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Spectrophotometric Determination of Methyldopa in Pharmaceutical Preparation Via Oxidative Coupling Organic Reaction with Para-Phenylenediamine in the Presence of Potassium Periodate

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Abstract

A simple, accurate and sensitive spectrophotometric method for the determination of Methyldopa in pure and pharmaceutical preparations has been developed .The proposed method uses Para-phenylenediamine as anew chromogenic reagent . The method is based on the oxidative coupling reaction of Methyldopa with Para-phenylenediamine with potassium periodate in neutral media to form a red water soluble dye product , that has a maximum absorption at λmax 494 nm . Linear calibration graph was in the range of (0.1–10.0) µg.ml^{-1} with molar absorptivity of (0.61 ×10^{5}) L.mol^{-1}.cm^{-1} ,a sandall sensitivity of (3.91 ×10^{-6}) µg.cm^{-2} , correlation coefficient of (0.9996) , detection limit (0.025) µg.ml^{-1} and the relative standard deviation of RSD% (0.99 %) . The method was applied successfully for the determination of Methyldopa in pharmaceutical preparations and the value of recovery % was better than (100.1%).

Keywords: Methyldopa drug, Spectrophotometric determination, Pharmaceutical preparation.

1. Introduction

Methyldopa (α-methyl-3,4-dihydroxyphenylalanine), is a catecholamine derivative widely used in the control of moderate and severe arterial hypertension. Methyldopa is considered a prodrug since it acts mainly due to its metabolism in the central nervous system to α-methylnorepinephrine, a a_2-adrenergic agonist [(1)]. Several methods have been proposed to quantify Methyldopa in pharmaceutical formulations, including high-performance liquid chromatography (HPLC) with fluorescence detection [(2)], colorimetry [(3,4)], GLC [(5)], titrimetry [(6)], electrophoresis [(7)] ,NMR[(8)], thin layer [(9)],voltammetry[(10,11)],spectrophotometry[(12-21)] and flow injection spectrophotometry [(22-24)]. Oxidative coupling organic reactions seems to be one of the most popular spectrophotometric methods for the determination of several drugs such as sulphonamids[(25)] , paracetamol[(26)], phenylephrine HCL[(27)],methyldopa[(28)] and folic acid[(29)].The proposed method is based on the reaction of the

methyldopa drug with Para-phenylenediamine in the presence of potassium periodate in neutral medium to form an red water soluble dye product which shows an absorption maximum at 494nm

2. Experimental Parts Apparatus

All spectral and absorbance measurement were carried out in a Double beam UV-Vis spectrophotometer-1800 . Equipped with a 1 cm quarts cell .
- Water bath(Lab. Companion , BS - 11) .
- Electronic balance (Sartorius AG GÖTTINGEN B2 2105 Germany) .

3. Reagents

All chemicals used were of analytical-reagent grade .
-Stock solutions from drug (100 $\mu g.ml^{-1}$) of Methyldopa(SDI - Iraq) were prepared by dissolving 0.01gm of Methyldopa in distilled water and diluting to the mark in 100 ml volumetric flask .Working solutions were prepared by diluting the solution in distilled water.
- Para-phenylenediamine (0.01M) stock solution was prepared by dissolving 0.0540gm of Para-phenylenediamine in distilled water and completed the volume to 50 ml in a volumetric flask with distilled water.
- potassium periodate(0.005M) stock solution was prepared by dissolving 0.115gm of KIO_4 in distilled water and diluting to the mark in 100 ml volumetric flask .

4. Recommended Procedure

In to a series of 25 ml volumetric flask , transfer increasing volume of Methyldopa solution(100$\mu g.ml^{-1}$) to cover the range of calibration curve (0.1– 10.0)$\mu g.ml^{-1}$, added (2.5) ml from (1.0 $x10^{-3}$M) of Para-phenylenediamine and shake well . Added (2.0)ml from (4.0$x10^{-4}$)M of KIO_4 , dilute the solution to the mark with distilled water , and allow the reaction to stand for 10 min at room temperature (25)^{o}c . measure the absorption at λmax(494 nm) against a reagent blank prepared in the same way but containing no Methyldopa.

5. Procedure for Pharmaceutical Preparations

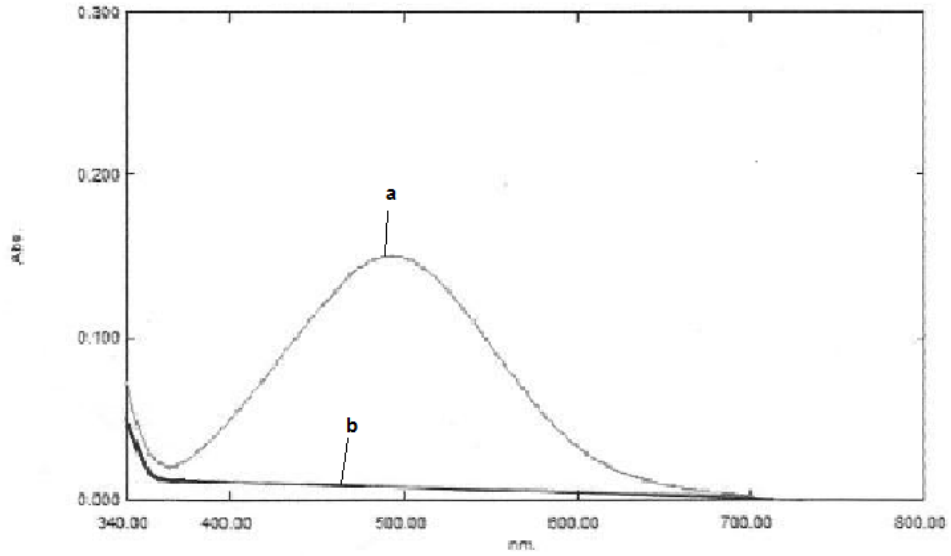
Aldomate tablets, provided from (SDI) Samara-Iraq and from ASIA - Syria (10) tablets were grinded well and acertain portion of the final powder was accurately weighted to give an equivalent to about 10 mg of Methyldopa was dissolved in distilled water . The prepared solution transferred to 100 ml volumetric flask and made up to the mark with measured against blank solution forming a solution of 100$\mu g.ml^{-1}$ concentration . The solution was filtered by using a Whitman filter paper No. 42 to avoid any suspended particles .These solution were diluted quantitatively to produce a concentrations in the range of calibration curve .

6. Results and Discussion

6.1. Absorption Spectra

It was found preliminary that the reaction of Methyldopa with Para-phenylenediamine and potassium periodate in neutral media forming an red water soluble dye product , that has a maximum absorbance at λmax (494 nm) Fig (1) . The reaction can be utilized for the determination of Methyldopa using spectrophotometric method . Initial studies were directed toward optimization of the experimental conditions , in order to establish the most favorable parameters for the determination of Methyldopa.

Fig-1. a-Absorption spectra of (1.25 µg.ml^{-1}) of Methyldopa with Para-phenylenediamine (1.00 x 10^{-3})M , and KIO_4(4.00 x 10^{-4})M at room temperature and measured against blank solution.
b- Para-phenylenediamine measured against distilled water .



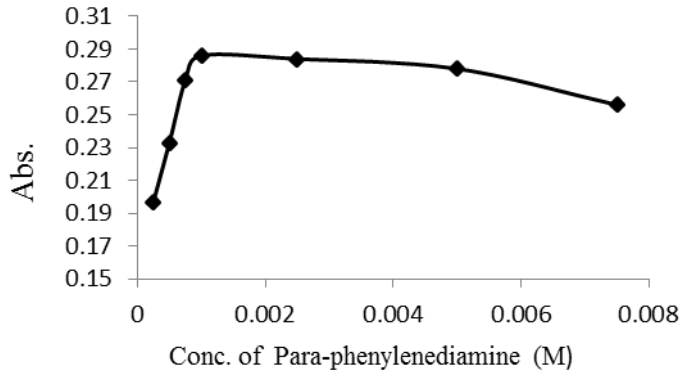
7. Optimization of the Experimental Condition

The influence of various reaction variables such as concentration of reactants , order of addition ,time and temperature were investigated .

8. Effect of Para-Phenylenediamine Concentration

The effects of different concentration of Para-phenylenediamine in the range of (7.5 x 10^{-3} – 2.5 x 10^{-4})M were investigated .A Concentration of (1.0 x 10^{-3})M give the higher absorption intensity at λmax 494 nm for 5.0µg.ml^{-1} of Methyldopa and(2.00x10^{-5}) M of KIO_4 Fig (2) and thus was chosen for further use .

Fig-2. Effect of Para-phenylenediamine Concentration on Absorption spectra of (5.0 µg.ml^{-1}) of Methyldopa .

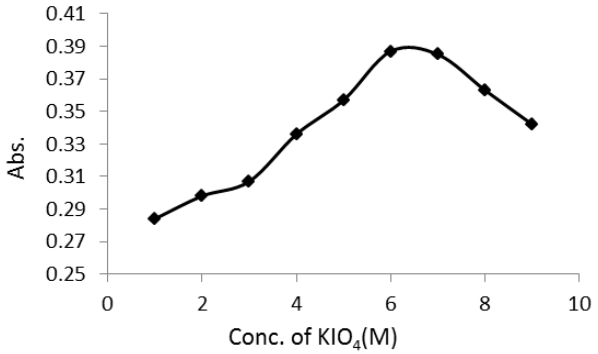


9. Effect of Potassium periodate KIO_4 Concentration

The effect of KIO_4 Concentration in the range of (7.0 x 10^{-4} - 2 . 0 x 10^{-5})M was similarly studied . A Concentration of (4 .0 x 10^{-4}) M of KIO_4 give the higher absorption intensity at λmax 494

nm for (5.00)µg.ml^{-1} of Methyl dopa and (1.0 x 10^{-3}) M Para-phenylenediamine.Fig (3) and thus was chosen for further use .

Fig-3. Effect of potassium periodate KIO_4 Concentration on Absorption spectra of (5.00 µg.ml^{-1}) of Methyl dopa .



10. Order of addition

The effect of order of addition on the absorption intensity of orange water soluble day was studied .

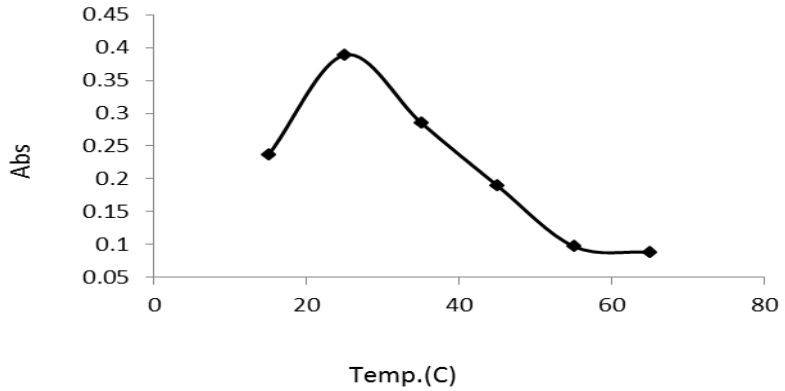
Table-1. Shows the order of addition could be followed , Drug : Para-phenylenediamine: KIO4 . Due to give the higher absorption intensity .

Order of addition	Absorbance at λmax(494)nm
Drug : Para-phenylenediamine: KIO4	0.384
Drug: KIO4 : Para-phenylenediamine	0.321
KIO4 : Para-phenylenediamine: Drug	0.281
KIO4: Drug : Para-phenylenediamine	0.311
Para-phenylenediamine: Drug : KIO4	0.378
Para-phenylenediamine: KIO4 : Drug	0.263

11. Effect of Temperature

The effect of Temperature on the color intensity of the product was studied in practice the highest absorption was obtained when the colored product was developed at room temperature (25ºc) . as shown in Fig (4)

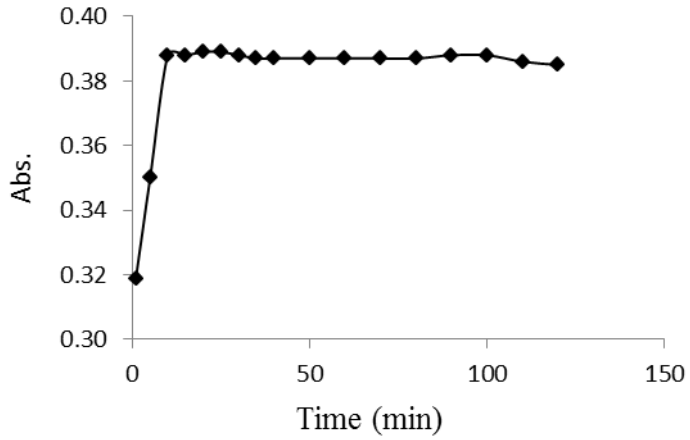
Fig-4. Effect of Temperature on Absorption spectra of (5.00 µg.ml^{-1}) of Methyldopa .



12. Effect of Time

The color intensity reached a maximum absorption after Methyldopa 5.00 µg.ml^{-1} has been reacted with Para-phenylenediamine and KIO_4 at 10 min . Therefore 10 min development time was chosen for further use . The results obtained are shown in Fig(5).

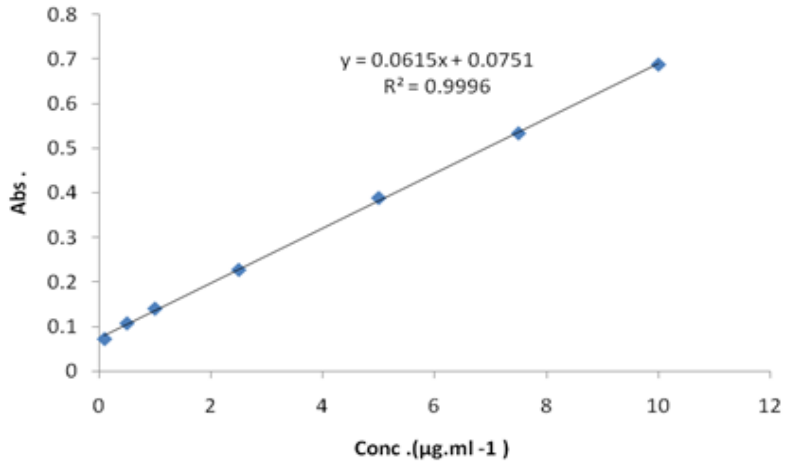
Fig-5. Effect of Time on Absorption spectra of (5.00 µg.ml^{-1}) of Methyl dopa .



13. Calibration Graph

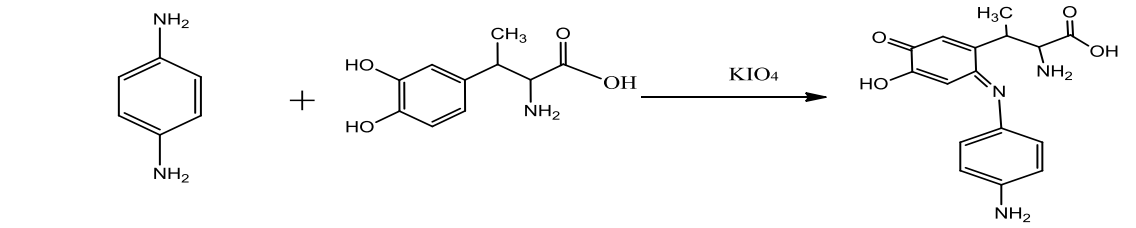
Under the optimum conditions , a linear calibration graph for the determination of Methyldopa was obtained over the concentration range of (0.1– 10.0) µg.ml^{-1}. The linear regression equation for the range of (0.1– 10.0) µg.ml^{-1} Methyldopa is Y=0.0615 X + 0.0751 and correlation coefficient of 0.9996 the linear calibration graph is shown in Fig (6) .

Fig-6. Calibration graph for the determination of Methyldopa.



14. Nature of the Dye Product

The stoichiometry of the reaction between Methyldopa and Para-phenylenediamine was investigated using the mole ratio and Slope ratio method[30-33] under the optimized conditions . The results obtained Fig (7,8) , show a 1:1 drug to reagent product was formed .The formation of the dye may probably be occur as follows :



Para-phenylenediamine Methyl dopa colored product

Fig-7. Mole ratio of reagent to drug .

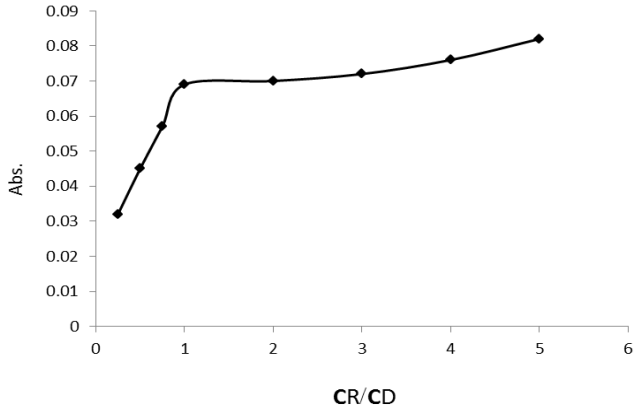
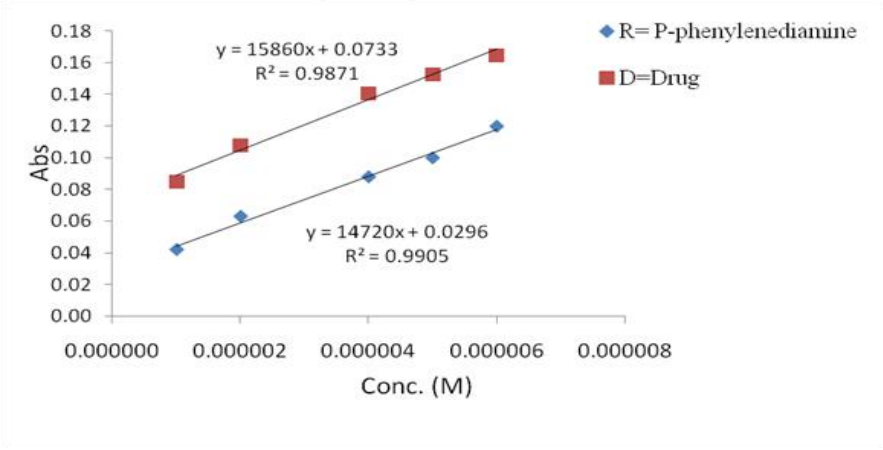


Fig-8. Slope ratio method



(R) Absorbance vers concentration of drug at constant concentration of Para-phenylenediamine .
(D)Absorbance vers concentration of Para-phenylenediamine at constant concentration of drug .

15. Interferences

Several pharmaceutical preparations are associated with flavoring agents, diluents and excipients. Table (2) shows the effect of interfering materials that may be present in pharmaceutical preparations, that indicate no influence effect on the proposed due to the value of recovery% change is less than (± 5.00%) .

16. Analytical Application

The proposed method was applied for the determination of Methyl dopa drug in pharmaceutical preparations. Good accuracy and precision were obtained for the studied drugs . The results obtained were

given in Table 1 which confirm Finally, the proposed method was compared successfully with the standard method Table(3).

Table-2. Influence of excipients and additives as interfering species in the determination of Methyldopa .

Foreign compound	**Recovery%* of 250 µg Methyldopa per µg compound added**				
	100	500	1000	2000	2500
Glucose	100.28	99.48	99.65	99.43	101.49
Lactose	101.23	101.21	100.34	99.77	102.92
Starch	101.38	99.75	101.73	99.63	98.65
Sucrose	102.15	100.38	101.39	101.65	100.60
Sodium chloride	100.19	100.23	101.36	99.80	99.46
EDTA	99.75	101.54	102.12	99.98	98.13
Citric acid	99.43	100.13	99.54	99.64	102.32
Magnesium setarate	102.50	100.38	101.29	101.46	103.11

Table-3. Application of the proposed method for the determination of Methyldopa in pharmaceutical preparations .

Drug sample	Amount of Methyl dopa(µg.ml^{-1})		Proposed Method			Standard Method
	Taken	Found	RSD %*	Error %*	Recovery %*	Recovery %[18]
Pure Methyldopa	2.50	2.55	1.31	+2.00	102.00	98.30
Aldomate (SDI) tablets	2.50	2.53	1.46	+1.20	101.20	
	5.00	4.96	0.63	-0.80	99.20	
	10.00	10.13	0.43	+1.30	101.30	
Aldomate (ASIA) tablets	2.50	2.48	1.42	-0.40	99.60	
	5.00	4.93	0.66	-1.40	98.60	
	10.00	10.16	0.41	+1.60	101.60	

*Average of five determinations .

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