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Development and validation of kinetic-spectrophotometric method for determination of methyl dopa in aqueous formulations and tablets

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ABSTRACT

The oxidation of Methyl dopa(MDP) by periodate in aqueous medium was used as basic reaction for developing new kinetic-spectrophotometric method for microgram estimation of MDP. The reaction was monitored by noting the increase in the absorbance of reaction mixture. The reaction is first order with respect to MDP and periodate each. The best fit conditions for for microgram determination of MDP in the range 51.30 µg/mL – 615.54 µg/mL are, [$NaIO_4$] = 1.0 ×10^{-5} mol dm^{-3}, Temperature = 35.0 ± 0.1°C, pH = 4.5, λmax = 485nm. The characteristics of various calibration curves, percentage recovery, effect of excipients, correlation coefficient and comparison with other reported methods etc. are presented.

Keywords: Kinetic-spectrophotometric method, Microgram Estimation, Methyl dopa, Periodate ion, Aqueous formulations, Tablets

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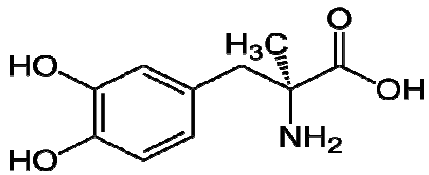
INTRODUCTION

Methyldopa or 3-(3,4-dihydroxy phenyl)-2 methyl-*L*-alanine sesquihydrate with molecular formula $C_{10}H_{13}NO_4$, 1½ H_2O, molecular weight 238.24 and structure [1] given below, is primarily used as the adrenergic blocker (central and peripheral adrenergic inhibitor) which reduces cardiac output or decreases the peripheral vascular resistance and helps in management of acute or severe hypertension [2-3].



The quantifying of catecholamines (like methyldopa, dopamine and levodopa) in physiological fluids has an importance in clinical chemistry as these biogenic amines serve as diagnostic marker molecules for a variety of metabolic and neurological disorders [4]. The determination of plasma catecholamine levels have been proven to be highly important for diagnosis, therapy and prognosis of cardiovascular diseases, catecholamine-secreting tumers arising from chromaffin cells of sympatoadrenalinal system such as neuroblastoma and pheochromocytoma, and active disorders [5]. Pharmaceutical preparations containing these catecholamines have been available for many years. On the basis of this background, the determination of trace amounts of catecholamines is becoming increasingly important [6]. In view of their importance considerable work has been done on their detection and quantification. A number of methods have been reported for the determination of catecholamines by spectrofluorimetry [7], gas chromatography [8], capillary electrophoresis [9], chemiluminiscence detection [10], HPLC [11], radio immuno assay [12] and voltammetric determination [13]. Estimation of some catecholamines after

their derivatization with organic and inorganic reagents and using stopped flow technique [14-17] or flow injection analysis [18-19] is already a part of literature. Kinetic study of oxidation of some catecholamines by digital simulation of cyclic voltagrammes was reported by Afkhami et al. [20-21].

Methyldopa is estimated in raw form and tablets after treatment with anhydrous formic acid, anhydrous acetic acid and dioxane and then titrating against perchloric acid using crystal violet as the indicator [1,22]. Currently, a method based on a lengthy pretreatment of tablets followed by spectrophotometric estimation of the product formed, is also in use [23]. Afkhami et al. [24] developed an indirect spectrophotometric method for determination of methyldopa based on its periodate oxidation in acidic media in the range 0.1 to 2.5 µg/ ml by measuring the absorbance of methylene blue indicator after its reaction with iodine produced by oxidation of iodide ion by the excess of periodate ion left. Berzas et al. [25] reported a stopped flow spectrophotometric determination of methyldopa based on its aerial oxidation in the range greater than 0.0002 M. Nevado et al. reported the determination of methyldopa in the range 2 x 10^{-5} to 20 x 10^{-5} mol/ lit. with detection limit 3.2 x 10^{-7} M by using the flow injection analysis of metaperiodate oxidation of methyldopa [26]. Nagaraja et al. [27] estimated methyldopa spectrophotometrically after its reaction with diazotized sulphanilamide in presence of molybdate ion. It was estimated by noting the absorbance of tris (o-phenanthroline) iron (II) or tris (bipyridyl) iron (II) obtained by its oxidation by fe(III) in presence of 1,10 – phenanthroline and 2, 2' – bipyridyl respectively in concentration range 0.5 – 1.0 and 0.04 – 1.3 µg/ ml [28]. Flow injection method has also been used for estimation of 20 – 200 p.p.m. of methyldopa after complexation of methyldopa with molybdate ion resulting in the formation of yellow product [29]. Madrakian et al. [6] estimated 0.1 to 20 mg/ ml of methyldopa using its periodate oxidation followed by oxidative coupling with 4-aminobenzoic acid at pH = 4 leading the formation of coloured o-benzoquinones. Tubino et al. [30] determined 40 to 360 µg/ ml of methyldopa after oxidizing it into quinone by ferric ion in presence of salicylic acid and HCl and measuring the absorbance of the blue coloured complex formed between ferric ion and salicylic acid.

Periodate ion oxidizes the compounds containing amino group [31-43]. Keeping this point in view, we have undertaken and reported the kinetics of periodate oxidation of MDP in aqueous medium [44]. Keeping in view the time consuming pretreatments, involvement of high cost and requirement of costly equipments for the estimation of MDP by the reported methods, and in continuation to our report on the kinetic study of periodate oxidation of MDP, this paper describes simple and cost effective kinetic-spectrphotometric methods for the estimation of MDP in aqueous formulations and tablets.

MATERIALS AND METHODS

Reagents

The pH of the reaction system was measured on Systronics digital pH meter. The pH of reaction mixtures was maintained by using Thiel, Schultz and Coch buffer [45] (in which different volumes of 0.05 M oxalic acid + 0.02 M boric acid + 0.05 M succinic acid + 0.05 M sodium sulfate + 0.05 M borax were used to maintain the pH values of reaction mixtures). Sodium metaperiodate (CDH, AnalaR grade), MDP of Glenmark make was used in pure form. To avoid the photooxidation the drug solutions were kept in dark bottles and fresh solutions were prepared as soon as they started getting coloured. All other chemicals of (CDH AnalaR) analytical reagent/ guaranteed reagent grade were used after redistillation/ recrystallization. Triply distilled water was used for preparation of the solutions.

Recommended Kinetic-spectrophotometric procedure

Reaction between methyl dopa (MDP) and periodate ion resulted in the development of light pink colour which disappears after about 2 hours. The reaction was studied in aqueous medium. The reaction was studied in a spectrophotometric cell and initiated by adding temperature equilibrated $NaIO_4$ solution of known concentration to the reaction mixture containing the MDP, and buffer and maintained at 35 ± 0.1^{0}C. The progress of the reaction was followed by recording the absorbance on Shimadzu double beam spectrophotometer (UV-Pharmaspec-1700), at 485 nm, i.e., the λ_{max} of the reaction mixture. λ_{max} was not found to change with change in time under experimental conditions. Desired temperature was maintained with the help of a high precision thermostatic control.

Following are the finally worked out conditions for running the kinetic sets for the purpose of determination of MDP in aqueous medium based upon the periodate oxidation of MDP: [MDP] = 51.30 µg/mL – 615.54 µg/mL, [$NaIO_4$] = 1.0 ×10^{-5} mol dm^{-3}, Temperature = 35.0 ± 0.1°C, pH = 4.5, λmax = 485nm.

A definite volume of stock solution of MDP in water was mixed with calculated volume of the stock solution of buffer and stirred a little with the help of the pipette. This mixture and stock solution of $NaIO_4$ were then clamped in a thermostat at 35 ± 0.1^{o}C. After 25 minutes, a required amount of the periodate solution was added to the mixture and stirred to start the reaction. All additions were made in amounts calculated for maintaining the concentrations of different reagents as mentioned above. Different sets were prepared in a similar manner varying the [MDP].

Aliquots were withdrawn from the reaction mixture after repeated intervals of 10 seconds and the absorbance was recorded on a double beam spectrophotometer. The absorbance vs time plots were then made for different sets. The initial rates [$(dA/dt)_{10}$] were evaluated after 10 seconds from the start of the reaction by applying plane mirror method on the absorbance vs time plots. The pseudo first order rate constants (k_1) were found by Guggenheim's method.

Using the method of least squares, linear calibration curves (Fig. 1,2), were obtained in terms of type 'A', type 'B', type 'C', type 'D', type 'E', type 'F' , type 'G', type 'H', type 'I'and type 'J' plot i.e. A_{10} or A_{20} or A_{30} or A_{40} or A_{50} or A_{60} or A_{70} or A_{80} or initial rate or k_1 vs [MDP] plots respectively (where A_{10}, A_{20}, A_{30}, A_{40}, A_{50}, A_{60}, A_{70} and A_{80} are the absorbance values after 10, 20, 30, 40, 50, 60, 70 and 80 seconds from the start of reaction respectively)

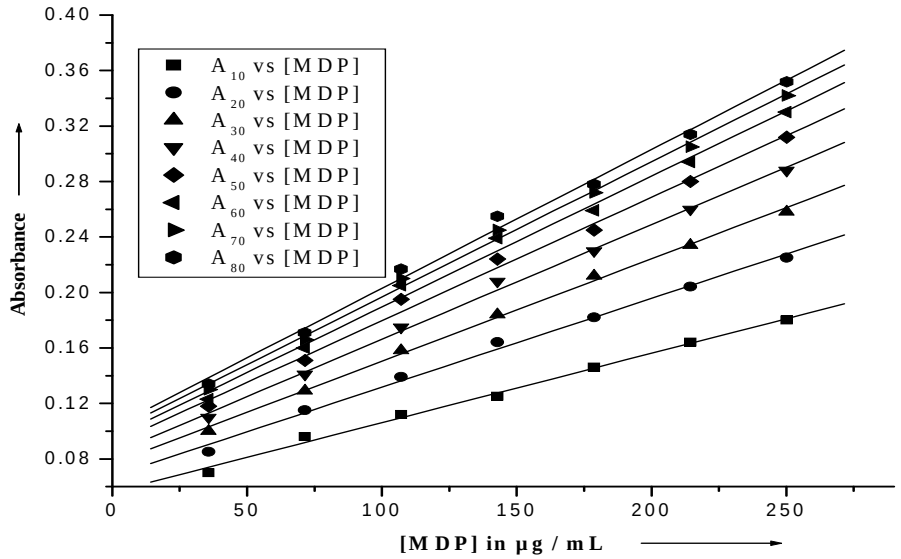


Fig. 1: Calibration curves in terms of absorbance vs [MDP] plots at [$NaIO_4$] $\times 10^5$ = 1.0 mol dm^{-3}, Temp. = 35 ± 0.1°C, pH = 4.5, λ_{max} = 485

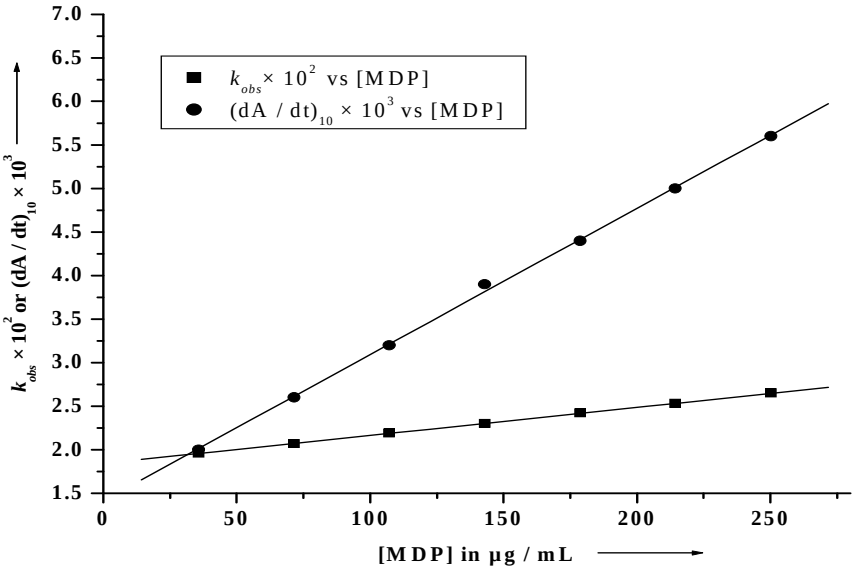


Fig. 2: Calibration curves in terms of initial rate or pseudo first order rate constant vs [MDP] plots at [$NaIO_4$] $\times 10^5$ = 1.0 mol dm^{-3}, Temp. = 35 ± 0.1°C, pH = 4.5, λ_{max} = 485 nm

RESULTS

Validity of Beer's law and other characteristics of the method

The range of [MDP] in which the Beer's law is obeyed, Sandell's sensitivity, correlation coefficient and the coefficient of determination, LOD, LOQ, value of 't' (at 0.01 significance level), relative standard deviation and % error for various calibration curves are given in Table 1 and 2. The characteristics of calibration curves were evaluated in the form of equations of straight line as follows:

$A_{10} = 5.61 \times 10^{-2} + 4.99 \times 10^{-4}$ [MDP] -----(1)
$A_{20} = 6.76 \times 10^{-2} + 6.41 \times 10^{-4}$ [MDP] -----(2)
$A_{30} = 7.67 \times 10^{-2} + 7.37 \times 10^{-4}$ [MDP] -----(3)
$A_{40} = 8.36 \times 10^{-2} + 8.26 \times 10^{-4}$ [MDP] -----(4)
$A_{50} = 9.07 \times 10^{-2} + 8.89 \times 10^{-4}$ [MDP] -----(5)
$A_{60} = 9.53 \times 10^{-2} + 9.42 \times 10^{-4}$ [MDP] -----(6)
$A_{70} = 9.93 \times 10^{-2} + 9.75 \times 10^{-4}$ [MDP] -----(7)
$A_{80} = 10.29 \times 10^{-2} + 10.00 \times 10^{-4}$ [MDP] -----(8)
$(dA/dt)_{10} = 1.41 \times 10^{-3} + 1.68 \times 10^{-5}$ [MDP] -----(9)
$K_1 = 1.84 \times 10^{-2} + 3.22 \times 10^{-5}$ [MDP] -----(10)

In Eq. (1) to (8), the slopes and intercept are in absorbance units. μg^{-1}. cm^3 and absorbance units respectively. For Eq. (9) and (10), the values of slope and intercepts are in absorbance units. μg^{-1}. cm^3. sec^{-1} and absorbance units. sec^{-1} respectively. The [MDP] are in µg/ml.

Effect of interferring ions

The method may be used in presence of the ions like Na^+, K^+, NO_2^-, ClO_4^{-2}, NO_3^-, and SO_4^{-2} as they do not interfere significantly in present case. It was observed that upto 100 µg/ml of Cl^-, PO_4^{-3}, SCN^-, NO_3^-, CO_3^-, CH_3COO^-, Ca^{++}, Mg^{++}, Na^+, K^+ and Zn^{++} do not interfere. Upto 50 µg/ml of Cu^{++} and Fe^{++} do not interfere.

However, the metals like Ag, As, B, Co, Cd, Cr, Hg, Mo, Ni, Pb, Sb, Se, and U are expected to interfere in this method. Cu^{++} and Fe^{++} interfere if present in amounts greater than 50 µg/ml. Therefore, a pretreatment is required for separating/ precipitating/ masking these ions before undertaking the proposed method. For this purpose, H_2S may be passed in presence of 0.3 M H^+ solution, followed by filtration and boiling off H_2S. After it, a dilute alkaline solution of α-nitroso-β-naphthol should be added and again the solution should be filtered [46]. Thereafter, the solution should be neutralized and the present method be applied. Fe may be removed by precipitation using basic formate method [47-48]. In absence of the above given interferrants, the proposed method may successfully be used for the determination of microgram quantities of MDP in aqueous medium.

Table 1: Characteristics of different Calibration curves for estimation of MDP

Parameter	'A' plot	'B' plot	'C' plot	'D' plot	'E' plot
Beer's law limits (µg/ml)	35.74-250.20	35.74-250.20	35.74-250.20	35.74-250.20	35.74-250.20
Sandell's sensitivity (µg. cm^{-2})	2.0	1.6	1.4	1.2	1.1
Slope x 10^4 absorbance units. $\mu g^{-1}.cm^3$ (from regression equation)	4.99	6.41	7.38	8.26	8.89
Intercept x 10^2 (abs.units) (from regression equation)	5.61	6.76	7.67	8.36	9.07
Correlation coefficient (r)	0.9974	0.9975	0.9989	0.9986	0.9969
Coefficient of determination (r^2)	0.9948	0.9950	0.9978	0.9972	0.9938
't' (at 0.01 significance level)	8.7266	8.4930	8.4547	8.3529	8.3687
LOD	3.7	3.97	3.65	2.78	3.55
LOQ	11.2	12.0	11.1	8.43	10.8
Relative Standard deviation (%) (Aqueous formulations)	2.2845 × 10^{-4}	3.2308 ×10^{-4}	3.2308 ×10^{-4}	2.2845 ×10^{-4}	8.2308 ×10^{-4}
Recovery (%)	99.9	99.8	99.8	99.9	99.9
Relative Standard deviation (%) (Alphadopa Tablet)	3.4163 ×10^{-3}	2.9552 ×10^{-3}	3.2195 ×10^{-3}	2.3902 ×10^{-3}	2.8569 ×10^{-3}
Recovery (%)	96.1	97.5	97.6	98.4	98.2

Table 2: Characteristics of different Calibration curves for estimation of MDP

Parameter	'F' plot	'G' plot	'H' plot	'I' Plot	'J' plot
Beer's law limits (µg/ml)	35.74-250.20	35.74-250.20	35.74-250.20	35.74-250.20	35.74-250.20
Sandell's sensitivity (µg. cm^{-2})	1.1	1.0	1.0	---	---
Slope x 10^4 absorbance units. $\mu g^{-1}.cm^3$ (from regression equation)	9.42	9.75	10.00	1.68 s^{-1}	3.22 s^{-1}
Intercept x 10^2 (abs.units) (from regression equation)	9.53	9.93	10.28	1.41 $x10^{-1}$ s^{-1}	1.84 s^{-1}
Correlation coefficient (r)	0.9963	0.9980	0.9974	0.9996	0.9999
Coefficient of determination (r^2)	0.9926	0.9960	0.9948	0.9992	0.9998
LOD	2.44	3.27	2.86	2.90	2.48
LOQ	7.40	9.89	8.68	8.70	7.45
't' (at 0.01 significance level)	8.3336	8.3712	8.4006	7.7826	24.4952
Relative Standard deviation (%) (Aqueous formulations)	7.9139 $\times10^{-4}$	7.9139 $\times10^{-4}$	3.2308 $\times10^{-4}$	2.2845 $\times10^{-2}$	1.3159 $\times10^{-2}$
Recovery (%)	99.5	99.5	99.9	99.6	99.2
Relative Standard deviation (%) (Alphadopa Tablet)	2.8569 $\times10^{-3}$	3.4638 $\times10^{-3}$	3.4163 $\times10^{-3}$	4.0402 $\times10^{-2}$	3.2883 $\times10^{-2}$
Recovery (%)	98.3	98.1	98.1	99.1	99.2

Effect of excipients

In order to examine the applicability of the proposed method to pharmaceutical analysis, the effect of various excipients was studied. It was found that Glucose, Fructose, Saccharose, starch, Polyethylene glycol, sodium chloride and sodium sulfite do not interfere and when these excipients were added 1 to 10 times the concentration of MDP, the recovery of MDP by using the proposed method was between 96 – 105%. Starch, talc, lactose, and magnesium stearate do not interfere in the proposed method.

Proposed method for determination of MDP* in *aqueous formulations

Dilute the sample with buffer solution in order to obtain a concentration of MDP between the range worked out for the proposed method and carry out the procedure described.

Proposed method for determination of MDP* in *tablets

Crush the tablet in mortar, dissolve one tablet in buffer solutions in order to obtain a concentration in the range worked out for the proposed method. By using mechanical shaker, the powder is completely disintegrated and the solution is clarified by passing it through Whatman filter paper no. 1, rejecting the first 10 ml and then appropriate dilutions are to be made. After it, the proposed method can be applied.

DISCUSSION

The proposed method was tested for many water samples containing known amounts of MDP in the range of the detection limits reported above. The results were found to be reproducible with reasonable standard deviation and low range of errors as calculated from six determinations (Table 1, 2). A change in absorbance by 0.001 unit is expected on changing the concentration of MDP by 1.0 – 2.0 µg/ml. Further, a change in concentration by 1.0 µg/ml will change the rate of reaction by 0.001 absorbance units/minute. This is much better than the sensitivity (14.92 µg/ml change causing a change in absorbance by 0.001 units in one minute) reported for the method proposed by Tubino et.al [30]. In addition, the value of k_1 will change by 0.00193 in 1 minute on changing [MDP] by 1 µg/ml i.e. a change sufficient enough to be observed easily. The range of determination (35.70 µg/ml to 250.2 µg/ml) is also considerably low and it is good for the general determination of MDP. The correlation coefficient (r) is in the range 0.9963 to 0.9999 which indicates the high precision involved in the determination and almost perfect correlation of the data. The value of coefficient of determination (r^2) suggests that 99.26% to 99.98% change in the value of A_{10} or A_{20} or A_{30} or A_{40} or A_{50} or A_{60} or A_{70} or A_{80} or initial rate or k_1 is caused by MDP and the rest 0.02% to 0.74% is the effect of unknown factors. The value of 't' as calculated for the calibration curves, are in the range 7.7826 to 24.4952 which are much higher than the tabulated critical value at 0.01 significance level. This suggests that there are less than 1% chances of error in drawing conclusions. The standard deviation is within reasonable limits. Percentage recovery on the basis of six parellel determinations is 99.2% to 99.9% in solutions and 96.1% to 99.2% in alphadopa tablets. Overall, the 'H' plot can be said to be the best calibration curve suited very well for estimation of MDP in aqueous medium in micrograms with Sendell's sensitivity of 1 µg/ ml.

The value of slope of the calibration curves and Sandell's sensitivity are not very good. However, as the MDP amount is to be estimated in tablets and other aqueous formulations in which it is present in the range 250-500 mg per tablet, the sensitivity in micrograms is sufficient enough for all practical purposes. Keeping this in view, it is not

required to have an ultra micro analytical quantitative method for its determination. On the contrary, a method workable in a higher concentration range is needed so that preparation of solutions etc becomes simple. It is encouraging that the proposed method is better than many of the reported ones [6, 21, 24, 26-30] as far as the simplicity, lesser facilities required for applying the methods, interference of aromatic amines or ions or excipients, validity of Beer's law, detection limits, correlation of data and reproducibility of results is concerned for the general estimation of MDP in aqueous formulations and tablets. Further the simplicity involved in the procedure and the low cost of determination go in favour of the proposed method.

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