Spectrophotometric Determination of Vitamin B₁ (Thiamin Hydrochloride) In Pharmaceutical Preparation by Coupling Reaction with Diazotized Sulfanilic acid.

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Abstract
A simple, accurate and sensitive spectrophotometric method for the determination of vitamin B₁ (thiamin hydrochloride) in aqueous solution is described. The method is based on the diazotization of sulfanilic acid followed by coupling with thiamin hydrochloride in the presence of sodium hydroxide to form reddish-brown water soluble dye that is stable and has maximum absorption at (λ max= 490nm). The molar absorptivity and sandell sensitivity were 7.74 x10³ L.mol⁻¹.cm⁻¹ and 0.045µg.cm⁻¹ respectively. The procedure is developed for bulk thiamin hydrochloride and some of their pharmaceutical preparations. The are accurate, precise and comparable to the standard method (British Pharmacopoeia).

طريقة طيفية لتعيين فيتامين B₁ (ثامين هايوروكواريد) في المستحضرات الصيدلانية باستعمال تفاعل الأطروحة مع سلفانيليك المؤزت.

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المستخلص
تم اقتراح طريقة طيفية بسيطة، دقيقة وحساسة لتحديد فيتامين B₁ (ثامين هايوروكواريد) في وسط مائي. تتضمن الطريقة تفاعل الأطروحة لحامض سلفانيليك يتيحها الإطروحة مع ثامين هايوروكواريد يوجد هيروكسيد الصوديوم لتكوين صبغة ذات لون أحمر-بني يمكن قياسها طيفيا عند الطول الموجي الأعظم 7.74 x10³ L.mol⁻¹.cm⁻¹ و كنايت الاختصاصية المولارية وحساسية سندي هي (λ max= 490nm) و (0.045µg.cm⁻¹) على التوالي. استخدم الطريقة لتحديد الثامين في بعض المستحضرات الصيدلانية وكانت النتائج دقيقة ومبنوطة عند مقارنتها مع الطريقة القياسية (B.P)
Introduction

Thiamin hydrochloride (vitamin B₁) (Fig.1) is the water-soluble B-group vitamins essential to the general health and well-being of animals. Compared with the requirements of the adult animal, there are also additional needs for the more demanding stages of the life cycle such as growth, pregnancy and lactation. Most of the B-group vitamins are involved with utilization of foods and the production or interconversion of energy in the body. In these processes the B-vitamins are used by the animal to form coenzymes (1).

![Thiamin hydrochloride (vitamin B₁) structure](image)

Vitamin B₁ is an essential nutrient for humans to maintain normal neutral activity and to prevent beriberi. Person usually obtains the nutrient from natural and fortified foods, when needed the vitamin can also be obtained from various pharmaceutical preparations containing thiamin hydrochloride (2).

Many methods for the determination of thiamin have been proposed and developed, most based on microbial (3), gas chromatographic (4), HPLC (5), spectrophotometric (6), flow injection spectrophotometric (7-9), flow injection turbidimetric (10), spectrophotometric (2,11), chemiluminescence (12,13) and potentiometric methods (14,15).

The present paper describes the development of a simple, versatile, and sensitive spectrophotometric method for the quantitative determination of mentioned vitamin in pharmaceutical preparation. The method is based on the diazotization of sulfanilic acid followed by coupling with thiamin in alkaline medium forming highly colored product. A comparative study of this technique with the official method was introduced.

Experimental

1. Apparatus

UV-Visible model 4085 UK Jenway spectrophotometer, pH meter 3305 Jenway, combined pH electrode, Gallenkamp magnetic stirrer were used.

2. Reagent and materials

All chemical used were of analytical grade reagent except otherwise mentioned, and double distilled water was used throughout. Sulfanilic acid (Schuchardt munchen Germany), sodium nitrite (Fluka), Urea (BDH Ltd Poole, England), sodium hydroxide (Merck Germany), Hydrochloric acid (Gainland chemical company U.K.) Thiamin hydrochloride (Fluka), and Dosage forms were obtained from local sources.

2. Standard preparations

A stock solution (500 µg.ml⁻¹) of thiamin hydrochloride was made up by weighing accurately 125 mg of the vitamin, dissolved in distilled water and diluted to 250ml in volumetric flask.

3. General procedure

Transfer 2ml of 1M hydrochloric acid into a 50ml volumetric flask, followed by 0.8ml of 0.4%sodium nitrite solution and cool in an ice-bath for 5min., add 4ml of 2%urea solution shake and allow to stand for 3min., add 5ml of 0.05M sulfanilic acid , shake, then add 0.1-3.5ml of thiamin hydrochloride (500µg.ml⁻¹) or suitable portion of a pharmaceutical preparation and add 5ml of 30%sodium hydroxide solution. Shake well and diluted to volume with distilled water. Measure the absorbance in a 1cm cell against a reagent blank at the wave length of...
maximum absorption ($\lambda_{\text{max}} = 490\text{nm}$). The absorbance is plotted against the final concentration to obtain a calibration graph.

4 Application

4.1. Analysis of tablet

Weigh and crush 4 tablets. Dissolve a known mass (87.5mg) of the powder, equivalent to 50mg of the pure drug, in distilled water, filter and dilute to 100ml in a volumetric flask. Transfer 1ml of this solution into 50ml volumetric flask and proceed as described under general procedure (sec. 2.3).

4.2. Analysis of injection

The content of 5 bottles of injection was mixed, then 1ml of this solution was transferred into a 100ml volumetric flask and make up to the mark with distilled water. Transfer 1ml of this solution into a 50ml volumetric flask and proceed as described under general procedure.

Fig (2) shows the spectra of the azo dye and of the reagent blank. The wavelength of maximum absorption ($\lambda_{\text{max}}$) at 490nm, characteristic of the azo dye was used in all subsequent experiments.

Results and discussion

1. Optimization of experimental variables

A series of experiment were conducted to establish the optimum analytical variables. The parameter optimized included reagent concentration and physical variable including the effect of time on the colored product.

1.1. Effect of sodium nitrite

The sulfanilic acid is diazotized with excess of nitrous acid, but the surplus affects the stability of the colour and must be removed with urea, which has no effect on the product $^{[16]}$. Table (1) shows that 1.4ml of 0.4% w/v sodium nitrite is sufficient for diazotization of sulfanilic acid.

1.2. Effect of sulfanilic acid

Fixing the sodium nitrite concentration at 1.4ml of 0.4% w/v. Different volumes of 0.05M sulfanilic acid solution were used, while other parameters were kept constant as in general procedure. The results obtained (Table (2)) indicates that 5ml of 0.05M sulfanilic acid is optimum and is used in all subsequent experiment.

1.3. Effect of sodium hydroxide

The concentration of the alkali used in the coupling reaction affects the accuracy. The reaction of the diazonium salt with vitamin B$_1$ requires 5ml of 30% sodium hydroxide for full colour development. Lower concentration gives incomplete colour evolvement, and higher once tends to decrease the stability of the colour. Table (3) demonstrate this effect.

1.4 Time of dye formation

Fig. (3) shows that the standing time of 20min. at room temperature is necessary for the most intense colour obtained. The colour produced was stable for more than 24h.

2. Calibration graph

Under the optimum conditions (2ml of 1M hydrochloric acid, 1.4ml of 0.4% w/v sodium nitrite, 5ml of 0.05M sulfanilic acid solutions containing various thiamin concentration are prepared by appropriate dilution of the stock solution converting the range (2-35µg.ml$^{-1}$) followed by 5ml of 30% sodium hydroxide). Linear regression line was obtained by plotting the concentration of the thiamin hydrochloride versus absorbance as shown in Fig.(4).

3. Stoichiometry of the product

Complementary volume of 0.05M solution of the diazotized sulfanilic acid and of thiamin hydrochloride (0+10, 1+9, 2+8, 3+7...... 9+1) respectively were examined with 4 ml of 2%w/v urea and 5ml of 30% w/v sodium hydroxide solution in 50ml volumetric flasks and the solution was
made up to the mark with distilled water. The absorbance of each solution was measured and plotted against the mole fraction of thiamin hydrochloride. The mole ratio of thiamin hydrochloride to sulfanilic acid was found to be 1:1 (Fig. (5)).

4. Possible mechanism
The possible mechanism for the reaction of diazotized sulfanilic acid with desired vitamin was explained in scheme (1). Since the diazotization reaction is exothermic and many diazonium salts are unstable, it is necessary to control the temperature carefully (17). Diazotization is normally carried out at 0°C. A low temperature is advantageous for two reasons, first, the stability of free nitrous acid is greater, and second, the moderate stability of most diazo compounds demands it. In cases where the diazo compound is relatively stable, higher temperature of diazotization may be used, such as 10°C to 15°C for sulfanilic acid (18).

5. Determination of Vitamin B₁ in pharmaceuticals
The results of the analyses of several dosage forms containing vitamin B₁ (thiamine hydrochloride) by both the proposed and official method (19) were made for comparison.

Statistical analysis (20, 21) of the results using student t-test and the variance ratio F-test, showed no significant difference between the performance of the two methods as regards to accuracy and precision (Table 4).

Vitamin B₁ is usually formulated in tablet and injection forms. Therefore, the effect of common tablet excipient and additive on the procedure was investigated, it was found that glucose, lactose sucrose, starch and acacia has no effect on the assay (Table 5). Moreover, the procedure was successfully applied to analysis of pharmaceutical preparations. The recoveries obtained were satisfactory and in reasonable agreement with those obtained by the British Pharmacopoeia (B.P.) method (Table 4).

Conclusion
A simple, accurate and sensitive spectrophotometric method is described for the determination of vitamin B₁ (thiamine hydrochloride) in pharmaceutical preparations. All solutions have a maximum absorption at 490nm.

Scheme (1): Possible mechanism of the reaction
Fig(2): Absorption spectra of (a) 10µg.ml⁻¹ of thiamin hydrochloride treated as described under general procedure and measured against a reagent blank, and (b) reagent blank against distilled water.

Fig. (3): Effect of reaction time of diazotized sulfanilic acid with thiamin hydrochloride.
Fig.(4): Calibration graph for determination of thiamin hydrochloride.

Fig.(5): Continuous Variations graph of thiamin hydrochloride with sulfanilic acid (each 0.05M)
**Table (1): Effect of volume 0.4% w/v sodium nitrite**

<table>
<thead>
<tr>
<th>Volume of sodium nitrite added (ml)</th>
<th>0.0</th>
<th>0.2</th>
<th>0.4</th>
<th>0.6</th>
<th>0.8</th>
<th>1.0</th>
<th>1.2</th>
<th>1.4</th>
<th>1.6</th>
<th>1.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>0.000</td>
<td>0.005</td>
<td>0.008</td>
<td>0.010</td>
<td>0.086</td>
<td>0.100</td>
<td>0.150</td>
<td>0.438</td>
<td>0.428</td>
<td>0.420</td>
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</table>

**Table (2): Effect of volume of 0.05M sulfanilic acid**

<table>
<thead>
<tr>
<th>Volume of sulfanilic acid (ml)</th>
<th>0.0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>0.00</td>
<td>0.108</td>
<td>0.231</td>
<td>0.321</td>
<td>0.403</td>
<td>0.440</td>
<td>0.425</td>
<td>0.410</td>
<td>0.406</td>
<td>0.402</td>
</tr>
</tbody>
</table>

**Table (3): Effect of volume 30% w/v sodium hydroxide**

<table>
<thead>
<tr>
<th>Volume of sodium hydroxide added (ml)</th>
<th>0.0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>0.000</td>
<td>0.016</td>
<td>0.256</td>
<td>0.386</td>
<td>0.412</td>
<td>0.442</td>
<td>0.430</td>
<td>0.321</td>
<td>0.302</td>
<td>0.290</td>
</tr>
</tbody>
</table>

**Table (4): Analysis of pharmaceutical preparations by proposed and official method. The figure in parentheses is coefficients of variation.**

<table>
<thead>
<tr>
<th>Pharmaceutical preparation</th>
<th>Mean recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proposed method</td>
</tr>
<tr>
<td>Solution injectable, Vrsac-Yougoslavie (100mg/ml)</td>
<td>102.25</td>
</tr>
<tr>
<td>Vitamin B1 Darou-Iran (100mg/Tablet)</td>
<td>96.83</td>
</tr>
</tbody>
</table>

*Mean of three determinations.

**Table (5): Effect of excipients on the recovery of thiamin hydrochloride by the proposed method.**

<table>
<thead>
<tr>
<th>Excipients</th>
<th>Amount Per 10mg of drug</th>
<th>Recovery%*</th>
<th>S.D%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0.5</td>
<td>99.3</td>
<td>0.30</td>
</tr>
<tr>
<td>Lactose</td>
<td>0.5</td>
<td>98.6</td>
<td>0.32</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.5</td>
<td>99.1</td>
<td>0.33</td>
</tr>
<tr>
<td>Starch</td>
<td>0.5</td>
<td>97.5</td>
<td>0.38</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>0.1</td>
<td>96.0</td>
<td>0.51</td>
</tr>
</tbody>
</table>

*Mean of three determinations.
References
1- Analytical Methods Committee, Analyst, 2000, 125,353.