New photometric method for the determination of methyl dopa via it is oxidation through periodate using Ayah 3Sx3-3D-solar micro FI photometer. Study &application

Issam M.A.Shakir AL-Hashimi & Mohammed Jassim Hamzaha AL-Kaffiji
Chemistry Department – College of Science – University of Baghdad – Iraq
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Abstract

A newly photometric analytical method characterized by it is speed and sensitivity was developed for the determination of methyl dopa in pure and pharmaceutical samples via its oxidation to orange-reddish colored complex through periodate reaction in alkaline media using homemade Ayah 3Sx3-3D-solar FI photometer . The orange-reddish species was determined using super bright green light emitting Diod (LED) as a source . A 230µl was taken as a reasonable sample volume for the determination of drug in pure and pharmaceutical formulations. The optimum conditions were1.4ml/min flow rate for both of sodium periodate(5mmole.L^{-1}) and sodium hydroxide (1mmole.L^{-1}) while allowed time for injection was 12 seconds. The linear dynamic range for the instrument response versus methyl dopa concentration was 0.1-1.4 mmole.L^{-1} while the L.O.D was 0.377nM/230µl sample from the stepwise dilution for the minimum concentration of lowest concentration in the linear dynamic range of the calibration graph . The correlation coefficient (r) was 0.9975 while the percentage linearity (%r^2) was %99.52 . The method was applied successfully for the determination of methyl dopa in pharmaceutical preparations . Using paired t-test it was shown that there was no significant difference between the proposed method and official method and on that basis the new method can be accepted as an alternative analytical method.

طريقة طيفية جديدة لتقدير عقار المثيل دوبا من خلال اكسدتة باستخدام بيرايودات الصوديوم ومنظومة Ayah3Sx3-3D-solar للتحليل بالحقن الجرياني الطيفي,دراسة وتطبيق.

مفتاح البحث: تقدير المثيل دوبا

الخلاصة

تم تطوير طريقة تحليلية طيفية سريعة وحساسة لتقدير المثيل دوبا في الصيغة النقية والمستحضرات الصيدلانية من خلال اكسدتة إلى ناتج ذو لون برتقالي- محمر بوساطة بيرايودات الصوديوم في وسط قاعدي. وتم قياس الناتج الملون باستخدام منظومة مصنعة محليا للتحليل بالحقن الجرياني الطيفي المستمر-Ayah3Sx3 3D solar FI photometer كما استخدم ثنائي الوصلة باعث ذي الضوء الأخضر والشدة العالية كمصدر للتشعيع , . استخدام حجم أنموذج 230 مايكروليتر لتقدير الدواء بصيغته النقية والمستحضرات الصيدلانية. الظروف المثلى التي تم التوصل إليها تتضمن استخدام سرعة جريان 1.4ml/min لكاشف بيرايودات الصوديوم (5 mmole.L^{-1}) وهايدروكسيد الصوديوم (1mmole.L^{-1}) . تم الحصول على علاقة لتغير الاستجابة الإلية مع تركيز الدواء وكانت حدود منحنى المعايرة 0.1-1.4 مللي مول.لتر$^{-1}$ إما حد الكشف 0.377 نانومولار/ 230 مايكروليتر من التخفيف التدريجي لأقل تركيز في منحنى المعايرة. بلغت قيمة معامل الارتباط(r) 0.9975 ومعامل التقدير(%r^2) 99.52% . طبقت الطريقة بنجاح لتقدير الدواء في المستحضرات الصيدلانية. أجريت مقارنة بين الطريقة المستحدثة والطريقة القياسية باستخدام اختبار t المزدوج وتبين أنة لا

يوجد فرق جوهري بين الطريقتين وعلى هذا الأساس بالإمكان استخدام الطريقة المستحدثة كبديل للطريقة القياسية

1- Introduction

Methyl dopa is sesquihydrate of (-)-β-3,4 dihydroxy phenyl-α-methyl L-alanine (fig.1).

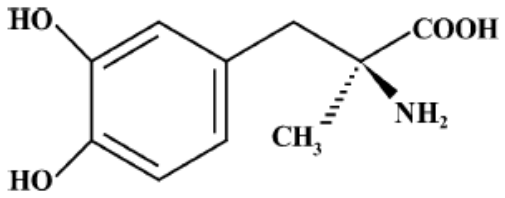

Fig.1- Structural formula of methyl dopa.

It is used in the treatment of hypertension whether it is moderate or even severe. It inhibits the conversion of dopa to dopamine by competing for the enzyme dopa decarboxylase[1]. It is a centrally acting α_2-adrenoreceptor agonist, which reduces sympathetic tone and produces a fall in blood pressure[2].

Several types of analytical procedures have been employed for the analysis of methyl dopa in pharmaceutical formulations and or biological samples. Among techniques used in several procedures most based on titrimetry[3] , spectrophotometery[4-10] , biomimetic sensor[11] , potentiometric[12] , gas and high pressure liquid chromatography[13-14] and fluorimetry[15]. Most of the methods described above are not simple for direct application in a large scale routine analysis and require expensive or sophisticated instruments or involve procedures with rigorous control of the experimental conditions. So far , little attention has been given to the use of the FIA system for on-line preparation of pharmaceutical samples for direct determination of methyl dopa . To the best our knowledge, there are a two reports on the use of FIA spectrophotometric method for the determination of methyl dopa[16-17]. In this present work , an FI spectrophotometric procedure for the determination of methyl dopa based on it is oxidation with sodium periodate in alkaline media is described. This procedure is involved using homemade[18] FI photometer which is equipped with three different light emitting Diod[blue(470nm) , green(525nm) and red(635nm)] as sources and solar cell detector. The performance of the proposed procedure was checked after analyzing commercial pharmaceutical formulations. This procedure is simple , rapid , inexpensive , dose not involve pretreatment procedure or heating steps and has smaller sample consumption and a higher analysis frequency.

2- Experimental

2-1- Chemicals

All used chemicals were of analytical reagent grade unless other wise stated. Distilled water was used throughout this work. Methyl dopa stock standard solution ($C_{10}H_{13}NO_4,1_{1/2}H_2O$, 238.2g/mol , SDI , 5mmole.L^{-1}) was prepared by dissolving 0.1191g/100ml warm distilled water. A stock solution of sodium periodate ($NaIO_4$, 213.89g/mol , BDH , 100mmole.L^{-1}):5.3472g/250ml distilled water. A 10mmole.L^{-1} sodium hydroxide (NaOH , 40g/mol , Fluka) was prepared by dissolving 0.1g/250ml of distilled water. A stock solution of sodium carbonate(Na_2CO_3 , 106g/mol , BDH, 10mmole.L^{-1}):0.1060g/100ml distilled water. A stock solution of ammonia (BDH ,

27% , 0.88 , 100mmole.L^{-1}) was prepared by dilution of 1.75ml in 250ml distilled water.

2-2- Apparatus &Reaction manifold

The flow injection system used for the determination of methyl dopa , shown in fig.2.

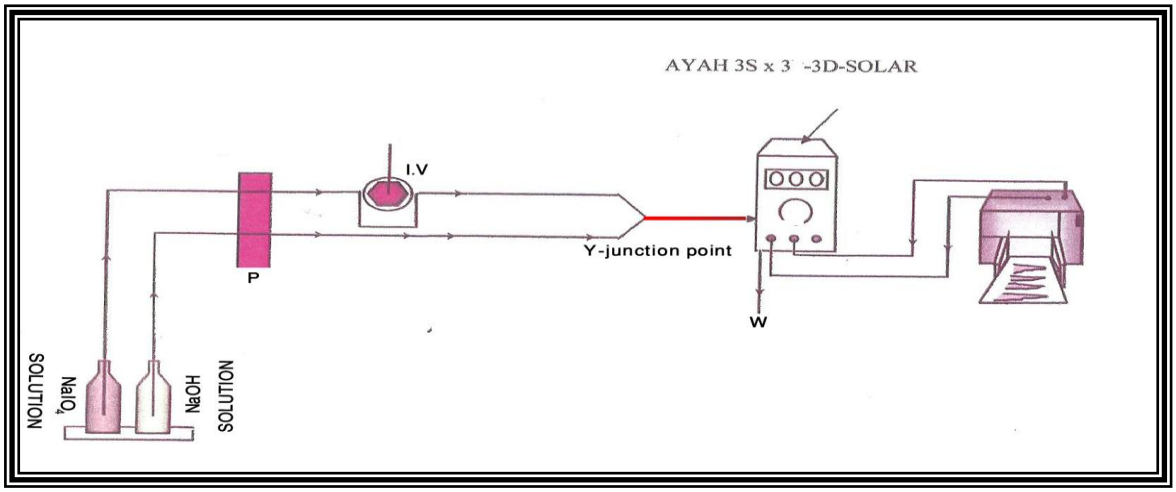

Fig.2- Schematic diagram of flow injection analysis system. P;peristaltic pump, I.V;injection valve , W;waste and Y-junction point.

Which comprises of a peristaltic pump: four channels , variable speed(Ismatic , USA) with a sample loop (0.7mm i.d , Teflon , variable lengths used for sample injection. The instrument response was measured by Ayah 3Sx3-3D solar FI photometer(homemade) using super bright blue, green and red light emitting Diod(LED) as source with a detection using solar cell. The output signal was recorded by voltage output potentiometric recorder(KOMPENSOGRAPH) model C-1032 recorder(Siemens, Germany); using the range of 1-500mV . Peak height was measured for each signals. A liquid junction point made of methylmethacrylate (organic glass) (Y-junction) for the combination of methyl dopa , sodium periodate and sodium hydroxide solutions. UV-Vis spectrophotometer digital double-beam type Optima (Japan) were also used to scan the spectrum of product of reactants using 1cm glass cell.

2-3- Methodology

The whole reaction manifold system for determination of methyl dopa via it is oxidation in alkaline media is shown in fig.2. The manifold system is composed of two lines: first line supplied with sodium periodate(5mmole.L^{-1})at 1.4ml/min, the same line leading to the injection valve, which allows the use 230µl of sample(loop length 60cm with 0.7mm I.D).While the second line is for sodium hydroxide solution at 1mmole.L^{-1} at 1.4ml/min flow rate. Both line meet at a junction (Y-junction); with an outlet for reactants product(methyl dopa- IO^- - OH^-) that produce orange-reddish species. The response peak of the resulting orange-reddish species is followed using Ayah 3Sx3-3D solar FI photometer , while the variation in response was monitored

using super bright green light emitting Diod(LED) throughout this reaction. Each solution was assayed in triplicate.

3- Results and discussion

3-1- Spectroscopic study

When a dilute aqueous solution of methyl dopa mixed with sodium periodate as oxidizing agent in alkaline media an intense orange-reddish species was formed immediately, the orange-reddish species shown a maximum absorption at 485nm against reagent blank as shown in fig.3.

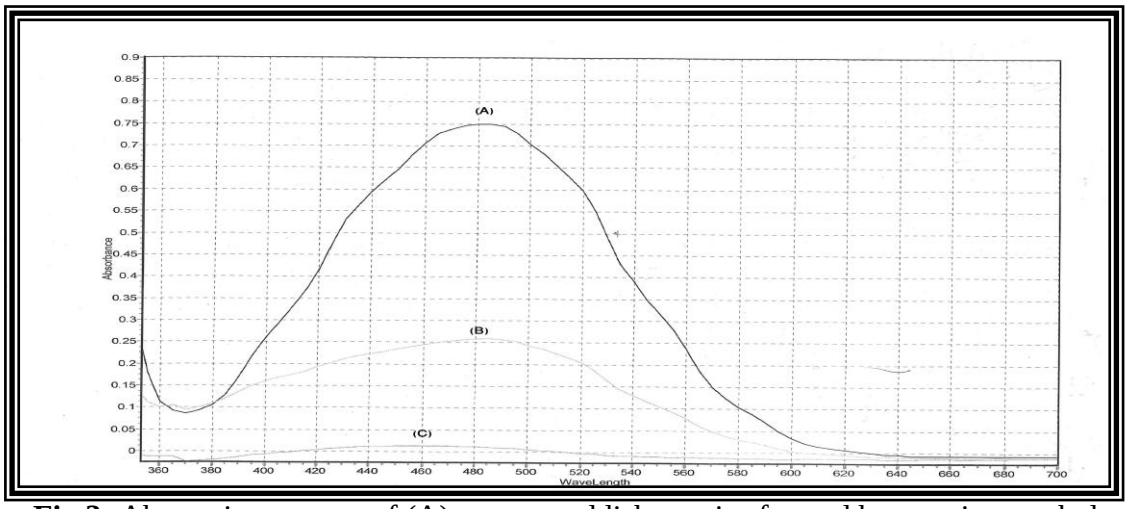

Fig.3- Absorption spectra of (A) orange-reddish species formed by reaction methyl dopa-IO^- - OH^- against reagent blank , (B) yellow species formed from methyl dopa-IO^- against reagent blank and (C) methyl dopa –OH^- against reagent blank.

The same orange-reddish species of methyl dopa as mentioned above was also measured using homemade Ayah3Sx3-3D solar FI photometer at three different super bright light emitting Diod(LED)[blue(470nm) , green(525nm) and red(635nm)]. A maximum response measured in mV obtained when using the high intensity blue or green light emitting Diod(LED) as source as shown in fig.4 , therefore in present work the high intensity green(525nm) light emitting Diod(LED) was used as source for next studies.

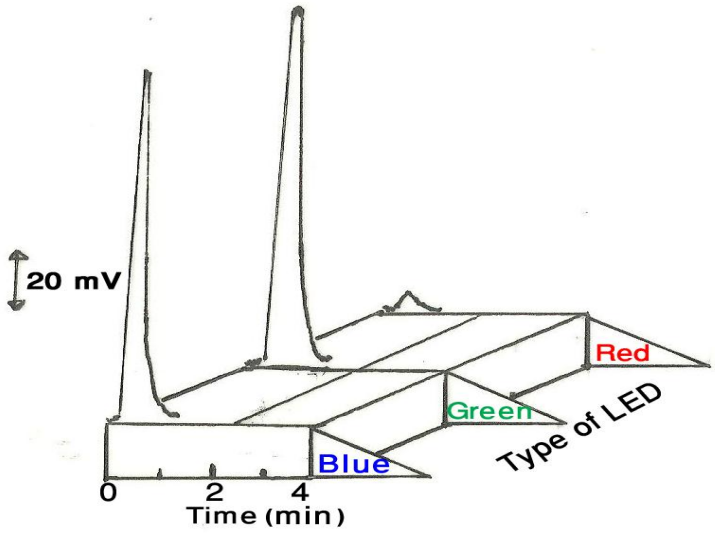

Fig.4-A maximum response measured in mV of orange-reddish species at three different light emitting Diod (LED),[blue(470nm),green(525nm) and red(635nm)].

The type of system that can be used for determination of methyl dopa were also investigated. The response of three different systems which including: (methyl dopa – IO_4^-) , (methyl dopa – OH^-) and (methyl dopa – IO_4^- - OH^-) was measured. A maximum response measured in mV obtained when using the systems (methyl dopa – IO_4^- - OH^-) as showm in fig.5. Which most attributed to the incomplete oxidation for methyl dopa without the presence of hydroxide ion , which proved that the elimination of the base(OH^-)completely from the reaction gave a quenching in response signal for the benefit of the formation of the orange-reddish species

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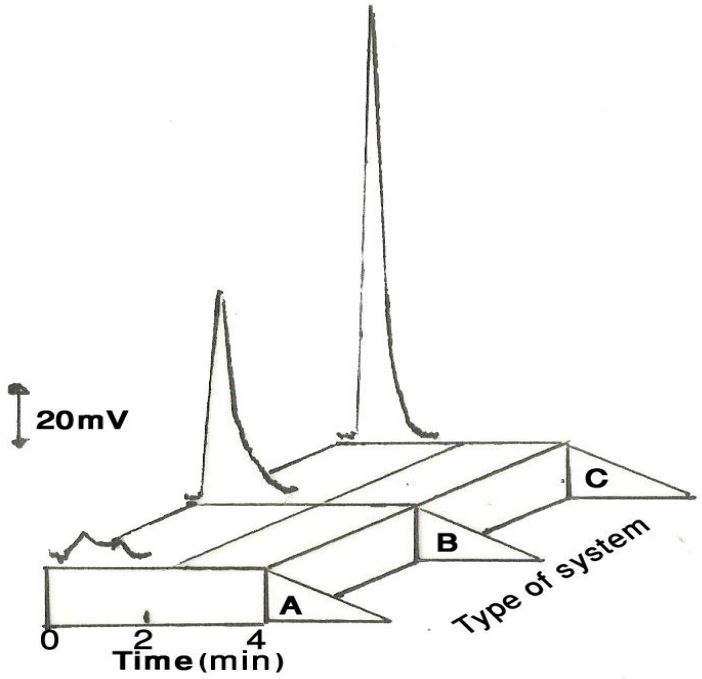

Fig.5- Variation the type of system versus energy transducer output response profile of Ayah 3Sx3-3D-solar FI photometer for the orange-reddish species.
(**A**) methyl dopa – OH^- , (**B**) methyl dopa – IO_4^- and (**C**) methyl dopa – IO_4^- - OH^-

3-2- Effect of basic medium

A set of experiments was carried out for the optimization the preferred basic medium(NaOH , NH_4OH , Na_2CO_3) using 90µl of 1mmole.L^{-1} of sample and 9 seconds as purge time for the sample segment. A maximum response measured in mV obtained when using sodium hydroxide as a basic medium for the whole oxidation of methyl dopa to the orange-reddish species as shown in fig.6 , therefore sodium hydroxide was chosen as best basic medium for the next studies.

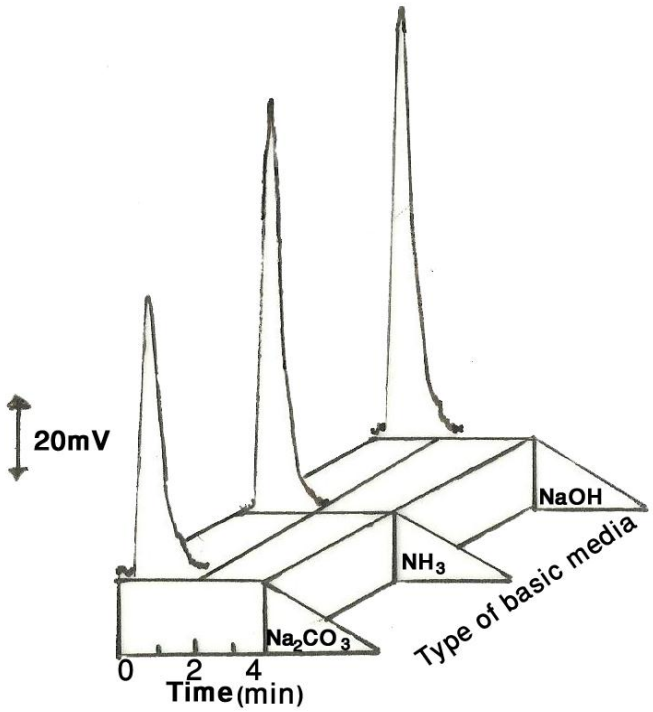

Fig.6- A maximum response measured in mV using different basic media for complete reaction and 90µl(1mmole.L^{-1}) sample volume.

3-3- Optimization of experimental conditions

A series of experiments were conducted to establish the conditions for the production of maximum well defined repeatability for the oxidation of methyl dopa. The physical variables including flow rate , sample volume and allowed permissible time were investigated, respectively and chemicals variables such as concentration of sodium periodate and sodium hydroxide were also investigated.

3-3-1- Physical variables

3-3-1-1- Effect of flow rate

A set of experiments were carried out for the optimization of preferred flow rate that extent from 0.57 to 2ml/min for both sodium periodate(4mmole.L^{-1}) and sodium hydroxide (1mmole.L^{-1}) using 90µl of 1mmole.L^{-1} of sample and 9 seconds as purge time for the sample segment and allowed for the sodium periodate to pass through the injection valve in the injection mode , after that allowed time the injection valve is returned to the load position. The results are tabulated in table no.1.

Table no.1- Effect of the variation of flow rate(ml/min) on the instrument response(mV).

Peristaltic pump (indication approximate)	Flow rate (ml/min)	Response n=3 $\acute{Y}_i$(mV)	Peak base width Δt_B(min)	t (sec)
10	0.57	127	2.8	48
13	0.75	132	2	36
17	0.97	138.2	1.8	30
20	1.1	154	1.6	24
23	1.3	155.7	1.4	18
25	1.4	160	1.2	12
30	1.7	182.3	1	12
35	2	193.2	0.8	12

t= the arrival time of sample segment to the flow measuring cell.

It was noticed that at low flow rate there is an increase in peak base width Δt_B shown in fig.7A, this might be due to dispersion and dilution which causes an irregular responses. While at higher speed > 20 (indication approximate), although the effect of physical parameter was very crucial on the response obtaining regular responses and very sharp maxima , therefore an indication approximate of 25 which correspond to flow rate 1.4ml/min was used to obtain regular responses , narrower Δt_B and minimized the consumption in the reactants solutions as shown in fig.7B.

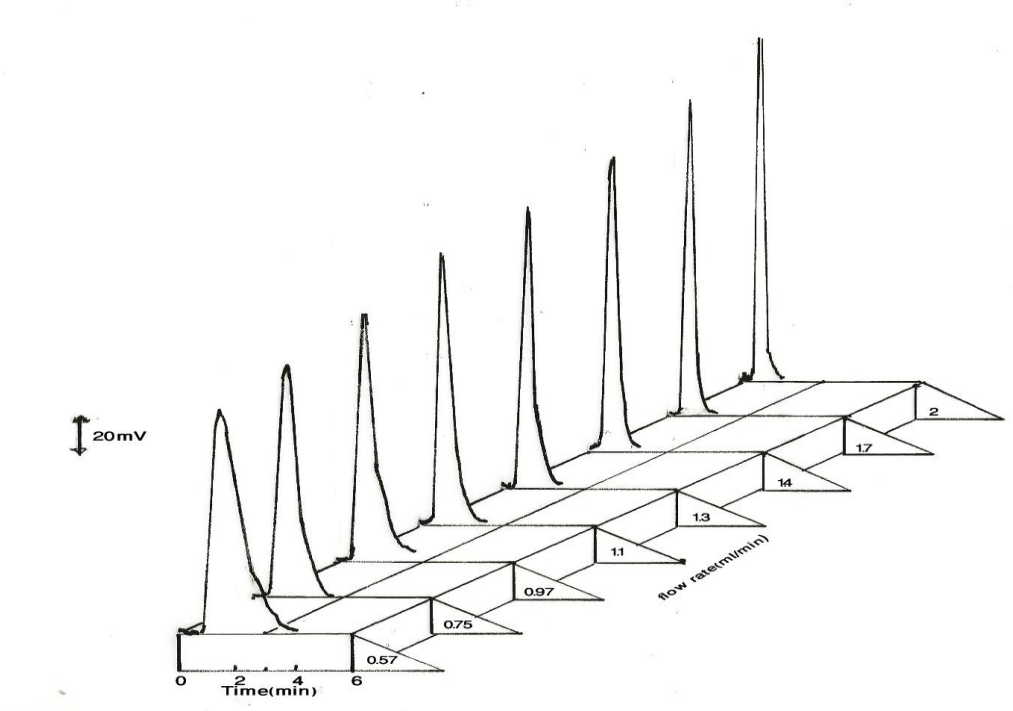

Fig.7A- Variation of flow rate versus energy transducer output response profile of Ayah 3Sx3-3D-solar FI photometer for the orange-reddish species.

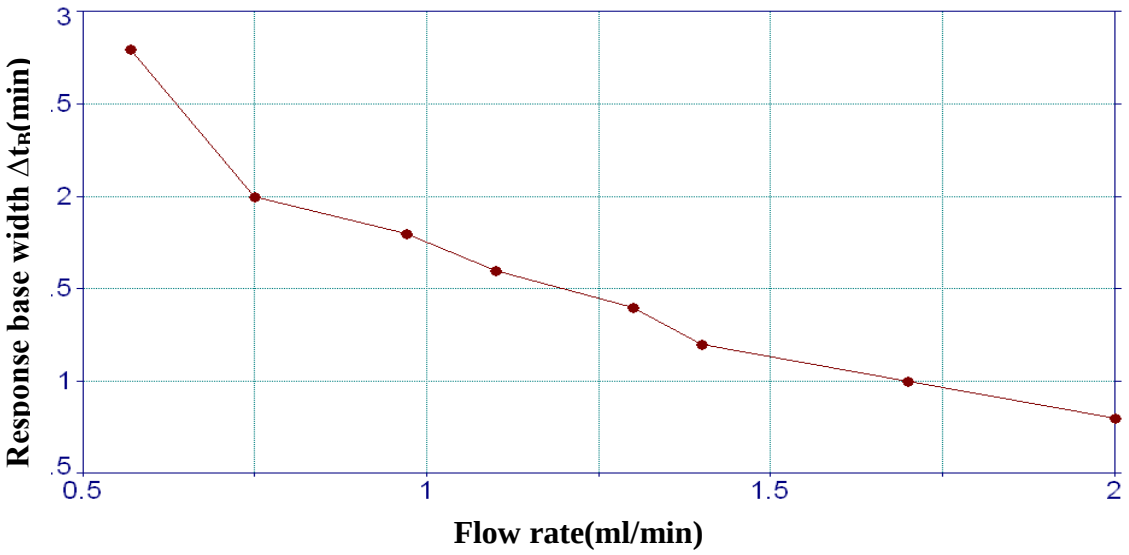

Fig.7B- Variation of response base width Δt_B versus flow rate.

3-3-1-2- Effect of sample volume

Using the optimum flow rate 1.4ml/min. Variable sample volume(40,90, 150,230,300µl)were injected using open valve mode i.e. allowance for continuous purge of sample from the sample loop in the injection valve. The data obtained were plotted as shown in fig.8A showing that the optimum sample volume is 230µl. Regular clear responses were obtained. Using larger volume i.e > 230µl even though it gave slight higher response but it was characterized by wider Δt_B which was probably attributed to the continuous relatively longer duration of sample segment in front of detector as shown in fig.8B.

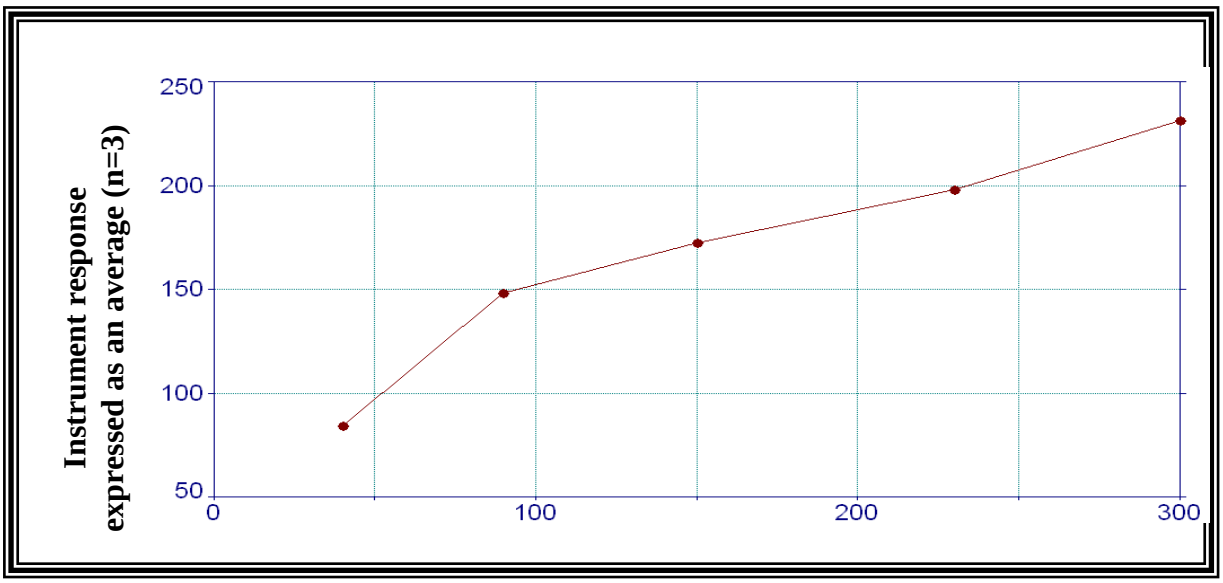

Fig.8A- Variation of injected sample volume versus energy transducer response.

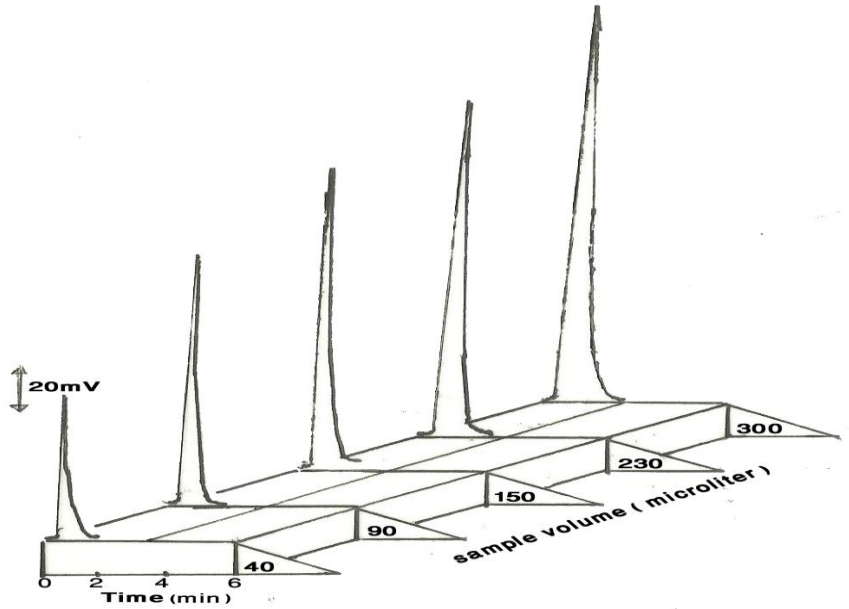

Fig.8B- Variation of sample volume versus energy transducer output response profile of Ayah 3Sx3-3D solar FI photometer.

3-3-1-3- Effect of purge time

Using different purge time for the sample segment i.e, using 3 to 18 seconds allowed time for the sodium periodate to passing through the injection valve in injection mode, followed by turning the injection valve to the load position. Sample volume of 230µl was used. Fig no 9 shows the continuation of the increase in response with increase of injection time up to 12 seconds , after that there was no significant differences in responses. The decrease in responses when using less than 12 seconds was attributed to the incomplete purge time of sample from sample loop in the injection valve. Therefore 12 seconds as purge time was chosen as optimum time to the complete purge of sample segment from sample loop for the next studies.

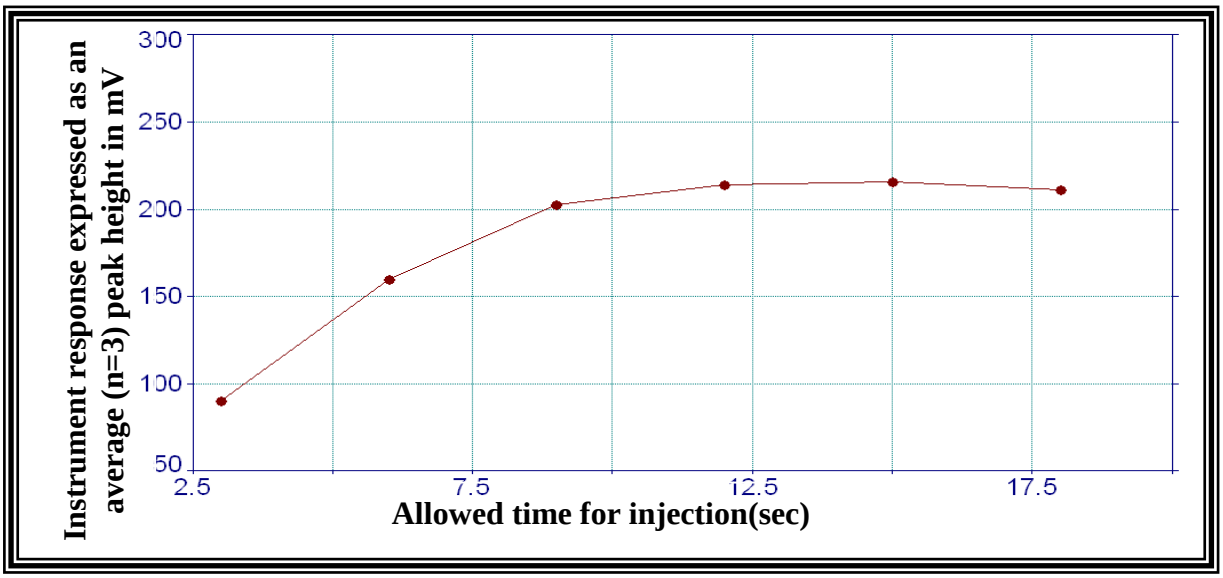

Fig.9- Variation of energy transducer response versus time of injection. A sample volume 230µl was used.

3-3-2- Chemical variables

3-3-2-1- Effect of sodium hydroxide concentration

Using the optimum variables achieved in previous sections. A series of sodium hydroxide solutions were prepared ranging 0.1-10mmole.L^{-1} to establish the optimum concentration that can be used. The study was carried out using 1mmole.L^{-1} of methyl dopa . Each measurement was repeated for three successive times. A repeatability of < 0.8% was obtained. Fig no 10 was obtained and it was noticed that 1mmole.L^{-1} was the optimum concentration of sodium hydroxide solution.

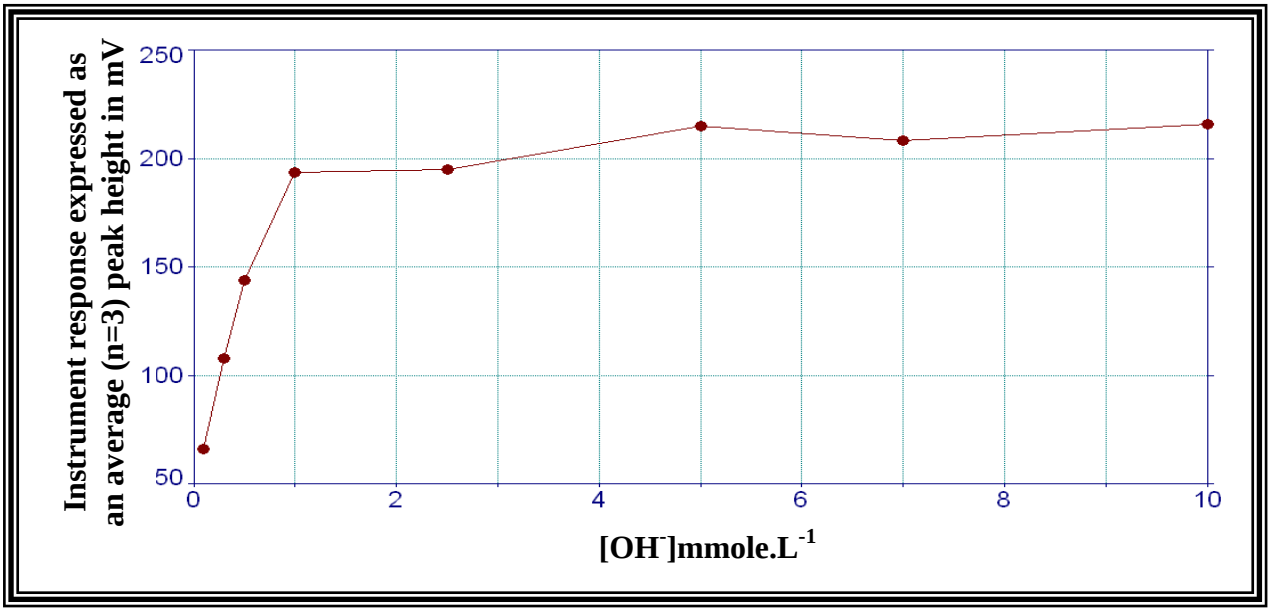

Fig.10- Variation of energy transducer response versus sodium hydroxide solution concentration.

3-3-2-2- Effect of sodium periodate concentration

A serious of sodium periodate solutions was prepared ranging from1-7mmole.L^{-1} using the optimum concentration of OH^- ion(1mmole.L^{-1}) and 1mmole.L^{-1} Of sample using 230µl as an injected sample volume with flow rate 1.4ml/min. The results shown in fig.11. It was noticed that the increase in sodium periodate concentration up to 5mmole.L^{-1} gave regular and sharp maxima with suitable peak height 200.6mV comparing with lower concentration of 5mmole.L^{-1} the responses were of low sensitivities(low response) .This might be due in not establishing the optimum level of the best concentration of oxidizing agent. While an increase of the sodium periodate concentration above 5mmole.L^{-1} , it was noticed that there were no significant differences on the height of the responses, this might attributed to the complete oxidation at level 5mmole.L^{-1} . On this basis 5mmole.L^{-1} was chosen as the optimum concentration for the sodium periodate.

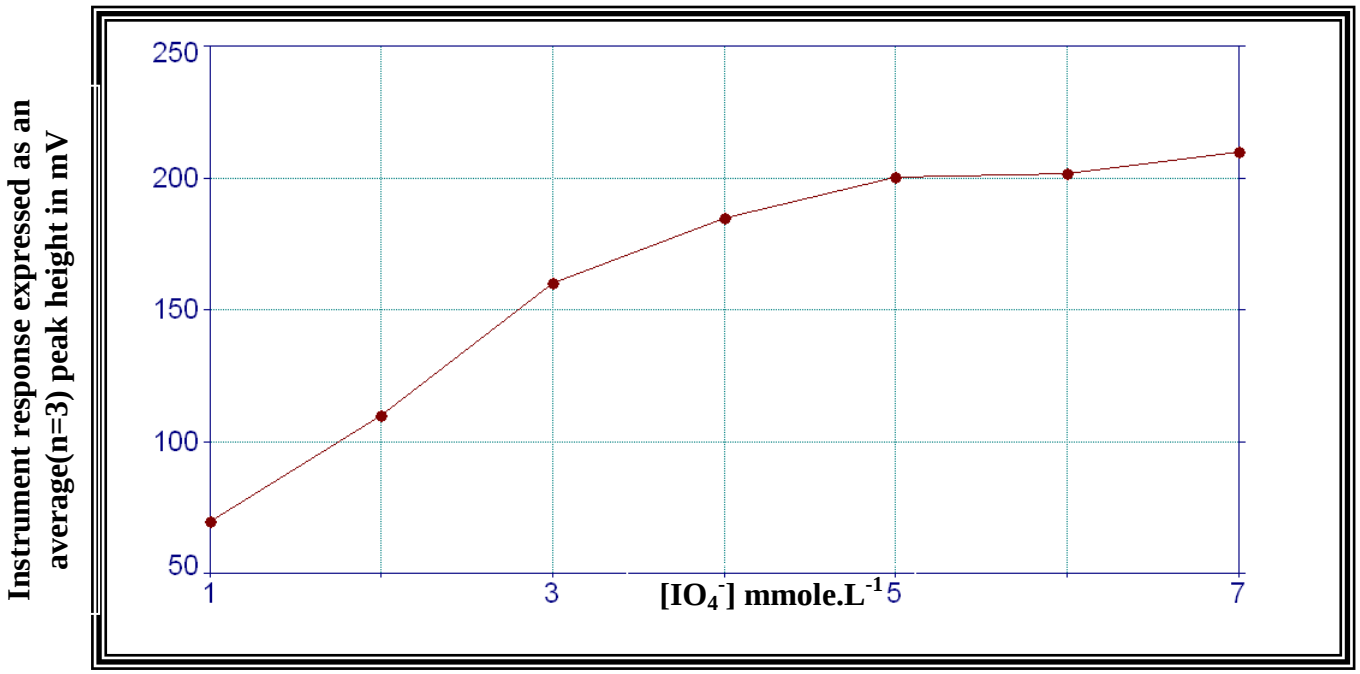

Fig.11- Variation in energy transducer response versus sodium periodate concentration.

3-4- Performance of methyl dopa measurements system

Fixing all the achieved parameters whether it is physical or chemicals. A series of solutions for methyl dopa 0.04-5mmole.L^{-1} were prepared, a calibration graph for the variation of instrument responses with methyl dopa concentration for 0.1-1.4mmole.L^{-1} as shown in fig.12. Above 1.4mmole.L^{-1} the value of correlation coefficient (r) will decrease most probably due to the unoxidized methyl dopa. The obtained results were tabulated in table no.2.

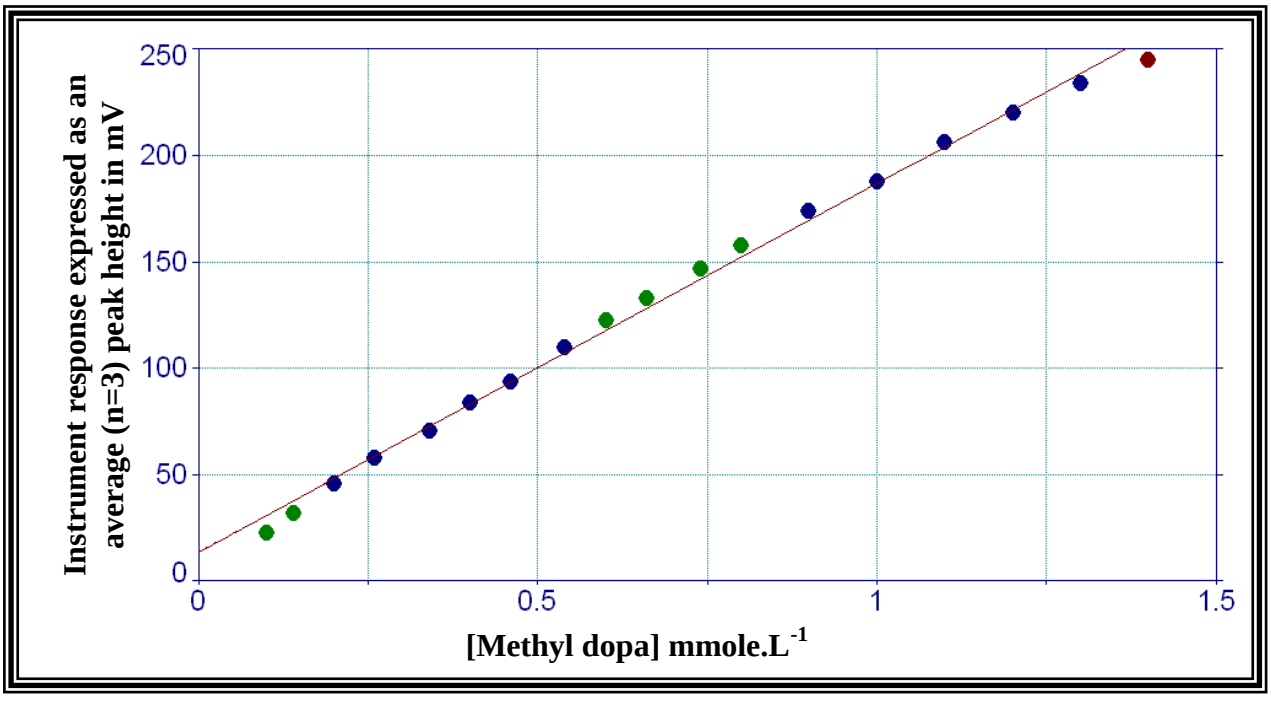

Fig.12- linear calibration graph for the variation of energy transducer response in mV versus methyl dopa concentration in mmole.L^{-1}.

Table no.2- summery of calibration graph results for the determination methyl dopa using sodium periodate as oxidizing agent in alkaline media.

Measured [methyl dopa] mmole.L^{-1}	**Linear dynamic range n=19**	**$\acute{Y}_{(mV)}=a\pm s_a t + b\pm s_b t$ [methyl dopa] mmole.L^{-1} at confidence interval 95%, n-2**	**r r^2 $\%r^2$**	**t_{tab}**	**$t_{cal}=/r/\sqrt{n-2}/\sqrt{1-r^2}$**
				at %95 , n-2	
0.04-5	0.1-1.4	13.57±13.57 + 173.16±173.16[methyl dopa] mmole.L^{-1}	0.9975 0.9952 %99.52	2.110<<59.36	

The limit of detection for methyl dopa was conducted through three methods as tabulated in table no.3 at injected sample volume of 230µl.

Table no.3- limit of detection of methyl dopa at optimum parameters.

Gradual dilution for the minimum concentration	**Based on the value of slope $x=3s_B$ / slope**	**Linear equation $\acute{Y}=Y_B+3s_B$**
0.377nM	0.0201µM	0.0201µM

X = value of L.O.D based on slope.
S_B = standard deviation of blank solution.
Y_B = average response for the blank solution(equivalent to intercept in straight line equation)
L.O.D = limit of detection.

3-5- The repeatability of methyl dopa results

The repeatability was carried out for the determination of methyl dopa via measurements of oxidized methyl dopa at concentration (0.54, 0.6mmole.L^{-1}) of five successively injected sample measurements as shown in fig.13.

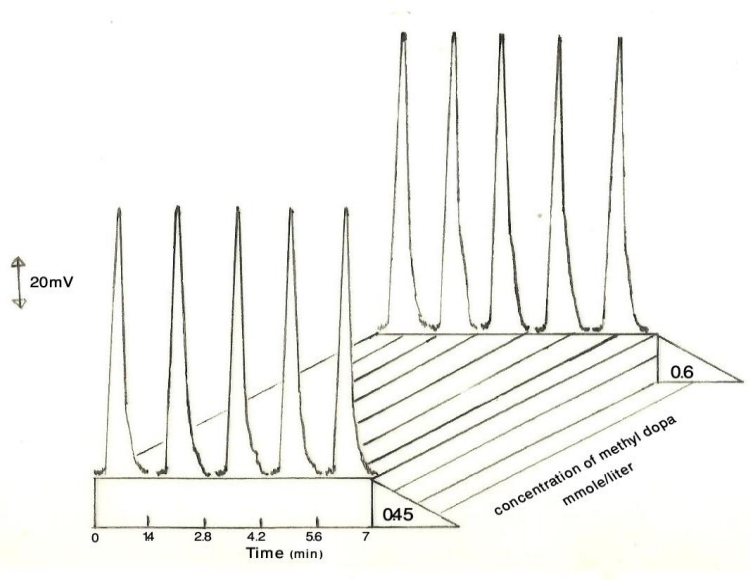

Fig.13- Successive repeatability measurements of methyl dopa (0.45, 0.6mmole.L^{-1}) Using Ahay 3Sx3-3D-solar FI photometer.

The results obtained are tabulated in table no.4.

Table no.4- Repeatability of methyl dopa results.

[methyl dopa] mmole.L^{-1}	$\acute{Y}_i$ (mV) n=5	σ_{n-1}	R.S.D%	Confidence interval of the mean $\acute{Y}_i \pm t_{0.05/2}\sqrt{\sigma}_{n-1}/\sqrt{n}$
0.54	110.4	0.89	0.81	110.4 ± 1.10
0.6	122.8	1.09	0.88	122.8 ± 1.35

3-6- Analysis of pharmaceutical preparations

The used of Ayah 3Sx3-3D solar FI photometer throughout this work was put into a test for it is efficiency of the measurements of methyl dopa in three different pharmaceutical preparations from different origin of supplier. Thirteen tablets from each pharmaceutical drug ; each tablets was weighted and an average of the tablets weights , standard deviation was measured ; these tablets were crushed , grinded then was dissolved what was equivalent to 1mmole.L^{-1} in tiny amount of warm distilled water then was filtered on a washed filter paper in order to get rid off the insoluble materials what were exist ; the residue was washed with distilled water and the volume was completed with 100ml in volumetric flask. Methyl dopa in each pharmaceutical drug was determined using the direct method in a direct calibration graph. The results are summarized in table no.5.

Table no.5- Determination of methyl dopa at different manufactures of pharmaceutical drugs in direct calibration graph by using methyl dopa – IO_4^- - OH^- system.

Pharmaceutical tablets , content & manufactures	Confidence interval of average weight at 95% , n=∞ $\acute{W} \pm t_{0.05/2}\sigma_{n-1}/\sqrt{n}$	Sample weight(0.0238g) equivalent to 1mmole.L^{-1} of active ingredient (g)	Theoretical content for active ingredient at 95%, n=∞ (mg)	Practical content for active ingredient at 95%, n=∞ (mg)	Recovery%
Aldosame,SDI Iraq(250mg)	0.3467±0.00415	0.0330	250±0.0029	252.04±2.26	100.81
Methyl dopa , AlGortham, Lebanon(250mg)	0.3481±0.00403	0.0331	250±0.0028	244.49±1.30	97.79
Methyl dopa , MBC , Syria (250mg)	0.3715±0.00210	0.0353	250±0.0014	258.13±1.136	103.32

Paired t-test was used as shown in table no.6. The obtained results indicated clearly that there was no significant differences between newly flow injection analysis method with official method at 95% confidence interval as the calculated t value is less than tabulated t value.

Table no.6- Paired t-test for flow injection analysis proposed method with official method for the determination of methyl dopa in pharmaceutical preparations.

Sample no	Practical content (mg)		d(mg)	$\bar{X}_d$	σ_{n-1}	Paired t-test $\bar{X}_d \sqrt{n} / \sigma_{n-1}$	t_{tab} at 95% confidence interval , n-1
	Proposed FIA method	Official method					
1	252.04	250	2.04	1.55	6.83	0.393<<4.303	
2	244.49	250	-5.51				
3	258.13	250	8.13				

3-7- Conclusion

In conclusion , the proposed method FI photometric procedure can be used for the analysis of methyl dopa in pharmaceutical preparations. This method is simple , fast , relatively inexpensive , precise , accurate , sensitive , using minimum number of reagents and reaction sequence .Then , the speed of analysis and the precision make this method also suitable for the quality control of formulations containing methyl dopa replacing tedious , expensive , slow official and chromatographic methods . Complex pre treatment of the samples is not necessary because the preparation of the pharmaceutical formulations and reagents is done simply by dissolving in water, in this manner , it not require the removal of usual excipients since they were found not to interfere with the determination of methyl dopa ,therefore this system is particularly useful for the implementation of routine analysis.

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