Metoclopramide as a coupling agent for the Kinetic - spectroscopic assay of methyldopa drug

Rusul Mazin Kaddouri[1], Sahar Rihan Fadhel[2]*
[1], [2]College of science/Diyala University/Iraq
Email: saharraihan@uodiyala.edu.iq

Abstract
A spectrophotometric method has been proposed for the determination of methyldopa in pharmaceutical dosage forms. The method is based on the diazotization reaction of metoclopramide hydrochloride (MCH) and coupling of the diazotized reagent with methyldopa (MDP) to give intense green colored product during this reaction is measured at 465 nm as a function of time. Variable parameters such as temperature, reaction time and concentration of the reactants have been analyzed and optimized. The kinetic study involves initial rate and fixed time (20 minutes) procedures for constructing the calibration graphs to determine the concentration of MDP. Under the proposed optimum condition, Beer's law was obeyed in the concentration range of 1-60 µg mL-1. The good correlation coefficients and low relative standard deviation assert the applicability of this method. The procedure was characterized by its simplicity with accuracy and precision.
Keywords: methyldopa, Diazotization coupling reaction, Kinetic - spectroscopic, pharmaceutical dosage form.

1. Introduction

Methyldopa (2 S) - 2 - Amino - 3 - (3, 4- dihydroxyphenyl) - 2 -methylpropanoic acid. fig. (1) is an alpha-adrenergic agonist (selective for α2-adrenergic receptors) psychoactive agent. [d as a sympatholytic or antihypertensive. Its use is now mostly deprecated following the introduction of alternative safer classes of agents. [1,2] However, it continues to have a role in otherwise difficult to treat hypertension and gestational hypertension (previously known as pregnancy-induced hypertension). Methyldopa is an important feature in pharmaceutical and clinical procedures Methyldopa is a catecholamine derivative. Methyldopa is an antihypertensive agent that is used in the treatment of high blood pressure or hypertension, especially when it is complicated with renal disease. Its antihypertensive properties are primarily due to its action on the central nervous system. Methyldopa inhibits the enzyme DOPA decarboxylase, which converts L-DOPA into dopamine, and is a precursor for norepinephrine and subsequently epinephrine. It is converted to α-methyl norepinephrine in adrenergic nerve terminals, and its antihypertensive action appears to be due to its stimulation of central adrenal receptors, which reduces sympathetic tone and produces a fall in blood pressure. The therapeutic concentration of methyldopa in human plasma is usually in the range of 0.1 to 0.5 mg.L−1, and its average terminal elimination half-life is (2 h).[2-4]

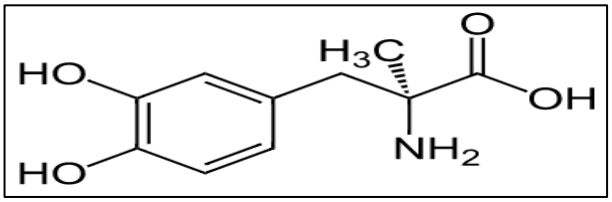


Figure. (1) The Chemical structure of Methyldopa

For the examination of methyldopa in bulk, pharmaceutical form, or biological fluids, a variety of analytical techniques have been reported. These techniques include: spectrophotometry [5-10] and flow injection spectrophotometry [11], high-performance liquid chromatography (HPLC) [12-14], thin-layer chromatography [15], GLC [16], Voltammetry [17,18] titrimetry [19,20], electrophoresis [21], NMR [22] , However, some of these methods are time consuming and/or require expensive equipment and conditions. The method that has been proposed is based on the reaction of the methyldopa drug with diazotized metoclopramide hydrochlorid (DMCH) to form a green water-soluble dye product, which has a maximum absorption at 465nm.

2. Experimental

2.1. Equipment and Materials

A double beam Jasco V-530 UV/vis spectrophotometer. Japan, was used for all spectral and absorbance measurements, Equipped with a 1cm quarts cell.
Hot plate (Lab. Companion, BS - 11).
Electronic balance (Sartorius AG GÖTTINGEN B2 2105 Germany).
Methyldopa and metochlopramide reference materials were obtained from SDI/Samarra/ Iraq, labeled to contain 99.86% w/w. methyldopa. Pharmaceutical formulations containing methyldopa were obtained locally from different sources.
All of the chemicals were analytical grade or general-purpose reagents obtained from various sources.

2.2. Solutions

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

A 0.1000 gm of pure methyldopa (SDI) was dissolved in distilled water then completed to 100 mL in a

doi.org/10.31838/hiv23.02.169 Received: 17.10.22, Revised: 15.11.22, Accepted: 16.12.22.

volumetric flask with the same solvent. More dilute solutions were prepared by suitable dilution of the stock standard solution with distilled water.

2.Diazotized metoclopramide reagent solution (0.04M) [23]:

1.198 g of metoclopramide was dissolved in 30 mL distilled water with continuous stirring and heating until complete dissolution. Then, 8.4mL of 11.8M HCl is added with continuous stirring, and the mixture is transferred into a brown 100mL-volumetric flask, cooled to 0 - 5 oC in an ice – bath. A 0.274g of NaNO2 is added and the mixture is stirred vigorously. ten minutes later, the solution is made up to final volume with cold water. The solution is stable for at least 48 hours when it is stored in a refrigerator.

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

The content of 10 formulated tablets was accurately weighed and powdered. A portion of the powder equivalent to 0.0860 g, 0.0882 and 0.0940 g for methyldopa (Iraq, UK and Lebanon) respectively, (containing 0.05g of methyldopa) was accurately and separately weighed. dissolved in 10ml distilled water and stirred for 10 min to ensure complete dissolution of the drug, then transferred into 100mL volumetric flask and diluted to the mark with some solvent to get 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.

2.3. General procedure for Calibration curve

In calibrated 10 mL volumetric flask, 1mL of different aliquots containing (10-1000)µg of MDP were added, 1mL of 0.04M of the diazotized metoclopramide (DMCH) solution with shaking and the flasks were left to stand for 5 minutes at 70 °C. The volumes were completed to the mark with distilled water. The absorbance of the colored azo dye was measured after 5 minutes at 465.0 nm against the reagent blank. The colour formed is stable for at least one hour.

2.4. General procedure for Kinetics of the reaction

Aliquot of 1 mL of the standard MDP solution containing various amount (4.0-240.0) µg of MDP was added conical flask 1mL of 0.04M diazonium salt (DMCH) was added. The flask was left to stand for 5 minutes at 70 °C, 2mL of distilled water has been added, then after transferred into 4 mL cuvette. The absorbance of this solution was immediately measured at 465nm. The value of absorbance was recorded at 1 s intervals for 20 minutes (0 and 1200)s.

3. Results and Disscution

1. Absorption spectra

The selection of analytical wavelength, the test was done by adding 1.0mL containing 200 µg of MDP in 10 mL-volumetric flask and 1.0mL of 0.02M diazotized metoclopramide. Then the mixture was left at 50 °C for 5 minutes, followed by dilution with distilled water. The colored product was recorded and scanned in the spectrum mode from (750 to 350) nm. The azo dye shows maximum absorption at 465nm in contrast to the reagent blank and it was used in all subsequent experiments, Figure (2).

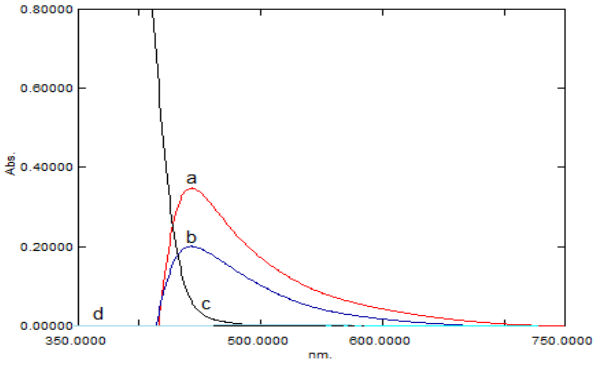


Figure (2): Absorption spectra: a. 20 µg. mL.-1 of MDP against reagent blank under the optimum conditions, b. 20 µg. mL.-1 of MDP under initial conditions, c. the reagent blank measured against distilled water, d. 20 µg.mL-1 of MDP. only against distilled water.

2. Optimization of the Experimental Conditions

2.1. Effect of Reagent Concentration

The effect of various concentrations of DMCH in the range of (0.005 to 0.07) M was investigated while keeping the other conditions constant. It was found that the absorbance increased as the concentration of DMCH increased up to 0.04 M. After this concentration, the absorbance was slightly changed Figure (3); therefore, concentration of 0.04 M was found to be the most suitable concentration for a maximum absorbance and was chosen for further use.

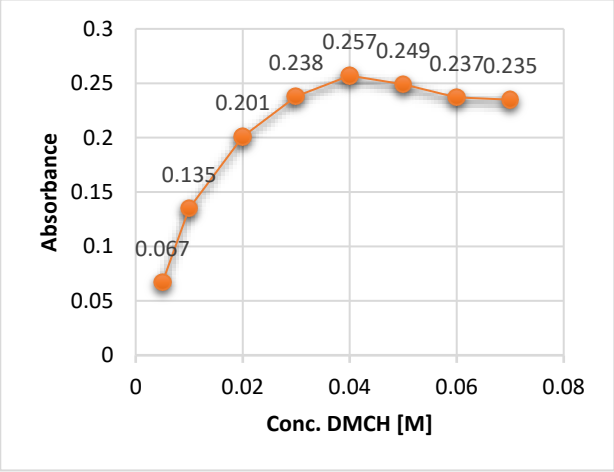


Figure (3): Effect of DMCH concentration on the absorbance of azo dye

2.2. Effect of time on the coupling reaction

A volume 1.0 mL containing 200µg of MDP were transferred in a series calibrated 10 mL volumetric flasks, then 1.0mL of 0.04M the DMCH solution were

add with shaking. The mixtures were left in different periods (0- 20) minutes.

Table (1) shows that the absorbance of the formed azo dye was maximum and stable when the diazotization reaction was let to stand for 5 minutes. Therefore, this period was adopted for diazotization in subsequent works.

Table (1): The effect time on the coupling reaction.	
Time (minute)	Absorbance
1	0.193
5	0.257
10	0.206
15	0.160
20	0.135

2.3. Effect of heating time

The effect of heating time on reaction was determined at different intervals of time. The result in Table (2) shows the coloured azo dye is developed and exhibits maximum intensity after 5minutes, then the absorbance value decreased at a high measure of time.

Table (2): Effect of heating time on absorbance	
Times(minute)	Absorbance
1	0.202
5	0.257
10	0.167
15	0.144

2.4. Effect of temperature on the coupling reaction

The resulting product of the proposed method was studied at different temperatures. It was observed that the reaction continued to increase till 50◦C and the reaction remains stable at 70◦C after which it decreased. The decrease in absorbance at higher temperature was probably due to thermal decomposition of azo-dye [24, 25]. As there was no significant difference between absorbance at 60◦C and 70◦C was chosen as the optimum temperature.Table (3)

Table (3): Effect of temperature on the development of dye	
Temperature	Absorbance
30	0.193
40	0.206
50	0.257
60	0.329
70	0.347
80	0.235
90	0.104

2.5. The stability of the developed dye

The effect of time on the development and stability period of the formed dye is investigated under the optimum conditions of the reaction. The maximum color intensity is reached after mixing the components of the reaction, and the absorbance of the formed dye remained constant for at least 60 minutes. Figure (4).

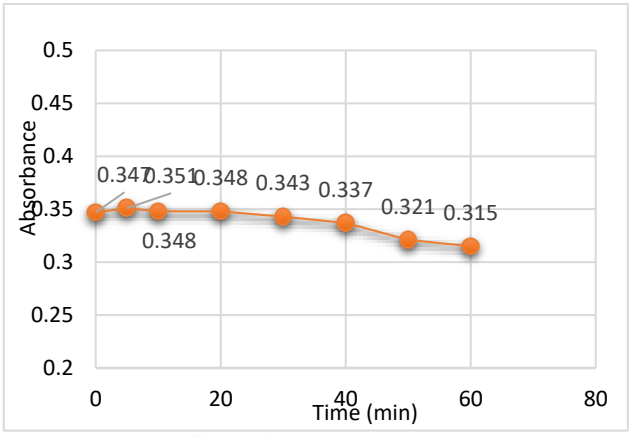


Figure (4): Effect of time on color development.

3. Calibration graph and analytical data

After optimization of all parameters effective on the product for determination of MDP, a linear relationship between the absorbance and concentration of the drug was obtained in the range of (1.0-60.0) µg.mL-1 Figure(5). The optical characteristics and statistical data of the regression equations were listed in Table (4).

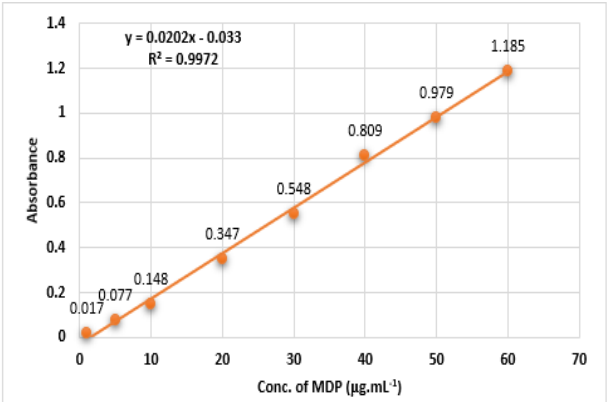


Figure (5): Calibration graph of MDP

Table (4): Optical characteristics and statistical data for the determination of MDP.	
Parameter	Value
λ_{max} (nm)	465
Color	green
Linearity range (µg. mL^{1})	1.0 -60.0
Regression equation	Y=0.0202[MDP.µg/mL]-0.033
Calibration sensitivity (mL. µg^{-1})	0.0202
Correlation coefficient (r)	0.9985
Correlation of linearity (r^2)	0.9972
Molar absorptivity (L.mol-1.cm-1)	4266.5430
Sandell's sensitivity (µg.cm 2)	0.0495
Detection limit (µg. mL 1)	0.2970
Quantification limit (µg. mL 1)	0.9100

3.4. The nature of the formed MDP-DMCH

The stoichiometry of the color dye is studied under the established conditions by applying Job's continuous variation methods and molar ratio. Figures (6) and (7) show the experimental data in both methods that the resulted product has been formed by a **1:1** combining ratio of MDP to the reagent. Scheme (1) shows the suggested mechanism of the reaction.

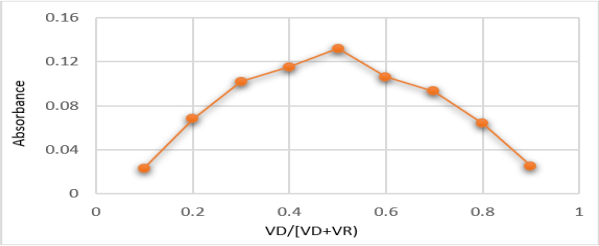


Figure (6): Continuous variation method

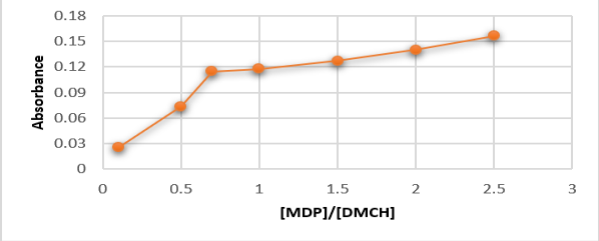
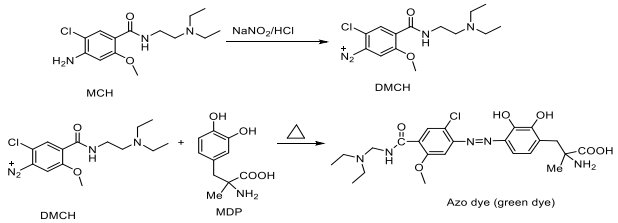


Figure (7): Mole ratio method.



Scheme (1): The suggested reaction mechanism

3.5. Kinetics of the reaction

Verification of reaction order

The rate of the reaction was studied to determine its order according to following equation:

$$\text{Rate} = k'(\text{MDP})^{n}$$

Where: k' and n represent the rate constant, and the order of the reaction.

This was accomplished by using different concentrations (5.0-60.0) µg.mL-1 of MDP solution and a constant concentration 0.04M of DMCH under the optimized conditions. It was found that the reaction rate is (MDP) dependent. Graphs on Figure (8) shown that reaction rate is increased with the increasing (MDP) concentration.

Measurements carried out by variable time method, could be used for rate (in terms of ΔA/Δt) estimation. The rate equation could also be expressed in logarithmic form as:

$$\log \text{rate} = \log\frac{\Delta A}{\Delta t} = \log k' + n\log(\text{MDP})$$

Where: A is the absorbance, and t is the measuring time.

Regression least square plot of log [MDP] versus log (rate) is shown in Figure (10). The regression equation is:

$\log(\text{rate}) = 0.1628\log[\text{MDP}] + 1.0505$, with correlation coefficient (r) = 0.9993. Accordingly, the value of k'= 79.3302 s-1 and the reaction is pseudo-first order since the value of n equals to 1.3019 (≈1).

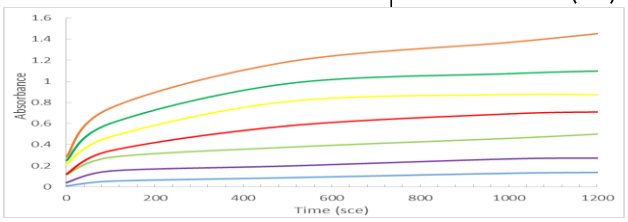


Figure (8): Absorbance versus time for the reaction of different concentration of MDP

3.6. Fixed time method

In the fixed time method, varying amounts of MDP were used for determination of reaction rate at a preselected fixed time (1, 5, 10, 15, and 20) minute and the absorbance values were measured and plotted against [MDP].

The low values of standard deviation (SD), limit of detection (LOD) and limit of quantification (LOQ) are given in Table (4). A fixed time of 10 minute indicates that this method could successfully applied for determination of MDP in its pure form and in pharmaceutical preparations, Figure (8).

3.7. Accuracy and Precision

The accuracy and precision of the determination of MDP were studied depending upon the value percentage of the Er%, and RSD % respectively. For three replicates of each concentration of MDP. The results in Table (8) show a good accuracy and precision.

Table (5): Accuracy and precision of the fixed time method			
Conc. of MDP µg. mL 1		accuracy and precision	
Taken	Found*	RE%	RSD%
5.00	5.016	0.320	0.050
10.00	10.008	0.080	0.089
20.00	20.015	0.075	0.046
*Average of three measurements			

3.8. Effect of Interferences

To evaluate the selectivity of the proposed method for the analysis of pharmaceutical preparations containing MDP, the interfering were examined by determining MDP in the presence of the interference and applying the proposed analytical procedure. The excipients studied were (starch, glucose, sucrose, lactose and magnesium stearate). For this study, solution was containing MDP and each one of the excipients was taken separately in concentrations ten-times greater than that of MDP were analyzed. Under the reaction conditions used all of them do not interfere as shown in table (6).

Table (6): Effect of excipients on the recovery of MDP of the fixed time method			
Excipients	Conc. of excipients. Taken (µg. mL-1)	MDP Conc. Taken (10.0 µg.mL-1) Recovery %	
		Found (µg. mL 1)	Recovery%
Starch	1000.0	10.044	100.440
Glucose		9.962	99.620
Sucrose		9,991	99.910
Lactose		9.970	99.700
Mg-stearate		10.050	100.500

3.9. Pharmaceutical Applications

The proposed method was successfully applied for the determination of MDP in tablets by the analysis of two concentrations for each sample Table (7) using the recommended procedure.

Table (7): Applications of proposed methods to determine MDP in tablet formulations of the fixed time method.

Pharmaceutical	Assay (mg/tablet)		Conc. (µg.mL-1)		Recovery*%	S.D*	RSD*%
	Spiked	Found	Taken	Found*			
Aldosam S.D.I.-Iraq	250	249.075	10	9.963	99.630	0.015	0.150
		249.400	20	19.952	99.760	0.017	0.085
Methyldopa Bristol- UK	250	250.750	10	10.030	100.300	0.006	0.059
		250.637	20	20.051	100.255	0.010	0.049
Aldomet Algorithm-Lebanon	250	249.55	10	9.982	99.820	0.014	0.140
		249.375	20	19.950	99.750	0.027	0.135
*Average of three measurements.							

References

The British Pharmacopeia (B.P) Medicinal and Pharmaceutical Subatance,Vol 1,(2020).

Elsevier, "Mosby's Medical Dictionary", 8th edition. (2016)

E. A. Gadkariem a, K. E. E. Ibrahim, N. A. A. Kamil a, M. E. M. Haga and H. A. El-Obeid "A new spectrophotometric method for the determination of methyldopa", King Saud University, Saudi Pharm. J., v. 17, p. 289 - 293, (2009).

M. A. AL-Da'amy and R. F. AL-Moswi, "Spectrophotometric Determination of Methyldopa in Pharmaceutical Preparation via Oxidative Coupling Organic Reaction", Chem. and Materials Research, v.3, No.5, (2013).

Mohamed Y. Dhamra and Theia'a N. Al-Sabha, Spectrophotometric Method for Indirect Determination Of Antihypertensive Drugs In Pharmaceuticals, Egypt. J. Chem. Vol 63, No. 10 pp. 3767 - 3777 (2020)

Qabas Naji Rashid, Mohsin Hamza Bakir, Shirwan Omar Baban. Spectrophotometric determination of Methyldopa in pure form
and in the pharmaceutical preparations. Tikrit Journal of Pharmaceutical Sciences 11(1), 67-77, (2016).

F. Rashwan and AL-Moswi, "Spectrophotometric Determination of Methyldopa in Pharmaceutical Preparation Via Oxidative Coupling Organic Reaction with Para-Phenylenediamine in the Presence of Potassium Periodate", Handbook on the Emerging Trends in Scientific Research 132, Malaysia, ISBN: 978-969-9347-16-0, (2014).

P. R. Ribeiro and R. M. Duarte, "Development and validation of a simple spectrophotometric method for the determination of methyldopa in both bulk and marketed dosage formulations", Brazilian J. of Pharm. Sci., v. 50, No. 3, (2014).

Gadkariem, E. A., Ibrahim, K. E., Kamil, N. A., Haga, M. E., & El-Obeid, H. A. A new spectrophotometric method for the determination of methyldopa. Saudi pharmaceutical journal: SPJ: the official publication of the Saudi Pharmaceutical Society, 17(4), 289–293. (2009).

Poliakov A.E. Dumshakova A.V. Muginova S.V. Shekhovtsova T.N.; A peroxidase-based method for the determination of dopamine, adrenaline, and α-methyldopa in the presence of thyroid hormones in pharmaceutical forms; Talanta; 84: 710–716. (2011)

M. Q. Al-Abachi and et al; Batch and Flow-Injection Spectrophotometric Determination of Methyldopa Using Metochlopramide as diazotized Chromogenic Reagent; Iraqi National Journal of Chemistry,2013, volume 49, 12- 24.

A. A. Majidano, N. A. Sodho, G. N. Rajper, M. Alamgir, M. Y. Khuhawr, "Liquid Chromatographic Determination of Dopamine, Methyldopa, L-dopa and Tyrosine in pharmaceutical preparations using 4-Dimethylaminebenzalaldehyde as a Derivatizing Reagent", Sindh Univ. Res. J. (Sci. Ser.) v. 46, No.1, p. 77-82 (2014).

M.V.L. Sahithi, V.P. Chandra K Sekhar, P. V. Babu, "Development and Validation of RP-HPLC method for simultaneous estimation of Methyldopa and Hydrochlorothiazide in Pharmaceutical Dosage Form", J. Adv. Pharm. Edu. & Res., v.3, Issue 4, (2013).

R. Ribeiro, J. Gasparetto, R. Vilhena, T. Francisco, C. Martins, M. Cardoso , R. Pontarolo, " Simultaneous determination of levodopa, carbidopa, entacapone, tolcapone, 3-O-methyldopa and dopamine in human plasma by an HPLC-MS/MS method", v. 7, Issus2, p 207-20, (2015).

Martens J. Guenther K. Schickedanz M.; Resolution of optical isomers by thin-layer chromatography: enantiomeric purity of methyldopa; Archiv der Pharmazie, (1986); 319: 572–574.

Watson J.R. Lawrence R.C.; Specific quantitative gas-liquid chromatographic analysis of methyldopa and some foreign related amino acids in raw material and commercial tablets; Journal of Chromatography; 103: 63–70. (1975)

H. Beitollahi, S. Tajik, M. H. Asadi and P. Biparva, "Application of a modified graphene nanosheet paste electrode for voltammetric determination of methyldopa in urine and pharmaceutical formulation", J. of Analytical Sci. and Technology, v. 5, No. 29, (2014).

E. Molaakbari, A. Mostafavi, H. Beitollahi, "First report for voltammetric determination of methyldopa in the presence of folic acid and glycine", Mater Sci. Eng. C Mater Biol. Appl. v. 1, No 36, p 168-72, (2014).

Pathak V.N. Shukla S.R. Shukla I.C.; Direct titrimetric determination of the antihypertensive drugs methyldopa and propranolol in pharmaceutical preparations; Analyst, (1982); 107: 1086–1087.
Bagheri, A. and I. Mesgarzadeh, Titrimetric and Spectrophotometric Methods for the Determination of β-blockers in Pharmaceutical Dosage Form. Journal of Applied Chemical Research. 6(2): p. 53-64(2012)
Ayad Kadhim Fadhila, Mohammed Jasim M. Hassan and Ashraf Saad Rasheed, A Comparative Review of Methods for Estimation of Some Antihypertensive Drugs in Pharmaceutical Production, Egypt. J. Chem. Vol. 64, No. 11 pp. 6301 - 6321 (2021).
Zahra Talebpoura, Soheila Haghgoob and Mojtaba Shamsipurc, 1H nuclear magnetic resonance spectroscopy analysis for simultaneous determination of levodopa, carbidopa and methyldopa in human serum and pharmaceutical formulations, Analytica Chimica Acta, Volume 506, Issue 1, 17, Pages 97-104. (2004)
Sahar Rihan Fadhel, et al, A Developed Method for the Estimation of Diclofenac Sodium via Coupling with Diazotized 4-Aminoacetophenone, Egypt. J. Chem. Vol. 64, No. 7 pp. 3703 - 3709 (2021).
M. M. Al-Rufaie, "New spectrophotometric method for the determination of sulfamethoxazole drug.", 172-183, (2016).
F. Shamsa, and L. Amani, "Determination of sulfamethoxazole and trimethoprim in pharmaceuticals by visible and UV spectrophotometry." Iranian Journal of Pharmaceutical Research; 31-36. (2010).