مجلة الدراسات التربوية والعلمية - كلية التربية - الجامعة العراقية
العدد الثاني والعشرون - المجلد الثالث - علوم الكيمياء - أيلول 2023 م
doi.org/10.52866/esj.2023.03.22.10

Spectrophotometric determination of Methyldopa using an organic reagent in pharmaceutical formulations

Mariam Khaled Amin
Tikrit University /College of Education for Pure Sciences - chemistry department

Qabas Naji Rashid
Tikrit University/ College of Pharmacy

Abstract :

A quick, simple, and sensitive method has been proposed for the determination of methyldopa in its pharmaceutical preparations: The method was based on the reaction of p-Nitrobenzaldehyde with Methyldopa and potassium hydroxide to form Schiff's base, where the highest absorption was 0.674 at laboratory temperature at a wavelength of about 462 nm. This reaction obeys Lambert-Beer law in the range of concentrations between (6-150) µg/ml and molar absorption of 1478.51 L/mol. cm. The limit of detection (LOD) for the method is 0.551µg/ml and the limit of quantity or quantification is 1.67µg/ml. Where this method was successfully applied in the determination of Methyldopa in its pharmaceutical preparations. As the materials that were used as additives to the drug, the results of this method were consistent with the known standard method.

Key words: Methyldopa, p-Nitrobanzaldehyde , Schiff's base.

التقدير الطيفي للمثيل دوبا باستخدام كاشف عضوي في مستحضراته الصيدلانية

أ.د قبس ناجي رشيد
جامعة تكريت/ كلية الصيدلة

مريم خالد أمين
جامعة تكريت/ كلية التربية للعلوم الصرفة - قسم الكيمياء

مستخلص

لقد تم إقتراح طريقة سريعة وبسيطة وحساسة لتقدير عقار المثيل دوبا في مستحضراته الصيدلانية:إعتمدت الطريقة على تفاعل بارا -نايتروبنزالديهايد مع مثيل دوبا هيدروكسيد البوتاسيوم كقاعدة لتكوين قاعدة شيف، حيث كان أعلى إمتصاص 0.674 في درجة حرارة المختبر عند طول موجي حوالي 462نانومتر .وهذا التفاعل يتبع قانون لامبرت-بير في نطاق تركيزات كانت بين(150-6مكغم/مل)وإمتصاص مولاري 1478.51لتر/مول .سم ،حد الكشف للطريقة (LOD)هو 0.551مكغم/مل وحد الكمية أوالقياس الكمي 1.67مكغم/مل. حيث تم تطبيق هذه الطريقة بنجاح في تقدير عقار المثيل دوبا في مستحضراته الصيدلانية. حيث أن المواد التي إستخدمت كمضافات للعقار كانت النتائج لهذه الطريقة متوافقة مع الطريقة القياسية.

المعروفة: مثيل دوبا ، بار-نايتروبنزالديهايد ، قاعدة شيف .

1.1 Introduction

Methyldopa (MD), is in the form of a white powder, which is odorless, its molar mass is 211.215 g/mole, MP=2900^{0}C, which is chemically known as alpha-methyl-4,3-dihydroxyphenylalanine, and it is a catechol derivative (catecholamine) Methyldopa is shown to result from stimulation of central alpha-adrenergic receptors by either alpha-methyl dopamine or alpha-methyl norepinephrine.(1) , as it is used to treat high blood pressure because it has the ability to expand blood vessels (2)

And that methyldopa reduces the sympathetic outflow from the motor centers in the brainstem but allows them to increase their sensitivity to control the receptors for pressure (3).

It is considered one of the central neurotransmitters, as it is considered particularly important in regulating movement and possesses important pharmacological properties. It is used to correct circulatory disorders associated with shock attacks(4).Usually, the therapeutic concentration of methyldopa in human plasma is about 0.1 to 0.5 mg. L and the terminal elimination half-life is (2 hours)(5). Several methods have been suggested for the determination of methyldopa in pharmaceutical preparations, including; Pulse differential potentiometer(6), High-performance liquid chromatography (HPLC)(7), Voltammetry(8), Colorimetry(9), as well as spectral determinations in pharmaceutical formulations(10), cyclic voltammetry(11), magnetic resonance spectroscopy(12), and kinetic methods(13).

2.1Research aims

The main objective of the research is to find a method that is economical, simple, and fast as well to estimate the drug methyldopa by using the reagent p-Nitrobanzaldehyde , which when linked to this drug gives a colored product. The equivalence of the reagent with the drug was achieved by relying on both the Job method and the molar ratio method, and the success of the method that was proposed in the drug estimation process in the pharmaceutical preparation for it.

1.2 Equipment

1- UV- VIS Spectrophotometer

-T92+ UV Spectrophotometer ‘’PG INSTRUMMENTS with (1cm) plastic cells’’.

- UV-VIS Spectrophotometer ‘’Single beam from Genesis UV10”.

- UV-VIS Spectrophotometer ‘’double beam from Shimadzu (model UV-1800”.

2- Balance Kern 770GS/GJ from Sartorius BL210S.

3- Semi-Micro Analytical Balances DIN 40050-IP20

4- Oven from Memmert, Schutzart

5- Hot Plate

2.2 Chemicals

Table (3-1): The chemicals used

Substance	Company	Molecular Formula	Molecular Weight (g/mol)	Purity%
Matheldopa	SDI Samarra. Iraq	$C_{14}H_{13}N_5O_5S_2$	395.416	99.9
p-Nitrobenzaldehyde	Merck	$C_7H_5NO_3$	151.121	99
Ethanol	Scharlau	C_2H_5OH	46.068	99.9
Sodium hydroxide	GCC	NaOH	40	98
Ammonium hydroxide	GCC	NH_4OH	35.05	25
Potassium hydroxide	GCC	KOH	56.105	98
Lactose Monohydrate	GCC	$C_{12}H_{22}O_{11}H_2O$	360.31	99
Cellulose	BDH	$(C_6H_{10}O_5)_n$	162.1406	99
Magnesium stearate	BDH	$Mg(C_{18}H_{35}O_2)_2$	591.27	96

3.2 Preparation of solutions

- Methyldopa standard solution 1000 mcg/ml

Methyldopa solution was prepared at a concentration of 1000 mcg/ml by dissolving 0.1000 gm in a small amount of distilled water and then completing the volume to 100 ml in a volumetric flask to the mark. From it, the rest of the diluted solutions were prepared.

- Reagent solution (p-Nitrobenzaldehyde) at a concentration of (0.01 M)

It was prepared by dissolving 0.075 grams of it in a quantity of ethanol and then filling the volume to 50 ml with the same solvent in a volumetric flask to the mark.

- Potassium hydroxide solution (1 M)

A potassium hydroxide solution was prepared by dissolving 1.4 gm of it in a quantity of distilled water and then completing the volume to 25 ml in a volumetric flask to the mark.

- The solution of the pharmaceutical preparation is 300 mcg/ml

A solution of the pharmaceutical preparation methyldopa and the preparation of the second company Aldomet (containing 10 tablets and each tablet containing 250 mg of methyldopa) was prepared at a concentration of 300 mcg/ml by dissolving 0.0425 gm of each of

the two powders in distilled water, after which filtering was done and each filtered solution was placed in 100ml glass bottle and fill the volume with distilled water to the mark.

1.3 Preliminary tests to find optimal conditions

Several preliminary experiments were conducted, and the optimal conditions for the determination of methyldopa were reached by the Schiff base formation reaction, as 1ml of methyldopa solution was added to a volumetric vial of 10 ml, then 0.5 ml of potassium hydroxide was added, and added 1 ml of p-Nitrobenzaldehyde reagent at a concentration of 0.01 M, then the volume was supplemented with distilled water to the mark against its mock solution.The highest absorption of the product was 0.674 at the laboratory temperature at 462 nm, while its mock solution did not give any absorption in this region, as shown in figures (1-3), (2-3).

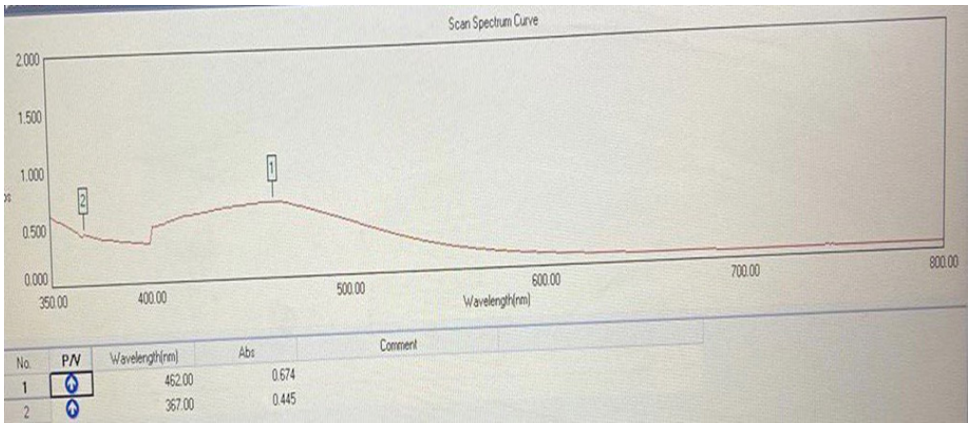

Fig. (1-3): The absorption spectrum of Meth – p-Nitro against Blank

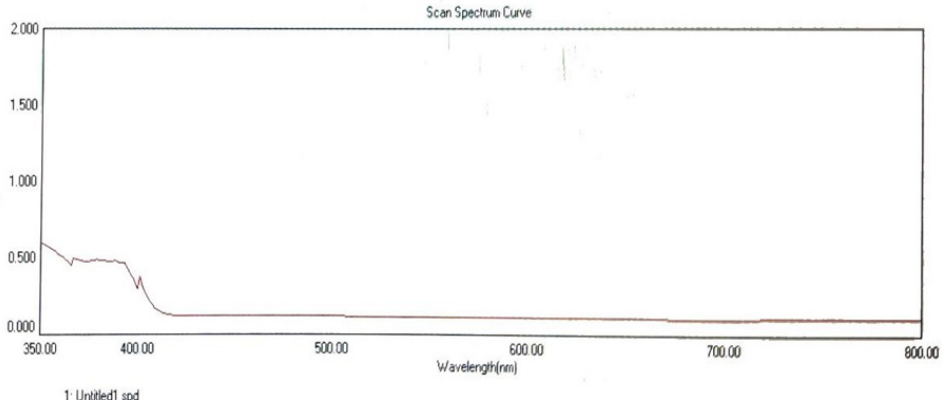

Fig. (2-3): Absorption spectrum of blank versus solvent

2.3 Study the optimal conditions for product formation

3.3 Effect of bases type

Different types of bases have been used, namely (NaOH, KOH, and NH_4OH) and they were all at a concentration of 1 molar and added a volume of 0.5 ml, in order to find out which of the bases used give the best absorption when forming the product, as shown in Table (2-3).

Table (3-2): The effect of the type of base on the absorption values of the product

Base	Abs.
KOH	0.678
NH_4OH	0.353
NaOH	0.561

From the table, it is clear that the use of Potassium hydroxide gives the highest absorption value.

4.3 Effect of the base volume

Increasing amounts of Sodium hydroxide solution were added with an initial concentration of 1 molar, to see its effect on the absorption values of the product formed, as shown in Figure (3-3), where it was found that the best volume added was 0.5 ml, which gave the best absorption value.

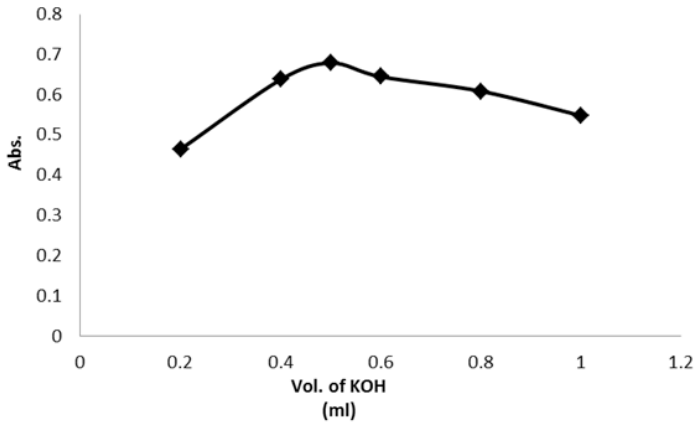

Fig. (3-3): Effect of the base volume

5.3 Effect of reagent volume

Different volumes of p-Nitrobenzaldehyde reagent were added at an initial concentration of 0.01 M.Where it was found that the best absorption is when adding a volume of 1 ml, as shown in Figure (3-4).

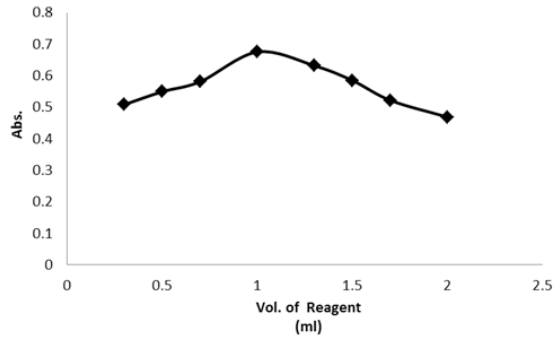

Fig. (3-4): Effect of reagent volume

6.3 Effect of time on the stability of the formed product

Due to the importance of the stability of the product formed from the interaction of the drug in an alkaline medium with the reagent, to know the period during which the product formed can remain stable, and through the experiments that were conducted, it was observed that the absorption values stabilized to approximately 50 minutes, and this time is sufficient to perform the required measurements And according to what is shown in Table (3-3).

Table (3-3): Stability of the formed product

Time (min.)	Direct	10	20	30	40	50	60
Abs.	0.668	0.671	0.673	0.675	0.674	0.670	0.667

7.3 Effect of Additives

The results obtained from studying the effect of adding an increase in concentrations of additives with the drug in the form of tablets indicated that these materials did not affect the absorption values when forming the product with a reagent p-Nitrobenzyldehyde , Where two concentrations were added to each of the additives, We conclude from this that the proposed method can be successfully applied to pharmaceutical preparations, as shown in Table (3-4).

Table (3-4) Effect of additives

RE%	Concentration (µg/ml)	RE%	Concentration (µg/ml)	Additives
-2.67	600	-0.74	300	**Lactose monohydrate**
-0.59	600	-2.75	300	**Magnesium stearate**
-2.89	600	-3.38	300	**starch**
-1.75	600	-1.23	300	**cellulose**

It was found that the detection limit value = 0.551 µg/mL, and the quantity limit = 1.67 µg/mL Determination of product stoichiometry 73-To determine the ratio of binding between methyldopa and the reagent p-Nitroe benzaldehyde in the product formed spectrophotometrically, two methods were applied, namely the molar ratio and the method of continuous changes, the first method was carried out by adding increasing volumes $(0.3\text{-}2)10^{-4}$ × ml of the p-Nitrobenzaldehyde reagent with an initial concentration of 1×10^{-3} M to a fixed volume of 1 ml of methyldopa with an initial concentration of 1×10^{-3} M in a volume of 10 ml, and this was done with the same steps previously installed and under the same optimal conditions for the standard curve, and the results shown in Figure (3-6) were obtained.

8.3 Calibration curve

It was shown from the calibration curve (MD) with the organic reagent (p-Nitrobenzaldehyde) and it was linear at concentrations between (6-150 mcg/ml). As shown in the figure below.

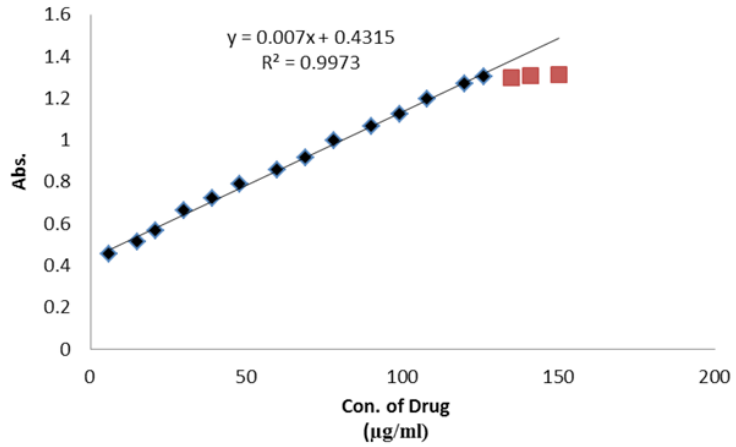

Fig. (3-5): Calibration curve for Meth-p-Nitro.

9.3 Accuracy and precision

The accuracy of the method represented by the relative error RE% and the precision of the method represented by the relative standard deviation %RSD were calculated using the following equations, by taking three different concentrations of the drug and at the same optimal conditions that were established in the working method and it was shown through the results and proven in Table (3-5) The method used has good accuracy and precision.

Table (3-5): Accuracy and precision

RSD%	RE%	Concentration (µg/ml)
.330	0.27-	15
0.28	0.31-	30
0.27	0.23	48

It was found that the detection limit value = 0.551 µg/ml, and the quantity limit = 1.67 µg/ml

10.3 Correlation ratio

Under optimal conditions, the "equivalent interaction" between (MD) and the (p-Nitro) reagent was studied by the correlation ratio at the initial concentration ($10^{-3}\times1$), where the valence of the drug with the reagent was 1:1 as shown in Figures (3-7).

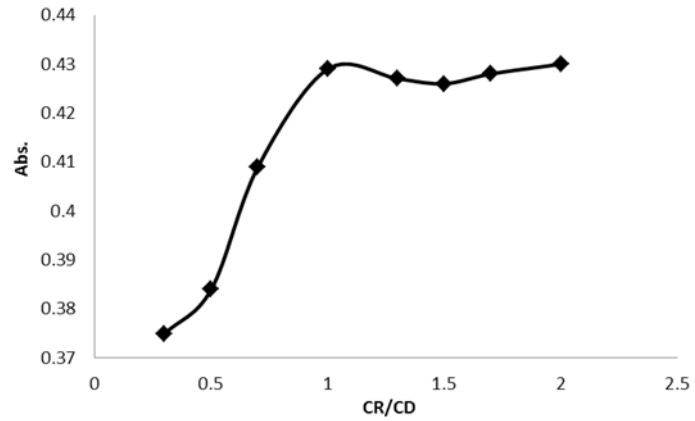

Fig. (3-6): Molar ratio curve for Meth-p-Nitro.

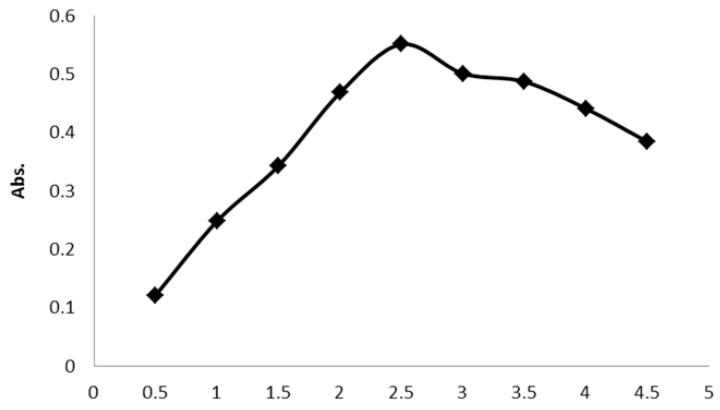

Fig. (3-7): Continuous Change Curve of Meth-p-Nitro product

11.3 Applications

The proposed method has been applied for the determination of methyldopa in a pharmaceutical preparation in tablet (3-4).

Table (3-4): Applications

Manufacture company	Rec.%	Obtained by the proposed method (mcg/ml)	intake (mcg/mL)	Pharmaceutical preparation
Accord	97.47	14.62	15	Mathyldopa
	100.8	30.24	30	
	99.75	47.88	48	
ALGORITHM S.A.L Lebanon	100.5	15.07	15	Aldomet
	97.40	29.22	30	
	99.02	47.53	48	

12.3 Conclusions

This method described is fast, simple and economical and does not require special conditions to work in contrast to other methods. This procedure showed a shorter reaction time. As well as stable colored species with an inexpensive reagent. The procedure can be done at room temperature without heating. Where it can be to apply the proposed method in order to determine (MD) in the pharmaceutical preparation in the form of (tablets).

References

1. Gotardo, M. A., Lima, L. S., Sequinel, R., Rufino, J. L., Pezza, L., & Pezza, H. R. (2008). A simple spectrophotometric method for the determination of methyldopa using p-chloranil in the presence of hydrogen peroxide. Eclética Química, 33, 7-12.
2. 2-Chobanian, A.V.; Bakris, G.L.; Black, H.R.; Cushman, W.C.; Green, L.A.; Izzo, J.L.; Jones, D.W.; Materson, B.J.; Oparil, S.; and Wright, J. M. A. Gotardo, L. S. Lima, R. Sequinel, J. L. Rufino, L. Pezza and H.

R. Pezza, A Simple spectrophotometric method for the determination of methyldopa using pchloranil in the presence of hydrogen peroxide., EcléticaQuímica, 2008, 33, 7-12T. (2003). Seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. Hypertension, 42(6), 1206-1252.

3. Tveden-Nyborg, P., Bergmann, T. K., & Lykkesfeldt, J. (2018). Basic & clinical pharmacology & toxicology policy for experimental and clinical studies. Basic & clinical pharmacology & toxicology, 123(3), 233-235.
4. Gilman, A. G., "The Pharmacological Basis of Therapeutics", 1996, 9th ed., McGraw-Hill, New York, 211.
5. Beitollahi, H., Tajik, S., Asadi, M. H., & Biparva, P. (2014). Application of a modified graphene nanosheet paste electrode for voltammetric determination of methyldopa in urine and pharmaceutical formulation. Journal of Analytical Science and Technology, 5(1), 1-9.
6. Ensafi, A. A., Saeid, B., Rezaei, B., & Allafchian, A. R. (2015). Differential pulse voltammetric determination of methyldopa using MWCNTs modified glassy carbon decorated with NiFe 2 O 4 nanoparticles. Ionics, 21, 1435-1444.
7. Emara, S., Masujima, T., Zarad, W., Kamal, M., Fouad, M., & El-Bagary, R. (2015). A combination of isocratic and gradient elution modes in HPLC with the aid of time-overlapping process for rapid determination of methyldopa in human urine. Journal of Liquid Chromatography & Related Technologies, 38(2), 153-162.
8. Molaakbari, E., Mostafavi, A., & Beitollahi, H. (2014). First report for voltammetric determination of methyldopa in the presence of folic acid and glycine. Materials Science and Engineering: C, 36, 168-172.
9. Al-Kalissy, R. S., & Mohammed, A. K. Spectrophotometric method for the determination of methyldopa by oxidizing coupling reaction with 4-aminoantipyrine.
10. Bahgat, E. A. (2018). Spectrophotometric Microdetermination of Methyldopa and Etilefrine Hydrochloride using Copper (II)-Neocuproine Reagent in Pure Form and Pharmaceutical Formulations. Scholars Research Library.
11. Ali, S. A., & Sami, M. A. (2005). Cyclic voltammetric study of alpha-methyldopa at carbon paste electrode. Pakistan Journal of Pharma-

ceutical Sciences, 18(1), 6-17.

12. Najafi, K., Asadpour-Zeynali, K., & Mollarasouli, F. (2020). Preparation of A Magnetic Nanosensor Based on Cobalt Ferrite Nanoparticles for The Electrochemical Determination of Methyldopa in The Presence of Uric Acid. Combinatorial Chemistry & High Throughput Screening, 23(10), 1023-1031.
13. Tubino, M., Batista, D. C., & Rodrigues, J. A. R. (2006). Kinetic Method for the Determination of α-Methyldopa in Pharmaceutical Preparations: Analytical Procedure and Reaction Mechanism Considerations. Analytical letters, 39(2), 327-339.