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Indirect Spectrophotometric Method for Determination of Methyldopa in Pure and Pharmaceutical Formulation

Sahar A. Fathi*, Nabeel S. Othman & Amer Th. AL-Taee

Chemistry Department, College of Science, University of Mosul, Mosul – Iraq

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ABSTRACT

Sensitive and accurate spectrophotometric method for the assay of methyldopa (MeD) as pure form and in it is formulations. The method was centred on the oxidation of methyldopa drug with Fe^{+2}ions. The free Fe^{+2} ions react with 4,7-diphenyl-1,10-phenanthroline chromogenic reagent. In the method, the formed Ferron complex is colorimetrically can be monitored at 533 nm versus reagent blank. The linear relationship between various MeD concentrations versus absorbance for each concentration was in the range of 0.25-2.5 µg.mL^{-1} with molar absorptivity 1.3272x10^5 L.mol^{-1}.cm^{-1} for the method. The determination coefficient (R^2) was found to be 0.9902, and the limit of detection (LOD) and limit of quantification (LOQ) were 0.011µg.ml^{-1}and 0.036 µg.ml^{-1}, respectively. The percent recovery from 99.84% to 102.40 %. The suggested procedure could be used for the estimation of MeD in dosage forms (two types of tablets from two companies) with satisfactory results.

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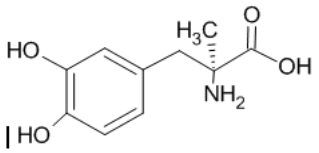
1. Introduction

Methyldopa is an antihypertensive that is supposed to have a main central action. It is decarboxylated in the CNS to alpha-methyl noradrenaline, which is used in the reduction of sympathetic tone and a drop in blood pressure. Methyldopa is commonly used to manage hypertension during pregnancy. There is little evidence of adverse effects on fatal development (Sweetman, 2007). Methyldopa as a chemical compound is widely a white or sometimes yellowish-white, crystalline powder, somewhat soluble in water, but in alcohol is very slightly soluble, almost insoluble in ether. MeD is freely soluble in dilute mineral acids MeD structure is shown in Fig.1.



M.wt 211.215 g/mol

Fig. 1. Chemical structure of methyldopa

Many different analytical methods were used in the estimation of the compound under study (MeD) and in its preparations or its presence in biological samples. These methods are the classical methods, including titrimetric. Fluorimetry (Kim & Koda, 1977). Electrochemical methods (Yanik et al., 2020). High-performance liquid chromatography (Zečević et al., 2001; da Silva et al., 2021) and flow injection analysis method (Ribeiro et al., 2005). Some of above techniques or methods are not for routine analysis, and the used instruments are expensive (Ribeiro et al., 2005). The spectrophotometric methods are simple, accurate, sensitive and inexpensive instrument needed. These methods including various types of reaction with various types of reagents. The oxidative coupling reaction has been used in estimation of MeD as pure and in it is formulations via using various reagents. Thiosemicarbazide

***Corresponding author:** Sahar A. Fathi, Chemistry Department, College of Science, University of Mosul, Mosul – Iraq

E-mail: **sahar.20scp129@student.uomosul.edu.iq**

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in presence of ferric nitrate to produce colored products (dark green) that have the highest absorbance at wavelength 460 nm (Abood et al., 2019). 1, 5-Diaminonaphthalene using ammonium Ceric (IV) nitrate.as an oxidant to produce a blue chromogen has the highest absorbance at 730 nm (Allah et al., 2022). 2,4-Dinitrophenylhydrazine reagent in the presence of potassium periodate form a yellow soluble product in acidic media, the absorbance was measured at 428 nm (AL-ghanam & AL-Enizzi, 2022).

p-Toluidine and sodium periodate in the determination of three compounds of catechol amine drugs included methyldopa (I), dopamine (II), and adrenaline (III)) via oxidative coupling reaction the reaction produced orange-water-soluble products, that have maximum absorption at 480 nm (Abdulrahman et al., 2005). Methyldopa and salbutamol have been estimated via cloud point extraction and flow injection spectrophotometric methods (Abood et al., 2019). The oxidation of MeD with an excess of N-bromosuccinimide and the residual of N-bromosuccinimide insert in bleaching the color of Eriochrom black-T (EBT). The absorbance of residual EBT is measured at 530 nm (Dhamra & Al-Sabha, 2020). Complexation reaction with molybdate also used in the estimation of MeD the absorbance of the yellow-colour product is estimated at 410 nm (Gadkariem et al., 2009). The electrochemical methods are used for the determination of methyldopa using modified glassy carbon electrodes (Antunes et al., 2019). Cyclic voltammetry (CV) technique using modified-GCE and PGE electrodes (Yanik et al., 2020), using nanocomposite graphene quantum dots (GQD) electrode for determination of methyldopa in biological samples (Sanati & Faridbod, 2017). Cyclic voltammetric technique using a modified glassy carbon electrode (GCE) was used for the determination of methyldopa (Erdoğdu, et al, 2019). MeD in human serum has been determined via clean-up and separation from serum by mixed-mode liquid chromatography (Zečević et al., 2001).

The present work involves the oxidation of methyldopa by adding ferric sulphate solution and the liberated ferrous ions subsequent coupling with 4,7-diphenyl-1,10-phenanthroline reagent as a novel coupling agent to form a highly coloured complex, that has been demonstrated successfully for the assay of methyldopa in its pharmaceutical preparation (tablets).

2. Materials and Methods

Apparatus

UV-Visible spectrophotometer Jasco model V-630, Japan, electronic balance KERN&Sohn GmbH, Germany and professional Bench top pH meter BP3001, Singapore were used in this study.

Chemical and solutions

All chemicals used were of analytical grade. The methyldopa was kindly supplied by Samara Drug Industry (SDI)-Iraq was prepared by MeD solution (100 µg .mL^{-1}) dissolving (0.0100)g of methyldopa in warm distilled water. The solution must be stored in a dark bottle. The solution was diluted as needed to 25 µg .mL^{-1}.

4,7-diphenyl-1,10-phenanthroline ; (2x10^{-3}M) this solution was prepared by dissolving 0.0664g of pure reagent in a small volume of ethanol(10 mL) with stirring then the volume was completed to the mark with ethanol in the 100 mL-calibrated flask.

Fe(III) solution (2x10^{-3}M) this solution was prepared by dissolving 0.0799 g of $Fe_2(SO_4)_3$ in 2mL of 0.5M of H_2SO_4 with stirring and heating, then diluted to 100 mL in a volumetric flask with distilled water to the mark. Fe(III) solution (2x10^{-3}M).

Pharmaceutical preparation

Solution of 100 µg.ml^{-1} Aldosam Tablets Formulation

5 Tablets (each containing 250 mg MeD) were weighed and finely powdered. 0.0138 g of powder equivalent to 0.0100 g of pure MeD was weighed and transferred into a 100 mL beaker. The powder was dissolved in 100 mL warm distilled water using calibrated flask to prepare a 100 µg MeD. mL^{-1}solution. Any solution needed in the procedure is prepared by diluting the original solution.

Solution of 100 µg.mL^{-1} from Methyldopa Tablets Formulation

5 Tablets (each containing 250 mg MeD) were weighed and finely powdered. 0.0143 g of powder equivalent to (0.0100 g) of pure MeD was weighed and transferred into a 100 mL beaker. The powder was dissolved in warm distilled water(100 mL) then transferred to in a calibrated flask to prepare 100 µg MeD. mL^{-1}solution and the solution was filtered through a filter paper. The filtrate solution was diluted as needed to (25 µg.mL^{-1}).

Recommended Procedure and Calibration Curve

Aliquots of standard drug solution of methyldopa 0.1 to 1 mL of (25µg mL^{-1}) were relocated into a series of 10 mL calibrated flasks. To each of these flasks 0.25 mL of $Fe_2(SO_4)_3$ (2x10^{-3} M) solution was added. The solutions were heated at 40°C for 10 minutes using a water bath, then cooling the solutions before adding 1.5 mL of 4,7-diphenyl-1,10-phenanthroline solution (2x10^{-3} M). The flasks were diluted with distilled water to the mark. The absorbance of the colour complex was measured at 533 nm versus the corresponding reagent blank. The linearity of the calibration curve was from 0.25 to 2.5 µg.ml^{-1} (Fig. 2), depending on the extent to which it obeys Beer's law (Nejres & Najem, 2023), with a value of the molar absorptivity equal to 1.3272×10^5 L.mol^{-1}.cm^{-1}, and the Sandell's sensitivity index value was equal to 0.00159 µg. cm^{-2}.

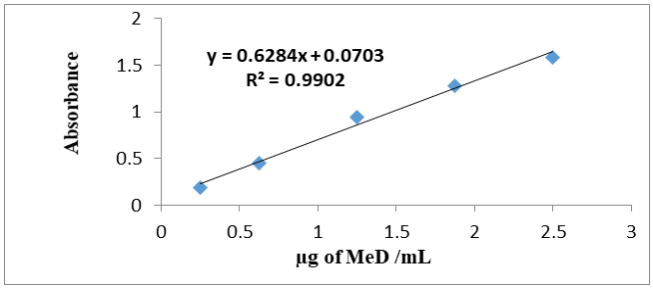


Fig. 2. Calibration curve for the determination of MeD according to the recommended procedure

3. Results and Discussion

Effect of Oxidizing Amount

Different volumes of ferric sulphate solution were used while keeping a fixed concentration of MeD and 4,7-diphenyl-1,10-Phenanthroline the absorbance of the complex was investigated giving maximum absorbance by using 0.25 mL of $Fe_2(SO_4)_3$ thus adopted as being optimal as shown in table1.

Table 1
The effect of oxidizing agent amount

mL of Fe (III) $2x10^{-3}$M	Absorbance of MeD(µg/ mL)				R^2
	0.625	1.25	2.5	3.75	
0.125	0.3755	0.6715	0.8388	1.1344	0.9569
0.25	**0.3928**	**0.7816**	**1.2637**	**1.5807**	**0.9713**
0.5	0.3724	0.6584	0.9973	1.1748	0.9528
0.75	0.2547	0.4453	0.6590	0.7899	0.9591
1.0	0.2256	0.4440	0.5627	0.8141	0.9595

Effect of Acid Type

Various acids (1M) have been tested, to gain high intensity of complex. The results in table 2 indicated that the oxidation of the drug takes place without acid, any acid added a decrease in absorbance.

Table 2
Choice of the acid type

Acid 1M	Absorbance of 1.25 µg/1mL MeD	pH
HCl	0.0296	2.07
H_2SO_4	0.0239	2.19
HNO_3	0.0474	2.11
CH_3COOH	0.7716	3.08
Without acid	**0.8097**	**3.30**

Effect of the Amount of Reagent

The influence of the concentration of 4,7-diphenyl-1,10-phenanthroline on the intensity of the colored product was examined in the range 1-1.75 mL of (2 $x10^{-3}$ M). It was found that the maximum absorbance was gated with 1.5 mL of the reagent. A decrease in absorbance occurs by increasing or decreasing the quantity of the reagent (4,7-diphenyl-1,10-Phenanthroline) from 1.5mL, therefore 1.5 mL was chosen in all next experiments (results shown in table3).

Table 3
The effect of the amount of the reagent

ml of R $(2x10^{-3})$ M	Absorbance of MeD µg/ mL				*R^2
	0.625	1.25	2.5	3.75	
1	0.3891	0.7918	1.2566	1.5496	0.9634
1.5	**0.4055**	**0.8187**	**1.3326**	**1.5995**	**0.9559**
1.75	0.3618	0.7412	1.3012	1.5608	0.9596

*Determination coefficient

Examine the Time Required for Oxidation

The reaction components were mixed in a number of 10 mL volumetric flasks and left for different periods of time before diluting and after dilution the absorbance was measurement. It was found that oxidation occurs instantaneously and the best time to obtain complete oxidation is10 minutes and it was confirmed in subsequent experiments (table 4).

Table 4
Choice of the reaction time

Time, min.	Immediately	5	10	15
Absorbance	0.6455	0.6580	0.8080	0.7777

The results in table 4 indicated that 10 min. development time was the optimal time to complete the oxidation , therefore selected for the general procedure.

Effect of Temperature

The effect of temperature on the oxidation reaction of (MeD) with Fe(III) was studied by continuous monitoring of the absorbance at 533 nm at different temperature (25-50°C). It was found that the reaction was affected on absorbance by increasing the temperature, the result shown in table 5.

Table 5
Effect of temperature on absorbance of colored product

Temp., oC	25 ±1*	30	40	50
Absorbance	0.8075	0.8290	1.0066	1.0048

* Room temperature

The results in Table 5 indicated that the maximum intensity of the complex was found after heating the mixture of reaction at 40°C for 10 min., therefore selected in the general procedure.

The Stability of the Product

The stability of the colored complex formed in the optimal conditions mentioned above was studied. The absorbance product formed was found stable at 40 C° the results are shown in table 6.

Table 6
Stability of colored product with time

Time, min.	Absorbance 1.25 µg mL^{-1}	Absorbance 2.5 µg mL^{-1}
Immediately	0.9407	1.5549
5	0.9476	1.5489
10	0.9449	1.5381
15	0.9417	1.5313
20	0.9401	1.5270
25	0.9440	1.5245
30	0. 9317	1.5263
35	0.9452	1.5230
40	0.9574	1.5270
45	0.9617	1.5363
50	0.9717	1.5370
55	0.9808	1.5370
60	0.9868	1.5370

The Final Absorption Spectra

Using the recommended procedure, the formed product shows a final absorption spectrum with maximum absorbance at 533 nm versus the blank solution (Fig. 3).

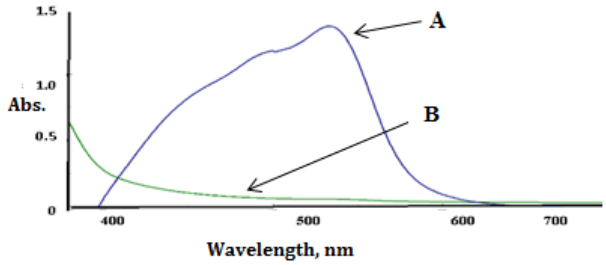


Fig. 3. Absorption spectra of (2.5µg.ml^{-1}) of MeD treated according to proposed method : (A) sample against blank solution, (B) blank against DW

Stoichiometry of the Reaction

Under the optimum conditions, the mole ratio of the reaction MeD with Fe (III) was examined by the mole–ratio method. The ratio of the reaction was found to be 1 MeD:2 Fe(III) the results are shown in Fig. 4.

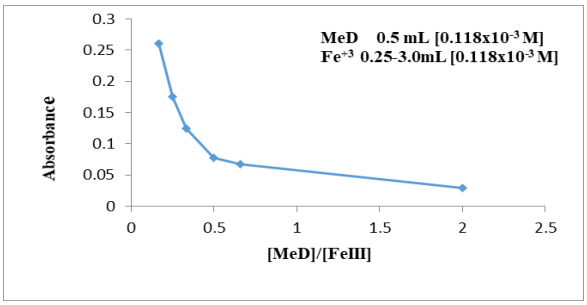
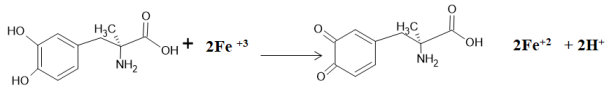


Fig. 4. Plot of mole ratio of MeD, Fe (III),



Then the reaction of Fe (II) with 4,7-diphenyl-1,10-Phenanthroline

The structure of the red complex product (Fig.5) was known (Nabeel, et al., 2017)

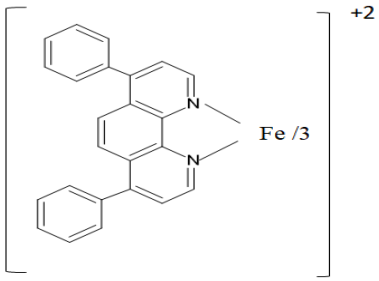


Fig. 5. The structure of red complex

Precision and Accuracy

Four replicate measurements were performed at two different concentrations of MeD. The relative standard deviation and relative error results showed that the suggested method has good precision and accuracy (table 7).

Table 7
The results of precision and accuracy

µg MeD/10 mL	Relative standard deviation * %	Relative error * %
6.25	0.42	-3.69
18.75	0.86	1.20

*Average of four determinations

Application of the Proposed Method.

To test the applicability of the recommended method, the estimation of methyldopa in its pharmaceutical preparation (tablet) has been applied. The results are shown in table 8.

Table 8
The results of Analytical applications

Pharmaceutical preparation	µg MeD Present/ 10 mL	µg MeD Measured/ 10 mL	Recovery*,%	Relative standard deviation*,%	Drug content (mg)
Aldosam 250 mg / Tablet (S.D.I Iraq)	6.25	6.24	99.84	0.266	249.60
	18.75	19.06	101.65	0.81	254.15
Methyldopa 250 mg / Tablet (Accord, UK)	6.25	6.35	101.60	0.94	254.00
	18.75	19.20	102.40	0.31	256.00

*Average of four determinations

Estimation by the Standard Addition Method

The results of the application of the standard addition method are recorded in Figs.6 and 7, and table 9. The consistency between the results obtained when estimating MeD in tablet formulation confirmed by the proposed analysis method by using the straight-line equation.

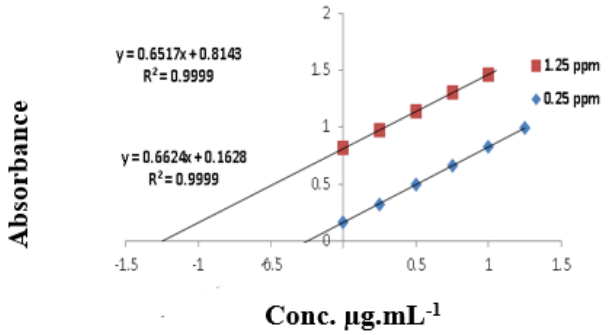


Fig. 6. Plot of standard addition method estimating of MeD in tablets SDI

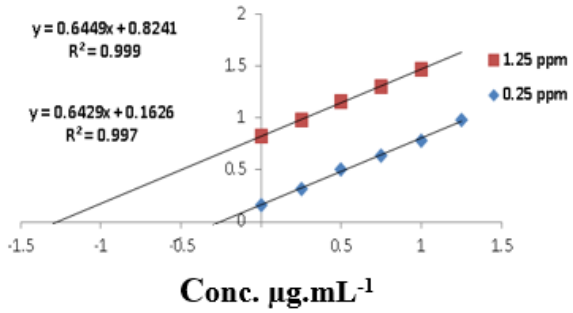


Fig. 7. Plot of standard addition method estimating of MeD in tablets Accord

Table 9
The results of MeD estimation in tablets

Pharmaceutical preparation	µg MeD present/mL	µg MeD measured/1mL	Recovery%	Drug content (mg)
Aldosam 250 mg / Tablet (S.D.I Iraq)	0.25	0.245	98.00	245.00
	1.25	1.249	99.92	249.80
Methyldopa 250 mg / Tablet (Accord, UK)	0.25	0.253	101.20	253.00
	1.25	1.278	102.24	255.60

Comparison of the Methods

Table10 contains the comparison of some analytical parameters of the present method with another method in literature.

Table10
Comparison of some analytical variables of the present method with the literature method

Analytical parameters	Proposed method	Reported method (Ribeiro et al., 2005)
Beer's law limit (µg.ml^{-1})	0.25 - 2.5	50- 200
Molar absorptivity (L.mol^{-1} cm^{-1})	1.3272 x10^5	1.134 × 10^3
λmax (nm)	533	410
Reagent used	4,7-diphenyl-1,10-phenanthroline	Ammonium molybdate
RSD%	0.42 -0.86	0.5 – 1.2
Stability of the color (min.)	60	35
Temperature (C°)	40	25±1

The results in table 10 indicate that the proposed method has the highest sensitivity and stability of the colour compared with the above literature method.

4. Conclusions

A spectrophotometric procedure was proposed for the determination of methyldopa. The method was based on the oxidation of MeD with Fe(III) to produce Fe(II). The Fe(II) reacts with 4,7-diphenyl-1,10-phenanthroline to produce a red-colored complex. The proposed method has been applied successfully for the determination of methyldopa in pharmaceutical preparation (Tablet).

Competing Interests

The authors have declared that no competing interests exist.

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