Evaluation of Vitamin B1, B2 and B6 Tablets in Bangladesh by UV-Vis Spectrophotometry

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ABSTRACT

Thiamine hydrochloride (vitamin B1), riboflavin (vitamin B2) and pyridoxine hydrochloride (vitamin B6) were assayed in pharmaceutical dosages form. Thiamine hydrochloride of 100 mg dose of six different companies, riboflavin of 5 mg dose of five different companies and pyridoxine hydrochloride 20 and 25 mg dosages of two different companies of Bangladesh were extracted from the tablets, cleaned up and their active ingredients were evaluated by UV-Vis spectrophotometer at 432, 445 and 292 nm, respectively. Evaluation was carried out with respect to calibration curves of their standard reference samples. Amount of thiamine hydrochloride in 100 mg tablets of six different companies were found to be in the range of 37.62±1.11 mg to 79.03±0.42 mg. For riboflavin in 5 mg tablets of five different companies, active ingredient was found in the range of 6.78±0.19 mg to 8.23±0.15 mg. In case of pyridoxine hydrochloride tablets, it was found that 20 mg tablet of one company contained 21.75±0.41 mg and 25 mg tablet of another company contained 29.72±0.59 mg of active ingredient. Recovery experiments were done by spiking excipients of the respective medicines at three different concentration levels with 5 replicate studies. Correlation coefficients ($r^2$) were found to be 0.99 for three vitamins and recoveries were 82.26± 0.10, 99.61± 0.06 and 109.91±0.12 for thiamine hydrochloride (vitamin B1), riboflavin (vitamin B2) and pyridoxine hydrochloride (vitamin B6) respectively.

Introduction

Vitamins are essential nutrients for performing necessary chemical and physiological functions in the human body [1]. On the basis of solubility, they are two types, solubility in fat (A, D, E, and K vitamins) and water (C and B vitamins) [2]. The B vitamins are named as thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), pyridoxine (vitamin B6), pantothenic acid (vitamin B5), biotin (vitamin B7), folate (vitamin B9) and cyanocobalamin (vitamin B12). The chemical structure of the thiamine hydrochloride molecule is shown in Figure 1(a). It helps to prevent from heart disease [3], brain disorder [4], mental illness [5, 6], autism [7], to alleviate dysmenorrhea [8], protect against uremia [9], renal disease [10], to the diabetic neuropathy [11], cures Beriberi [12] and helps to the elderly population by improving sleep and increase energy [13] etc. Vitamin B6 (Figure 1b) is riboflavin whose IUPAC name is 7, 8-dimethyl-10-(1’-dimethylamino)isoalloxazine. It has been used in several clinical and therapeutic situations including prevention of migraine [14, 15], gastroplasty [16], helps to ensure proper growth of unborn babies [17], protect against gingivitis [18], beneficial for premature babies [19], antiretroviral side effects [20] etc. Vitamin B6 (Figure 1c), chemically pyridoxine hydrochloride is 5-hydroxy-6-methylpyridine-3,4-dimethanol hydrochloride. Its intake is necessary for relieving the nausea and vomiting associated with early pregnancy [21], help to alleviate neuropathy [22], infant health growth [23], reduce stress and anxiety [24], reduce risk of heart disease [25, 26], reduce blood pressure in patients with hypertension [27], reduce risk of lung cancer [28] etc. Vitamins have a variety of uses in industrial and clinical sectors. They uses in foods as colorants, antioxidants and especially nutritive additives [29]. Although the vitamin requirement of the human body can be easily satisfied by a balanced diet, but there is still a chance of susceptibility to low micronutrient intakes and hence a higher risk of vitamin deficiency. Enrichment and fortification of vitamins in foods such as infant foods, fruit juices, milk, cereal, etc. have helped address this issue. For planning a healthy and balanced diet, the proper information on vitamin...
content in food sources consumed frequently is critical to assessing dietary adequacy. That is where the significance of vitamin analysis comes into play. As many vitamins are unstable and easily degraded, monitoring their loss during processing is important in the development of appropriate processing and storage schemes for optimal nutrient content in the final food products [30]. Moreover, addition of vitamins into food needs to be properly controlled to satisfy the guidelines set by the governmental authorities [29]. Sometimes, the deficiency of vitamins in human body causes a lot of diseases. Therefore, rapid and reliable analysis of vitamins is in high demand by food manufacturers and drug administrators. In continuation of our research group to work on contaminants of foodstuff, the objective of the present work was to develop simple, precise, rapid, sensitive and accurate methods to assay vitamin B1, B2 and B6 by UV-visible spectrophotometer.

Materials and Methods

Sample collection

Commercial tablets of thiamine hydrochloride (B1), riboflavin (B2) and pyridoxine hydrochloride (B6) were collected from different pharmacies in Dhaka city. Six different types of commercial thiamine hydrochloride (coded as T1 to T6), five different types of commercial riboflavin (coded as R1 to R5) and 2 different types of commercial pyridoxine hydrochloride (coded as P1 and p2) samples were collected for analysis. All of them were in tablet form. Standard sample of vitamin B1, B2, B6 and 4-aminophenol were obtained by courtesy of New life and Co.Pvt.Ltd., Bangladesh.

Chemicals and Reagents

Methanol, acetone, acetic acid, ammonia solution, chloroform (purchased from BDH England) and HPLC grade water were used to carry out the study.

Instruments

A double beam Ultraviolet-visible spectrophotometer (Shimadzu, UV-1800), an analytical balance (AL 104, Mettler Toledo), a vortex machine (Kebo LabRex-2000), anoven (G-1020, Salvis) and a centrifuge machine (Cowbell) were used.

Preparation of standards solutions

Thiamine hydrochloride (vitamin B1)

The primary standard solution (1000μg/mL) of riboflavin (vitamin B2) was made by dissolving 100 mg of riboflavin in 100.0 mL volumetric flask by HPLC grade water. Primary standard solution was diluted to 500, 100 and 50 μg/mL with HPLC grade water. Preparation steps were performed in the subdued light condition using glass wares covered with foil to prevent vitamins from degradation. 20 mL of the thiamine hydrochloride solution (50 μg/mL) was taken in a separating funnel. Then 10 mL of previously prepared 100 μg/mL 4-aminophenol solution, 20 mL concentrated ammonia solution and 40 mL of chloroform was added and mixed well. Then the whole mixture was kept for settle down and a yellow colored complex and two layers were formed. The yellow colored complex was extracted in chloroform layer. Thiamine hydrochloride in 50 μg/mL 20 mL solution is extracted in 40 mL chloroform layer. So, the final concentration of thiamine hydrochloride was 25 μg/mL. The working standard solutions were prepared by dilution of 25 μg/mL thiamine hydrochloride and 4-aminophenol complex solution by chloroform. The working standard solutions of thiamine hydrochloride and 4-aminophenol complex were prepared in concentration 0.1, 0.5, 1, 2.5, 5, 10, 12.5, 15, 17.5, 20 and 25 μg/mL. Absorptions of working standard solutions of the respective medicines were measured; calibration curves were drawn by plotting absorption vs concentration graph (Fig. 2a) and limit of detections were found out.

Riboflavin (B2)

The primary standard solution (1000μg/mL) of riboflavin (vitamin B2) was made by dissolving 100 mg of riboflavin in a 100 mL volumetric flask by HPLC grade water. The working standard solutions of riboflavin (0.5, 1, 5, 10, 20, 40, 50, 100 and 500 μg/mL) were made from primary standard solution by dilution with the same solvent. Absorbance (deionized water was taken as reference) of these solutions were measured by a double beam UV spectrophotometer to draw a calibration curve of absorbance vs. concentration (Fig 2b).

Pyridoxine hydrochloride (B6)

The primary standard solution (500μg/mL) of pyridoxine hydrochloride (vitamin B6) was made by HPLC grade water. After dilution by the same solvent, working standard of pyridoxine hydrochloride solutions (0.5, 1, 2.5, 5, 10, 12.5, 25, 50, 80, 100, 120, 150, 200 and 250 μg/mL) were made. 10 mL from each solution was taken in 50 mL volumetric flask separately. Then 25 mL of methanol was added followed by the addition of 3.5 mL of glacial acetic acid. Then the solution was up to the marked by deionized water. As 10 mL of each solution was transferred and up to the marked in 50 mL volumetric flask the final concentration of each solution was 5 times diluted. So the final concentration of 0.5, 1, 2.5, 5, 10, 12.5, 25, 50, 80, 100, 120, 150, 200, 250 μg/mL converted to 0.1, 0.2, 0.5, 1, 2, 2.5, 5, 10, 16, 20, 24, 30, 40, 50 μg/mL respectively. A blank solution was prepared in a 50 mL volumetric flask by adding 25 mL of methanol, 3.5 mL of acetic acid and then up to the marked by deionized water. Absorbance (blank solution was taken as reference) of the prepared working standard solutions were measured by a double beam UV spectrophotometer to draw a calibration curve (Fig 2c) of absorbance vs. concentrations.

Extraction and cleanup of active ingredients

Thiamine hydrochloride (vitamin B1) sample solutions

Ten tablets of riboflavin of each company were weighted, homogenized by making powder (using mortar and pestle). Three replicate studies were done with the homogenized powder of the medicine. By considering the actual weight of tablet and written active constituent (100 mg of thiamine hydrochloride) required amount of homogenized sample powder was suspended in HPLC grade water for preparing 50 μg/mL thiamine hydrochloride solution in 100.0 mL volumetric flask. The suspended materials were vortex (2
min), centrifuged (3000 rpm; 5 min) and the supernatants were collected. The solution was filtered by passing through syringe filter for removing any un-dissolved particles. Then 20 mL of the 50 μg/mL thiamine hydrochloride tabletsolution was taken in a separating funnel by the addition of 20 mL ammonia solution, 10 mL previously prepared 4-aminophenol solution (100 μg/mL) and 40 mL of chloroform. Couple of minute later a nice yellow coloured solution was formed. The lower part of the two layers was chloroform layer containing 1:1 complex of thiamine hydrochloride and 4-amino phenol. 3 mL solution from the chloroform layer was taken in a 10 mL volumetric flask and then up to the marked by chloroform. The final concentration was7.5μg/mL. The active ingredients present in the supernatants were cleaned up by filtering through HPLC grade syringe filters (0.45 μm). Absorptions of the cleaned extracts (three replicates for each company of each medicine) were measured and their concentrations were calculated using calibration curves. Relative Standard Deviations were calculated and the data are presented in Table 2.

Riboflavin (vitamin B2) sample solutions
Weight of ten tablets of riboflavin of each company was measured, homogenized by making powder (using mortar and pestle). Three replicate studies were done with the homogenized powder of the medicine. Considering the average weight of riboflavin (5 mg) tablets, the required amount of powder was suspended in HPLC grade water to obtain 20 μg/mL active ingredients of the medicines. A yellow-orange solution was formed. The solution was cleaned up by filtering through HPLC grade syringe filters (0.45 μm). Three replicates for each brand were done and absorptions of the cleaned extracts were measured(Table 3).

Pyridoxine hydrochloride (vitamin B6) sample solutions
Weight of ten tablets of pyridoxine hydrochloride of each company was measured, homogenized by making powder (using mortar and pestle). Three replicate studies were done with the homogenized powder of the medicine. Considering the average weight of pyridoxine hydrochloride (20 mg and 25 mg of pyridoxine hydrochloride) tablets, the required amount of powder was suspended in HPLC grade water to obtain 10 μg/mL active ingredients of the medicines. The suspended materials were vortex (2 min), centrifuged (3000 rpm; 5 min) and the supernatants were collected. The active ingredients present in the supernatants were cleaned up by filtering through HPLC grade syringe filters (0.45 μm). 10 mL from this solution was transferred in a 50 mL volumetric flask where 3.5 mL of acetic acid and 25 mL of methanol were added earlier. The solution was then up to the marked by deionized water. A blank solution was also prepared in a 50 mL volumetric flask by adding 25 mL of methanol, 3.5 mL of acetic acid and then up to the marked by deionized water. Absorptions of the cleaned extracts (three replicates for each company of each medicine) were measured and their concentrations were calculated using calibration curves. Relative Standard Deviations were calculated and the data are presented in the Table 4.

Recovery experiment

Recovery experiments were done by spiking the respective medicines to the excipients at three different concentration levels (6, 7.5 and 9 μg/mL of thiamine hydrochloride; 10, 20, 30 μg/mL of riboflavin; and 6, 10, 12 μg/mL of pyridoxine hydrochloride). Five Replicates were done for each concentration. Relative Standard Deviations were calculated and the results are presented in Table 1.

Results and Discussion

The active ingredients of thiamine hydrochloride, riboflavin and pyridoxine hydrochloride were evaluated by UV-Vis spectrophotometer at 432, 445 and 292 nm, respectively. The wavelength of maximum absorption (λmax) of the cleaned-up extracts of thiamine hydrochloride, riboflavin and pyridoxine hydrochloride tablets (Fig. 3b, 4b and 5b) were fitted very well with their respective standard spectra (Fig. 3a, 4a and 5a) and overlain spectra (3c, 4c and 5c). In these studies the methods were modified and validated by 5 replicate studies. The correlation coefficients (r²) values 0.9993, 0.9941 and 0.9974 (Fig. 2a, 2b and 2c) of thiamine hydrochloride, riboflavin and pyridoxine hydrochloride were showed accuracy of experiments. Low limit of detection (LOD) were 0.1, 1 and 0.1 μg/mL (Fig. 3c, 4c & 5c) showed sensitivity of the modified methods. The method was validated from recovery experiments (Table 1) and their low respective relative standard deviations were indicated that the method was satisfactory. The results also showed acceptable repeatability and reproducibility of the methods. The % of mean recovery was 82.26±0.10 for thiamine hydrochloride, 99.61±0.06 for riboflavin and 109.91±0.12 for pyridoxine hydrochloride.

In case of thiamine hydrochloride the number of sample analyzed was showed (Table 2) a range of results (37.62±1.11 to 79.03±0.42 mg). All samples were found to be contained lower amount of active ingredients than the amount written on the label (100 mg). For riboflavin tablets (Table 3), active ingredient was found in the range of 6.78±0.19 to 8.23±0.15 mg. All of them contained higher amount of active ingredients than written value (5 mg). For pyridoxine hydrochloride tablets (Table 4), it was found that sample P1 (20 mg, written on the label) contained 21.75±0.41 mg and sample P2 (25 mg, written on the label) contained 29.72±0.59 mg of pyridoxine hydrochloride. The methodology for determination of thiamine hydrochloride [31] and riboflavin [32] were partially according to the published methods with some modifications but for pyridoxine hydrochloride, the method was new. The described methods for the determination of thiamine hydrochloride, riboflavin and pyridoxine hydrochloride were satisfactory with a wide range of concentration. Our goal was to determine thiamine hydrochloride, riboflavin and pyridoxine hydrochloride easily in dosage forms with sufficient precision and accuracy. As the relative standard deviation values (0.53-7.51 for thiamine hydrochloride, 0.43-5.56 for riboflavin and 1.89-1.99 for pyridoxine hydrochloride) obtained from the analysis were below 20, so the methods were satisfactory.
pyridoxine hydrochloride of pharmaceutical dosage forms can be done within a short period of time.

Table 1: Accuracy studies in standard thiamine hydrochloride, riboflavin and pyridoxine hydrochloride

<table>
<thead>
<tr>
<th>Standard with matrix</th>
<th>Spiking concentration (ppm, n=5)</th>
<th>% Recovery</th>
<th>Mean recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamine hydrochloride</td>
<td>6</td>
<td>84.47±0.04</td>
<td>82.26±0.10</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>75.69±0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>86.62±0.14</td>
<td></td>
</tr>
<tr>
<td>Riboflavin</td>
<td>10</td>
<td>91.43±0.05</td>
<td>99.61±0.06</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>103.90±0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>103.49±0.06</td>
<td></td>
</tr>
<tr>
<td>Pyridoxine hydrochloride</td>
<td>6</td>
<td>115.07±0.07</td>
<td>109.91±0.12</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>106.70±0.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>107.95±0.08</td>
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</tr>
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</table>

Table 2: Assay of thiamine hydrochloride (vitamin B₁)

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Amount of active ingredients written in tablet (mg)</th>
<th>Estimated amount, (mg; n=3)</th>
<th>Relative standard deviation (%)</th>
<th>Percentage of active ingredient (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>100</td>
<td>60.62±0.84</td>
<td>1.38</td>
<td>60.62</td>
</tr>
<tr>
<td>T2</td>
<td></td>
<td>79.03±0.42</td>
<td>0.53</td>
<td>79.03</td>
</tr>
<tr>
<td>T3</td>
<td></td>
<td>37.62±1.11</td>
<td>2.95</td>
<td>37.62</td>
</tr>
<tr>
<td>T4</td>
<td></td>
<td>66.83±1.22</td>
<td>1.82</td>
<td>66.83</td>
</tr>
<tr>
<td>T5</td>
<td></td>
<td>71.02±1.22</td>
<td>1.72</td>
<td>71.02</td>
</tr>
<tr>
<td>T6</td>
<td></td>
<td>48.42±3.63</td>
<td>7.51</td>
<td>48.42</td>
</tr>
</tbody>
</table>

Table 3: Assay of riboflavin

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Amount of active ingredients written in tablet (mg)</th>
<th>Estimated average amount (mg; n=3)</th>
<th>Relative standard deviation (%)</th>
<th>Percentage of active ingredient (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>5</td>
<td>6.78±0.19</td>
<td>2.77</td>
<td>135.6</td>
</tr>
<tr>
<td>R2</td>
<td></td>
<td>7.73±0.03</td>
<td>0.43</td>
<td>154.6</td>
</tr>
<tr>
<td>R3</td>
<td></td>
<td>7.60±0.42</td>
<td>5.56</td>
<td>152.0</td>
</tr>
<tr>
<td>R4</td>
<td></td>
<td>7.74±0.09</td>
<td>1.16</td>
<td>154.8</td>
</tr>
<tr>
<td>R5</td>
<td></td>
<td>8.23±0.15</td>
<td>1.80</td>
<td>164.6</td>
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</table>

Table 4: Assay of pyridoxine hydrochloride

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Amount of active ingredients written in tablet (mg)</th>
<th>Average amount, (mg; n=3)</th>
<th>Relative standard deviation (%)</th>
<th>Percentage of active ingredient (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>20</td>
<td>21.75±0.41</td>
<td>1.89</td>
<td>108.75</td>
</tr>
<tr>
<td>P2</td>
<td>25</td>
<td>29.72±0.59</td>
<td>1.99</td>
<td>118.87</td>
</tr>
</tbody>
</table>
Fig 1: Structures of vitamin B1; thiamine hydrochloride (a), B2; riboflavin (b) and B6; pyridoxine (c).

Fig 2: Calibration curves of standard thiamine hydrochloride (a), riboflavin (b) and pyridoxine hydrochloride (c).

Fig 3: Spectra of standard thiamine hydrochloride (a), cleaned extract of tablet (b) and overlain.

Fig 4: Spectra of standard riboflavin (a), cleaned extract of tablet (b) and overlain spectra (c).
Fig 5: Spectra of standard pyridoxine hydrochloride (a), cleaned extract of tablet (b) and overlain spectra (c).

Conclusion

Vitamin B₁ (thiamine hydrochloride), vitamin B₂ (riboflavin) and B₆ (pyridoxine hydrochloride) are very common medicines for the treatment of vitamin deficiency diseases. Though vitamins are essentials, but insufficient or excessive amount can bring harmful situation. The present spectrophotometric method for the determination of thiamine hydrochloride (vitamin B1), riboflavin (vitamin B2) and pyridoxine hydrochloride (vitamin B6) was successful for a wide range of concentration and was cheap, easy and rapid. As the standard deviation values obtained from the analysis were satisfactory, it can be concluded that the method is sufficiently sensitive and reproducible in the routine analysis of vitamin B₁, B₂ and B₆.

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References