Application of Spectrophotometric Methods for the Determination of Thiamine (VB1) in Pharmaceutical Formulations Using 7-Chloro-4-Nitrobenzoxadiazole (NBD-Cl)

Abstract

Simple, swift and sensitive method for Spectrophotometric determination of thiamine (VB1) in pharmaceutical tablets has been described. The proposed method is based on the reaction of thiamine (VB1) and 7-Chloro-4-nitrobenzoxadiazole (NBD-Cl) at alkaline medium (pH 10.5) to develop a deep brown adduct that bears maximum absorption at 434 nm. Beer’s law is obeyed in the concentration range 5-35 µg/ml of thiamine at the selected wavelength. Under optimized reaction conditions, the linear regression coefficients were \( a = 0.033 \), \( b = -0.009 \), and \( r^2 = 0.999 \) calculated for the general equation of the calibration curve \( y = ax + b \).

The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.667µg/ml and 2.020µg/ml respectively. The method has been successfully applied to the determination of thiamine (VB1) in pharmaceutical formulations, and can be used as an alternative of the existing sophisticated method used in drug laboratories and factories.

Keywords: Thiamine (VB1); Spectrophotometric; Pharmaceutical formulation; 7-Chloro-4-nitrobenzoxadiazole (NBD-Cl)

Introduction

Since Thiamine - the first discovered vitamin among B complex vitamins - is considered as an essential nutrient [1], and used for treatment of many disorders and diseases [2]; and because it is included as a component of different multi-vitamins, a variety of analytical methods have been proposed for its determination in pharmaceutical formulations. Those methods include flow injection analysis (FIA) [3-7], spectrophotometric chromatographic methods [9,10], fluorometry [11], high-performance liquid chromatography (HPLC) [12,13], chemiluminescence [2], and electrochemical techniques [14,15].

Most of those methods, though sensitive and reliable, are associated with several disadvantages. FIA methods require a pre-oxidation step of thiamine and extraction with chloroform [7]. Other techniques such as HPLC and electrochemical techniques require highly sophisticated instrumentation besides tedious and time-consuming experimental steps. This sheds light to the need of alternative simple and sensitive methods for thiamine analysis.

Spectrophotometry is considered the most convenient analytical technique, because of its inherent simplicity, low cost, and wide availability in most quality control laboratories [16].

7-Chloro-4-nitrobenzoxadiazole (NBD-Cl) is a well known fluorogenic and color-forming reagent that is widely used in the analysis of a variety of amino group containing drugs [17-20]. Its use in pharmaceutical analysis was reviewed by Elbashir et al. [17] and proved to be a convenient low-cost derivatizing reagent [21,22].

Thus far, the reaction between Thiamine and NBD-Cl has not been reported yet. In a previous paper, we reported a sensitive and simple Spectrophotometric procedure for determination of Thiamine using NQS as color-developing reagent [8]. In this context we report the determination of Thiamine using NBD-Cl as a reagent, and we establish the optimum reaction conditions necessary for reliable measurements in dosage forms.

Experimental

Apparatus

Absorbance measurements were carried out using Shimadzu UV-Visible Spectrophotometer model 1800 with quartz cells of 1cm optical path length, pH measurements were performed in pH meter model HI 255 (Hanna Instruments, Mumbai, India). Masses were taken using a digital Analytical Balance.

Material and reagent

Thiamine hydrochloride was obtained from Sigma Aldrich Ltd, Khartoum, Sudan, and used as received; its purity was 99.15%. A solution of 7-Chloro-4-nitrobenzoxadiazole (NBD-Cl) 0.2% (w/v) was prepared by dissolving 0.2g in distilled water, transferred...
into a 100mL volumetric flask and diluted to the mark with distilled water then mixed well. The solution was freshly prepared and protected from light during use. A series of buffer solutions of pH range from 7-12 were prepared according to the literature methods [23].

An optimum buffer solution of pH 10.5 was prepared by mixing 100mL of 0.025M aqueous solution of Borax with 36.5mL of 1M solution of sodium hydroxide and adjusted to pH 10.5 with 1M Sodium hydroxide. All other chemicals were of analytical grade.

Preparation of Standard and Sample Solution

**Stock standard solution of thiamine (1000µg/mL)**

An accurately 250mg of thiamine hydrochloride standard was dissolved in distilled water, transferred into 250 ml volumetric flask, diluted to the mark with same solvent and mixed well. This stock solution was further diluted to 200µg/mL to obtain working solutions in the range of 5-35µg/mL.

**Tablets sample solution**

Five capsules (Thiamine 100mg/capsule) were weighted and finely-grinded. A portion of the powder equivalent to 25mg of the drug was weighted and dissolved in distilled water, filtered and then transferred into 250 volumetric flask, completed to the mark with distilled water to give a solution of 100 µg/mL. The solution was then subjected to chemical analysis according to the following procedure.

Procedure of analysis

Aliquots of 200µg/mL of Thiamine was transferred into 10ml volumetric flask to give final concentrations of 5-35µg/mL. 1.5mL of 7-Chloro-4-nitrobenzoxadiazole (NBD-Cl) 0.2% (w/v) was added and followed by 1.5mL pH 10.5 buffer solution. The reaction was completed to volume with distilled water, and the resulting solution was measured at 434nm against reagent blank treated similarly.

Stoichiometry of the chemical reaction

The method of continuous variation (Job’s method): The Job’s method of continuous variation was employed to determine the stoichiometric ratio of the reaction between thiamine and NBD-Cl [23]. Equimolar (7.5×10-4M) aqueous solution of Thiamine hydrochloride and NBD-Cl were prepared. A series of 10 mL portions of Thiamine standard and NBD-Cl were made up comprising different complementary proportions (1:9,...:9:1, inclusive) in 10 mL volumetric flask containing 1.5 mL of buffer solution (pH=10.5). The solution was further manipulated as described under the general recommended procedures [24].

Results and Discussion

Absorption spectra

The absorption spectrum of thiamine was recorded against water (Figure 1), it was found that thiamine exhibits a maximum absorption peak (λ\text{max}) at 235nm. Because of highly blue-shifted λ\text{max} of thiamine, its determination in the dosage form based on the direct measurement of its absorption for ultraviolet is susceptible to potential interferences from the common excipients. Therefore, derivatization of thiamine to attain visible-range absorbing species was undoubtedly necessary. Thus, derivatization of thiamine with NBD-Cl was performed, and the absorption spectrum of the product was recorded against reagent blank (Figure 1). It was found that the product is brown colored exhibiting λ\text{max} at 434nm, and the λ\text{max} of Thiamine-NBD-Cl was 342nm. The λ\text{max} of thiamine-NBD-Cl derivative was red-shifted, eliminating any potential interference. The wavelength 434 nm therefore was fixed as optimum.

Optimization of the reaction conditions

The optimum conditions for the developed method were established by varying the parameters one at a time while keeping the other parameters constant and following the effect exerted on the absorbance of the colored product. In order to establish experimental conditions, the effect of various parameters such as pH, time, buffer volume and concentration of NBD-Cl were investigated.

Effect of pH

The effect of pH on the reaction between thiamine and NBD-Cl was tested by varying the pH form 7.0 to 12.0. As shown in Figure 2, the absorbance of the product is low at pH 7.0, indicating that thiamine cannot react with (NBD-Cl) in neutral media. This was possibly due to the existence of the amino group of thiamine in the form of hydrochloride salt, which hampers nucleophilic substitution capability. As the pH increased from 7 to 12, the readings increased dramatically, releasing the amino group of thiamine and facilitates the nucleophilic substitution. The maximum absorption was attained at pH value of 10.5. At pH values more than 10.5, a decrease in the absorption occurred. This was attributed probably to the increase in the amount of hydroxide ion that increases the rate of the backward reaction of thiamine with NBD-Cl.

Effect of reaction time

The absorbance of the reaction product was monitored at different times (Figure 3). Keeping other conditions intact, the absorbance of the reaction product was followed after standing for different time spans at 25°C. The results show that thiamine reacts with NBD-Cl at 25°C and the absorbance begins to increase.
gradually and reach a maximum after 25 min. For longer reaction times, a slight drop in the absorbance was observed. Accordingly, 25 min was set as the convenient reaction time for determination.

**Effect of amount of the buffer**

From the above parameters-adjusting experiments, the optimized conditions used for the assay were: pH 10.5, NBD-Cl concentration 0.2% (w/v), volume of the buffer 1.5 mL, reaction time 25 min and temperature 25°C.

**Effect of NBD-Cl concentration**

The study of the effect of NBD-Cl concentrations showed that the reaction was dependent on the reagent concentration. The highest absorption intensity was attained at NBD-Cl concentration of 0.2% (w/v), and higher concentration of NBD-Cl leads to a decrease in the absorbance (Figure 5).

**Validation of the method**

The method was validated for the following parameters: specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), and robustness according to the International Conference on Harmonization (ICH) guidelines [25].

**Linearity, limit of detection (LOD) limit of quantification (LOQ)**

The linearity was evaluated by linear regression analysis determined by constructing seven concentrations of thiamine, in the range of 0.5–35 μg/mL, which was calculated by the least square regression method to calculate the calibration equation and the correlation coefficient. The calibration curves were constructed by plotting concentration versus absorbance, using linear regression analysis. The regression equation for the results

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**Figure 2:** Effect of pH on the reaction of Thiamine with NBD-Cl. Thiamine (20 μg/ml): 1 ml, NBD-Cl conc. 0.2% (w/v), reaction time 20 min.

**Figure 3:** Effect of reaction time on the reaction of Thiamine with NBD-Cl. Thiamine (20 μg/ml): 1 ml, Buffer (pH 10.5): 1.5 ml, NBD-Cl conc. 0.2% (w/v).

**Figure 4:** Effect of Buffer amount (ml) on the reaction of Thiamine with NBD-Cl. Thiamine (20 μg/ml): 1 ml, Buffer pH: 10.5, NBD-Cl conc. 0.2% (w/v), reaction time 25 min.

**Figure 5:** Effect of NBD-Cl concentration on its reaction with Thiamine. Thiamine (20 μg/ml): 1 ml, Buffer (pH 10.5): 1.5 ml, reaction time 25 min.
was A=0.033x - 0.009 (r²=0.999), where A is the absorbance at 434 nm, x is the concentration of thiamine in μg/mL in the range of 0.5-35 μg/mL, and r is correlation coefficient (Table 1). It was found that the linear concentration range is comparable with our previous method using NQS [8]. The limit of detection (LOD) and limit of quantification (LOQ) were determined according to the following formula LOD=3.3×SDa/b, and LOQ=10×SDa/b, SDa is the standard deviation of the intercept; b is the slope under the ICH guidelines [25]. The LOD and LOQ were found to be 0.667 and 2.020 μg/mL, respectively (Table 1).

### Table 1: Parameters for the performance of the proposed method.

| Parameter                  | Value  |
|----------------------------|--------|
| Measurement wavelength (nm)| 434    |
| Linear range (μg/mL)       | 5-35   |
| Regression equation        | y = 0.033x - 0.009 |
| Intercept                  | -0.00986 |
| Standard deviation of intercept | 0.00667 |
| Slope                      | 0.0330 |
| Standard deviation of slope | 0.000298 |
| Correlation coefficient (r²)| 0.9995 |
| Limit of detection, LOD (μg/mL) | 0.667 |
| Limit of quant., LOQ (μg/mL) | 2.0207 |

### Accuracy

The accuracy of the proposed method was carried out by applying 3 different concentrations 10, 20, and 30 μg/mL of thiamine drug within linear range calculated as the percentage of the drug recovered from the samples (Table 2).

### Table 2: Recovery studies for the determination of thiamine by the proposed method.

| Sample No | Sample Content (μg/mL) | Thiamine Standard Amount (μg/mL) | Amount Found (Total) (μg/mL) | Recovery + SD* |
|-----------|------------------------|---------------------------------|-----------------------------|----------------|
| 1         | 1                      | 10                              | 14.5                        | 96.29±0.015    |
| 2         | 5                      | 20                              | 24.72                       | 98.90±0.018    |
| 3         | 5                      | 30                              | 35.5                        | 101.47±0.148   |

*Values are mean of three determinations.

Relative error (RE) was within 0.24% with corresponding standard deviation within 0.004 for three different determinations (Table 3).

### Table 3: Evaluation of accuracy and precision.

| Sample No | Concentration (μg/mL) | Concentration Found (μg/mL) | Recovery (% ± SD) | Relative Error (%) |
|-----------|-----------------------|-----------------------------|-------------------|--------------------|
| 1         | 5                     | 4.70                        | 95.5±0.004        | 0.224              |
| 2         | 20                    | 19.29                       | 96.46±0.002       | 0.15               |
| 3         | 30                    | 29.25                       | 97.50±0.003       | 0.24               |

### Robustness

**Reaction mechanism:** It has been reported that NBD-Cl reacts with amino group of primary or secondary amine derivatives [26-29]. Similarly, amino group of thiamine can act as a nucleophile due to the lone pair of electrons on the nitrogen atom, trending to attack on the electron–deficient center in NBD-Cl (Table 4 & Figure 6). At the same time, it has been proved that the composition of the product is 1:1 of thiamine and NBD-Cl (Figure 7). So it is concluded that amino group of thiamine react with NBD-Cl to form a brown adduct. The reaction equation is shown in Figure 7.

### Table 4: Influence of small variation in the assay condition on the analytical performance of the proposed spectrophotometric method for determination of Thiamine using NBD-Cl reagent.

| Parameter                  | Recovery (% ± SD) |
|----------------------------|-------------------|
| Recommended condition      | 97.50±0.003       |
| NBD-Cl concentration (0.22%) | 98.75±0.002     |
| NBD-Cl concentration (0.180%) | 96.76±0.002     |
| Buffer PH(10.7)            | 96.90±0.002       |
| Buffer PH(10.3)            | 95.79±0.022       |
| Reaction Time min (23)     | 96.09±0.009       |
| Reaction Time min (27)     | 98.01±0.003       |

Figure 6: The Job's method plot for the stoichiometry of the reaction of Thiamine with NBD-Cl Vr: Volume of NBD-Cl (7.5×10^-4 mol/L), Vt: Volume of Thiamine (7.5×10^-4 mol/L), Vr + Vt=10 ml.

### Figure 6: The Job's method plot for the stoichiometry of the reaction of Thiamine with NBD-Cl.

Vr: Volume of NBD-Cl (7.5×10^-4 mol/L), Vt: Volume of Thiamine (7.5×10^-4 mol/L), Vr + Vt=10 ml.
Application of the proposed method to analysis of thiamine dosage form

Thiamine tablets were subjected to the analysis by the proposed and the label claim agrees well with our new method as shown in Table 5. The proposed method has the advantage of being virtually free from interferences by excipients.

Table 5: Analysis of Thiamine-containing dosage form by the proposed method.

| Brand Name of Label Claim (Mg) | Amount Found (Mg) | (% Found ± SD) |
|-------------------------------|------------------|---------------|
| Thiamine tablets (100 mg) | 99.96 | 99.9±0.025 |

*values are mean of five determinations.

Figure 7: Reaction of thiamine with NBD-Cl showing 1:1 stoichiometry.

Conclusion

The present paper described the evaluation of NBD-Cl as analytical reagents in the development of simple, sensitive, and accurate spectrophotometric method for the determination of thiamine in pharmaceutical formulations. The proposed method is simple, reliable, specific, accurate, reproducible, and highly sensitive, for the determination of thiamine in commercially available dosage forms. The procedure presented here does not need necessitate any expensive apparatus; and can be used advantageously as a routine method for the determination of thiamine in quality control labs and industry. The method may be applied to the determination of other secondary amine derivatives as well.

References

1. Barbara SS (2013) A Simple and Sensitive Analytical Method for the Determination of Thiamine in Pharmaceutical Preparations. Journal of Analytical Chemistry 68(3): 218-222.
2. Chengxiao Z, Guojun Z, Zhujun Z, Akawa M (1999) Highly sensitive electrochemical luminescence determination of thiamine. Analytica Chimica Acta 394(2-3): 165-170.
3. Rocha FR, Fatibello FO, Reis BF (2003) A multicommuted flow system for sequential spectrophotometric determination of hydrosoluble vitamins in pharmaceutical preparations. Talanta 59(1): 191-200.
4. Pérez RT, Martínez LC, Sanz A, Guillelín A (2004) Successive determination of thiamine and ascorbic acid in pharmaceuticals by flow injection analysis. J Pharm Biomed Anal 34(3): 551-557.
5. Alfonso A, Almendral MJ, Porras MJ, Carto Y (2006) Flow-injection solvent extraction without phase separation: Fluorimetric determination of thiamine by the thiocchrome method. J Pharm Biomed Anal 42(2): 171-177.
6. Oliveira CNC, Vicente PA, Aniceto C, Fatibello O (1999) Flow injection turbidimetric determination of thiamine in pharmaceutical formulations using silicotungstic acid as precipitant reagent. Talanta 48(3): 659-667.
7. Andrei F, Martínez J (1994) FIA-Spectrophotometric determination of thiamine after uv-irradiation. Talanta 41(12): 2147-2151.
8. Abdel RST, Elbashir AA, El-Mukhtar M, Ibrahim MM (2015) Development and Validation of Spectrophotometric Method for Determination of Thiamine (VB1) in Pharmaceutical Formulations using 1, 2-Naphthoquinone-4-Sulphonate (NQS). Enliven: Bio analytical Techniques 2(1): 1-6.
9. Marsza ML, Lebiedzinska A, Czarowski W, Szefer P (2005) High-performance liquid chromatography method for the simultaneous determination of thiamine hydrochloride, pyridoxine hydrochloride and cyanocobalamin in pharmaceutical formulations using coulometric electrochemical and ultraviolet detection. J Chromatogr A 1094 (1-2): 91-99.
10. Tang X, Cronin DA, Brunton NP (2006) A simplified approach to the determination of thiamine and riboflavin in meats using reverse phase HPLC. J Food Compos Anal 19(8): 831-837.
11. Mohsen Z, Mohammad RG, Parviz N (2010) Dispersive liquid-liquid microextraction followed by spectrophotometry as a simple and accurate technique for determination of thiamine (vitamin B1). Microchem Acta 168: 317-324.
12. Hassan O, Chee MJ (2001) Sensitivity of UV detection in simultaneous separation and detection of B-vitamins using HPLC. Malaysian Journal of Analytical Sciences 7(1): 251-255.
13. Losa R, Sierra MI, Fernandez A, Blanco D, Buesa JM (2005) Determination of thiamine and its phosphorylated forms in human plasma, erythrocytes and urine by HPLC and fluorescence detection: a preliminary study on cancer patients. J Pharm Biomed Anal 37(5): 1025-1029.
14. Halvatizis SK, Timotheou-S-Potamia M (1989) Kinetic-potentiometric determination of ascorbic acid, biotin, pyridoxine hydrochloride and thiamine hydrochloride with n-bromosuccinimide. Analytica Chimica Acta 227(1989): 405-419.
15. Hassan SSM, Elhenna E (1989) Selective determination of thiamine (Vitamin B(1)) in pharmaceutical preparations by direct potentiometric argentometric titration with use of the silver-silver sulphide ion-selective electrode. Talanta 36(10): 1011-1015.

16. Sara AM, Abdalla AE, Hassan YAE (2011) Spectrophotometric methods for the determination of gemifloxacin in pharmaceutical formulations. Acta Pharmaceutica Sinica B 1(4): 248-253.

17. Elbashir AA, Krieger S, Schmitz OJ (2014) Simultaneous determination of polyamines and acetylpolyamines in human urine by capillary electrophoresis with fluorescence detection. Electrophoresis 35(4): 570-576.

18. Elbashir AA, Alfadil AAB (2013) Development and Validation of Spectrophotometric Method for Determination of Penicillamine (PA) in Pharmaceutical Formulation Using 4-Chloro-Nitrobenzo-2-Oxa-1, 3-Diazol (NBD-CL). World Journal of Analytical Chemistry 1(2): 18-22.

19. Basheir BEA. Elbashir AA (2015) Spectrophotometric Method for Determination of L-Dopa in Pharmaceutical Formulation Using 7-Chloro-4-Nitrobenzoxadiazole (NBD-CL) as A Chromogenic Reagent. European Journal of Pharmaceutical and Medical Research 2(1): 304-316.

20. Mohammed TO, Elbashir AA (2015) Spectrophotometric Method for Determination of Gabapentin in Pharmaceutical Formulation by Derivatization with 4-Chloro-7-Nitrobenzo-2-Oxa-1, 3-Diazole (NBD-CL). International Journal of Drug Development and Research 7: 1-4.

21. Elbashir AA, Suliman FO, Aboul EHY (2011) The application of 7-chloro-4-nitrobenzoxadiazole (NBD-CL) for the analysis of pharmaceutical-bearing amine group using spectrophotometry and spectrofluorimetry techniques. Applied Spectroscopy Reviews 46(3): 222-241.

22. Elbashir AA, Suliman FO, Aboul EHY (2011) The Application of 7-Chloro-4-Nitrobenzoxadiazole and 4-Fluoro-7-Nitro-2,1,3 Benzoxadiazole for The Analysis of Amines and Amino Acids Using High-Performance Liquid Chromatography. Source Gazi University Journal of Science 24(4): 679-697.

23. Robinson RA, Stokes RH (1968) "Electrolyte Solutions" (2nd Edn), Butterworths, London.

24. Skoog DA, West DM, Crouch SR (2004) Fundamentals of Analytical Chemistry, (8th edn), Cole Cengage Learning, USA, pp.805.

25. ICH (2005) Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of analytical procedures: Text and methodology Q2 (R1). ICH Harmonised Tripartite Guideline, p: 1-17.

26. Klimisch HJ, Stadler L (1974) Fluorimetric Bestimmung of nitrosamines after acid catalysed denitrification and obtaining of a derivative with 7 chloro 4 nitrobenzo 2 oxa 1,3 diazole. J Chrom 90(1): 223-225.

27. Murray GM, Sepaniak MJ (1983) HPLC laser fluorometric determination of amines in beer. Journal of Liquid Chromatography 6(5):931-938.

28. Tosunoglu S, Ersoy L (1995) Determination of baclofen in human plasma and urine by high-performance liquid chromatography with fluorescence detection. Analyst 120(2): 373-375.

29. Knabe J (1974) Colorimetric and Fluorimetric Analysis of Organic Compounds and Drugs. Wiley online library 308(10): 803-804.