Continuous Flow Injection Analysis,Turbidmetric and Photometric Determination of Methyldopa Using a New Long Distance Chasing Photometer (NAG-ADF-300-2)

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Abstract

A specific, sensitive and new simple method was used for the determination of methyldopa in pure and pharmaceutical formulations by using continuous flow injection analysis. This method is based on formation of ion pair compound between methyldopa and potassium hexacyanoferrate in acidic medium to obtain a yellow precipitate complex using long distance chasing photometer (NAG-ADF-300-2). The linear range for calibration graph was 0.05-35 mmol/L for cell A and 0.05-25 mmol/L for cell B, and LOD 1.4292 µg /200 µL for both cells with correlation coefficient (r) 0.9981 for cell A and 0.9994 for cell B, RSD% was lower than 0.5 % for n=8 for. The results were compared with classical method UV-Spectrophotometric at λ_{max}=280 nm and turbidmetric method by using the standard addition method via the use of t-test, at 95% confidence level. The comparison of data explain that long distance chasing photometer (NAG-ADF-300-2) is the choice with excellent extended detection and wide application.

***Keywords*:** *Methyldopa, Attenuation of light, Continuous flow injection analysis, Turbidity.*

Introduction

Methyldopa (Aldomet), the L- isomer of alpha-methyldopa, is levo-3-(3,4-dihydroxyphenyl)-2-methylalanine.Molecular Weight 238.2 g/mol, Molecular Formula $C_{10}H_{13}NO_4.1.5\ H_2O$.It is a white to yellowish white, odorless and soluble in water, methanol and ethanol. Methyldopa widely used in the control of moderate and severe arterial hypertension, structure of methyldopa in figure 1[(1-4)]. Several methods depend on continuous flow injection analysis [(5-15)] and several analytical methods have been proposed to quantify methyldopa in pharmaceutical formulations such as UV- VIS Spectrophotometry and FTIR [(1)], UV- Vis spectrophotometry [(2-4, 16-18)], continuous flow injection analysis [(19)], voltammetry method [(20)], HPLC [(21)], capillary zone electrophoresis [(22)], Gas chromatography [(23)].

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In this work using flow injection analysis turbidmetric method, the turbidity is measured via reflection of the incident light from the surfaces of particulate formed between methyldopa and potassium hexacyanoferrate in acidic medium at $0\text{-}180^0$ by using homemade long distance chasing photometer (NAG-ADF-300-2) [(24)].

Chemicals and Apparatus

Reagents and chemicals

Every chemicals were used of analytical reagent grade and all the solutions dissolved by distilled water. A standard solution of 70 mmol/L of methyldopa, was prepared by dissolving 1.6674 g in 100 ml. A series of potassium hexacyanoferrate solutions were prepared from the dilution of standard solution 200 mmol/L with distilled water.

Apparatus

Using the manifold homemade NAG-ADF-300-2 is a long distance chasing photometer as a flow cell will have 300 mm as a distance with 2 mm as a path length to chaise and to accumulate the output resulted from attenuation of incident light 0-180^0 and diverged or fluorescence light at 0-90^0 via a flow cell. The first flow cell is of 110 mm length covered with 11 white snow LED (WSLED) followed by uncovered distance of 100 mm length then attached to another with 2 solar cell at each side of (0-180^{0}and 0-90^0) cell (cell number 2) which is covered by 6 WSLED and a single photo cell (solar) of 60 mm length at each side was used with peristaltic pump (Ismatec, Switzerland) and six-port medium pressure injection valve (IDEX Corporation, USA) with sample loop (1 mm i.e. Teflon, variable length). Potentiometric recorder to estimate the output signals (Siemens, Germany). UV-Spectrophotometric (RF-1501, shimadzu, Japan) was use for classical methods. A turbidimetry instrument, HANNA Company (Hungary), which is used for classical method measurement of turbidity at 0-180^o.

Results and Discussion

Study of the optimum intensity used for cell A and cell B

A study was conducted for the effect of intensity of incident light for the irradiation sources on the response of the energy transducer. The selector switch gives 0-1-2-3-4 four choices plus the off position for both cell individually controlled. The optimum intensity of the measuring cell A, I=3 and I=2 for cell B which was adopted in subsequent studies.

Optimization of variable

Chemical variables

-Potassium hexacyanoferrate concentration

A series of multiple concentration of precipitated reagent 3-20 mmol/L were prepared 100 µL volume of sample was injected through the carrier stream distilled water. 20 mmol/L concentration of methyldopa was injected with 3.2, 4.6 mL/min flow rate for carrier stream and reagent respectively. It was found that an increase in peak height with increase of potassium hexacyanoferrate concentration. It is possible that might be attributed to the increase of coloured precipitate particulate which in turn work on attenuation of part of the incident irradiation light plus it's absorption due to its coloration. While at higher concentration (i.e. > 18, 15 mmol/L respectively to for cell A and cell B) might lead to increase agglomeration of precipitated particulate and an increase of inter spatial distances which help to increase penetration light toward the detector and a decrease in the response height, Therefore 18, 15 mmol/L was selected as optimum concentration for cell A and cell B respectively.

-Effect of different medium

Various solutions were used as a carrier stream and the effect on the reaction between methyldopa (20 mmol/L) with potassium hexacyanoferrate. Different solution media (NH_4Cl, CH_3COONH_4, NaCl, KBr, Na_2SO_4, CH_3COOH, H_2SO_4, HNO_3, HCl) at 50 mmol/L concentration in addition to aqueous medium. It was noticed for the studied; different media causes to a decrease of S/N-response; this might be attributed leads to its effect in an increasing the agglomeration i.e.; increase the density of aggregates and compactness with each other than increase the intensity of transmitted light as there will be more vacant spaces in between agglomerates of particulate except H_2SO_4 which leads to an increase of S/N-response; this might be because increase of particles size leads to increase the space between the particles and increase of transmitted light toward the solar cell, therefor H_2SO_4 medium was used as a carrier stream in the next studied.

-Effect of variable concentration of sulphuric acid

Variable concentration of sulphuric acid at ranging 10-90 mmol/L were used to dilute the prepared solutions, 100 µL sample volume at 3.2 ml/min and 4.6 ml/min flow rate for carrier stream and reagent solution respectively. It was noticed that an increase of sulphuric acid concentration after 30 mmol/L causes a decrease in S/N- transducer response, Therefore 30 mmol/L of H_2SO_4 concentration was chosen as optimum carrier stream. Figure 1 shows a proposed expected mechanism for the reaction of methyldopa with potassium hexacyanoferrate in acidic medium [1, 25-27].

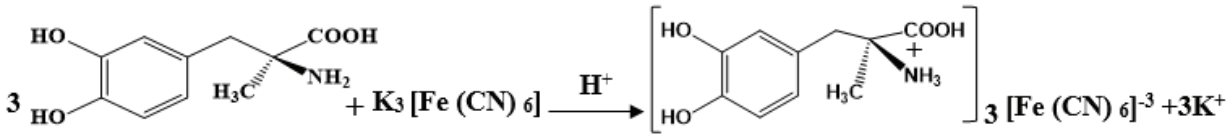

Figure1: Probable mechanism pathway for the reaction of methyldopa with potassium hexacyanoferrate.

Physical variables

-Flow rate

Fixing all previous experimental parameters concerning chemical variation for methyldopa- potassium hexacyanoferrate system. Variable flow rate study were carried out using 0.7- 4.8 ml/min for carrier stream line while 1-7.4 ml/min was used for precipitating reagent solution line. It was noticed for figure 2. A and B that up to 2.6 ml/min of carrier stream for cell A gave good S/N peak profile While up to 1.4 ml/min of carrier stream for cell B causes a decrease in S/N- transducer response because the reaction is slowly and needing some time to complete so we observe responses to the distorted for cell A. Therefore; 3.2 & 1.4 ml/min flow rate for carrier stream and 4.6, 2 ml/min flow rate for reagent respectively to for cell A and cell B was selected as optimum flow rate for either cells.

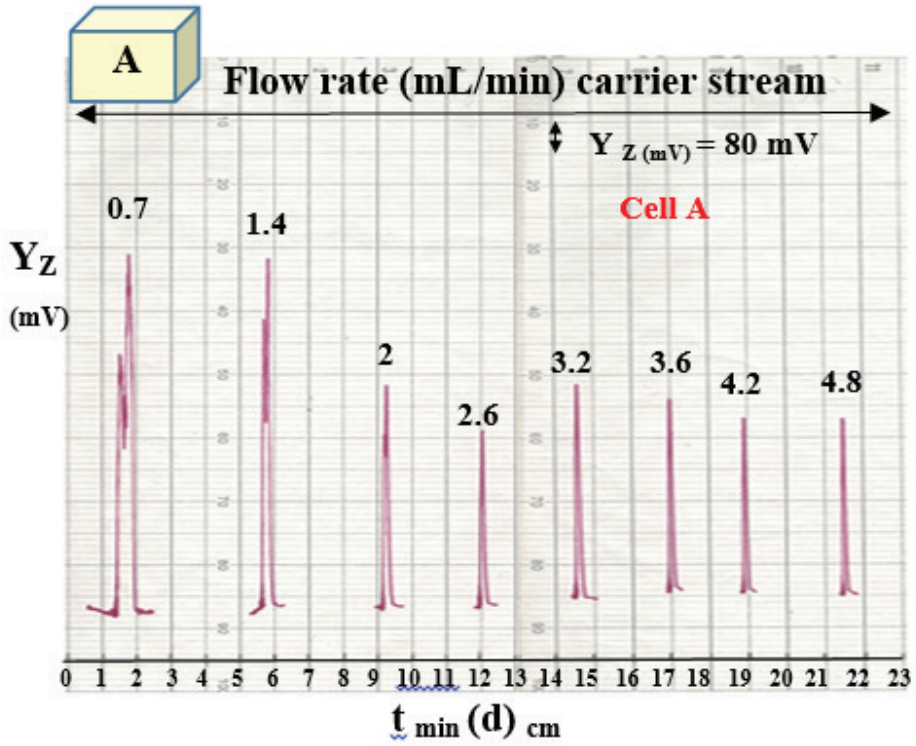

Figure 2: Effect of the variation of flow rate on: A; S/N energy transducer response versus $t_{min}(d)_{cm}$ using methyldopa–potassium hexacyanoferrate system, 100 µL of sample volume.

-Sample volume

Using methyldopa–potassium hexacyanoferrate system and Variable length of Teflon tube ranging (2.6-25.5) cm of diameter (D) 1 mm that is equivalent to (20-200) µL of sample volume. It was noticed that an increase of sample volume reaching to 200 µL lead to a significant increase in response height. Therefore 200 µL was chosen as optimum volume of sample.

Estimating the linear dynamic range from scatter plot for the variation of methyldsopa

versus S/N energy transducer response

A series of methyldopa solution (0.05-40 & 0.05-37 mmol/L) for cell A and cell B respectively were prepared this will represent the x-axis (Independent variable) using the optimum chemical and physical parameters. Table 1 explains the variance ranges for each cells.

Table 1: Summary of result for linear regression for the variation of S/N energy transducer response with methyldopa concentration using first degree equation of the form $\hat{Y}$=a + b x at optimum conditions

Type of mode	Range of [methyldopa] mmol/L(n)	$\hat{Y}$Zi(mV)= a mV± Sat+ b(Δ y mV/Δxmmol/L)± Sbt [Methyldopa] mmol/L at confidence level 95%, n-2	r, r2, R2%	t tab at 95%,n-2	Calculated t-value tcal=/r/√n-2/ √1-r2
Cell A					
Cell B					
Scatter plot	0.05-40 (24)	186.4920±54.2718+59.8484±2.8040 [Methyldopa]mmol/L	0.9944, 0.9889, 98.89		2.074 << 44.2701
	0.05-37 (22)	159.8407±99.0871+48.3620±5.8548 [Methyldopa] mmol/L	0.9679, 0.9369, 93.69		2.086 << 17.2318
Dynamic range or analytical range	0.05-37 (23)	167.7994±34.5164+62.1480±1.9257 [Methyldopa] mmol/L	0.9977, 0.9954, 99.54		2.080 << 67.4110
	0.05-30 (20)	91.0043±48.0221+58.1070±3.5272 [Methyldopa] mmol/L	0.9926, 0.9852, 98.52		2.101 << 34.6162
Working range or calibration range	0.05-35 (22)	159.7006±30.2236+63.2344±1.8269 [Methyldopa] mmol/L	0.9981, 0.9962, 99.62		2.086 << 72.4098
	0.05-25 (19)	66.5374±12.8752+62.1258±1.0592 [Methyldopa] mmol/L	0.9994, 0.9989, 99.89		2.110 << 124.2417
Linear range or linear dynamic range	0.05-35 (22)	159.7006±30.2236+63.2344±1.8269 [Methyldopa] mmol/L	0.9981, 0.9962, 99.62		2.086 << 72.4098
	0.05-25 (19)	66.5374±12.8752+62.1258±1.0592 [Methyldopa] mmol/L	0.9994, 0.9989, 99.89		2.110 << 124.2417

n: no. of measurement, $\hat{Y}_{Zi(mV)}$: estimated value of cell A and cell B in mV, r: correlation coefficient, r^2: coefficient of determination, R^2% (percentage capital R-squared): explained variation as a percentage / total variation and t_{tab}= $t_{0.05/2,n-2}$.

-Limit of Detection

The limit of detection was studied of methyldopa through as an injected sample volume of 200 µL. Limit of

detection equal to 1.4292 µg / 200 µL for both cells.

-Repeatability

The value of the RSD % for methyldopa 2, 14 and 20 mmol/L less than 0.5 % was obtained indicating a reliable measurement can be achieved using this method.

Assessment of NAG – ADF – 300 – 2 analyser using two cell and multi solar cells for the determination of methyldopa in drugs

The newly developed methodology (NAG-ADF-300-2) was used for the determination of methyldopa in three different samples of drugs from three different of companies. The continuous flow injection analysis used of homemade NAG-ADF-300-2 was compared with two methods which includes UV-spectrophotometric at λ_{max}=280 [28, 29] nm and turbidmetric method at 0 -180° for yellow precipitate particles of methyldopa-potassium hexacyanoferrate system. Results were mathematically treated for the standard addition method. The obtained results indicate clearly that there was no significant differences between newly developed method, UV- Spectrophotometric and turbidmetric method (classical method) at 95% (α = 0.05) confidence level as the calculated t_{cal} (0.8993, 0.7940 and 0.0303, 0.3032) is less than t_{tab} (4.303) for each cell (i.e.; cell A & cell B).

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Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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