Flow Injection Turbidimetric Determination of Vitamin B$_1$ using LEDs as a Source of Irradiation and Two Solar Cells as an Energy Transducer

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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 homemade Ayah-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 (0.1,1.5) 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 $\lambda_{max}$=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$_1$at 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 organic sulfur compound with a chemical formula C$_{12}$H$_7$N$_2$O$_5$. 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 name: 3-((4-Amino-2-methyl-5-pyrimidinyl) methyl)- 5-(2-hydroxyethyl)-4-methylthiazolium chloride](image)

There are various analytical methods for determination of VB$_1$. These analyses include: HPLC [7-9], Liqchromatography –Tandem mass spectrophotometry [10], spectrophotometric [11-16], electrochemical technique [17], fluorimetry [18] and Chemiluminescence [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$_7$C$_{1}$N$_2$O$_5$, molar mass 330.8076 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$_4$)$_2$Mo$_7$O$_{24}$-4H$_2$O, 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$_1$ were 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 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 [21], which used 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

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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\textsubscript{1} using 4 cm quartz cell.

![Figure 2: Flow system used for determination of VB\textsubscript{1} using homemade Ayah 6SX1- ST-2D solar cell CFIA at 210\textmu l, open valve mode & applied voltage to the LEDs 1.686volt DC](image)

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\textsubscript{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\textsubscript{1} (no other chemicals were involved)in the reaction pattern; the vitamin B\textsubscript{1} sample (210\textmu l) 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\textsubscript{1} with ammonium molybdate and the reaction come into being. The measurements were accomplished by Ayah 6SX1- TS - 2D Solar cell-CFIA analyzer triplicate successive measurements were used. The proposed reaction for vitamin B\textsubscript{1} with Am is represented in scheme 1 [22,23].

![Scheme 1- Probable reaction between vitamin B\textsubscript{1} (Thiamin. Cl) and Ammonium molybdate (Am).](image)

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

A series of the precipitating reagent (Am) having the concentration of 0.1-3 mMol.L\textsuperscript{-1} were prepared at constant concentration of vitamin B\textsubscript{1} (2mMol.L\textsuperscript{-1}), 251.2\textmu l sample volume, 1.8 & 2 ml.min\textsuperscript{-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\textsuperscript{-1}. More than ( > 0.7mMol.L\textsuperscript{-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\textsuperscript{-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 B₁ 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 B₁

<table>
<thead>
<tr>
<th>[Am] mMol.L⁻¹</th>
<th>Energy transducer response expressed as an average peak heights (n=3) ȝ in mV</th>
<th>RSD%</th>
<th>Confidence interval at 95% ȝ±t₀.05/2,ₙ₋₁σn⁻¹/√ₙ</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>520</td>
<td>0.159</td>
<td>520 ± 2.062</td>
</tr>
<tr>
<td>0.3</td>
<td>1008</td>
<td>0.091</td>
<td>1008 ± 2.286</td>
</tr>
<tr>
<td>0.5</td>
<td>1168</td>
<td>0.088</td>
<td>1168 ± 2.599</td>
</tr>
<tr>
<td>0.7</td>
<td>1328</td>
<td>0.083</td>
<td>1328 ± 2.708</td>
</tr>
<tr>
<td>1</td>
<td>912</td>
<td>0.123</td>
<td>912 ± 2.782</td>
</tr>
<tr>
<td>2</td>
<td>856</td>
<td>0.130</td>
<td>856 ± 2.758</td>
</tr>
<tr>
<td>3</td>
<td>504</td>
<td>0.232</td>
<td>504 ± 3.155</td>
</tr>
</tbody>
</table>

\[ t₀.05/2,ₙ₋₁=4.303 \]

Physical variables:

Table 2: Effect of flow rate on the measurement of energy transducer response via reflection of incident light for the determination of vitamin B₁

<table>
<thead>
<tr>
<th>Physical variables</th>
<th>Range of flow rate (mL.min⁻¹)</th>
<th>Energy transducer response expressed as an average peak heights (n=3) ȝ in mV</th>
<th>RSD%</th>
<th>Confidence interval at 95% ȝ±t₀.05/2,ₙ₋₁σn⁻¹/√ₙ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate of carrier stream (mL.min⁻¹)</td>
<td>0.2 - 2.8</td>
<td>520</td>
<td>0.159</td>
<td>520 ± 2.062</td>
</tr>
<tr>
<td>Flow rate of carrier stream (mL.min⁻¹)</td>
<td>0.4 - 3</td>
<td>1008</td>
<td>0.091</td>
<td>1008 ± 2.286</td>
</tr>
<tr>
<td>Flow rate of carrier stream (mL.min⁻¹)</td>
<td>0.6 - 4</td>
<td>1168</td>
<td>0.088</td>
<td>1168 ± 2.599</td>
</tr>
<tr>
<td>Flow rate of carrier stream (mL.min⁻¹)</td>
<td>0.8 - 5</td>
<td>1328</td>
<td>0.083</td>
<td>1328 ± 2.708</td>
</tr>
<tr>
<td>Flow rate of carrier stream (mL.min⁻¹)</td>
<td>1 - 6</td>
<td>912</td>
<td>0.123</td>
<td>912 ± 2.782</td>
</tr>
<tr>
<td>Flow rate of carrier stream (mL.min⁻¹)</td>
<td>2 - 10</td>
<td>856</td>
<td>0.130</td>
<td>856 ± 2.758</td>
</tr>
<tr>
<td>Flow rate of carrier stream (mL.min⁻¹)</td>
<td>3 - 15</td>
<td>504</td>
<td>0.232</td>
<td>504 ± 3.155</td>
</tr>
</tbody>
</table>

\[ t₀.05/2,ₙ₋₁=4.303 \]

Figure 4: Influence of variation of flow rate on:

A: Response profile for Am-Vitamin B₁ system.
B: Energy transducer response, using 2mMol.L⁻¹ vitamin B₁ concentration.
Table 2: Effect of flow rate on the measurements of energy transducer response for determination of VB₁

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

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

Effect of Sample Volume

Using 2 mMol.L⁻¹ vitamin B₁, 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 (\( \Delta t \)), 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 \( \Delta t \). 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 B₁

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 B₁

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

\( t^* \): Arrival time from injection valve reaching to measuring cell (Sec), \( \Delta t \): Base width of peak response (Sec), \( t_{0.05/2,2} = 4.303 \)
Effect of Reaction Coil

Variable coil length 0-50 cm was studied. These lengths comprise a volume (0 – 392.5) µl which connected after Y-junction directly in flow system. While keeping all other changeable constant (vitamin B₁: 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 turn 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](image)

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

<table>
<thead>
<tr>
<th>Coil Length Cm</th>
<th>Coil Volume µl</th>
<th>Energy Transducer Response expressed as an average peak heights( n=3) $\bar{y}_j$ in mV</th>
<th>RSD %</th>
<th>Confidence interval at 95% $\bar{y}<em>j \pm t</em>{0.05/2, n-1}\sigma_{n-1}/\sqrt{n}$</th>
<th>$t^*$ sec</th>
<th>Base width $At_n$ sec</th>
<th>$V_{at}$ ml at flow cell</th>
<th>Concentration mMol.L⁻¹ At flow cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>0*</td>
<td>0*</td>
<td>1500</td>
<td>0.072</td>
<td>1500±2.708</td>
<td>18</td>
<td>78</td>
<td>3.980</td>
<td>0.105</td>
</tr>
<tr>
<td>10</td>
<td>78.5</td>
<td>1432</td>
<td>0.075</td>
<td>1432±2.683</td>
<td>21</td>
<td>90</td>
<td>4.560</td>
<td>0.092</td>
</tr>
<tr>
<td>20</td>
<td>157.0</td>
<td>1424</td>
<td>0.069</td>
<td>1424±2.460</td>
<td>24</td>
<td>96</td>
<td>4.850</td>
<td>0.087</td>
</tr>
<tr>
<td>30</td>
<td>235.5</td>
<td>1400</td>
<td>0.094</td>
<td>1400±3.279</td>
<td>24</td>
<td>102</td>
<td>5.140</td>
<td>0.082</td>
</tr>
<tr>
<td>40</td>
<td>314.0</td>
<td>1440</td>
<td>0.099</td>
<td>1440±3.553</td>
<td>27</td>
<td>105</td>
<td>5.285</td>
<td>0.079</td>
</tr>
<tr>
<td>50</td>
<td>392.5</td>
<td>1440</td>
<td>0.105</td>
<td>1440±3.776</td>
<td>27</td>
<td>108</td>
<td>5.430</td>
<td>0.077</td>
</tr>
</tbody>
</table>

For Coils selected, $t^*$: Arrival time from injection valve reaching to measuring cell (Sec) $At_n$: 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 VB₁ at 2 mMol.L⁻¹ was studied. While keeping all other variables fixed (210 µl sample volume, Ammonium molybdate 0.7 mMol.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 5 which 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 response.
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**

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

$ 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 8B). 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 particulates 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 8C shows the calibraton curve of classical method. In classical spectrophotometric method [11] via the measurement at $\lambda_{max}$ = 246 nm, in which observed that, the linear calibration curve was ranged from 0.1-10 mMol.L$^{-1}$.
Figure 8 A: Profile for the variation of vitamin B₁ concentration versus time

Figure 8 B: Calibration graph for the variation of vitamin B₁ 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 \(\lambda_{max} = 246\) nm
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 B1 through three methods as tabulated in Table 7.

### Table 6: Summary of linear regression for the variation of energy transducer response with vitamin B1 concentration using first degree equation of the form \( \hat{Y} = a + bx \) at optimum conditions

<table>
<thead>
<tr>
<th>Type of measurement</th>
<th>Measured ([B_1] ) mMol.L(^{-1})</th>
<th>Linear Range of ([B_1] ) mMol.L(^{-1})</th>
<th>( \bar{y} = a + bX ) Linear Regression at optimum Conditions (95%, n=2)</th>
<th>( t_{(0.025)} )</th>
<th>( t_{(0.025)} ) at 95% , n=2</th>
<th>Calculated t-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Developed method</td>
<td>0.1 – 10</td>
<td>0.1 – 2</td>
<td>(-2.818\pm2.754+862.009\pm173.642[B_1])mMol.L(^{-1})</td>
<td>0.9708</td>
<td>94.25%</td>
<td>2.306&lt;11.448</td>
</tr>
<tr>
<td>Classical method UV-SP ( \lambda_{max}=246nm )</td>
<td>0.05 – 10</td>
<td>0.1-10</td>
<td>0.04\pm0.010+0.02</td>
<td>0.0024 [B_1]mMol.L(^{-1})</td>
<td>0.9836</td>
<td>96.74%</td>
</tr>
</tbody>
</table>

\( \bar{y} \): 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 \( \bar{y} = a + bx \), \( r \): 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 background 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 = \( \hat{Y}_b + 3S_b \) 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. \( =3S_b/slope S_b = \sigma_{\text{mean}} \) (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 B1 through three methods as tabulated in Table 7.

### Table 7: limit of detection for vitamin B1 at optimum parameters in different approaches using210µl sample volume

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

\( X \): 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 B1 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 less than 0.5% indicate clearly, the high efficiency of the new method.
Figure 9: Response profile of repeatability of vitamin B₁ in different concentration:

A: 0.9 mMol.L⁻¹
B: 1.5 mMol.L⁻¹

Table 8: Repeatability of vitamin B₁ at optimum parameters with 210µl sample volume of VB₁.

<table>
<thead>
<tr>
<th>[B₁] mMol. L⁻¹</th>
<th>Energy transducer Response expressed as an average peak heights (n=3) ȳ [mV]</th>
<th>RSD %</th>
<th>ȳ ± t₀.₀₂₅,₅σ/√n [mV] at confidence interval 95 %</th>
<th>Number of injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9</td>
<td>752</td>
<td>0.136</td>
<td>752±0.853</td>
<td>8</td>
</tr>
<tr>
<td>1.5</td>
<td>1408</td>
<td>0.103</td>
<td>1408±1.522</td>
<td>6</td>
</tr>
</tbody>
</table>

t₀.₀₂₅,₅ =2.365 ,  t₀.₀₂₅,₅ =2.571, n=number of injection

Analysis of vitamin B₁ in 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 achieved in this work were used for the analysis of VB₁ 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 λₘₐₓ at 246 nm and developed method (using Ayah 6SX1-ST-2D solar cell CFI Analyzer).

A series 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 VB₁ (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 standardVB₁ (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 B₁.
Figure 10: Effect of variation of vitamin B<sub>1</sub> 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: Developed method  
E: Classical method.

Table 9A was shown a practical concentration of vitamin B1 in 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 ($\mu_i$) and quoted value ($\mu$)

  $H_0: \mu_i = \mu$

  Against

- **Alternative hypotheses:** There is a significant difference between the means and quoted value

  $H_1: \mu_i \neq \mu$

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 9B (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** ($H_0$): $\mu_{uv} - \mu_{sp} = \mu_{Ayah 6SX1-ST-2D}$

- **Alternative hypothesis** ($H_1$): $\mu_{uv} - \mu_{sp} \neq \mu_{Ayah 6SX1-ST-2D}$

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 ($\mu$) of the two different methods for analyzing two drugs. Since calculated t-value less than $t_{tab}$ (i.e. 5.006 < 12.706), therefore $H_0$ is accepted against $H_1$. So, any one of them can be used for analysis vitamin B1.

### Table 9A: Standard addition results for the determination of B1 in two pharmaceutical preparation using Am-VB1 system.

<table>
<thead>
<tr>
<th>No. of sample</th>
<th>Commercial name , Company Content Country</th>
<th>Type of method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Developed method using Ayah 6SX1-ST-2D Solar cell CFIA (mV)</td>
<td>Uv.Sp Classical method Absorbance measurement at $\lambda_{max}$=246 nm</td>
</tr>
<tr>
<td></td>
<td>Vitamin B1 mMol.L$^{-1}$</td>
<td>Equation of standard addition at 95% for n=2</td>
</tr>
<tr>
<td></td>
<td>0.25 ml</td>
<td>0.5 ml</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>1.5 ml</td>
<td>1.75 ml</td>
</tr>
</tbody>
</table>

![Figure 10: Standard addition calibration curve for Switzerland drug using two methods](image-url)
Table 9B: Summary of results for practical content, efficiency(Rec%) for determination of vitamin B₁ in two samples of pharmaceutical and paired t-test preparation using two methods

<table>
<thead>
<tr>
<th>No.</th>
<th>Type of method</th>
<th>Developed using Ayahb 650xl – ST-2D solar cell CFIA</th>
<th>Uv-sp classical method Absorbance measurement at λ&lt;sub&gt;max&lt;/sub&gt;=246nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Practical concentration (nmol L&lt;sup&gt;-1&lt;/sup&gt;) in 25 ml</td>
<td>Weight of B₁ in each sample (g)</td>
<td>Efficiency of determination</td>
</tr>
<tr>
<td></td>
<td>Practical concentration (nmol L&lt;sup&gt;-1&lt;/sup&gt;) in 100 ml</td>
<td>Practical weight of B₁ in (g)</td>
<td>Rec%</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.483, 0.5094</td>
<td>0.390±0.007</td>
<td>96.55%</td>
</tr>
<tr>
<td></td>
<td>0.290, 0.303</td>
<td>0.261±0.007</td>
<td>87.884%</td>
</tr>
<tr>
<td></td>
<td>0.429, 0.551</td>
<td>87.884±2.462</td>
<td>87.884%</td>
</tr>
<tr>
<td></td>
<td>0.511, 0.583</td>
<td>10.21±0.002</td>
<td>102.1%</td>
</tr>
<tr>
<td></td>
<td>10.21±0.002</td>
<td>204.239±1.452</td>
<td>102.1%</td>
</tr>
<tr>
<td>2</td>
<td>0.478, 0.511</td>
<td>0.31±0.002</td>
<td>95.627%</td>
</tr>
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