Academic Science Journal

Development of a Sensitive Spectrophotometric Determination of Methyldopa Drug

Rusul Mazin Kaddouri*, Sahar Rihan Fadhel

Department of Chemistry – College of Science – University of Diyala

scichems2113@uodiyala.edu.iq

Received: 21 August 2022 **Accepted: 10 March 2023**

DOI: https://doi.org/10.24237/ASJ.01.04.677B

Abstract

A sensitive spectrophotometric approach for measuring methyldopa (MDP) in both its pure and pharmaceutical forms was recommended by the study. The suggested method relies on combining MDP with 4-aminopyridine with N- Bromosuccinimide and sodium hydroxide are present to produce a colorful product. The conditions were improved and factors affecting the reaction yield were investigated. By measuring its absorbance at 445 nm, the colored product was tracked spectrophotometrically. In the concentration ranges of 1.0 to 70.0 µg.mL^{-1}, the plot was linear. The approach that was developed was effectively utilized in the process of determining the amount of MDP present in its pharmaceutical form.

Keywords: Methyldopa 4-amino pyridine, N-Bromo succinimide and Sodium hydroxide

تطوير طريقة طيفية حساسة لتقدير عقار المثيل دوبا

رسل مازن قدوري ,سحر ريحان فاضل

قسم الكيمياء – كلية العلوم – جامعة ديالى

الخلاصة

تهدف الدراسة إلى إقتراح طريقة طيفية حساسة لتقدير المثيل دوبا(MDP) في حالته النقية والصيدلانية. الطريقة المقترحة تعتمد على إقتران عقار المثيل دوبا مع كاشف 4-أمينو بيريدين بوجود N-بروموسكسينيميد في وسط قاعدي من هيدروكسيد الصوديوم لأنتاج معقد ملون. تمت دراسة العوامل المؤثرة على التفاعل بهدف تحديد الظرف الفضلى للتفاعل. تمت متابعة

Academic Science Journal

الناتج الملون المقاس طيفيا من خلال متابعة امتصاصه عند 445نانومتر وكانت خطية التفاعل في نطاقات التركيز(1.0 إلى 70.0)مايكروغرام.مل$^{-1}$ تم تطبيق الطريقة المقترحة بنجاح في تحديد العقار في مستحضراته الصيدلانية.

الكلمات المفتاحية: عقار المثيل دوبا، 4-أمينو بيريدين، N-بروموسكسينيميد و هيدروكسيد الصوديوم

Introduction

Methyldopa (MDP) (S)-2-amino-3- (3,4-dihydroxyphenyl) -2-methyl-propanoic acid- is a catechol derivative (catecholamine) often used to treat hypertension. As illustrated in figure 1it has the chemical formula $C_{10}H_{13}NO_4$. MDP is a centrally active agonist of the 2-adrenoceptor that reduces sympathetic tone and lowers blood pressure [1,2]. Its range of activity lies between that of stronger medications like guanethidine and that of the milder antihypertensive reserpine [3]. Dihydroxyphenyl alanine (dopastructural)'s equivalent is methyldopa. The sole difference is whether or not the side chain's carbon is home to a methyl group. A chiral center can be found in methyldopa. As a result, it can exist as a S or R-isomer. The S-isomer of methyldopa is responsible for methyldopa's antihypertensive action [4].

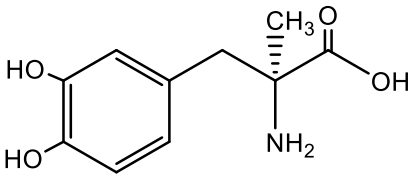


Figure 1: Chemical structuer of methyldopa

There are several analytical methods that have been published for the analysis of methyldopa in biological fluids, pharmaceutical form, or bulk. High-performance liquid chromatography with fluorescence detection [5], colorimetry [6,7], GLC [8], titrimetry [9] electrophoresis [10] ,NMR [11] ,thin layer [12],voltametry[13,14], spectrophotometry [15-21] and flow injection spectrophotometry [22-25] are a few of these methods. Some of these techniques, meanwhile, take a long time and/or need for expensive tools and setups. The process is based on the formation of a reddish water-soluble dye product with a maximum absorption at 445 nm when the drug methyldopa reacts with 4-amino pyridine in the presence of N-bromosuccinmid in an alkaline media.

Academic Science Journal

Experimental

Equipment and Materials

A double beam Jasco V-530 UV/vis spectrophotometer, Japan, was used for every spectral and absorbance mesrmunt Equipped with a 1cm quartz cell. (Lab Companion, BS - 11) Water bath. Digital balance (Germany's Sartorius AG Gttingen B2 21105). SDI/Samarra/Iraq provided methyldopa reference material that was labeled to contain 99.86% w/w methyldopa. Locally, pharmaceutical formulations containing methyldopa were obtained from various sources. The chromogen reagent, 4-aminopyriden, was obtained from, Scharlau Chemie, S.A., Spain. The chemicals were all analytical grade or general-purpose reagents obtained from a variety of sources.

Solutions

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

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

4-Aminopyrdine (1×10^{-2}M)

Daily prepared, 0.0941gm of 4-Aminopyrdine was dissolved in a 100mL volumetric flask filled with distilled water.

N-Bromosuccinimide ($4\times 10^{-2}M$)

0.7119gm of N-Bromosuccinimide was dissolved in 100mLvolumetric flask with distilled water.

Sodium Hydroxide Solution (≈0.8 M)

In a 100mL volumetric flask filled with distilled water, 3.200 gm of NaOH was dissolved.

Methyldopa tablets in stock solution (500µg. mL^{-1}))

The content of 10 formulated tablets was accurately weighed and powdered. A portion of the powder equivalent to (0.0860, 0.0882 and 0.0940) gm for methyldopa (Iraq, UK and Lebanon) respectively, (containing 0.05g of methyldopa) was accurately and separately weighed, dissolved in 10 mL of distilled water, agitated for 10 minutes to ensure complete drug dissolution, transferred to a 100 mL volumetric flask, and diluted to the proper concentration with a solvent to obtain 500µg.mL^{-1} MDP. The sample solution was centrifuged at a rate 4000 rpm for five minutes and filtered through Whatman filter paper. More dilute solutions were prepared by suitable dilution with distilled.

Procedure

1. Calibration curve

A series of 10 mL volumetric flasks were filled with 1.0 mL aliquots of the MDP standard solution that contained (10 - 700) µg. Each flask received 1.0 mL of (1×10^{-2}M) 4-amino pyridine, followed by 1.0 mL of (4×10^{-2}M) N-bromosuccinmid and 0.8 M sodium hydroxide. After 10 minutes, the substance was diluted to the desired concentration with distilled water, and then measured at 445 nm against the reagent blank.

2. Assay of methyldopa tablets

1.0mL of 100, 200 and 500 µg.mL^{-1} of MDP. tablets solutions were transferred into a series of 10 mL volumetric flasks.1.0 mL of 1×10^{-2}M 4-amino pyridine was added to each flask, and then 1.0 mL of 4×10^{-2} N-Bromosuccinimide and 1ml of 0.8 M of sodium hydroxide were added. The contents were diluted to the mark with distilled water after 10 minutes, and the absorbance was then at 445nm in comparison to the reagent blank.

Academic Science Journal

Results and discussion

1. Absorption spectrum

Preliminary findings revealed that the reaction 1mL of 200µg.mL^{-1} methyldopa with 1mL of 0.001M 4-amino pyridine in the presence of 1mL of 0.001M N-Bromosuccinimide in alkaline medium, 1mL of 0.1M sodium hydroxide forming an reddish-water soluble dye product, that maximal absorption occurs at 445 nm figure 2, this wavelength was thus employed for all measurements. The absorbance of the comparable reagent blank is essentially insignificant at this wavelength.

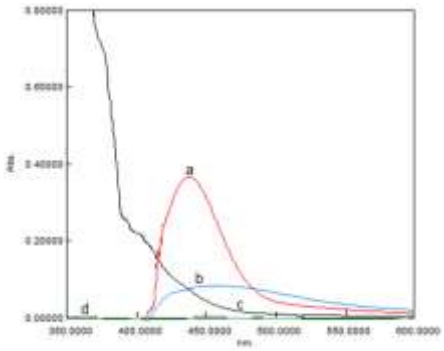


Figure 2: UV/VIS spectra a. 20µg. mL.$^{-1}$ MDP versus reagent blank under optimum conditions , b. 20 µg.mL.$^{-1}$ MDP under initial conditions, c. the reagent blank, which was compared to distilled water. d. 20 µg.mL^{-1} MDP alone aginast distilled water.

2. Optimization of reaction variables:

The effects of different reaction factors, such as reactant concentration, oxidant agent type, addition order, and time, were examined.

- **Effect of Reagent concentration**

1mL aliquots standard solution of 200 µg.mL^{-1} MDP was transferred into a series of 10mL standard volumetric flasks. To each flask then, 1mL of different concentration (1×10^{-3}- 6×10^{-2})M of 4-aminopyridine solution was add followed by 1 mL of (1×10^{-2})MN-Bromosuccinimide and 1mL of 0.1M sodium hydroxide with shaking , the volume was made up to the mark with distilled water. The absorbance of the formed dye was measured versus reagent blank. Figure

(3) demonstrates that 0.01M of the reagent solution was sufficient to achieve the highest absorbance and this solution was employed in the studies that followed.

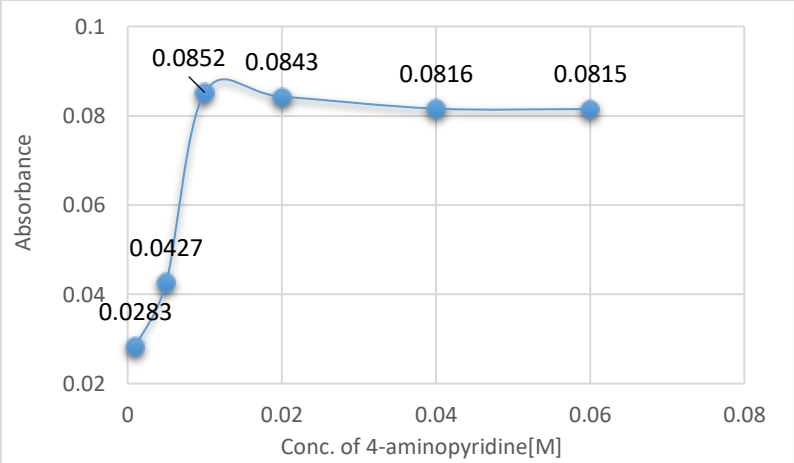


Figure 3: Effect of reagent concentration**.**

- **Effect of oxidizing agent type**

A series of 10mL standard flasks, containing1.0 mL of 200µg.mL-¹ MDP. solution, 1mL of 1×10-²M 4-amino pyridine was add, then 1.0mL of 1×10^{-2}M of different oxidizing agents (N-Bromosuccinimide,potassium periodate ,potassium iodate,sodium nitroprusside and hydrogen peroxide) and 1.0 mL of 0.1 M sodium hydroxide were added to each flask with shaking. The solutions were made up to the final volume with distilled water before making the absorbance measurements vs the corresponding reagent blank. Table (1) shows that N-Bromosuccinimide solution was optimum and was used in the subsequent experiments.

Table 1: Effect of oxidizing agent type

Reagent concentration	Absorbance
C4H4BrNO2	0.0852
KIO3	0.0420
KIO4	0.0388
Na2[Fe(CN)5NO] · 2H2O	0.0391
H2O2	0.0056

- **Effect of Oxidizing agent concentration**

The effect of the amount of oxidizing agent was studied. 1.0 mL aliquots of 200µg.mL-¹ MDP standard solution was transferred in a series of 10 mL standard flasks 1.0 mL (1×10^{-2}) M4-amino pyridine was added to each flask, the mixture was then diluted to the specified volume with distilled water and thoroughly mixed after the addition of 1.0 mL of (1×10^{-3}- 6×10^{-2})M N-Bromo succinmide and 1.0 mL of 0.1 M sodium hydroxide. Comparing the colored product's absorbance to the reagent blank allowed for measurement. Based on the maximum intensity of the colored product, the results displayed in figure 4, suggested that 1.0 mL of [0.04M] N-Bromosuccinimide solution was the best and should be used in following trials.

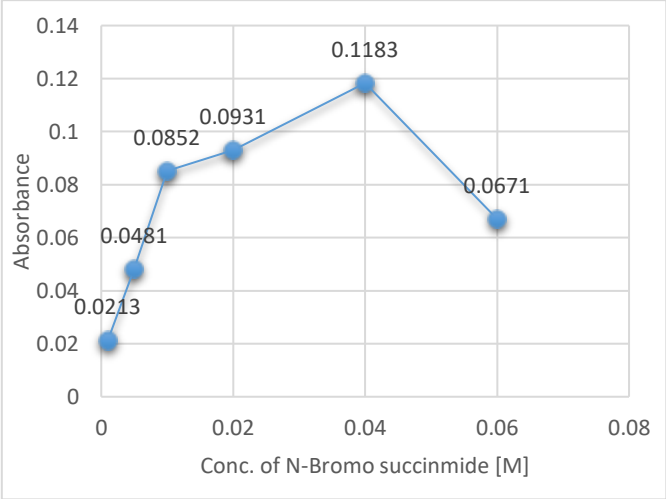


Figure 4: Effect of Oxidizing agent concentration

- **Effect of different bases**

In to a series of 10 mL volumetric flasks, 1.0 mL aliquots of 200µg.mL-¹ MDP. standard solution 1mL of $1x10^{-2}$ M 4-aminopyridine were added to all the flasks, then 1.0 mL of $4x10^{-2}$ M N-Bromosuccinimide 1.0 mL of 0.1 M from (sodium carbonate, potassium carbonate, ammonium hydroxide sodium hydroxide and potassium hydroxide) base solutions, were added to each flask. With distilled water, the mixture was properly diluted to the appropriate level. In comparison to the reagent blank, the colored product's absorbance was assessed. The most effective basic medium for a maximum absorbance was discovered to be NaOH, which was employed in all ensuing studies, table 2.

Table 2: Effect of different bases

Base type	Absorbance
Sodium hydroxide	0.1183
Sodium carbonate	0.0135
Potassium carbonate	0.0083
Ammonium hydroxide	0.0160
Potassium hydroxide	0.0134

- **Effect of sodium hydroxide concentration**

1.0 mL aliquots of 200µg.mL-¹ MDP standard solution were transferred in a series of 10mL volumetric flasks, 1.0mL of (1×10^{-2}) M 4-amino pyridine of was added to each flask followed by 1.0 mL of 4×10^{-2} M N-Bromo succinimide and 1.0ml of different concentrations of sodium hydroxide the contents were diluted to the mark with distilled water and mixed well. The absorbance of the colored product was measured against the reagent blank. Figure (5) shows that 1 mL 0f 0.8M sodium hydroxide gave the maximum absorbance of the coupling yield and no more increase in absorbance was noted after the addition of that volume. This may be due to the attribution of the partial decolorization of the dye at higher volume of sodium hydroxide [26] or can be explained by considering the acid–base equilibriums of the resulting compound. Further increase in alkalinity leads to subsequent deprotonation to form the oxidative coupling complex [27].

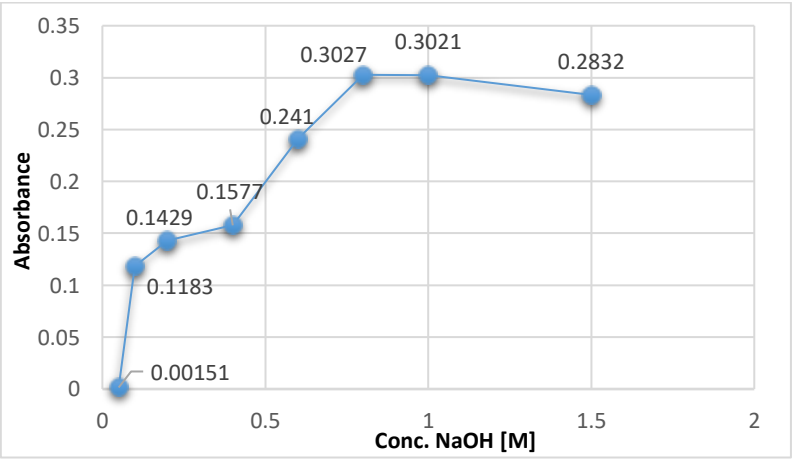


Figure 5: Effect of Sodium hydroxide

- **The effect of reaction time**

Following the color development allowed us to determine the reaction time. The absorbance was followed by allowing the reactants to stand for different time intervals (1-20) min before dilution. The optimum reaction time is determined spectrophotometrically at λmax 445 nm. Table (3) shows that complete colour development is attained at 10 minutes.

Table 3. Effect of reaction time

Time (min.)	Absorbance
Immediately	0.3027
5	0.3080
10	0.3230
15	0.3149
20	0.3107

- **Effect of order addition**

Optimal sequencing was investigated, the variation in hue and maximum absorbance when medication, reagent, oxidizing agent, and base additions are made in a different order for the greatest absorption, the ideal sequence was [Reagent -oxidizing- drug- base solution], table (4).

Table 4: Effect of order addition

Order No.	Components	Abs.
1	4-amino pyridine +Oxidizing agent +drug+ NaOH	0.3746
2	Drug+4-aminopyridine+oxidizing agent +NaOH	0.3230
3	Oxidizing agent+4-amino pyridine +NaOH+Drug	0.1573
4	NaOH+4-amino pyridine +Oxidizing agent+Drug	0.0523
5	Drug+ NaOH+4-aminopyrdine+Oxidizing agent	0.0478

- **The stability**

Stability study of the coloured product formed upon reaction of drug solution with 4-aminopyridine was carried out by measuring its absorbance at different time intervals. As it is obvious from the results in Figure (6) there was no effect of standing time on the absorbance of the coloured product after diluting the reaction mixture to the final volume. The absorbance readings at 445 nm was stable up to 10 min.

Academic Science Journal

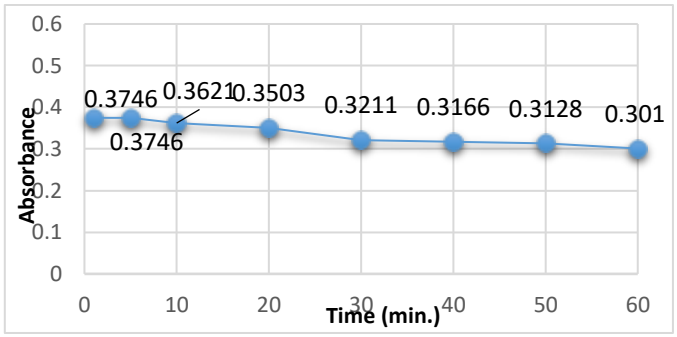


Figure 6: Effect of time on colour development.

Analytical data and the calibration curve

A series of 10 mL volumetric flasks were filled with 1.0 mL aliquots of the MDP standard solution that contained (10-700) µg. Each flask received 1.0 mL of (1×10^{-2}M) 4-amino pyridine, (1.0 mL) of (4×10^{-2} M) N-Bromosuccinimide, and 1.0 mL of 0.8M sodium hydroxide. After 10 minutes, the contents were diluted with distilled water to the proper concentration, and the absorbance was assessed in comparison to the reagent blank. Figure (7) shows that the regression equation has a high correlation coefficient, which suggests that it is linearly appropriate for the operating concentration range. The statistical processes used to analyze the analytical data are summarized in table (5).

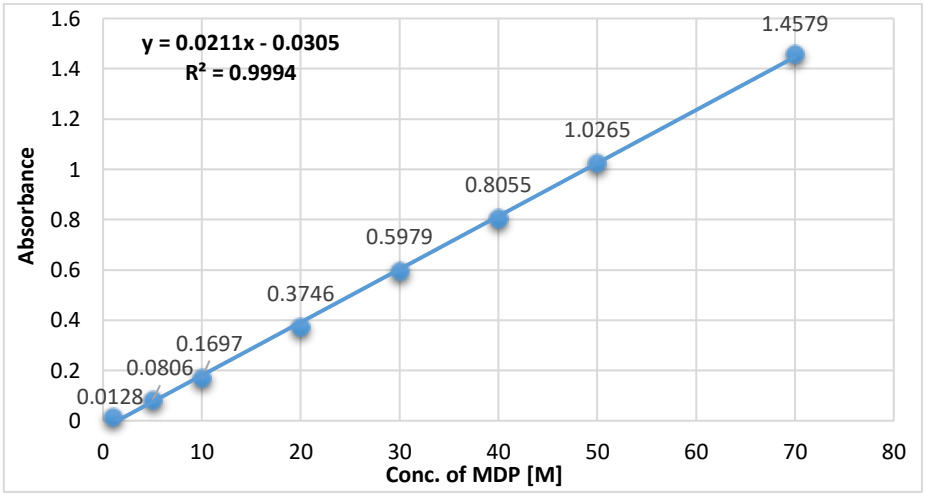


Figure 7: Calibration graph of MDP

Table 5: Analytical values of statistical treatments for the calibration graph

Parameter	Value
λ_{max} (nm)	445
colure	Reddish
Linearity range (µg.mL^{-1})	1.0-70.0
Regression equation	Y=0.0211[MDP µg/mL]- 0.0305
Calibration sensitivity (mL.µg^{-1})	0.0211
Correlation coefficient (r)	0.9997
Correlation of linearity (r^2)	0.9994
Molar absorptivity (L.mol^{-1}.cm^{-1})	4456.63
Sandell's sensitivity (µg.cm^{-2})	47.3934
Detection limit (µg.mL^{-1})	0.1990
Quantification limit (µg.mL^{-1})	0.5687

Structure of the product

Under the optimum conditions, the stoichiometry of the reaction between MDP and reagent was investigated by Job's continuous variation methods and molar ratio. The results obtained of both methods were plotted and are shown in Figure (8 and 9) which indicated the existence of **1:1** (MDP: reagent). The formation of dye may probably occur as given in scheme (1).

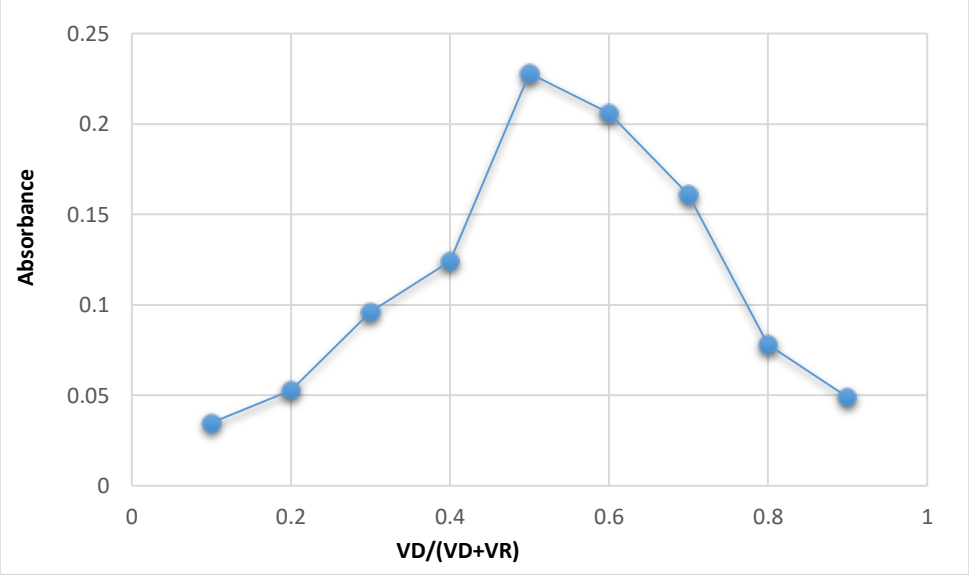


Figure 8: Job's method of continuous variation

Academic Science Journal

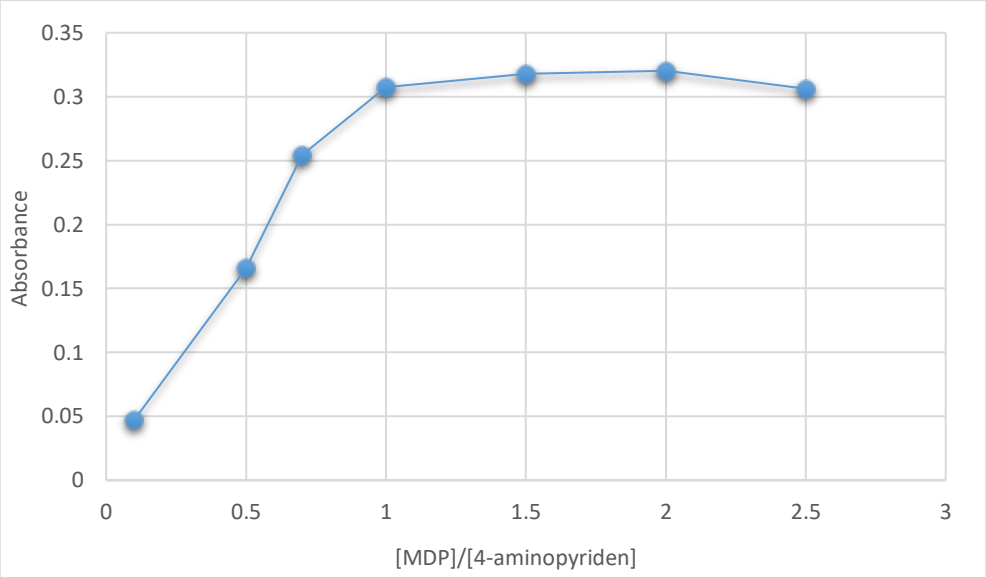
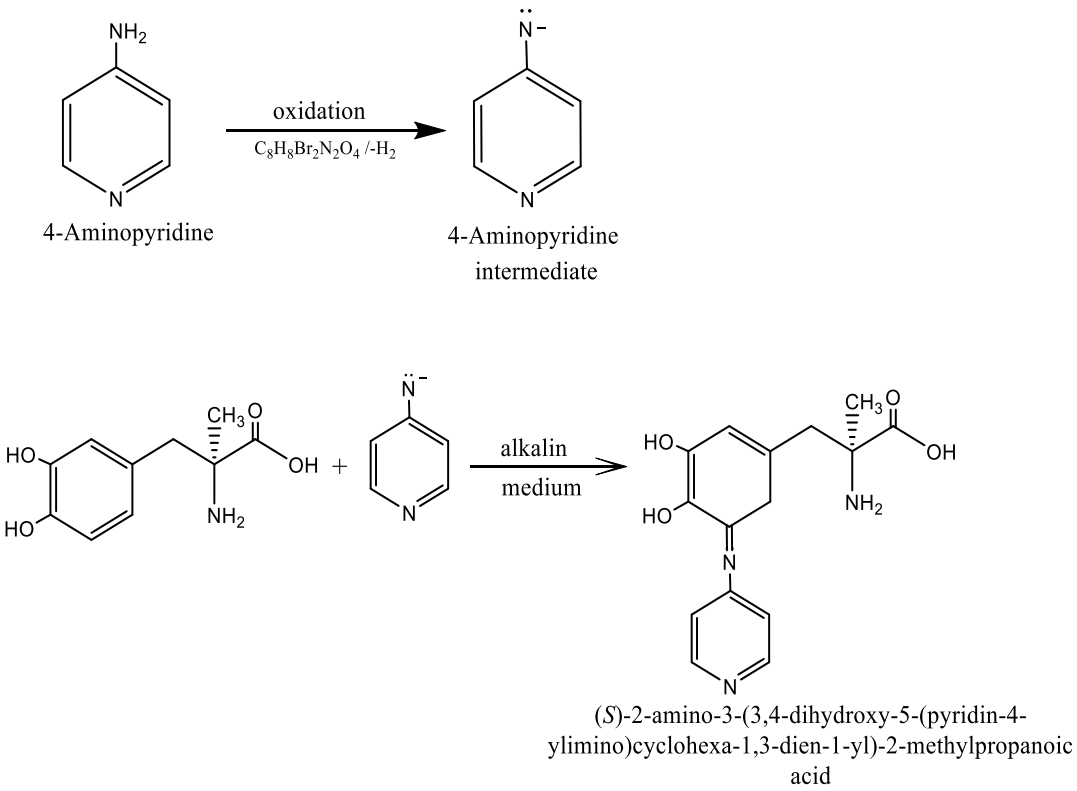


Figure 9: Mole ratio method



Scheme 1: Oxidizing coupling reaction of MDP with 4-aminopyridine

The method's precision and accuracy

The method's precision and accuracy were investigated. Precision was expressed as a percentage relative standard deviation (RSD%), while accuracy was expressed as a percent error (relative error) (RE%). The relative error (RE%) and relative standard deviations (RSD%), as shown in Table (6), demonstrated the high precision and accuracy of the described MDP assay method.

Table 6: Evaluation of RE% and RSD% of accuracy and precision.

Conc. of MDP µg.mL^{-1}		accuracy and precision	
Taken	*Found**	*RE%*	*RSD%*
5.000	5.040	0.800	0.209
10.000	9.975	-0.250	0.052
20.000	19.992	-0.040	0.300

*Average of three measurements

Effect of common excipients

The effect of common excipients used in the pharmaceutical preparation was studied by analyzing MDP drug containing the quantity mentioned in Table (7) in presence of 500µg.mL-¹for (Glucose, Starch, Sucrose, Lactose, Mg-stearate). It was observed that the studied foreign species did not interfere at this concentration. On the other hand, glucose, lactose, sucrose, starch and Mg-stearate interfere at higher concentration levels.

Table 7: 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}) Recovery %	
		Found (µg.mL^{-1})	Recovery%
Glucose	500.0	10.031	100.310
Starch		10.060	100.600
Sucrose		10.105	101.050
Lactose		10.028	100.028
Mg-stearate		9.9770	99.770

Application in pharmaceutical sample

The proposed method was successfully applied to the determination of MDP in a tablet. The percent recovery of pure MDP added was (99.470- 100.510) % with relative standard deviation of (0.079- 0.808) % (n=3). The results are summarized in Table 8.

Table 8: 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	251.275	10	10.051	100.510	0.043	0.427
		250.450	20	20.036	100.180	0.016	0.079
Methyldopa Bristol- UK	250	249.200	10	9.968	99.680	0.072	0.722
		284.675	20	19.894	99.470	0.105	0.527
Aldomet Algorithm- Lebanon	250	250.425	10	10.017	100.170	0.081	0.808
		249.312	20	19.945	99.725	0.103	0.516

*Average of three mesurmented

References

1. Indian Pharmacopoeia , published by the controller of publications , Delhi, on behalf of Government of India , Ministry of health and family welfare , 1996, 1 , 274 , 477 .
2. F. T. Fraunfelder, F. W. Fraunfelder, Drug - induced ocular side effects, 5th ed., (Butterworth - Heinemann , Boston, 2001) , 36 , 314
3. S. Laurent, Pharmacological research, 124, 116-125(2017)
4. J. L. LeóN-Romo, C. I. Virues, J. Aviña, I. Regla, E. Juaristi, Chirality, 14(2-3), 144-150(2002)
5. G. Bahrami, A. Kiani, S. Mirzaeei, Journal of Chromatography B, 832(2), 197-201(2006)
6. M. A. AL-Da'amy, R. F. AL-Moswi, kerbala journal of pharmaceutical sciences, (6), (2013)

7. M. E. Rani, Spectrophotometric determination of methyldopa in pharmaceuticals dosage forms by bromination method, 20(32),24-27(2013)
8. J. R. Watson, R. C. Lawrence, Journal of Chromatography A, 103(1), 63-70(1975)
9. A. A. Al-Majed, A. H. Bakheit, H. A. A. Aziz, F. M. Alajmi, H. AlRabiah, Propranolol. In Profiles of drug substances, excipients and related methodology, (Vol. 42, Academic Press, 2017), 287-338
10. A. H. Mohammed, N. A. Numan, G. F. Al-Temimi, N. N. Al-Shawi, H. A. Fawzi, International Journal of Research in Pharmaceutical Sciences, 10(1),444- 452(2019)
11. Z. Talebpour, S. Haghgoo, M. Shamsipur, Analytica chimica acta, 506(1), 97-104(2004)
12. K. Günther, P. Richter, K. Möller, Chiral Separations,12(2) 29-59(2004)
13. H. Beitollahi, S. Tajik, M. H. Asadi, P. Biparva, Journal of Analytical Science and Technology, 5(1), 1-9(2014)
14. A. A. Ensafi, B. Saeid, B. Rezaei, A. R. Allafchian, Ionics, 21(5), 1435-1444(2015)
15. E. A. Gadkariem, K. E. E. Ibrahim, N. A. A. Kamil, M. E. M. Haga, H. A. El-Obeid, Saudi pharmaceutical journal, 17(4), 289-293(2009)
16. K. Upadhyay, A. Asthana, R. K. Tamrakar, Research on Chemical Intermediates, 41(8), 5521-5528(2015)
17. Q. N. Rashid, M. H. Bakir, S. O. Baban, Tikrit Journal of Pharmaceutical Sciences, 11(1), 67-77(2016)
18. A. I. Shihab, N. T. Al-Sabha, Pak. J. Anal. Environ. Chem, 21(1), 10-18(2020)
19. O. E. Thomas, A. K. Mustapha, Nigerian Journal of Pharmaceutical Research, 11(1), 147-153(2015)
20. K. Sharma, S. P. Sharma, S. C. Lahiri, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 92, 212-224(2012)
21. J. J. Berzas Nevado, J. M. Lemus Gallego, P. Buitrago Laguna, Fresenius' journal of analytical chemistry, 353(2), 221-223(1995)
22. P. R. S. Ribeiro, J. G. Neto, L. Pezza, H. R. Pezza, Talanta, 67(1), 240-244(2005)

23. J. Upan, S. Themsirimongkon, S. Saipanya, J. Jakmunee, Chiang Mai J. Sci., 46, 537-546(2019)
24. P. R. D. S. Ribeiro, R. M. Duarte, Brazilian Journal of Pharmaceutical Sciences, 50, 573-582(2014)
25. Q. N. Rashid, M. H. Bakir, S. O. Baban, Tikrit Journal of Pharmaceutical Sciences, 11(1), 67-77(2016)
26. H. D. Revanasiddappa, H. N. Deepakumari, S. M. Mallegowda and K. B. Vinay, Analele Universitatii Bucuresti, Chimie, 20(2), 189-196(2011)
27. M. S. Sudhir, R. V. Nadh Oriental Journal of Chemistry, 29(4), 1507-1514(2014)