Spectrophotometric method for the determination of methyldopa by oxidizing coupling reaction with 4-aminoantipyrine

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Abstract

A simple, sensitive, accurate and low cost effective spectrophotometric method has been developed for the determination of methyldopa in pure and pharmaceutical formulations. The method is based on the reaction of methyldopa with 4-aminoantipyren (4-AAP) in presence of potassium ferriecyanide (PFC) in an alkaline medium.

Two optimization methods were applied to determine the optimum conditions of oxidizing coupling reaction variables; univariate and design of experiment (DOE) method. The conditions effecting the reaction; pH, buffer Volume, reagent concentration, oxidant concentration, type of buffer, order of addition, time of reaction and stability were optimized . Under univariate and design of experiment (DOE) method; the colored product having (λmax) at 468nm and 466nm respectively. Beer's law was obeyed in concentration range of (1-60 $\mu g.mL^{-1}$) and (1-50$\mu g.mL^{-1}$) with correlation coefficient of 0.9995 and 0.9997 respectively. The assay limits of detection quantification were 0.0962 and 0.0633$\mu g.mL^{-1}$, respectively. The method was successfully applied to the analysis of some drug tablets formulation.

Keywords: Methyldopa drug (MED), Spectrophotometric determination, oxidizing coupling reaction, 4-aminoantipyren (4-AAP).

طريقه التقدير الطيفي الكمي للمثيل دوبا بتفاعل الازدواج التاكسدي مع 4- امينو انتي بايرين

طورت طريقة طيفية بسيطة وحساسة ودقيقة وواطئة الكلفة للتقدير الكمي للمثيل دوبا (MED) بشكله النقي وفي الشكل الصيدلاني. تعتمد الطريقة على تفاعل المثيل دوبا مع 4- امينو انتي بايرين بوجود(PFC) في وسط قلوي. وقد تمت دراسة العوامل التي تؤثرفي إتمام تفاعل الازدواج التاكسدي بعناية للحصول على الظروف الفضلى بطريقتين. وذلك باتباع نمط المتغير الواحد وبالاعتماد على وطريقة المتغيرات المتعددة (تصميم التراكم المركزي لثلاثة متغيرات). المتغيرات التي تؤثر على التفاعل هي pH , تركيز الكاشف, تركيز العامل المؤكسد, تسلسل الاضافه, زمن التفاعل والاستقراريه. الامتصاص الأعظم (λmax) للمركب الملون الناتج حسب الطريقتين 468, 465 نانومتر بالتسلسل، وجد أن قانون بير ينطبق على مدى من التراكيز يتراوح بين $\mu g.mL^{-1}$ (1-60) و $\mu g.mL^{-1}$ (1-50) و بمعامل ارتباط مساوي لـ 0.9995 و0.9997 وكان حد الكشف يساوي $\mu g.mL^{-1}$ 0.0962 و $0.0633\mu g.mL^{-1}$ لكلا الطريقتين بالتتابع . طبقت الطريقة المقترحة بنجاح لتقدير للمثيل دوبا في نماذج دوائيه.

الكلمات المفتاحية: مثيل دوبا, التقدير الطيفي, تفاعل الأزدواج التاكسدي,4-امينو انتي بايرين.

Introduction

Methyldopa is an important feature in pharmaceutical and clinical procedures Methyldopa (α-methyl-3, 4-dihydroxyphenylalanine), is a catecholamine derivative (1). 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)]. Several methods have been proposed to quantify Methyldopa in pharmaceutical formulations, including; voltammetry[(2,3)] , Differential pulse voltammetry[(4)] high-performance liquid chromatography(HPLC)[(5,6)], colorimetry[(7)], titrimetry[(8)] flow injection spectrophotometry[(9-11)] and spectrophotometry.[(1,2,12-14)]

The aim of the present work is to provide an optimized spectrophotometric method using the univariate and multivariate design of experiment (DOE) method. In the design of experiment method three-interest factors, buffer volume, reagent concentration and oxidant concentration were designated as independent variables and absorbance as response.

Experimental

Apparatus

All absorption measurements were obtained by using a double beam Shimadzu 1800, Kyoto, Japan UV-Visible Spectrophotometer with 40 mm matched quartz cells and the pH measurements were performed by using i-Trans, BP 3001, Sangapor pH meter.

Reagent

All Chemicals used are of analytical-reagent grade

1-A pure Methyldopa powder received in pure form (99.99%) was provided as a gift from the State Company for Drug Industries and Medical Appliances Samara-Iraq (SDI). The standard solution of MED were prepared by dissolving accurate weighted 100 mg of pure drug in 50 mL of distilled water with mild heating and brought to 100 mL of volumetric flask. Each working standard solution was freshly prepared by diluting the stock solution with distilled water.

2.4-aminoantipyrine (4-AAP) stock solution [0.3% (m/v)]: prepared by dissolving 0.3g of 4-AAP in distilled water and diluting to 100 mL in a dark volumetric flask at stored in a refrigerator. Each working standard solution was freshly prepared by diluting the stock solution with distilled water.

3. Potassium ferricyanide (PFC) stock solution [3 % (m/v)]: prepared by dissolving 3 g of PFC in distilled water and diluting to 100 mL in a volumetric flask. Each working standard solution was freshly prepared by diluting the stock solution with distilled water.

4. Borax [0.1 % (m/v)]: prepared by dissolving 0.1 g of Borax in distilled water and diluting to the mark in a 100 Ml volumetric flask.

5.Sodium hydroxide [~ 0.5 M]: prepared by dissolving 42.4 g of NaOH in 100 mL of distilled water.

6. Glucose [10000 $\mu g.mL^{-1}$]: prepared by dissolving 0.1 g of glucose in 10 mL distilled water.

7. Lactose [10000 $\mu g.mL^{-1}$]: prepared by dissolving 0.1 g of lactose in 10 mL distilled water.

8. Acacia [10000 $\mu g.mL^{-1}$]: prepared by dissolving 0.1 g of acacia in 10 mL distilled water.

9. Soluble Starch [10000 $\mu g.mL^{-1}$]: Triturate 0.1 g of soluble starch with a little cold water into a thin paste, and add boiling water. Boil until a clear solution is obtained (5 minutes) then let it cold and diluting to 10 mL in a volumetric flask. This solution should be freshly prepared as required. [(15)]

10. Dihydrogen Potassium Phosphate [0.1 %]: prepared by dissolving 0.1 g of KH_2PO_4 in 100 mL of distilled water.
11. Hydrochloric acid [~0.5) M]: prepared by taking 4.17 mL of concentrated HCl and diluted to 100 mL with distilled water.
12.Potassium hydroxide [~0.5 M]:prepared by dissolving 2.8 g of KOH in 100 mL of distilled water.

Analysis of tablets

Ten tablets were grinded to a homogenous fine powder, weighed and average mass per capsule was determined. The amount of powder equivalent to 0.3440 g of Iraqi tablet and 0.3524 g of Lebanon tablet, (MED) was dissolved in distilled water then diluted to the mark of 100 mL volumetric flask. Each solution was filtered using whatman filter paper No.41 to dispose of any Insoluble materiel before used. The solution prepared freshly by subsequent dilution with distilled water and analyzed by the recommended procedure.

General standard procedures

Two procedures were recommended for the determination of MED via the proposed methods. The first was carried out following the conditions obtained by univariate optimization, while the second base of those conditions was obtained by chemometric multivariate design of experiment method optimization.

Univariate

0.15mL of the standard solution contain (150 µg) of MED was transferred via a micropipette directly into quartz cuvette, followed by the addition of 0.9 mL of the borax buffer solution (with pH=9), 0.45 mL of 1% (m/v) Potassium ferricyanide solution, 0.45 mL of 0.15% (m/v) 4-AAP solution and 1.05 mL of ethanol of MED to make the volume to 3 mL. As soon as the cuvette was covered after that the solution was quickly shaken, and leaved to stand in dark for 5 min at room temperature then pleased in the spectrophotometer cell holder, the spectra of the colored compound were measured against the reagent blank and λmax was extracted at 468 nm.

Multivariate (design of experiment method)

0.45 mL of 0.75% (m/v) Potassium ferricyanide solution, was directly put into quartz cuvette, followed by the addition of 0.45 mL of 0.175% (m/v) 4-AAP solution, 0.15mL of the standard solution contain (150 µg) of Methyldopa and 0.75 mL of the borax buffer solution (0.1% pH=10), After zero min the mixture diluted by ethanol to make the volume to 3 mL. As soon as the cuvette was covered, the solution was quickly shaken, leaved to stand for 30 min at room temperate then pleased in the spectrophotometer cell holder; the spectra of the colored compound were measured for Methyldopa against the reagent blank at 466nm.

Results and Discussion

Optimization of reaction variables

A systematic study of the effects of various parameters on development of product color was taken by varying the parameters one at a time and controlling all others fixed.

1- Effect of Buffer pH

To study the effect of buffer pH on the development of maximum color, different range (7.0-11.0) of 0.1% borax buffer solution was prepared. The maximum color intensity was observed in the pH value 10.0 and was used throughout the experiment Fig. (1).

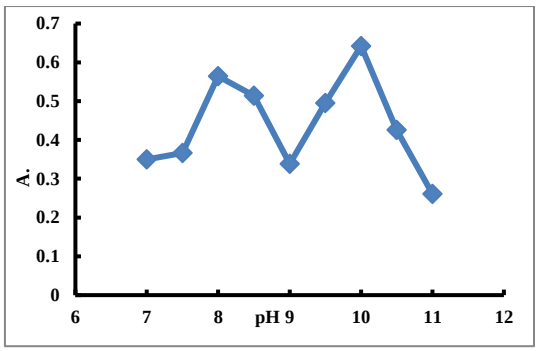


Fig. (1): Effect of pH on the absorbance of color product, [Methyldopa] =50µg.mL^{-1}, [Borax] =0.1%,[PFC]=1%, [AAP]=0.15% .

2- Effect of Buffer Volume

The influence of the volume of 0.1% borax buffer solution on the intensity of the color development was examined in the range (0.3-1.2) mL0.1% borax buffer solution. It is clear from Fig. (2) That the maximum absorbance 0.75 mL of borax buffer solution; above and below this volume, the intensity of the color product decreased, therefore, 0.75 mL was selected as the optimum volume.

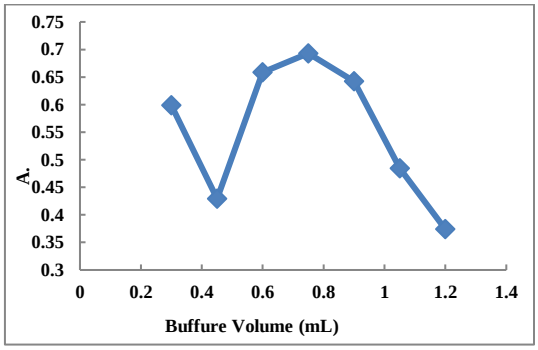


Fig. (2): Effect of Buffer Volume on the absorbance of color product, [Methyldopa] =50 µg.mL^{-1}, Buffer pH=10.0, 0.75 mL of [Borax] =0.1%, [PFC] =1%, [AAP]=0.15% .

3- Effect of the Reagent (4-aminoantipyrene (AAP)) Concentration

The influence of concentration of 4-AAP on the absorbance the color product was investigated in the range (0.1-0.3% (m/v)) Fig. (3) it was found that the maximum absorbance of the dark orange color was reached with 0.2% (m/v) of the reagent. Above and below this value causes a decrease in absorbance, therefore 0.2% was chosen in all experiments.

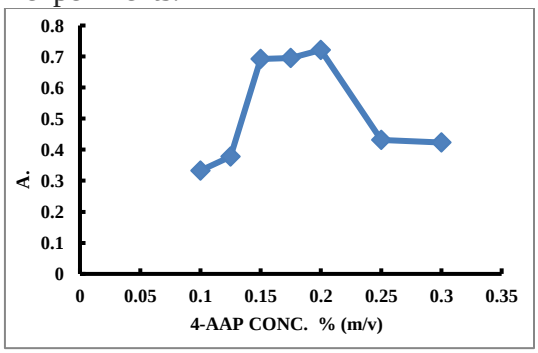


Fig. (3) Effect of [AAP] on the absorbance of color product, [Methyldopa] = 50 µg.mL^{-1}, Buffer pH=10.0, 0.75 mL of [Borax] =0.1%, [PFC] =1%.

4- Effect of Potassium Ferricyanide Concentration

The studying of PFC concentration revealed that the reaction was depending on PFC as an oxidant agent. The highest absorbance was attained when the concentration of PFC was 0.75 %. Above this value a decreased in the absorbance reading occurred. Fig. (4).

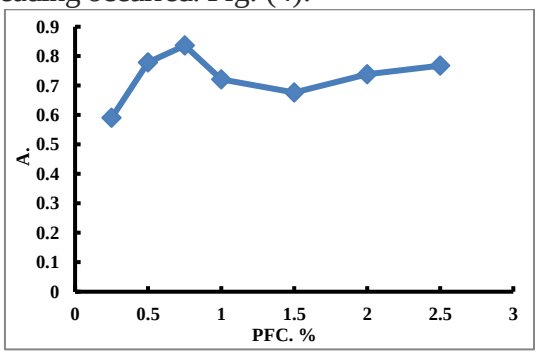


Fig. (4) Effect of [PFC] on the absorbance of color product, [Methyldopa] =15 µg.mL^{-1}, Buffer pH=10.0, 0.75 mL of [Borax] =0.1%, [AAP] =0.2%.

5- Effect of the type of Buffer

Tow Buffer solutions (Borax and Di hydrogen Potassium phosphate) were examined to achieve maximum color intensity. Borax buffer proved to be the most favorable one due to it's highly absorbance value in addition to instantaneously formation of the color product Fig (5).

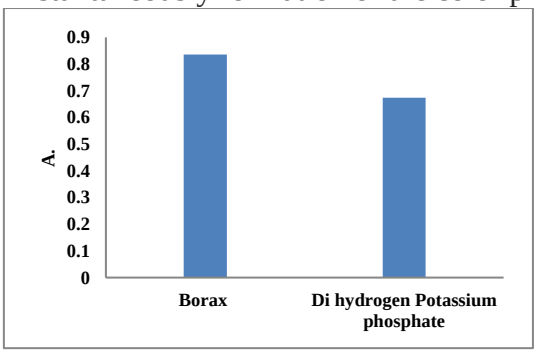


Fig. (5) Effect of Buffer kind on the absorbance of color product, [Methyldopa] =50 µg.mL^{-1}, Buffer pH=10.0, 0.75 mL of [Buffer] =0.1%, [4-AAP] =0.2%, [PFC] =0.75%.

6- Effect of order of addition

The optimum sequence was defined by following the color intensity and maximum absorbance on changing the sequence of addition of drug, reagent, oxidizing agent and buffer. The best condition was {the oxidizing agent solution first – reagent solution– drug solution –and the buffer solution} for the maximum absorbance Table (1).

Table (1): Show the order of addition

Order No.	Reaction component	Absorbance
1.	Drug + Buffer + PFC + 4-AAP	0.836
2.	Drug + PFC + 4-AAP + Buffer	0.947
3.	PFC + 4-AAP + Drug + Buffer	1.023
4.	4-AAP + PFC + Buffer + Drug	0.762
5.	Drug + Buffer + 4-AAP + PFC	0.937
6.	PFC + Buffer. + Drug + 4-AAP	1.006

* [MED] = 50 µg.mL^{-1}, Buffer pH=10.0, 0.75 mL of [Buffer] =0.1%, [4-AAP] =0.2%, [PFC] =0.75%.

7- Effect of Reaction Time

The reaction time is determined by following the color development at different time intervals when the reaction component where leave in dark place at room temperature under the optimum conditions. It was found that maximum absorbance is attained directly (after 0 min), that mean the reaction was very fast, almost constant shown in the absorption intensity, therefore 0 min was chosen as the optimum reaction time, Fig. (6).

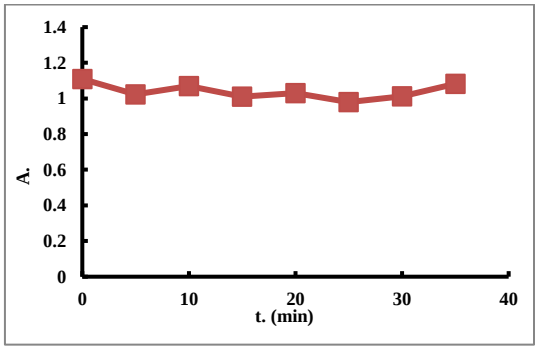


Fig. (6) Effect of Reaction Time on the absorbance of color product, [Methyldopa] =50 µg.mL^{-1}, Buffer pH=10.0, 0.75 mL of [Borax] =0.1%, [4-AAP] =0.2%, [PFC] =0.75%.

8- Effect of Stability

Under the aforementioned optimum condition, it was found that the reaction product was taken place instantaneous and the absorbance takes increase and constant at 30 min, therefore 30 min was chosen as the optimum reaction time Fig. (7).

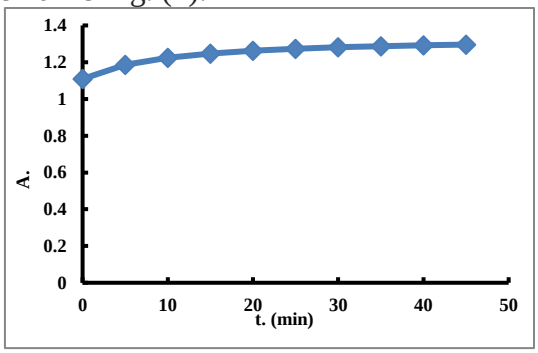


Fig. (7): Effect of Stability by the absorbance, [Methyldopa] =50 µg.mL^{-1}, Buffer pH=10.0, 0.75 mL of [Borax] =0.1%, [4-AAP] =0.2%, [PFC] =0.75%.

To develop the product color and found the optimum experimental conditions, Multivariate Method Experimental Design were used. The most three critical variables (buffer volume, reagent concentration and oxidant concentration) were examined using a central composite design while the other variables obtained from univariate method namely, effect of buffer pH, reaction time, effect of the buffer kind, effect of order of addition, effect of reaction time and effect of stability, were kept constant, to test the curvature of the response three levels of each selected variable were required.

The number of experiments needed to investigate the previously noted three parameters at three levels would be 27 (3^3). However, this was reduced to 20 using a central experimental design. Response surface model was applied to study the effect of the three variables, and generate an optimal response surface.

A second order polynomial equation was used to express the absorption as a function of independent variables (buffer volume, reagent concentration and oxidant concentration).

Absorbance = $\beta_0 + \beta_1 \times$ (buffer volume) + $\beta_2 \times$ [4-AAP] + $\beta_3 \times$ ([PFC]) + $\beta_4 \times$ (buffer volume × [4-AAP] + $\beta_5 \times$ (buffer volume× ([PFC]) + $\beta_6 \times$ [4-AAP] × [PFC]) + $\beta_7 \times$ (buffer volume)^2 + $\beta_8 \times$ [4-AAP]^2 + $\beta_9 \times$ ([PFC])$^{2.}$

The result of optimum conditions according to central composite design and the experimental points used according to the design listed in Table (2).

Table (2): The central composite design with three independent variables (un coded variables) and their experimental absorption values of 50 μg.mL^{-1} Methyldopa oxidation coupling product

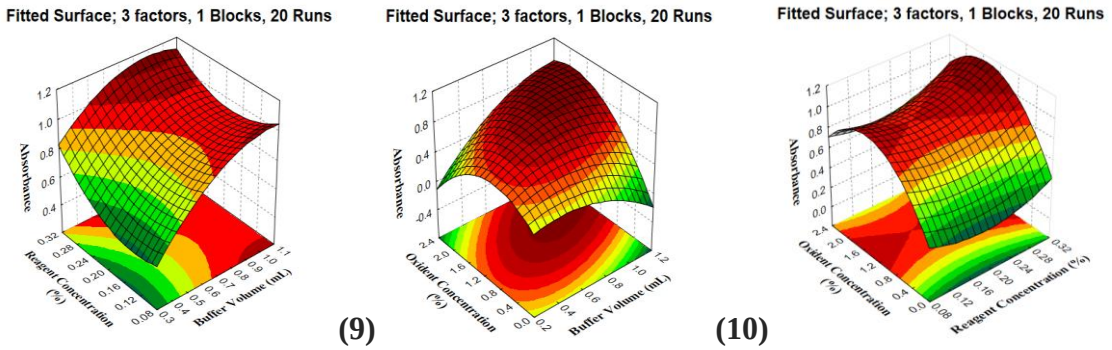
Exp. no.	*Buffer volume (mL)*	*Reagent Con. (%)*	*Oxidant Conc. (%)*	*Abs.*
1	0.75	0.3	2.00	0.774
2	0.75	0.1	2.00	0.385
3	0.75	0.2	2.00	0.679
4	0.75	0.3	2.00	1.074
5	0.75	0.2	3.50	0.862
6	0.75	0.2	0.50	0.861
7	1.20	0.3	3.50	0.905
8	0.30	0.2	3.50	0.288
9	0.75	0.2	2.00	1.034
10	0.30	0.2	0.50	0.354
11	1.20	0.2	2.00	0.864
12	1.20	0.1	3.50	0.62
13	0.30	0.1	2.00	0.399
14	0.30	0.2	0.50	0.86
15	0.30	0.2	3.50	0.847
16	0.75	0.2	2.00	0.873
17	1.20	0.3	0.50	0.47
18	1.20	0.1	0.50	0.429
19	0.75	0.3	2.00	0.273
20	0.75	0.1	2.00	0.971

The coefficients of the response surface equation were determined by **STATISTICA 8.0** software **(StatSoft. Inc, release 2007)**, the results are listed in Table (3).

Optimum conditions that are developed from central composite design for the determination of Methyldopa via oxidation coupling reaction with 4-AAP were calculated mathematically and the results are0.788 mL of buffer volume, 0.23% of reagent concentration and 1.537% of oxidant concentration). Figures 8, 9 and 10 show the response surface model if one of the three variables is remained constant.

Table(3): Regression coefficients,P or probability for absorption of MET oxidation coupling product

Variable	Regression coefficient	St. E of coefficient	t-value	P
Constant	917.42	200.920	4.56608	0.001032
Buffer volume	-953.45	563.594	-1.69172	0.121573
(Buffer volume.)^2	200.43	383.758	0.52229	0.612842
Reaction time	-2911.45	1492.900	-1.95020	0.079722
(Reaction time)^2	1821.75	3453.822	0.52746	0.609378
[Oxidant]	-300.71	107.965	-2.78531	0.019274
([Oxidant])^2	17.83	34.538	0.51619	0.616939
Buffer volume* Reaction time	1659.62	674.994	2.45871	0.033751
Buffer volume.* [Oxidant]	166.68	67.499	2.46942	0.033138
Reaction time * [Oxidant]	498.49	202.498	2.46169	0.033579



(8) **(9)** **(10)**

Fig. (8): The response surface for the absorbance of Methyldopa oxidation coupling product as a function of buffer volume and reagent concentration (at constant optimum value of oxidant concentration: 1.537%).

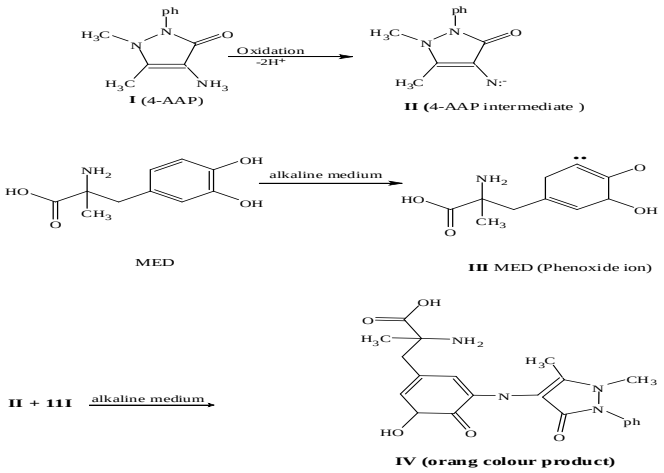
Fig. (9) The response surface for the absorbance of Methyldopa oxidation coupling product as a function of buffer volume and oxidant concentration (at constant optimum value of reagent concentration: 0.23%).

Fig. (10) The response surface for the absorbance of Methyldopa oxidation coupling product as a function of reagent concentration and oxidant concentration (at constant optimum value of buffer volume: 0.788mL).

Reaction scheme [16] and absorption spectra

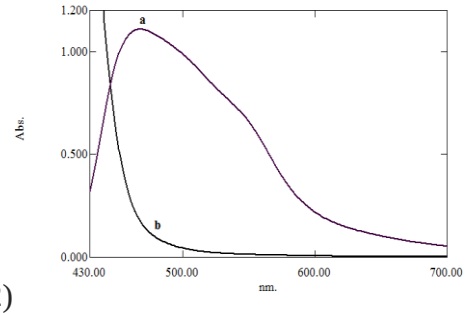
Oxidizing coupling reaction includes reaction of two or more organic materials in the existence of oxidizing agent in suitable conditions, as a result of oxidizing, intermediate compound which reacts with others to produces colored product can be measured spectrophotometric.

Under the oxidizing coupling reaction condition, 4-AAP (I) upon oxidation with PFC loses two protons forming a nucleophilic intermediate (II), which has been postulated to be an active coupling species. MED has a free ortho position to the hydroxyl group, hence the intermediate of 4-AAP (II) undergoes nucleophilic substitution with phenolic moieties (III) of MED in alkaline medium, to form a colored quinonoid type product (IV) [16-18] represented in Scheme (1).

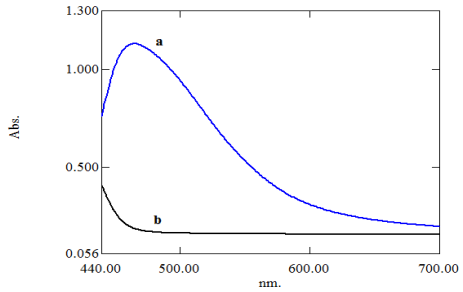


Scheme (1): Oxidizing Coupling reaction of Methyldopa with 4-aminoantipyrene.

Absorption spectrum was recorded under the optimum conditions (for univariate and Experimental Design method) and showed the maximum absorption (468 and 466) nm sequentially for the dark orange compound against the reagent blank as shown in Figure (12) and (13).



(12)



(13)

Figure (12): Absorption spectrum of color product (A) 50 µg.mL^{-1}Methyldopa against reagent blank, (B) blank solution against distilled water under the optimum conditions.

Figure (13): Absorption spectrum of color product: (A) 50 Methyldopa against reagent blank, (B) blank solution against distilled water under the Experimental Design Method conditions.

Calibration Curves and Analytical Data

I- Univariate Method

Employing the experimental condition, two scale of linear calibration curve plotted according to Beer's law (Fig. (14), the absorbance at λmax (466) of set of solution containing varying amounts of Methyldopa (1 -60) µg.mL^{-1} with specified amount of reagent were recorded against the blanks solution, Optical characteristics such as a maximum absorption, Beer's Law Limits, molar absorptivity, correlation coefficient and Sandell's sensitivity are listed in Table(4).

Table (4): Optical characteristics and statistical data for the determination of Methyldopa by univariate method.

Parameter	***Value***
λmax (nm), Color	468 nm, Dark Orange
Linearity range (µg.mL^{-1})	1-60
Regression equation	Y=0.0217 [MED. µg.mL^{-1}]-0.0022
Calibration sensitivity (mL. µg $^{-1}$)	0.0217
Correlation coefficient (r)	0.9995
Correlation of linearity (r^2)	0.9991
Molar absorptivity (L. mol $^{-1}$.cm $^{-1}$)	$\varepsilon = 4.583 \times 10^{+3}$
Sandell's sensitivity (µg.cm^{-2})	0.0461
Detection limit (µg.mL^{-1})	0.0962
Quantification limit (µg.mL^{-1})	0.2915

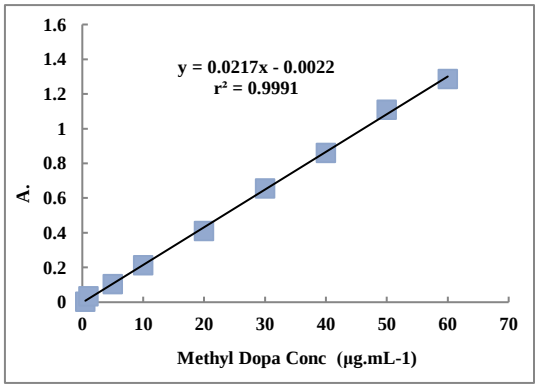


Fig. (14) Calibration curve for the determination of Methyldopa by univariate optimal condition.

II- Design of Experiment Method

The Optical characteristics such as a maximum absorption, Beer's Law Limits, molar absorptivity, correlation coefficient and Sandell's sensitivity for the proposed method are summarized in Table 5 and Fig. (15).

Table (5): Optical characteristics and statistical data for the determination of Methyldopa by Design of Experiment Method.

Parameter	***Value***
λmax (nm), Color	466 nm, Dark Orang
Linearity range (µg.mL^{-1})	1-50
Regression equation	Y = 0.0233 [MEDµg.mL^{-1}]- 0.0341
Calibration sensitivity (mL. µg $^{-1}$)	0.0233
Correlation coefficient (r)	0.9997
Correlation of linearity (r^2)	0.9995
Molar absorptivity (L. mol $^{-1}$.cm $^{-1}$)	$\varepsilon = 4.921\times10^{+3}$
Sandell's sensitivity (µg.cm^{-2})	0.0429
Detection limit (µg.mL^{-1})	0.0633
Quantification limit (µg.mL^{-1})	0.1919

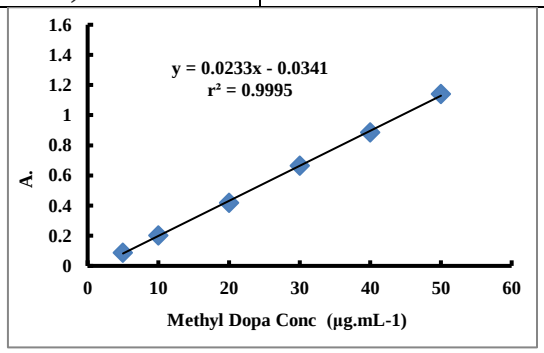


Fig. (15) Calibration curve for the determination of Methyldopa by DOE optimal condition.

Precision and Accuracy

The accuracy (in term of relative error percent) and precision (in term of coefficient of variation) of the proposed methods was tested by analyzing three replicate samples of MED in four different concentration levels. The values of the relative error % and precision are summarized in Table 6. These values indicate good accuracy and precision of the method.

Table (6): Evaluation of accuracy and precision for the determination of MED by proposed methods.

MED Conc.(µg.mL^{-1})	**Taken**	**Found***	**R. E. %**	**R. S. D. %**
For univariate	10	10.0270	-0.2710	0.3084
	30	30.2470	-0.8230	3.1075
	60	59.8330	0.2789	1.0926
For DOE	10	9.8731	1.2690	3.0185
	30	29.9550	0.1511	0.2870
	50	50.0800	-0.1590	0.2606

***Average of three determinations.

Interference Study

The extent of interfering by some excipients which often accompanied pharmaceutical preparations were studies by measuring the absorbance of solution containing 40 µg.mL^{-1} of MED

and 1000 µg.mL^{-1}. It was found that the studied excipients do not interfere in the determination of MED in its dosage forms, Table (7).

Table (7): Percent recovery for 50 µg.mL^{-1} of MED in the presence of 1000 µg.mL^{-1} of excipients. (MED conc. taken (50 µg.mL^{-1}))

Excipients	Excipients	[MED]found µg.mL^{-1}	Recovery%
Acacia	1000 µg.mL^{-1}	51.2274	97.5452
Glucose		48.8843	102.2315
Lactose		50.7812	98.4378
Starch		50.3350	99.3305

Application in pharmaceutical Sample

In order to demonstrate the applicability of the proposed method for the determination of MED, the method was successfully applied to the analysis of MED in three concentration levels of two type of drug. The results summarized in Table (8).

Table (8) Application of the DOE method to the MED concentration measurements in synthetic sample.

Sample	Weight* found (250mg/100mL)	Conc. taken (µg.mL^{-1})	Conc.* found (µg.mL^{-1})	Recovery* %	R.S.D* %
250mg of MED Iraqi capsule	254.4492	10	10.1780	101.7797	1.5913
	253.5676	20	20.2854	101.4270	2.9583
	254.0607	30	30.4873	101.6243	1.0874
250mg of MED Lebanon capsule	257.3920	10	10.2957	102.9568	0.9174
	257.4425	20	20.5954	102.9770	0.3445
	256.9901	30	30.8388	102.7961	1.9932

*Average of three determination.

Conclusions

Oxidizing coupling reaction of MED with 4-AAP presence of potassium ferriecyanide (PFC) in alkaline medium was found to be a simple, sensitive and accurate spectrophotometric method for quantitative determination of (MED) in pure form and Pharmaceutical. The classical univariate and Design of Experiment method have been used for optimizing the different variable affecting the completion of the reaction. The proposed method offers good linearity and precision. The proposed method can applied in many samples like food samples and biological fluids.

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