ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

DEVELOPMENT AND VALIDATION OF VISIBLE SPECTROSCOPY METHOD FOR THE DETERMINATION OF THIAMINE (VITAMIN B1)IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple spectrophotometric method has been described for the determination of thiamine. The method is based on the derivatization of thiamine with MBTH (0.2%w/v) in alkaline medium 2 mLNaoH (1N) was performed. It was found that the product is Yellowcolored exhibiting λ_{max} at 434nm.The λ_{max} of thiamine-MBTH derivative was red-shifted, eliminating any potential interference. The wavelength 434 nm therefore was fixed as optimum.Beer's law was obeyed over the concentration range from 1-50 µg/mL thiamine. The relative standard deviation,< 1.5%;correlation coefficient,0.9998; molar absorptivity0.96x10 ^{4},Sandels sensitivity 35ng/cm^2 ;The limit of detection, 0.0076 µg/mL; the limit of quantification, 0.0231 µg/mL and recovery, 101.47% thiamine. Finally, the developed method was applied to the determination of thiamine in pharmaceutical formulations.

KEY WORDS: Thiamine (vitamin B1), MBTH, Validation, visible spectroscopy, pharmaceutical formulations.

1. INTRODUCTION

Thiamine (Figure 1), known as vitamin B1 (a water soluble vitamin) is a natural nutrient present in many foods and is also added as an essential nutrient in food products. It has been used for the prevention and treatment of beriberi, neuralgia, etc. It is also added to medical doses or vitamin B1 enriched food or drinks. It is necessary for carbohydrate metabolism and for the maintenance of neutral activity [1].

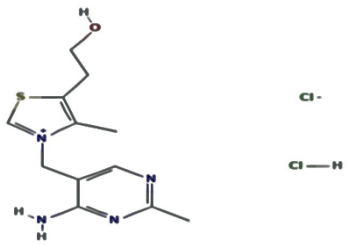

Figure 1.Chemical structure of Thiamine Hydrochloride

The chemical method most widely used for determination of thiamine, involves the reaction between vitamin B1 and potassium hexacyanoferrate(III) in alkaline solution, followed by extraction of the thiochrome (TC) formed in aqueous phase into an organic phase, which is then measured florimetrically[2]. This procedure is the official U.S.P. method and has been automated by flow injection (FI) with fluorimetric and chemiluminescence detection [3, 4]. Other FI methodsaccomplish the oxidation of thiamine to fluorescent TC using Hg(II) [5],Cu(II) [6] strong anionic resins loaded with hexacyanoferrate(II) [7] and electrochemical oxidation [8]. The on-line UV irradiation of thiamine with photometric [9] and fluorimetric detection [10] and the derivatization reaction of the primary amine group with o-phthalaldethude in presence of 2-meracapto ethanol using fluorimetric detection [11] have also been proposed. Thiamine hydrochloride was determined by using the chromatographic method depends on HPLC [12]. Thiamine has been determined gravimetrically by precipitation with silictungestic acid as recommended by British Pharmacopeia [13]. The precipitate as insoluble in water and its molecular formula was described as

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[C12H17N4OS]2[Si(W3O10)]4. However, this gravimetric procedure is tedious, once it involves several time consuming steps such as digestion, filtration, heating to dryness and weighting. Also thiamine determined based on the precipitation reaction of thiamine with silicotungstic acid in acidic medium to form a thiamine silicotungstate suspension that is measured at 420 nm [14]. Thiamine was determined by a spectrofluorimetric method based on the catalytic activity of horseradish peroxidase in the presence of hydrogen peroxide [15]. Two simple and sensitive spectrofluorimetric methods were developed for determination of thiamine by oxidized to thiochrome by iodine in an alkaline medium [16] or oxidized with potassium iodate(V) to the colourless product and a stoichiometric amount of iodide ions was formed. The latter reacted with the excess of iodate(V) ions in acidic medium, to form free iodine which oxidized leucocrystal violet to the crystal violet dye [17].

This work describes a simple, rapid and sensitive spectrophotometric method for the determination of thiamine in pharmaceutical preparations. The method is based on the derivatization of thiamine with MBTH (0.2%w/v) in alkaline medium 2 mLNaoH (1N) was performed.It was found that the product is Yellowcolored exhibiting λ_{max} at 434nm.

2. EXPERIMENTAL WORK

2.1. Apparatus

A Single beam UV-Vis Spectrophotometer, thermoscientific ,model Aqua mate plus equipped with 1 cm quartz cells. The spectral bandwidth was 2 nm and the wavelength scanning speed was 2000 nm/min.

2.2. Preparation of solutions

2.2.1.Preparation of Working standard solutions:

Accurately weighed and transferred 100mg of pure drug in 100mL of volumetric flask and final volume was made upto 100mL with diluent,further 10 mL was transferred into100 mL volumetric flask and volume was made upto 100mL from this Working standard solutionfive different standard solutions containing 1-50 µg/mL were prepared by suitable dilution with distilled water.

2.2.2.Preparation of sample solution for Assay:

Ten 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.

2.2.3.Selection of Wavelength:

The absorption spectrum of thiamine was recorded against water, it was found that thiamine exhibits a maximum absorption peak (λ_{max}) at 235nm. Because of highly blue-shifted λ_{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.

3.EXPERIMENTAL PROCEDURE:

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 reaction time, concentration of NaOH and concentration of MBTH were investigated.

3.1.Effect of NaOH concentration

The effect of NaOH concentration (0.5, 1.0, 1.5, 2.0, 2.5 and3.0 N) on the absorbance is shown in Figure. The amount of sodium hydroxide solution for maximum color intensity was examined. The maximum constant color intensity was reached when using 2 mL of 1 N sodium hydroxide solution. This amount was selected for subsequent experiments.

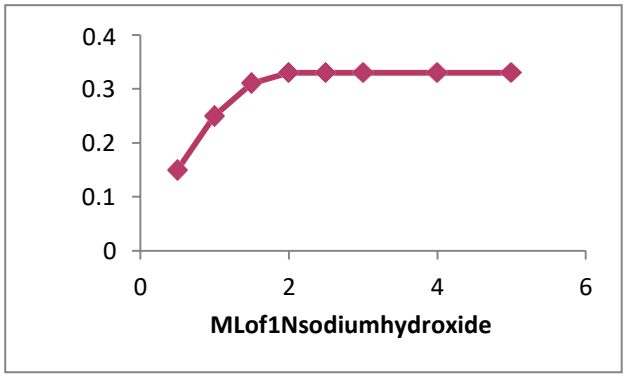

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Figure 2. Effect of NaOH concentration

3.2.Effect of reaction time

The absorbance of the reaction product was monitored at different times in figure 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 MBTH at 25°C and the absorbance begins to increase gradually and reach a maximum after 25min. For longer reaction times, a slight drop in the absorbance was observed. Accordingly 25min was set as the convenient reaction time for determination.

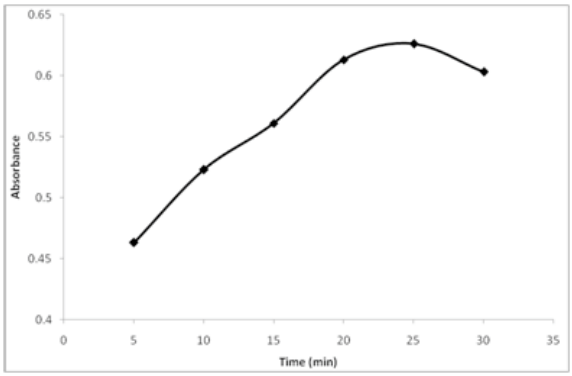

Figure 3. Effect of reaction time

3.3.Effect of MBTH concentration:

The study of the effect of MBTHconcentrations showed that the reaction was dependent on the reagent concentration. The highest absorption intensity was attained at MBTHconcentration of 0.2% (w/v), and higher concentration of MBTH leads to a decrease in the absorbance.

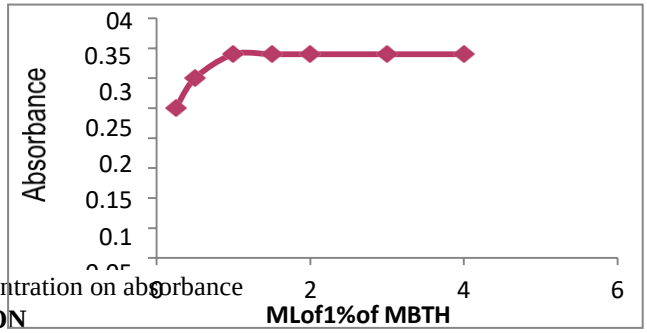

Figure 4. Effect of MBTH concentration on absorbance

4.RESULTS AND DISCUSSION

4.1.SPECIFICITY

The specificity of the UV-VISIBLE method is where complete separation of Thamine was noticed for the presence of impurities. In addition there was no any interference of impuritites at the absorbance of Thamine in the spectrum using blank sample and placebo . This shows that the absorbance peak of analytes was pure and excipients in the formulation did not interfere the analyte.

4.2. PRECISION

A standard solution of thamine of concentration of 2µg/ml was prepared and then the absorbance studies were conducted for the solution by using uv-visible spectrophotometry. Blank solution and six replicates of repeatability solutions were seen for absorbances. The inter-day precision (24 hr lag phase) was also determined by the same procedure. The results indicated the good precision of the developed method given in Table no1.

Table no1Method Precision and Intermediate Precision

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Sample No.	Method Precision or intraday	Intermediate Precision	Over all % RSD (n=6)
1	98.3	101.8	1.0
2	100.2	101.3	
3	100.1	98.6	
4	99.9	100.2	
5	99.9	100.3	
6	100.5	101.1	
Mean	99.8	100.6	
% RSD	0.8	1.1	

4.3.LINEARITY AND RANGE:

The linearity was evaluated by linear regression analysis determined by constructing seven concentrations of thiamine, in the range of 1–50µg/mL. The molar absorptivities, regression equations and correlationcoefficients were calculated.The least square method was used to derive the regression equations for the suggested procedures, and the values of the correlation coefficient ranged from 0.9998.

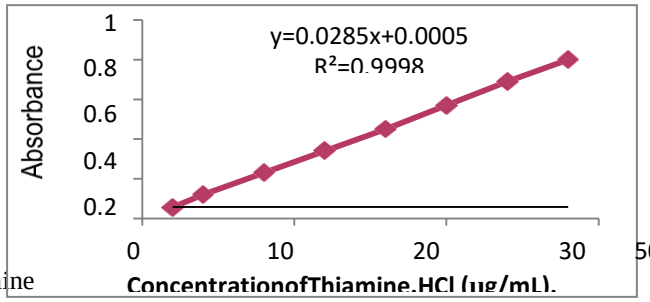

Figure 5. Linearity of Thiamine

4.4.ASSAY OF THIAMINE CAPSULES

Preparation of sample Solution:

Ten capsules (Thiamine 100mg/capsule) were weighed and finely-grinded. A portion of the powder equivalent to 25mg of the drug was weighed 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.from the above solution 7.5µg/ml test solution was prepared and absorbance was measured. The amount was calculated by the formula.% Label claim = concentration of sample practically/concentration of samplefoundtheoritically X 100.The amount was given in Table no 2.

Table no2 Assay of thiamine capsules

Formulation	Amount	Amountfound	recovery	%RSD
Thiaminecapsule	**100mg**	**100.8mg**	**100.8%**	**1.3**

4.5.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 .By the formulaand results are given in Table no3

%Recovery = Analytical Result/ True Value x 100%

Table no3Accuracy

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Sample no	Sample content µg/ml	Thiamine standard amount µg/ml	Amount found (total) µg/ml	Recovery+/-SD
1	5	10	14.70	98.80+/-0.05
2	5	20	24.72	98.92+/-0.018
3	5	30	35.5	101.47+/-0.148

4.6.Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. To determine the robustness of the method, the final experimental conditions were altered and the results were examined. The λ max was varied by (±) 0.1 nm. Reaction time (±) 2min, concentration of MBTH (±)0.1%.

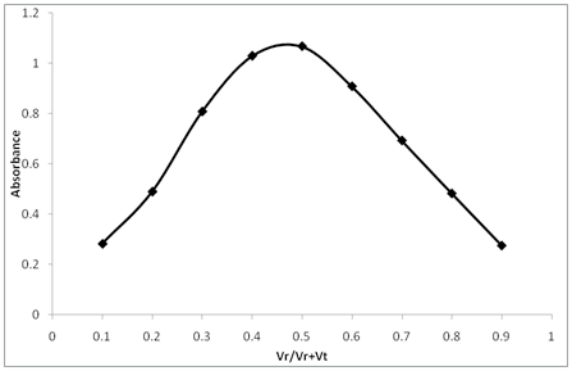

Figure 6.Robustness

4.7.LOD&LOQ:

LOD's is calculated based on the standard deviation of the response (Sy) of the curve and the slope of the calibration curve (S) at levels approximating the LOD,The calculation method is again based on the standard deviation of the response(SD) and the slope of the calibration curve (S) according to the formula: LOQ = 10(Sy/S)according to the formula: LOD = 3.3(Sy/S). The limit of detection (LOD) and limit of quantification (LOQ) are 0.0076 and 0.0231 mg/100 ml, respectively.

5.SUMMARY AND CONCLUSION

Derivatization of thiamine with MBTH was performed, and the absorption spectrum of the product was recorded against reagent blank. It was found that the product is Yellow colored exhibiting λ_{max} at 434nm,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 reaction time, concentration of NaOH and concentration of MBTH were investigated.From the above parameters-adjusting experiments, the optimized conditions used for the assay were thiamine concentration 1.0% (w/v), volume of the NaOH 2mLof 1N, reaction time 25min and temperature 25ºC.Analytical method validation was performed according to ICH guidline(ICH Q2 (R1) Validation of analytical procedures),All the parameters satisfies the acceptance criteria.Spectrophotometry is considered the most convenient analytical technique, because of its inherent simplicity, low cost, and wide availability in most quality control laboratories,The developed method is found to be sensitive, accurate, simple, precise, economical, and can be used for routine quality control analysis of thiamine hydrochloride in pure form and pharmaceutical formulations.

6. ACKNOWLEDGEMENT

I thank Nirmala college of pharmacy for proving the facilities.

7. CONFLICT OF INTEREST

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There is no conflict of interest

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