

## Sensitive-Spectrophotometric Determination of Methyl Dopa and Adrenaline

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A Simple, precise, sensitive and accurate method for determination of trace quantities of methyl dopa and adrenaline is described which is based on their reaction with Fe(III) in the presence of 1,10-phenanthroline at pH 5.4. There is a linear relation between the absorbance of produced ferroin at 510 nm and the concentration of methyl dopa and adrenaline in the rang of 0.01–0.8  $\mu\text{g ml}^{-1}$  and 0.03–8.0  $\mu\text{g ml}^{-1}$ , respectively. The relative standard deviation for ten determinations of 0.5  $\mu\text{g ml}^{-1}$  of methyl dopa and adrenaline was 0.85 and 1.06% respectively. The  $3\sigma$  limit of detection of the method was 0.009 and 0.017 for methyl dopa and adrenaline, respectively. The method was applied to the determination of methyl dopa and adrenaline in plasma and pharmaceutical preparations.

**Key Words:** Spectrophotometric, Determination, Methyl dopa, Adrenaline

### INTRODUCTION

Catecholamines (CAs) are compounds containing an *o*-catechol nucleus and amine group on a chain of two-carbon atom *m*<sup>-</sup> or *p*<sup>-</sup> to the phenolic hydroxyl groups. Methyl dopa and adrenaline are two of the catecholamines. Methyl dopa is converted to  $\alpha$ -methyl dopamine and  $\alpha$ -methyl norepinephrine and used in the treatment hypertension. Adrenaline (epinephrine) is a very potent vasoconstrictor and cardiac stimulant<sup>1</sup>. Pharmaceutical preparations containing these catecholamines are available for many years. On the basis of this background, the determination of trace amounts of catecholamines is becoming increasingly important.

Different methods have been used for determination of catecholamines, such as high performance liquid chromatography<sup>2</sup>, fluorimetry<sup>3</sup> and spectrophotometry<sup>4–10</sup>. Difference spectrophotometric method has been used to the determination of 1,2-diphenolic drugs<sup>4</sup>. The method is base on the measurement of the difference in absorbance between two equimolar solutions of the drug in phosphate buffer of pH 7.0 one of which also contains 0.1 M  $\text{H}_3\text{BO}_3$ . This difference in absorbance, which is maximum at 292 nm, is due to the difference spectral characteristics of the  $\text{H}_3\text{BO}_3$  ester of the drug and of the unesterified drug and is proportional to drug concentration. Dual wavelength spectrophotometry

has been used<sup>5</sup> for the determination of 20–100 µg/ml of benserazide. Berzas *et al.*<sup>6</sup> have reported a stopped flow spectrophotometric determination of dopamine and methyl dopa. The method is based on the aerial oxidation of these catecholamines in 0.6 M NaOH at 60°C and determination of the variation in absorbance at 360 nm at 30 sec after injection of the sample calibration graphs were linear up to  $2.0 \times 10^{-4}$  M dopamine and  $2.08 \times 10^{-4}$  M methyl dopa. Peroxidase based spectrophotometric method has been used for the determination of ascorbic acid, norepinephrine, epinephrine, dopamine and levodopa<sup>7</sup>. The method is based on the inhibitory action of the named compounds on the oxidative coupling of *p*-chlorophenol and 4-aminoantipyrine at pH 6.6 in the presence of H<sub>2</sub>O<sub>2</sub> and horseradish peroxidase catalyst. Calibration graphs were linear in the range of 0.4–12.0 µg/ml for ascorbic acid, 1.0–20.0 µg/ml for epinephrine, 0.4–4.0 µg/ml for dopamine and 0.075–1.2 µg/ml for L-dopa. Gowada *et al.*<sup>10</sup> determined catecholamines by spectrophotometry after their oxidation by K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> followed by oxidative coupling with sulfanilic acid.

In this paper a spectrophotometric method for the determination of trace amounts of these methyl dopa and adrenaline has been introduced based on their reaction with Fe(III) in the presence of 1,10-phenanthroline at pH 5.4. The proposed method is simple, reliable and sensitive and is suitable for the determination of trace quantities of methyl dopa and adrenaline in plasma and in pharmaceutical preparations.

## EXPERIMENTAL

All solutions were prepared using reagent grade substances and triply distilled water. Methyl dopa and adrenaline stock solutions ( $1000 \mu\text{g ml}^{-1}$ ) were prepared from Sigma products in water and stored in a dark bottle in refrigerator. Working solutions were prepared by diluting the stock solutions with water. A  $5.18 \times 10^{-3}$  M Fe<sup>3+</sup> solution was prepared by dissolving 0.3500 g FeCl<sub>3</sub> 6H<sub>2</sub>O (Merck) in a 100 ml volumetric flask. A  $5.56 \times 10^{-3}$  M solution of 1,10-phenanthroline (Merck) was prepared by dissolving 0.1002 g in a 100 mL volumetric flask. Acetate-acetic acid buffer solution was used.

A Cecil model CE 1020 spectrophotometer with an 1-cm glass cell was used for absorbance measurements.

### Procedure

A suitable aliquot of the sample solution was transferred into a 10 mL volumetric flask. Then 1 mL of  $5.18 \times 10^{-3}$  M Fe<sup>3+</sup> solution and 1 mL of pH 5.4 acetate buffer solution was added. The solution was diluted to ca. 9 mL with water and heated in a water bath at 40°C for 1/2 h subsequent by cooling to room temperature (30°C). Then 1 mL of  $6.06 \times 10^{-3}$  M 1,10 phenanthroline solution was added and the solution was diluted to the mark with water. A portion of solution was transferred into a 1-cm glass cell to measure the absorbance at 510 nm against a blank solution that was prepared in the same method except that distilled water was used instead of methyl dopa or adrenaline solution.

## RESULTS AND DISCUSSION

Methyl dopa and adrenaline are oxidized by  $\text{Fe}^{3+}$  to produce  $\text{Fe}^{2+}$ . The produced  $\text{Fe}^{2+}$  reacts with 1, 10-phenanthroline to produce ferriin. The amount of produced ferriin is then proportion with the methyl dopa or adrenaline concentration. Ferriin concentration could be determined spectrophotometrically by measuring the absorbance of the solution at 510 nm ( $\lambda_{\text{max}}$  for absorption spectra of ferriin).

### Effect of variables

To take full advantage of the procedure, the reagent concentrations and reaction conditions must be optimized. These parameters were optimized by placing all parameters constant and optimizing one each time. Methyl dopa.

The effect of  $\text{Fe}^{3+}$  concentration in the range of  $7.4 \times 10^{-5} - 5.18 \times 10^{-4}$  M on the absorbance was studied. As Fig. 1 shows the absorbance increased by increasing  $\text{Fe}^{3+}$  concentration up to  $3.0 \times 10^{-4}$  M and remained constant at higher concentrations. Therefore, a final concentration of  $5.18 \times 10^{-4}$  M  $\text{Fe}^{3+}$  was used for routine works.

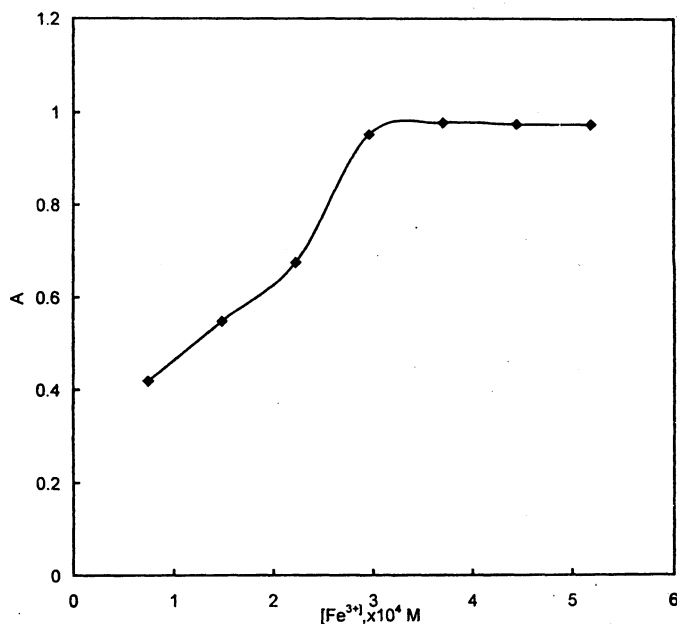


Fig. 1. Effect of  $\text{Fe}^{3+}$  concentration on the reaction

The effect of 1,10-phenanthroline on the absorbance was studied in the range of  $0.00 - 1.11 \times 10^{-3}$  M. The results are shown in Fig. 2. As Fig. 2 shows the absorbance increased by increasing 1,10-phenanthroline concentration up to  $6.0 \times 10^{-4}$  M and remained constant at higher concentrations. Therefore a final concentration of  $1.11 \times 10^{-3}$  M 1,10-phenanthroline was used for routine works.

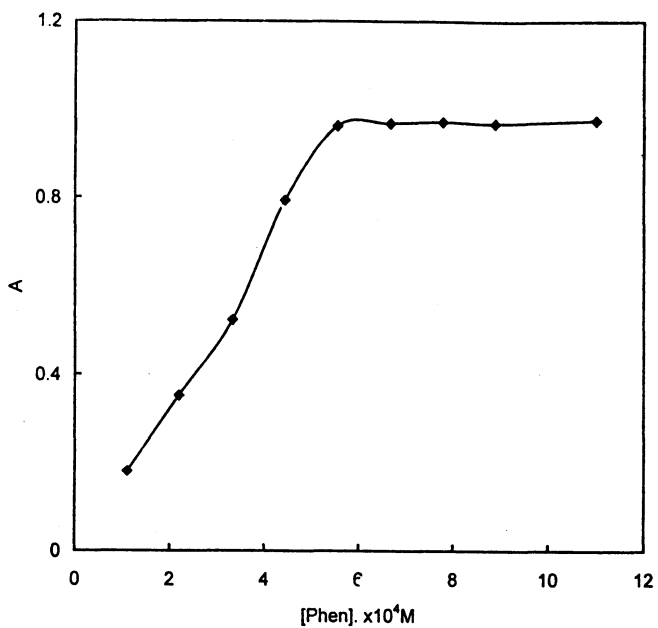


Fig. 2. Effect of phenanthroline concentration on the reaction

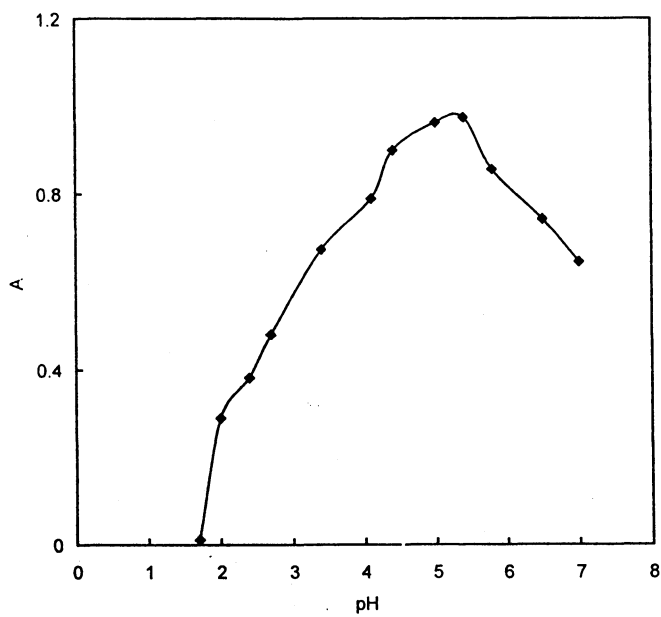


Fig. 3. Effect of pH on the reaction

The effect of pH on the absorbance was studied in the range of 1.7–7.0 using HCl and NaOH solutions for pH adjustment. As Fig. 3 shows the absorbance of the reaction mixture increased by increasing pH up to 5.4 and decreased at higher pH values. Therefore pH of 5.4 was optimum.

The effect of temperature in the range of 5–70°C was studied by heating the reaction mixture in the water bath of desired temperature for 1/2 h. The results are shown in Fig. 4. The absorbance increased by increasing temperature up to 40°C and remained constant at higher temperatures. Therefore 1/2 h heating in 40°C water bath is sufficient for maximum color production.

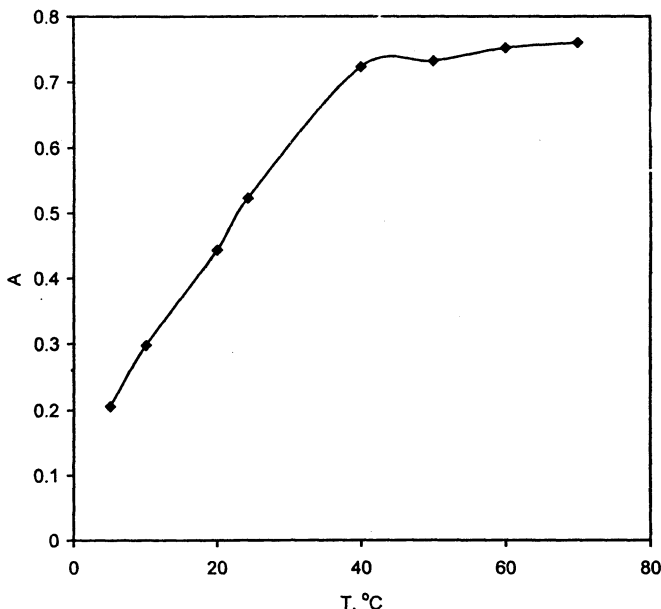


Fig. 4. Effect of temperature on the reaction

### Analytical parameters

The calibration graphs were obtained under the optimum conditions described above. For methyl dopa, the calibration graph was linear in the range of 0.01–0.80  $\mu\text{g/mL}$ . The regression equation is  $A = 0.0858 + 0.557 C$ , with a correlation coefficient of 0.9992, where A is sample absorbance and C is methyl dopa concentration in  $\mu\text{g/mL}$ .

For adrenaline, the calibration graph was linear in the range of 0.03–8.00  $\mu\text{g/mL}$ . The regression equation is  $A = 0.054 + 0.181 C$ , with a correlation coefficient of 0.9990, where A is sample absorbance and C is adrenaline concentration in  $\mu\text{g/mL}$ .

The relative standard deviation for ten determinations of 0.5  $\mu\text{g mL}^{-1}$  of methyl dopa and adrenaline was 0.85 and 1.06% respectively.

The limit of detection which can be calculated on the basis of  $Y_{\text{LOD}} = Y_{\text{B}} + 3S_{\text{B}}$ , in which  $Y_{\text{LOD}}$ ,  $Y_{\text{B}}$  and  $S_{\text{B}}$  are signal of limit of detection, signal of blank,

standard deviation of blank, signal of blank and standard deviation of blank<sup>11</sup>, respectively, was 0.009  $\mu\text{g ml}^{-1}$  for methyl dopa and 0.017  $\mu\text{g ml}^{-1}$  for adrenaline.

### Applications

To evaluate the analytical applicability of the proposed method, it was applied to the determination of methyl dopa and adrenaline in pharmaceutical preparations and in plasma. Catecholamines in aqueous formulations were determined after proper dilution of the sample with water, for determination of catecholamines in tablets, 20 tablets were weighed carefully and powdered. An accurately weighed quantity of powder was shaken with water. After filtration and proper dilution of the solution the recommended procedure was followed. The results are given in Table 1. As Table-1 shows, there is a good agreement between the results of the proposed and official method.

The plasma was spiked with different amounts of methyl dopa or adrenaline and then analyzed by proposed method. The results are given in Table-2. The recoveries approached to 100%.

TABLE-1  
DETERMINATION OF METHYL DOPA AND ADRENALINE  
IN PHARMACEUTICAL PREPARATIONS

Analyte	Formulation	mg of analyte/tablet or aliquot		
		Declared amount	Proposed method <sup>a</sup>	Official method
Methyl dopa	tablet	500	507	503
	tablet	250	255	251
Adrenaline	injection	10.0/1 mL	10.8	10.2
	injection	1.0/1 mL	1.10	0.98

<sup>a</sup>average of 5 determinations

TABLE-2  
DETERMINATION OF METHYL DOPA AND ADRENALINE IN PLASMA

Analyte	Amount of Analyte ( $\mu\text{g mL}^{-1}$ )		Recovery (%)
	Added	Found	
Methyl dopa	0.200	0.215	107.5
	0.500	0.530	106.0
Adrenaline	0.800	0.760	95.0
	1.000	0.920	92.0

### Conclusion

The proposed spectrophotometric method is economical, simple, rapid, precise and sensitive for determination of methyl dopa and adrenaline in pharmaceutical preparations or in plasma. It necessitates no prior hydrolysis or prior extraction.

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