Spectrophotometric determination of Thiamine.HCl in pharmaceutical preparations using Prussian blue reaction

Alaa Frak Hussin, Muneer A.AL Da‘amy and Abd-almutalb bader manhy Alkhalily
Department of chemistry, science College, Karbala University

Abstract
A simple, sensitive, rapid and accurate spectrophotometric method was developed for the determination of Thiamine.HCl in pharmaceutical preparations and in pure form. The method is based on the reduced Fe(III) salt by Thiamine.HCl to form Fe(II) salt which subsequently react with potassium ferric cyanide forming a soluble Prussian blue dye which has a maximum absorption at \( \lambda_{\text{max}} = 747 \text{nm} \) linear calibration graph was in the range of \((0.2 - 14) \mu \text{g.mL}^{-1}\) with molar absorptivity of \((2.42 \times 10^3 \text{L.mol}^{-1}.\text{cm}^{-1})\), a sandall sensitivity of \((139.38 \times 10^{-6} \mu \text{g.cm}^{-2})\), correlation coefficient of 0.999, detection limit \((0.106 \mu \text{g.mL}^{-1})\) and the relative standard deviation of RSD\% \((0.763)\). The method was applied successfully for the determination of Thiamine.HCl in pharmaceutical preparations. Recovery was in the range of \((97.8 - 104.4)\%\). The proposed method can be carried out at \(40^\circ\text{C}\) temperature with no need for solvent extraction step or pH control.

Introduction
Thiamine.HCl is a water-soluble vitamin of the B complex (vitamin B1), whose phosphate derivatives are involved in many cellular processes. Its structure contains a pyrimidine ring and a thiazole ring linked by a methylene bridge.\(^{(1)}\)

(Structures of Thiamine Hydrochloride)

The coenzyme, thiamine pyrophosphate or cocarboxylase is intimately connected with the energy releasing reactions in carbohydrate metabolism.\(^{(2)}\) Various methods has been reported for the determination of Thiamine.HCl.\(^{(3,4,5,6,7)}\) There are include chromatographic, spectrophotometric,\(^{(8,9,10,11)}\) Electrochemical, \(^{(12)}\) Flow Injection.\(^{(13)}\)
The method of formation of Prussian blue complex is used to determine of many drug such as Amoxicilline\(^{(14)}\), cephalosporine antibiotics\(^{(15)}\), tinidazol\(^{(16)}\), nifedipine\(^{(17)}\), folic acid\(^{(18)}\), adrenaline\(^{(19)}\), Diclofenac sodium\(^{(20)}\), Metoclopramide.HCl\(^{(21)}\) and Rantiden.HCl\(^{(22)}\).

**Experimental**

**Apparatus:**

All spectral and absorbance measurement were carried out in a Double beam UV-Vis spectrophotometer-shimadzu-1800. Equipped with a 1cm quarts cell.

**Reagents:**

All chemicals used were of analytical-reagent grade.

- Stock solutions (1mg/ml) of Thiamine. HCl (vitamin B1) were prepared by dissolving 0.1000 gm Thiamine HCl in deionized water and diluting to the mark in a 100 ml volumetric flask. Working solutions were prepared by diluting the standard solution in deionized water.

- Hydrous ferric nitrate (0.1M) stock solution was prepared by dissolving 4.0384 gm of Fe(NO\(_3\))\(_3\).9H\(_2\)O in sufficient deionized water containing 1ml of nitric acid and the solution made up to the mark in 100 ml volumetric flask with deionized water.

- Potassium hexacyanoferrate(III) (0.1M) stock solution was prepared by dissolving 3.2900 gm of K\(_3\)[Fe(CN)]\(_6\) in deionized water and diluting to the mark in 100 ml volumetric flask.

**Recommended Procedure:**

In to a series of 25 ml volumetric flask, transfer increasing volume of Thiamine.HCl solution (100 µg.ml\(^{-1}\)) to cover the range of calibration curve (0.2–14 µg.ml\(^{-1}\)), added 0.19 ml (0.1M) of Fe(NO\(_3\))\(_3\).9H\(_2\)O and shake well. Added 0.6 ml (0.1M) of K\(_3\)[Fe(CN)]\(_6\), dilute the solution to the mark with distilled water, and allow the reaction to stand for 20 min in water bath at 40ºC. measure the absorbance at 747 nm against a reagent blank prepared in the same way but containing no Thiamine.HCl.

**Procedure for Pharmaceutical Preparations:**

- Vitamin B1 Tablets:

  provided from (SDI) Samarra-Iraq. 10 tablets were grinded well and a certain portion of the final powder was accurately weighted to give an equivalent to about 10 mg of vitamin B1 was dissolved in deionized water. The prepared solution transferred to 100 ml volumetric flask and made up to the mark with deionized water forming a solution of 100 µg/ml concentration. The solution was filtered by using a whatmann filter paper No.42 to avoid any suspended particles.

**Results and Discussion:**

**Absorption spectra:**

It was found preliminary that the reaction of Thiamine .HCl (vitamin B1) with Ferric Nitrate and Potassium hexacyanoferrate produced highly coloured prussian blue soluble dye that has a maximum absorption at \(\lambda_{\text{max}}\) (747nm) Fig (1). The above reaction can be utilized for the determination of Thiamin.HCl using spectrophotometric method. Initial studies were directed toward optimization of the experimental conditions, in order to establish the most Favorable parameters for the determination of Thiamine.HCl.
Fig (1): Absorption spectra of (6µg.ml⁻¹) of Thiamine.HCl treated with Fe(NO₃)₃.9H₂O(7.5×10⁻⁴ M), K₃Fe(CN)₆ (2.5×10⁻³ M) at room temperature and measured against blank solution.

**Optimization of the Experimental Condition:**

The influence of various reaction variables such as concentration of reactants, order of addition, time and temperature were investigated.

**Effect of Iron (III) Nitrate Concentration:**

The effects of different concentration of Iron (III) Nitrate in the range of (1×10⁻⁴–7.5×10⁻³ M) were investigated. A concentration of (7.5×10⁻⁴ M) gives the highest absorption Fig. (2) and thus was chosen for further use.

![Effect of Iron (III) Nitrate Concentration](image)

**Effect of K₃Fe(CN)₆ Concentration:**

The effect of potassium Hexacyanoferrate(III) Concentration in the range of (2.5×10⁻⁴–1×10⁻² M) was similarly studied. A (2.5×10⁻³ M) of K₃Fe(CN)₆ solution gave the best results. The results obtained are shown in Fig (3).
Order of addition:
The effect of order of addition on the absorption of Prussian blue color dye was studied. Table (1), shows the order of addition could be followed, Drug : Fe(NO$_3$)$_3$.9H$_2$O : K$_3$Fe(CN)$_6$. Due to gave the highest absorption.

Table (1) Effect of order of addition

<table>
<thead>
<tr>
<th>Order of addition</th>
<th>Absorbance at $\lambda_{\text{max}}$(747nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug : Fe(NO$_3$)$_3$.9H$_2$O : K$_3$Fe(CN)$_6$</td>
<td>0.375</td>
</tr>
<tr>
<td>Drug : K$_3$Fe(CN)$_6$ : Fe(NO$_3$)$_3$.9H$_2$O</td>
<td>0.255</td>
</tr>
<tr>
<td>K$_3$Fe(CN)$_6$ : Fe(NO$_3$)$_3$.9H$_2$O : Drug</td>
<td>0.262</td>
</tr>
<tr>
<td>K$_3$Fe(CN)$_6$ : Drug : Fe(NO$_3$)$_3$.9H$_2$O</td>
<td>0.221</td>
</tr>
<tr>
<td>Fe(NO$_3$)$_3$.9H$_2$O : Drug : K$_3$Fe(CN)$_6$</td>
<td>0.336</td>
</tr>
<tr>
<td>Fe(NO$_3$)$_3$.9H$_2$O : K$_3$Fe(CN)$_6$ : Drug</td>
<td>0.281</td>
</tr>
</tbody>
</table>

Effect of Temperature:
The effect of Temperature on the color intensity of the product was studied in practice the highest absorption was obtained when the colored product was developed when the calibration flask was placed in an water bath(40$^\circ$C). as shown in Fig(4)
The color intensity reached a maximum absorption after Thiamin.HCl has been reacted with Iron (III) Nitrate and K$_3$Fe(CN)$_6$ at 20 min. Therefore 20 min development time was chosen for further use. The results obtained are shown in Fig(5).

**Effect of Time:**

Under the optimum conditions, a linear calibration graph for the determination of Thiamine.HCl was obtained over the concentration range of (0.2 – 14)µg.ml$^{-1}$. The linear regression equation for the range of (0.2 – 14) µg.ml$^{-1}$ Thiamine.HCl is $Y = 0.0709x + 0.0047$ and correlation coefficient of 0.9999 the linear calibration graph is shown in Fig(6).
Mechanism of Reaction:

Thiamine.HCl reduce iron(III) ion in aqueous medium to form iron(II) ion, which subsequently chelate with potassium hexacyanoferrate(III) forming a soluble Prussian blue (23). This substance so-called Turn bulls blue result from the interaction of 1:1 molar proportion of Fe(II) and K₃[Fe(CN)₆], which has the approximate composition (24) KFe^{II}[Fe^{III}(CN)₆].XH₂O as in the following equation:

\[
\text{Thiamin.HCl + Fe}^{+3} \rightarrow \text{Fe}^{+2}
\]

\[
\text{Fe}^{+2} + K₃[\text{Fe}^{III}(\text{CN})₆] \rightarrow \text{KFe}^{II}[\text{Fe}^{III}(\text{CN})₆]
\]

(Potassium Ferro Firric cyanide)

The intense colour is due to charge transfer (25) from Fe^{II} to Fe^{III}.

Accuracy and Precision:
The accuracy of the proposed method tested by determining the recoveries of different amount of Thiamine.HCl and the precision of the method was investigated by determining the relative standard deviation of five determinations at three concentration level of Thiamine.HCl 3.5 and 10 µg.ml⁻¹. The results obtained are shown in Table (2).

<table>
<thead>
<tr>
<th>Conc. Of vitamin (µg.ml⁻¹)</th>
<th>RSD*%</th>
<th>Recovery*%</th>
<th>Error*%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taken</td>
<td>Found</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.000</td>
<td>3.130</td>
<td>0.550</td>
<td>104.330</td>
</tr>
<tr>
<td>5.000</td>
<td>5.088</td>
<td>0.413</td>
<td>101.760</td>
</tr>
<tr>
<td>10.000</td>
<td>9.850</td>
<td>0.217</td>
<td>98.500</td>
</tr>
</tbody>
</table>

*Average of five determinations
Analytical Application:
The application of the proposed method for the assay of the pharmaceutical tablets was investigated using Tablets from SID (10mg) containing Thiamine.HCl. A good precision and recovery were obtained according to the results obtained in Table (3).

Table (3): Application of the proposed method for the determination of Thiamine.HCl in pharmaceutical preparations

<table>
<thead>
<tr>
<th>Drug sample</th>
<th>Conc.B1 µg.ml⁻¹</th>
<th>Proposed method</th>
<th>Standard (26) method</th>
</tr>
</thead>
<tbody>
<tr>
<td>vitamin B1 (10µg) SID</td>
<td>Taken</td>
<td>Found</td>
<td>R.S.D*%</td>
</tr>
<tr>
<td>3.000</td>
<td>2.860</td>
<td>0.617</td>
<td>-4.66</td>
</tr>
<tr>
<td>5.000</td>
<td>5.220</td>
<td>0.450</td>
<td>4.40</td>
</tr>
<tr>
<td>10.000</td>
<td>9.780</td>
<td>0.362</td>
<td>-2.20</td>
</tr>
</tbody>
</table>

*Average of five determinations

Conclusion:
A rapid, simple, sensitive and accurate spectrophotometric method was developed for the determination of Thiamine.HCl in pharmaceutical preparations. The method is based on the reaction of Thiamine.HCl with Fe(III) ion to produce Fe(II) ion which is upon further reaction potassium hexacyanoferrate(III) to produce a soluble Prussian blue dye. It has several advantages, do not needs farther steps such as solvent extraction, PH control and expensive reagent.

References
22- Mohammed J. Hussan PDH Thesis Application of new flow injection spectrophotometric and HPLC methods for determination of same drugs in pharmaceutical preparations peg (87-88).