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SPECTROFLUOROMETRIC METHODS FOR DETERMINATION OF THIAMINE (VITAMIN B1) IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT
Two simple and sensitive methods for determination of Thiamine in pharmaceutical formulations have been reported. The first method was based on the reaction of Thiamine with 1,2-Naphthoquinone-4-sulphonate sodium salt (NQS) in basic medium (pH = 11) giving a fluorescent product which shows excitation and emission maxima at 390 and 460 nm respectively. The second method was based on the reaction of Thiamine with 7-chloro-4-nitro-2,1,3-benzoxaldizole (NBD-Cl) in buffered medium (pH = 10.5) leading to the formation of highly fluorescent adduct which is measured at 562 nm after excitation at 472 nm. All factors that affect the reaction was studied and optimized, and under optimum conditions linear relationship with good correlation coefficient (0.999) relating to both reagents was obtained in the concentration ranges 0.1 – 1.0 and 0.2 – 1.0 µg/mL for NQS and NBD-Cl methods respectively. The limit of detection was found to be 0.020 µg/mL for the two methods. The Precision of the methods were satisfactory with relative standard deviation (RSD) of less than 2. The proposed methods were successfully applied to the analysis of Thiamine in pharmaceutical formulations with good accuracy; the recovery percentages ranged from 98.90±0.49 –101.39±1.02 %. The results were in good agreements with those of the reported methods.

KEYWORDS: Thiamine (Vitamine B1), Spectrofluorometry, 7-Chloro-4-nitrobenzoxadiazole (NBD-Cl), pharmaceutical formulations.

1. INTRODUCTION

Thiamine is a sulfur-containing vitamin and it is a member of the B complex group. Thiamine was discovered by two Dutch scientists, Jansen and Donath in 1926, and isolated in pure form by Williams in 1934.[1,2] Thiamine has various names in the literature, it is called vitamin B_1, aneurin and antineuritic vitamin, but it is most commonly called vitamin B1.

Thiamine was used by all living organisms, and only bacteria, fungi, and plants can synthesize it from natural resources. Other animals obtain it from their diet, and it is considered to be an essential nutrient for human beings.[3]

Thiamine also is a necessary redox coenzyme and function in the metabolism of carbohydrates including glucose, and works in the pentose shunt, and citric acid catalytic cycles.[2,4,5] Thiamine diphosphate, or pyrophosphate (TPP), is involved in oxidative decarboxylation in the mitochondrion[6], and is involved in more than 24 enzymatic reactions in the body.[3] It is also acts as a coenzyme in pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase, and transketolase (TK) catalytic cycles.[7]

Many different methods were reported for the analysis of Thiamine in pharmaceutical formulations. Among them flow injection analysis (FIA)[8-12], spectrophotometric methods[13], chromatographic methods[14,15], high-performance liquid chromatography (HPLC)[16,17], electrochemical techniques[18,19], and chemilumenescence.[20,21]

Although those described methods are sensitive and reliable, yet, they are accompanied by some difficulties. These difficulties may be a pre-oxidation step, or tedious extraction procedures by different organic solvents, or require highly sophisticated instrumentation which limits their use in normal quality control laboratories. Spectrofluorometry is considered to be one of the most convenient analytical techniques because of its simplicity, low cost and wide availability in quality control labs.

Both 1,2-Naphthoquinone-4-sulphonate sodium salt (NQS), and 7-Chloro-4-nitrobenzoxadiazole (NBD-Cl) are well known as chromogenic and fluorogenic reagent that is widely used in the analysis of a variety of amino group- containing drugs[22-28] Their use in pharmaceutical analysis were reviewed by Elbashir et al. and proved to be convenient low-cost derivatizing reagents.[29-31]

To the best of our knowledge, there were no reports considering the determination of Thiamine spectrofluorometrically using those two reagents appeared in the literature. For this reason, the present study describes two simple spectrofluorometric methods for the analysis of Thiamine in pure and pharmaceutical dosage forms.

2. EXPERIMENTAL

2.1 Apparatus

Spectrofluorometric measurements were recorded using RF-5301 PC spectrofluorimeter (Shimatzu, Japan) equipped by 1-cm quartz cell. pH measurements were recorded in pH meter model HI 255 (Hanna Instuments, Mumbai, India). Masses were taken using a digital analytical balance.

2.2 Chemicals and reagents

All chemicals used are of analytical grade (AR). Thiamine hydrochloride (99.15%), Sodium-1,2 naphthoquinone-4-sulphonate (NQS), and 4-Choro-7-nitrobenzo-2-oxa-1,3 diazole (NBD-Cl) 98% were obtained from Sigma Aldrich, and were used as received without further purification. Water was doubly distilled.

2.3 Standard stock solution of Thiamine (100µg/ml)

An accurately weighted 25.0 mg of thiamine hydrochloride standard was dissolved in distilled water, transferred into 250 ml volumetric flask, diluted to the mark with distilled water and mixed well. This stock solution was further diluted to obtain working solutions in the range of 0.1-1.0 µg/mL.

2.4 Stock standard solution of NQS (0.6% weight/volume)

A solution of sodium-1,2-naphthoquinone-4- sulfonate (NQS) 0.6% (w/v) was prepared by dissolving 0.6 g in distilled water, transferred into a 100 mL volumetric flask and diluted to the mark with distilled water and mixed well. The solution was freshly prepared and protected from light during use.

2.5 Stock standard solution of NBD-Cl (0.2% weight/volume)

An accurately weighted 0.2g of NBD-Cl was dissolved in methanol (HPLC grade), transferred to 100 ml volumetric flask, and diluted to the mark with methanol.

2.6 Buffer solutions

Buffer solution of pH 11.0 was prepared by mixing 100 ml of 0.1M aqueous solution of sodium bicarbonate with 50 ml of 0.1M solution of sodium carbonate and adjusted to pH 11.0 with 1M sodium hydroxide.

Buffer solution of pH 10.5 was prepared by mixing 100 ml of 0.025M Borax with 36.5 ml of 1M sodium hydroxide solution and adjusted to pH 10.5 with 1M sodium hydroxide solution.

Buffer solutions of different pH values were prepared according to methods stated in the literature.[28]

2.7 Sample solutions

Five capsules (Thiamine 100 mg capsules) were weighted and finely grinded. A portion of the powder equivalent to 25 mg of the drug was weighted, and dissolved in distilled water, filtered and then transferred to 250 ml volumetric flask, and then completed to the mark with distilled water to give a solution of 100µg/mL.

2.8 Analysis procedure

Aliquots of standard thiamine solution was transferred into 10 ml volumetric flask to give ultimate concentrations in the range (0.1 – 1.5 µg/mL), 2.0 ml of buffer solution (pH 11.0) was added followed by 2.0 ml of NQS solution (0.6% w/v). The reaction was allowed to stand at room temperature for 25 min. The fluorescent intensity of the resulting solution was measured at 460 nm (λ_{ex} 390 nm) against reagent blank treated similarly.

The same procedure was repeated using 1.0 ml of NBD-Cl (0.2% w/v) and reaction time of 15 min at room temperature and pH of 10.5. Excitation was performed at 562 nm where emission was being at 472 nm.

2.9 Stoichiometry of the chemical reaction

The limiting logarithmic method was used to determine the stoichiometric ratio of the reaction between thiamine and NQS or NBD-Cl. Two sets of experiments were carried out employing the general recommended conditions. The first set of experiments was carried out using increasing NQS concentrations (from 6.25×10^{-5}M – 5.0×10^{-4}M) at fixed thiamine concentration (5.0×10^{-4}M), or increasing NBD-Cl concentration (from 6.13×10^{-5}M – 4.91×10^{-4}M) at fixed thiamine concentration (5.0×10^{-4}M). The second set of experiments was carried out using increasing thiamine concentration (from 1.85×10^{-5}M – 1.48×10^{-4}M) at fixed NQS concentration (1.0×10^{-3}M) or NBD-Cl concentration (1.5×10^{-3}M). The logarithms of the obtained absorbances were plotted as function of the logarithms of the NQS/NBD-Cl concentrations in the first set, and as a function of thiamine concentrations in the second set. The slopes of the fitting lines in both sets of experiments were calculated and compared.

3. RESULTS AND DISCUSSION

3.1 Optimum reaction conditions

Since the optimum conditions used in the Spectrophotometric determination of thiamine with NQS and NBD-Cl described on our previously published

papers[13, 33] are arising mainly from the complex adduct that formed between thiamine and each of NQS or NBD-Cl, the same optimum parameters were selected in the spectrofluorimetric assays. Those conditions show good results when the relative fluorescence intensity was measured with excitation at 472 nm and emission at 562 for the reaction of thiamine with NBD-Cl. Beer's law was obeyed in the concentration range of 0.2 – 1.0 µg/mL. The relative fluorescence intensity (RFI) was increased linearly with increasing concentration of thiamine. The linear regression was a = 328.78, b = 357.38 and r^2 = 0.9997 calculated for the general equation of the calibration curve (y = ax + b) (Figure 1)

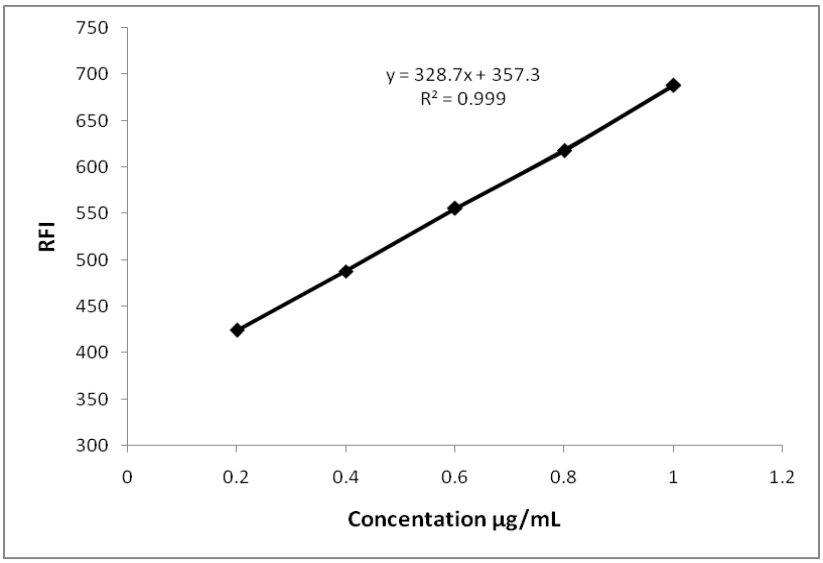


Figure 1: Calibration curve of Thiamine with NBD-Cl.

In case of Thiamine with NQS, good results were obtained when the measurement was done at 460 nm after excitation at 390 nm. The calibration curve was linear over the concentration range 0.1 – 1.0 µg/mL and the linear regression was a = 445.98 b = 408.83 and r^2 = 0.9996 as shown in figure (2)

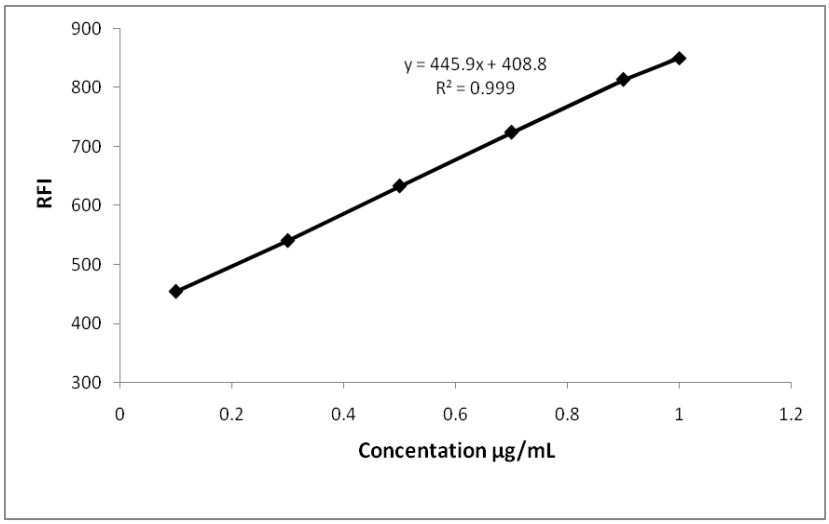


Figure 2: Calibration curve of Thiamine with NQS.

3.2 Validation of the method

Calibration curve for the determination of thiamine with NBD-Cl and NQS was constructed by plotting the fluorescence intensity as a function of concentration. Five concentrations of thiamine in the range (0.2 – 1.0 µg/mL) in case of NBD-Cl reagent were selected in which a proportional increase in fluorescence intensity was observed and a linear plot with good correlation coefficient was obtained. The limit of detection (LOD) was calculated based on the standard deviation and the slope of the calibration curve. For NQS reagent six concentrations of Thiamine in the range (0.1 – 1.0 µg/ml) were selected in which a proportional increase in fluorescence intensity was observed and a linear plot with good correlation coefficient results. The limit of detection (LOD) was calculated based on the standard deviation and the slope of the calibration curve. The results were shown in table (1)

Table 1: Analytical parameters for the reaction of Thiamine with NBD-Cl and NQS reagents.

Parameter	NBD-Cl	NQS
Excitation (nm)	472	390
Emission (nm)	562	460
Concentration range (µg/ml)	0.2 – 1.0	0.1 – 1.0
Limit of detection (µg/ml)	0.02	0.020
Limit of quantitation (µg/ml)	0.06	0.067
Regression equation	y = 382.78x + 357.38	y = 445.98x + 408.83
Correlation coefficient (r^2)	0.9997	0.9996
Standard deviation of the intercept (S_a)	2.32	3.02
Standard deviation of the slope (S_b)	3.50	4.55

3.3 Accuracy and precision

The accuracy and precision of the method was estimated by three replicate analytical samples of thiamine. The assays gave satisfactory results and the relative standard deviation (RSD) was less than 2. The level of precision of the proposed method was adequate for analysis of thiamine in pharmaceutical dosage forms. The results were tabulated in tables (2 and 3)

Table 2: Accuracy results of Thiamine with NBD-Cl.

Concentration taken µg/ml	Concentration found µg/ml	Recovery % ± RSD
0.2	0.19	97.89±0.61
0.4	0.39	98.89±0.51
0.8	0.80	100.14±0.58

Table 3: Accuracy results of Thiamine with NQS.

Concentration taken µg/ml	Concentration found µg/ml	Recovery % ± RSD	Relative error
0.2	0.208	104.136±0.34	0.001
0.6	0.626	104.355±0.18	0.002
0.8	0.837	104.678±0.14	0.001

3.4 Robustness

Robustness of the procedure was assessed by evaluating the influence of small variations in the experimental variables, concentration of NDB-Cl or NQS reagents, reaction time and pH on the analytical performance of the method. In this experiment, one experimental parameter was changed while the other parameters were kept constant and the recovery percentage was calculated each time. It was found that small variation in any of the variables did not significantly affect the results. This gave an indication of the reliability of the proposed method during routine work.

The results were shown in table (4).

Table 4: The effect of the variation of analytical parameters on the proposed method, values between brackets are for NQS reagent.

Variation	Recovery %±SD
pH	
10.3 (10.8)	95.01±1.25 (100.5±1.464)
10.7 (11.2)	97.74±1.04 (104.5±0.731)
NBD-Cl or NQS concentration (w/v)	
0.22 (0.062)	105.26±2.71 (105.99±1.97)
0.18 (0.058)	97.99±1.79 (98.55±1.57)
Reaction time (min)	
23 (13)	95.5±1.42 (99.916±1.06)
27 (17)	102.8±2.24 (105.116±0.86)

3.5 Recovery of the method

The recovery of the proposed method was carried out by applying standard addition technique. A different amount of standard solution was added to known concentration of the drug sample. The recovery percentages obtained were tabulated in table (5)

Table 5: Recovery for the determination of Thiamine with NBD-Cl and NQS, values between brackets are for NQS reagent.

Sample Concentration	Concentration added (STD) (µg/ml)	Concentration found (µg/ml)	Recovery±SD
0.2	0.2	0.38 (0.421)	96.36±4.10 (103.196±0.24)
0.2	0.4	0.60 (0.621)	100.35±5.76 (103.64±0.15)
0.2	0.6	0.79 (0.846)	99.57±1.17 (105.809±0.14)

3.6 Application of the method on real dosage form

Thiamine tablets were subjected to analysis by the proposed method and the label claim agrees well with the method as shown in table (6)

The proposed method has the privilege of being virtually free from interferences by excipients.

Table 6: Analysis of thiamine-containing dosage form by the proposed method. Values between brackets are for NQS reagent.

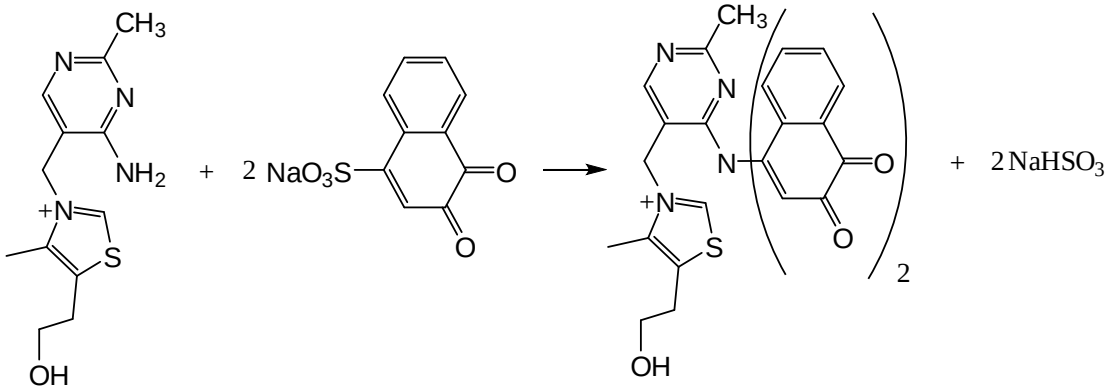
Dosage form	Amount found	%found ± RSD
100 mg	98.90 (101.00)	98.90±0.49 (101.39±1.02)

3.7 Stoichiometry of the reaction

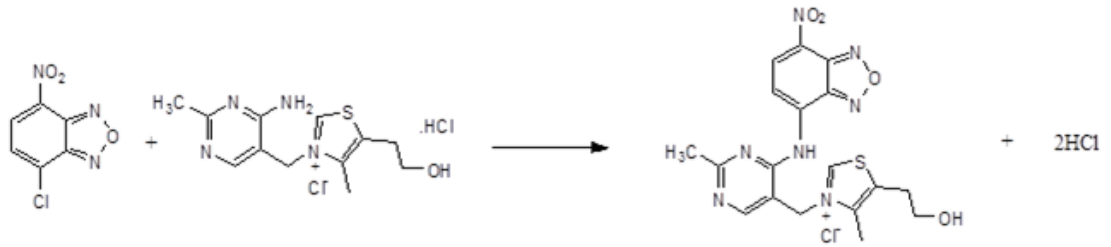
The stoichiometry of the reaction between Thiamine and NBD-Cl was studied utilizing the limiting logarithmic method.[34] Plots of log RFI versus log [NBD-Cl] and log [Thiamnine] gave straight lines, with the slopes of 0.913 and 0.932 respectively. Hence it was concluded that the reaction proceeds in the molar ratio of 1:1 which is consistent to what was found in the spectrophotometric method described previously.[33]

Job method was used to determine the stoichiometry of the reaction between Thiamine and NQS, which is found to be 1:2.[13]

Reaction equations were shown in schemes 1 and 2.



Scheme 1: The reaction pathway of thiamine with NQS.



Scheme 2: Reaction of thiamine with NBD-Cl showing 1:1 stoichiometry.

4. CONCLUSION

Thiamine can be determined successfully and reliably in pharmaceutical formulations via spectrofluorometry using NQS and NBD-Cl reagents. The methods are simple, sensitive and accurate and can be applied in routine work. The new methods has the privilege over other methods in terms of simplicity and sensitivity and low cost. The methods have comparable analytical performances and are free from any potential interference. We recommend that they can be used as alternative methods for the routine analysis of Thiamine in quality control laboratories.

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