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Research Article

A NOVEL SPECTROPHOTOMETRIC DETERMINATION OF METHYLDOPA THROUGH TERNARY COMPLEXATION PROCEDURE USING FE(III), MN(II), AND CO(II) WITH 2-AMINOPYRIDINE

MUSTAFA JAMAL KHALEEL BICHAN, FADAM MUTEB ABDOON*

Department of Chemistry, College of Science, Tikrit University, Tikrit, Iraq. *Email: Fadamabdon@tu.edu.iq

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ABSTRACT

Objective: The present study is aimed to find a three simple, low cost, accurate, rapid, and sensitive spectrophotometric methods based on the formation of ternary complexes to assay methyldopa (MTD) in both pure and pharmaceutical dosage forms.

Methods: The suggested complexation procedure is based on the formation of ternary complex among MTD, 2-aminopyridine (2-Amp), and different metal cations such as [Fe(III), Mn(II), and Co(II)] to form three complexes of Fe(III)-MTD-2-Amp (A), Mn(II)-MTD-2-Amp (B), and Co(II)-MTD-2-Amp (C) in an aqueous medium.

Results: The obtained colored complexes are spectrophotometrically measured for the previously mentioned complexes at 572, 473, and 465 nm, respectively. Under optimum conditions, the complexes exhibited apparent, molar absorptivities of 1810.62, 2954.18, and 2596.8 l/mol/cm, Sandell's sensitivity of 0.132, 0.08, and 0.092 μg/cm^2, and Beer–Lambert's law is obeyed over the ranges 4–40, 4–32, and 4–40 μg/ml for the three developed methods, respectively.

Conclusion: The developed spectrophotometric methods showed excellent results in regard to accuracy and precision with recovery of 99.48±1.62%, 100.24±1.76%, and 100.72±1.65% of the complexes A, B, and C, respectively. The obtained results are compared statistically with a reported method with respect to *t*- and *F*-tests and the calculated results displayed no significant difference.

Keywords: Methyldopa, Ternary complex, Mixed-ligand, Spectrophotometry.

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INTRODUCTION

Methyldopa (MTD) is a catecholamine derivative that recommended for the treatment of hypertensive disease. It is one of the most preferred antihypertensive medications in pregnancy, particularly in complicated cases of pregnancy and renal failure. Due to, it does not affect both uterine and placental circulations, it maintains the renal blood flow [1,2], chemically, known as 3-hydroxy-a-methyl-L-tyrosine sesquihydrate (Fig. 1). Its antihypertensive characteristic is primarily assigned to mislead the alpha-adrenoreceptors in the lower brain stem to accept its metabolitea-methyl-norepinephrine instead of norepinephrine and causes neurotransmitter stimulation to reduce nervous sympathetic and leads to lower of the blood pressure [3].

The literature survey reported several analytical methods to determine MTD. Among these methods, high-performance liquid chromatography [4], liquid chromatography coupled with mass spectrometry [5], electrochemical methods [6-9], chemiluminescence [10], and other techniques [11,12]. The ternary complex formation has been considered to be of an important role in the field of spectrophotometric analysis [13,14]; they are divided into two types, ion-association and a mixed-ligand complex. Different pharmaceutical compounds have been analyzed using ion-pair complexes [15,16]. Meanwhile, the formation of ternary complexes starts to get much attention [17,18]. By increasing the demand for the more sensitive reagents, the attempts have been made to intensify the color using a third component like metal ions to form a ternary complex. Large bathochromic shift due to deep color formation is observed by the addition of specific metal ions to decolorized solutions, and high absorbance at a shifted wavelength will result in an increase in molar absorptivity and sensitivity.

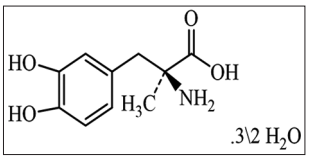

Fig. 1: Structure of methyldopa

Although the reported methods indicated that different analytical techniques have been employed to determine MTD, the present study aims to suggest a simple and fast spectrophotometric method based on the formation of ternary Complexes of MTD, 2-Amp and different metal cations such as Fe(III), Mn(II), and Co(II) in an aqueous medium. The suggested method is exploited to determine the MTD in its bulk powder and pharmaceutical dosage forms.

EXPERIMENTAL

Physical measurements

All spectrophotometric measurements are carried out using a Shimadzu spectrophotometer (Model UV-1800) dual beam with 1.0 cm matched quartz cells. Infrared spectra data are recorded on a Shimadzu FTIR-8400S (4000-400 cm-1) using KBr discs.

Chemicals and reagents

All chemicals used are of analytical grade and used without further purification. Deionized water is used throughout all the experiments. A stock solution of 1000 μg/ml, 4.19×10^{-3} mol/l) of MTD standard is kindly supplied by the state company of Drugs Industry and Medical Appliances (Samarra, Iraq). A freshly prepared solutions of each (1000 μg/ml, 6.16×10^{-3} mol/l) of Iron(III) chloride (BDH, pool, UK) (1000 μg/ml, 5.05×10^{-3} mol/l) of Manganese(II) chloride hexahydrate (Fluka, Switzerland), (1000 μg/ml, 4.2×10^{-3} mol/l) of Cobalt(II)

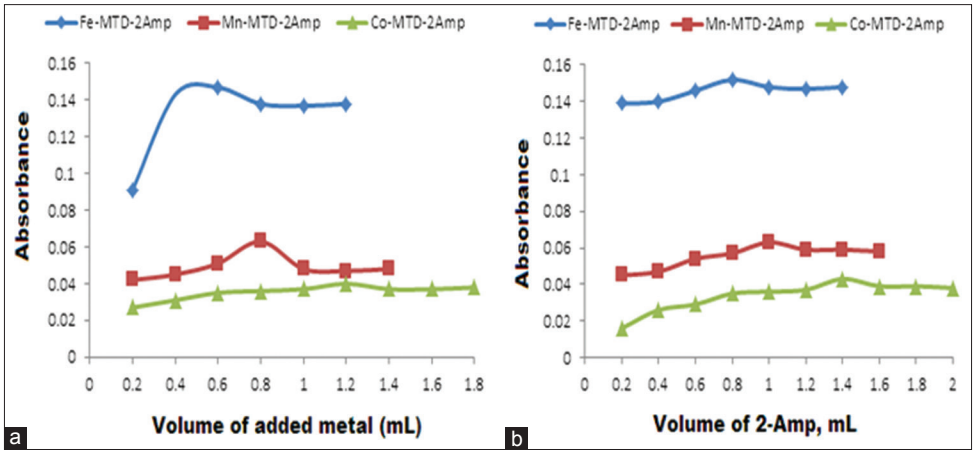

Fig. 2: (a and b) Selection of the suitable volumes of reacting reagent 2-aminopyridine and the added metal ion

chloride hexahydrate (Merck), and (0.1 mol/l) of 2-Amp (Merck) are used. Furthermore, 0.1 mol/l of sodium hydroxide (BDH, pool, UK) and 37% of hydrochloric acid (Fluka, Switzerland) are also prepared. All the materials for the interference study are from (Sigma-Aldrich, Hamburg, Germany). A solution (1000 µg/ml, 4.19×10^{-3} mol/l) of two brands of Aldosam (SDI, Samarra, Iraq) and Aldomet (Algorithm pharmaceutical company, Lebanon) labeled 250 mg/tablet, each one is prepared by dissolving an amount equivalent to 100 mg of MTD in a 100 ml of volumetric flask and diluted to mark with water, then filtered. Solutions of less concentrations are prepared by appropriate dilution.

Standard procedure for the assay of methyldopa

*For complex (A), a series of solutions containing 4.0–40 µg/mlof MTD, 0.8 ml of 6.16×10^{-3} mol/l Fe(III) solution, and 0.8 ml of 0.1 mol/lof 2-Amp solution, the mixture is diluted to 10 ml with deionized water, mixed well, and kept at room temperature for 10 min.

*For complex (B), a series of solutions containing 4.0–32 µg/mlof MTD, 0.8 ml of 5.05×10^{-3} mol/lMn(II) solution, and 1.0 ml of 0.1 mol/l2-Amp solution is prepared. The mixture is diluted to 10 ml with deionized water, mixed well, and kept at 60°C for 30 min in a thermostatically controlled water bath.

*For complex (C), a series of solutions containing 4.0–40 µg/mlof MTD, 1.2 ml of 4.2×10^{-3} mol/l Co(II) solution, and 1.4 ml of 0.1 mol/l2-Amp solution, the mixture is diluted to 10 ml with water, mixed well, and kept at 60°C for 20 min in a thermostatically controlled water bath. After thermostatic control, solutions of complex B and C are cooled down in water to room temperature. The absorbance of these solutions is measured at 572, 473, and 465 nm, respectively.

RESULTS AND DISCUSSION

Optimization of analytical conditions

The analytical conditions for the proposed spectrophotometric method are investigated and optimized. The suitable volumes of each reacted 2-Amp reagent and the added metal solution are tested in the range of 0.2–2.0 ml for the proposed systems A, B, and C, using fixed MTD concentrations 20, 4, and 4 µg/ml, respectively. The recorded data revealed that the suitable volumes of added metals are 0.6, 0.8, and 1.2 ml of Fe(III), Mn(II), and Co(II), respectively (Fig. 2a). Additionally, the highest absorbance is observed using 0.8, 1.0, and 1.4 ml of the reagent 2-Amp for the previously mentioned systems (Fig. 2b).

The effect of the order of the reagent addition on the performance of the spectrophotometric measurement is investigated. As presented in Table 1, the best absorbance is obtained when using the order MTD + Metal + 2-Amp.

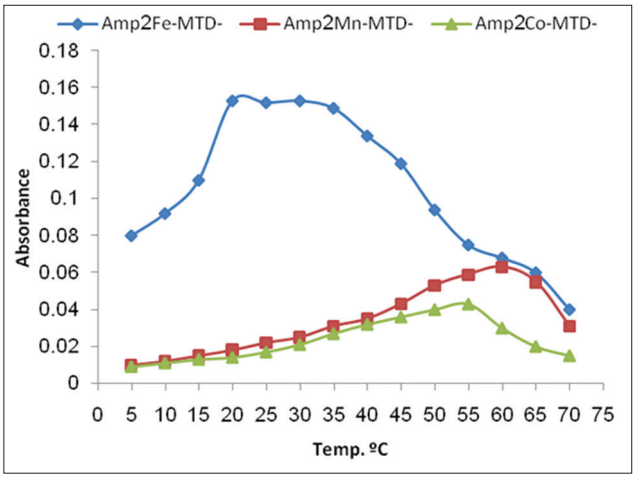

Fig. 3: Effect of temperature on the complexes formation

Table 1: The obtained results using different orders of addition

Order addition	Fe, MTD, 2-Amp	Mn, MTD, 2-Amp	Co, MTD, 2-Amp
MTD+metal+2-Amp	0.153	0.063	0.042
2-Amp+MTD+Metal	0.080	0.053	0.019
Metal+2-Amp+MTD	0.021	0.059	0.025

MTD: Methyldopa, 2-Amp: 2-aminopyridine

Since the addition of a small amount of acid or base is found to decrease the absorbance value which can be attributed to the decomposition or change the structure of a ternary complex so the addition of any acid or base is avoided.

Furthermore, the influence of temperature on the absorbance measurements is studied. Fig. 3 shows that at 5–20°C, the absorbance of Fe-MTD-2Amp complex is increased and a plateau between 20 and 35°C, then the decrease in absorbance is observed between 35 and 70°C. For the Mn and Co complexes, an increase in absorbance is noticed with increasing the temperature.

Finally, the stability time of the formed complexes is studied and it is noticed that the absorbance of the complex A is decreased up to 10 min and still constant for 24 h. Meanwhile, in both of complex B and complex C, the color does not develop instantaneously at room temperature. Thus, the effect of temperature is also investigated by incubating the formed complexes B and C at 40, 45, 50, 55, and 60°C for

15–40 min; a constant absorbance is obtained at 55°C for 30 min for the complex band at 60°C for 20 min for the complex C (Fig. 4).

Under optimum conditions, the determination of MTD using the suggested spectrophotometric method is performed using a fixed concentration of 20, 4, and 4 µg/ml of MTD for A, B, and C systems, respectively (Fig. 5).

Calibration curve

The calibration graphs of the three suggested systems A, B, and C are constructed by plotting the absorbance *vs.* the MTD concentrations (Fig. 6). The critical response data are presented in Table 2. The percentage recoveries are 99.48, 100.24, and 100.72% with limits of detection [19] 0.152, 0.256, and 0.282 µg/ml and quantification limits 0.46, 0.776, and 0.853 µg/mlfor systems A, B, and C, respectively.

Interferences

The effect of some foreign inorganic ions and the most common excipients used in the dosage formulations on the assay of a fixed concentration of MTD (20 µg/ml) is studied. As indicated in Table 3, no significant interferences are recorded. Therefore, the suggested spectrophotometric systems are suitable for the determination of the investigated drug.

Analytical applications

The proposed spectrophotometric systems are applied for the determination of MTD in pharmaceutical tablets (Aldosam, Aldomet).

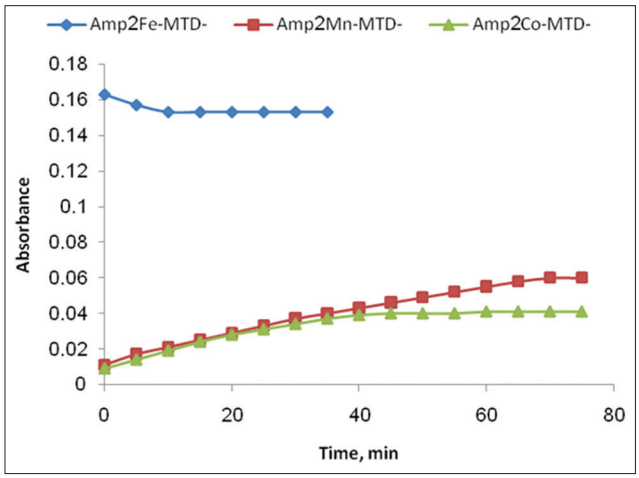

Fig. 4: Effect of the stability time on the formed ternary complexes

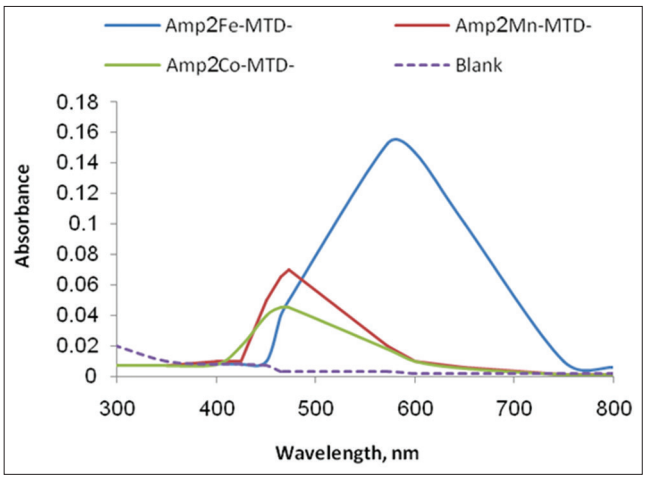

Fig. 5: Ultraviolet-Vis spectra of the formed complexes under optimum conditions

The results are presented in Table 4 and compared with the results obtained from a previously published method [20] using Student's *t*-test and variance ratio *F*-test. To prove the accuracy of the suggested method, the standard addition method (Figs. 7 and 8, Table 5) is also employed to analyze the investigated drug. This method included the addition of fixed amount (8 µg/ml) of either Aldosam or Aldomet and 0, 4, 8, and 12 µg/ml of standard MTD solution in a series of 10 ml volumetric flasks. The solutions are treated as in calibration graph procedure. The obtained results showed excellent accuracy. Moreover, intraday and interday assays are applied for studying the precision of the present method by the determination of three different concentrations

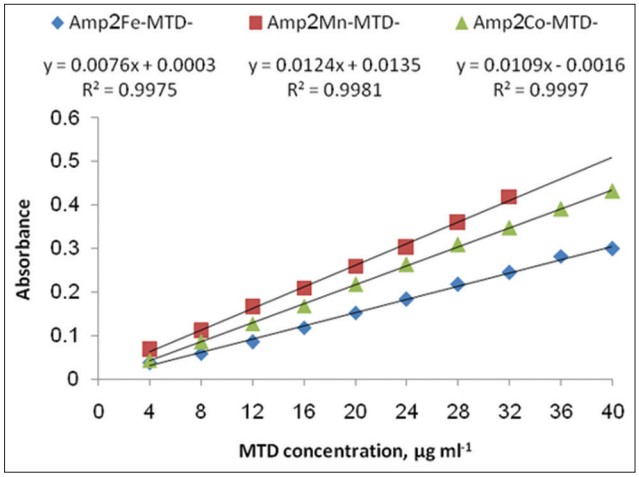

Fig. 6: Calibration graphs of the suggested systems

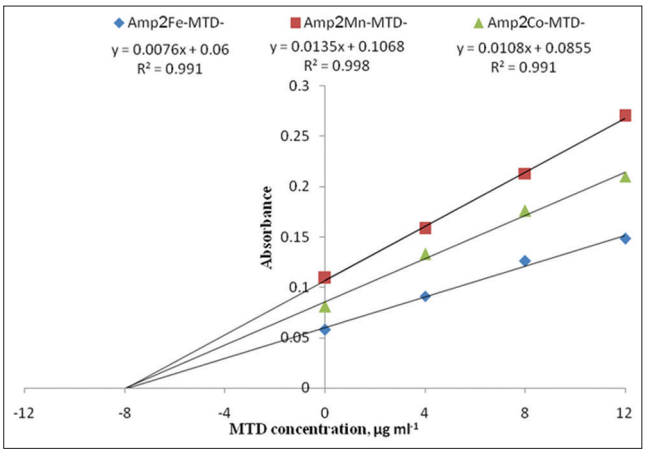

Fig. 7: Standard additions method for assay of Aldosam

Table 2: Analytical data obtained from the assay of methyldopa using the proposed spectrophotometric methods

Parameter	Complex A	Complex B	Complex C
Linearity, µg/ml	4–40	4–32	4–40
Slope, a	0.0076	0.0124	0.0109
Intercept, b	0.0003	0.0135	0.0016
Correlation coefficient, R^2	0.9975	0.9981	0.9997
Molar absorptivity (ε) l/mol/cm	1810.62	2954.18	2569.80
Sandell's sensitivity, µg cm^2	0.132	0.080	0.092
RSD %	1.64	1.67	1.65
Mean recovery %	99.48	100.24	100.72
LOD, µg/ml	0.152	0.256	0.282
LOQ, µg/ml	0.460	0.776	0.853

LOD: Limit of detection, LOQ: Limit of quantitation, RSD: Relative standard deviation

Table 3: The effect of foreign substances on the assay of methyldopa

Substance	Molar ratio MTD: Substance	Recovery percentage		
		For Fe-MTD-2-Amp	For Mn-MTD-2-Amp	For Co-MTD-2-Amp
Ca^{+2}	87.4	95.25	96.5	95
Zn^{+2}	87.4	100	100	100
Na^{+}	203.7	100	100	100
Cd^{+2}	65	100	100	100
Ascorbic acid	90	100	100	100
Glucose	100	100	100	100
Fructose	100	100	100	100
Lactose	190	100	100	100
Sucrose	180	100	100	100
Starch (mg)	1.0	100	100	100
Talc	31.4	100	100	100
Polyethylene glycol (mg)	10	100	100	100
Microcrystalline cellulose (mg)	1.0	100	100	100
Croscarmellose sodium	12	100	100	100
Polyvinylpyrrolidone (mg)	1.0	100	100	100
Magnesium stearate	20	100	100	100

MTD: Methyldopa, 2-Amp: 2-aminopyridine

Table 4: Assay of methyldopa in pharmaceutical tablets

Pharmaceutical preparation	Complex A	Complex B	Complex C	Reported method
Aldosam				
Recovery %±SD, (*n*=6)	100.25±0.47	99.93±0.44	100.73±0.42	99.1±0.26
Percentage SE	0.2	0.4	0.3	SE=0.4
t-test*	2.05	0.13	1.09	*n*=6
F-test*	1.19	1.43	1.50	
Aldomet				
Recovery %±SD, (*n*=6)	100.27±0.38	100.11±0.22	100.21±0.39	100.1±0.29
Percentage SE	0.4	0.3	0.1	SE=0.7
t-test*	1.77	0.23	1.42	*n*=6
F-test*	2.42	0.55	1.76	

*t_{tab} is equal to 2.78 for 4° of freedom at 95% confidence level, F_{tab} is equal to 19.0 for 2° of freedom at 95% confidence level. SD: Standard deviation, SE: Standard error

Table 5: Results of standard additions method for the determination of methyldopa in pharmaceutical tablets

Pharmaceutical preparations	Fe-MTD-2-Amp		Mn-MTD-2-Amp		Co-MTD-2-Amp	
	Found, µg/ml	Recovery %	Found, µg/ml	Recovery %	Found, µg/ml	Recovery %
Aldosam Taken: 8 µg/ml	7.89	98.63	7.91	98.88	7.92	99
Aldomet Taken: 8 µg/ml	7.94	99.25	7.95	99.45	7.94	99.25

MTD: Methyldopa, 2-Amp: 2-aminopyridine

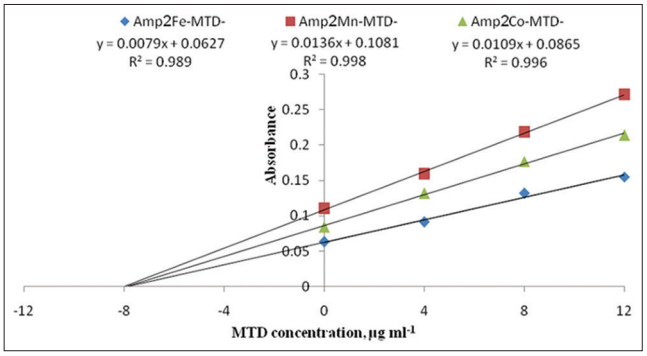

Fig. 8: Standard additions method for assay of Aldomet

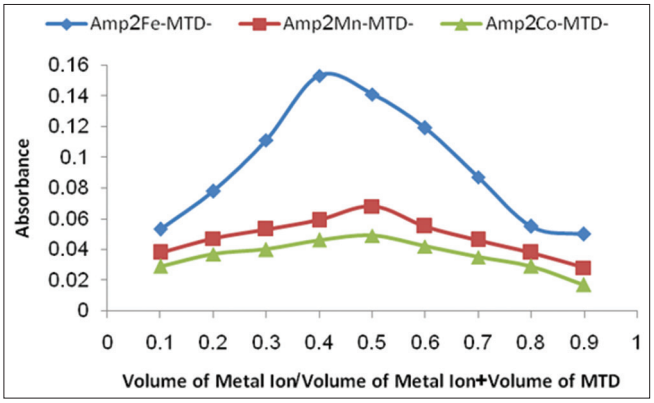

Fig. 9: Plots of job's method of continuous variation

of MTD in pure form through three consecutive occasions or by replicating the analysis for a period of 3 consecutive days. The value of % relative standard deviation ranged from 0.3 to 0.5% for intraday and 0.2%–0.7% for interday assays, revealing good precision for the proposed method and it can be applied for the assay of MTD.

Stoichiometry of the reaction

Job's method of continuous variation is used to study the stoichiometry of the reaction; the ratio of MTD to the metal is estimated. The results

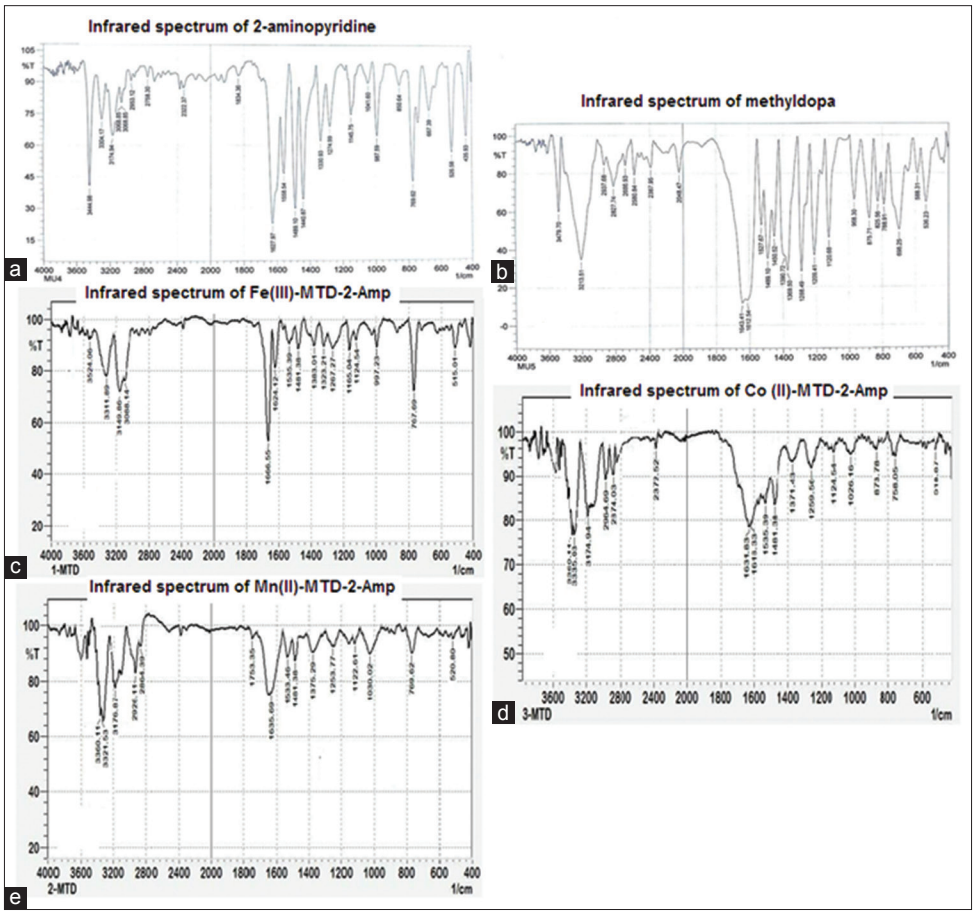

Fig. 10: Infrared spectra of (a) methyldopa, (b) 2-aminopyridine, (c) Fe(III)-methyldopa-2-aminopyridine, (d) Mn (II)-methyldopa-2-aminopyridine, and (e) Co(II)-methyldopa-2-aminopyridine

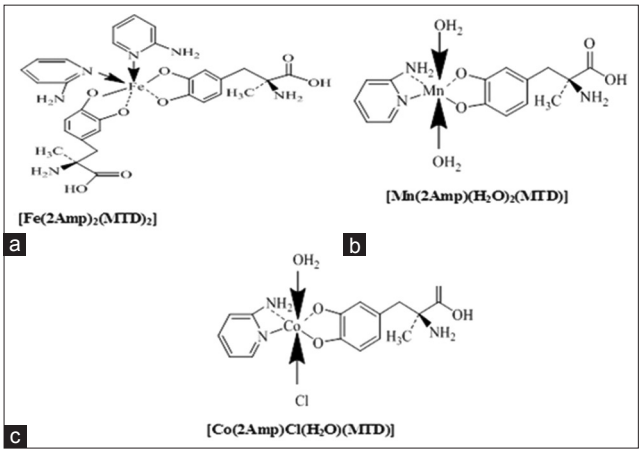

Fig. 11: The proposed chemical structure of the formed complexes (a) [Fe(2-Amp)$_2$(MTD)$_2$], (b) [Mn(2-Amp)(H$_2$O)$_2$(MTD)], and (c) [Co(2Amp)Cl(H$_2$O)(MTD)]

(Fig. 9) showed that the ratio of MTD to Fe(III) (complex A) is 2:1, and for both complex B and complex C, the ratio of MTD: Mn and MTD: Co is 1:1.The structure of these complexes is clarified by infrared spectra of the three complexes and their free ligands. In the spectra of these complexes, the band of the OH group of the catechol nucleus (Fig. 10a) in MTD molecule is disappeared. Therefore, MTD in all these complexes is coordinated to metal as a bidentate ligand, on the other hand, the 2-Amp molecule (Fig. 10b) is coordinated to metal by two groups, first is linked through (–C=N) of 2-Amp ring. This coordination bond is found in all these complexes (Fig. 10c-e), where its position in free ligand (1595 cm^{-1}) is shifted to lower wave number (1481.31 cm^{-1}). The second coordination bond of 2-Amp is attributed to NH_2 group of 2-Amp, and its band is disappeared in both of complex (B) and complex (C), another data are found for molecules of water at 3360 cm^{-1} in both of complex (B) and complex (C). Finally, complex (C) displayed two bands for (Co–Cl) [21] at (330 cm^{-1}) and (345 cm^{-1}), so the structures of the formed complexes can be deduced (Fig. 11a-c) for complex (A) as [Fe(2-AMP)$_2$(MTD)$_2$], Complex (B) as [Mn(2-AMP)(H$_2$O)$_2$(MTD)], and Complex (C) as [Co(2-AMP)Cl(H$_2$O)(MTD)].

CONCLUSION

The present study focused on the development of a new spectrophotometric method for determination of MTD based on the formation of ternary complex using three different metal ions by a mixed-ligand type. The results obtained revealed high accuracy and good reproducibility. The method has been successfully applied for determination of MTD in the pharmaceutical tablets (Aldosam and Aldomet).

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AUTHOR CONTRIBUTIONS

M. Bichan carried out the experimental part and wrote the paper. F, Abdoon supervised the practical study and approved the measurements of all parameters. Both authors reviewed, read, and approved the final version of the manuscript.

CONFLICT OF INTEREST

No conflict of interest associated with the present work**.**

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