Indirect Spectrophotometric Determination of Thiamine in Pharmaceutical Preparations

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An indirect, sensitive spectrophotometric method for the assay of thiamine is described. The procedure is based on the formation of a mercaptide by thiamine, with a known excess of silver ions in a buffered medium of pH 9.0±0.1. The unreacted silver ions are determined by the formation of an ion-pair complex with 1,10-phenanthroline and 2,4,5,7-tetrabromofluorescein, which shows an absorption maximum at 550 nm. The absorbance is found to decrease linearly with increasing concentrations of thiamine, which is corroborated by the calculated correlation coefficient value of −0.998. The system obeys Beer's law for 0—25 μg of thiamine in an overall aqueous volume of 10 ml. The molar absorptivity and relative standard deviation (RSD) were calculated to be 7.8×10^4 l mol^{-1} cm^{-1} and 2.5% ($n=10$), respectively. The proposed method was applied successfully to the determination of thiamine in pharmaceutical preparations. The reliability of the assay was established by parallel determination by the standard thiochrome method and recovery studies.

Key words thiamine; ternary ion-pair complex; pharmaceutical preparation; indirect spectrophotometry; mercaptide formation

Thiamine (or vitamin B_1) plays a key role in cellular carbohydrate metabolism and is also required for the metabolism of fats and alcohol.[1] Its deficiency causes beri-beri which is characterized by anorexia, cardiac enlargement, lassitude, muscular weakness and nerve degeneration to name some of the important symptoms.[2]

There are several reports available in the literature for the determination of thiamine by techniques as varied as titrimetry,[3] spectrofluorimetry,[4-6] spectrophotometry,[7-11] electroanalytical,[12-14] chromatography,[15,16] chemiluminescence,[17] molecular emission cavity analysis,[18] atomic absorption spectrometry (AAS),[19] atomic emission spectrometry (AES),[20] NMR spectrometry[21] and thiamine-selective optrode.[22]

The standard thiochrome method is specific and sensitive with poor reproducibility. In this study, thiamine was indirectly assayed by a simple spectrophotometric method with good sensitivity and reproducibility.

Experimental

A Carl Zeiss PMQ II spectrophotometer with 1 cm pathlength glass cells was used for absorbance measurements.

Reagents All reagents were of analytical-reagent grade and distilled water was used to prepare the reagent solutions.

Stock Standard Thiamine Solution (1000 μg ml^{-1}): A 0.1 g of thiamine hydrochloride was dissolved in 100 ml of distilled water to obtain a 1000 μg ml^{-1} solution. Suitable dilutions were made to obtain a 10 μg ml^{-1} working standard solution.

Silver Solution (1000 μg ml^{-1}): This was prepared by dissolving 0.1575 g silver nitrate in 100 ml of water. Suitable dilutions were made to obtain a 15 μg ml^{-1} working solution and were stored in amber coloured bottles.

Ammonia Solution (1 mol l^{-1}): This was prepared by diluting 7 ml of liquor ammonia ($d=0.91$) to 100 ml. Suitable dilutions were made to obtain a 0.005 mol l^{-1} ammonia solution.

Borate-Ammonia Buffer Solution (0.013 mol l^{-1} $Na_2B_4O_7 \cdot 10H_2O$ in 0.005 mol l^{-1} Ammonia Solution): A 0.5 g of $Na_2B_4O_7 \cdot 10H_2O$ was dissolved in 100 ml of 0.005 mol l^{-1} ammonia solution.

1,10-Phenanthroline Solution (0.01%): This was prepared by dissolving 0.01 g 1,10-phenanthroline monohydrate in 100 ml of distilled water.

2,4,5,7-Tetrabromofluorescein Solution (0.01%): A 0.01 g of 2,4,5,7-tetrabromofluorescein (or eosin) was dissolved in 100 ml of distilled water to obtain a 0.01% solution. Suitable dilutions were made to obtain a 0.002% dye solution and were stored in amber coloured bottles.

Glycerol Solution (0.5%): This was prepared by diluting 1 ml of glycerol ($d=1.261$) to 250 ml.

Hydrogen Peroxide Solution (1%): A 3.4 ml of 30% hydrogen peroxide was diluted to 100 ml to obtain a 1% solution. Twenty ml of this solution was diluted to 100 ml to give a 0.2% solution. These solutions were stored in plastic containers under refrigeration.

8-Hydroxyquinoline Solution (0.5%): A 0.25 g of 8-hydroxyquinoline (or oxine) was dissolved in 50 ml of chloroform to obtain a 0.5% solution, which was stored in an amber coloured bottle.

EDTA Solution (0.01%): A 0.01 g amount of EDTA disodium salt was dissolved in 100 ml of water to obtain a 100% solution.

Light Petroleum: The fraction of bp 60—80 °C was used.

Procedure Calibration Graph: In each of a series of 10 ml calibrated flasks was placed 0.5—2.5 ml of 10 μg ml^{-1} thiamine solution and the aqueous volume was adjusted to 3 ml by the addition of the requisite amount of distilled water. Subsequently, 1 ml of borate-ammonia buffer solution and 1 ml of 15 μg ml^{-1} silver solution were added and the mixture was allowed to stand for 15 min. This was followed by the addition of 1 ml of 0.01% 1,10-phenanthroline solution, 2.5 ml of 0.002% eosin solution and 1 ml of 0.5% glycerol solution. The overall aqueous volume was made up to 10 ml. The reagent blank containing the optimum concentrations of all the reagents was prepared simultaneously and all absorbance measurements were made at 550 nm against water using 1 cm pathlength glass cells.

Procedure for Multi-vitamin Preparations: Representative samples of finely ground tablets or cut-open capsules were stirred with 30—50 ml of distilled water for 10 min. The residual solid was filtered and washed with water. The filtrate and washings were collected and made up to a known volume.

Pharmaceutical preparations containing fat-soluble vitamins were partitioned between 10 ml of light petroleum and water. The organic layer was equilibrated twice with 10 ml of water and the combined aqueous extracts were made up to a known volume.

Appropriate dilutions were made before analysis, so that 3 ml of the sample solution contained not more than 25 μg of thiamine and 2.5×10^{-4} M (100 μg) EDTA which was added to complex interfering metal ions such as Cu^{2+}, Ca^{2+}, Mn^{2+} and Zn^{2+}.

The aliquot taken for analysis was treated with 1 ml of 0.2% hydrogen peroxide solution to oxidise ascorbic acid prior to the addition of silver solution. Suitable volumes of sample solutions containing up to 500 μg Fe^{2+} were treated with 1 ml of 0.2% hydrogen peroxide solution and 1 ml of 0.005 mol l^{-1} sulphuric acid, followed by equilibration with a 5 ml volume of a solution of 0.5% oxine in chloroform. The organic layer containing the iron (III) complex was drained, the aqueous phase treated with 1 ml of 0.1 mol l^{-1} ammonia and reequilibrated with 5 ml of

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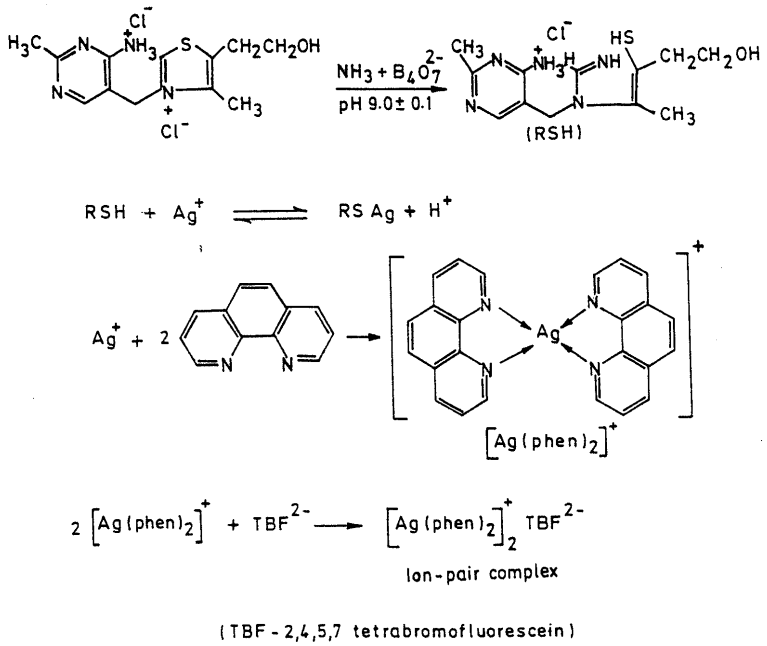

Fig. 1. Reaction Scheme

chloroform to extract the free oxine. The chloroform layer was drained and the aqueous layer diluted to a suitable volume such that 3 ml of the final solution contained not more than 25 μg thiamine.

The pH of the diluted solutions was adjusted to 7.0 $\pm$ 0.1 with ammonia before making up to a known volume.

Results and Discussion

Free thiamine is a basic substance containing pyrimidine and thiazole rings. The thiazole ring readily opens under high pH conditions yielding a colourless thiol which can be oxidised to a dimer or converted to a mercaptide by treatment with a suitable metal ion. This ability of thiamine to form a mercaptide with silver ion has been used to develop an indirect, spectrophotometric method for its determination.

Trace amounts of silver ions have been determined by the formation of an intensely coloured ternary complex with 1,10-phenanthroline and 2,4,5,7-tetrabromofluorescein.[23] In the presence of increasing amounts of thiamine, in a medium of high pH (9.0 $\pm$ 0.1) proportionally increasing amounts of added silver ions are fixed as silver mercaptide by the thiol (generated from thiamine). As a result there is a decrease in the silver ion concentration for the formation of the ternary complex (Fig. 1). This causes a concomitant decrease in the absorbance of the solutions which is proportional to the thiamine concentration.

The various parameters involved in the formation of the mercaptide and the ion-pair complex were optimised. It was found that 1 ml of a 15 μg ml^{-1} solution of silver was required to obtain the desirable maximum absorbance. A fraction of silver ions was fixed by the thiol form of thiamine which was generated by the addition of 1 ml of borate–ammonia buffer reagent (pH 9.0 $\pm$ 0.1). Although 10 min standing time was found to be adequate for the formation of the mercaptide, 15 min was allowed for the reaction. One ml of 0.01% 1,10-phenanthroline and 2.5 ml of 0.002% eosin were found to be optimum for the formation of the ternary ion-pair complex with the silver

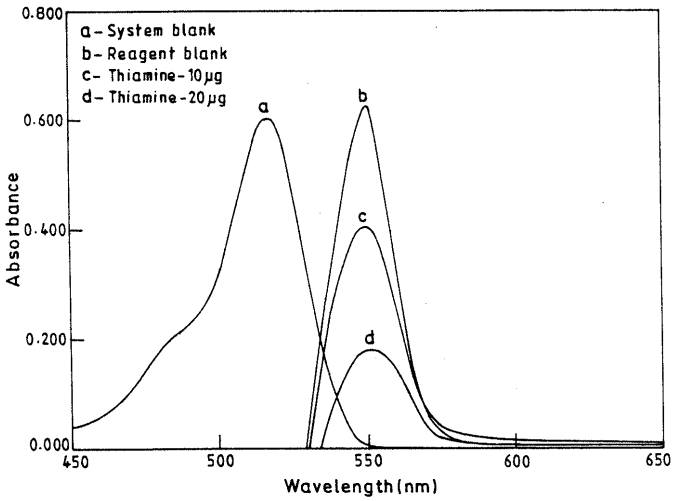

Fig. 2. Absorption Spectra Recorded against Water

ions. In the presence of excess of 1,10-phenanthroline, the mercaptide dissociates and silver ions are released, causing a slow increase in the absorbance values. An excess of eosin causes the slow precipitation of the ion-pair complex.

Since the pH of the medium is in the alkaline range, the ion-pair, which has maximum stability at neutral pH, has a limited stability of 15 min which can be increased to 30 min by the addition of 1 ml of 0.5% glycerol. Glycerol helps in maintaining a uniform particle size, retarding the precipitation of the ion-pair in the reagent blank.

Two blanks were prepared for this system. The reagent blank which contained optimum concentrations of all the reagents except thiamine gave maximum absorbance (*cf.*, Fig. 2). The other blank was prepared in the absence of silver ions and thiamine to determine the contribution of the other reagents to the absorbance of the system. As the absorbance of this second blank was comparable to that of water, the absorbance of the ion-pair was measured

against water. The decrease in absorbance values at 550 nm was plotted against the increasing concentrations of thiamine to obtain the calibration graph. Beer's law was obeyed over the range 0—25 μg of thiamine in an overall aqueous volume of 10 ml, the equation of the line being $y=-0.02214x+0.632$, where y is the absorbance and x (μg) is the amount of thiamine.

It was observed that the silver mercaptide is not formed quantitatively from the ternary ion-pair complex of silver. Hence, 1,10-phenanthroline and eosin must be added to the unreacted silver ions after the formation of silver mercaptide. As the generation of the thiol is essential for mercaptide formation, the buffer reagent must be added prior to the addition of silver ions.

The absorbance spectrum of the system is shown in Fig. 2 and the calculated molar absorptivity was found to be $7.8\times10^4\,\mathrm{lmol^{-1}\,cm^{-1}}$ at 550 nm. The developed colour was stable for 30 min. The precision of the method was established at the 10 μg level of thiamine and the RSD was 2.5% ($n=10$). The correlation coefficient of the calibration plot was calculated as -0.998, confirming a linear decrease in absorbance with increasing concentration of thiamine.

Interference Study The effects of various vitamins, organic compounds and inorganic ions usually associated with thiamine in tablets and capsules on the colour development were studied at the 10 μg level of thiamine. A variation in the absorbance of the solution of more than ±0.01 in the presence of any co-existing matrix constituent was taken as an indication of interference. The results are given in Table 1. Common excipients such as talc, starch and magnesium stearate do not interfere in the determination. Pharmaceutical preparations containing fat soluble vitamins were subjected to pre-treatment as described earlier.

The interfering ions Cu^{2+}, Ca^{2+}, Mn^{2+} and Zn^{2+} were masked up to 10 μg level by the addition of 2.5×10^{-4} M (100 μg) EDTA to the sample solution. Fe^{2+} was present in large amounts in some pharmaceutical preparations. Since hydrogen peroxide was tolerated up to 2000 μg in the proposed method, interference of Fe^{2+} up to 500 μg was overcome by oxidising it to Fe^{3+} with 1 ml of 0.2% hydrogen peroxide in the presence of 1 ml of 0.02 M sulphuric acid. The formed Fe^{3+} was complexed with oxine and the complex was extracted into chloroform. The unreacted oxine (present in the aqueous phase) was subsequently extracted into chloroform after the addition of 1 ml of 0.1 M ammonia solution.

Ascorbic acid is a major interferent in the proposed method as it reduces Ag^+ ions to metallic silver. The interference of up to 500 μg of ascorbic acid was overcome by oxidising it with 1 ml of 0.2% hydrogen peroxide prior to thiamine analysis.

Application of the Method The proposed method was applied for the assay of thiamine in multi-vitamin preparations (Table 2) and pharmaceutical preparations containing essential minerals along with the vitamins (Table 3). The results compared well with those obtained by standard thiochrome method.[24]

Table 1. Interference Study

Thiamine 10 μg	
Interferents	Tolerance limit (μg)
Urea, starch, tartarate, Mg^{2+}	10,000.0
Sucrose, ethanol, nicotinic acid, calcium pantothenate	5,000.0
MoO_4^{2-}, $B_4O_7^{2-}$	2,500.0
Hydrogen peroxide, nitrite	2,000.0
Glucose, F^-	1,000.0
Pyridoxine, citrate, ascorbic acid,[a] (Fe^{2+})[b]	500.0
Oxalate, vitamin B_{12}, EDTA, sulphamic acid	100.0
SeO_3^{2-}	50.0
Ca^{2+}, Cl^-	25.0
Riboflavin, Ni^{2+}	10.0
Folic acid	5.0
Co^{2+}	2.5
Zn^{2+}, Cu^{2+}, Mn^{2+}	1.0
I^-	0.1

a) Oxidised with 1 ml 0.2% hydrogen peroxide. *b*) Oxidised with hydrogen peroxide to Fe^{3+} and separated as the oxine complex with chloroform.

Table 2. Determination of Thiamine in Multi-vitamin Preparations

Sample	Co-existing vitamins (concentration)	Thiamine content per tablet (mg)	Thiamine found[a]	
			Present method (mg)	Thiochrome method (mg)
Macraberin (Allenburys)	Vit B-6 (3 mg), B-12 (15 μg)	10	9.9	9.7
Beplex fore (AFD)	Vit B-2 (10 mg), B-6 (3 mg), B-12 (15 μg) C (150 mg), nicotinic acid (15 mg), niacinamide (45 mg), calcium pantothenate (25 mg), folic acid (1.5 mg)	10	10.9	11.0
Becozules (Pfizer)	Vit B-2 (10 mg), B-6 (3 mg), B-12 (15 μg), C (150 mg), niacinamide (50 mg), calcium pantothenate (12.5 mg), folic acid (1 mg)	10	10.9	10.7
Becozym C Forte (Roche)	Vit B-2 (10 mg), B-6 (3 mg), B-12 (10 μg), C (150 mg), nicotinamide (50 mg), calcium pantothenate (16.3 mg), biotin (0.15 mg)	10	10.9	11.0
Cobadex Forte (Glaxo)	Vit B-2 (10 mg), B-6 (3 mg), B-12 (15 μg), C (150 mg), nicotinamide (100 mg), calcium pantothenate (50 mg), folic acid (1.5 mg)	10	10.0	9.9

a) Average of three determinations.

Table 3. Determination of Thiamine in Pharmaceutical Preparations Containing Minerals and Vitamins

Sample	Co-existing vitamins and minerals (concentration)	Thiamine content per tablet (mg)	Thiamine found[a]	
			Present method (mg)	Thiochrome method (mg)
Revo-B [Revopharm (India) Pvt. Ltd.]	Vit. B-2 (10 mg), B-6 (3 mg), C (75 mg), niacinamide (50 mg), calcium pantothenate (10 mg), molybdenum oxide (150 μg), chromium oxide (100 μg)	10	10.9	10.7
Zevit [Remidx Pharma Pvt. Ltd. India]	Vit. B-2 (10 mg), B-6 (2 mg),B-12 (5 μg), C (150 mg), E-acetate (15 mg), nicotinamide (50 mg), calcium pantothenate (12.5 mg), $ZnSO_4 \cdot H_2O$ (61.8 mg)	10	10.1	10.0
Fourts B [Fourts (India) Laboratories Pvt. Ltd.]	Vit. B-2 (10 mg), B-6 (3 mg), C (150 mg), niacinamide (50 mg), $ZnSO_4$ (80 mg), Se as sodium selenite (100 μg), Cr as chromic chloride (150 μg)	10	10.3	10.5
Fesovit Spansule [Eskaylab]	Vit. B-2 (2 mg), B-6 (1 mg), C (50 mg), niacinamide (15 mg), pantothenic acid (2.5 mg), dried $FeSO_4$ (150 mg)	2	1.9	1.8
Supradyn[b] [Roche]	Vit. A acetate (10,000 I.U), D_3 (1,000 I.U), B-2 (10 mg), B-6 (3 mg), B-12 (15 μg), C (150 mg), nicotinamide (100 mg), calcium pantothenate (16.3 mg), α-tocopheryl acetate (25 mg), biotin (0.25 mg), $Ca_3(PO_4)_2$ (129 mg), MgO (60 mg), dried $FeSO_4$ (32.04 mg), $MnSO_4$ (2.03 mg), $CuSO_4$ (3.39 mg), $ZnSO_4$ (2.2 mg), sodium molybdate (0.25 mg), sodium borate (0.88 mg)	10	10.0	9.9
Becadexamine[b] (Glaxo)	Vit. A (5000 I.U), D3 (400 I.U), E (15 mg), B-2 (5 mg), B-6 (2 mg), B-12 (5 μg), C (75 mg), nicotinamide (45 mg), D-panthenol (5 mg), folic acid (100 μg), ferrous fumarate (50 mg), $Ca_3(PO_4)_2$ (70 mg), $CuSO_4$ (0.1 mg), $MnSO_4$ (0.01 mg), $ZnSO_4$ (50 mg), KI (0.025 mg) MgO (0.15 mg)	5	4.9	4.8

a) Average of three determinations. *b*) Partitioned between light petroleum and water before analysis.

Conclusion

The salient features of the developed method are high sensitivity and simplicity for routine analysis of thiamine in complex matrices.

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