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DIAZOTISED SULPHANILIC ACID REAGENT FOR THE DETERMINATION OF THIAMINE IN AQUEOUS SOLUTION – APPLICATION TO PHARMACEUTICAL PREPARATIONS

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ABSTRACT

A simple, precise, accurate, high reproducible and economical visible spectrophotometric method of analysis for the determination of thiamine was developed and validated. The proposed method involves diazotization of sulphanilic acid under acidic conditions in presence of sodium nitrite, followed by its coupling with thamine in alkaline medium. The absorption spectra of the yellow colored formed between thiamine and positive diazonium ion has absorption maximum at 405 nm. The linear regression analysis data for the calibration plot showed good linear relationship (r = 0.9969) with in the concentration range of (2 –26)µg mL^{-1}. With molar absorption1.0253×10^4 $L.mol^{-1}.cm^{-}1$. The method was successfully applied in the evaluation of thymine in pharmaceutical preparation**.**

INTRODUCTION

Vitamins are essential organic molecules that function as cofactors for enzymatic reactions. Thiamine (vitamin B_1) chemically known as 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]- 5-(2-hydroxyethyl)-4-methylthiazolium.[1]

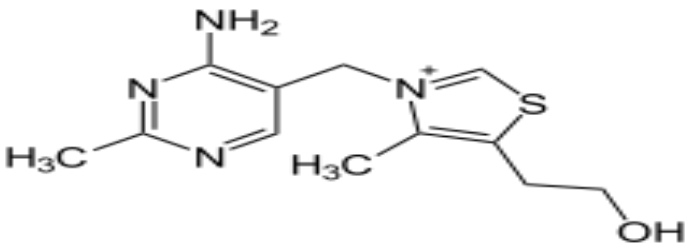

Figure. 1.

Found in foods such as cereals, whole grains, meat, nuts, beans, and peas. Thiamine is important in the breakdown of carbohydrates from foods into products needed by the body. It

has been used for the prevention and treatment of beriberi, neuralgia, etc. Various analytical techniques have been reported in the literature for the analysis of thiamine including, spectrophotometry[2,3,4,5,6,7], High-performance liquid chromatography[8,9,10,11], Ion exchange[12,13] Gas chromatography.[14]

Experimental

Apparatus

A Shimadzu UV-VIS 1800 digital double-beam recording spectrophotometer (Kyoto, Japan) was used for all spectral and absorbance measurements with matched 1cm quartz cells.

Reagents

All chemicals and reagents used were of analytical grade and used without further purification.

Thiamine solution (100µgml^{-1}): This solution is prepared by dissolving 0.01g of thiamine and diluted to the mark with distilled water in 100 ml-volumetric flask. These solutions were further diluted with water to required concentrations for working solutions.

Diazotised sulphanilic acid reagent solution(50 mM)

A 0.865 g of sulphanilic acid is dissolved in about 75 ml of distilled water and the mixture is heated until the clear solution is obtained, , then 1 ml of concentrated hydrochloric acid is added, the mixture is then cooled to 0 -5°C in an ice bath, and a 0.345g sodium nitrite is added and stirred vigorously. After 5 minutes the solution is made up to volume in 100 ml volumetric flask with cooled distilled water, and is kept in a brown bottle in a refrigerator. This solution is prepared freshly each day.

Sodium hydroxide solution(1M): The solution was prepared by dissolving 4.0 g of sodium hydroxide in in distilled water and diluting to the marked in 100 mL volumetric flask.

Procedure for Pharmaceutical Preparations

Vitamin B1 Tablets: 10 tablets were grinded well and a certain portion of the final powder was accurately weighted to give an equivalent to about 10 mg of vitamin B1 was dissolved distilled water. The prepared solution transferred to 100 ml volumetric flask and made up to the mark with distilled water forming a solution of 100 µg ml^{-1} concentration. The solution was filtered by using a filter paper to avoid any suspended particles**.**

RESULTS AND DISCUSSION

Study of the optimum reaction conditions

The various parameters affecting and related to the yellow azo-dye have been studied and optimum conditions have been selected.

Effect of diazotized sulphanilic acid reagent amount

The effect of the amount of the diazotized sulphanilic acid on the maximum absorbance of the azo-dye formed with thiamine has been investigated.

Table (1): The effect of diazotised sulphanilic acid amount on absorbance.

ml of Diazotised sulphanilic acid (50 mM)	Absorbance / min standing time				
	1	3	5	7	10
0.5	0.096	0.097	0.097	0.098	0.098
1.0	0.132	0.132	0.133	0.132	0.134
1.5	0.173	0.174	0.175	0.174	0.174
2.0	0.207	0.206	0.207	0.208	0.208
2.5	0.225	0.226	0.225	0.224	0.225
3.0	0.183	0.183	0.182	0.181	0.180

The results show that 2.5 ml of diazotized sulphanilic acid (50mM) reagent solution gives the highest intensity, therefore 2.5ml is recommended for the subsequent experiments.

Effect of base

The preliminary experiments have shown that the azo-dye develops only completely in alkaline medium. Different amounts of base (IM) have been used (Table2).

Table (2): The effect of base on absorbance.

Base used 1 M	Absorbance / ml of base used				
	0.5	1.0	1.5	2	2.5
Na OH	0.153	0.172	0.228	0.222	0.209
Na_2CO_3	0.179	0.207	0.219	0. 198	0.175
$NaHCO_3$	0.223	0.191	0.180	0.163	0.145

The experimental data show that 1.5 ml of 1M NaoH is recommended for the subsequent experiments.

Effect of surfactant

The effect of surfactant on the colour intensity has been examined. The results given in table (3).

Table (3): Effect of surfactant.

Surfactant	Absorbance / ml of surfactant used				
	0.5	1.0	1.5	2	2.5
SDS (1×10^{-3} M)	0.225	0.226	0.226	0.227	0.228
Triton X-100 (2%)	0.223	0.225	0.231	0.228	0.224
CTAB (1×10^{-3} M)	0.227	0.227	0.228	0.227	0.228

It was observed that 1.5 ml of Triton X-100 solution at 2 % Gives the highest absorbance and therefore this amount was adopted in subsequent experiments.

Effect of time on colour development

A study of the time effect on colour development showed that the colour formed practically within about one minute. The azo-dye formed from lower concentrations of thiamine gives good stability for at least45 min (Table4).

Table (4): The effect of time and thiamine amount on absorbance.

Thiamine 8µg. ml^{-1}	Absorbance/minute standing time									
	0	5	10	15	20	25	30	35	45	50
Absorbance	0.229	0.229	0.230	0.232	0.231	0.230	0.229	0.231	0.230	0.213

Interference: The effects of foreign compounds on the determination of thiamine have been examined and the results are given in Table (5).

Table (5): Effect of foreign compounds for assay of thiamine.

Foreign compounds	Recovery% ,of 8 $\mu gml^{-1}$of , thiamine per $\mu gml^{-1}$of foreign compounds added		
	100 μgml^{-1}	300 μgml^{-1}	500 μgml^{-1}
sucrose	101.73	101.3	99.13
Lactose	100.43	99.56	102.17
Starch	98.69	102.17	98.26
Gelatin	101.3	101.73	99.56

The results in Table 5 indicate that none of these compounds interfered seriously in the determination of thiamine.

Order of addition

2ml of ($100\mu gml^{-1}$) solution thiamine, 2.5ml of diazotised sulphanilic acid reagent solution(50 mM) , 1.5ml of 1M NaoH and followed by 2ml of Triton X-100 solution(2%): were mixed in various orders as is shown in Table (6).

Table (6): Effect of Order of addition.

Order of addition	Order number	Absorbance
Thiamine+ sulphanilic acid+ Sodium hydroxide + Triton -100	I	0.231
Thiamine+ sulphanilic acid+ Triton -100 +Sodium hydroxide	II	*0.*227

It is noted from the above table that the first(I) order gave a high absorption intensity so it continued to be adopted in subsequent experiments**.**

Absorption spectra

When thiamine in aqueous solution is treated with diazotized sulphanilic acid reagent solution, an absorption peak is obtained showing intense absorption at 405nm characteristic of the yellow dye. this wavelength has been used in all subsequent experiments.

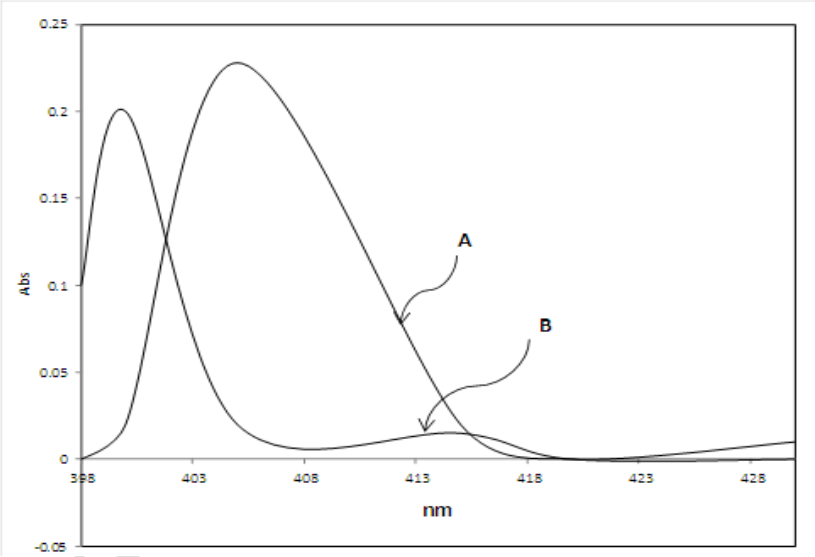

Fig. (2): Absorption spectrum of (A) complex against (B) reagent blan.

K solution

Calibration curves and analytical data

Aliquots of thiamine standard solution containing 50 -650 µg were transferred into a series of 25ml volumetric flasks ,to each , 2.5 ml of Diazotised sulphanilic acid reagent solution , 1.5 mL of Sodium hydroxide solution(1M)and 2 ml. triton X-100 solution(2%)were added, then the volumes are made to the mark with distilled water. The absorbance of each solution was measured at 405nm versus blank prepared in the same manner but without thiamine(Fig.3).

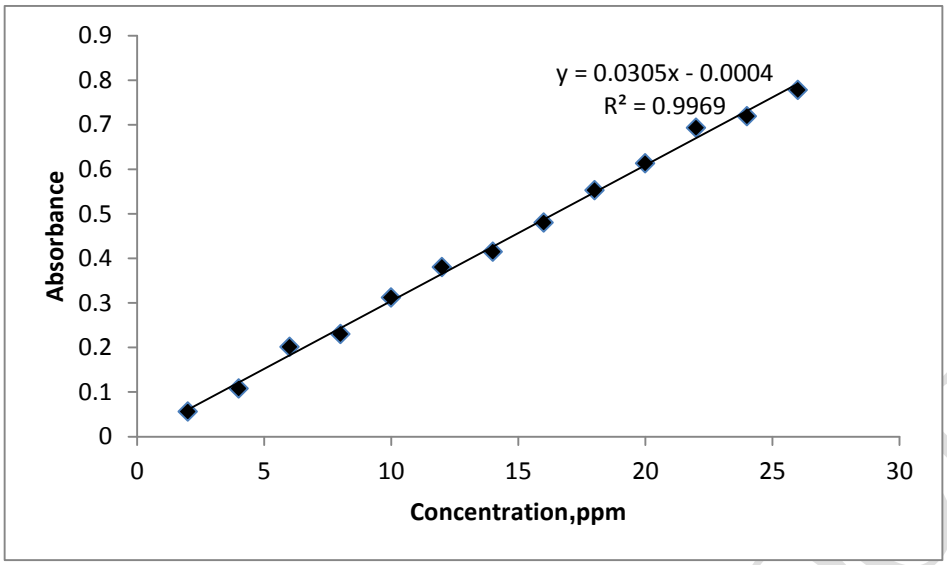

Fig. (3): Calibration graph for the determination of thiamine.

Accuracy and precision

The accuracy and precision of the proposed method was estimated by measuring the content of thiamine in pure form at three different concentration levels. within the Beer's law limit in five replicates , (Table7). The relative standard deviation and mean percent recovery obtained by the proposed method can be considered to be satisfactory.

Table. (7): Accuracy and Precision of the method.

Amount of thiamine taken µg/ml	Amount of thiamine found	Relative error, %*	Recovery%	Relative standard deviation, %*
10	10.09	-0.9	100.9	0.363
16	15.96	0.25	99.75	0.109
22	22.04	-0.23	100.21	0.078

*Average of five determinations

Nature of the dye

The stoichiometry of the reaction between thiamine and diazotised sulphanilic acid in the presence of sodium hydroxide was investigated applying the continuous variation Job's method and mole –ratio method. The results obtained in fig (4)and fig(5) indicated that the product is formed in the ratio of 1:1.

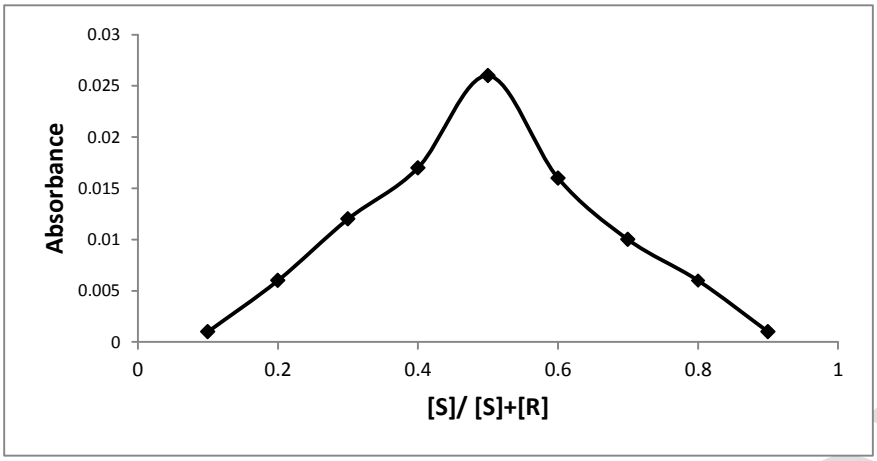

Fig. (4): Job's method.

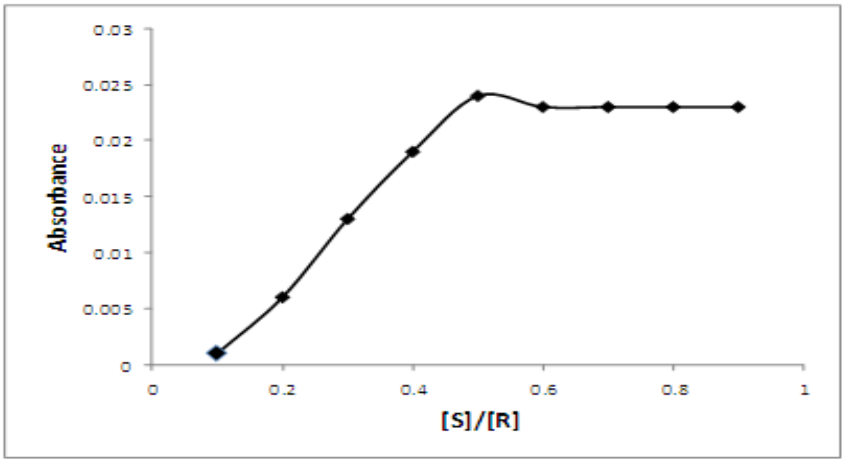

Fig (5) mole – ratio methods.

Applications

The application of the proposed method for the assay of the pharmaceutical tablets was investigated using Tablets from SID (10mg) containing Thiamine. A good precision and recovery were obtained according to the results obtained in Table (8).

Table (8): Accuracy and Precision of Applications.

Drug sample Vitamin B1 (10µg) SID	Conc.B1 µg.ml^{-1}		Proposed method			Standard method
	Taken	Found	R.S.D*%	Error%*	Recovery%*	Recovery%*
	8	7.97	0.375	0.375	99.62	101.53
	16	16.02	-0.125	-0.125	100.12	
	24	24.01	-0.041	-0.041	100.4	

* Average of five determinations

CONCLUSION

The proposed method for determination of thiamine is simple, rapid and economical when compared with already reported methods do not require any pretreatment of the drugs. The proposed method is applied for the determination of thiamine in pharmaceutical preparation.

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