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CFIA-Turbidimetric 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, sensitive and simple method was used for the determination of: vitamin B_9 (Folic acid) in pure and pharmaceutical formulations using continuous flow injection analysis. The method is based on formation of ion pair compound between folic acid and ammonium molybdate in an aqueous medium to obtain a gray precipitate complex, using homemade; Ayah-6SX1-ST-2D solar cell CFI Analyzer. Optimum parameters was studied to increase the sensitivity for developed method. The linear range for the calibration graph was 0.01-0.6 $mMol.L^{-1}$ of vitamin B_9 and LOD was 131.994 ng/sample with correlation coefficient (r) of 0.9810, RSD% was lower than 0.1%, (n=9) for the determination of vitamin B_9 at concentration (0.07and 0.5) $mMol.L^{-1}$ respectively. The developed method was applied successfully for the determination of vitamin B_9 in pharmaceutical tablets. A comparison was made between two methods: developed method and the classical UV spectrophotometric method at λ_{max}=255 nm, by using the standard addition method via the use of paired t-test. It showed that there was no significant difference between the developed method and the classical method for determination vitamin B_9 at 95% confidence level.

Keywords: Vitamin B_9, flow injection analysis, turbidity, homemade instrument.

Introduction:

Folate, forms of which are known as folic acid and vitamin B_9, is one of the B vitamins[1]. It 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 (FA) also known as vitamin M. Pale orange-yellow crystals or flakes. About 250°C 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, 1gm of 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 pteridine aminobenzoyl 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 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

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single molecule of L-glutamic acid as shown in Figure .1[5].

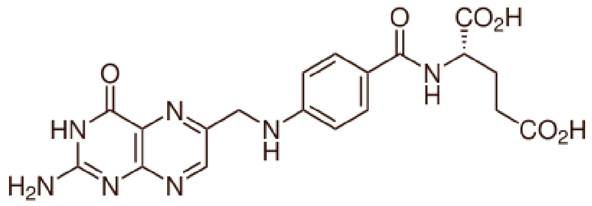


Figure 1. The structure formula of folic acid.

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}

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 turbidimetric method ,the turbidity is measured via reflection of incident light from the surfaces of particles formed (ion pair complex of vitamin B_9-ammonium molybdate (Am) system) at 0-180° 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].

Experimental:

Reagents and chemicals

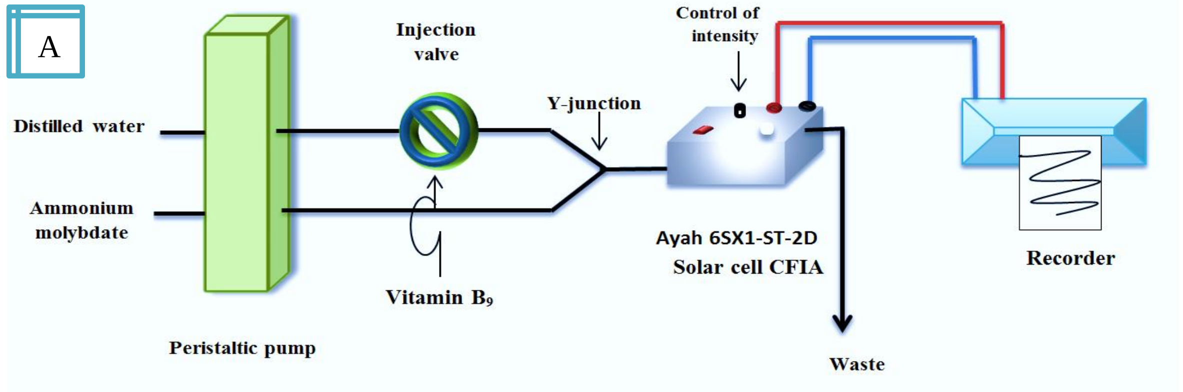
Every chemicals were used of analytical-reagent grade and all the solutions dissolved by distilled water. A standard solution 0.01Mol.L^{-1} of Vitamin B_9 $C_{19}H_{19}N_7O_6$, molar mass 441.42g.mol^{-1} Hopkins& Williams was prepared by dissolving 1.10355g folic acid in 10ml of 1Mol.L^{-1} Na_2CO_3 and complete the volume to 250ml with distilled water. A stock solution (0.01 Mol.L^{-1}) of ammonium molybdate$(NH_4)_6Mo_7O_{24}.4H_2O$, molar mass1235.58 g.mol^{-1} CABLO EBBA- England was prepared by dissolving 6.178 g in 500 ml of distilled water.

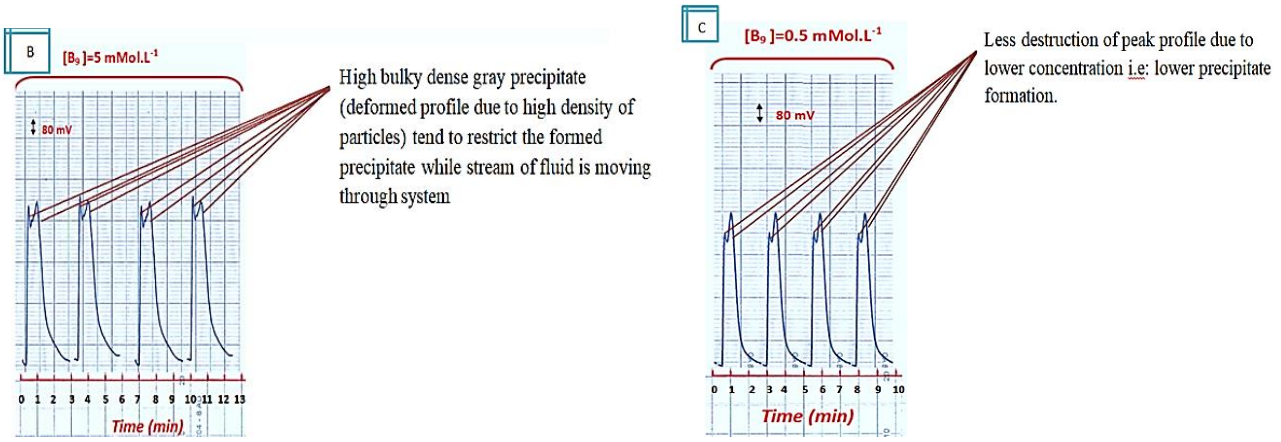
Sample Preparation

Twenty tablets were weighted then crushed and grinded .Tablets containing (5,5,1) mg of vitamin B_9 were weighted 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

The manifold flow system which is used for the determination of vitamin B_9 consisting of two lines which were used to conduct this work as shown in Fig. 2A.The determination of vitamin B_9 was carried out by the reaction between folic acid and ammonium molybdate (Am) 0.7mMol.L^{-1} in aqueous medium to form a gray color precipitate as an ion pair complex form. The first line represents the carrier stream (distilled water) at 1.4 ml.min^{-1} flow rate which leads to the injection valve to carry a sample volume (110µl of vit.B_9 uses open valve mode, while the second line supplies Ammonium molybdate solution at 1.5 ml .min^{-1} .Both lines met at a Y-junction ,with an outlet for reactants product from complex, which passes through a homemade Ayah 6SX1-ST-2D solar cell CFI Analyzer, applied voltage to the LEDs source (six snow white light emitting diodes) was 2.08 volt DC. The response profile of each was recorded on x-t potentiometric recorder to measure energy transducer response expressed as peak heights in mV that is shown in Fig.2 B, C using (0.5 and 5 mMol.L^{-1}) concentration of vit.B_9. A proposed reaction in alkaline medium between vit. B_9 and Am is shown in Scheme.1 [18,19]

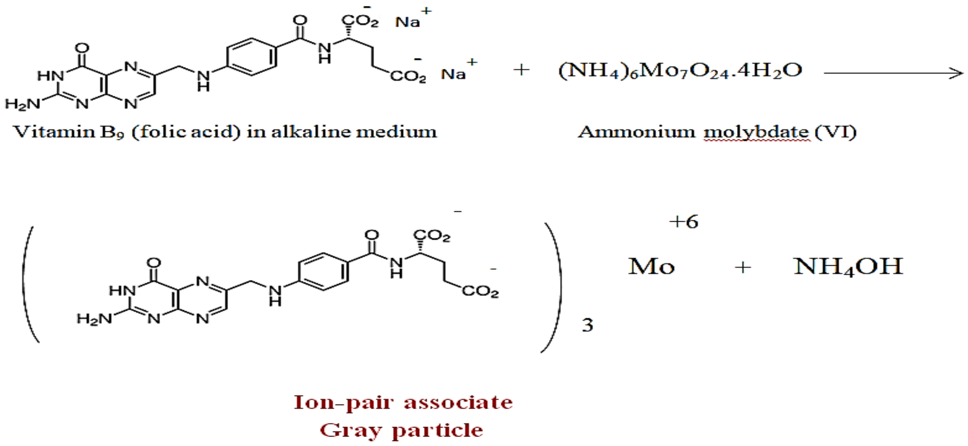




High bulky dense gray precipitate (deformed profile due to high density of particles) tend to restrict the formed precipitate while stream of fluid is moving through system

Less destruction of peak profile due to lower concentration i.e: lower precipitate formation.

Figure 2. A- Flow diagram manifold system used for determination of vitamin B_9 using homemade Ayah 6SX1-ST-2D CFIA, Ammonium molybdate 0.7mMol.L^{-1}.
B- Response profile for vit. B_9 (5mMol.L^{-1}), C-Response profile for vit. B_9 (0.5mMol.L^{-1})



Scheme 1. Probable ion –pair complex product species for proposed reaction of turbidity system between vitamin B_9 and Am in basic medium.

Response profile as explained in Fig .2B and C shows that there is a gradient in the distribution of precipitated particles within precipitate plug, and this in turn leads to formation of three regions.

The first region (head region) : represents a region of dispersed light due to the presence of inter particles spaces that gives the allowance for light reflection ,refraction, dispersion and also a diffused light that will cause an increased signal profile as shown in part - A (Fig.3).

The second region (center region): A dense bulky plug of precipitated particles that holdup the incoming incident light, which is a drop in signals. This is represented as shown in Fig .3- part B, the yellow arrows.

The third region (Tail region): An elongated dispersed region that precipitated particles are far away from each other causing with the effect of carrier stream an increase in the incident light i.e: in the peak height. These are represented in blue arrow as shown in Fig .3-part C.

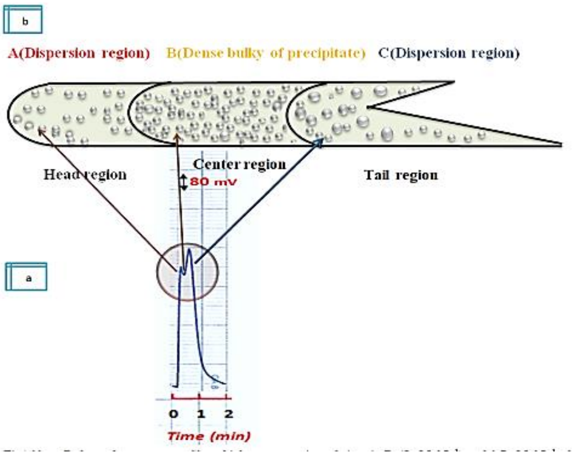


Figure 3. a- Deformed response profile at concentration of vitamin B_9 (0.5mMol.L^{-1}) and .7mMol.L^{-1} of Ammonium molybdate (Am). b- Schematic representation for the distribution of precipitate particles as it is pass through flow cell.

Study of the optimum parameters for determination of vitamin B_9 Chemical parameters Effect of variable concentration of Ammonium molybdate [Am]

A series of the precipitating reagent [Am]solutions (0.005 -0.7) mMol.L^{-1} were prepared at constant concentration of vitamin B_9 0.5mMol.L^{-1}, 110 µl sample volume at (1.4&1.5)ml.min^{-1}flow rate for carrier stream and reagent respectively .The intensity of incident light of LEDs 2.08 V was used .Fig .4A shows the increase in concentration of Am leads to increase in the shoulder of response up to 0.1 mMol. L^{-1}and became wider. At 0.1mMol.L^{-1} of Am concentration steady movement of sample segment in steady equilibrium with no destruction of peak profile. While more than 0.1 mMol. L^{-1} might be due to resistance of sample segment to movement due to bulky and density of formed precipitate .So, at high concentration (> 0.1 mMol. L^{-1}) does not really reflect actual structural deformation; therefore, it is not a reliable to choose from, as shown in Fig .4B .A 0.1 mMol. L^{-1} Am concentration was chosen as the optimum concentration that is used for further experiments.

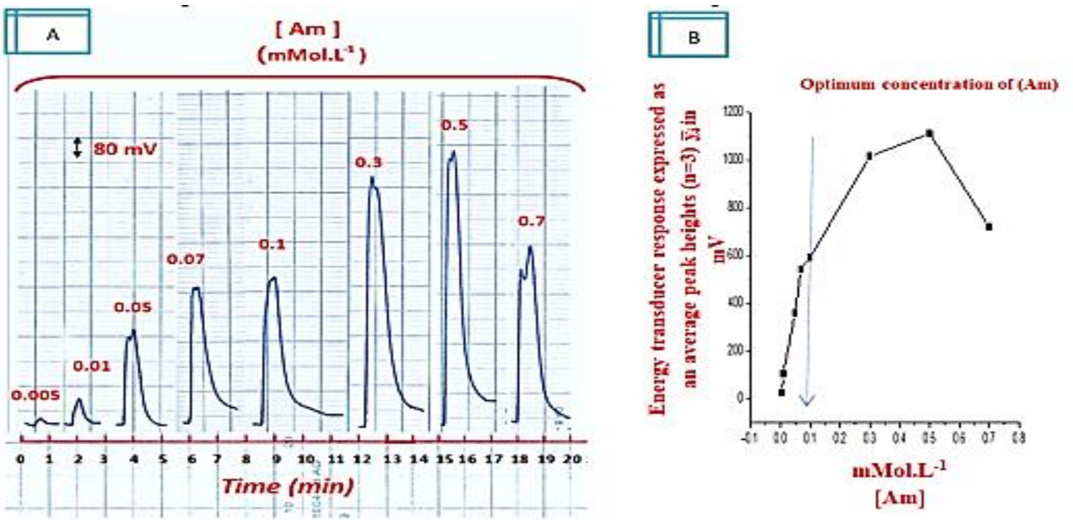


Figure 4. A: Response profile at variable concentrations of Am.
B: Influence of [Am] on peak height.

- Physical parameters

- Effect of variable flow rate

Variation of the flow rates (0.2- 2.8) & (0.4- 3) ml.min^{-1} for carrier stream and reagent respectively, controlled by the peristaltic pump for determination of vitamin B_9 at 0.5 mMol.L^{-1} concentration was studied . While keeping all other variables constant (i.e. 110µl sample volume, 0.1mMol.L^{-1}(Am) concentration and intensity of light is 2.08 V). Fig .5A, shows the obtained response, it was noticed that at low flow rate (< 2.5ml.min^{-1}flow rate for carrier stream) increased resistance due to bulky weight of precipitate formed while at higher flow rate > 2.5 ml.min^{-1}, overcome the steady lessness sample segment movement that causes from dense precipitate which in turn to cause agglomerate of precipitate particulate.Therefore, the best flow rate was 2.5 and 2.7ml.min^{-1} (Fig.5B). The results obtained were summarized in Table 1.

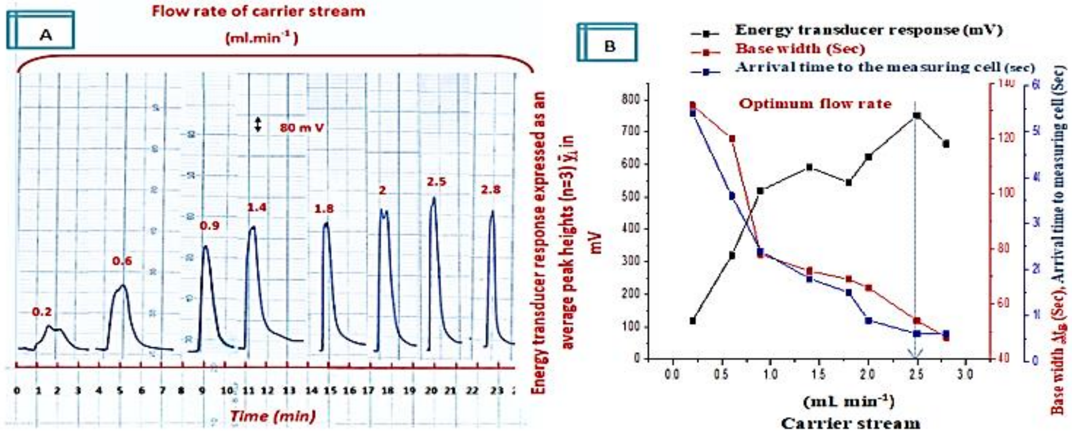


Figure 5. Effect of flow rate on: A- Response profile vs. time.
B - Energy transducer response.

Table 1. Influence of flow rate on the measurement of energy transducer response using 110µl sample volume

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	$t_B\Delta$ Sec	V_{add} ml	Concentration M Mol.L^{-1} at flow cell
	F$_1$	**F$_2$**							
5	0.2	0.4	120	0.575	120 ± 1.714	54	132	1.43	0.0385
10	0.6	0.7	320	0.259	320 ± 2.062	36	120	2.71	0.0203
15	0.9	1.0	520	0.162	520 ± 2.087	24	78	2.58	0.0213
20	1.4	1.5	592	0.157	592 ± 2.310	18	72	3.59	0.0153
25	1.8	2.0	596	0.182	596 ± 2.459	15	69	4.48	0.0123
30	2.0	2.2	624	0.165	624 ± 2.559	9	66	4.73	0.0116
35	**2.5**	**2.7**	**752**	**0.145**	**752 ± 2.708**	**6**	**54**	**4.79**	**0.0115**
40	2.8	3.0	664	0.161	664 ± 2.658	6	48	4.75	0.0116

t: Arrival time from injection valve reaching to measuring cell (Sec), Δt_B :Base width of peak (Sec), $t_{0.05/2,\ 2}$ = 4.303, vs.: versus, F$_1$: Flow rate of carrier stream (distilled water), F$_2$: Flow rate of Reagent(Am).

Variation of Sample loop volume and its effect on response sensitivity and response profile with the sake for decrease signal –Noise(S/N) ratio.

Using the manifold system is shown in Fig.6A, a series of measurements were carried out to establish the most suitable optimum sample volume that will be used to conduct the rest of the research work concerning the determination of vitamin B$_9$ (folic acid). A solution of 0.5mMol.L^{-1} folic acid of variable volume extended from 60-up to 310µl. Other variables were kept at their optimum. It was noticed that at larger sample volume broadening was the apparent features (Fig.6A). Therefore, a small size volume was the adopted volume to be used throughout this work. The causes of broadening might be attributed to the reverse effect of precipitate weight that the carrier streams tend to push it through the manifold unit. While less difficulty occurred when a small volume is used. (Fig.6B) shows all the practically obtained response. So, 60µl of sample volume is the best.

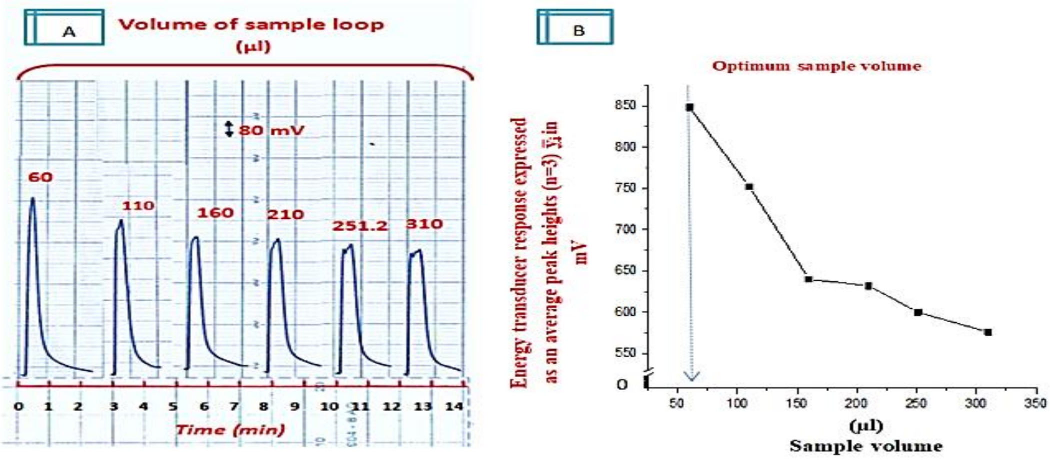


Figure 6. Effect of sample volume on: A- Response profile vs. time. B- Energy transducer response.

-Effect of variable coil length

Variable coil length 0-50 cm were studied. These length comprises a volume (0 – 392.5) µl which connected after Y-junction directly in flow system. While keeping all other changeable constant: vitamin B$_9$ 0.5mMol.L^{-1}, (Am) concentration 0.1 mMol.L^{-1} , flow rate 2.5 & 2.7 ml.min^{-1} for carrier stream (distilled water) and reagent (Am) respectively, sample volume 60µl and applied voltage of LEDs was 2.08 volt DC. Fig. 7A shows the increase of coil volume which leads to decrease of peak height with increase of base width Δt_B (which is measured by taking the tangential for base of peak). This might be attributed to diffusion and dispersion of precipitate particulate due to increase of dispersion regions and mostly lead to accumulation of precipitate particles causing loss of some of the reflecting surface. So, it can be seen clearly that no reaction coil was selected for further work, Fig. 7B shows the best choice.

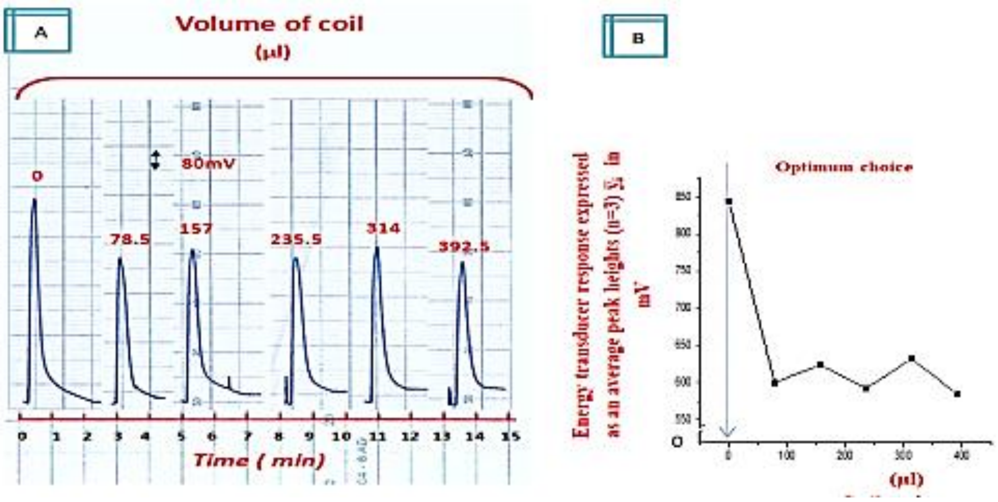


Figure 7. Variation of coil length on: A- Response profile vs. time
B- Energy transducer response by reflection, diffused and dispersed of incident light

-Effect of purge time

A study was carried out to determine the optimum duration of the injection time, i.e. allowed permissible time for purging of the sample segment from the injection valve. Variable purge time extended from 3-up to 15 sec and open valve mode were used in this study. The optimum physical and chemical parameters achieved in previous section were kept constants. Fig .8A shows the continuation of the increase of the height of response with increase of purge time up to 10sec, after that there was no longer significant difference in peak height; therefore, 10sec as a purge time was chosen as an optimum to completely purge of sample segment from sample loop (Fig.8B).

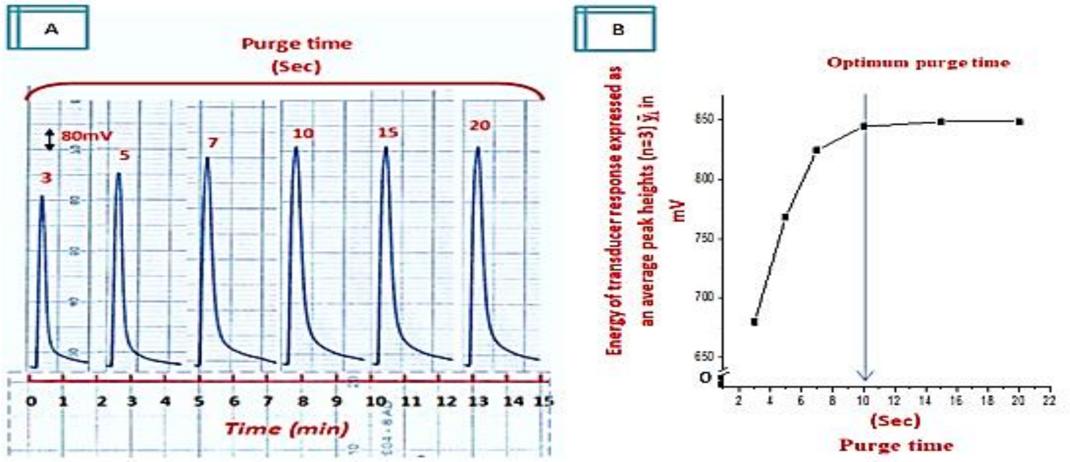


Figure 8. Effect of the purge time on: A- Response profile vs. time.
B- Energy transducer response

-Intensity of incident light on the signal-Noise (S/N) ratio and the efficiency of peak profile responses and reliability.

Fixing all previous experimental parameters leading to monitor the new methodology approach for the determination of vitamin B_9. Another parameter which quite important in dealing with extension or restriction of calibration graph with a linear plot representing the simple straight line equation y= a+bx that was born from initial scatter plot. Lambert –beer law uses a fixed intensity and representing -Log I□ /I as the unitless absorbance. While have intensity of incident light can be varied according to the course of the nature of the formed precipitate. (i.e; more intense incident light is required to determination highly populated dense precipitate; while less intensity is required for a more transparent or less dense of precipitate). Therefore, a compromise should be made between extension of linear range of calibration graph and the capability of maneuverability according to the nature of agglomerate precipitated grains that will form a dense highly populated precipitate. Reliability of measurements and its repetition will be a major concern.

To a certain upper limits of applied Dc-voltage to the light source it was used starting from a weak intensity 0.62 VDC up to just about a bright light source reaching 2.13 VDC. Care was considered not to use an over voltage that might shorten the life time of the light sources. Figure no. 9A shows the kind of responses obtained. It is noise free while Fig 9B shows the variation of responses vs. applied VDC; it is kind of growth curve. 2.10 VDC was the choice.

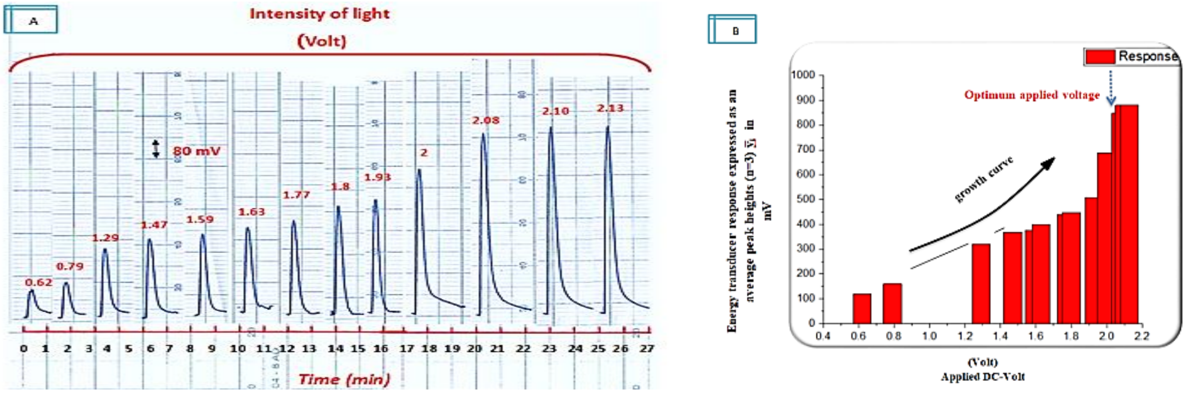


**Figure 9. Effect of variation intensity of light on: A- Response profile vs. time.
B- Energy transducer response by reflection of incident light Scatter plot for determination of vitamin B_9**

-Construction of calibration graph obeying linear response for a given range of concentration of vitamin B_9 from a scatter plot.

A study was carried out to establish a calibration graph that is linearity relating responses obtained using Ayah 6SX1 -ST - 2D- Solar cell CFI Analyzer versus concentration of vitamin B_9.

A series of solutions ranging from 0.005-up to 5 mMol.L^{-1} were prepared; and all measurements were conducted while optimum parameters that were established previously were fixed at their values. Scatter profiles of various concentrations were shown in Fig .10A. A clear linear response having a correlation coefficient of 0.9810 with a coefficient of determination of 0.9623 with r^2 % (squared-R) =96.23% ; i.e; the linear range from 0.01-0.6 mMol.L^{-1} can be explained with 96.23 % (Fig.10B) by the chosen linear equation of the form of:

Response = intercept + slope [B_9] mMol.L^{-1}. Summary of results using Ayah 6SX1 -ST - 2D- Solar cell CFI Analyzer tabulated in Table 2. Limit of detection for vitamin B_9 calculated by three different methods. Table 2 tabulated all the practically and theoretically value of detection limit. Repeatability of the measurements were studies at fixed concentration of vitamin B_9 (0.07 and 0.5) mMol.L^{-1}and the obtained results were summed up in Table 2 with RSD% less than 0.1%. Fig. 11Aand B shows the profile versus time, which repeated for nine successive injections.

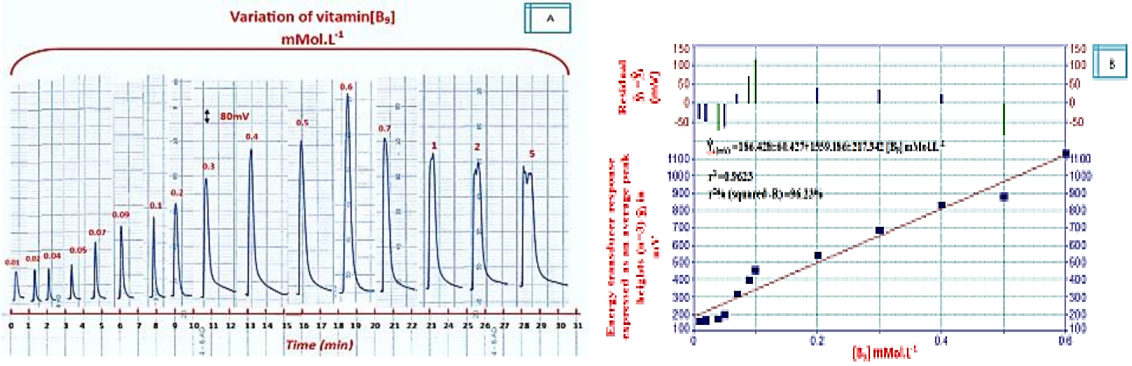


Figure 10. A: Profile for the variation of vitamin B_9 concentration versus time using vitamin B_9- Am system and 60µl., B: Calibration carve for the variation of vitamin B_9 concentration on the energy transducer response expressed by linear equation using Ayah 6SX1- ST-2D solar cell CFI Analyzer New Developed method).

Table 2. Summary of linear regression for the variation of vitamin B_9 concentration using simple straight line equation with Ayah 6SX1 -ST - 2D- Solar cell CFI Analyzer limit of detection using three different methods and repeatability for nine successive injections, 60 µl sample volume loop.

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 $\frac{/r/\sqrt{n-2}}{\sqrt{1-r^2}}$
0.005-5	12	0.01 - 0.6	$\hat{Y}_i$=186.428±60.427+1559.186 ±217.342$[B_9]mMol.L^{-1}$	0.9810 0.9623 96.23%	2.228< 15.977	

Limit of detection

Practically based on the gradual dilution for the minimum concentration(0.005)$mMol.L^{-1}$	Theoretical (slope method)based on the value of slope $X=3S_B/slope$	Theoretically (linear equation)based on the value of $\hat{Y}=Y_b+3S_b$
131.994 ng/sample	15.290 ng/sample	3.355µg/sample

Repeatability

$[B_9]$ m Mol. L^{-1}	Energy transducer response expressed as an average peak heights $\bar{y}_i$ in mV	RSD%	Reliability (two tailed) at 95% $\bar{y}_i \pm t_{0.05/2,n-1}\sigma_{n-1}/\sqrt{n}$
0.07	320	0.078	320 ± 0.060
0.5	876	0.095	876 ± 0.638

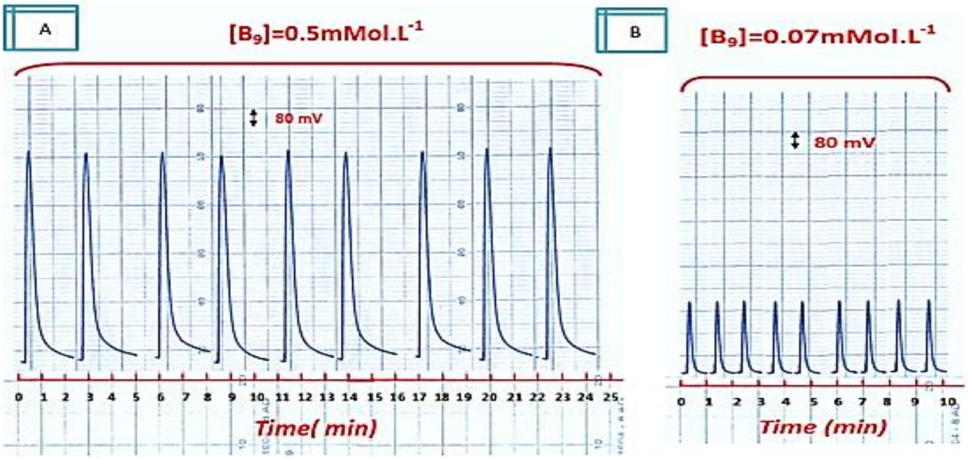


Figure 11. Response profile of repeatability of vitamin B_9 in different concentration: A: 0.5mMol.L^{-1} B: 0.07mMol.L^{-1}

-Study of the absorption spectrum of vitamin B_9 and the preferentiation of selected band and its usage

An ultra violet scanning of vitamin B_9 was carried out (0.04mMol.L^{-1}). It was noticed that a three peaks absorbance at three different wavelengths (255nm (2.334), 286nm (2.301) and 363nm (0.812)). The full spectrum is shown in Fig .12. On the above basis a calibration graph was constructed at all three different wavelengths as shown in Table no.3.in which two concentrations were not detected at 363nm(mainly 0.005 and 0.007mMol.L^{-1}). They gave no reading (i.e., zero absorbance).

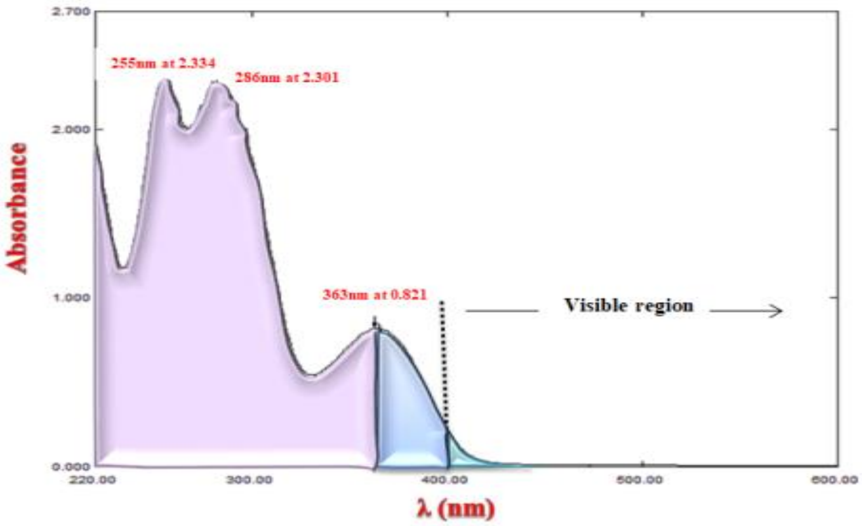


Figure12. Absorbance spectra of the vitamin B_9 at 0.04mMol.L^{-1} concentration vs. distilled water as a blank.

Table 3. Summary of linear regression equations for determination of vitamin B_9 using classical spectrophotometry.

Type of wavelength (nm) (Range, mMol.L^{-1})	[vit.B9] mMol.L^{-1}												$\hat{Y}_i=a\pm S_a t+b\pm S_b t\,[B_9]$mMol.L^{-1} at 95 % confidence level, n-2	r r^2 r^2%
	0.005	0.007	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1		
	Absorbance($\bar{y}_i$)													
255 (0.005-0.1)	0.09	0.12	0.21	0.39	0.58	0.81	1.00	1.21	1.42	1.58	1.82	1.98	-0.006±0.019+ 20.081±0.331[B_9]mMol.L^{-1}	0.9997 0.9994 99.94 %
286 (0.005-0.1)	0.10	0.19	0.25	0.49	0.62	0.79	1.05	1.25	1.52	1.65	1.82	1.93	0.051±0.057+ 19.664±1.001[B_9]mMol.L^{-1}	0.9974 0.9948 99.48 %
363 (0.01-0.1)	0	0	0.10	0.14	0.32	0.49	0.69	0.89	1.02	1.46	1.52	1.66	-0.162±0.094+ 18.262±1.655[B_9]mMol.L^{-1}	0.9918 0.9837 98.37 %

A comparative study will be conducted to establish the most suitable wavelength that will be based upon to compare the achieved methodology with the most sensitive most reliable wavelength to be compared with. As different researcher [7, 9] used different wavelengths.

Two methods of comparison will be carried out that will depends on the results tabulated in Table 3.

a- Paired t-test [20] between all three wavelengths. Table no.4 tabulated all results.

The Null hypothesis H_o: $\mu_{\lambda 255nm} = \mu_{\lambda 286nm} = \mu_{\lambda 363nm}$ i.e.: There is no significant difference between the mean of the absorbance at three wavelengths.

The Alternative hypothesis H_1: $\mu_{\lambda 255nm} \neq \mu_{\lambda 286nm} \neq \mu_{\lambda 363nm}$

Using SPSS version 20 gave the summary of data that is tabulated in Table 4, taking two tailed and three levels of confidence i.e., α =0.1, 0.05 and 0.001.

Table 4. Paired t-test results for comparison between three wavelengths at 90%, 95% & 99.9% confidence level.

Type of paired (mean)	Correlation coefficient	Paired Differences						t_{cal}	Sig*
		Mean	Std. Deviation	Std. Error Mean	Confidence interval at 90% t_{tab}=1.796 Lower-upper	Confidence interval at 95% t_{tab}=2.201 Lower-upper	Confidence interval at 99.90% t_{tab}=4.437 Lower-upper		
1-2 0.9344-0.9717	0.998	-0.0373	0.0461	0.01331	-0.0612 - -0.0133	-0.0666 - -0.0079	-0.0963 - 0.0218	/-2.798/	0.017
1-3 0.9344-0.6929	0.991	0.2415	0.1036	0.02992	0.1878 - 0.2952	0.1757 - 0.3073	0.109 - 0.3742	8.073	0.00
2-3 0.9717-0.6929	0.988	0.2788	0.1089	0.03145	0.2223 - 0.3352	0.2095 - 0.3479	0.139 - 0.418	8.864	0.00

No. of measurements= 12 , Std: standard *: All have
1: The results of Absorbance at λ= 255nm df= degree of freedom=11 significant
2: The results of Absorbance at λ= 286nm difference
3: The results of Absorbance at λ= 363nm

The comparison shows a good correlation coefficient between the three wavelengths. While t_{cal} when compared with t_{tab} it shows that there is a significant difference at three probabilities level (α=0.1, 0.05and 0.001) or at 90,95 and 99.9% confidence level.

b- The second method was to choose a new approach of plotting the obtained result and compared according to their slope. Table no.5 tabulates all the obtained results while Figure no.13 shows the plot of responses versus response of another wavelength. The figures indicate that a slope biased to any axis is the optimum.

All results are in favour of λ at 255nm.

On the above arrived decision the absorbance at λ= 255nm will be our aim for comparison With New developed method.

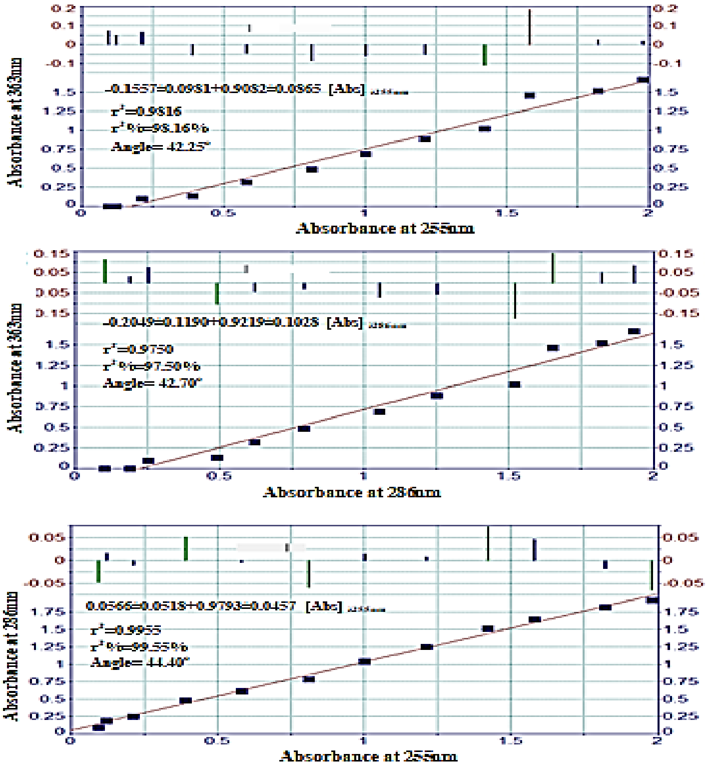


Figure 13. Calibration graph for the variation of absorbance at three different wavelengths on the same of concentration at range (0.005 → 0.1mMol.L^{-1}).

Table 5. Summary of results for comparison between three wavelengths using 12 point of concentration (0.005- 0.1mMol.L^{-1})

Type of relation expressed as an average of Abs. $\bar{y}_i$	$\hat{Y}_i= a\pm S_a t+b\pm S_b t$ [Abs]$_{\lambda(nm)}$ at 95%, n-2	b (slope) Angle	r r^2 r^2%	t$_{tab}$ at 95%, n-1, (n=12)	t$_{cal}$
Abs$_{\lambda 363nm}$ vs. Abs$_{\lambda 255nm}$	-0.1557±0.0981+ 0.9082±0.0865[Abs]$_{\lambda 255nm}$	(0.9082) 42.25	0.9908 0.9816 98.16%	2.201<	23.0958
Abs$_{\lambda 363nm}$ vs. Abs$_{\lambda 286nm}$	-0.2049±0.1190+ 0.9219±0.1028 [Abs] $_{\lambda 286nm}$	(0.9224) 42.70	0.9874 0.9750 97.50%	2.201<	19.9454
Abs$_{\lambda 286nm}$ vs. Abs$_{\lambda 255nm}$	0.0566±0.0518+ 0.9793±0.0457 [Abs] $_{\lambda 255nm}$	(0.9793) 44.40	0.9977 0.9955 99.55%	2.201<	47.1986

- New methodology for the analysis and assessment of B$_9$–Application for determination a available drugs

Two methods were used for the determination of vitamin B$_9$ .The first method was the use of Ayah 6Sx1-ST-2D Solar CFI Analyzer and the second method was the classical measurement for absorbance at λ= 255nm.

A series of solutions were prepared of each pharmaceutical drug (1 mMol.L^{-1}) by transferring 0.5 ml to each five volumetric flask (10 ml), followed by the addition of gradual volumes of standard vitamin B$_9$ (0.01Mol.L^{-1}) (0, 0.02, 0.05 ,0.07 ,0.1) ml to obtain (0, 0.02, 0.05, 0.07, 0.1) mMol.L^{-1} for each developed method and classical method. Taking into consideration that the first flask is for the sample. The measurements were conducted by both methods. Results were mathematically treated for the standard addition method. Fig 14 profile for the three drugs of Folic acid in each pharmaceutical drug by developed method. Table 6A,B shows a practical content of active ingredient at 95% confidence level & efficiency of determination in addition to paired t-test which shows a comparison at two difference paths.

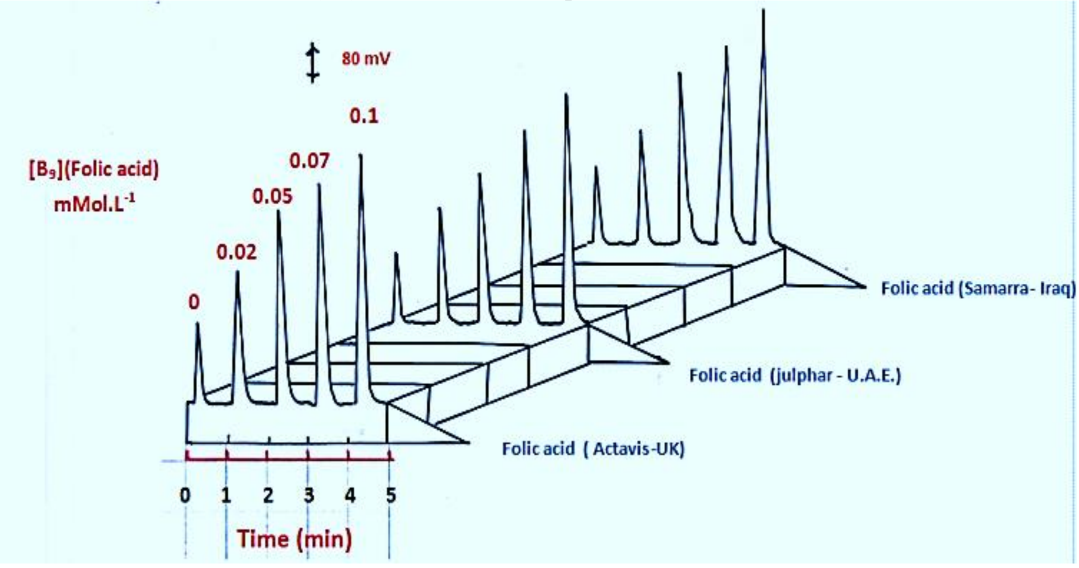
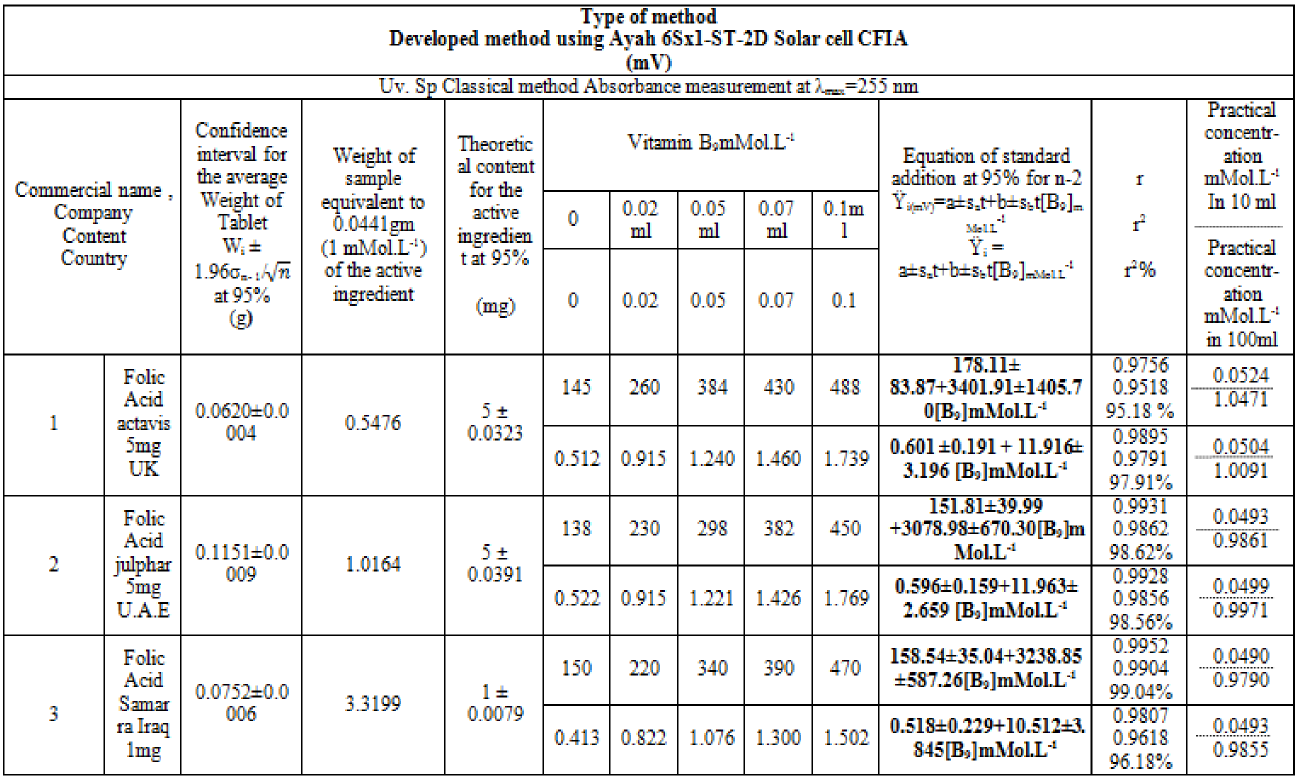


Figure 14. Samples of response profile using standard addition method for three samples of drugs.

Table 6A. Standard addition results for the determination of vitamin B_9 in three pharmaceutical reparations using two methods.



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												
Commercial name , Company Content Country		Confidence interval for the average Weight of Tablet $W_i \pm 1.96\sigma_{n-1}/\sqrt{n}$ 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 $\hat{Y}_{i(mV)}=a\pm s_at+b\pm s_bt[B_9]_{mMol.L^{-1}}$ $\hat{Y}_i = a\pm s_at+b\pm s_bt[B_9]_{mMol.L^{-1}}$	r r^2 r^2%	Practical concentration mMol.L^{-1} In 10 ml Practical concentration mMol.L^{-1} in 100ml
					0	0.02 ml	0.05 ml	0.07 ml	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	145	260	384	430	488	**178.11±83.87+3401.91±1405.70[B_9]mMol.L^{-1}**	0.9756 0.9518 95.18 %	0.0524 1.0471
					0.512	0.915	1.240	1.460	1.739	**0.601 ±0.191 + 11.916± 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	138	230	298	382	450	**151.81±39.99 +3078.98±670.30[B_9]mMol.L^{-1}**	0.9931 0.9862 98.62%	0.0493 0.9861
					0.522	0.915	1.221	1.426	1.769	**0.596±0.159+11.963± 2.659 [B_9]mMol.L^{-1}**	0.9928 0.9856 98.56%	0.0499 0.9971
3	Folic Acid Samarra Iraq 1mg	0.0752±0.0006	3.3199	1 ± 0.0079	150	220	340	390	470	**158.54±35.04+3238.85 ±587.26[B_9]mMol.L^{-1}**	0.9952 0.9904 99.04%	0.0490 0.9790
					0.413	0.822	1.076	1.300	1.502	**0.518±0.229+10.512±3.845[B_9]mMol.L^{-1}**	0.9807 0.9618 96.18%	0.0493 0.9855

$\hat{Y}$: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 vitaminB_9 in three samples of pharmaceutical preparations 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**						
No. of sample	**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 $_{i(g)}$ $\pm 4.303\sigma_{n-1}/\sqrt{n}\bar{w}$** **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 methods**	
					$t_{cal}=\bar{X}d\sqrt{n}/\sigma_{n-1}$	**t_{tab} at 95% confidence level and n-1degree of freedom (Df)**
1	0.052 1.047 0.046	0.046±0.009 5.233±0.988	104.66%	1.015< 4.303	$\bar{X}$d: 0.043 σ_{n-1}: 0.1318 0.565<4.303	
	0.050 1.009 0.045	0.045±0.004 5.043±0.462	100.87%	0.400< 4.303		
2	0.049 0.986 0.044	0.044±0.006 4.926±0.678	98.52%	0.470/< 4.303/-		
	0.050 0.997 0.044	0.044±0.005 4.984±0.532	99.69%	/-0.129/<4.303		
3	0.049 0.979 0.043	0.043±0.038 0.979±0.865	97.85%	-0.104/<4.303/		
	0.049 0.986 0.044	0.044±0.028 0.986±0.624	98.55%	0.097/<< 4.303/-		

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

-First path: Conduct t-test for each sample of folic acid using both methods (classical and new developed methodology) on the following basis:

Null hypothesis (H_o) against Alternative hypothesis (H_1) for comparing between the mean for three samples from different companies using two methods, a hypothesis can be estimated as follow:

For actavis-UK: H_o: $\mu_{quoted} = \overline{w}_{i\ class}$.
H_1: $\mu_{quoted} \neq \overline{w}_{i\ class}$. (5mg)
and H_o: $\mu_{quoted} = \overline{w}_{i\ New\ methodology}$
H_1: $\mu_{quoted} \neq \overline{w}_{i\ New\ methodology}$

The above assumption will be repeated and reconsidered for both used drugs (i.e; julphar-U.A.E and Samarra-Iraq). Since all values obtained (t_{cal}) are less than t_{tab}; null hypothesis will be accepted and reject the alternative hypothesis. This means that there is no significant difference between the measurements of the mean of the three samples using two methods. This is clearly indicated in column no.5 in Table 10B.

-Second path: Paired t-test was used in order to compare between two methods. Taking into a consideration neglecting the effect of different drug suppliers and manufactures.

Therefore, another hypothesis should be carried out as follows:

Null hypothesis: There is no significant difference between the means obtained from each method.(i.e: : H_o: $\mu_{class.} = \mu_{New\ developed\ method}$)
against

Alternative hypothesis (H_1): There is a significant difference between the means of the two methods (i.e: H_1: $\mu_{class.} \neq \mu_{New\ developed\ method}$)

Since the value of t_{cal} less than t_{tab}; this means that there is no significant difference between the two methods.

Therefore, the assessment of vit.B_9; any of the mentioned methods can be used equally as there was no significant difference that could be accounted for.

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التحليل بالحقن الجرياني المستمر للقياس الفوتوني والتعكري لتقدير فيتامين B_9 (حامض الفوليك) بأستخدام ثنائيات وصلة باعثة للضوء كمصدر تشعيع واثنان من الخلايا الشمسية كمحولة طاقة

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الخلاصة:

استخدمت طريقة جديدة حساسة وبسيطة لتقدير فيتامين B_9 (حامض الفوليك)في شكلها النقي وفي المستحضرات الصيدلانية تعتمد على تكوين معقد مزدوج ايوني راسب رصاصي من تفاعل حامض الفوليك و موليبدات الامونيوم في المحلول المائي بأستخدام الجهاز المحلي الصنع الموسوم Ayah-6SX1-ST-2D Solar Cell CFIA. تم دراسة الظروف المثلى لزيادة حساسية التقدير وتحسين حدود الكشف بأستخدام الطريقة المطورة فكان مدى الخطية لمنحني المعايرة لفيتامين B_9 (0.01-0.6) ملي مول. لتر$^{-1}$ ومعامل الارتباط r=0.9810 تم الحصول على حدود كشف 131.994 نانوغرام / النموذج من التخفيف التدريجي لأقل تركيز في منحني المعايرة كانت RSD% للتراكيز (0.07,0.5) مللي مول. لتر$^{-1}$ > 0.1% لـ (n = 9). الطريقه اثبتت نجاحها لتقدير فيتامين B_9 في المستحضرا ت الصيدلانية فقد اجريت مقارنة بين الطريقة المطورة والطريقة التقليدية عند طول موجي λ_{max}=255nm تم التحليل باستخدام منحني الاضافات القياسية واجري اختبار الـ paired t-test للمقارنة بين القيم المستحصلة لتقدير الفيتامين بالطريقة المطورة والطريقة التقليدية والنتائج اثبتت عدم وجود فرق جوهري عند مستوى ثقه 95%وبذلك يكون كلا الاختبارين ناجحين لتقدير فيتامين B_9.

الكلمات المفتاحية: فيتامين B_9، التحليل بالحقن الجرياني، التعكرية، الجهاز المحلي الصنع.