RESEARCH ARTICLE

NEW, SIMPLE AND VALIDATED KINETCS SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF METHYLDOPA IN ITS PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A new kinetic and thermodynamic studies of spectrophotometric method for the quantitative analysis of micrograms of methyldopa (MD) in pure and pharmaceutical dosage forms has been adopted.The method is based on reaction of MD with ferric chloride and nitroso-R-salt (NRS). MD reduces ferric ion, resulting in the formation of ferrous ion which finally forms a green color complex with NRS which is measured spectrophotometrically at 708 nm. The initial rate and fixed time methods were adopted for constructing the calibration curves. The linearity ranges were found to be 0.5-10 and 0.3-10 µg mL-1 for initial rate and fixed time methods, respectively. Thermodynamics' parameters like free energy change, the enthalpy of formation as well as the entropy were determined for the reaction product .The method was applied successfully for the estimation of MD in commercial dosage forms. Statistical comparison between the proposed and reference spectrophotometric methods showed excellent agreement between the accuracy and precision of the two methods.

INTRODUCTION

Methyldopa(LαMethyl3,4dihydroxyphenylalanine;$C_{10}H_{13}NO_4$, $1^1/_2H_2O$) (1993) is an alpha adrenergic agonist (selective for α_2-adrenergic receptors)psychoactive drug used as a sympatholytic or antihypertensive (Hoffman and Lefkowitz,1996). Its molecular weight is 238.2 gm moL^{-1}(Fig.1). Methyldopa is used in the clinical treatment of hypertension (or high blood pressure) and gestational hypertension (or pregnancy-induced hypertension) and pre-eclampsia (2008)
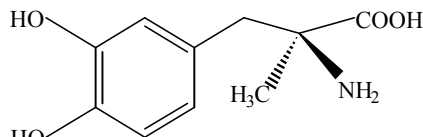
Fig.1 Chemical structure of methyldopa

Various methods have been reported for determining MD in pharmaceutical preparations. These methods included titrimetry (Salem,1987), fluorimetry (Salem,1993), amperometry (Ali and Sami, 2005), potentiometry (Badawy et. al. ,1996), capillary zone electrophoresis (Xiangdong *et. al.,* 2012), voltammetry (Leite *et. al.,* 2012), gas chromatography (de Jong *et. al.,* 1988), high-performance liquid chromatography (HPLC) (Bugamelli *et. al.,* 2011)and spectrophotometry (Gotardo *et. al.,* 2008; AL-Esawati, 2002; Ribeiro *et. al.,* 2006). Kinetic methods have certain advantages in pharmaceutical analysis regarding selectivity and elimination of additive interferences, which affect direct spectrophotometeric methods .The literature survey reported very little and insensitive kinetic methods for determination of MD (Chamsaz *et. al.,* 2007; Tubino *et. al.,* 2006).Therefore, there is a need for another kinetic approach to estimate the drug in commercial dosage forms. This paper describes a simple and sensitive kinetic spectrophotometric method for the determination of MD in bulk and drug formulations. The aim of the present work was to study the simple reaction between MD with ferric chloride and NRS kinetically in an attempt to evaluate this drug in its dosage forms. The kinetic study and thermodynamic parameters of the drugs with reagent were also determined.

EXPERIMENTAL DESIGN
Apparatus

All spectral and absorbance measurements were carried out on a UV-visible-260 digital double beam recording spectrophotometer using 1-cm silica cell. Immersion thermostat, 220 V (PHYWE, made in germany).

Reagents and materials

- *Standard methyldopa solution (Industries and Medical Appliance, SDI, Samara. Iraq):*Stock solution (500 µg mL^{-1}) was prepared by dissolving 0.0500 g of the pure compound in a sufficient amount of hot distilled water and complete to 100 mL in a volumetric flask with the same solvent. Working

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solution (100 µg mL^{-1}) was prepared by a simple dilution with the same solvent.

- *Nitroso-R-Salt solution (BDH, England, 0.025 M):* Prepared by dissolving 0.9432 g of reagent in 100 mL of distilled water.
- *Ferric chloride solution (Fluka,Switzerland, 0.02 M):*Prepared by dissolving 0.3244 g of ferric chloride in 100 mL of 0.1 M hydrochloric acid .

Preparation of dosage forms sample solution

An accurately weighed amount (20 powdered tablets) equivalent to 10 mg of the pure drug was dissolved into a 100 mL of hot distilled water and completed to the mark with the same solvent to obtain 100 µg mL^{-1} of MD. The flask with its contents was shaked well and filtered. A sample of 30 and 50 µg of MD in a final volume of 10 mL was taken and the measurements were carried out as described under general procedure.

General analytical procedures and data treatment

Aliquots of standard MD solution (0.25–5.00 mL, 20 µg mL^{-1}) were transferred into a series of 10 mL calibrated volumetric flasks. Then 0.5 mL of 20 mM ferric chloride solution and 1 mL of 25 mM NRS solution were added and the volume was made up to the mark with distilled water at 25°C. After mixing, the contents of each flask were immediately transferred to the spectrophotometric cell and the increase in absorbance was recorded at 708 nm as a function of time against reagent blank treated similarly. The initial rate of the reaction at different concentrations was obtained from the slope of the tangent to the absorbance–time curve.The calibration curve was constructed by plotting the logarithm of the initial rate (log k') versus the logarithm of the molar concentration of MD(log C). Alternatively, the calibration curve was constructed by plotting the absorbance measured after a fixed time of 5 min.

DETERMINATION OF MOLAR RATIO OF THE REACTIONS

For MD with ferric chloride

The job's method was applied by placing 1 to 4.5 mL of 4.198×10^{-4} M ferric chloride solutions into a series of 10 mL volume flasks; this was followed by placing 4 to 0.5 mL of 4.198×10^{-4} M MD, and 1 mL of 25 mM of NRS ,the volumes were diluted to the mark with distilled water, allowed to stand for 20 min; and the absorbances were measured versus the reagent blank.The results were plotted as shown in Fig.2 a, which indicated the existence of 1:2(MD : Fe(III)).

For metal with NRS

The job's method was applied by placing 1 to 4.5 mL of 8 mM ferric chloride this was followed by placing 4 to 0.5 mL of 8 mM of NRS in the presence of fixed amount of 1mL of 4.198×10^{-4}M of MD, the volumes were diluted to the mark with distilled water, allowed to stand for 20 min; and the absorbances were measured versus the reagent blank. The results were plotted as shown in Fig.2 b, which indicated the existence of 1: 3 (Metal: Reagent).

RESULTS AND DISCUSSION

Involved reaction and absorption spectra

The reaction involved in the present study was based on the formation of a colored complex [Fe(NRS)$_3$]$^{2+}$,resulted from the redox reaction between MD and ferric chloride in the presence of NRS reagent. The formation of this colored product was monitored spectrophotometrically at its maximum absorption peak (708 nm). The absorption spectrum for the reaction product is given in Fig.3. The following sections describe the optimization of different factors affecting the reaction, kinetics, and the use of the optimized conditions in the development of the assay procedures.
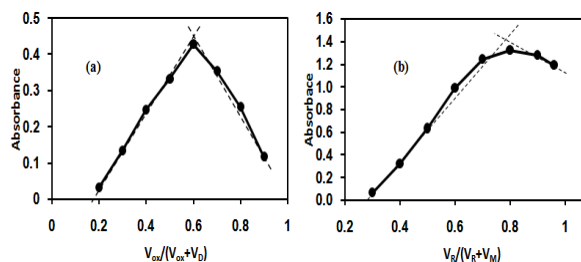

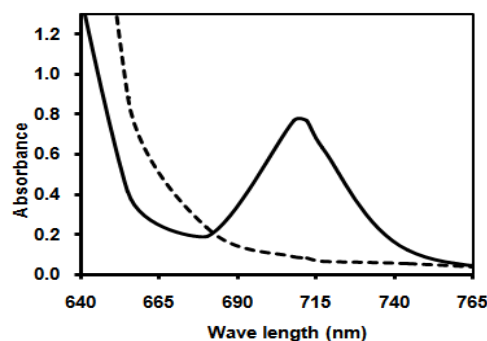**Fig.2** Continuous variation plots for: (a) MD with ferric chloride, (b) metal with NRS

Fig.3 Absorption spectra of 5 µgmL^{-1} of (—) MD treated as described under procedure and measured against reagent blank and (----) the reagent blank measured against distilled water

Optimization of reaction conditions

The factors affecting reaction conditions (concentrations of ferric chloride and NRS reagents and temperature) were studied by altering each variable in turn while keeping the others constant. It was found that the color intensity was dependent on the concentration of both reagents. The highest color intensity was attained when the concentrations of FeCl$_3$ and NRS in the final reaction solution were 1 and 2.5 mM respectively; these concentrations were used in all the subsequent experiments. The reaction was carried out at room temperature (25± 5°C).It was found that the color intensity decreased significantly when the reaction temperature increased. Therefore, the further experiments were carried out at room temperature.

Stoichiometry, mechanism, and kinetics of the reaction

The stoichiometry of the reaction between MD and ferric chloride and other between ferrous ion and NRS were investigated by job's method (de Levie, 1997). Based on these ratios, the reaction pathway was postulated to proceed as shown in Fig.4.Under the reaction conditions MD was

oxidized with Fe^{3+} to produce Fe^{2+}. The produced Fe^{2+} is then complexed by NRS to form the green color complex, $[Fe(NRS)_3]^{2+}$, which exhibits an absorption maximum at 708 nm.

Under the optimum conditions, the absorbance–time curves for the reaction of varying MD concentrations(2×10^{-6} to 4.19×10^{-5} M) with a fixed concentration of ferric chloride (0.5 mL of 20 mM) in presence of NRS(1 mL of 25 mM)were generated (Fig.5). The initial reaction rates (K) were determined from the slopes of these curves.
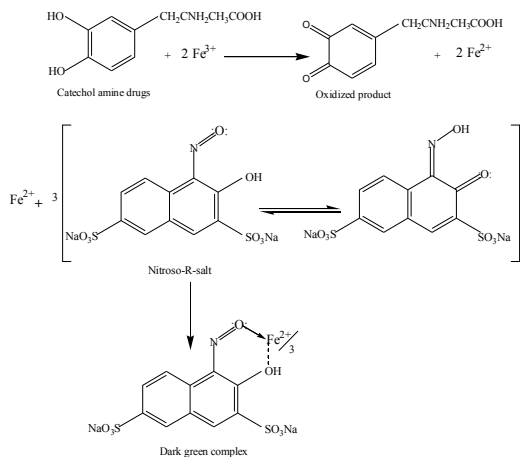

Fig.4 Reaction scheme

The logarithms of the reaction rate (log K) were plotted as a function of logarithms of MD concentrations (log C) (Fig.6). The regression analysis for the values was performed by fitting the data to the following equation: $\log K = \log k' + n\log C$ where K is reaction rate, k' is the rate constant, C is the molar concentration of MD, and n (slope of the regression line) is the order of the reaction. A straight line with slope values of 0.9959 ($\approx$1) was obtained confirming that the reaction was first order. However under the optimized reaction conditions, the concentrations of ferric chloride and NRS were in much more excess than that of MD in the reaction solution. Therefore, the reaction was regarded as a pseudo-first order reaction.
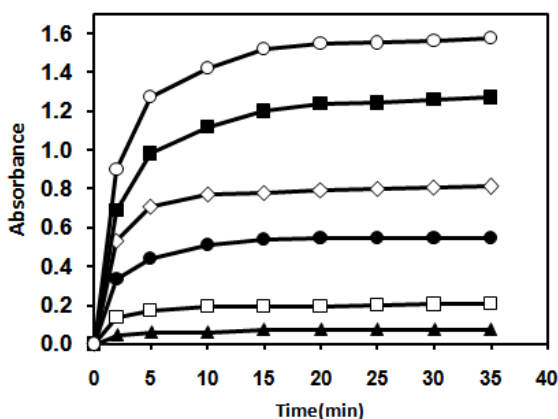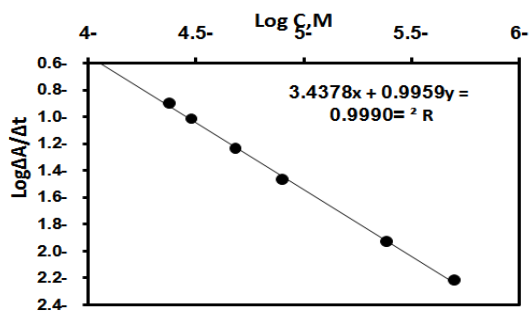

Fig.5 Absorbance versus time graphs for MD (▲ , 2.0×10^{-6}M); (□ , 4.1×10^{-6}M) ;(● , 1.25×10^{-5}M); (■ , 2.09×10^{-5}M); (○ , 3.35×10^{-5}M) ;(◇ 4.19×10^{-5}M)

Quantitation methods

Initial rate method

The initial rates of the reaction for MD followed a pseudo first order and were found to obey the following equation:

$K = \Delta A/\Delta t = K'C^n$ where K is the reaction rate, A is the absorbance, t is the measuring time, K' is the pseudo-first order rate constant, C is the molar concentration of MD and n is the order of the reaction. The logarithmic form of the above equation is written as follows:$\log K = \log\Delta A/\Delta t = \log K' + n \log C$Regression analysis, using the method of least square was performed for the data. The value of n (slope) was 0.9959 ($\approx$1) in the regression equation, confirmed that the reaction was first order with respect to MD concentration, the relation is linear in the range of 0.5-10 $\mu g\ mL^{-1}$, with good correlation coefficient (0.9990).

Fixed time method

In this method, the absorbance of the reaction solution containing varying amounts of MD was measured at a pre-selected fixed time. Calibration plots of absorbance versus the concentrations of MD were established at fixed periods of time for the reaction. The regression equations, coefficients of correlation, and limits of detection are given in Table 1.

The widest linear ranges were obtained at 15 and 20 min, however poor linearity and correlation coefficient were obtained at 15 min, as compared with that at 20 min. Therefore, on the basis of wider concentration range and less time of analysis, the fixed time of 20 min was recommended for analytical procedure. The limit of detection (LOD) and limit of quantification (LOQ) at fixed time of 20 min were calculated and found to be 0.13 and 0.43 $\mu g\ mL^{-1}$, respectively.

Table 1 Regression equations for MD drug at different fixed time over range 1×10^{-6} to 4.19×10^{-5} M

Time(min)	Regression equation	Correlation coefficient	Linear range ($\mu g mL^{-1}$)
5	A=0.0981C+0.1575	0.9914	2-15
10	A=0.1328C+0.0865	0.9969	1-10
15	A=0.1497C+0.0190	0.9991	0.5-10
20	A=0.1531C+0.0187	0.9996	0.3-10
25	A=0.1544C+0.0143	0.9994	0.3-8
30	A=0.1573C+0.0121	0.9991	0.3-8
35	A=0.1557C+0.0290	0.9984	0.5-8

Validation of the proposed methods

Accuracy and precision

The accuracy and precision of the proposed kinetic spectrophotometric method were determined at three concentration levels (3, 5 and 8 µg mL^{-1}) of MD by analyzing five replicate samples of each concentration by both the initial rate and fixed time methods. The relative standard deviations (RSD) for the results did not exceed 2.3% (Table 2), proving the high reproducibility of the results and the precision of the method. This good level of precision was suitable for quality control analysis of MD in its pharmaceutical tablets.

Table 2 Accuracy and precision of the initial-rate and fixed time methods

Method	Conce. of MD (µg mL^{-1})		Error %*	Reccovery%*	RSD%*
	Present	Found			
Initial rate	3.00	2.98	-0.72	99.28	1.94
	5.00	4.96	-0.82	99.18	2.09
	8.00	8.20	+2.53	102.53	2.29
Fixed time	3.00	3.05	+1.60	101.60	1.49
	5.00	5.05	+0.96	100.96	0.96
	8.00	7.98	-0.29	99.70	0.75

*Average of five determinations

Application to pharmaceutical formulations

The proposed methods were applied for the determination of MD in its tablets where, good recoveries of 98.2-102% were obtained. These results were statistically compared with those obtained by a manufacturer method (1993) proving no significance difference with respect to accuracy and precision (Table 3).
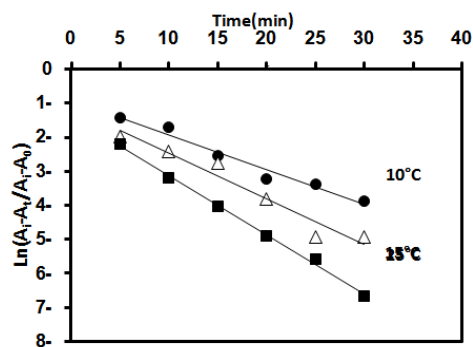

Fig.7 Pseudo first order plots for reaction of MD

Table 4
Rate constants and half life times of the studied reaction of MD at different temperatures using Arrhenius plot

Temperature(K)	Slop (k',min^{-1})	Intercept	t$_{1/2}$ (min)
283	0.1018	-0.7974	6.81
288	0.1330	-0.1218	5.21
298	0.1733	-0.1416	3.99

The relationship between the reaction rate and temperature is determined by the Arrhenius equation:

$$\ln k' = Ae^{-E_a/RT}$$

$$\ln k' = -\frac{E_a}{RT} + \ln A$$

E_a is the activation energy, R= 8.314 J moL^{-1}, T is the absolute temperature in Kelvin, and A is frequency factor (Atkins, 2006). The other activation parameters such as enthalpy, entropy and free energy of activation were

Table 3 Application of initial-rate and fixed time methods for determination of MD in tablets

Pharmaceutical form (Tablets 250mg)	Present Conce. (µgmL^{-1})	Initial rate			Fixed time			Official method recovery
		Found conce. (µgmL^{-1})	Recovery[a] (%)	RSD[a] (%)	Found conce. (µgmL^{-1})	Recovery[a] (%)	RSD[a] (%)	
Kadomet	3.00	2.95	98.47	1.11	2.95	98.19	2.41	99.5
	5.00	4.93	98.56	2.92	4.96	99.09	0.95	
Aldomate -SDI	3.00	2.97	99.00	2.93	2.98	99.48	2.15	98.3
	5.00	4.91	98.16	1.93	5.00	100.05	1.31	
Aldomate-ASIA	3.00	2.96	98.69	2.20	3.05	101.76	1.59	101.0
	5.00	5.10	102.04	2.79	5.01	100.18	0.84	
			t =0.49)[b]			t =0.19)		
			F=1.39)[b]			F=1.38)		

[a] For five determinations., [b] Critical values at *P*=0.95, *t*=2.447 and *F*=9.28

Kinetic and thermodynamic parameters

The kinetic studies of the reaction of MD drug with ferric chloride and NRS were carried out at different temperatures (10°C, 15°C and 25°C) within 35 min. The plot of ln{(A$_\infty$ - A$_t$)/(A$_\infty$- A$_0$)} as a function of time produced straight line with slope equal to –K', for a first order or pseudo first order reaction. The equation that gave the best fit for the experimental data corresponding to first order (Zhou *et. al.*, 2004) (Fig. 7) and the slope represents the rate constant. Table 4 summaries the calculated k and t$_{1/2}$ at different temperatures, from which it is evident that as the temperature increases, the t$_{1/2}$ decreases i.e. the reaction was less time consuming. Linear Arrhenius curves were obtained by plotting *ln k'* versus 1/T , and E$_a$ for MD was calculated from the slope to be 24.083 kJ moL^{-1} ,indicating that the reaction need low activation energy, and lnA calculated from the intercept to be 7.986 .

calculated using Eyring equation (Stevens and Phil, 1970) which was applied in the following form:

$$\ln \frac{k'}{T} = \left(\frac{-\Delta H^*}{R}\right)\left(\frac{1}{T}\right) + \ln \frac{k_B}{h} + \frac{\Delta S^*}{R}$$

where k$_B$ is Boltzmann's constant [1.381×10^{-23} J K^{-1}], h is Plank's constant [6.626×10^{-34} J s] and ln k$_B$/h equals 10.76, ΔH* (kJ moL^{-1}) is activation enthalpy, while ΔS* (J moL^{-1} K^{-1}) is activation entropy and ΔG* (kJ moL^{-1}) is the free activation enthalpy (Gibb's free energy).

Table 5 Thermodynamic parameters for the determination of MD at different temperatures using Eyring equation

Temperature (K)	ΔS* (J moL^{-1} K^{-1})	ΔH* (kJ moL^{-1})	ΔG* (kJ moL^{-1})
283			45.069
288	-78.535	21.666	44.284
298			43.892

The Eyring plot of ln(k'/T) versus 1/T produced a straight line , its slope $=-\Delta H^*/R$ .The ΔG^* (kJ mol^{-1}) which is the free activation enthalpy was calculated from the Gibbs-Helmholtzequation (Martin,1996): $\Delta G^* = \Delta H^* -T\Delta S^*$ All thermodynamic parameters were summarized in Table 5.

The negative values of ΔS^* at test reduce the freedom of motion in the transition state and relatively slow reaction that can be followed spectrophotometrically. The free energy ΔG^* is the key parameter, because its value under a particular set of reactant concentrations dictates the direction of molecular equilibrium. The free energy is a balance between enthalpy and entropy. The positive values of ΔG^* indicate that the reaction of both drugs with ferric chloride and NRS is non-spontaneous. The enthalpy change reflects the amount of heat energy required achieving a particular state, and the entropy measures how easily that energy might be distributed among various molecular energy levels. A positive value of ΔH^* indicates that the reaction is accompanied by absorption of heat and the process is endothermic (Cooper and Johnson, 1994).

CONCLUSION

The proposed method is superior to other reported methods by showing good sensitivity, and low detection limit. In addition to competitive precision and sensitivity, the proposed procedures show relevant selectivity allowing analysis without separation steps. The proposed methods are advantageous when they are compared with colorimetric methods, in having higher sensitivity (Tubino *et. al.,* 2006; Gotardo, 2008; Salem, 1987).Furthermore, the proposed method does not require pH control nor elaboration of treatment and procedure, which are usually associated with chromatographic methods and other colorimetric methods (Gadkariem *et. al.,* 2009; Issopoulos, 1989).The relationship between the reaction rate and temperature is determined by the Arrhenius equation. Linear Arrhenius curve indicates that the reactions need low activation energy.

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