International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

CFIA-Turbidimetric and Photometric Determination of Vitamin B_2 using LEDs as a Source of Irradiation and Two Solar Cells as an Energy Transducer

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Abstract: ***A new, sensitive and simple method has been used for the determination of vitamin B_2 (Riboflavin) in pure and pharmaceutical formulations by continuous flow injection analysis. The method is based on oxidation vitamin B_2 by sodium periodate to release formic acid then coupled with 2,4 dinitrophenylhydrazin in an aqueous medium to obtain a yellow precipitate 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_2concentration was (0.05-5.5)mMol.L^{-1}while the L.O.D was 1.615µg/sample, the correlation coefficient (r) was 0.9825while percentage linearity (r^2%) was 96.52%. RSD% for the repeatability (n=8) was less than 0.5%for the determination of vitamin B_2 at concentration (1,4) mMol.L^{-1} respectively .The method was applied successfully for the determination of vitamin B_2 in pharmaceutical preparation .A comparison was made between two methods: newly proposed method and the reference claimed method UV-SP spectrophotometry at λ_{max}=445 nm, of analysis using the standard addition method via the use paired t-test .It showed that there was no significant difference between the two methods proposed (developed) method and the reference claimed (classical method)for determination of vitamin B_2at 95% confidence level.***

Keywords: Vitamin B_2, flow injection analysis, turbidity, homemade instrument

1. Introduction

Vitamin B_2 (Riboflavin) (fig. 1) belongs to the group of water soluble vitamins. It has yellow color. It is very stable at high temperatures, so that at 120 °C can remain stable and active over a period of five to six hours. In acidic medium the stability is still increasing, while in alkaline medium activity decreases. Vitamin B_2 is very sensitive to visible light, and if it is exposed to it for a longer period it can lead to its breakdown [1]. The name "riboflavin" (often abbreviated to Rbf or RBF) comes from "ribose" (the sugar whose reduced form, ribitol, forms part of its structure) and "flavin", the ring-moiety which imparts the yellow color to the oxidized molecule (from Latin *flavus*, "yellow"). The reduced form, which occurs in metabolism along with the oxidized form, is colorless [2].

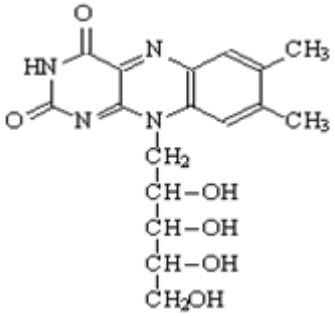


Figure 1: Structure of vitamin B_2(Riboflavin) IUPAC name7, 8-Dimethyl-10-[(2S, 3S, 4R)-2,3, 4,5-tetra hydroxypentyl benzo pteridine-2,4-dione.

All B vitamins are water soluble, meaning the body does not store them [3]**.**Vitamin B_2 is a water-soluble vitamin that is flushed out of the body daily, so it must be restored each day. The best way to get this vitamin is by eating foods that are rich in riboflavin. Riboflavin is found in eggs, nuts, dairy products, meats, broccoli, brewer's yeast, Brussels- sprouts, wheat germ, wild rice, mushrooms, soybeans, green leafy vegetables and whole grain and enriched cereals and bread[4].There are many various analytical technique methods for determination of vitamin B_2: HPLC [5-8], Spectrophotometric [9], electrochemical technique [10,11], flourometry[12], Chemilumenescence [13,14], extraction [15]

In this new work using flow injection turbidimetric method, the turbidity is measured via reflection of incident light from the surfaces of particles formed when oxidation vitamin B_2 by sodium periodate then coupled with 2,4 dinitrophenylhydrazin (2,4 DNPH) at 0-180° by homemade Ayah -6SX1-ST-2D solar cell CFIA provide with six snow-white light as a source with two solar cells as a detector.

2. Experimental

Reagent and chemicals

All chemicals were used of analytical-reagent grade and distilled water was used to prepare all the solutions. A standard solution 2mMol.L^{-1} of vitamin B_2(Riboflavin -5-phosphate sodium 2-hydrate);chemical formula $C_{17}H_{20}N_4NaO_9P \cdot 2H_2O$, molar 514.36 g.$mol^{-1}$& SDI-Iraq was prepared by dissolving 0.103 g of riboflavin -5-phosphate sodium 2-hydrate in 100 ml of distilled water. stock solutions:0.1 Mol.$L^{-1}$of sodium periodatemolecular formula $NaIO_4$, molar mass 213.89g mol^{-1} & BDH- England was prepared by dissolve 5.347 g in 250 ml of distilled water and 0.05Mol.L^{-1} 2,4dinitrophenylhydrazin molecular formula $C_6H_6N_4O_4$,molar mass 198.14 g mol^{-1}& BDH- England was prepared by dissolve 4.954 gm in (500 ml distilled water involve 15ml sulpharic acid (H_2SO_4)0.1Mol.L^{-1}).

Sample Preparation

Twenty capsule were weighted by extract the substance from its gelatin capsule . containing (2) mg of vitamin B_2 for

each sample 1.4313g,1.5944g,1.5828g (equivalent to 0.0103g of active ingredient,0.2 mMol.L^{-1}) for B-plex Samarra Iraq & Safaplex Diala Iraq, B-plexin Haryana India respectively .Dissolved in distilled water.The solution was filtered to get rid of undissolved materials, the residue was washed with distilled water and completed the volume to 100ml with the same solvent(distilled water).

Apparatus

The response was measured by a homemade Ayah 6SX1-ST-2D Solar cell CFI Analyzer, which used a six snow-white light emitting diode LEDs for irradiation of the flow cell at 2 mm path length. The flow system used for the determination of vitamin $B_2$is shown as flowgram in Figure 2, Peristaltic pump – 2 channels variables speed (Ismatec, Switzerland), Injection valve with valve 6port medium pressure (IDEX corporation, USA) with sample loop (1 mm i.d.Teflon, different length). Two solar cells are used as detector for collecting signals via sample travel for 60 mm length. The readout of the system composed of x-t potentiometric recorder (Kompenso Graph C-1032) Siemens (Germany), this recorder measured by (1-500) mV or voltage and digital AVO-meter (auto range)AM666AL (200mV-20 volt) (China). UV-Vis spectrophotometer single beam type PU 8720, philips, Japan was used to scan the spectrum of vitamin B_2 using 4 cm quartz cell.

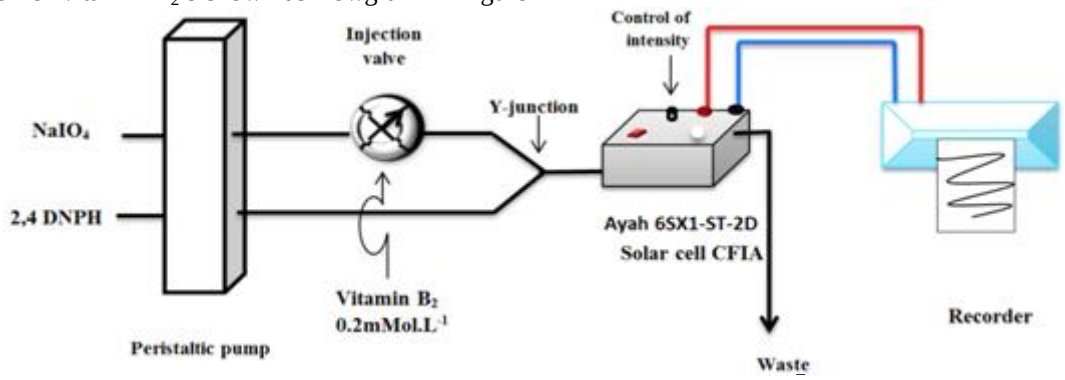
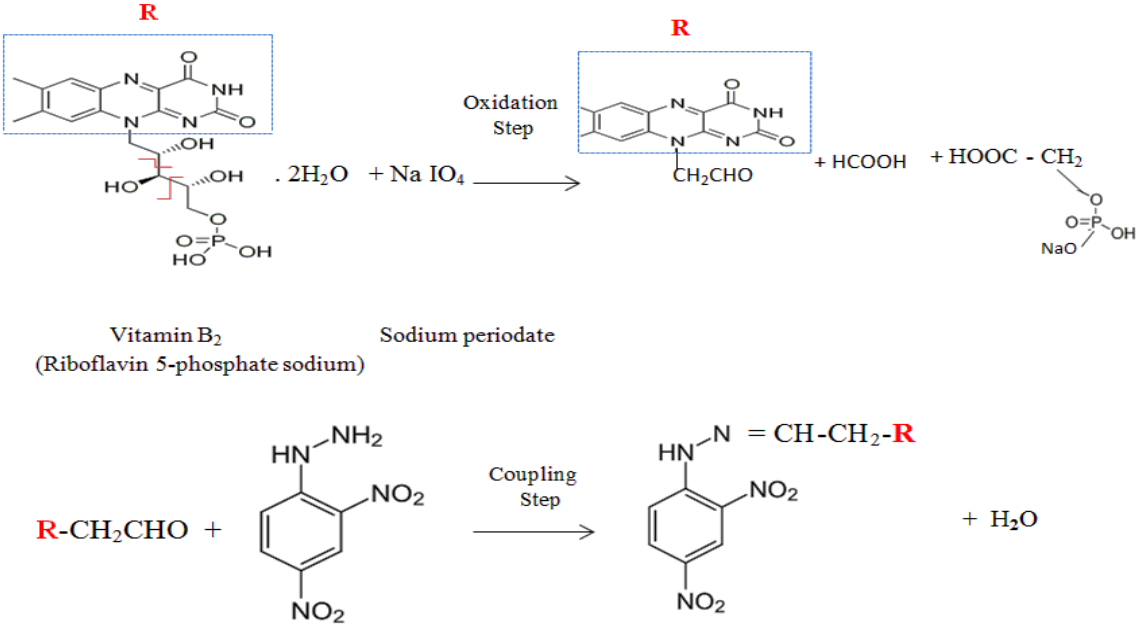


Figure 2: Manifold design system for determination of vitamin B_2 using Vit.B_2-IO_4^- - 2,4-DNPH system - Carrier stream : Sodium periodate((0.1Mol.L^{-1} at flowrate 1.4ml.min^{-1}) . - Reagent : 2,4 DNPH (10mMol.L^{-1} at flow rate 1.5ml.min^{-1})

3. Methodology

Two line system was used (fig 2). Sodium periodate(0.1Mol.L^{-1})was passed in the first line as a carrier stream(1.4 ml.min^{-1} flow rate); the same line leading to the injection valve for carrying 210µl sample volume of vitamin B_2 (4.5mMol.L^{-1}) which in turn to reactant with sodium periodate (as an oxidation reagent) to form the oxidant species inside injection valve.While the second line supplies 2,4-dinitrophenylhydrazin (2,4DNPH)(10mMol.L^{-1}) as a reagent (1.5 ml.min^{-1}flow rate).Both lines meet at a Y-junction point (methyl methacrylate) with an outlet for reactant product (form a yellow precipitate particles).A proposed successive reactions [16 ,17]for vit.B_2-IO_4^- -2,4-DNPH system can be represented by the following scheme1.



Scheme 1: Aproposed successive reactions for vit.B_2-IO_4^--2, 4 DNPH system

4. Result and Discussion

- Study of the optimum parameter for determination of vitamin B_2

Physical and chemical variables were studied based on single variable optimization.

-Chemical parameters

- Effect of Variable Concentration of 2,4 dinitrophenylhydrazin (2, 4-DNPH)

A series of solutions (1 -10) mMol.L^{-1} 2,4DNPH were prepared; which is used as a reagent at flow rate of 1.5 ml.$min^{-1}$and Sodium periodate 0.1 Mol.L^{-1}as a carrier stream at flow rate of 1.4 ml.min^{-1}, 210µl sample volume of 4.5mMol.L^{-1} of vitamin B_2& applied voltage of LEDs was 1.686 volt DC. It can be seen that the best of peak height at 5mMol.L^{-1}. More than 5mMol.$L^{-1}$of 2,4DNPH causes peak responses profile to be distorted or deformed shape which create a difficulty in recognizing the apex of the response which causes erroneous peak height (fig 3A) measurements. This might probably attributed to high density of precipitate particles that formed in a rapid local concentration which in turn to irregularities in particles shape. The results summarize in table 1. Fig 3B shows the energy transducer response via reflection of incident light for determination of vitamin B_2.

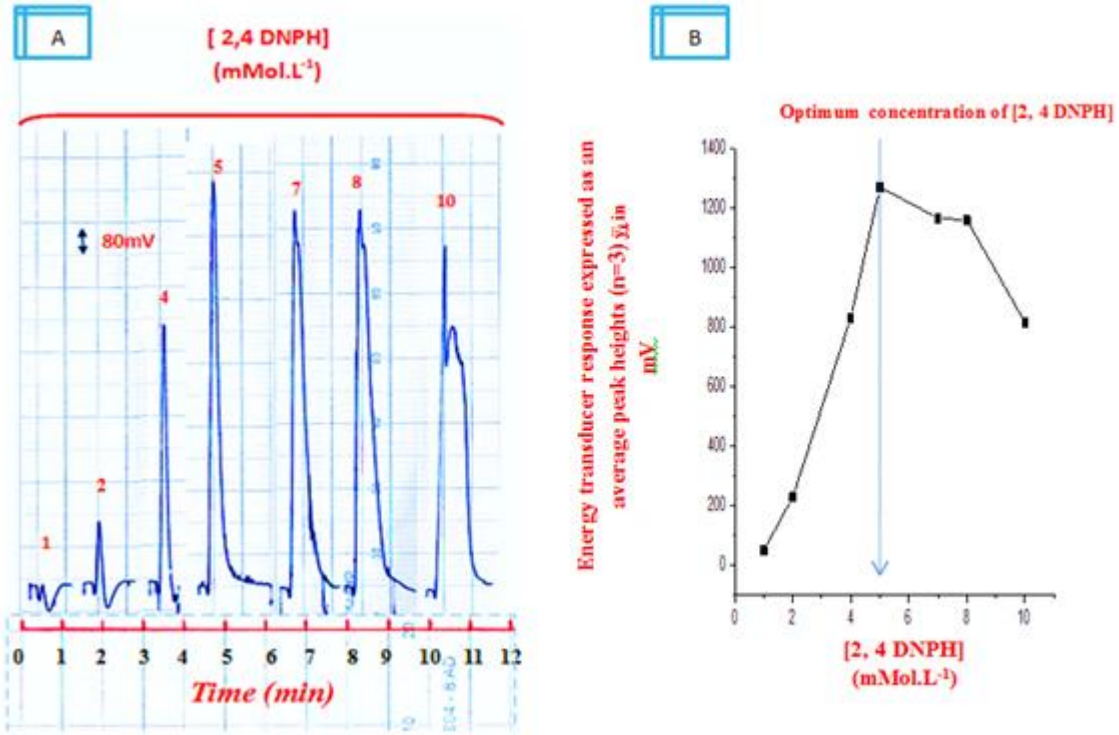


Figure 3: Effect of variation of 2,4DNPH concentration on:
A- Response profile versus time.
B - Energy transducer response by reflection of incident light

Table 1: Results for effect of 2, 4 DNPH on the measurements of energy transducer responseforthe determination of vitaminB_2

[2,4 DNPH] mMol.L^{-1}	Energy transducer response expressed as an average peak heights (n=3) $\bar{y}_i$in mV	RSD%	Confidence interval at 95% $\bar{y}_i \pm t_{0.05/2,n-1}\sigma_{n-1}/\sqrt{n}$
1	48	0.938	48±1.118
2	228	0.364	228±2.062
4	832	0.118	832±2.435
5	1272	0.080	1272±2.534
7	1168	0.092	1168±2.683
8	1160	0.106	1160±3.056
10	816	0.113	816±2.286

$t_{0.05/2,2}$ **= 4.303**

- Variation of add sulpharic acid concentration

In order to select the suitable medium used in the precipitation using vitamin B_2(4.5mMol.L^{-1})-IO_4^- (0.1Mol.L^{-1})-2,4DNPH(5mMol.L^{-1}) system for determination of vitamin B_2; a series of constant concentrations of 2,4 DNPH were prepared from a stock solution.To these solutions, a variable volume of 1 Mol.L^{-1} –H_2SO_4 were added ranging (0, 0.01, 0.02, 0.03 and 0.05)Mol.L^{-1}.It can be seen that an increase of acid concentration causes a decrease in peak height obtained (fig 4A).Most probably due to solubilizing the formed precipitate. All results were tabulated in table 2.An aqueous medium (i.e., Distilled water)was regarded as the optimum medium for the use in this work (fig 4B).

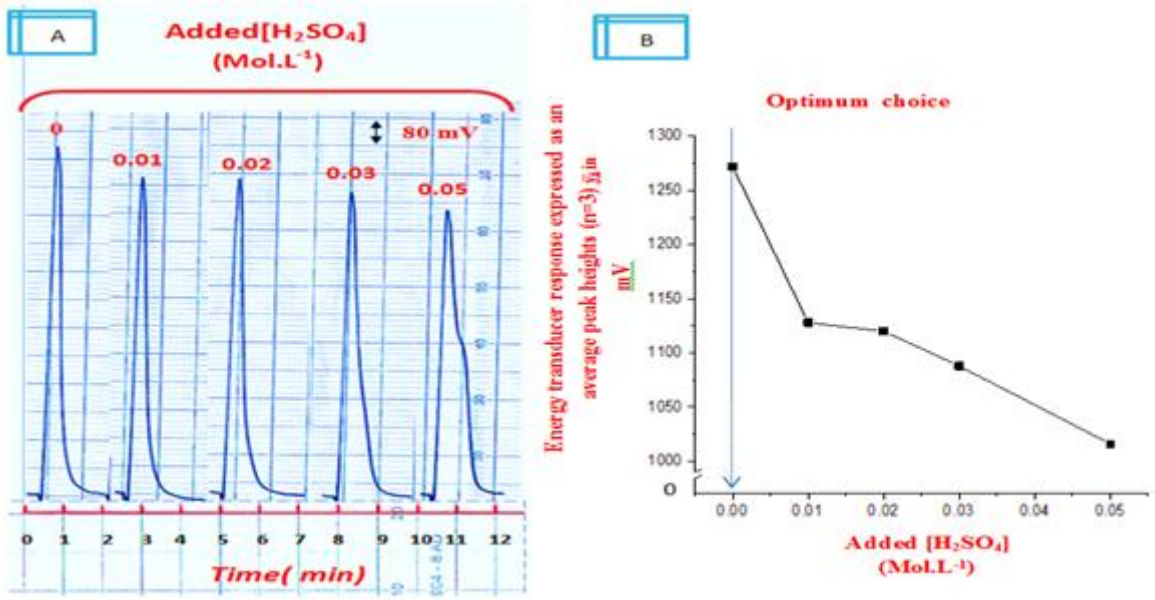


Figure 4: Effect of variation ofadd sulphuric acid concentration on:
A-Response profile versus time
B-Energy transducer response by reflection of incident light

-Effect of variable concentration of Sodium periodate on determination of Vitamin B_2

The effect of theoxidizing agent (IO_4^- ion) concentration on the sensitivity was studied. A series of solutions (0.03 -0.2) $Mol.L^{-1}$ from sodium periodate were prepared at 1.4 $ml.min^{-1}$ flow rate. 4.5$mMol.L^{-1}$ of vitamin B_2 was used as the injected concentration and 210µl sample volume with a constant concentration of 2,4-DNPH (5$mMol.L^{-1}$) at 1.5$ml.min^{-1}$ flow rate and applied voltage of LEDs was 1.686 volt DC. Fig 5A shows that 0.15$Mol.L^{-1}$ is the optimum concentration .An increase in concentration (> 0.15$Mol.L^{-1}$) leads to the prevention of the part of incident light causing the increase in the attenuation of incident light. While at low concentration (i.e. ;< 0.15$Mol.L^{-1}$) smaller tiny particles were obtained having spaces between them causing scattering of light in an irregular way leading to distorted profile. Table 2 summarizes the total results obtained, while the best concentration of sodium periodate can be seen in fig 5B.

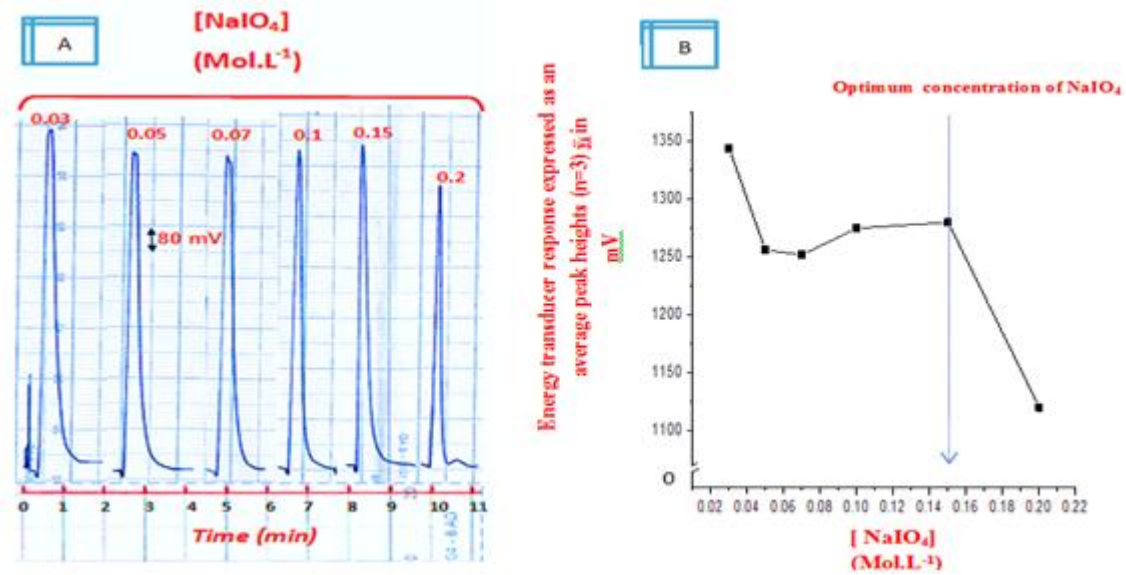


Figure 5: Effect of variable concentration of Sodium periodate on:
A- Response profile versus time
B- Energy transducer response by reflection of incident light

Table 2: Effect of Sodium periodate on the measurement of energy transducerresponse

[$NaIO_4$] Mol.L^{-1}	Energy transducer response expressed as an average peak heights (n=3) $\bar{y}_i$ in mV	RSD %	Confidence interval at 95% $\bar{y}_i \pm t_{0.05/2,n-1}\sigma_{n-1}/\sqrt{n}$
0.03	1344	0.061	1344±2.0371
0.05	1256	0.088	1256±2.7327
0.07	1252	0.097	1252±3.0060
0.10	1275	0.070	1275±2.5588
0.15	1280	0.081	1280±2.3601
0.20	1120	0.091	1120±2.5340

$t_{0.05/2,2}$ **= 4.303**

-Physical parameters

-Effect of Flow rate

Using optimum parameters achieved in previous section .A variable flow rate expressed an indication approximate as shown in table 3column no.1 represented the variable flow rate of 0.2-2.8 ml.min^{-1}for sodium periodate (0.15Mol.L^{-1}) as a carrier stream and o.4-3.0ml.min^{-1} for 2,4DNPH(5mMol.L^{-1})was studied .From fig 6A; it can be seen that there are an increase in the response height at low flow rate (less than 1.4ml.min^{-1}for carrier stream) but shows a kind of deformed peak response which could be attributed to the increase of dispersion due to diffusion that might overcome convection at this stage causing wide of base peak ,also peak apex suffer deformed distorted maxima .While at higher flow rate (>1.4,1.5 for carrier stream and line no.2 (2,4DNPH respectively) less time relatively is given for diffusion that causes dispersion at halt state while convection is the dominant factor this will definitely will increase volume that is fed to the system relatively in comparison with low flow rate causing the dilution of sample segment that is precipitated; and for the purpose of compromising the consumption of chemicals with the reality of peak response profile 1.4 &1.5 ml.min^{-1} were the choice for each line with the manifold used as shown in fig 6B.

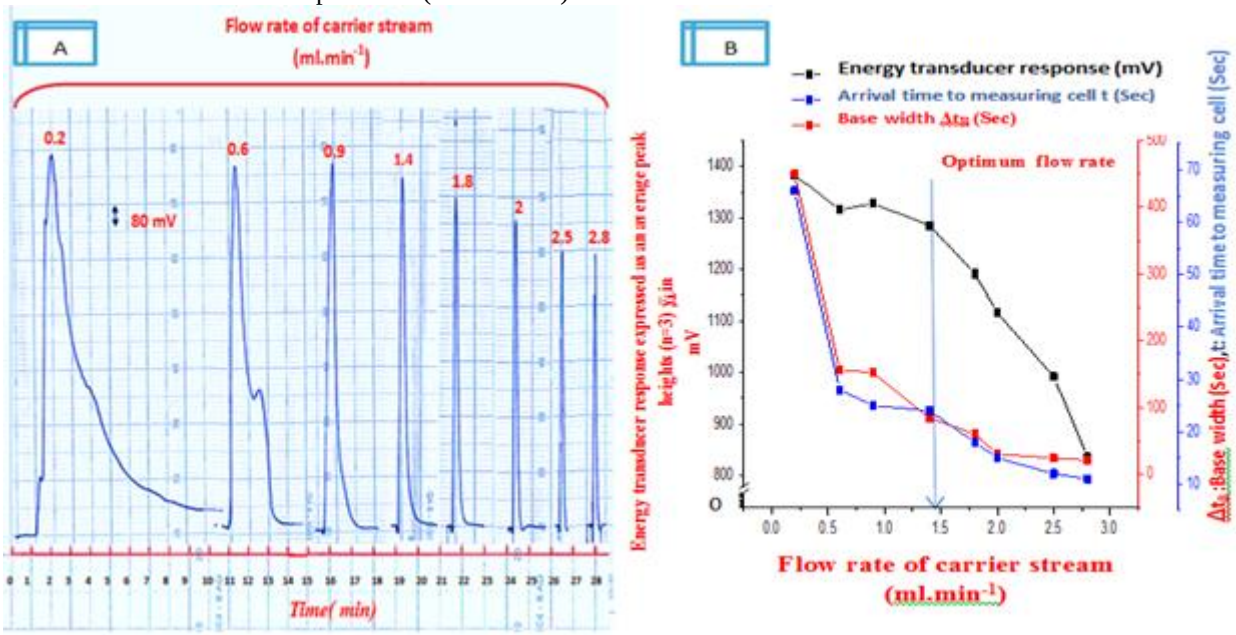


Figure 6: Effect of flow rate on: A- Response profile versus time.
B - Energy transducer response by reflection of incident light.

Table 3: Effect of flow rate on the measurements of energy transducer response

Pump speed	Flow rate (ml.min^{-1})		Energy transducer response expressed as an average peak heights (n=3) $\bar{y}_i$ in mV	RSD%	Confidence interval at 95% $\bar{y}_i \pm t_{0.05/2,n-1}\sigma_{n-1}/\sqrt{n}$	t Sec	$t_B\Delta$ Sec	V_{add} ml	Concentration (mMol.L^{-1}) at flow cell
	Line 1 $NaIO_4$	**Line 2 2,4 DNPH**							
5	0.2	0.4	1384	0.066	1348 ± 2.2856	66	450	4.71	0.201
10	0.6	0.7	1316	0.074	1316 ± 2.4346	28	156	3.59	0.263
15	0.9	1.0	1328	0.076	1328 ± 2.5340	25	152	5.02	0.188
20	1.4	1.5	1285	0.080	1285 ± 2.5837	24	84	3.91	0.242
25	1.8	2.0	1192	0.094	1192 ± 2.8073	18	60	4.01	0.236
30	2.0	2.2	1116	0.100	1116 ± 2.8569	15	30	2.31	0.409
35	2.5	2.7	992	0.120	992 ± 3.0309	12	24	2.29	0.413
40	2.8	3.0	836	0.150	836 ± 3.1799	11	21	2.24	0.422

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 volumes

The effect of variable sample volume(110-310)µl using optimum flow rate (1.4&1.5)ml.min^{-1}for carrier stream and reagent respectively) and vitamin B_2 (4.5 m.Mol.L^{-1})-IO4 (0.15Mol.L^{-1})-2,4DNPH(5mMol.L^{-1})system ,open valve mode with applied voltage to the six snow white light emitting diodes as a source for irradiation (1.686 volt) were studied .It was noticed that an increase in sample volume led to an increase in the height of response without affecting to the profile of obtained response up to the sample volume of

251.2µl (fig 7A)..Above 251.2 µl there was a slightly decrease in the height of peak with an increase in base width with irregular output response. Therefore, when sample segment is increased, it causes a kind of agglomerates and forming a dense precipitate having irregular shapes due to the speed of formation of the precipitate .This dense precipitate due to its formation in a speedy way form a gel, formed amorphous particulate that may be adsorbed on the flow cell wall causing a more than one reason for signal deformation .Therefore the above most probably explanation might explain the deformed peak response profile .Also another factor that might add to the above reason that the dense precipitate might more difficult to move on without distraction in the flow of liquid in the flow cell .So the 251.2µl was the best sample volume as shown in fig 7B.

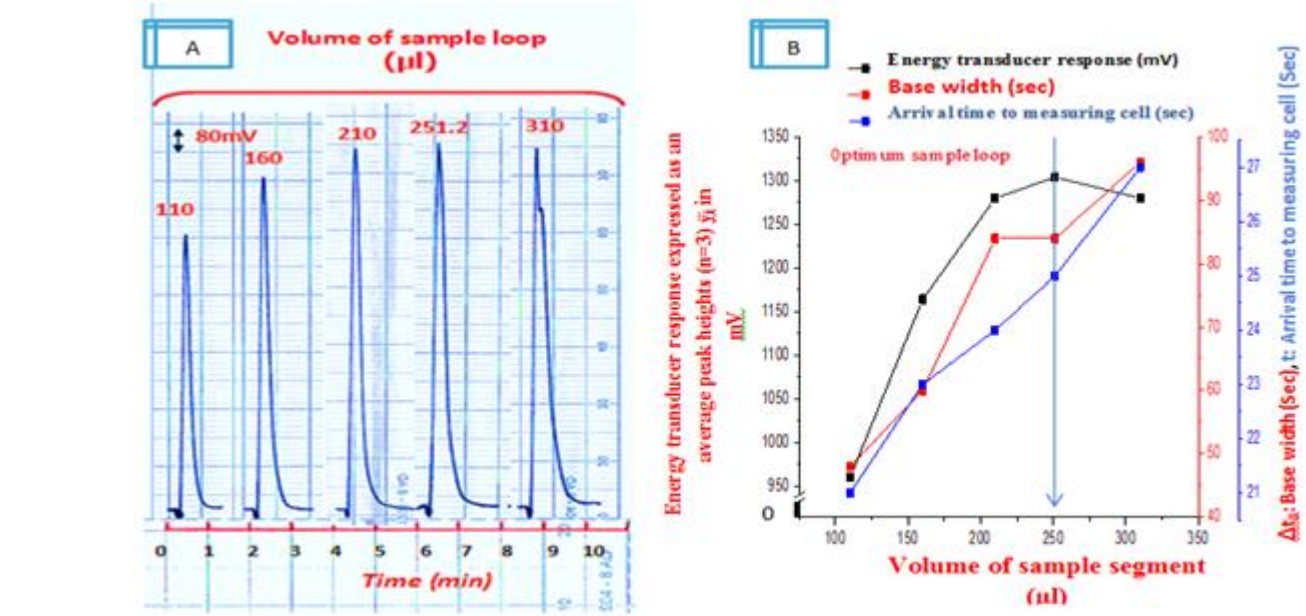


Figure 7: Effect of sample volume on: A- Response profile versus time.
B - Energy transducer response by reflection of incident light.

-Allowed permissible time (purge time) for sample injection

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(2-24)seconds, in addition to 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 the height of response with increase of purge time up to 18 sec, after that there was no longer significant difference in peak height but increase of Δt_B ,which might be attributed to the resistance of flowdue to the continuous passage of carrier stream through the injection valve which leads to the slow movement of reflecting particles , therefore 18sec as a purge time was chosen asoptimum to completely purge of sample segment through sample segment (fig 8B) .

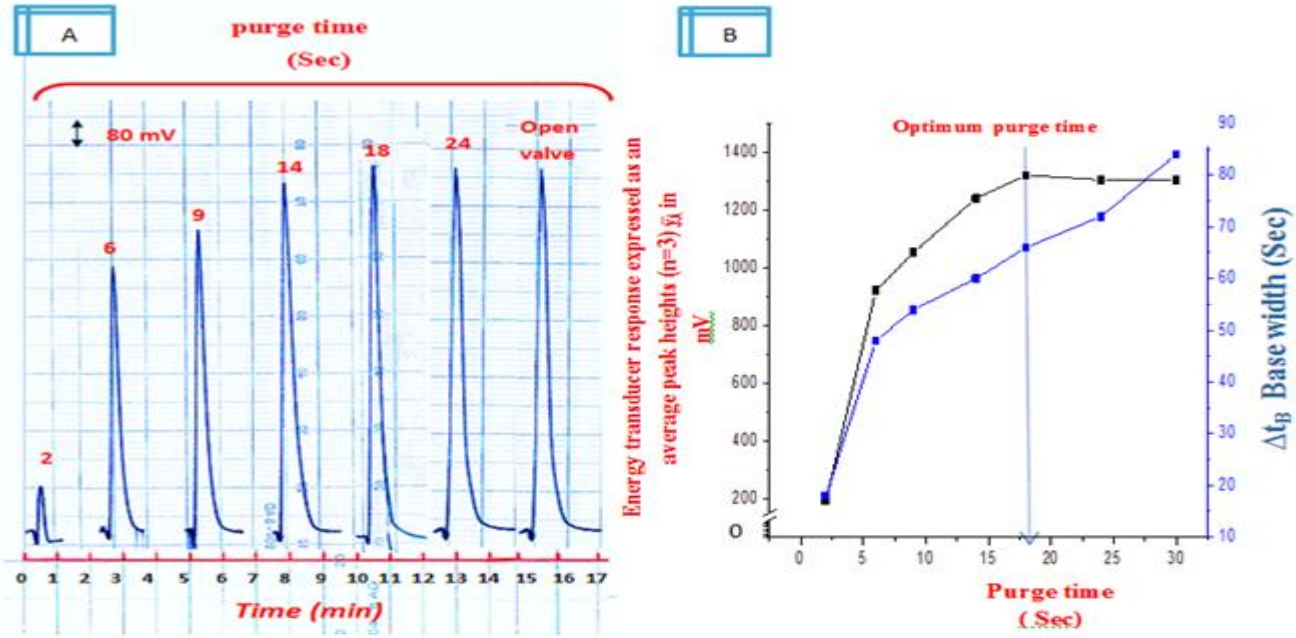


Figure 8: Variation of purge time on:A- Response profile versus time.
B- Energy transducer response by reflection of incident light

-Effect of mixing coil

Variable coil length 0-40 cm was studied. These length comprises a volume (0 –314) µl which connected after Y-junction directly in flow system. While keeping all other changeable constant (vitamin B_2 4.5 mMol.L^{-1},flow rate (1.4 & 1.5) ml.min^{-1} for carrier stream (Sodium periodate 0.15Mol.$L^{-1}$and reagent (2, 4 DNPH 5mMol.L^{-1}) respectively, sample volume 251.2µl and applied voltage of LEDs was 1.686 volt DC. Fig. 9 A shows the increase of coil volume lead to increase of peak height even extreme 157µl, then shows the decrease of peaks heights with increase length of mixing coil leads toslowing down of precipitate particles causing kind of accumulation while the precipitate segment moves up to exit position of the flow cell, this will affect the liquid movement through the cell. Fig 9B shows the effect of mixing coil length on energy transducer response,.

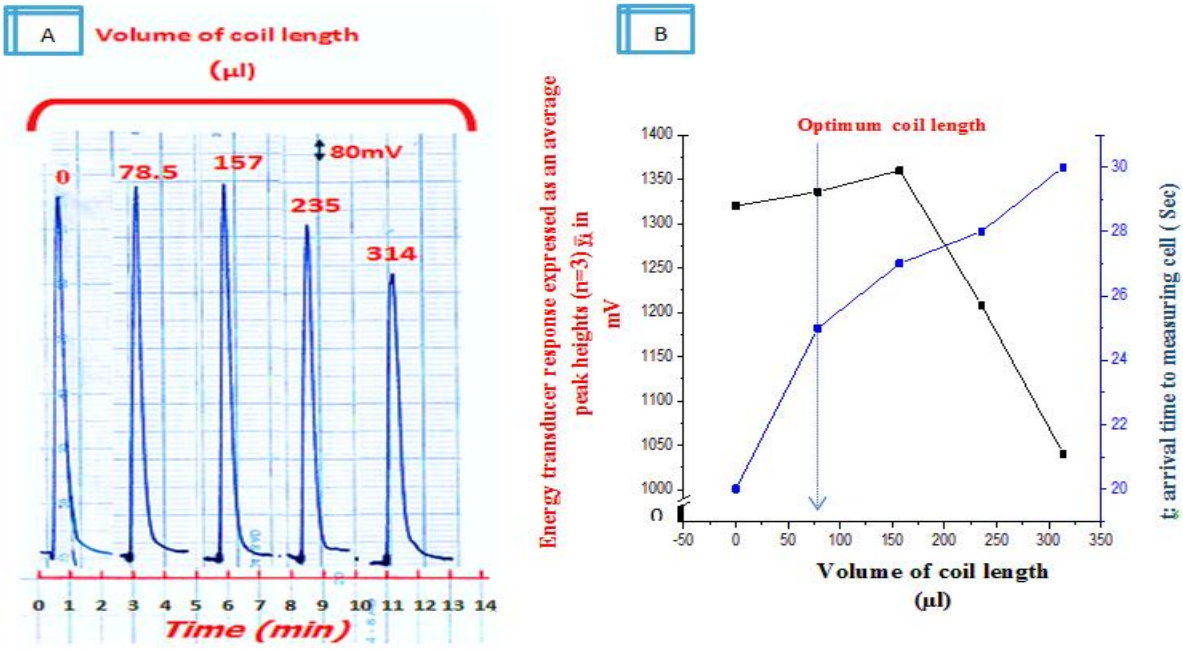


Figure 9: Effect ofmixing coil length on:A- Response profile versus time
B- Energy transducer response by reflection of incident light

-Intensity of light

Variation of light source intensity on the efficiency for determination of vitamin B_2 at 4.5 mMol.L^{-1} concentration was studied while keeping all other changeable fixed (i.e: Sodium periodate (0.15 Mol.L^{-1}) as a carrier stream , 2,4DNPH(5 mMol.L^{-1}) as a reagent,251.2 µl sample volume , flow rate (1.4&1.5) ml.min^{-1} for carrier stream and reagent respectively ,purge time 50sec & volume of coil length 78.5µl, the applied voltages to the LEDs were used with ranging (0.51-1.98) volt DC, by variation of light intensity read by AVO- meter. Fig10A shows the profile.Fig.10B showing the relation between energy transducer response outputs versus applied voltage; which shows that an increased light intensity of irradiation via increased voltage supply; causes increased reflection of light until supply voltage reach 1.95 volt. Above this (>1.95volt), no more gain of response is achieved. Therefore; the intensity of 1.95volt was selected as the best voltage to give a better outcome of response.

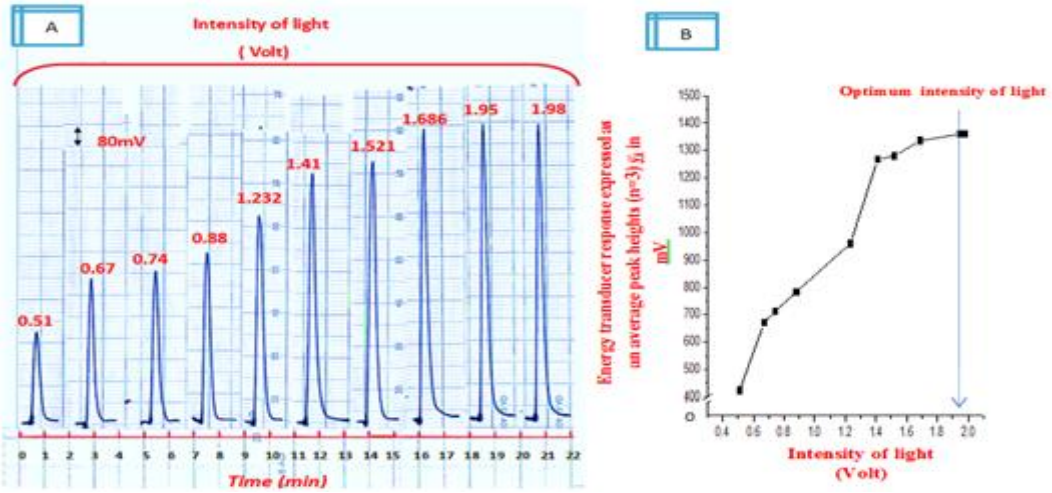


Figure 10: Effect of intensity of light on: A- Response profile versus time
B- Energy transducer response by reflection of incident light

-Scatter plot Calibration Curve

Under the established optimum condition, the calibration curve of continuous flow injection analyses coupled with Ayah 6SX1-ST-2D-Solar cell CFI Analyzer via reflection of incident light were estimated. Fig 11Ashows a profile of this study. While fig 11B a scatter plot diagram shows that a linear calibration graph range for the variation of the energy transducer response with vitamin B_2 concentration was ranging from 0.05-5.5 mMol.L^{-1} with correlation coefficient r : 0.9825 .It was noticed that when high concentration of vitamin B_2 up to ,i.e.; indicating a maximum amount of 5.5 mMol.L^{-1} .Adeformed peak response profile causingindecisiveness i.e.; lacking definition in determined the heights of obtained responses which in turn causes a misleading results.

This deformation could be most probably due to irregularities concerning the flow of the chemical through the flow cell. The results were compared with the traditional classical method [18,19]using spectrophotometric method.All results tabulated in table 4..Linear responses extended from 0.001-0.025 mMol.L^{-1} as shown in fig 12.

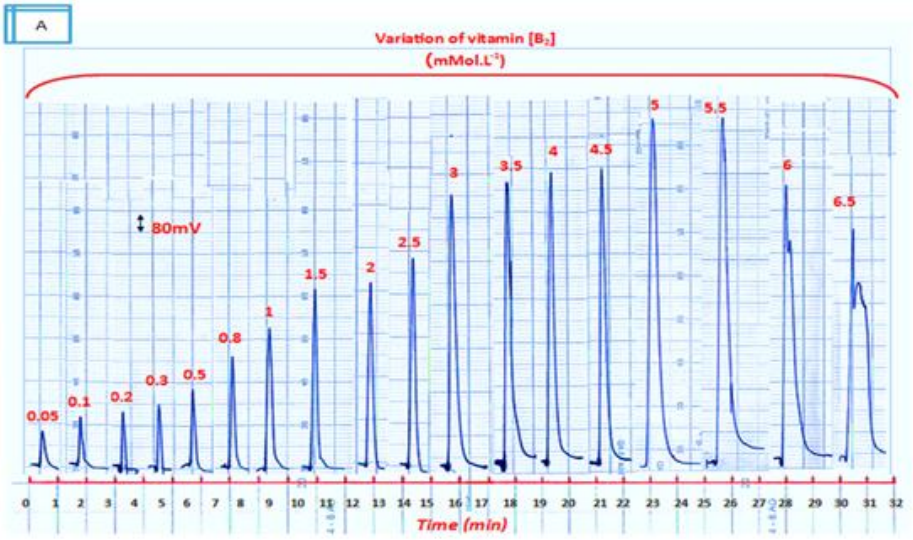


Figure 11A: Profile for the variation of vitamin B_2 concentration on the peak heightversus time, using 251.2µlassample volume.

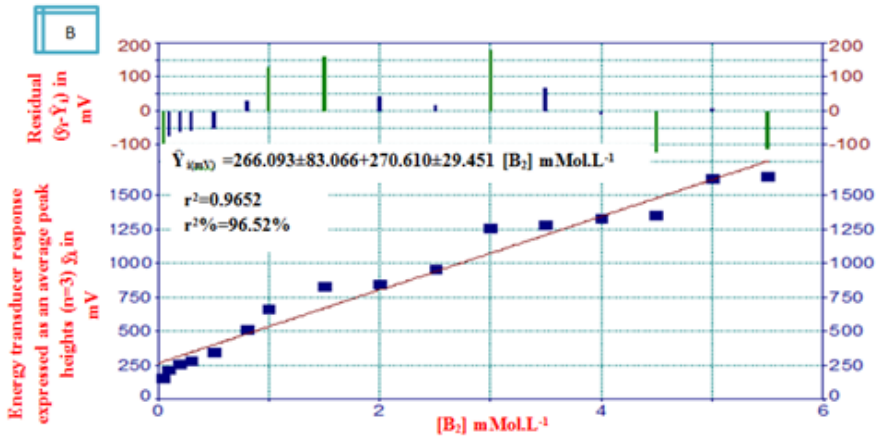


Figure 11 B: Linear calibration graph for the variation of vitamin B_2 concentration on the energy transducer response by reflection of incident light expressed by linear equation using Ayah 6SX1-TS-2D solar –CFI Analyzer (Developed method), $\bar{y}_i$:practical value,$\hat{Y}_i$:estimated value

Table 4: Summary of linear regression equation results for the variation of instrument response with vit.B_2 concentration.

Type of measurement	Measured [B_2] mMol.L^{-1}	n	Range of [B_2] mMol.L^{-1}	$\hat{Y}_i=a\pm s_a t+b\pm s_b t$ [B_2]mMol.L^{-1} at confidence interval 95%,n-2	r r^2 r^2%	t_{tab} at 95% ,n-2	Calculated t-value $\frac{/r/\sqrt{n-2}}{\sqrt{1-r^2}}$
Developed method	0.05-10	16	0.05-5.5	266.093±83.066+270.610±29.451[B_2]mMol.L^{-1}	0.9825 0.9652 96.52%	2.145<19.710	
Classical method uv-vissp λ_{max}= 445nm	0.001- 6	10	0.001-0.025	0.022±0.010+40.068±0.737[B_2]mMol.L^{-1}	0.9997 0.9995 99.95%	2.306<126.451	

Ŷ: Estimated response mV for n=3 expressed as an average peaks heights of linear equation of the form or absorbance value, Ŷ=a+bx, r: Correlation coefficient, r^2 : Coefficient of determination, r^2%: Linearity percentage
sp: Spectrophotometry, Developed method: using 6SX1-ST-2D CFIA.

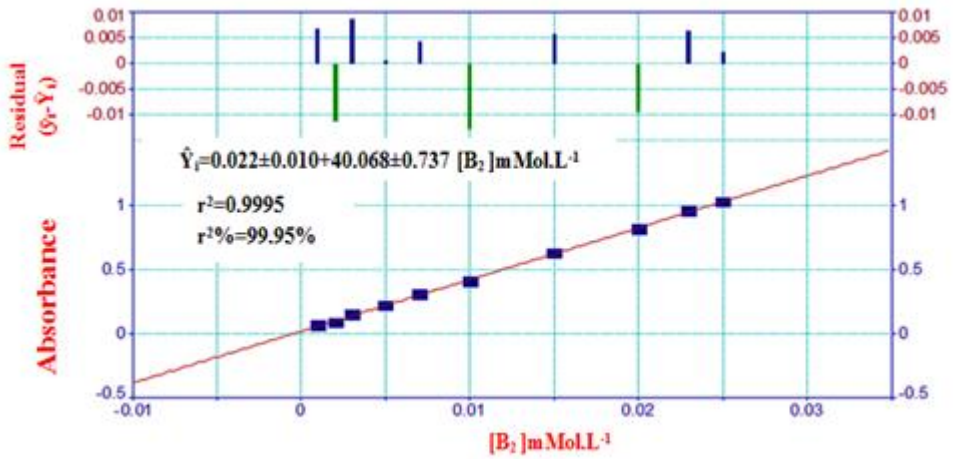


Figure 12 Linear calibration graph for the variation of vitamin [B_2] on the absorbance (Classical method).

- A limit of detection (L.O.D) was carried out based on three different approaches. Gradual dilution of lowest concentration in the calibration graph which was 0.0125 mMol.L^{-1}, based on the numerical value of slope and from the linear regression plot. Table 5 summarizes the results of vitamin B_2using251.2µl sample volume.

Table 5: Limit of detection for vitamin B_2 at using 251.2µl as an injection sample and optimum parameters

L.O.D. (µg/smple		
Practically based on the gradual dilution for the minimum concentration of $[B_2]$=0.0125mMol.L^{-1}	Theoretical based on the value of slope x=$3S_B$/slope for n=13	Theoretical (linear equation) based on the value of =Y_b +$3S_b$Ŷ
1.615	0.429	143.322

X:value of L.O.D. 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 was studies depend on the relative standard deviation expressed as percentage which is equally to the repeatability of the measurements. A successive injections for eight times of $[B_2]$= 1mMol.L^{-1} and 4mMol.L^{-1}were measured . The obtained results are tabulated in Table 6 which shows that the percentage relative standard deviation was less than 0.5% . Fig.13is shown response profile of repeatability at 1 and 4 mMol.L^{-1}respectivelyof concentration of vitamin B_2.

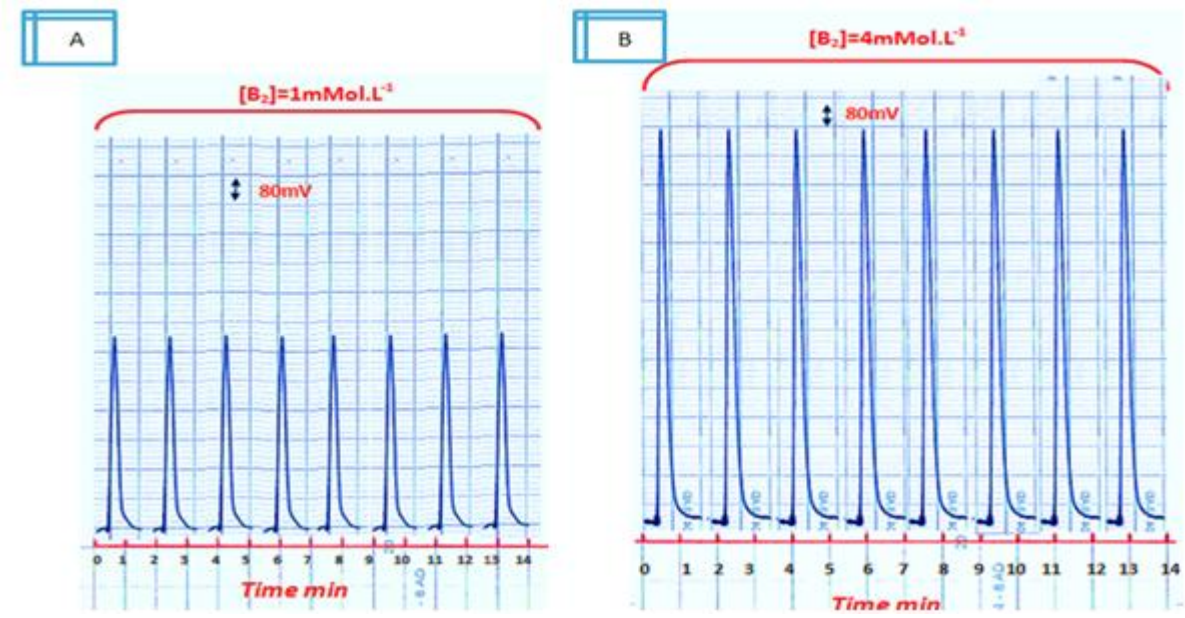


Figure 13: Response profile of repeatability of vitamin B_2 in different concentration: A: 1 mMol.L^{-1}
B: 4 mMol.L^{-1}

Table 6: Repeatability of vitamin B_2 at optimum parameters with 251.2µl sample volume via vit B_2-IO_4^- -2,4DNPH system.

$[B_2]$ mMol. L^{-1}	Energy transducer response expressed as an average peak heights $\bar{y}_i$ in mV	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2,n-1}\sigma_{n-1}/\sqrt{n}$	Number of injection
1	664	0.133	664±0.736	8
4	1336	0.077	1336± 0.861	8

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

-Analysis of vitamin B_2 and treatment of data

Flow injection analysis using homemade Ayah 6SX1-ST-2D solar cellCFI Analyzer with optimum parameters that were achieved in previous section for the vit. B_2 –IO_4^- (0.15Mol.L^{-1})-2,4 DNPH(5mMol.L^{-1})system which form a yellow precipitate particles will be used for the application of method to analyze vitamin B_2 in three different pharmaceutical formulations from different origins of suppliers. The proposed method (continuous flow injection analysis with turbidity measurements expressed as an energy transducer response) was compared with claimed method for absorbance measurements at λ_{max}=445nm [18,19].

Series of solutions were prepared of each pharmaceutical drug (0.2mMol.L^{-1}) by transferring 5 ml to each six volumetric flask (10 ml), followed by the addition of gradual volumes of standard B_2 2mMol.L^{-1} (0, 3, 4 ,5 ,6,7) ml to obtain (0, 0.6, 0.8, 1, 1.2,1.4) mMol.L^{-1} for proposed method. And take (0, 0.2, 0.3,0.4 ,0.5,0.6)ml volumes of standard vitamin B_2(0.2 mMol.L^{-1})for claimed method to obtain(0 ,4 ,6 ,8 ,10,12)µMol.L^{-1} . Flask no.1 is the sample .The measurements were conducted by both methods. Results were mathematically treated forthe standard addition method. Fig 14; shows the kind of profile obtained for compared between three drugs .

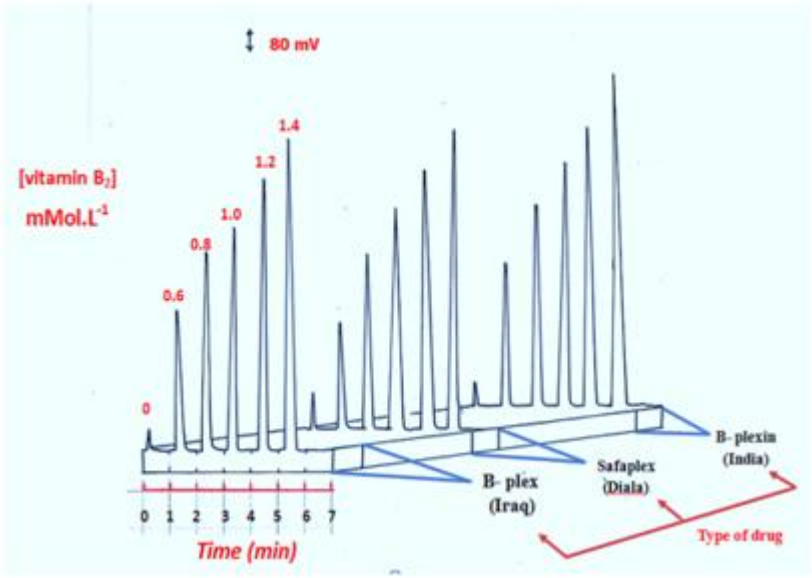


Figure 14: Profile of three drugs

Table no. 7A collected all results which obtained by newly developed (Ayah 6X1-ST-2D solar cell CFI –Analyzer) versus reference method as used for absorbance measurements using standard addition method and equation of the form $\hat{Y}$= a+bx ;in addition to practical values obtained from graph in term of concentration (column 14-table 7A).

Table 7B represent the form of two types of the t-test which is an analysis of two population means through the use of statistical examination for mean comparison were used.

-First comparison : based on the quoted value (µ) on the commercially available
(Individual t-test) drug sold in the market with the mean of analysis ($\bar{w}_i$)conductedusing the recently developed method.
a hypothesis can be estimated as follow:
Null hypothesis : There is no significant difference between the means ($\bar{w}_i$) obtained in all measured data with quoted value (µ)of officially British pharmacopeia[20].
i.e. ; H_0 : $\mu_{quoted\ value}$ (2 mg)=$\bar{w}_i$(for each different company).
Against
Alternative hypothesis: There is a significant difference between the means with quoted value
i.e. ; H_1: $\mu_{quoted\ value}$ (2 mg)≠$\bar{w}_i$(for each different company).
Since all values obtained are t_{tab}($t_{0.025,2}$(4.303))<$t_{value}$for each individual t-test ; these indicating clearly that there was a significant difference between the value was obtained from both methods and quoted value may be due to interferences effect.
Second comparison (scheme 2) : based on paired t-test for mean comparison i.e. ;
(paired t-test) based on the analysis of the same samples by two methods namely
the recently developed method and the reference method
The postulation is as follows:
H_0 = Null hypothesis =There is no significant difference between the means of both methods i.e. ; $\mu_{Ayah6SX1\text{- }ST\text{-}2D\ CFIA}$ = $\mu_{reference\ method}$
Against
H_1 (Alternative hypothesis):At leastone mean is different from the others
i.e. ; $\mu_{Ayah6SX1\text{- }ST\text{-}2D\ CFIA} \neq \mu_{reference\ method}$

From the results obtained that were tabulated in table 9 B shows that there is no significant difference between the means obtained in all data (i.e. ; measurements of the means of the two methods ; since the value of $t_{tab}(4.303) > t_{cal}(0.915)$.Therefore Null hypothesis will be accepted and will be rejected the alternative hypothesis.

Table 4.23 A: Results of Standard addition for the determination of vitamin B_2in three pharmaceutical preparations.

	Commercial name , Company Content Country	Type of method: Developed method using Ayah 6Sx1-ST-2D Solar cell CFIA $\bar{y}_i$(n=3)inmV; uv. vis Sp. Classical method Absorbance measurement at λ_{max}=445 nm $\bar{y}_i$(n=3)											
		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 0.010287gm (0.2mMol.L^{-1}) of the active ingredient	Theoretical content for the active ingredient at 95% (mg)	Vitamin B_2mMol.L^{-1} / Vitamin B_2µMol.L^{-1}						Equation of standard addition at 95% for n-2 $\hat{Y}_{i(mV)}=a\pm s_at+b\pm s_bt[B_2]_{mMol.L^{-1}}$ $\hat{Y}_i = a\pm s_at+b\pm s_bt[B_2]_{\mu Mol.L^{-1}}$	r r^2 $r^2\%$	Practical concentr-ation mMol.L^{-1} In 10 ml Practical concentr-ation mMol.L^{-1} in 100ml
					0	3ml	4 ml	5ml	6ml	7ml			
					0	0.6	0.8	1	1.2	1.4			
					0	0.2ml	0.3ml	0.4ml	0.5ml	0.6ml			
					0	4	6	8	10	12			
1	B-Plex B_2=2mg B_1=5mg B_6=1mg B_{12}=10mcg Samarra-Iraq	0.2783±0.0082	1.4313	2 ± 0.059	80	510	720	810	980	1120	82.162± 58.776+745.405±61.955[B_2]mMol.L^{-1}	0.9982 0.9964 99.64 %	0.1102 0.2204
					0.158	0.253	0.332	0.354	0.468	0.546	0.138 ±0.059+ 0.032± 0.008 [B_2]µMol.L^{-1}	0.9855 0.9712 97.12%	0.0043 0.2149
2	Safaplex B_2=2mg B_1=5mg B_6=1mg B_3=10mg ديالى / الصفاء	0.3099±0.0029	1.5944	2 ± 0.019	125	380	670	800	930	1080	75.541±159.831+706.351±168.477[B_2]mMol.L^{-1}	0.9856 0.9713 97.13%	0.1069 0.2139
					0.136	0.250	0.320	0.373	0.442	0.529	0.127± 0.026+ 0.032± 0.003 [B_2]µMol.L^{-1}	0.9972 0.9944 99.44%	0.0039 0.1972
3	B-Plexin B_2=2mg B_1=5mg B_6=1mg B_3=10mg B_{12}=10mcg Haryana-India	0.3077±0.0052	1.5828	2 ± 0.034	90	520	730	880	1010	1200	78.649±55.573+791.622±58.579[B_2] .L^{-1}	0.9986 0.9972 99.72%	0.0993 0.1987
					0.140	0.272	0.340	0.420	0.470	0.550	0.139±0.015+0.034±0.002[B_2]µMol.L^{-1}	0.9991 0.9982 99.82%	0.0041 0.2037

Ŷ:Estimated response in mV for developed method and absorbance for uv- vis spectrophotometric method, r:correlation coefficient, r^2:coefficient of determination ,r^2% :linearity percentage,sp : spectrophotometric method,$t_{0.025,\infty = 1.96\ at\ 95\%}$,t=2.776 for n-2.

Table 4.23B : Summary of results for paired t –test ,practical content and efficiency for determination ofvitamin B_2in three samples of pharmaceutical preparation using vit.B_2 -IO_4^- -2,4 DNPH system.

No.of sample	Type of method: Developed method using Ayah 6Sx1 –ST-2D solar cell CFIA(; uv.vissp classical method for Absorbance measurement at λ_{max}=445nm					
	Practical concentration mMol.L^{-1} in 10 ml Practical concentration mMol.L^{-1} in 100 ml Weight of B_2 In(g)	Weight of B_2 in tablet $_{i(g)} \pm 4.303\sigma_{n-1}/\sqrt{n}$w Weight of B_2 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 $(\bar{x}_i - \mu)\sqrt{n}/\sigma_{n-1}$	Paired t –test: Compared between two methods: $t_{cal}=\bar{X}d\ \sqrt{n}/\sigma_{n-1}$	t_{tab} at 95% confidence level(n-1)
	0.110 0.220	0.011±0.206	110.24%	21.811> 4.303	$\bar{X}d$ =0.0573	

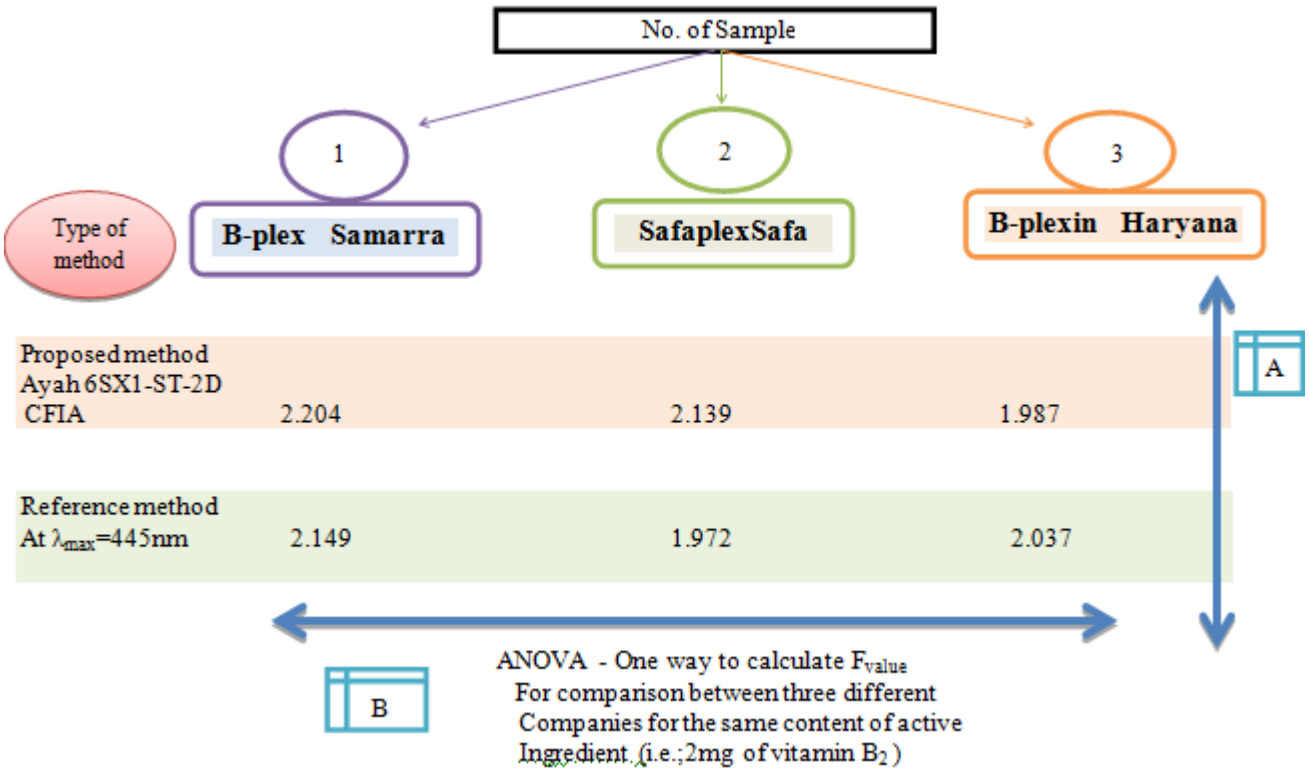
	0.011	2.204±0.040			σ_{n-1}=0.1085
1	0.004 0.215 0.011	0.011±0.1888 2.149±0.037	107.45%	17.321 > 4.303	
2	0.107 0.214 0.011	0.011±0.075 2.139±0.015	106.95%	40.806 > 4.303	0.915 < 4.303
	0.004 0.197 0.010	0.010±0.066 1.972±0.013	98.60%	/-9.268/ >4.303	
3	0.099 0.199 0.010	0.010±0.056 1.987±0.011	99.35%	-5.085/ >4.303/	
	0.004 0.204 0.011	0.011±0.102 2.037±0.020	101.85%	8.041 > 4.303	

μ:quated value 2 mg ,$\bar{w}$:practical content (mg) ,$\bar{x}$d:average of difference between two methods(proposed &reference methods),$t_{tab}=t_{0.05/2,n-1}$=4.303 for n(No.of samples)=3for paired t-test, σ_{n-1}:standard deviation of difference.

One way ANOVA (F-test)[21,22] was carried out at α =0.05 (95% confidence level)for comparing between three different samples (as the same active ingredient) from different companies . This test (i.e.; ANOVA) depend on calculated F-value for comparing three or more means.

The first estimated is called between group variance while the second estimated based on the within group variance.

The following hypothesis should be used.
H_0 (Null hypothesis): $\mu_{B\text{-plex-samarra}}=\mu_{safaplex\text{-}safa}=\mu_{B\text{-plexin-Haryana}}$
Against
H_1 (Alternative hypothesis): $\mu_{B\text{-plex-samarra}}\neq\mu_{safaplex\text{-}safa}\neq\mu_{B\text{-plexin-Haryana}}$
From the results obtained that were tabulated in table 8shows that the kind of three samples supplied by different companies is no significant difference between the means, which in concerning to the three different samples (i.e. ;different companies). Since the value of Sig >0.05 and F_{cal}(2.6098) <F_{tab}(9.55)and sig (0.221>0.05), therefore null hypothesis will be accepted and will rejected the alternative hypothesis.



Scheme 2
A: Paired t-test for comparison between new adopted method(Ayah 6SX1-ST-2D CFIA)and reference method atλ_{max}=445nm

B: Represent if there is a significant difference between three different companies for the same sample(vitamin B_2)

Table 8: Construction of ANOVA-One way for comparison between samples (drug) supplied by three different companies

Source of variation	Sum of squares SSq	df	Mean square (M_{sq})	F_{cal}	F_{tab}	S_{ig} at 95% confidence level
Between group	SSq_B=0.0291	2	0.01453	2.61<<9.55		0.221 i.e. ;S_{ig}>0.05 no significant difference
Within group	SSq_w=0.0167	3	0.00557			
Total	0.0458					

F_{tab}= $F_{0.95,\gamma1,\gamma2}$= $F_{0.95,2,3}$= 9.55, df = degree of freedom

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