Spectrophotometric evaluation of methyldopa in pure and pharmaceutical formulation using Ecological-friendly Method

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Abstract. A simple, low cost, precise and fast spectrophotometric methods development for estimation of methyldopa are described. The primary method which includes conversion of methyldopa to colored complex with Fe (II) in the alkaline medium (PH=12). The colored product has a violet color with absorbance at λmax 555 nm. Between the concentration range (5-50 μg.mL⁻¹), the Beer’s law is obeyed with correlation coefficient (R²= 0.9994), limit of detection as 0.1641 μg.mL⁻¹, limit of quantification as 0.541 μg.mL⁻¹ and molar absorptivity as 1562.22 L.mol⁻¹.cm⁻¹. The other technique, cloud point extraction was utilized to determination of a trace amount of the colored product in the previous method followed by measuring with a UV-Vis spectrophotometer. The linearity of calibration curve was above the range of (1-50 μg.mL⁻¹), the correlation coefficient (R²= 0.9991) and molar absorptivity was 6080 L.mol⁻¹.cm⁻¹. The detection limit (LOD) and quantification limit (LOQ) were based to be 0.0486 and 0.160 μg.mL⁻¹ respectively. This technique was successfully employed for methyldopa detection within the pure and pharmaceutical preparations.

Key word: Cloud point extraction, Determination, Ecological – friendly, Spectrophotometry, Methyldopa.

1. Introduction

Methyldopa, known as [2-amino-3-(3,4-dihdroxy-phenyl)-2-methyl propionic acid] (fig.1). It is a catecholamine derivative and utilized as an antique antihypertensive agent for treatment of moderate to slight high blood pressure 1-4. Many of methods were proposed to determination of methyldopa in pharmaceutical formulations containing colorimetry 5,6, electrophoresis 7, thin layer 8, spectrophotometry9-16, voltammetry 17,18, titrimetry 19, HPLC with fluorescence detection 1, flow injection spectrophotometry 9 and GLC 20. Complexation reactions seems to be one of the most famous technique to evaluation of numerous drugs like cefotaxime21, glibenclamide 22, salbutamol 23 and ciprofloxacin hydrochloride 24.

The cloud point extraction have several advantages like safety, rapid and low cost, consequently it has applied as one of the estimation and pre-concentration methods in analytical chemistry 25-27. In this work, the proposed procedure is based totally on the complexation reaction of methyldopa with Fe(II) metal in the presence alkali media to form a violet solution, then assessment and pre-concentration the usage cloud point extraction (
CPE) which suggests an absorbance at 555nm. The aim of the current study is to estimate and to find the optimal conditions for estimation the methyldopa medication in two methods first through complexity with iron II at the maximum wavelength of 555 nm and the second method extraction by cloud point using Triton-X-100 as a surfactant and then compare of the two methods.

Figure 1. Methyldopa Structure

2. Material and Methods
Single beam UV-Vis spectrophotometer (160) was utilized for all spectral and absorption intensity measurements with corresponded 1 cm quartz cells. The chemicals substances and reagents had been of analytical grade. However, methyldopa was purchased from (SDI) and (FeCl₂·H₂O) from Merck company. A stock iron solution (250 μg/mL) was prepared by dissolving (0.056 g) of FeCl₂·H₂O in D.W and dilution to (100 mL) volumetric flask. Stock methyldopa solution (250 μg/mL) was prepared by dissolving (0.025 g) in D.W and dilution to the mark in (100 mL) volumetric flask. (0.01 M) of hydrochloric acid, (0.01 M) sodium hydroxide, (10 %) of TritonX-100 (Ph12).

2.1. Procedure of Methyldopa complexation
A 1 mL of (250 μg/mL) of methyldopa were transferred into (10 mL) volumetric flask, 1 mL of (250 μg/mL) FeCl₂·H₂O were added and followed by (1.2 mL) of pH 12 (Na₂HPO₄+NaOH). The volume were completed to volume (10 mL) with D.W. The mixture kept in the thermostatic bath (35 °C) for 30 min. The resulting solution were measured at 555 nm against blank treated similarly but without methyldopa drug (fig.1).

Figure 2. Mechanism reaction of MD-Fe(II) complex

2.2. General procedure of CPE for methyldopa
Specific concentrations (1-50 μg/mL) of methyldopa complex put within the (10 mL) centrifuge tubes, then (1 mL) of Triton-X-100(10%) and D.W was added to complete the volume of solution to 10 mL. The mixture solution stored within the thermostatic bath (80 °C) for 30 min. Separation of two phases was carried out via centrifugation for 4 min at 1000 rpm. The mixture become cooled to increase the viscosity of the surfactant-rich phase and aqueous phase was easily disposal by decantation. The rich-surfactant phase from this
technique was diluted with 2 mL of MeOH and transferred into quartz cell to measure its absorption intensity at 555 nm.

2.3. Pharmaceutical preparations procedure
Aldomet (Lebanon) and Aldosam (SDI) tablets 250 mg have been carefully weighed, then average tablets weight (0.321 gm) and (0.333 gm) for respectively was extracted. The valet weight was dissolved in D.W to make certain the entire solubility, then made up to volumetric flask (100 mL) and the solution was filtrated.

2.4. Procedure for stoichiometric ratio
The reaction stoichiometry between methyldopa and Fe(II) has been estimated spectrophotometrically, then extraction and pre-concentration using cloud point extraction. The molar ratio and continuous variation techniques had been utilized. In the former procedure, equimolar solutions of methyldopa and Fe(II) \(1 \times 10^{-3} \text{ M}\) have been used. Various aliquots of Fe(II) were added to constant aliquots of methyldopa solution, total volume (10 mL) and the absorbance have been accomplished at 555 nm against the blank treated similarly. While in the other method, a series of methyldopa- Fe(II) solutions were kept at 10 mL (0.1:0.9, 0.2:0.8, 0.3:0.7, 0.4:0.6, 0.5:0.5, 0.6:0.4, 0.7:0.3, 0.8:0.2, 0.9:0.1). The absorbances of these solutions were measured at 555 nm.

3. Results and Discussion
Absorption intensity of methyldopa-Fe(II) system against blank in pH 12 at temperature (35 °C) have been produced violet colored product which absorbs at 555 nm, the results showed in (fig.3).

3.1. Optimization of complexation reaction
In order to establish optimized conditions, essential for quantitative and fast formation of violet colored with stability and sensitivity, the influence of various parameters like, pH, type and volume of pH, metal concentration, temperature and time of reaction. The influence of pH on the absorbance with a fixed concentration of complex was completed in the variety pH between (1-13). Two types of buffer solutions (sodium hydrogen orthophosphate/sodium hydroxide and potassium chloride/sodium hydroxide) were examined. The best one is phosphate buffer and optimum value was pH 12 (fig.4). Hence, (1.2 mL) of buffer solution pH 12 was selected in the subsequent experiments.
The influence of the concentration metal Fe(II) was studied through various concentrations of Fe(II) used between (5-75 μg/mL) in the complexation process and it was found that 25 μg/mL gave the choicest absorption intensity as shown in (fig.5). The temperature and reaction time was achieved. It was found that the temperature 35 °C and reaction time 30 min were the optimum conditions to get the heights absorbance for methyldopa as proven in (fig.6 and fig.7). The continuous variation and mole ratio procedures were investigated to assess the stoichiometry of methyldopa: Fe(II) ratio. The results observed that the ratio of 1:1 (drug: Fe^{2+}) (fig.8 and fig.9).
3.2. Calibration Curve

Underneath the optimization, a linear calibration curve for evaluation of methyldopa was investigated in the range of concentration (5-50) μg.mL\(^{-1}\). The linear regression equation of methyldopa is \(Y=0.0074X-0.0077\) and \(R^2 = 0.9994\) of the linear calibration graph is examine in (fig.10).

![Figure 7. Effect of Time on the complex formation](image7.png)

![Figure 8. Continuous variation method of MD-Fe(II) complex](image8.png)

3.3. Accuracy and precision

Accuracy of the measurements of the proposed process was estimated using the calibration curve methyldopa drug, the mean percentage for methyldopa was received indicating high accuracy of the procedure. Precision and reproducibility had been estimated by way of calculating relative standard deviation and recoveries of five replicate determinations using three different concentrations of drug. As result, the obtained results by the proposed procedure were existed to be acceptable. The results are listed in the table.

3.4. Optimization of cloud point extraction (CPE)

The formation of the complex methyldopa with Fe(II) and its chemical stability are the two vital effect factors for cloud point extraction. The influence of type and volume of surfactant (Triton-100, TritonX-114, Tween 80, Tween 20, CTAB and SDS) at the analytical responses become investigated. The influence the volume of TritonX-100 on the extraction and estimation of methyldopa was investigated in the range between (0.2-3 mL). The absorbance of the procedure increased by increasing volume of TritonX-100 up to at least 1 mL and decrease the absorbance at higher volume. So, 1 mL of TritonX-100 was selected in further works. The results are shown in (fig.11). It was desirable to utilize the lowest possible temperature and the shortest equilibration time as a compromise between separation performance and completion of extraction of phases. The efficient of extraction upon temperature and time above, the cloud point in the variety of (60-90 °C) and (10-50 min) had
been thoroughly achieved respectively. It was found that an equilibration temperature of 80 °C and time of 30 min were selected in the subsequent work(fig.12 and fig.13). The results showed the experiment in the optimized Fe(II) concentration after heating for 30 min at 80 °C and centrifuging by 1 min in 1000 rpm and cooling in the 20 min lead to high recovery of methyldopa in brief time. After completed the extraction process (CPE), the aqueous phase was removed by decantation and ethanol was added to the surfactant-rich phase to lower the viscosity of surfactant-rich phase and ease its transfer into spectrophotometric cell. 2 mL of ethanol was selected in the subsequent work.

3.5. Calibration Curve
Under the optimization, a linear calibration curve for evaluation of methyldopa drug was investigated within the variety of concentration (1-50) μg.mL⁻¹. The linear regression equation of methyldopa is Y=0.0288X-0.0142 and R² = 0.9991 of the linear calibration curve is observe in (fig.14).

3.6. Accuracy and precision
Accuracy of all measurements of the proposed procedure was evaluated using the calibration curve methyldopa drug, the mean percentage for methyldopa was obtained indicating excessive accuracy of this procedure. Precision and reproducibility have been predicted via calculating relative standard deviation (RSD %) and recoveries of 5 replicate determinations.
using three different concentrations of drug. Hence, the obtained results by using the proposed procedure were existed to be appropriate. The results are listed in the table.

### Table 1: Analytical parameter of cloud point extraction method.

| Parameters                      | Before CPE       | After CPE        |
|---------------------------------|-----------------|-----------------|
| $\lambda_{\text{max}}$ nm       | 555             | 555             |
| Color                           | violet          | deep violet     |
| Regression equation             | $Y=0.0074X-0.0077$ | $Y=0.0288X-0.0142$ |
| Linearity range ($\mu g/mL^{-1}$)| 5-50            | 1-50            |
| Correlation Coefficient ($R^2$) | 0.9994          | 0.9991          |
| $\varepsilon$ ($\text{L.mol}^{-1}.\text{cm}^{-1}$) | 1562.22         | 6080            |
| Sandell’s sensivity ($\mu g . \text{cm}^{-2}$) | 0.135           | 0.0347          |
| Slope (b)                       | 0.0074          | 0.0288          |
| Intercept(a)                    | 0.0077          | 0.0142          |
| Limit of detection ($\mu g/mL^{-1}$) | 0.164           | 0.0486          |
| Limit quantification ($\mu g/mL^{-1}$) | 0.541           | 0.160           |
| C.L. for the slope(b±tsb) at 95% | 0.0074±8.3016×10^{-4} | 0.0288±13.8×10^{-4} |
| C.L. for the intercept(a±tsa) at 95% | 0.0077±11.53×10^{-3} | 0.0142±0.037   |
| Standard error for regression line ($Sy/x$) | 0.0166          | 0.035           |
| C.L for Conc. 10 $\mu g$ mL^{-1} at 95% | 9.8±52×10^{-4} | 9.6±6×10^{-4}   |
| C.L for Conc. 20$\mu g$ mL^{-1} at 95% | 19.6±48×10^{-4} | 20.01±56×10^{-4} |
| C.L for Conc. 30 $\mu g$ mL^{-1} at 95% | 30.1±47×10^{-4} | 29.9±55×10^{-4} |
| Enrich Factor                   | 389             | 14.285          |
| Preconcentration Factor         |                 |                 |

### Table 2: Application of the proposed CPE for the evaluation of Methyldopa

| drug          | Conc. of drug $\mu g/mL^{-1}$ | Relativ e Error | Recover % | Averag e Recovery % | RSD % (n=5) |
|---------------|-------------------------------|-----------------|----------|---------------------|-------------|
| **Before cloud point extraction** |                               |                 |          |                     |             |
| (Aldosam)     |                               |                 |          |                     |             |
| Taken         | 10 9.88                       | -1.2            | 98.8     | 1.376               |             |
| Found         | 20 19.84                      | -0.8            | 99.2     | 1.129               |             |
|               | 30 29.68                      | -1.06           | 98.93    | 4.85                |             |
| (Aldomet)     |                               |                 |          |                     |             |
| Taken         | 10 9.91                       | -0.9            | 99.1     | 4.15                |             |
| Found         | 20 19.93                      | -0.35           | 99.65    | 1.26                |             |
|               | 30 29.79                      | -0.7            | 99.3     | 2.3                 |             |
| **After cloud point extraction** |                               |                 |          |                     |             |
| (Aldosam)     |                               |                 |          |                     |             |
| Taken         | 10 9.67                       | -3.3            | 96.7     | 4.71                |             |
| Found         | 20 19.68                      | -1.6            | 98.4     | 0.82                |             |
|               | 30 29.79                      | -0.7            | 99.3     | 0.72                |             |
| (Aldomet)     |                               |                 |          |                     |             |
| Taken         | 10 9.8                        | -2              | 98       | 1.36                |             |
| Found         | 20 20.15                      | 0.75            | 100%     | 5                   | 1.83        |
|               | 30 30.03                      | 0.1             | 100%     | 0.516               |             |
Table 3: Compassion the values of LOD and LOQ of the CPE method with various methods reported in literature to methyldopadetermination.

| Method                              | LOD  μg/mL | LOQ  μg/mL | Ref. |
|-------------------------------------|------------|------------|------|
| Spectrophotometric method           | 0.152 μg/mL| 0.460 μg/mL| [28] |
| Colorimetric method                 | 0.38 μg/mL | -          | [29] |
| Flow injection method               | 0.769 μg/mL| -          | [30] |
| HPLC method                         | 0.027 μg/mL| -          | [31] |
| electrochemical sensor              | 9.0 nM     | -          | [32] |
| Electrochemical method              | 0.01 μg/mL | -          | [33] |
| Nanostructured TiO2 Carbon Paste Based Sensor | 1μM    | -          | [34] |
| Electrochemical method              | 8 μM       | -          | [35] |
| HPLC method                         | -          | 2 ng/mL    | [1]  |
| Cloud point extraction              | 0.0486 μg/mL| 0.160 μg/mL| Present work |

4. Conclusion

The proposed method for the evaluation of methyldopa in pharmaceutical preparations has the gain of high sensitivity, low cost, simplicity, repeatability and reproducibility. In addition to, it is important for practical quality control analysis of methyldopa in pure and pharmaceutical preparations without interference from general additives.

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