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CFIA- Colorimetric assessment and photometric determination of vitamin B_9 (Folic acid)using LEDs as a source of irradiation and two solar cells as an energy transducer

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Abstract: *A specific, new, sensitive and simple method has been used for the determination of vitamin B_9(Folic acid)in pure and pharmaceutical formulations by continuous flow injection analysis .The method is based on oxidation vitamin B_9 by Ce(IV) sulphate in sulphuric acid to obtain a red complex, using homemadeAyah-6SX1-ST-2D solar cell CFIA. Optimum parameters have been studied to increase the sensitivity and limit of detection for this developed method. The linear range for the instrument response versus vitamin B_9 concentration was (0.1-5) $mMol.L^{-1}$ while the L.O.D was 5.544µg/sample, the correlation coefficient (r) was 0.9953 while percentage linearity (r^2%) was 99.07%. RSD% for the repeatability (n=8) was less than 0.5%for the determination of vitamin B_9 at concentration (0.9, 5) $mMol.L^{-1}$ respectively .The method was applied successfully for the determination of vitamin B_9 in pharmaceutical preparation .A comparison was made between two methods: newly proposed method and the reference claimed method UV-SP spectrophotometry at λ_{max}=255 nm, of analysis using the standard addition method, via the use paired t-test and there was no significant between the two methods at 95% confidence level.*

Keywords: *Vitamin B_9, flow injection analysis , Colorimetry ,homemade instrument.*

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I. Introduction

Folate, forms of which are known as folic acid and vitamin B_9, is one of the B vitamins[1].That is normally found in foods such as dried beans, peas, lentils, oranges, whole-wheat products, liver, asparagus, beets, broccoli, brussels sprouts, and spinach. Folic acid helps your body produce and maintain new cells, and also helps prevent changes to DNA that may lead to cancer.

As a medication, folic acid is used to treat folic acid deficiency and certain types of anemia (lack of red blood cells) caused by folic acid deficiency. The recommended daily intake level of folate is 400 micrograms from foods or dietary supplements[2] .It is also used as a supplement by women during pregnancy to prevent neural tube defects (NTDs) in the baby [3].

The physical and chemical properties of folic acid

Folic acid also known as vitamin M. Pale orange-yellow crystals or flakes. About 250 ℃ darken not melt occurs carbonization. Dissolved in hot dilute hydrochloric acid and sulfur, slightly soluble in acetic acid, phenol pyridine, alkali hydroxide and alkali carbonate solution, slightly soluble in methanol, insoluble in ethanol and butanol, and insoluble in ether, acetone, chloroform and benzene. About dissolved 1% in a 25 ° C water solubility of only 0.0016mg/mL, boiling, 1G folic acid in 10mL of water suspension, pH of 4.8-4.8. But folic acid sodium salt easily soluble in water, but its sodium salt dissolved in water by light decomposes pteridineaminobenzoyl sodium glutamate. Folic acid is stable in the air, but by the ultraviolet light that the decomposition of losing its vitality. Thermally unstable in acidic solution, but in the neutral and alkaline environment is very stable and heated under 100 ° C for 1 hour will not be damaged[4].

Folic acid (FA) chemically: (s)-2-(2-((2-amino-4-hydroxy pteridine-6-yl) methyl amino) benzamido) pentandioic acid one of the water soluble B vitamins. It is degraded in aqueous solution by sunlight, ultraviolet and visible light. It has little native fluorescence, it can be turned into a strongly fluorescene compound by oxidation. Folic acid is made up of bicyclic joined by peptide linkage to a single molecule of L-glutamic acid as shown in Figure .1[5].

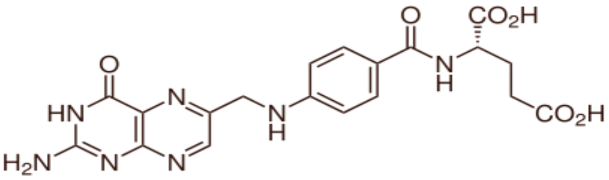


Figure. 1- The structure formula of folic acid.

IUPAC name : (2*S*)-2-[[4-[(2-Amino-4- oxo-1*H*-pteridin-6- yl) methylamino]benzoyl]amino]pentanedioic acid.

Formula $C_{19}H_{19}N_7O_6$

Molar mass: 441.40 g·mol^{-1}

Solubility in water 1.6 mg/L (25 °C).

A deficiency of folate in the diet is closely linked to the presence of neural tube defects in new borns and to an increase rise of megaloblastic anemia, cancer, alzheimer's disease and cardiobascular disease in adults [6]. There are various analytical methods for determination of Vitamin B_9 . These analysis include :HPLC[7], spectrophotometric[8-10].flourimetry [11,12] , Chemilumenescence [13,14] Chemilumenescence with Flow injection analysis [15] and Voltammetry [16].

In this work using flow injection colorimetric method ,the measured done via attenuation of incident light after oxidation vitamin B_9 (folic acid) by Ce (IV)sulphate in a sulphuricacid to obtain a clear red complex,by homemade Ayah -6SX1-ST-2D solar cell provide with six snow-white light as a source with two solar cells as a detector[17]

II. Experimental

Reagent and chemicals

All chemicals were used of analytical-reagent grade and distilled water was used to prepare all thesolutions . A standard solution 0.01Mol.L^{-1}ofVitamin B_9(Folic acid) molecular formula$C_{19}H_{19}N_7O_6$,molar mass441.42g.mol^{-1}&Hopkins&Williams was prepared by dissolving1.10355g folic acid in 10ml of 1Mol.L^{-1}$Na_2CO_3$and complete the volume to 250ml With distilled water.A stock solution of (0.1 Mol.L^{-1}) Ce $(SO_4)_2$ 332.298g/molHopkins&Williamswas prepared by dissolving8.3075g/250min sulphuric acid 1Mol.L^{-1}

Sample Preparation

Twenty tablets were weighted then crushed and grinded .Tablets containing (5,5,1) mg of vitamin B_9 were weight 0.5476g, 1.0164g, 3.3199g (equivalent to 0.0441g of active ingredient,1mMol.L^{-1}) for Folic acid (actavis UK, julphar U.A.E & Samarra- Iraq) respectively and dissolved in approximately 10ml from 1Mol.L^{-1} Na_2CO_3. The solution was filtered to get rid of undissolved materials, the residue was washed with distilled water and completed the volume to 100ml with distilled water.

Apparatus

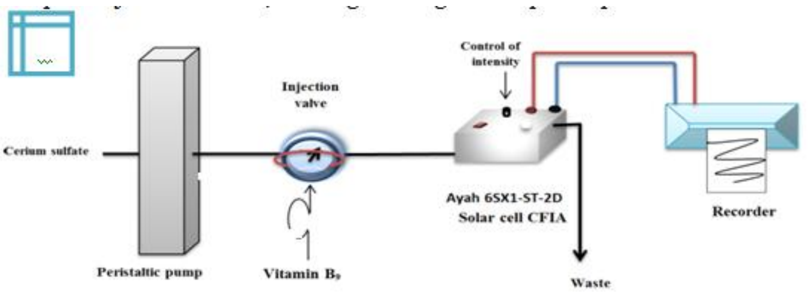
-Colorimetric assessment of vit.B_9 via the use of Ce(IV) ion as an oxidizing agent.

- Manifold Reaction Design for oxidation of vit.B_9 by Ce (IV) ion and its spectrophotometric assessment.

The study carried out using experimental conditions for vitamin B_9 (folic acid) (5 mMol.L^{-1}), Ce (IV) sulphate (0.03Mol.L^{-1}) dissolve in 1 Mol.L^{-1} H_2SO_4 and complete the volume with distilled water, sample volume 210 µl , applied voltage to the LEDs was 1.95 volt DC & open valve mode (leaving the injection valve in the inject mode until the response output comes to back ground levels). These experiments were conducted with a homemade Ayah 6SX1-ST-2D Solar cell CFI Analyzer, which work with a six snow white light emitting diode will be used as a source. Each solution injected Six times. The purpose behind this study was to optimize the best manifold system to choose the most representative S/N profile due to absorption of portion of incident light by colored species produced from the oxidation of folic acid by Ce(IV) sulphate; and this in turn leads to weaken the transmitted light passing to the detector which results in having negative responses relative to the back ground signals. The response was recorded on x-t potentiometric recorder.

First manifold design:

One line system Fig .2a was used , which supplied Ce(IV) sulphate (0.03Mol.L^{-1}) at 1.5 ml .min^{-1} flow rate and then passes through injection valve for carrying vit.B_9 (5 mMol.L^{-1}),to form the colored species inside loop of injection valve , leading to irregular response profile as shown in Fig.2b



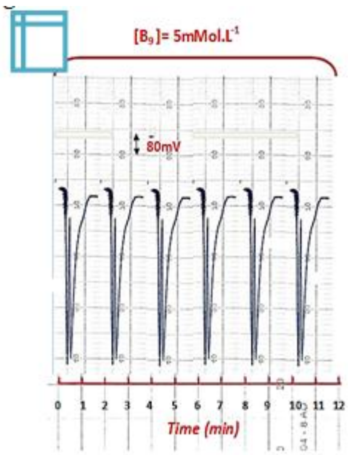
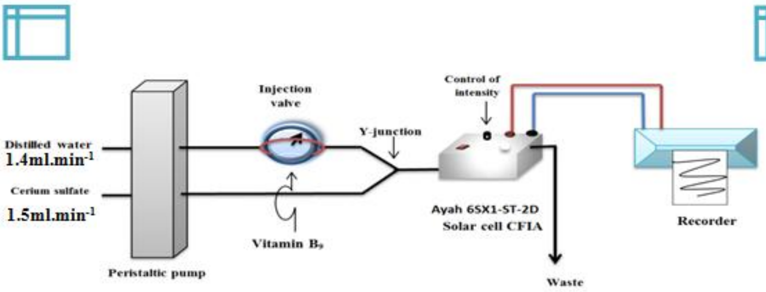


Fig.2 **a: The manifold designs which include one line;**
-Carrier stream: Ce(IV) sulphate (0.03Mol.L^{-1})
b : Response profile of first manifold design.

Second manifold design:
The manifold is of two lines (Fig. 3a): The first line supplied with distilled water as a carrier stream at 1.4 ml .min $^{-1}$ which leads to the injection valve for carrying 210 µl sample volume of vit. B_9 (5 mMol.L^{-1}), the second line supplied Ce(IV) sulphate (0.03Mol.L^{-1}) at 1.5 ml .min $^{-1}$ as a reagent. Both of lines met at Y-junction point with an outlet for reactants product from red colored species which passes through Ayah 6SX1-ST-2D Solar cell CFI Analyzer. Two lines system gave a regular reproducible & smooth response profile (Fig.3b).



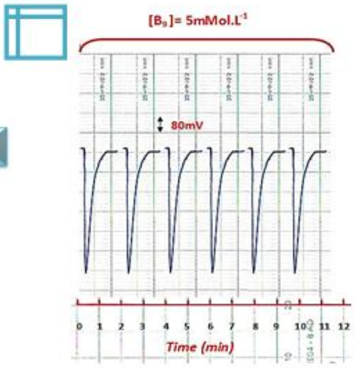
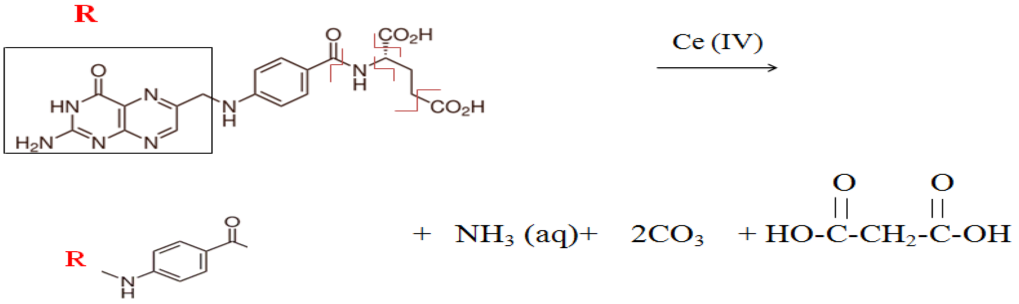


Fig .3 **a: Manifold design include two lines;**
-Distilled water as carrier stream
-Ce (IV) sulphate (0.03Mol.L^{-1})as a reagent
b: Response profile of second manifold design

On the above mentioned basis the two line manifold is the most appropriate manifold design. due to completion of mixing and oxidation to form the colored plug in comparison with one line manifold system as in here the mixing occur in the injection valve via sample plug loop which in reality a short area-volume to obtain a distinct mixing (i.e., homogenous reaction –mixing)for a complete coloured specimen. A deformed profile of response-time was the output; therefore, it was cancelled throughout this process of research piece of work.
A proposed mechanism for the oxidation of vit.B_9 with Ce(IV)sulphate in sulphuric acid can be represented according to the following steps[18 -21].
Step 1: The primary reactive species of Ce(IV) sulphate in acidic medium can be resultfrom the equation:

$$Ce(SO_4)_2 + H_2SO_4 \rightleftharpoons HCe(SO_4)_3 + H^+ \rightleftharpoons H_2Ce(SO_4)_3$$

Step 2& Step 3 which are gives carboxylic acid & carbonate ion.



$$+ \ NH_3\,(aq) + \ 2CO_3 \ + HO\text{-}\overset{O}{\overset{||}{C}}\text{-}CH_2\text{-}\overset{O}{\overset{||}{C}}\text{-}OH$$

Schematic.1: Proposed mechanism of vit.B_9–Ce(IV)Sulphate –H_3O^+system.

–Selection of optimum chemical and physical parameters leading to highestpossible S/N profile and signal that will relayed on for next comingexperiments .

-Chemical parameters

-Effect ofCe (IV) sulphate Concentration on absorbance of colouredspecies.

Using different concentration of Ce (IV) sulphate 0.001-0.1 Mol.L^{-1} while 5mMol.L^{-1} of B_9 and 210µl sample volume was injected on the stream (distilled water) at a flow rate 1.4 ,1.5 ml.min^{-1} for carrier stream and reagent respectively . The applied voltage to the LEDs was 1.95 volt DC. Each measurement was repeated for three times. The response profile for this study as shown in Fig.4a . It was noticed ; that the height response profile of coloured species increase when the incident light decrease with increase Ce(IV)sulphate concentration up to 0.07 Mol.L^{-1}, while at higher concentration > 0.07Mol.L^{-1} lead to decrease of response height .It might be due to increase of the density of the colored product which work as an internal filter that prevent the remaining light intensity after absorption process by the colored species from passes to the solar cell detector . Fig 4b shows the plot of results using Ayah 6SX1-ST-2D solar cell CFI Analyzer. Therefore 0.07Mol.L^{-1} Ce(IV) sulphate concentration was chosen as the optimum concentration for next coming experiments .

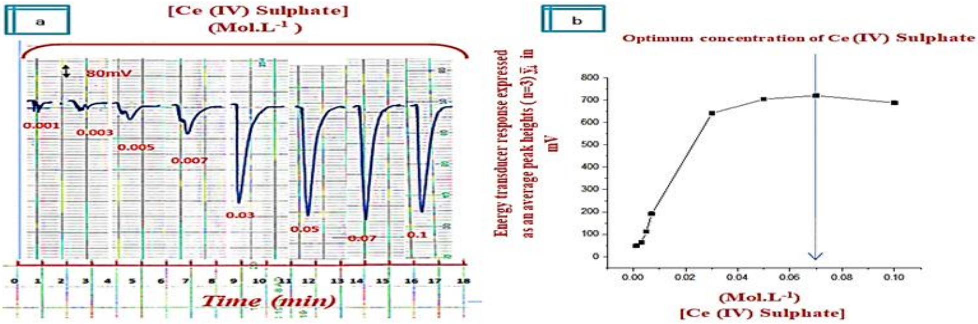


Fig 4 :Effect of variation of Ce(IV)Sulphate concentration on:
a- Response profile versus time
b- Energy transducer response by attenuation of incident light

-Effect of added sulphuric acid

A series of sulphuric acid solutions were prepared (0.1-1) Mol.L^{-1} in addition to distilled water, which is used as a carrier stream to study the effect of added H_2SO_4 concentration on Ce(IV)-vit.B_9-H_3O^+system and just to kept the oxidizing agent in its soluble form. 5mMol.L^{-1} of vit.B_9, 210µl of sample volume was used for this study. Fig.5a shows the effect of H_2SO_4 concentration on height of response profile. Fig.5b was obtained, in which that the increase of absorbance expressed as an energy transducer response of colored species with increasing concentration of sulphuric acid. A slight increment was noticed in absorbance reading 7.78% relative to distilled water back ground when using 0.7 Mol.L^{-1} concentration of H_2SO_4.In order to compromise in having increased sensitivity maintaining Ce(IV) sulphate in its soluble form and catalyst action of the reaction; 0.7 Mol.L^{-1} sulphuric acid was the most satisfactory choice.

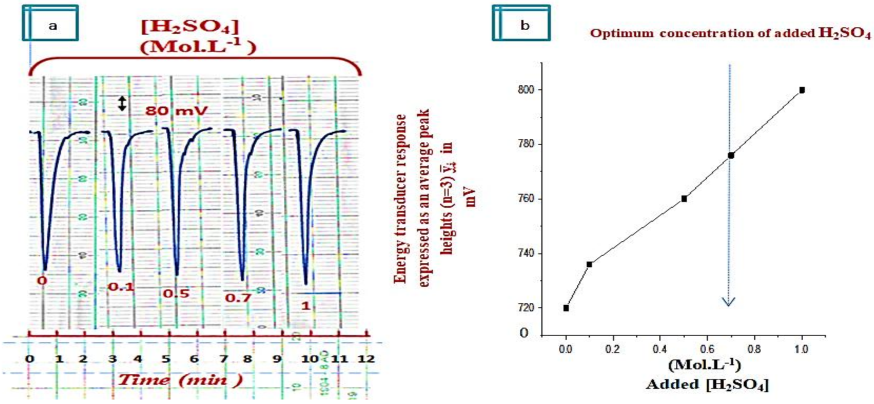


Fig.5: Effect of added sulphuric acid on:
a-Response profile versus time
b-Energy transducer response by attenuation of incident light using 210µl and 1.4,1.5ml.min^{-}flow rate for carrier stream and reagent respectively.

-physical parameters

-Effect of flow rate

Variation of flow rates 0.2- 2.8 ml.min^{-1} for carrier stream (H_2SO_4 0.7Mol.L^{-1} was chosen to be a good carrier stream instead of distilled water from the last study before) and 0.4- 3ml.min^{-1} for reagent; (Ce(IV) sulphate 0.07Mol.L^{-1}). Controlled by the peristaltic pump for determination of vit. B_9 at 5 mMol.L^{-1} concentration was studied; While keeping other changeable constant.i.e.; 210µl sample volume , open valve mode, and applied voltage to the LEDs was 1.95 volt DC .Fig.6a shows that at low flow rate there was an increase in peak height & wider base width this might be attributed to the increase dispersion & area of colored segment in flow cell that causes a longer time duration of colored species in front of detector. While at higher pump speed > 25; (1.8, 2) ml .min $^{-1}$ for carrier stream and reagent respectively; causing irregular S/N response profile might be due to complete of coloured species formation in the flow cell; therefore the speed of 25(1.8ml.min^{-1}flow rate for carrier stream)will be used for the more clear response. Table.1 summarized the result, Fig.6b shows the optimum flow rate.

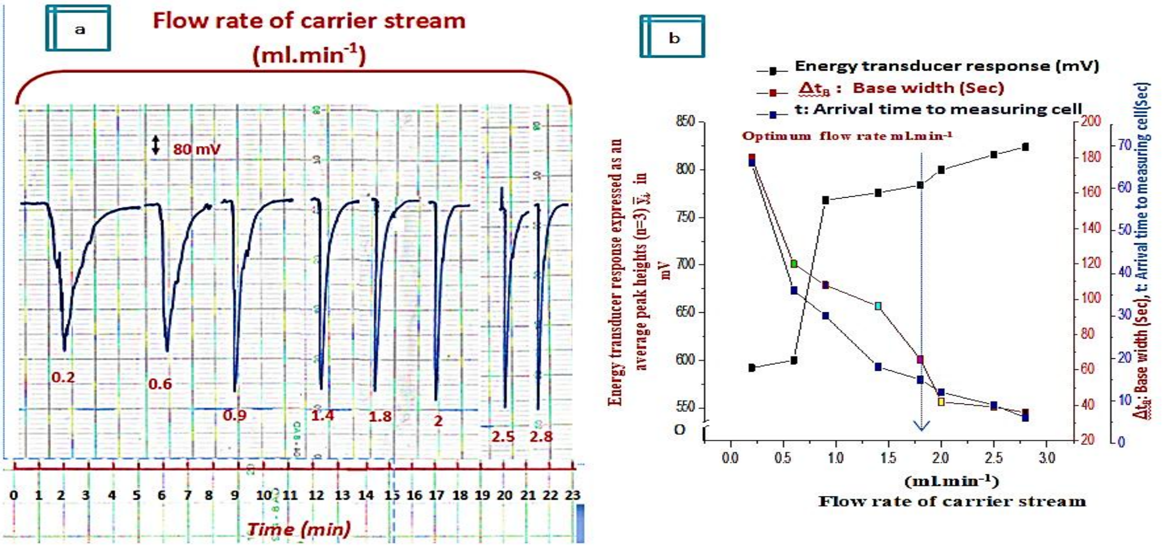


Fig.6: Effect of flow rate on: a- Response profile versus time.
b- Energy transducer response by attenuation of incidentlight.

Table.1: Effect of flow rate on the measurement of energy transducer response using 210µl sample volume and 5 mMol.L^{-1} of vit.B_9.

Pump speed	Flow rate ml.min^{-1}		Energy transducer response expressed as an average peak heights (n=3) $\bar{y}_i$ in mV	RSD%	Reliability (two tailed at 95% $\bar{y}_i \pm t_{0.05/2,n-1}\sigma_{n-1}/\sqrt{n}$	t Sec	Δt_BSec	V_{add} ml	Concentrationat flow cell mMol.L^{-1}
	F_1	F_2							
5	0.2	0.4	592	0.211	592±3.105	66	180	2.01	0.522
10	0.6	0.7	600	0.188	600±2.807	36	120	2.81	0.374
15	0.9	1	768	0.133	768±2.534	30	108	3.63	0.289
20	1.4	1.5	776	0.139	776±2.683	18	96	4.85	0.155
25	1.8	2	784	50.12	784±2.435	15	66	4.39	0.239
30	2	2.2	800	0.099	800±1.963	12	42	3.15	0.333
35	2.5	2.7	816	0.126	816±2.559	9	39	3.59	0.292
40	2.8	3	824	0.124	824±2.534	6	36	3.69	0.285

$F_{1:}$ line no. 1 for carrier stream, F_2: line no.2 for reagent, t: Arrival time from injection valve reaching to measuring cell (sec),Δt_B :Base width of response (sec), $t_{0.05/2,2}$=4.303

–Effect of sample volume

The optimum flow rate 1.8, 2 ml .min $^{-1}$ for carrier stream(H_2SO_4 0.7Mol.L^{-1}) & reagent (Ce(IV) sulphate 0.07Mol.L^{-1}) respectively and vit. B_9 of 5mMol.L^{-1} concentration was used .The injected volume of sample was varied in the range 110-310 µl by changing the length of the sample loop in the injection valve , applied voltage to the LEDs was 1.95 volt DC. It was found that an increase in the volume led to a significant increase in sensitivity and more perceptible than low volumes as shown in Fig.7a which shows that the optimum sample volume of 251.2 µl gave a regular responses for the absorption of incident light, this indicate to complete reaction and consumption of the chemicals when 251.2 µl will be used as an optimum sample volume as shown in fig .7b. The obtained results were tabulated in Table.2.

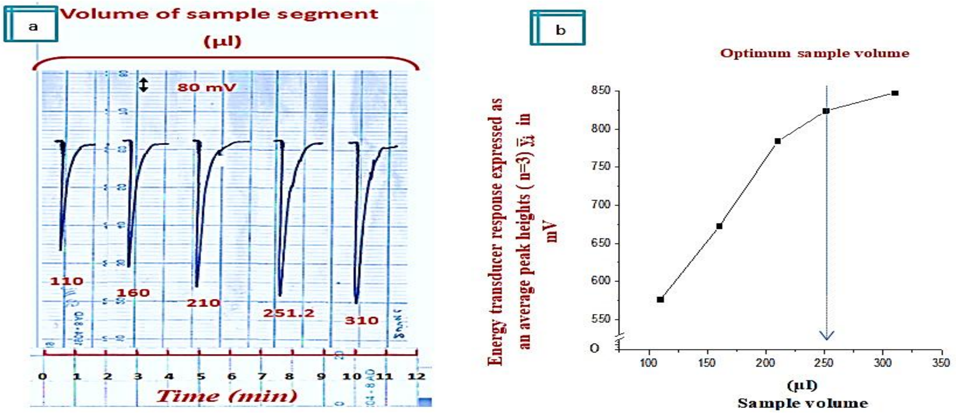


Fig.7: Effect of sample volume on: a- Response profile vs. time.
b - Energy transducer response by absorbance of incident lightusing coloured species.

Table.2: Effect of sample volume on the measurement of energy transducer response using open valve

Length of loop Cm r=0.5mm	Sample volume µl	Energy transducer response expressed as an average peak heights (n=3) $\bar{y}_i$ in mV	RSD%	Reliability (two tailed) at 95% $\bar{y}_i \pm t_{0.05/2,n-1}\sigma_{n-1}/\sqrt{n}$	Δt_B Sec
14.0	110.0	576	0.177	576±2.534	60
20.4	160.0	672	0.144	672±2.410	63
26.8	210.0	784	0.138	784±2.683	66
32.0	251.2	824	0.125	824±2.559	68
39.5	310.0	848	0.120	848±2.534	74

mode. **$t_{0.05/2,n-1}=4.303$ for n=3**

4.3.2. B.2.2.3-Effect of purge time

A study was carried out to determine the optimum duration of the injection time . Allowed permissible time for purging of the sample segment from the injection valve ranging (3-24 Sec in addition to open valve mode (30 Sec))was used in this study. The optimum parameters achieved in previous section were kept at constant . Fig.8a,b shows the continuation of the height response with increase of injection time up to 13 second . The decrease in height of response when using less than 13 sec which might be attributed to the incomplete purge time of sample from sample loop in the injection valve, after 13 sec the peak heights became irregular when the response output comes to back ground levels. Therefore; 13 second chosen as an optimum purge time for the departure of the sample segment completely from the injection valve.

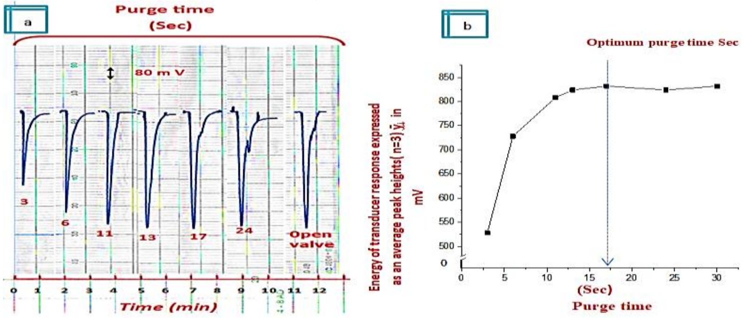


Fig .8: Effect of the variation purge time on: a- Response profile versus time
b- Energy transducer response by absorbance of incident lightusing colourd species.

- Effect of coil length

Using B_9(5mMol.L^{-1})-Ce (IV) Sulfate (0.07 Mol.L^{-1})-H_2SO_4 (0.7 Mol.L^{-1}) system and purge time is 13 sec , the effect of reaction coil was studied .The reaction coil length has a large role in the homogenization and completion of chemical reaction. Different coil length (0 – 50) cm was used , this range of lengths comprises a volume of 0-392.5 µl which connected after Y-junction directly in flow system. Fig.9a,b shows that a decrease in peak height with increase coil length ,at the same time increase of the base width(Δt_B) . Therefore; two lines manifold system without reaction coil necessary for completion of oxidation vit. B_9 by Ce (IV) sulfate in acidic medium.

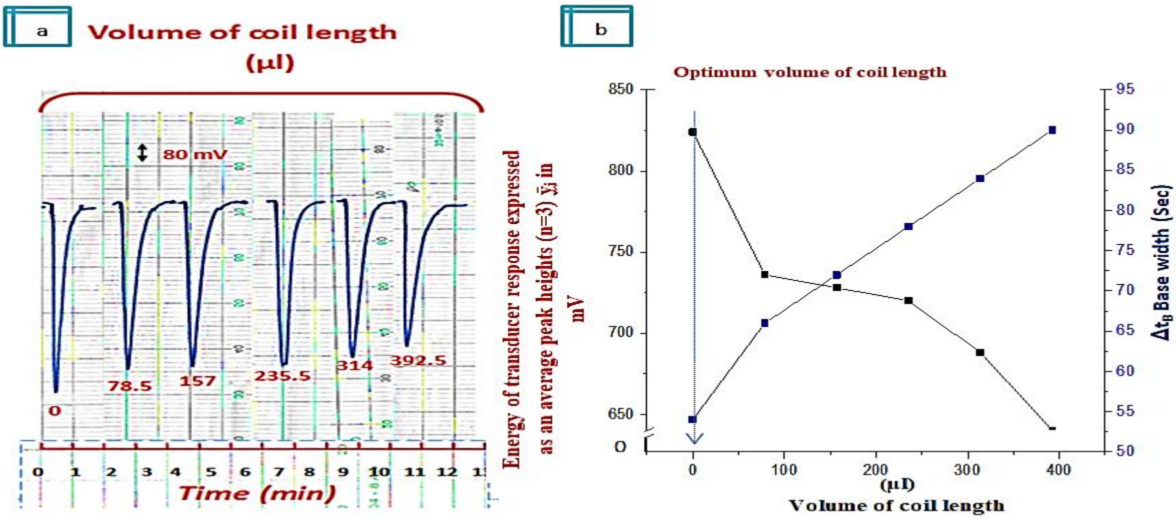


Fig .9: Effect of variation coil length on: a- Response profile versus time.
b- Energy transducer response by absorbance of incident light
using colour species.

-Effect of Intensity of incident light

Variation of light source intensity on the efficiency for determination of vit.B_9 5 mMol.L^{-1} was studied while keeping all other changeable fixed (Ce (IV) sulphate 0.07Mol.L^{-1} as a reagent , H_2SO_4 0.7Mol.L^{-1} as a carrier stream ,251.2 µl sample volume , flow rate 1.8,2 ml.min^{-1} for carrier stream and reagent respectively &purge time 13 sec , the applied voltages to the LEDs were used (1.27-2.10) volt DC, by variation of light intensity reads by AVO- meter. Fig.10a shows the response height.Fig.10b shows that an increase in the peak height with increase intensity of light source So 2.08 volt DC. was selected as the optimum voltage for the snow white light emitted diodes(LEDs)to prolong the efficiency of the instrument.

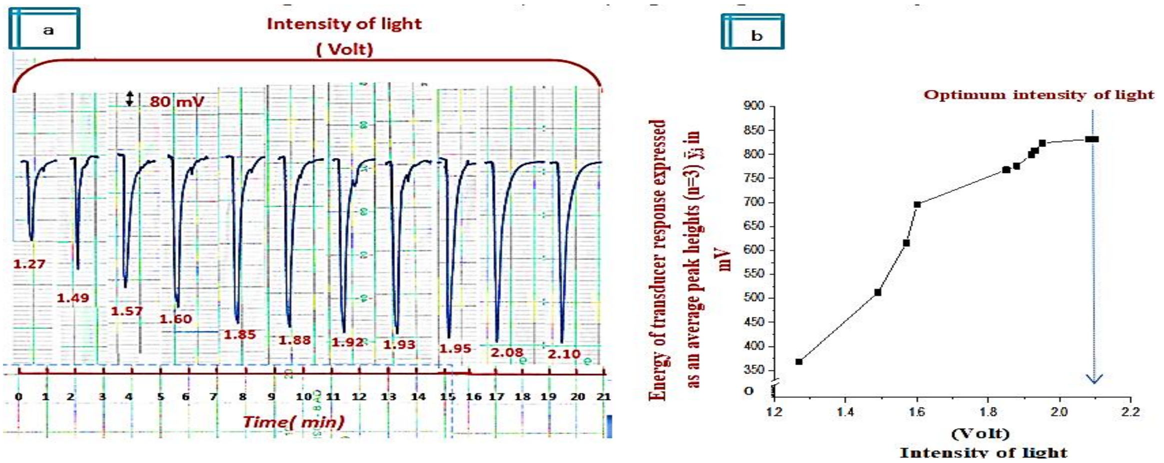


Fig.10: Effect of variation Intensity on : a- Response profile versus time
b- Energy transducer response by attenuation of incident light

- Scatter plot calibration curve for variation of vitamin B_9 versusenergy transducer response

Using the optimum chemical and physical parameters, a series of vit.B_9 solutions ranging 0.1 -8 mMol.L^{-1}were prepared, each measurement was repeated three times successive. Fig.11 a shows response profile & height for each vit. B_9 concentration. A scatter plot diagram shows that a linear calibration graph range for the variation of the energy transducer response of Ayah 6SXI-ST-2D solar cell CFI Analyzer with vit.B_9 concentration was ranging from 0.1 -5 mMol.$L^{-1}$with correlation coefficient (r):0.9953 as shown in Fig.11b. The results obtained were tabulated in Table.3. It was noticed, above 5 mMol.L^{-1} a broad in the peak maxima was observed and increase of the base width (Δt_B) , this cause a deviation of correlation coefficient (deviate from linearity) ; most probablydue to the high intensity of the colored species in front of detector and due to the effect of inner filter of colored species present in solution, which were results from the oxidation of vit.B_9 by Ce(IV)sulfate in acidic medium causing a decrease in transmitted light to the solar cell .

Table .3 Summed up the data of linear regression for the variation of energy transducer response with vitamin B_9 concentration using first degree equation of the form $\hat{y}=a+bx$ at optimum conditions . In addition to calculate t-value at 95% confidence level which larger than tabulated t-value indicating clearing that the linearity against non -linearity is accepted

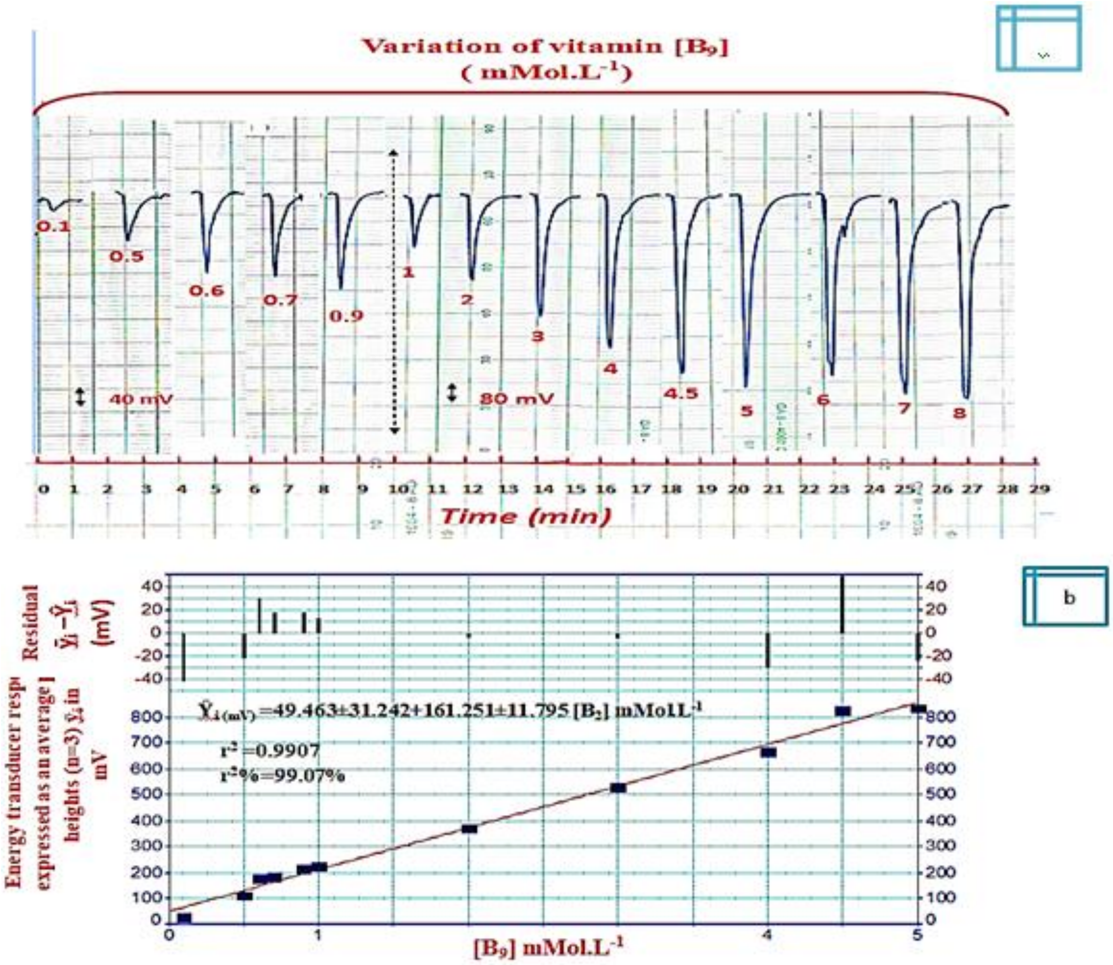


Fig 11: a- Profile of calibration curve for the variation of vitamin B_9 concentration on the responseversus time.

b- Linear calibration graph for the variation of vitamin B_9 concentration on the energy transducer response by reflection of incident light expressed by linear equation usingAyah 6SX1-ST-2D solar cell CFI Analyzer (Developed method), $\bar{y}_i$:practical value,$\hat{Y}_i$:estimated value

Table.3 : Summary of linear regression equation results for the variation of instrument response with vitamin B_9 concentration.

Type of measurement	Measured [B_9] mMol.L^{-1}	n	Range of [B_9] mMol.L^{-1}	$\hat{Y}_{i(mV)}=a\pm s_at+b\pm s_bt$ [B_9]mMol.L^{-1} at confidence interval 95%,n-2	r r^2 r^2%	t_{tab} at 95% ,n-2	Calculated t-value $\frac{\lvert r\rvert\sqrt{n-2}}{\sqrt{1-r^2}}$
Newly Developed methodology	0.1-8	11	0.1-5	49.463±31.242+161.251± 11.795[B_9]mMol.L^{-1}	0.9953 0.9907 99.07%	2.262< 30.922	

Ŷ: Estimated response (mV) for n=3 expressed as an average peaks heights of linear equation of the form Ŷ=a+bx or absorbance value, r: Correlation coefficient, r^2 : Coefficient of determination, r^2%: Linearity percentage,

Developed method: using 6SX1-ST-2D CFIA.

- Limit of detection (LOD)

A study was carried out to calculate the limit of detection of vitamin B_9 through three methods:
Practically: based on the gradual dilution for the minimum concentration .
Theoretical: based on the value of slope and from the linear regression plot. Table .4summarizes the results of vitamin B_9using251.2μl sample volume.

Table .4: Limit of detection for vitamin B_9 at optimum parameter using 251.2μl as an injection sample.

L.O.D. (μg/sample)		
Practically based on the gradual dilution for the minimum concentration of $[B_9]$=0.05mMol.L^{-1}	Theoretical based on the value of slope x=$3S_B$/slope for n=13	Theoretical (linear equation) based on the value of $\hat{Y}=Y_b+3S_b$
5.544	0.618	60.817

X: value of LOD based on slope, S_B: standard deviation of blank repeated for 13 times, Y_b: average response for blank = intercept, S_b: standard deviation equal to $S_{y/x}$(residual).

-Repeatability

The relative standard deviation expressed as percentage which is equally to the repeatability of the measurement. A repeated measurements for eight successive injections were measured at fixed concentrations of vitamin B_9 , while mainly two concentrations were used (0.9 ,5) mMol.L^{-1}. Fig. 12 is shown response profile of repeatability at 0.9 and 5 mMol.L^{-1} respectively the obtained results is tabulated in Table.5which shows that the percentage relative standarddeviation was less than 0.5% **.**

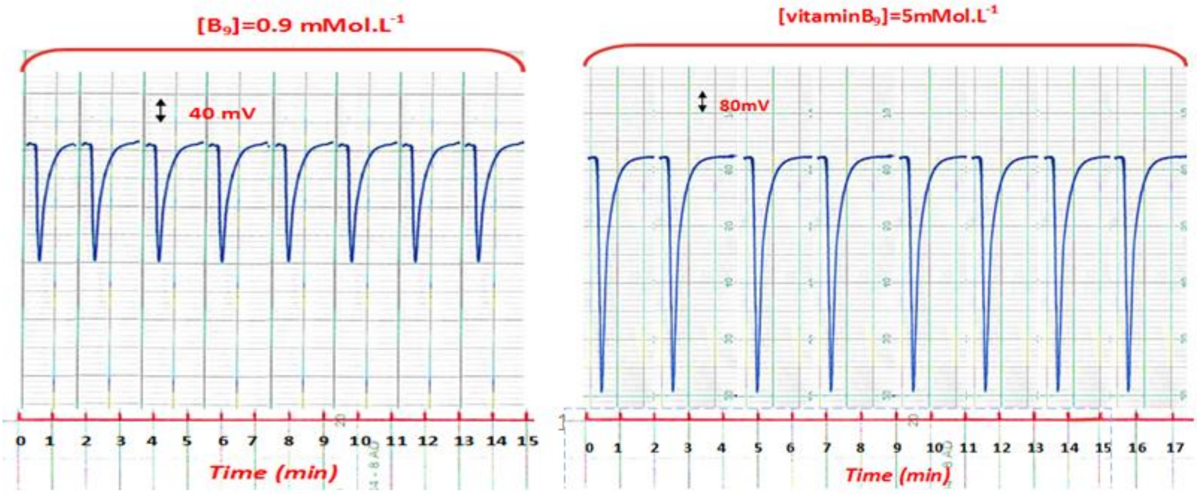


Fig .12: Profile of repeatability response for both vitamin B_9 concentration0.9&5mMol.L^{-1} respectively forn=8.

Table .5: Repeatability of vitamin B_9 at optimum parameters with 215.2μl samplevolume via absorbance of incident light by color species expressedas an energy transducer response for eight successive of injected.

$[B_9]$ mMol. L^{-1}	Energy transducer response expressed as an average peak heights ($\bar{y}_i$) in mV	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2,n-1}\sigma_{n-1}/\sqrt{n}$
0.9	212	0.111	212±0.196
5	832	0.107	832± 0.744

$\bar{y}_i$ Average response (mV), n=number of injection, $t_{0.05/2,7}$ =2.365 .

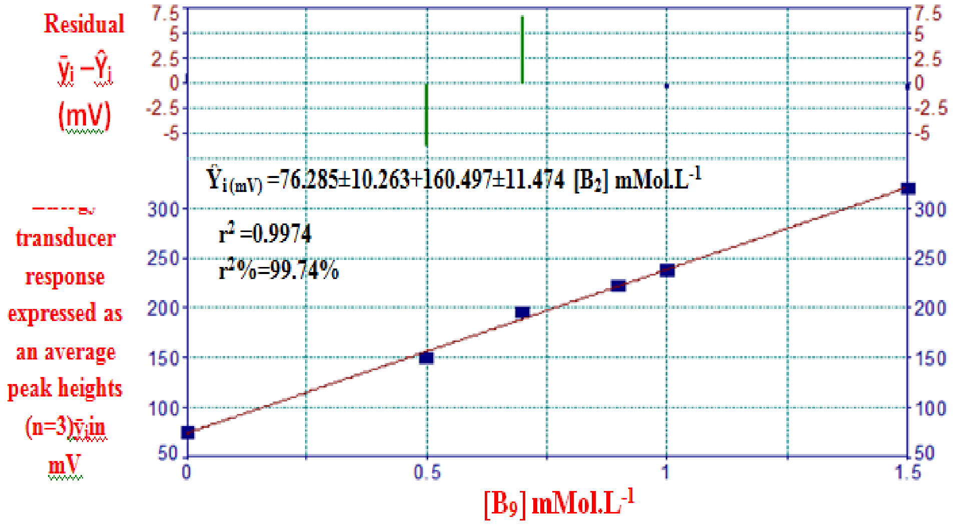
-Analysis of vitamin B_9 and treatment of data

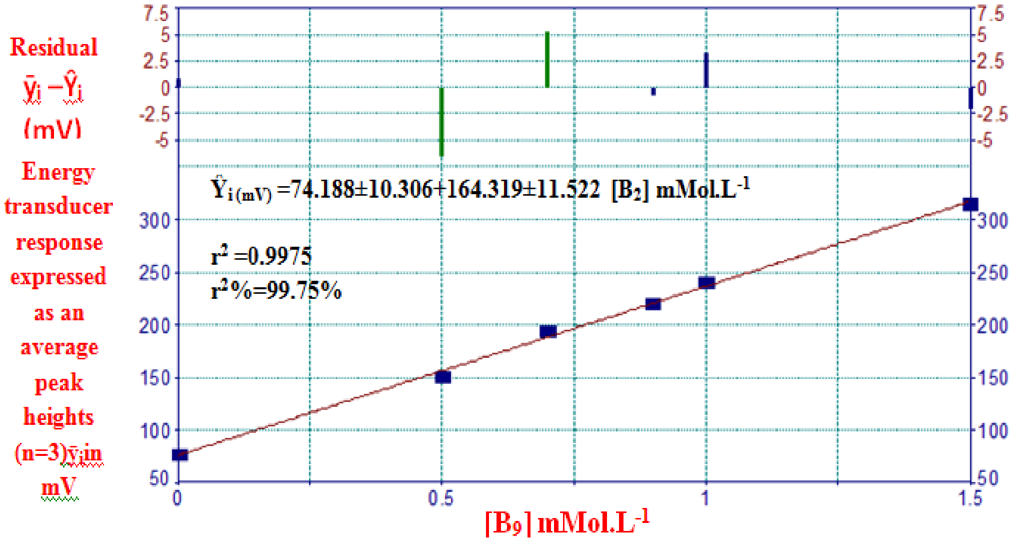
Flow injection analysis using homemade Ayah 6SX1-ST-2D solar cellCFI Analyzer with optimum parameters that were a achieved in previous section for thevit B_9 – Ce(IV) (0.07Mol.L^{-1}) – H_2SO_4 (0.7Mol.L^{-1}) system which form a red solution which used for the application of method to analyze vitamin B_9 in three different pharmaceutical formulations from different origins of suppliers. The Newly developed methodology that measured the absorbance of incident light by coloured species expressed as an energy transducer response was compared with classical method which based on the measurement of absorbance at λ=255nm.

A series of solutions were prepared of each pharmaceutical drug (1mMol.L^{-1}) by transferring 5 ml to each six volumetric flask (10 ml), followed by the addition of gradual volumes of standard B_9 0.01Mol.L^{-1} (0, 0.5, 0.7, 0.9 ,1.0 ,1.5) ml to obtain (0, 0.5, 0.7, 0.9, 1.0, 1.5) mMol.L^{-1} for proposed method. Flask no.1 is the sample and the same data for classical method. Results were mathematically treated for the standard addition method. (Table.6a). Fig.13a,b&c shows a calibration curve for three samples for Newly developed methodology using Ayah 6SX1-ST-2D solar cell CFI Analyzer.

Paired t-test [22,23,24] was carried out in two approaches as shown in table .6b. The first includes; individual t-test was made between the mean of weight ($\overline{w}_i$) from Newly developed method with quoted value of officially British pharmacopeia [25].

It was found that there is no significant different between two values. Second: paired t-test was used in order to compare a Newly developed method with the classical spectrophotometric method (at λ=255nm). The obtained results are shown in table 6b, indicating clearly that there was no significant differences between two methods, Since the calculated t-value was less than critical tabulated t-value





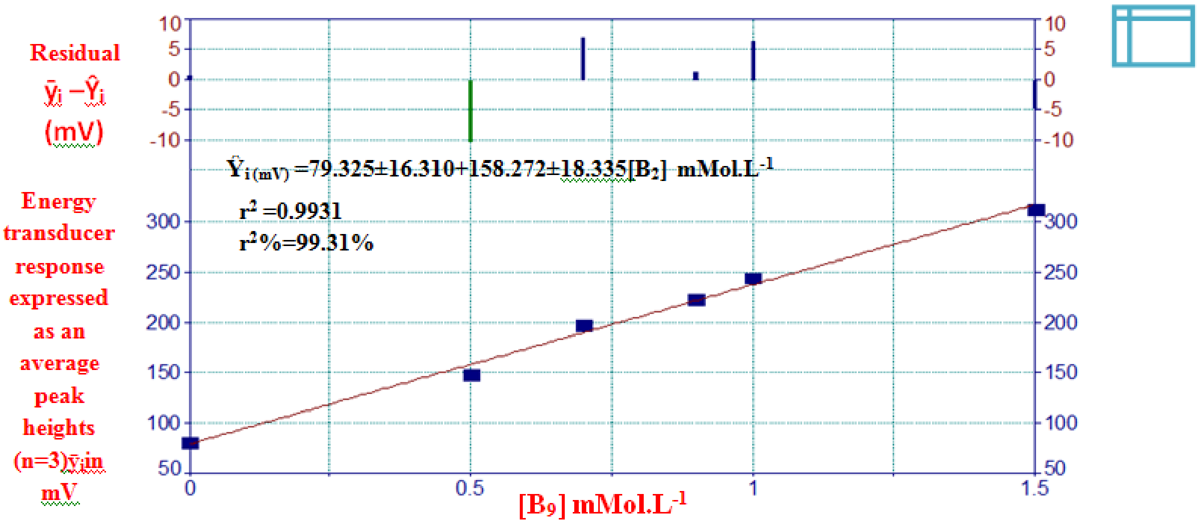


Fig .13: Standard addition curve using Newly developed methodology for the determination of vit.B_9– Ce(IV)– H_2SO_4 system for three samples :

- **a : Actavis – UK**
- **b: Julphar – U.A.E.**
- **c: Samarra- Iraq .**

$\bar{y}_i$: practical value, $\hat{Y}_i$: estimated value, Residual=$\bar{y}_i$-$\hat{Y}_i$in mV.

Table.6a: Standard addition results for the determination of B_9 in three pharmaceutical preparation using vit.B_9– Ce(IV)– H_2SO_4 system.

	Commercial name , Company Content Country	Type of method Developed method using Ayah 6Sx1-ST-2D Solar cell CFIA (mV) Uv.Sp Classical method Absorbance measurement at λ_{max}=255 nm											
		Confidence interval for the average Weight of Tablet $\bar{W} \pm 1.96\sigma_{n-1}/\sqrt{n}$ at 95% (g)	Weight of sample equivalent to 0.0441gm (1 mMol.L^{-1}) of the active ingredient	Theoretical content for the active ingredient at 95% (mg)	Vitamin B_9mMol.L^{-1}						Equation of standard addition at 95% for n-2 $\hat{Y}_{i(mV)}=a\pm s_a t+b\pm s_b t[B_9]_{mMol.L^{-1}}$ $\hat{Y}_i = a\pm s_a t+b\pm s_b t[B_9]_{mMol.L^{-1}}$	r r² r²%	Practical concentr-ation mMol.L^{-1} In 10 ml Practical concentr-ation mMol.L^{-1} in 100ml
					0	0.5ml	0.7ml	0.9ml	1 ml	1.5 ml			
					0	0.5	0.7	0.9	1	1.5			
					0	0.02ml	0.05ml	0.07ml	0.1ml				
					0	0.02	0.05	0.07	0.1				
1	Folic Acid actavis 5mg UK	0.0620±0.0004	0.5476	5 ± 0.0323	77	150	194	220	240	315	$76.285\pm10.263+160.497\pm11.474\,[B_9]$ mMol.L^{-1}	0.9987 0.9974 99.74 %	0.4753 0.9506
					0.512	0.915	1.240	1.460	1.739		$0.601\pm0.191 + 11.916\pm 3.196\,[B_9]$mMol.L^{-1}	0.9895 0.9791 97.91%	0.0504 1.0091
2	Folic Acid julphar 5mg U.A.E	0.1151±0.0009	1.0164	5 ± 0.0391	75	150	196	222	238	320	$74.188\pm10.306+164.319\pm11.522\,[B_9]$ mMol.L^{-1}	0.9987 0.9975 99.75%	0.4515 0.9030
					0.522	0.915	1.221	1.426	1.769		$0.596\pm0.159+11.963\pm 2.659\,[B_9]$mMol.L^{-1}	0.9928 0.9856 98.56%	0.0499 0.9971
3	Folic Acid Samarra 1mg Iraq	0.0752±0.0006	3.3199	1 ± 0.0079	80	148	197	223	244	312	$79.325\pm16.310+158.272\pm18.335[B_9]$ mMol.L^{-1}	0.9965 0.9931 99.31%	0.5012 1.0024
					0.413	0.822	1.076	1.300	1.502		$0.518\pm0.229+10.512\pm3.845[B_9]$mMol.L^{-1}	0.9807 0.9618 96.18%	0.0493 0.9855

* draw 5ml for new developed method and 0.5 ml for classical method from (1 mMol.L^{-1}-sample), $\hat{Y}$:Estimated response in mV for developed method and absorbance for Uv-sp method, r:correlation coefficient, r^{2}:coefficient of determination ,r^{2}% :linearity percentage, Uv –Sp : Uv–spectrophotometric mehod,$t_{0.025,\infty} = 1.96$ at 95% ,t_{tab}=$t_{0..025,n-2}$ = 3.182 for n=5 , $\bar{W}_i$:Mean of weight for n=20.

Table. 6b: Summary of results for practical content, efficiency for determination of vit.B$_9$ in three samples of pharmaceutical preparation and paired t –test

	Type of method Developed method using Ayah 6Sx1 –ST-2D solar cell CFIA (mV)					
	Uv.sp classical method Absorbance measurement at λ_{max}=255nm					
	Practical concentration mMol.L^{-1} in 10 ml Practical concentration mMol.L^{-1} in 100 ml Weight of B$_9$ in(g)	Weight of B$_9$ in tablet $\bar{w}_{i(g)} \pm 4.303\sigma_{n-1}/\sqrt{n}$ Weight of B$_9$ in tablet $\bar{w}_{i(mg)} \pm 4.303\sigma_{n-1}/\sqrt{n}$	Efficiency of determination Rec.%	Individual t-test for compared between quoted value& practical value $(\bar{w}_i - \mu)\sqrt{n}/\sigma_{n-1}$	Paired t –test Compared between two method	
					t_{cal}= $\bar{X}d\sqrt{n}/\sigma_{n-1}$	t_{tab} at 95% confidence level(n-1)
1	0.475 0.951 0.042	0.042±0.007 4.753±0.711	95.062%	/-1.495/< 4.303	Xd= -0.2477 σ_{n-1}= 0.2453 / -1.749 /<4.303	
	0.050 1.009 0.045	0.045±0.004 5.043±0.462				
2	0.452 0.903 0.040	0.040±0.008 4.515 ±0.824	90.298%	/-2.533/< 4.303		
	0.050 0.997 0.044	0.044±0.005 4.984±0.532				
3	0.501 1.002 0.044	0.044±0.028 1.002±0.642	100.238%	0.013< 4.303		
	0.049 0.986 0.044	0.044±0.028 0.986±0.624				

μ: quated value (mg) ,$\bar{w}_i$:practical content(mg) ,$\bar{x}$d : average of difference between two type of method(developed&classical),t_{tab} =$t_{0.05/2,n-1}$=4.303 for n(No.of samples)=3.

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