Flow Injection Turbidimetric Determination of Vitamin B_1using LEDs as a Source of Irradiation and Two Solar Cells as an Energy Transducer

Proph. Nagam Shakir Al-Awadi[1], Rana Adnan Kamal Aldeen[2]

Department of chemistry, College of Science, University of Baghdad, Baghdad, Iraq

Abstract: ***A sensitive and simple method has been used for the determination of vitamin B_1 (thiamine chloride) in pure form and pharmaceutical tablets by flow injection turbidimetry. The method is based on formation of ion pair compound between thiamin chloride and ammonium molybdate in an aqueous medium to obtain a white precipitate complex, using homemadeAyah-6SX1-ST-2D solar cell CFI Analyzer. Optimum parameters have been studied to increase the sensitivity for developed method. The linear range for the calibration graph was 0.1-2 mMol.L^{-1} vitamin B_1 (VB_1) and LOD was 1.579µg/sample with correlation coefficient (r) was 0.9708, RSD% was lower than 0.5% the repeatability (n=8,6) for the determination of VB_1 at concentration (0.9,1.5) mMol.L^{-1} respectively .The developed method was applied successfully for the determination of VB_1 in pharmaceutical tablets. A comparison was made between two methods: developed method and the classical method UV spectrophotometry at λ_{max}=246 nm, by using the standard addition method via the use paired t-test .It showed that there was no significant difference between the developed method and the classical method for determination vitamin B_1at 95% confidence level.***

Keywords: Vitamin B_1, flow injection, turbidimetry, homemade instrument

1. Introduction

Vitamin B_1 (Thiamin) (figure 1) was named B_1 because it was the first B complex vitamin to be discovered [1,2] . Vitamin B_1 (VB_1) is a colorless organo sulfur compound with a chemical formula $C_{12}H_{17}N_4OS^+$. Its structure consists of an aminopyrimidine and a thiazole ring linked by a methylene bridge. The thiazole is substituted with methyl and hydroxylethyl side chains [3].

Thiamin is soluble in water, methanol, and glycerol. and practically insoluble in less polar organic solvents. It is stable at acidic pH, but is unstable in alkaline solutions [4]. Thiamin, which is a N-heterocyclic carbene, can be used in place of cyanide as a catalyst for benzoin condensation [5]. Thiamine is unstable to heat, but stable during frozen storage. It is unstable when exposed to ultraviolet light and gamma irradiation[6]. Thiamin reacts strongly in Maillard-type reactions [3].

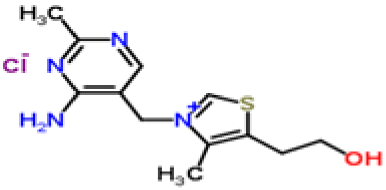


Figure 1: Structure of VB_1 (Thiamin Chloride) IUPAC name3-((4-Amino-2-methyl-5-pyrimidinyl) methyl)- 5-(2-hydroxyethyl)-4-methylthiazolium chloride

There are various analytical methods for determination of VB_1 . These analysisinclude :HPLC[7-9], Liqchromatography –Tandem mass spectrophotometry[10] ,spectrophotometric [11-16], electrochemical technique[17], flourimetry [18] and Chemilumenescence [19], Luminescence emitting nano rods[20]

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 VB_1-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.

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 0.01 Mol.$L^{-1}$of vitamin B_1(thiamin chloride): molecular formula $C_{12}H_{17}ClN_4OS$, molar mass 300.8076 g mol^{-1} & SDI-Iraq was prepared by dissolving 0.3008 g of thiamin chloride in 100 ml of distilled water. A stock solution 0.01 Mol.$L^{-1}$of Ammonium molybdate (Am) molecular formula $(NH_4)_6Mo_7O_{24}.4H_2O$, molar mass 1235.58g mol^{-1} & CABLO EBBA- England was prepared by dissolve 6.1779 g in 500 ml of distilled water.

Sample Preparation

Twenty tablets were weight then crushed and grinded .Tablets containing (100,200)mg of vitamin B_1were weighted 0.5098g,0.6947g(equivalent to 0.3008g of active ingredient,10mMol.L^{-1})for Samavit Samarra Iraq &Neurorubi ne-fort Switzerland, respectively and dissolved in distilled water.The solution was filtered to get rid of undissolvedmaterials,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[21], which used a six snow-white light emitting diodes LEDs for irradiation of the flow cell at 2 mm path length. The flow system used for the determination of vitamin $B_1$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_1 using 4 cm quartz cell.

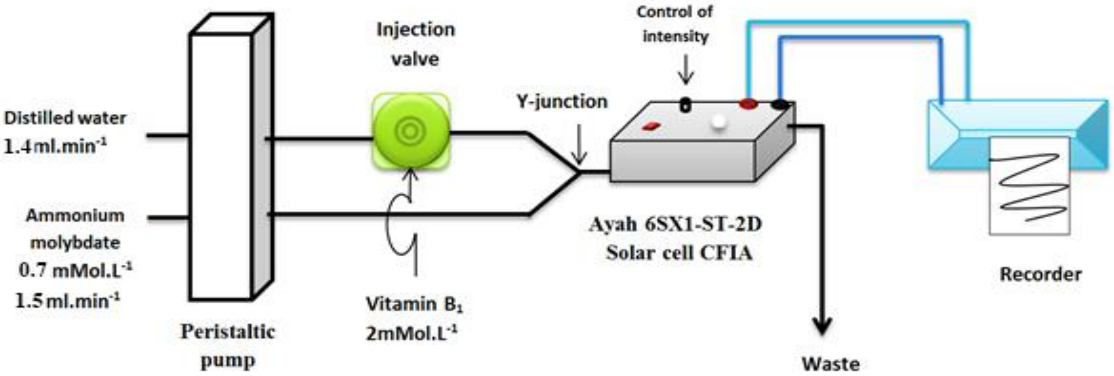
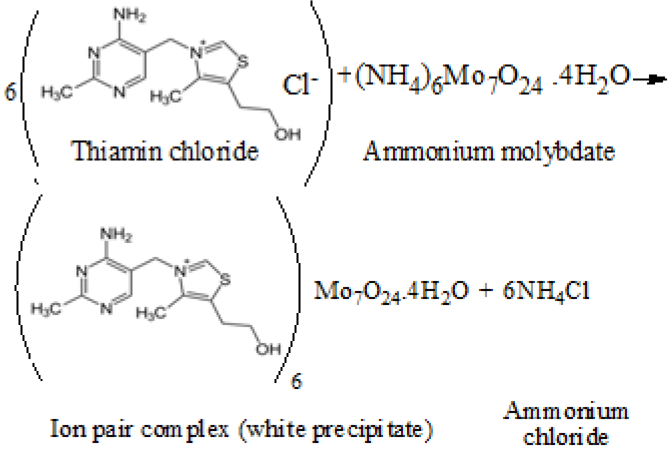


Figure 2: Flow system used for determination of VB_1using homemade Ayah 6SX1- ST-2D solar cell CFIA at 210µl, open valve mode & applied voltage to the LEDs 1.686volt DC

3. Methodology

Two lines manifold system was used as shown in Figure no.2.The design pattern show in the mentioned figure was used for the determination of VB_1 .The main expected chemical reaction is the formation of ion pair complex as a white precipitate. Via the reaction of ammonium molybdate with vitamin B_1 (no other chemicals were involved)in the reaction pattern; the vitamin B_1 sample (210µl)was carried out via the distilled water (which constitute the first line feed in the used system while ammonium molybdate was fed through the second line. Used flow rate and concentration depicted in figure no.2 .To induce the chemical reaction a Y-junction (mode of PMMA plexiglass) was connected for the reaction of vitamin $B_1$with ammonium molybdate and the reaction come into being. The measurements were accomplished by Ayah 6SX1- TS - 2D Solar cell-CFIAnalyzertriplicate successive measurements were used. The proposed reaction for vitamin B_1 with Am is represented in scheme 1[22,23].



Scheme 1- Probable reaction between vitamin B_1 (Thiamin. Cl) and Ammonium molybdate (Am).

4. Result and Discussion

Optimization of reaction pattern parameters

To maximize the efficiency of adopted methodology .A chemical as well as physical constrains that will affect on the sensitivity of a clear profile of response .The chemical variable is the concentration of ammonium molybdate with physical variables include: Flow rate, sample volume, coil length & intensity of light.

Chemical variable

Ammonium molybdate (Am) concentration

Aseries of the precipitating reagent (Am) having the concentration of 0.1-3 mMol.L^{-1}wereprepared atconstant concentration of vitamin B_1 (2mMol.L^{-1}),251.2µl sample volume , 1.8& 2 ml.min^{-1} flow rate for carrier stream & reagent respectively .The intensity of incident light of LEDs 1.650 V was used .Fig 3 A shows the increase in Am concentration leads to increase in the peak height due to the effect of formation of tiny solid particulate that might increase the reflection of incident light ,that at the end will cause increase on incident light intensity up to 0.7mMol.L^{-1}.More than (> 0.7mMol.L^{-1}), at can be seen attenuation of incident light due to coagulation of precipitate particles ,which in turn to decrease in the reflection of incident light .Therefore 0.7mMol.L^{-1} Am concentration was regarded as the optimum concentration .Table 1 summarizes the total results obtained .Fig 3B shows the effect of variable Am concentration on energy transducer response.

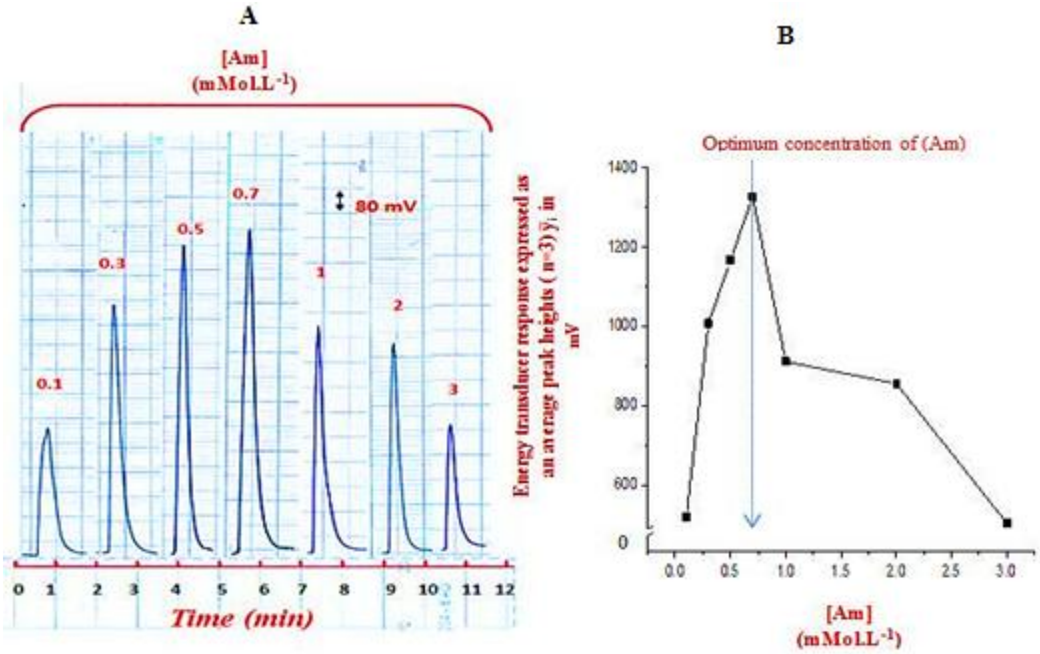


Figure 3: Variation of Am concentration on:

A: Profile of Am –vitamin B_1 system.
B: Height of energy transducer response expressed as an average peak heights (n=3).

Table 1: Effect of Am concentration on the measurement of energy transducer response via reflection of incident light for the determination of vitamin B_1

[Am] mMol.L^{-1}	Energy transducer response expressed as an average peak heights (n=3) $\bar{y}_i$ in mV	RSD%	Confidence interval at 95% $\bar{y}_i \pm t_{0.05/2,n-1}\sigma_{n-1}/\sqrt{n}$
0.1	520	0.159	520 ± 2.062
0.3	1008	0.091	1008 ± 2.286
0.5	1168	0.088	1168 ± 2.559
0.7	1328	0.083	1328 ± 2.708
1	912	0.123	912 ± 2.782
2	856	0.130	856 ± 2.758
3	504	0.252	504 ± 3.155

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

Effect of 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 the determination of vitamin B_1 at 2 mMol.L^{-1} was studied . While keeping all other variables constant (i.e . 251.2µl sample volume, Am concentration 0.7mMol.L^{-1}) .From fig 4 A, even though that slow flow rate (<1.4 ml.min^{-1} for carrier stream) enhance sensitivity in a clear way ,but a wider in base of response (increase Δt_B)was obtained while a sharp peak was obtained at higher flow rate. Acompromise between enhanced sensitivity and good profile response, therefore 1.4&1.5 ml.min^{-1} for carrier stream and Am line respectively was chosen as an appropriate flow rate for this specific work as shown in table 2 and fig 4 B.

Physical variables:

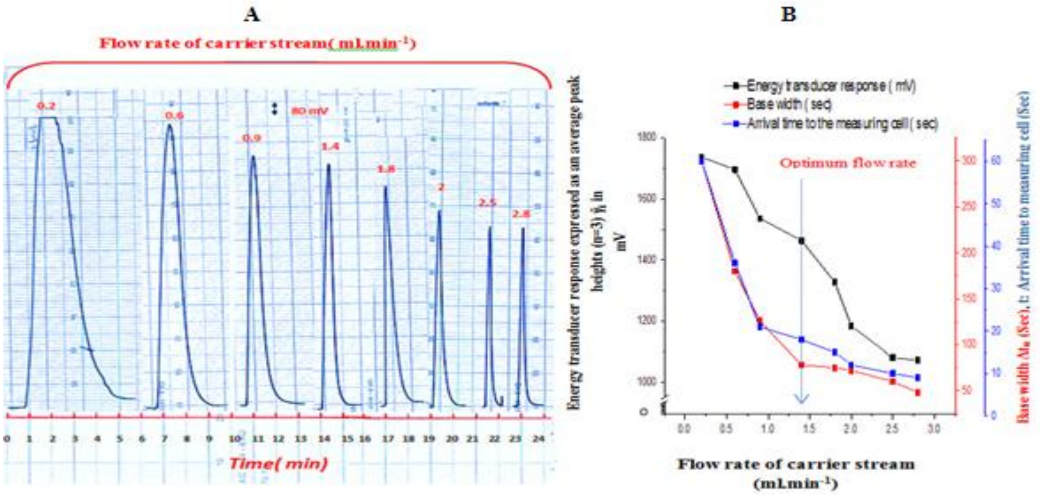


Figure 4: Influence of variation of flow rate on:

A: Response profile for Am-Vitamin B_1system.
B: Energy transducer response, using 2mMol.L^{-1} vitamin B_1 concentration.

Table 2: Effect of flow rate on the measurements of energy transducer response for determination of VB_1

Pump speed	Flow rate ml.min^{-1}		Energy transducer response expressed as an average peak heights(n=3) $\bar{y}_i$ in mV	RSD%	t* Sec	Base width Δt_B Sec	Confidence interval at 95% $\bar{y}_i \pm t_{0.05/2,n-1}\ \sigma_{n-1}/\sqrt{n}$
	Line 1 (Carrier Stream)	Line 2 (Am)					
5	0.2	0.4	1736	0.022	60	300	1736±0.969
10	0.6	0.7	1696	0.050	36	180	1696±2.111
15	0.9	1.0	1536	0.069	21	126	1536±2.658
20	1.4	1.5	1464	0.074	18	78	1464±2.707
25	1.8	2.0	1328	0.084	15	75	1328±2.782
30	2.0	2.2	1184	0.097	12	72	1184±2.857
35	2.5	2.7	1080	0.110	10	60	1080±2.956
40	2.8	3.0	1072	0.187	9	48	1072±4.993

t*:Arrival time from injection valve reaching to measuring cell (Sec), Δt_B : Base width of peak response(Sec), $t_{0.05/2,2}$ =4.303

Effect of Sample Volume

Using 2 mMol.L^{-1} vitamin B_1, 0.7mMol.L^{-1}Am concentration, flow rate 1.4&1.5 ml.min^{-1} for carrier stream(distilled water) and reagent (Am) respectively and applied voltage of LEDs was 1. 650 volt DC. The injected volume of sample was varied in the range of 110-310µl using open valve mode .It was noticed (fig 5 A) that an increasing in the sample volume led to a significant increase in peak height up to 210µl.All results are tabulated in table 3.Using sample volume more than 210µl, a slight decrease was observed and increase of base width (Δt_B), probably due to along duration of carrier stream to passes through injection valve causing a restriction of the flow, which lead to increase of dispersion of the precipitate particles segment and increase of Δt_B. So, 210µl was the best sample volume (fig 5 B).

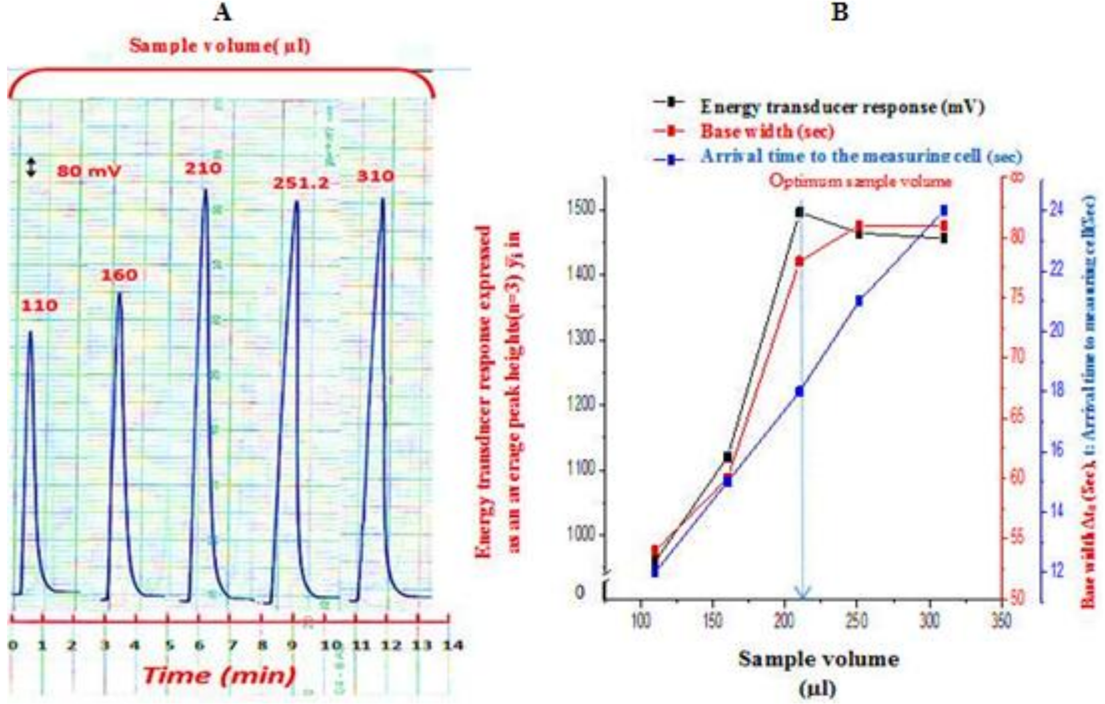


Figure 5: Variation of sample volume on:

A: Response profile versus time

B: Variation of energy transducer response by reflection of incident light for vitamin B_1

Table 3: Effect of the variation of sample volume loop on the measurement of energy transducer response via reflection of incident light for determination of vitamin B_1

Length of Sample segment Cm r=0.5mm	Sample Volume µl	Energy transducer response expressed as an average peak heights (n=3) $\bar{y}_i$ in mV	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2,n-1}\ \sigma_{n-1}/\sqrt{n}$	Base width Δt_B sec	t* sec
14.0	110.0	960	0.093	960±2.211	54	12
20.4	160.0	1120	0.070	1120 ±2.112	60	15
26.8	210.0	1496	0.069	1496±2.584	78	18
32.0	251.2	1464	0.070	1464± 2.534	81	21
39.5	310.0	1456	0.078	1456± 2.807	81	24

t*:Arrival time from injection valve reaching to measuring cell (Sec), Δt_B : Base width of peak response(Sec), $t_{0.05/2,2}$=4.303

Effect of Reaction Coil

Variable coil length 0-50 cm was study. 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_1: 2mMol.L^{-1}; Am concentration 0.7 mMol.L^{-1}, flow rate 1.4 &1.5 ml.min^{-1} for carrier stream(distilled water) and reagent(Am) respectively, sample volume 210µl and applied voltage of LEDs was 1.650 volt DC.Fig 6A shows the increase of coil volume lead to decrease of peak height,with increase of base width and departure time for sample segment from injection valve to measuring cell, this might be attributed to affect of diffusion & dispersion on the precipitate particulate segment causing increase of dispersion regions and mostly lead to accumulation of precipitate particles which in turne to cause lose of some of the reflecting surface. So, it can be seen clearly that no reaction coil was selected for further work (Table 4 & fig 6B).

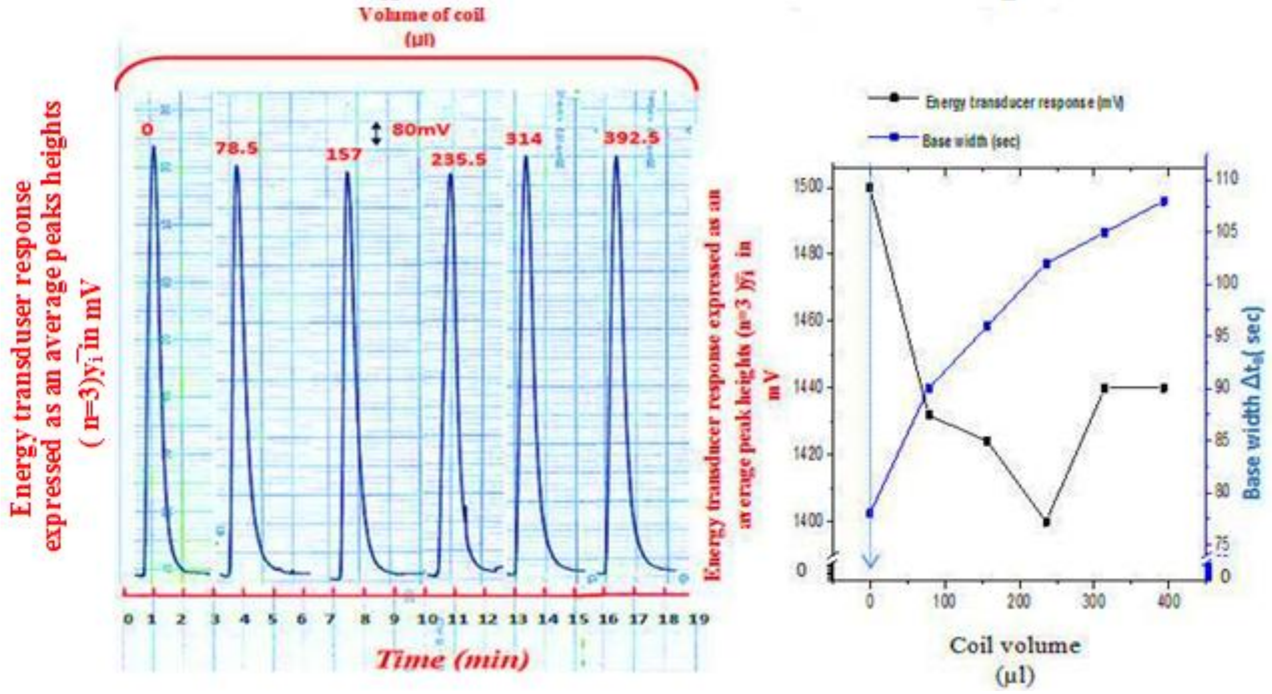


Figure 6: Influence of variation of coil volume on the:

A: Instrument response.

B: Output of energy transducer response.

Table 4: Effect of coil volume on reflection of incident light

Coil Length Cm r=0.5mm	Coil Volume µl	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	Base width Δt_B sec	V_{add} ml at flow cell	Concentration mMol.L^{-1} At flow cell
0*	0*	1500	0.072	1500±2.708	18	78	3.980	0.105
10	78.5	1432	0.075	1432 ± 2.683	21	90	4.560	0.092
20	157.0	1424	0.069	1424 ± 2.460	24	96	4.850	0.087
30	235.5	1400	0.094	1400 ± 3.279	24	102	5.140	0.082
40	314.0	1440	0.099	1440 ± 3.553	27	105	5.285	0.079
50	392.5	1440	0.105	1440 ± 3.776	27	108	5.430	0.077

0* Without coil, t*: Arrival time from injection valve reaching to measuring cell (Sec), Δt_B : Base width of peak response(Sec), $t_{0.05/2,2}$=4.303 .

Effect of light Intensity

Variation of light source intensity on the efficiency for determination of VB_1 at 2 mMol.L^{-1} was studied .While keeping all other variables fixed (210 µl sample volume , Ammonium molybdate 0.7mMol.L^{-1} ,1.4& 1.5 ml.min^{-1} flow rate for carrier stream and reagent line respectively . The applied voltage to the LEDs was ranged (0.593 -1.852)volt DC by variation of light intensity . The whole process was monitored by AVO-meter.Fig 7A shows a profile and the results were tabulated in Table 5which shows that an increase on the energy transducer response with increase intensity of light source.Therefore the intensity of 1.686 volt DC was selected as the optimum voltage that can be supplied to give a better peak height and for the sake of the compromise between sensitivity and instrument life time. Fig 7B shows the effect of intensity of light on the measurement of energy transducer respon.

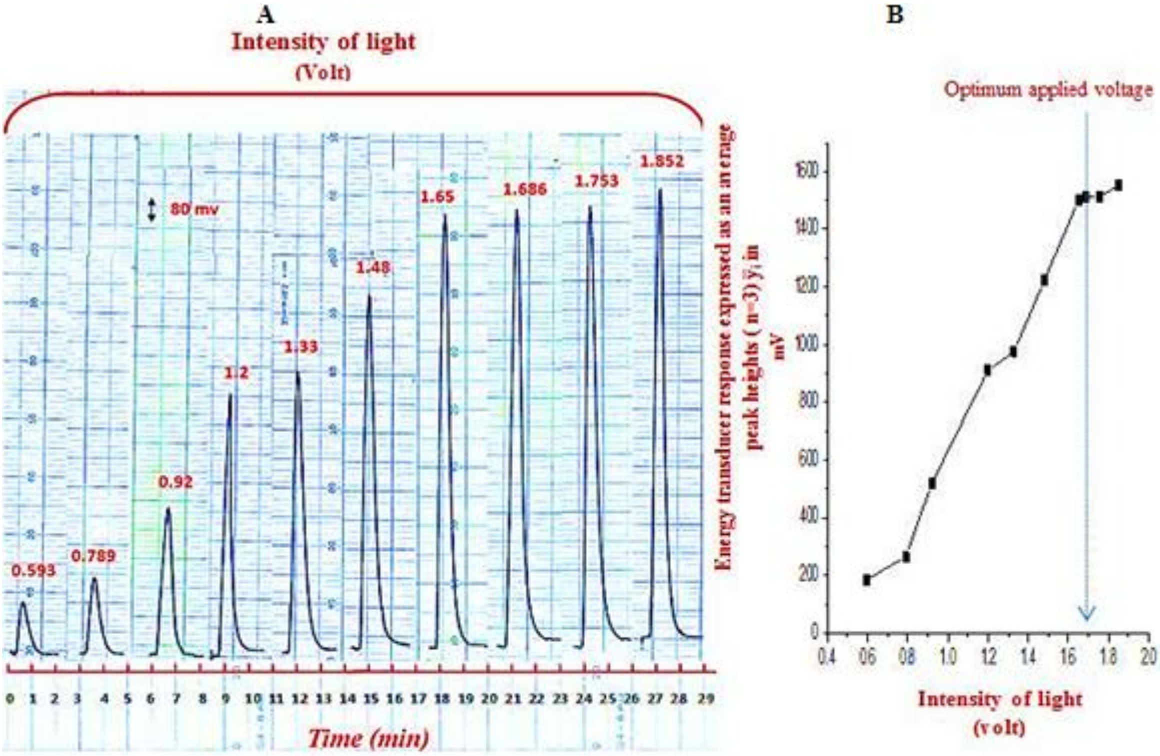


Figure 7: Effect of intensity of light on:

A : Instruments response profile.

B : Variation of energy transducer response by reflection of incident light

Table 5: Effect of coil volume on reflection of incident light

Intensity of light (volt)	Energy transducer response expressed as an average peak heights (n=3) $\bar{y}_i$ in mV	RSD%	Confidence interval at (95%) $\bar{y}_i \pm t_{0.05/2,n-1}\sigma_{n-1}/\sqrt{n}$
0.593	184	0.511	184± 2.335
0.789	264	0.371	264± 2.435
0.920	520	0.158	520± 2.037
1.200	912	0.080	912± 1.814
1.330	976	0.050	976± 1.217
1.480	1224	0.053	1224± 1.615
1.650	1500	0.073	1500± 2.720
1.686	1512	0.079	1512± 2.967
1.753	1512	0.083	1512± 3.118
1.852	1552	0.085	1552± 3.277

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

Study of the variation of vitamin B_1 concentration on the energy transducer response:

A series of vitamin B_1 solutions ranging (0.1-10) mMol.L^{-1} were prepared with fixed optimum parameters Fig 8A shows response profile for this study. A scatter plot diagram explain that a linear calibration graph range for the variation of the energy transducer response of Ayah 6SX1-ST-2D solar cell CFI Analyzer with B_1 concentration was ranging from 0.1- 2 mMol.L^{-1} (Fig 8 B). It was noticed, above 2 mMol.L^{-1} a broad in the maxima of the peak , this cause a deviation of correlation coefficient . Its attributed to the an increase in precipitate particulats and its compactness, thus leading to decrease interstitial spaces and reflecting surface , in addition to an increase of particle size causing a slow movement of precipitate particles leading to a longer time duration of precipitate segment in front of the detector. Table 6 summarizes the results including: correlation coefficient ,linearity percentage,straight line equation and the calculated t-value at 95% confidence level ,in which t_{cal} larger than t_{tab} at optimum conditions indicating that the linearity of calibration curve is accepted .Fig 8 C shows the calibraton curve of classical method.In classical spectrophotometric method [11] via the measurement at λ_{max} = 246 nm ,in which observed that, the linear calibration curve was ranged from 0.1-10 m Mol.L^{-1}.

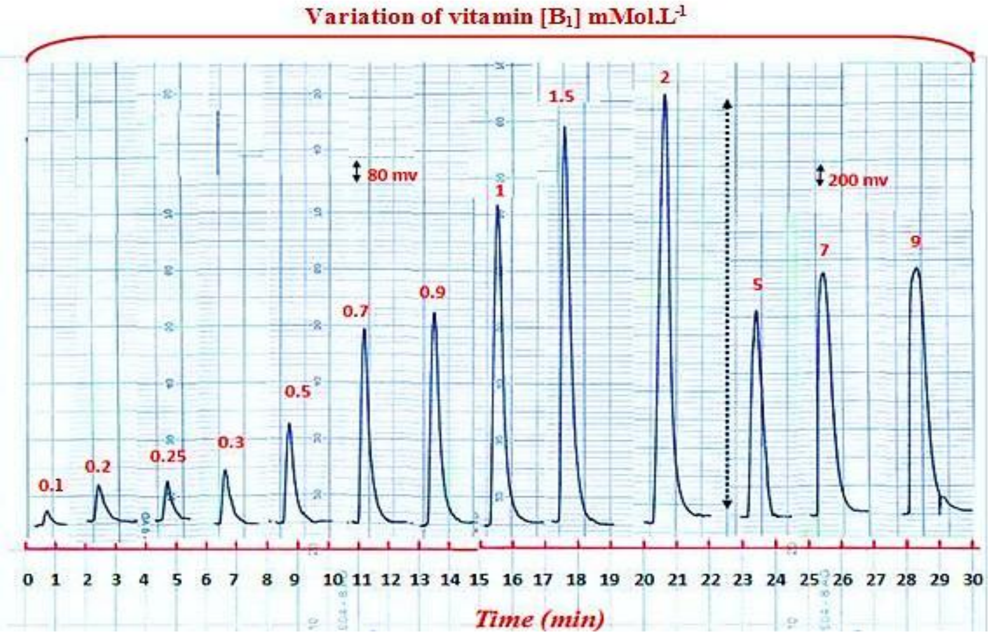


Figure 8 A: Profile for the variation of vitamin B$_1$ concentration versus time

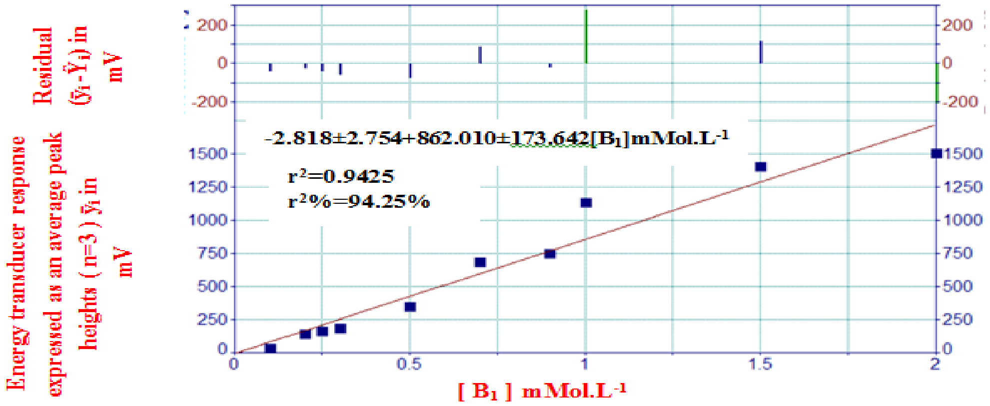


Figure 8 B- calibration graph for the variation of vitamin B$_1$ concentration on the: energy transducer response by reflection of incident light expressed by linear equation using Ayah 6SX1-TS-2D solar cellCFI Analyzer.(Developed method), Residual($\bar{y}_i$ - $\hat{Y}_i$);$\bar{y}_i$: Practical value,$\hat{Y}_i$: estimated value.

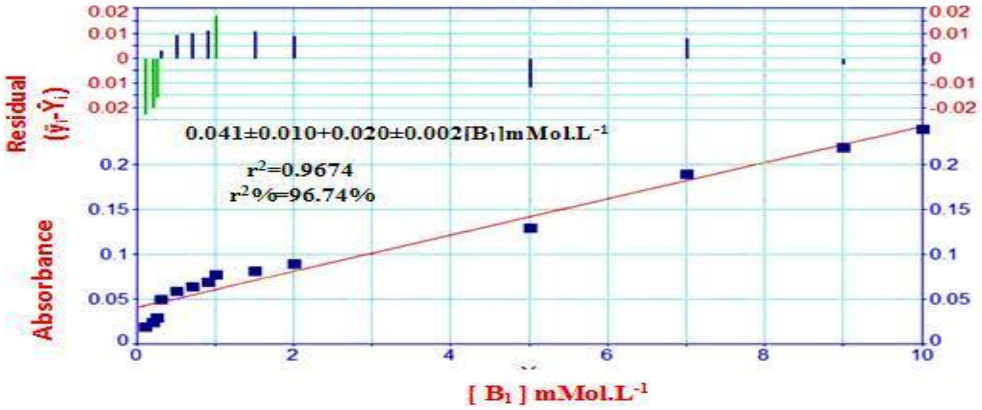


Figure 8 C: calibration graph of classical method (uv-sp) at λ_{max} = 246 nm

Table 6: Summary of linear regression for the variation of energy transducer response with vitamin B_1concentration using first degree equation of the form $\hat{Y} = a + bx$at optimum conditions

Type of measurement	**Measured $[B_1]$ $mMol.L^{-1}$**	**n**	**Linear Range of $[B_1]$ $mMol.L^{-1}$**	**$\bar{y}_i = a \pm s_a t + b \pm s_b t[B_1] mMol.L^{-1}$ 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	0.1 – 10	10	0.1 – 2	$-2.818 \pm 2.754 + 862.009 - \pm 173.642 [B_1] mMol.L^{-1}$	0.9708 0.9425 94.25%	2.306<11.448	
Classical method UV- SP λ_{max}=246nm	0.05 –10	14	0.1-10	$0.041 \pm 0.010 + 0.020 \pm 0.0024 [B_1] mMol.L^{-1}$	0.9836 0.9674 96.74%	2.179 <18.869	

$\hat{Y}$: Estimated response in mV for n=3 expressed as an average peak heights or absorbance value for classical method of linear equation of the form $\hat{Y}=a+bx$, r: correlation coefficient , r^2: coefficient of determination, r^2% : linearity percentage. sp: spectrophotometry. Developed method :using 6SX1-ST-2D solar cell CFI Analyzer, n: no.of measurements.

5. Limit of detection (L.O.D)

L.O.D of an analyte described as that : concentration which gives an instrument signal (y) significantly different from the blank or back ground signal . This description gives the exact definition of L.O.D . There is an increasing trend to define the L.O.D. as: the analyte concentration giving a signal equal to the blank signal, depend on the following : L.O.D = $\bar{Y}_b + 3S_b$ using three approaches for the expression of L.O.D

1) Gradual dilution: based on gradual dilution of the lowest concentration used in calibration graph, this should be regarded as the real, and trustable value of D.L. (i.e . reliable D.L. for the proposed method).
2) Theoretically (slope method) L.O.D. =$3S_B$/slope $S_B = \sigma_{(n-1)B}$ (standard deviation of blank n=13)
3) Theoretically (Linear equation) method $\hat{Y} = Y_b + 3S_b$, Y_B (average response for the blank solution, this is equivalent to intercept (a) in straight line equation y=a+bx) [24]. The last two methods are an output of a linear regression graph treatments where the obtained (real results are subjected to statistical treatments), these method can be used as an approximate indication but should not unless otherwise defined. A study was carried out to calculate the limit of detection of vitamin B_1 through three methods as tabulated in Table 7.

Table 7: limit of detection for vitamin B_1 at optimum parameters in different approaches using210µl sample volume

Practically based on the gradual dilution for the minimum concentration in calibration curve (0.025)$mMol.L^{-1}$	**Theoretical (slope method) based on the value of slope $X=3S_B$/slope**	**Theoretically (linear equation)based on the value of $\hat{Y}=Y_b+3S_b$**
1.579 µg/sample	65.949 ng/sample	30.758µg/sample

X :value of L.O.D based on the slope ,S_B :standard deviation of blank repeated for 13 times , Y_b: average response for blank = intercept (a) , 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 and six successive injections were measured at fixed concentrations of vitamin B_1 for two concentrations were used (0.9, 1.5)$mMol. L^{-1}$ respectively in optimum parameters.Fig 9 A, B and the obtained results is tabulated in Table 8. The percentage of relative standard deviation lessthan 0.5% indicate clearly, the high efficiency of the new method.

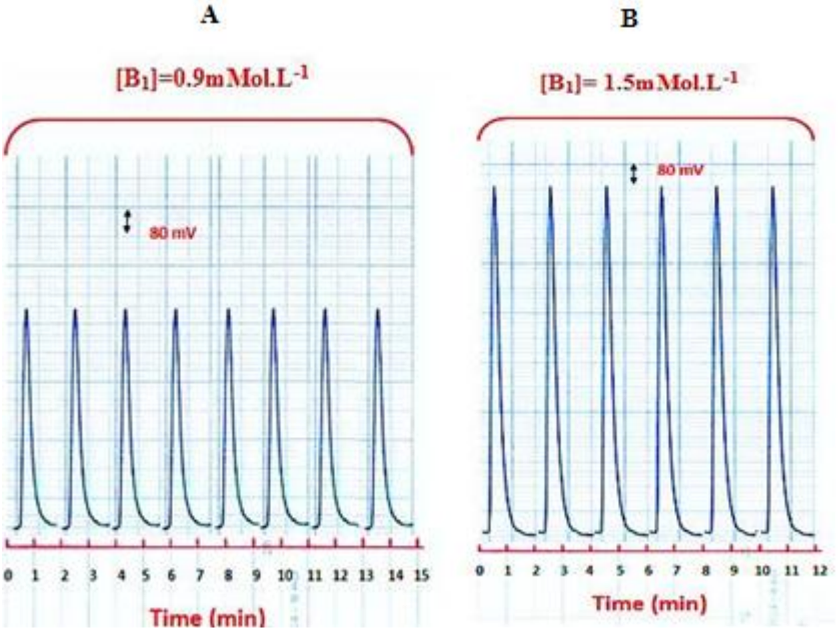


Figure 9: Response profile of repeatability of vitamin B_1 in different concentration:

A: 0.9mMol.L^{-1}
B:1.5 mMol.L^{-1}

Table 8: Repeatability of vitamin B_1at optimum parameters with 210µl sample volume of VB_1 .

$[B_1]$ mMol. L^{-1}	Energy transducer Response expressed as an average peak heights(n=3) $\bar{y}_i$in mV	RSD%	$\bar{y}_i \pm t_{0.05/2,n-1}\sigma_{n-1}/\sqrt{n}$ at confidence interval 95%	Number of injection
0.9	752	0.136	752±0.853	8
1.5	1408	0.103	1408±1.522	6

$t_{0.025,7}$ =2.365 , $t_{0.025,5}$ =2.571, n=number of injection

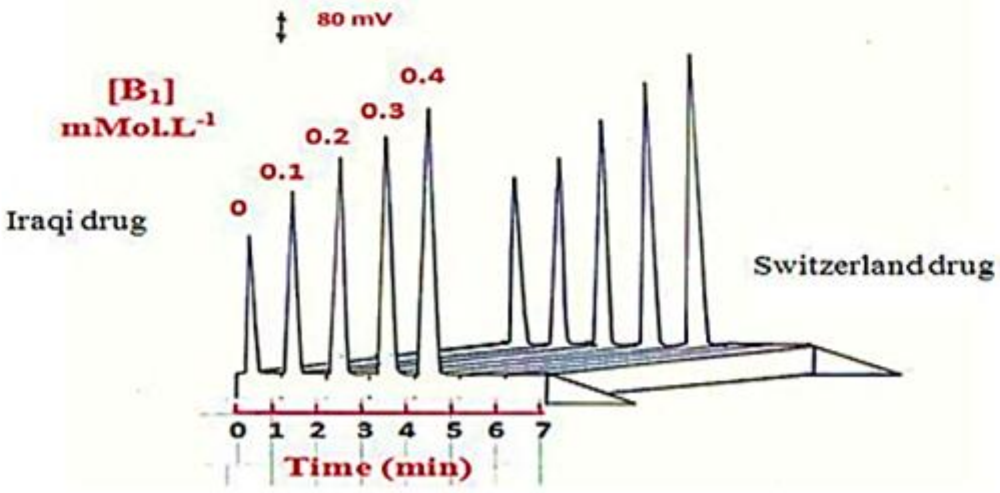
Analysis of vitamin B_1in pharmaceutical tablets

The CFIA via turbidity measurement expressed as an energy transducer response by reflection of incident light at 0-180° using Ayah 6SX1-ST-2D solar cell CFI Analyzer a achieved in this work were used for the analysis of VB_1 in two different of pharmaceutical preparations (Samavit-Samarra-Iraq &Neurorubine-forte-Switzerland). The results were compared by UV-Spectrophotometric method [11]via the measurement of λ_{max} at 246 nm and developed method (using Ayah 6SX1-ST-2D solar cell CFI Analyzer) .

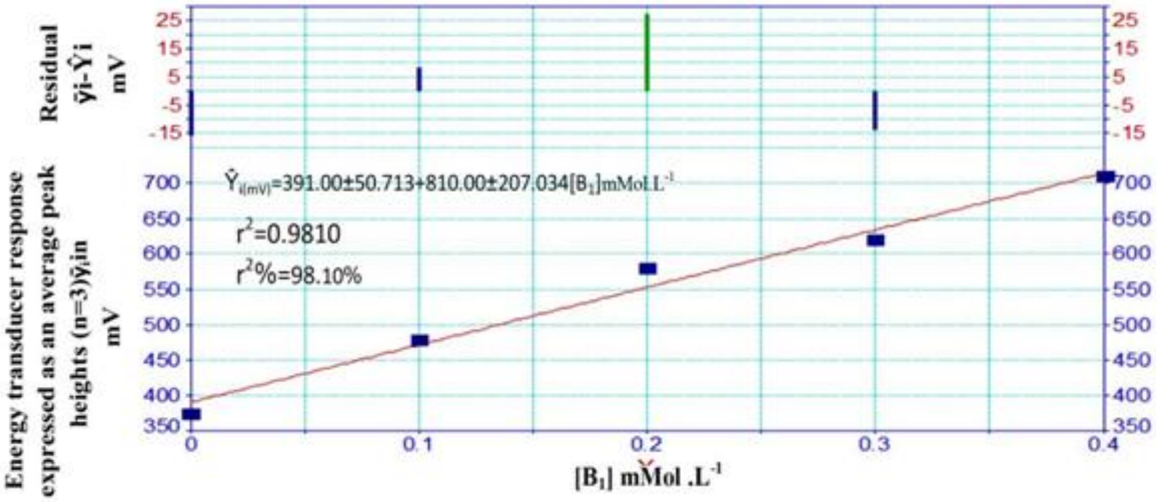
Aseries of solutions were prepared of each pharmaceutical drug (0.01Mol.L^{-1}) by transferring 1.25 ml to each five volumetric flask (25 ml), followed by the addition of gradual volumes of standard VB_1(0, 0.25, 0.5 , 0.75 ,1) ml of 0.01Mol.L^{-1} to obtain (0, 0.1, 0.2, 0.3, 0.4) mMol.L^{-1} for developed method. And (0, 1.5, 1.75 ,2 ,2.25)ml volumes of standerdVB_1(0.01 Mol.L^{-1})for classical method to obtain(0,0.6 ,0.7 ,0.8 ,0.9)mMol.L^{-1} . Flask no.1 is the sample .The measurements were conducted by both methods. Results were mathematically treated for the standard addition method.

Fig 10 A,B&D: Was shown responses profile and calibration graphs for standard addition method using developed method .

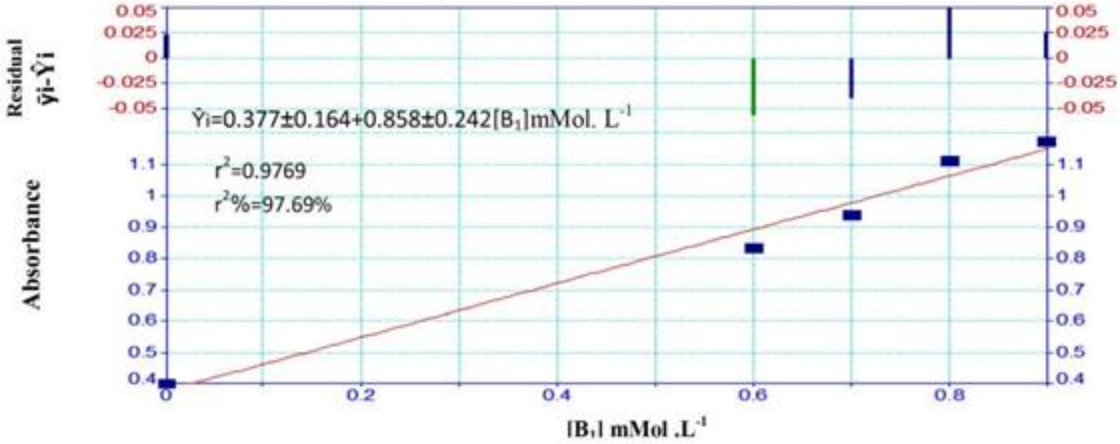
Fig 10 C, E: Calibration curve s using classical method for different two drugs of vitamin B_1.



A



B



C

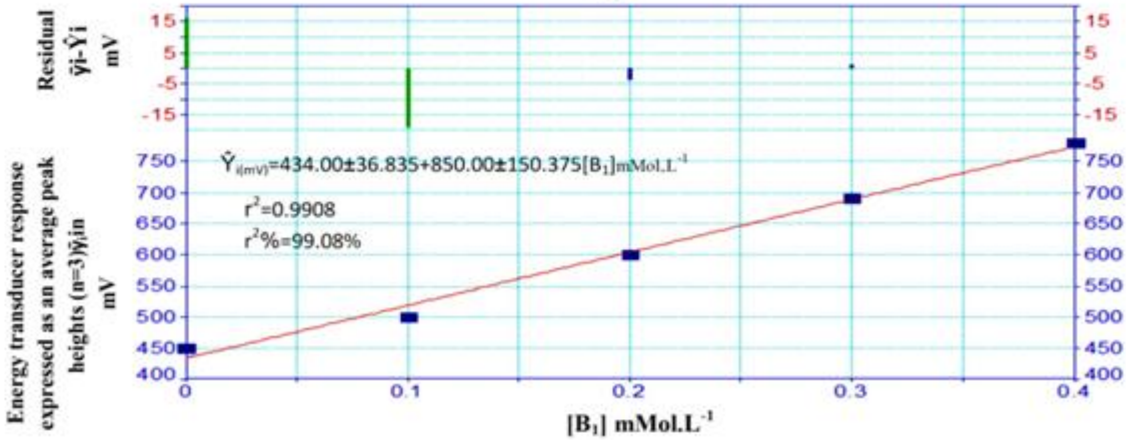
Figure 10: Effect of variation of vitaminB_1 concentration (using standard addition method)on:

A: Sample of response profile .

B: Energy transducer response (developed method).

C: Absorbance (classical method). For Iraqi drug.

D



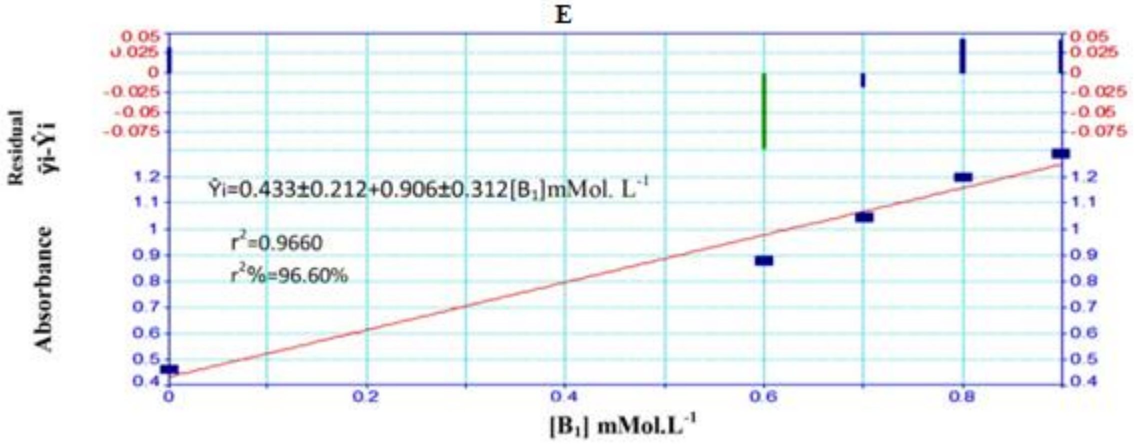


Figure 10: Standard addition calibration curve for Switzerland drug using two methods:

D: Developed method
E: Classical method.

Table9A was shown a practical concentration of vitamin B_1in 25 ml &100 ml respectively while table 9B showing a practical content of active ingredient in mg at 95% confidence level and efficiency of determination in addition to paired t-test at confidence interval 95% .From the results obtained can be a comparison at two different paths:

First path: comparison of value from newly developed method with official quoted value[25] (100mg&200mg)as shown in table 9B by calculated t_{value} of each individual company and these comparison with tabulated t-value (4.303).
Assumption as follow:

Null hypotheses: There is no significant difference between mean obtained from each drug from two different companies ($\overline{x}_i$) and quoted value (µ)

i.e$H_0 : \bar{x}_i = \mu$
Against
Atternative hypotheses: There is a significant difference between the means and quoted value
i.e$H_0 : \bar{x}_i \neq \mu$

It was noticed (column 5 in table 9B)that there were a significant difference between the quoted value and the measured value ,Since calculated t_{value} more than t_{tab}(4.303)at confidence level 95% of each individual t-test using two methods. So, null hypothesis will be reject and accepted Alternative hypothesis, it may be attributed to interferences effect .

Second path : paired t-test was used in order to compare between two methods of analysis as shown in table 9 B (column 6),taking in to consideration that two drugs are of the same population standard, i.e: neglecting individual differences between one and another manufacturer (scheme 2).
Assumption:

Null hypothesis(H_o): $\mu_{uv\text{-}sp} = \mu_{Ayah\ 6SX1\text{-}ST\text{-}2D}$
Against

Alternative hypothesis(H_1)= $\mu_{uv\text{-}sp}$# $\mu_{Ayah\ 6SX1\text{-}ST\text{-}2D}$

6. Conclusion

From the results, it was noticed that two methods of analysis proved to indicate that there is no significant difference between the means (µ)of the two different methods for analyzing two drugs
Since calculated t_{value} less than t_{tab} (i.e: 5.006<12.706),therefore ,H_o is accepted against H_1 .So, any one of them can be used for analysis vitamin B_1.

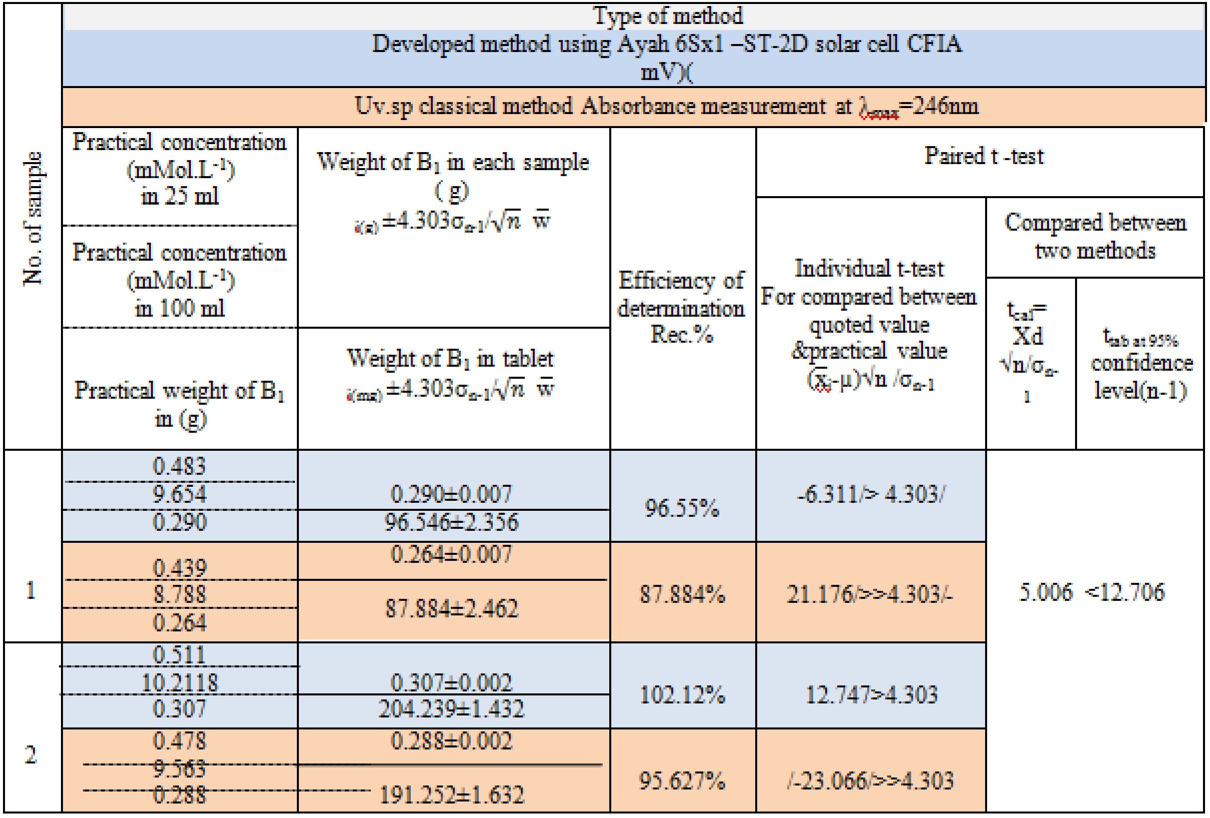
Table 9 A: Standard addition results for the determination of B_1in two pharmaceutical preparation using Am-VB_1 system.

No. of sample	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}=246 nm										
		Confidence interval for the average Weight of Tablet $\overline{W} \pm 1.96\sigma_{n-}$	Weight of sample equivalent to 0.3008 gm(10 $mMol.L^{-1}$)of the active	Theoretical content for the active ingredient at 95% (mg)	Vitamin B_1mMol.L^{-1}					Equation of standard addition at 95% for n-2 $\hat{Y}_{i(mV)}=a\pm s_a t+b\pm s_b t[B_1]_{mMol.L^{-1}}$ $\hat{Y}_i = a\pm s_a t+b\pm s_b t[B_1]_{mMol.L^{-1}}$	r r^2 r^2%	Practical concentration mMol.L^{-1} In 25 ml Practical concentrationmMol.L^{-1}
					0	0.25 ml	0.5ml	0.75ml	1ml			
					0	0.1	0.2	0.3	0.4			
					0	1.5ml	1.75 ml	2ml	2.25 ml			

		$_1/\sqrt{n}$ at 95% (g)	ingredient		0	0.6	0.7	0.8	0.9			in 100ml
1	Samavit B_1 100 mg Samarra Iraq	0.1695±0.00199	0.5098	100±1.1741	375	480	580	620	710	391.0± 50.713+810.0±207.034[B_1]mMol.L^{-1}	0.9905 0.9810 98.10 %	0.4827 9.6543
					0.400	0.834	0.937	1.113	1.176	0.377 ±0.164 + 0.858± 0.242 [B_1]mMol.L^{-1}	0.9884 0.9769 97.69%	0.4394 8.7882
2	Neurorubine-forte 200 mg B_1 Switzer Land B_6=50mg B_{12}=1mg	0.4619±0.0021	0.6947	200±0.9093	450	500	600	690	780	434.0± 36.835+850.0± 150.375[B_1]mMol.L^{-1}	0.9954 0.9908 99.08%	0.5106 10.2118
					0.465	0.879	1.048	1.200	1.290	0.433± 0.212+ 0.906 ± 0.312 [B_1]mMol.L^{-1}	0.9829 0.9660 96.60%	0.4781 9.5627

Ŷ: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% ,$t_{0.025,3}$=3.182 for n=5.

Table 9 B: Summary of results for practical content ,efficiency(Rec%) for determination of vitamin B_1in two samples of pharmaceutical and paired t-test preparation using two methods



No. of sample	Type of method					
	Developed method using Ayah 6Sx1 –ST-2D solar cell CFIA mV)(					
	Uv.sp classical method Absorbance measurement at λ_{max}=246nm					
	Practical concentration (mMol.L^{-1}) in 25 ml Practical concentration (mMol.L^{-1}) in 100 ml Practical weight of B_1 in (g)	Weight of B_1 in each sample (g) $_{i(g)} \pm 4.303\sigma_{n-1}/\sqrt{n}\ \bar{w}$ Weight of B_1 in tablet $_{i(mg)} \pm 4.303\sigma_{n-1}/\sqrt{n}\ \bar{w}$	Efficiency of determination Rec.%	Paired t -test		
				Individual t-test For compared between quoted value &practical value $(\bar{x}_i-\mu)\sqrt{n}/\sigma_{n-1}$	Compared between two methods	
					t_{cal}= Xd $\sqrt{n}/\sigma_{n-1}$	$t_{tab\ at\ 95\%}$ confidence level(n-1)
1	0.483 9.654 0.290	0.290±0.007 96.546±2.356	96.55%	-6.311/> 4.303/	5.006	<12.706
	0.439 8.788 0.264	0.264±0.007 87.884±2.462	87.884%	21.176/>>4.303/-		
2	0.511 10.2118 0.307	0.307±0.002 204.239±1.432	102.12%	12.747>4.303		
	0.478 9.563 0.288	0.288±0.002 191.252±1.632	95.627%	/-23.066/>>4.303		

μ:quated value (100,200)mg ,$\bar{w}$:practical content (mg) ,$\bar{x}$d:average of difference between two type of methods (developed&classical),$t_{tab}=t_{0.05/2,n-1}$=12.706 for n(No.of samples)=2for paired t-test, σ_{n-1}:standard deviation, $\bar{x}_i$:practicaly weight in mg ,$t_{0.025,2}$=4.303,$\bar{X}$d=(Xd_1 +Xd_2)/2 =10.825 ; Xd_1 =96.546-87.884=8.662 ; Xd_2 = 204.239-191.252=12.987,n=2 , σ_{n-1}=3.058.

References

[1] Alina Bradford,**2015**, Live Science Contributor, 1sdEd, New York.

[2] Wikipedia *the free Incyclopedia*, **1998**,2ndEd, Weily, New York.

[3] Mahan, L. K. andEscott-Stump, S.,**2000**,Food, nutrition, and diet therapy, 10th ed.,*Philadelphia: W.B. Saunders Company*,pp: 7216-7904.

[4] Tanphaichitr,V.,Shils, ME, Olsen, JA.and Shike, M.,**1999**, *ThiaminModern Nutrition in Health and Disease*. 9th ed. Baltimore: Lippincott Williams & Wilkins, pp: 749-766.

[5] Lohmann, K.; Schuster, P.,**1937,***Untersuchungenüber die Cocarboxylase Biochem.* Vol(294), p:188–214.

[6] Luczak, M. and Zeszyty, P.P.V., **1968**, Spectrophotometric for the determination of thiamin in pharmaceutical preparation,vol (80),p: 497,*ChemAbstr***1969**,vol(71),p: 2267.

[7] Rada,A.,Jasmina,B.,Olivera,C.and Sote,V., **2005**, RP-HPLC determination of vitamins B1,B3,B6,folic acid and B12 in multivitamin tablets, *Journal Serb.Chem.Soc. JSCS* ,vol.(10),pp:1229-1235.

[8] Junaid,A.,Mohammed, S.,M., Saeed,A.,K. and Abdul,Q.,K.,**2008**,HPLC analysis of water –solublevitamins(B1,B2,B3,B5,B6)in invitro and exvitro germinated chickpea (CicerarietinumL.),*African Journal of Biotechnology* ,vol.7(14),pp:2310-2314.

[9] Saad,A.,Nazeera,S.and Tereza,S.,**2015,** Determination of water-soluble vitaminsB1.B2,B3,B6,B9,B12 and C on C18 column with particle size 3µM in some manufactured food products by HPLC with UV-DAD/FLD detection, *International Journal of Pharmacy and Pharmaceutical Science*,vol.(7),pp:219-224.

[10] Cun, L., Yin-Liang, w., Ting, Y. and Wei-Guo,H.,**2012,** Rapid determination of fumonisins B1andB2 in Corn by liquid chromatography-tandem mass spectrometry with ultrasonic extraction ,*Journal of Chromatographic Science*,vol.(50),pp:57-63.

[11] Ortega Barrales, P., Fernandez de Cordova,M.L. and Molina Diaz,A.,**1998**,Aselective optosensor for UV spectrophotometic determination of thiamin in the presence of other vitamin B,*AnalyticaChimicaActa* ,vol.(376),pp:227-233.

[12] Alaa,F.,H.,Muneer,A.,A.and Abd-almutalb, B.,M.,A**,** **2010**, Spectrophotometric determination of thiamine. HCl in pharmaceutical preparations using Prussian blue reaction, *Journal of KerbalaUniversity*,vol.(8),pp:854-864.

[13] Gaurav,K.,R., Ashok,K.,Amit,K. and Gaurav,S., **2011**, To develop a simple (UV-VIS spectrometric)method for the estimation of water soluble vitamins,*International Journal of Drug Formulation and Research*,vol.(2),PP:511-523.

[14] Hind, S.,A. and Saba, Z.,H., **2015**, Spectrophotometric method for the determination of thiamin hydrochloride in pure form, pharmaceutical and biological fluids, *International Journal of pharmaceutical Sciences and Research*,vol. (8),pp:1814-1829.

[15] Abdel RST, Elbashir AA, El-Mukhtar M, and Ibrahim MM., **2015**, Development and validation of spectrophotometric method for determination of thiamine (VB1) in pharmaceutical formulations using 1, 2-naphthoquine-4-sulphonate (NQS) *.Enliven :BioanalyticalTechniques*,vol.(1),pp:1-6.

[16] Abdalla ,E.,**2016**, Application of spectrophotometric methods for the determination of thiamin (VB1)in pharmaceutical formulations using 7-chloro-4-nitrobenzoxadiazole (NBD-CL),*Journal of analytical & pharmaceutical research*, vol.(2),pp:1-6.

[17] Norouzi, P.,Mirzaei, T.,H.Rashedi, G.,H.A.Zamani and Ganjali, M., 2010 ,Ultrasensitive flow-injection electrochemical method using fast fourier transform square-wave voltammetry for detection of vitamin B1,International journal electrochemical sci.,vol.(5),pp:639-652.

[18] Mohsen, Z., Mohammad, R., Parviz, N.,**2010**, Dispersive liquid-liquid microextraction followed by spectrofluorimetry as a simple and accurate technique for determination of thiamin (vitamin B$_1$).*MicrochimActa*vol.(168), pp:317-324.

[19] Jianxiu, D.,Yinhuan, L. and Jiuru, Lu.,**2002** ,Flow injection chemiluminescence determination of Thiamin based on its enhancing effect on the luminol-hydrogen peroxide system, *AnalyticachimicaActa*,vol.(4),pp:661-5.

[20] Yan, L.,Peng, W.,Xin W.,Ming, C.,YunSheng, X., Chun, C.,MeiGui, L. and Chang qing, Z., **2010**, An immediate Luminescence enhancement method for determination of vitamin B$_1$using long-wavelength emitting water soluble CdTenanorods,*MicrochimicaActa* ,vol.(169),pp:65-71.

[21] Issam, M. A.andNagam, S.,T.,**2014**, Ayah 6SX1-ST-2D solar cell CFI Analyzer ,Patent, Present to central organization for standardization and quality control-Baghdad-Iraq, GO1N21/00.

[22] Vogel, A., I., **1979**, Text book of Macro and Semimicro qualitative inorganic analysis, 5thEd., Longman,London,P:214.

[23] Amina, M., E., Mohamed, E., M. and Fawzi,A., E., **2005**, Spectrophotometric determination ofsome fluoroquinolone anti bacterialsby ion-pair complex formation with Cobalt(II)tetra thiocyanate,Journal of the chinese chemical society vol.(52), P: 77-84.

[24] Miler ,J.C.and Miller,J.N.,**1988**,*Statistics for Analytical Chemistry* ,2nd,Ed.,John Wiley andN.Y.Sons, p:56.

[25] Bluman ,A.G.,**1997**,*Elementary Statistics*,3rd,Ed.,WCB/MC Graw-Hill,NewYork, p:38.