A New Visible Spectrophotometric Approach for Determination of Methyldopa in Pharmaceuticals

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Abstract

The investigation's objective is to develop a new spectrophotometric method for determining methyldopa in both pure and pharmaceutical forms. The proposed process produces a colorful product by combining methyldopa with anisidine in the presence of potassium nitroprusside and sodium hydroxide. The effects of several factors on reaction yield were investigated, including the reagent concentration, reaction time and color stability period and the settings were optimized. The absorbance at 597 nm of the colored product was monitored spectrophotometrically. In concentration ranges of 0.50 to 80.0 µg. mL^{-1} the plots were linear, sandell's sensitivity was 0.0218µg·cm^{-1}, the correlation coefficient was found to be (r = 0.9992). The detection limit was 0.0353µg·ml^{-1}, and the limit of quantitation was 0.2691µg·ml^{-1}. The reaction ratio between methyldopa and anisidine was studied and found to be 1:1. The proposed approach was validated and results obtained for the assay of three different brands of methyldopa tablets were compared with the BP method.

Keywords: Anisidine, Methyldopa, Oxidative coupling reaction, Potassium nitroprusside, Spectrophotometry.

Introduction

Methyldopa (MDP), (α-methyl-3,4-dihydroxy phenylalanine), is a catechol derivative (catecholamine) often used to treat hypertension. It has the chemical empirical formula $C_{10}H_{13}NO_4.1.5H_2O$ as shown in Fig. 1[1]. Methyldopa is a centrally acting 2-adrenoceptor agonist that decreases blood pressure and reduces sympathetic tone[2]. Its activity is intermediate between that of more potent drugs like guanethidine and that of the milder antihypertensive reserpine[3]. Dihydroxyphenyl alanine's structural equivalent is methyldopa. It only varies if the side chain's carbon has a methyl group. In methyldopa, there is a chiral center. As a result, it might take on the form of an S or R-isomer. The S-isomer of methyldopa is responsible for the drug's antihypertensive action[4,5]. At present, there has been a great need for rapid and easy methods for the detection of all drugs, including methyldopa, in pharmaceutical preparations because routine quality control in the analysis of manufactured drugs is essential. Although the official technique described in the United States Pharmacopeia explains the aqueous titration of the MDP test, it is time-consuming and laborious despite being employed in standard analysis[6].
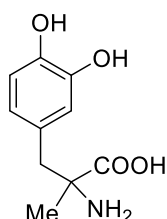
Figure 1. Chemical structure of methyldopa[1].

For the analysis of methyldopa in bulk, pharmaceutical form, or biological fluids, a range of analytical techniques has been published. HPLC[7],

Materials and Methods

Equipment and Materials

A Jasco V-530 (Japan) UV/VIS spectrophotometer with two beams was used to measure all spectra and absorbance, A 1cm quartz cell is included. Water bath (BS-11, Lab Companion). Sartorius AG GTTTINGEN B2 2105 Germany, electronic balance.

SDI/Samarra/Iraq provided methyldopa reference material that was labeled to contain 99.86 percent w/w methyldopa. Locally, methyldopa-containing pharmaceutical formulations were obtained from a variety of sources. Anisidine, the chromogen reagent, was obtained from Scharlau Chemie, S.A. in Spain.

All of the substances were analytical grade or general-purpose reagents that came from various sources.

Solutions

Methyldopa Stock Solution (1000 μg.ml.$^{-1}$)

A 0.1000gm solution of pure methyldopa (SDI) was dissolved in distilled water and concentrated to 100mL in a volumetric flask using the same solvent. More dilute solutions were made by diluting the stock standard solution with distilled water to the required concentration.

Anisidine (1×10^{-2}M)

The daily dose was made by dissolving 0.1232gm of anisidine in a little amount of ethanol and topping it up with distilled water in 100 mL standard flask.

HILIC-MS/MS[8], electrochemical[9,10], electrophoresis[11], NMR[12], mass spectrometry[13], voltammetry[14,15], cloud point analysis[16,17] and spectrophotometry[18-22] are some of the techniques used. However, several of these approaches take time and/or necessitate costly equipment and circumstances. In an alkaline solution, methyldopa is combined with anisidine in the presence of potassium nitroprusside to form a crimson water-soluble dye with a maximum absorption wavelength of 597nm.

Potassium Nitroprusside Solution (4×10^{-2} M)

In a 100 mL volumetric flask with distilled water, 1.1760gm of potassium nitroprusside (PNS) was dissolved.

Sodium Hydroxide Solution (≈0.4M)

0.8000gm amount of NaOH was dissolved in a 50 mL volumetric flask with distilled water.

Methyldopa Tablets Stock solution (500 μg.mL^{-1})

The contents of ten prepared tablets were weighed and powdered with precision. A quantity of the powder (containing 0.0500gm of methyldopa) weighing 0.0860 gm, 0.0882 gm, and 0.0940 gm (Iraq, UK, and Lebanon) respectively was accurately and independently weighed, dissolved in 10mL distilled water and agitated for 10minutes to ensure thorough solubility of the medication, then transferred to a 100 mL voluqazzwaq2metric flask and diluted to the mark with some solvent to obtain 500μg.mL^{-1} methyldopa. The sample solution was centrifuged at a rate of 4000 rpm for five minutes and filtered through Whatman filter paper.

Dilution with distilled water resulted in more dilute solutions.

Procedure:

Calibration Curve

1.0 mL aliquots of MDP. (5.0 – 800.0) μg/mL of the standard solution was transferred into a series of 10mL volumetric flasks. Each flask received 1.0 mL of 1×10^{-2} M anisidine, followed by 1.0 mL of 4×10^{-2} M potassium nitroprusside and 1.0mL of 0.4 M

sodium hydroxide. After 5 minutes, the contents were diluted to the desired concentration with distilled water and allowed to stand for 5 minutes, the contents

were diluted with distilled water to the appropriate concentration and rested for 5 minutes before being tested against a reagent blank.

Results and Discussion

Initial Absorption Spectrum:

The reaction of methyldopa with anisidine in the presence of potassium iodate in an alkaline medium produced a crimson water-soluble dye product that has a maximum absorption at 597nm which was used

in all assays Fig. 2. At this wavelength, the comparable reagent blank exhibits essentially no absorbance. The research concentrated on fine-tuning the experimental settings to select the optimum parameters for measuring methyldopa.
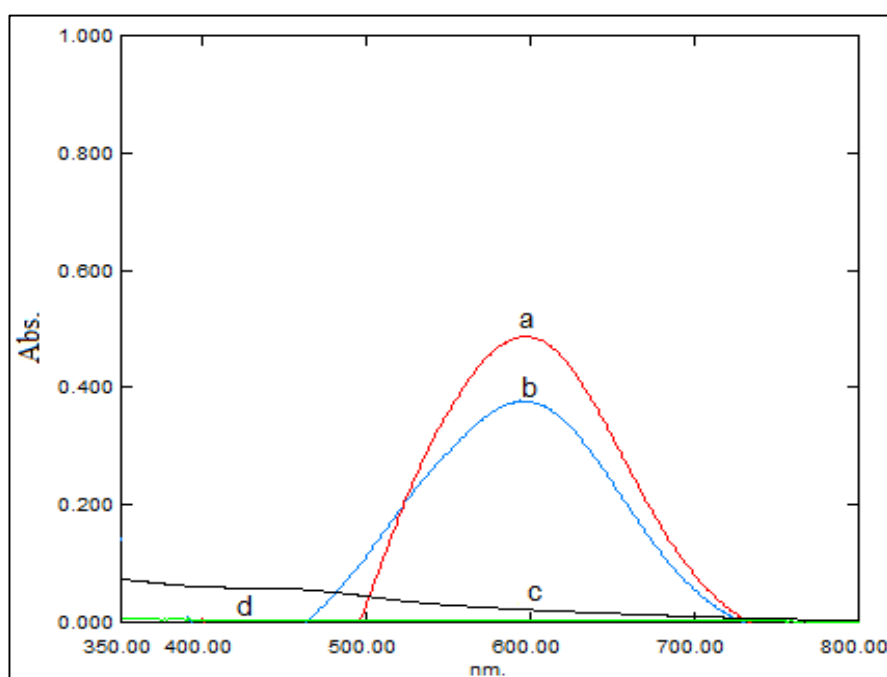
Figure 2. UV/VIS spectrum a. 10 μg mL.$^{-1}$ MDP versus reagent blank under ideal circumstances, b. 10 μg mL.$^{-1}$ MDP under initial conditions, c. the reagent blank, which was compared to distilled water. d. 10 μg mL^{-1} MDP alone against distilled water.

Optimization of Reaction Variables:

The effects of several reaction factors such as reactant concentration, oxidant agent type, sequence of addition, and time were studied.

Anisidine Concentration Effect

Using 1.0 mL of reagent solution with concentrations ranging from $(5\times10^{-3} - 6\times10^{-2})$M, the influence of anisidine concentrations on the observed

absorbance of the produced colored product was investigated. The results showed that the concentration of the reagent is directly related to the intensity of the color of the product formed. Fig. 3, shows that 1×10^{-2}M anisidine produced the highest absorption. The absorbance value fell when the concentration of the reagent was increased by more than 0.01M. Therefore, the recommended amount of anisidine was chosen to be 1.0mL of 1×10^{-2}M and used for all following experiments.
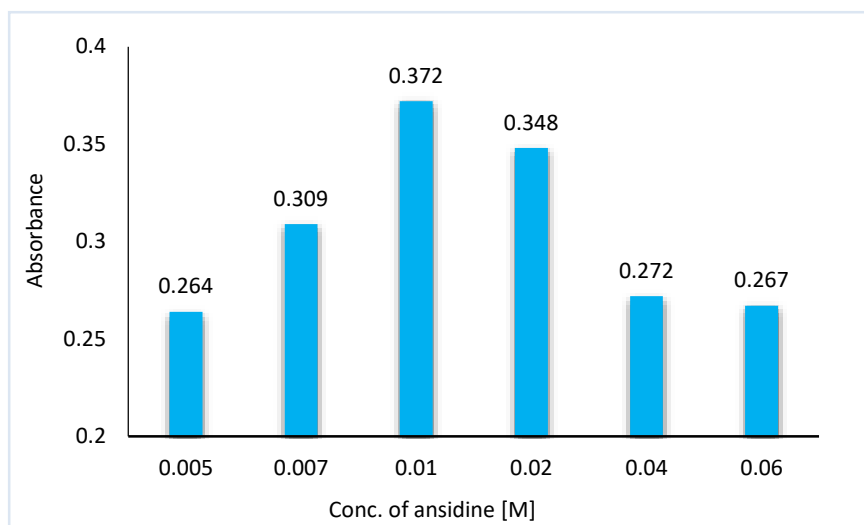
Figure 3. Effect of reagent concentration.

Selection of the Oxidizing Agent

By adding 1.0 ml of several types of oxidizing agents 1×10^{-2}M, the effect of the oxidizing agent type was investigated. Four types of oxidizing agents were tested, namely, potassium nitroprusside, Potassium iodate, Potassium periodate and Sodium nitroprusside as listed in Table 1.

Potassium nitroprusside was discovered to have the highest absorption intensity of the colored product; hence it was utilized in the following experiments.
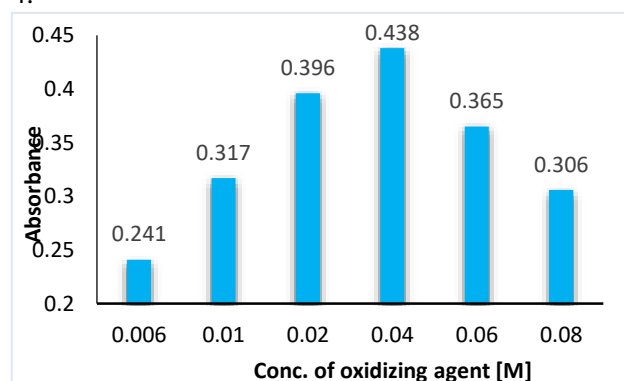
Table 1. Effect of oxidizing agent type.

Oxidizing agent	Absorbance
potassium nitroprusside	**0.396**
Potassium iodate	0.372
Sodium nitroprusside	0.035
Potassium periodate	Turbid

Effect of Oxidizing Agent Concentration

1.0mL of various potassium nitroprusside concentrations were tested to determine the optimal amount of the oxidizing agent potassium nitroprusside. Due to the greatest color intensity of the product at 597nm, the results show that applying 4×10^{-2}M of the oxidizing agent was sufficient. This concentration is regarded as optimum, as seen in Fig. 4.

Figure 4. Effect of oxidizing agent concentration.

Effect of Different Bases

The impact of utilizing 0.1mL of 0.1M solution of various bases on the production of colorful complexes was investigated by looking at the intensity of the colored product. Fig. 5 reveals that sodium hydroxide produced the best results, hence it was employed in the subsequent studies.
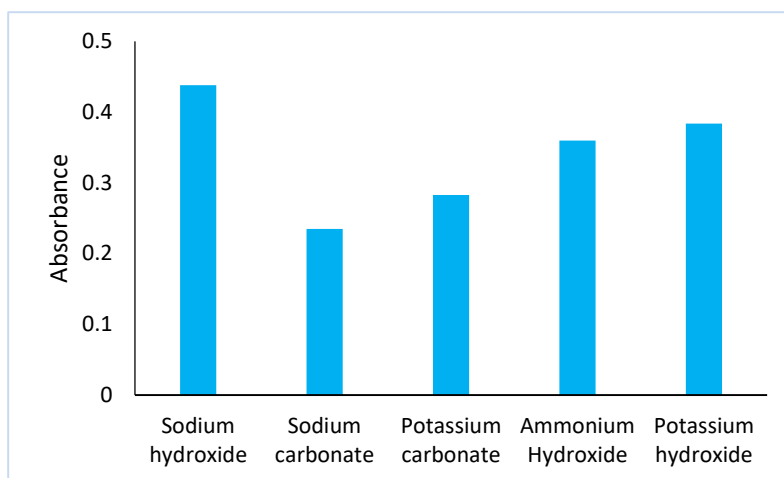
Figure 5. Effect of the base type.

Effect of Sodium Hydroxide Concentration

The effect of different concentrations of NaOH was tested using 1.0ml of (0.05-2.0) M NaOH. The color intensity of the reaction product between the reactants increased with increasing base concentration, this may be due to the attribution of the partial decolorization of the dye at a higher volume of sodium hydroxide[23]. As shown in Fig. 6. It was discovered that at concentrations more than 0.4M, the resultant complex has low absorption. As a result, for determination studies, 1.0 mL of 0.4M NaOH was chosen as the best concentration.
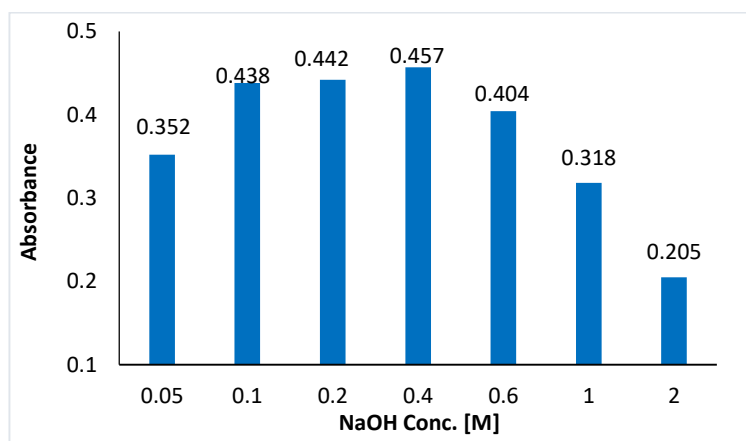
Figure 6. Effect of NaOH concentration.

The Effect of Reaction Time

The effect of time on the coupling reaction step for the maximum formation of the resulting dye was investigated by allowing the reaction to proceed for varying time intervals since the concentration of the product will change over time until they reach equilibrium[24,25]. When the reaction components were left in a dark place under optimum conditions, the reaction time was calculated by watching the color develop at different time intervals. The maximum absorbance was found after 5 minutes, indicating that the reaction was fast and almost continuous, as seen by the absorption intensity, hence 5 minutes was chosen as the optimum reaction time, Table 2.

Table 2. Effect of reaction time.

Time (min.)	Absorbance
Immediately	0.457
5	**0.486**
10	0.480
15	0.463
20	0.461
25	0.453
30	0.438

Effect of Order Addition

The effects of changing the order of adding the drug, reagent, oxidizing agent, and base on color intensity

and maximum absorbance were investigated. The best condition was [drug solution -reagent solution -the oxidizing agent solution and base] for the maximum absorbance, Table 3.

Table 3. Show the order of addition.

Order No.	Components	Absorbance
I.	**Drug + Anisidine + Oxidizing agent + NaOH**	**0.486**
II.	Drug + Oxidizing agent + Anisidine + NaOH	0.427
II.	Anisidine + Oxidizing agent + Drug + NaOH	0.361
IV.	Drug + NaOH + Anisidine + Oxidizing agent	0.258
V	Anisidine + Oxidizing agent + NaOH+ Drug	0.206

Stability of the Colored Product

The absorption of the reaction product steadily diminished when it was left at room temperature for

varying durations of time. Because of the gradual loss of color, it is best to measure the absorbance 5 minutes after dilution, which results in a fall in absorbance after 40 minutes, as illustrated in Fig.7.
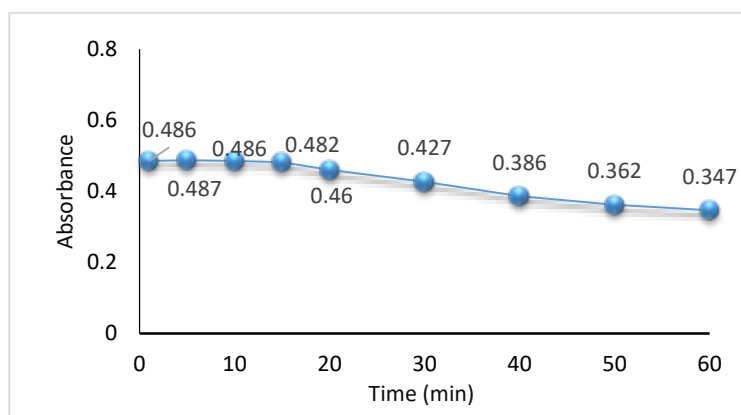

Figure 7. The time stability of a colored reaction product.

Analytical Data and the Calibration Curve

The final absorption spectrum was obtained under the established optimum conditions, and the maximum wavelength was found to be 597nm (see Fig. 2) Over the concentration range of 0.5 – 80

μg.mL^{-1}, a calibration curve for MDP was created and confirmed to be linear. Fig. 8 illustrates that the regression equation has a strong correlation coefficient, indicating good linearity over the operating concentration range. Table 4. summarizes the statistical treatments of the analytical data.
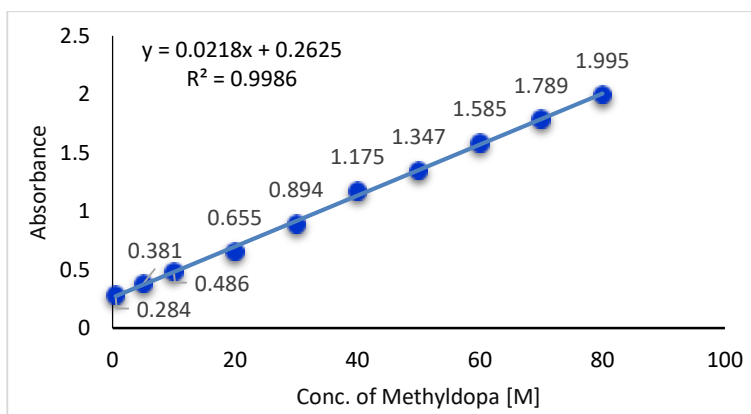
**Figure 8. MDP calibration curve in optimum conditions.**

Table 4. Shows the optical properties and statistical data used to calculate MDP.

Parameter	Value
λ_{max} (nm)	597
Color	Crimson
Linearity range ($\mu g.mL^{-1}$)	0.5-80
Regression equation	Abs=0.0218[MDP.μg/mL]+0.2625
Calibration sensitivity ($mL.\mu g^{-1}$)	0.0218
Correlation coefficient (r)	0.9992
Correlation of linearity (r^2)	0.9986
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	2957.0
Sandell's sensitivity ($\mu g.cm^{-2}$)	0.0169
Detection limit ($\mu g.mL^{-1}$)	0.0353
Quantification limit ($\mu g.mL^{-1}$)	0.2691

The Nature of the Formed MDP-Anisidine

The stoichiometry of the color dye has been studied under the established conditions by applying Job's continuous variation method and molar ratio method[26]. Figs. 9 and 10 show the experimental data in both methods and demonstrate that the resulting product was formed by a 1:1 combining ratio of MDP to the reagent. Based on this ratio, the reaction pathway was postulated to proceed as shown in Scheme 1.
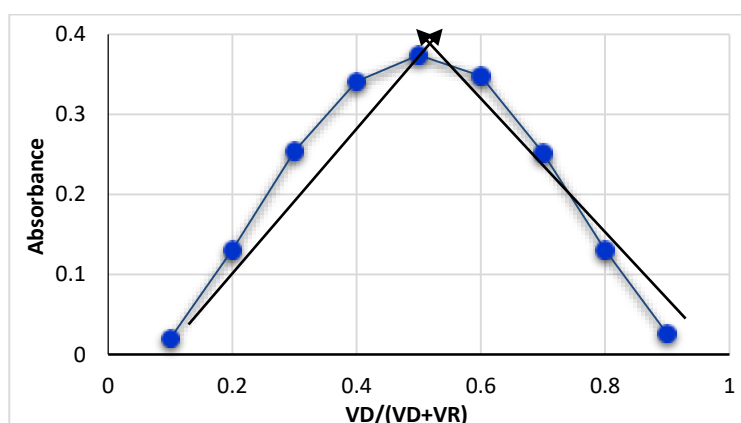
Figure 9. Continuous variation method for reaction (MDP) with ansidine.
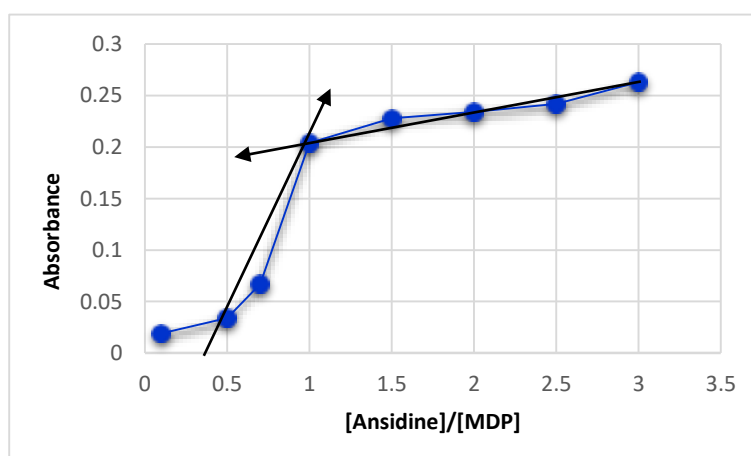
Figure 10. Mole ratio method for MDP with ansidine.

Scheme 1. Suggested steps of the main reactions between MDP and anisidine.[19]

Precision and Accuracy of the Method

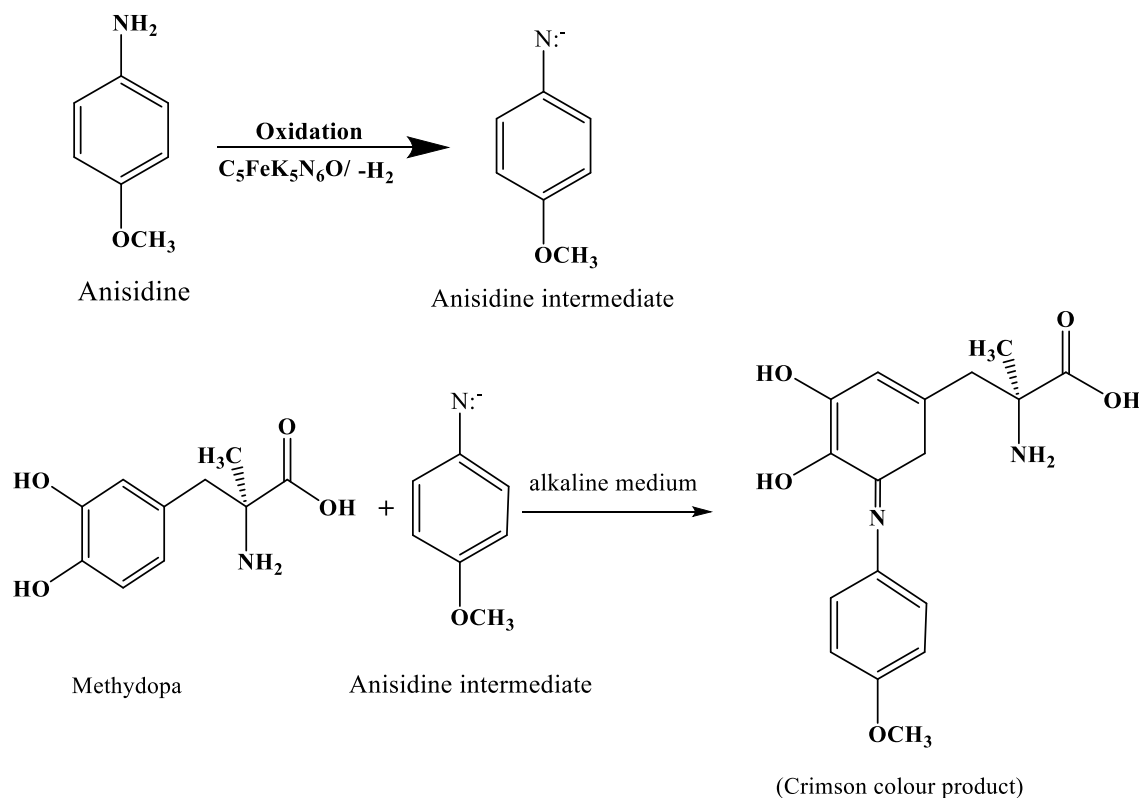Three replicate studies containing MDP solution at three varying concentrations were performed within Beer's law limits to assess the method's precision and accuracy. Table 5, shows the percent error (RE percent) and relative standard deviation percent (RSD percent) values, revealing the recommended method's high accuracy and precision.

Table 5. Evaluation of RE% and RSD% of accuracy and precision.

	Conc. of MDP $\mu g.mL^{-1}$	Accuracy and precision	
Taken	*Found**	*RE%*	*RSD%*
5.00	4.996	-0.080	0.050
10.00	10.013	0.130	0.089
20.00	20.016	0.080	0.046

*Average of three measurements.

Common Excipients Effect

By measuring the absorbance of solutions containing $10g.mL^{-1}$ of MDP and various concentrations of various excipients in a final volume of 10mL, the level of interference by some excipients, which frequently accompany pharmaceutical preparations, was investigated. The tested excipients were shown to not effect on the current approach, even when they were present in considerable quantities. Table 6. summarizes the findings.

Table 6. Percent recovery of MDP solution in the presence of excipients.

Excipients	Conc. of excipients. Taken(μg.mL^{-1})	MDP Conc. Taken (10.0 μg.mL^{-1})	
		Found (μg.mL^{-1})	Recovery%
Glucose	1000.0	10.065	100.560
Starch		9.963	99.630
Sucrose		10.041	100.410
Lactose		10.055	100.550
Mg-stearate	500.0	10.073	100.730

Application in the Pharmaceutical Sample and Statistical Evaluation

To demonstrate the usefulness of the suggested method for the determination of MDP in tablets, the drug was analyzed at varied concentration levels of three pharmaceutical tablet dosages (containing 250 mg of the active ingredient). The recovery ranged from 99.530% – 100.520% for the analysis of the tablet and the results are summarized in Table 7.

Table 7. Applications of proposed methods to determine MDP in tablet formulations.

Pharmaceutical	Assay (mg/tablet)		Conc. (μg.mL^{-1})		Recovery*%	S.D*	RSD*%
	Spiked	Found	Taken	Found*			
Aldosam S.D.I.-Iraq	250	249.425	10	9.977	99.770	0.008	0.080
		249.825	20	19.986	99.930	0.017	0.085
Methyldopa Bristol- UK	250	246.825	10	9.873	98.730	0.021	0.212
		248.825	20	19.906	99.530	0.016	0.080
Aldomet 999999Algorithm-Lebanon	250	251.300	10	10.052	100.520	0.008	0.079
		250.887	20	20.071	100.355	0.015	0.074

*Average of three measurements.

The Student's t- and F-values at 95.0 percent confidence level did not surpass the tabulated values, according to the results in Table 8 which confirms that the proposed methods' results and the reference method's results[1] are in good agreement in terms of precision and accuracy.

Table 8. t- and F- values for analysis of 10μg.mL^{-1} MDP in pharmaceutical compounds.

Pharmaceutical	Proposed method	BP method	t_{cal} (tab)*	F_{cal} (tab)*
Aldosam S.D.I.-Iraq	99.770	99.900	0.361 (2.353)	0.327 (9.277)
Methyldopa Bristol- UK	98.730	101.620	1.724 (2.353)	0.480 (9.277)
Aldomet Algorithm- Lebanon	100.520	99.620	0.528 (2.353)	1.629 (9.277)

*MDP 250mg/tablet. n = the number of three replicates.

Conclusion

The proposed method offered clear advantages for the fast determination of methyldopa in pure form and pharmaceutical preparation. It was found to be simpler, and faster. All the parameters enabled the rapid quantitative and qualitative estimation of MDP in three brands of MDP tablets, the proposed method does not require temperature control. In addition, it was important for practical quality control analysis of methyldopa in pure and pharmaceutical preparations without interference from general additives.

Authors' Declaration

- Conflicts of Interest: None.

- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.

- Ethical Clearance: The project was approved by the local ethical committee in University of Diyala.

Authors' Contribution Statement

S.R.F. conceived and supervised the project, performed the analysis of the results, and wrote the manuscript. R.M.K. carried out the experiment. Both authors discussed the results and contributed to the final manuscript.

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طريقة طيفية جديدة لتقدير الميثيل دوبا في المستحضرات الصيدلانية

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قسم الكيمياء، كلية العلوم، جامعة ديالى، بعقوبة، العراق.

الخلاصة

الهدف من البحث هو تطوير طريقة طيفية جديدة لتقدير عقار الميثيل دوبا بشكليه النقي والصيدلاني تعتمد الطريقة المقترحة على التفاعل بين المثيل دوبا و كاشف الانسدين بوجود نيتروبروسيد البوتاسيوم وهيدروكسيد الصوديوم لتكوين ناتج ملون لتكوين ناتج ملون تم دراسة تأثير العديد من العوامل المؤثرة على ناتج التفاعل تتضمن تركيز الكاشف و زمن التفاعل و استقرارية الناتج الملون مع ضبط هذه العوامل. متابعة التفاعل تمت بقياس الامتصاص عند 597 نانومتر للناتج الملون. في مدى من التركيز من (0.50 إلى 80.0) ميكروغرام.مل ⁻¹ كانت حساسية ساندل 0.0218 مايكروغرام.سم⁻¹و حد الكشف 0.0353 مايكروغرام.مل⁻¹ و الحد الكمي 0.2691 مايكروغرام.مل⁻¹. تم تطبيق الطريقة المقترحة بنجاح لتقدير المثيل دوبا في المستحضرات الصيدلانية.

الكلمات المفتاحية: الانسدين، المثيل دوبا، تفاعل الازدواج التاكسدي، نايتروبروسيد البوتاسيوم، المطيافية.