A NOVEL SPECTROPHOTOMETRIC DETERMINATION OF METHYLDOPA THROUGH TERNARY COMPLEXATION PROCEDURE USING Fe(III), Mn(II), AND Co(II) WITH 2-AMINOPYRIDINE

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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 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/cm², 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.

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 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. Large bathochromic shift due to deep color formation is observed by the use of more sensitive reagents, the attempts have been made to intensify the color using a third component like metal ions to form a ternary complex.

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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 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.

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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/ml of MTD, 0.8 ml of 6.16×10^{-3} mol/l Fe(III) solution, and 0.8 ml of 0.1 mol/l 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/ml of MTD, 0.8 ml of 5.05×10^{-3} mol/l Mn(II) solution, and 1.0 ml of 0.1 mol/l 2-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/ml of MTD, 1.2 ml of 4.2×10^{-3} mol/l Co(II) solution, and 1.4 ml of 0.1 mol/l 2-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.

**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/ml for 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).

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 (0 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.

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**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    | 0.997    |
| Molar absorptivity (\(\varepsilon\) l/mol/cm) | 1810.62  | 2954.18  | 2569.80  |
| Sandell’s sensitivity, µg/cm² | 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         | 180                        | 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                    | 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      | t-test*=         |
| F-test*                    | 1.19      | 1.43      | 1.50      | n=6             |
| Aldomet                     | 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      | t-test*=         |
| F-test*                    | 2.42      | 0.55      | 1.76      | n=6             |

* 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                     | 7.89         | 98.63        | 7.91         | 98.88        | 7.92         | 99           |
| 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

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
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As shown in Fig. 9, 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. In the spectra of these complexes, the band of the OH group of the catechol nucleus in the 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 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 is shifted to lower wave number.

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) 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.

REFERENCES
1. Fouladgara M, Ahmadzadeh S. Application of a nanostructured sensor based on NiO nanoparticles modified carbon paste electrode for determination of methyldopa in the presence of folic acid. Appl Surf Sci 2016;379:150-5.
2. Emara S, Masujima T, Zarad W, Kamal M, Fouad M, El-Bagary R, et al. An eco-friendly direct injection HPLC method for methyldopa determination in serum by mixed-mode chromatography using a single protein-coated column. J Chromatogr Sci 2015;53:1353-60.
3. Perez-Mella B, Alvarez-Lueje A. Development of a carbon nanotube modified ionic liquid electrode for the voltammetric determination of methyldopa levels in urine. Electroanalysis 2013;25:2193-9.
4. Emara S, Masujima T, Zarad W, Kamal M, Fouad M, El-Bagary R. A Combination of isocratic and gradient elution modes in HPLC with the aid of time-overlapping process for rapid determination of methyldopa in human urine. J Liq Chromatogr Relat Technol 2015;38:153-62.
5. Viase L, Mihu D, Popa DS, Popa A, Briciu C, Loghin F, et al. Determination of methyl dopain human plasma by LC/MS-MS for therapeutic drug monitoring. Studia UBB Chem 2013;1:31-41.
6. Shahrokhiiana S, Rastgara S. Electrodeposition of Pt–Ru nanoparticles on multi-walled carbon nanotubes: Application in sensitive voltammetric determination of methyldopa. Electrochim Acta 2011;58:125-33.
7. Karimi-Maleh H, Khalilzadeh MA, Ranjbarha Z, Beitollahi H, Ensafid AA, Zareyeeb D. p-chloranil modified carbon nanotubes paste electrode as a voltammetric sensor for the simultaneous determination of methyldopa and uric acid. Anal Methods 2012;4:2088-94.
8. Molaakbari E, Mostafavi A, Beitollahi H. First report for voltammetric determination of methyldopa in the presence of folic acid and glycine. Mater Sci Eng C Mater Biol Appl 2014;36:168-72.
9. Ramirez C, Del Valle MA, Isaacs M, Armijo F. Electrochemical oxidation of catecholamines on fluoride-doped SnO2 substrates. Square-wave voltammetric method for methyldopa determination in pharmaceutical dosage forms. Electrochim Acta 2016;199:227-33.
10. Chaichi MJ, Khajyand T, Mehrzad J, Asghari S, Qandalee M. Electrochemical oxidation of catecholamines on fluoro-doped SnO2 substrates. Square-wave voltammetric method for methyldopa determination in pharmaceutical dosage forms. Anal Sci 2013;29:815-21.
11. Ghasemi F, Hormozzi-Nezhad MR, Mahmoudi M. Identification of catecholamine neurotransmitters using fluorescence sensor array. Anal Chim Acta 2016;917:85-92.
12. Vardini MT, Mardani L. Surface imprinting of silica gel by methyldopa and its application in the solid phase extraction procedure. J Braz Chem Soc 2018;29:310-9.
13. Kamino S, Mitani S, Asano M, Yamaguchi T, Fujita Y, Hoshino M. Spectrophotometric determination of titanium with o-carboxyphenyl fluorone in cationic micellar media, and its equilibrium and kinetic studies. Talanta 2011;85:2339-43.
14. Hoshino M, Kamino S, Mitani S, Asano M, Yamaguchi, T, Fujita Y. Spectrophotometric determination of quinolone antibiotics by an association complex formation with aluminum(III) and erythrosin. Anal Sci 2009;25:125-8.
15. Ramadan AZ, Mandal H, Alsayed-Ali R. Spectrophotometric determination of rosuvastatin in pure form and pharmaceutical formulations through ion-pair complex formation using bromoresol green. Int J Pharm Pharm Sci 2015;7:191-8.
16. Rahman H. Utilization of eosin dye as an ion pairing agent for determination of pharmaceuticals: A brief review. Int J Pharm Sci 2017;9:1-9.
17. Rauf MA, Ikram M, Ahmad M. Spectrophotometric studies of ternary complexes of lead and bismuth with o-phenanthroline and eosin. Dyes Pigments 2002;52:183-9.
18. Belsare GW, Zade AB, Kalbende PP, Belsare PU. Spectrophotometric study of ternary complex forming systems of some rare earths with bromopyrogallol red in presence of cetyltrimethyl ethylammonium bromide for micro determination. Pharm Chem 2012;4:1226-38.
19. Lavanya G, Sunil M, Eswaraiah MM, Eswaraiah MC, Harisudha K, Spandana BN. Analytical method validation: An updated review. Int J Pharm Sci Res 2013;4:1280-6.
20. Upadhyay K, Asthana A, Tamrakar RK. Extractive spectrophotometric determination of u-methyldopa in bulk dosage and in its formulations. Res Chem Intermed 2014;41:5521-8.
21. Venkatalakshmi N, Rajasekharan MV. EPR of the Co(II) complexes of phenanthraquinonedioxide and benzoquinonedioxide. Formation of dioxygen adducts in the solid state. J Chem Sci 1995;107:327-33.