mg/L. Table 1 presents several characteristics derived from the curve.

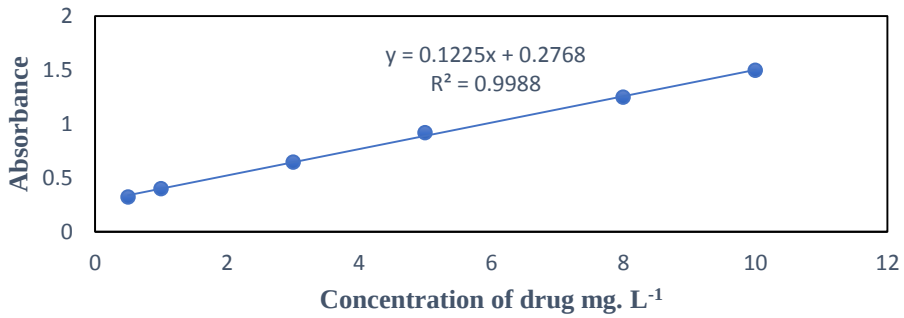

Figure 12: NBS and DAB reagents are used to form the calibration curve for the M. dopa molecule.

Table 1: the Properties of the M. dopa compound calibration curve using a combination of NBS and DAB as reagent.

Drug	Linearity mg. L^{-1}	Correlation coefficient (R^2)	Limit of Detection (LOD) mg. L^{-1}	Limit of Quantitation (LOQ) mg. L^{-1}	Sensitivity Slope	Sandell sensitivity (S) μ g cm^{-2}	Molar absorptivity (Ɛ) L $mol^{-1}cm^{-1}$
M.dopa	(0.5-10)	0.9988	0.171	0.571	0.1225	0.008	25.87×10^3

The equations provided below are used to calculate the limit of detection (LOD) and the limit of quantification [26]:

$$LOD = \left(\frac{3\times SD\ of\ blank}{Slope}\right) \quad (1)$$

$$LOQ = \left(\frac{10\times SD\ of\ blank}{Slope}\right) \quad (2)$$

SD refers to the standard deviation of the blank, while Slope represents the slope of the calibration curve.

3.2.10 The effect of interference ions

It is investigated how various cations and anions, at both low and high concentrations, affect the measurement of M. dopa. The concentrations of foreign ions were determined at 5 and 50 mg L^{-1}. Throughout the trial, the concentration of the M. dopa drug remained consistent at 5 mg L^{-1}. "Ascorbic acid, glucose, $ZnCl_2$, $MgCl_2$, $CaCl_2.2H_2O$, and $AlCl_3.6H_2O$" were among the interferences. The results show that the M. dopa drug's determination was not affected by any cation or anion used, regardless of concentration.

3.2.11 The applications of M. dopa drug

The recommended techniques are used to the analysis of five samples: methyl dopa, Aldosam, Aldomet, methyl dopa Safa, and methyl dopa Moroccan. For various pharmaceutical samples, the drug concentration at 5 mg/L is measured using a spectrophotometric technique. The conventional technique is the pure value. Good accuracy may be seen when comparing the recommended approach with the pure value; Table 2 shows the recovery percentages. HPLC is the standard procedure used to determine the true value measurement.

$$\mathbf{R.E\%} = \frac{\mathbf{Measured\ value - Pure\ value}}{\mathbf{Pure\ value}} \times \mathbf{100} \ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

$$\mathbf{Recovery\%} = \mathbf{100} \pm \mathbf{R.E\%} \ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Table 2: comparing the pure value's accuracy and the suggested method's accuracy in identifying M. dopa.

No	The Sample	The Company	The true value mg. L^{-1}	The measured value mg. L^{-1}	(E%)	The Recovery%
1	**Methyl dopa**	Accord - UK	5	5.156	3.119	103.119
2	**Aldosam**	The General Company for Pharmaceutical Industries and Medical Supplies in Samarra, Iraq.	5	5.039	0.780	100.780
3	**Aldomet**	Algorithm from Lebanon.	5	5.731	14.620	114.620
4	**Methyl dopa Safa**	The Al-Safaa Pharmaceutical Production Company located in Diyala, Iraq.	5	5.712	14.245	114.245
5	**Methyl dopa Moroccan**	Pharma 5	5	5.325	6.501	106.501
*Average of three Times The starting dosage for each tablet containing the five drugs is 250 mg.						

4. Conclusion

This study proposes a novel spectrophotometric method that is simple, cost-effective, accurate, highly sensitive, and selective for detecting M. dopa. It utilizes a mixture of NBS and DAB as a new reagent system. The suggested spectrophotometric method has proved to be simple, rapid and sensitive for determination of Methyl dopa in pure and pharmaceutical preparations. The proposed method obeyed Beer's law over a wide range

of concentrations, 0.5-10 mg. L-1, therefore it is suitable for quantitation of Methyl dopa in pharmaceutical samples which have trace levels of Methyl dopa, as well as, those with high levels of Methyl dopa. The interference of ZnCl2, $MgCl_2$, $CaCl_2.2H_2O$, $AlCl_3.6H_2O$, glucose, ascorbic acid, and zinc. The outcomes demonstrate that neither cation nor anion utilized at any concentration interfered with the M. dopa drug's determination. The proposed method was more sensitive compared with other studies which used the same method but different reagents. The results showed that the Sandell's sensitivity, the limit of quantification (LOQ), and the limit of detection (LOD) were 0.008 µg/cm², 0.571 mg/L, and 0.171 mg/L, respectively.

. The proposed method achieved a recovery percentage ranging from 100.780% to 114.620% for various pharmaceutical samples. The results obtained in this study were favorable across all parameters compared to previously published research [27, 28].

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