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Methods of Analysis of Thiamine : A Review

Wajiha Gul[1], Saima Zahid[2], Shaheen Perveen[1], Iqbal Ahmad[2]*

[1]Faculty of Pharmacy, Jinnah University for women, Karachi - 74600, Pakistan
[2]Baqai Institute of Pharmaceutical Sciences, Baqai Medical University, Toll Plaza, Super Highway, Gadap Road, Karachi-74600, Pakistan

ABSTRACT

Thiamine (B1) is a component of B vitamins and multivitamin preparations. Several analytical methods have been developed for the assay of thiamine in pharmaceutical preparations and biological samples. These methods are based on the chemical, physical and microbiological characteristics of the molecule and include UV and visible spectrometry, fluorimetry, chromatography, injection flow, light scattering and microbiological methods. The applications of these methods depend on the level of concentration of the vitamin in the samples to be determined. Fluorimetric methods are more sensitive than the other methods used for the assay of thiamine. The microbiological methods based on the promotion of microbial growth are more specific and provide better results compared to the physical methods. The problem of the assay of thiamine in combination with other vitamins can be performed by the application of chromatographic methods.

Keywords: Thiamine, UV and Visible Spectrometry, fluorimetry, chromatogrphy

INTRODUCTION

Thiamine [2-3-(4-amino-2-methyl-pyrimidine-5-yl)methyl]-4-methyl-thiazol-yl]ethanol)], also known as vitamin B1, is a water-soluble vitamin used for the prevention and cure of beri beri. It was the first water soluble vitamin discovered in 1926, but the complete determination of its structure and synthesis were not accomplished until 19361. Many natural foods such as the germ of cereals, brans, egg yolk, yeast extracts, peas, beans and nuts provide enough thiamine for human requirement.2 Chemically thiamine consists of a thiazole ring attached to the pyrimidine ring with a methylene bridge.2 It is degraded by hydrolytic and oxidation reactions to

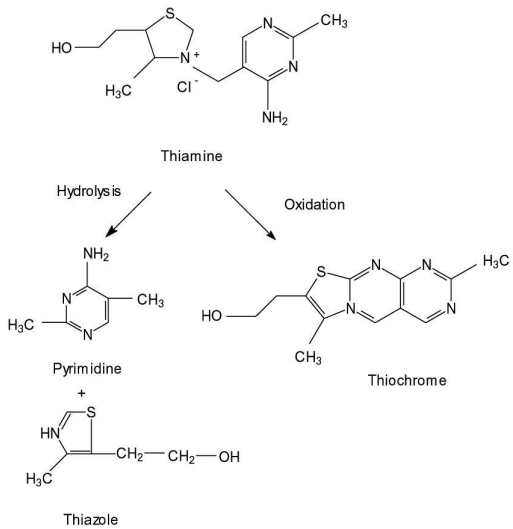

Figure 1: Chemical structure of thiamine and its degradation products

*Corresponding author: wajihanasheed_05@hotmail.com

inactive products (Figure 1).

Studies comprising the structure, biochemical functions, bioavailability, absorption, transport, distribution and requirements of thiamine have been reviewed by Bates2.

Thiamine, available as thiamine hydrochloride and thiamine mononitrate, is a colorless, crystalline, hygroscopic and highly water-soluble substance. Thiamine mononitrate is less hygroscopic than the chloride and is often preferred for use in food fortification. Thiamine is moderately soluble in alcohol and acetone (from which it can be crystallized) and practically insoluble in acetone, ether, chloroform, and benzene. It is stable at acidic pH, but is unstable in alkaline solutions1,3,4, as shown in Figure 1. The salts of Thiamine can be affected by light, pH, heat and reducing and oxidizing agents and should be stored under specified conditions. 5,6,7

ANALYTICAL METHODS FOR THE ASSAY OF THIAMINE

Several methods have been used for the assay of Thiamine and its salts in pharmaceutical preparation and biological fluids. These methods are reviewed in the following sections.

Spectrometric Methods

1. UV and visible spectrometric methods

Thiamine hydrochloride exhibits absorption maxima at 246nm (A11 450) in acidic and at 232 nm (A11 566) and 336 nm in alkaline solutions.8 The spectral characteristic have been used for the early assay of thiamine HCl in pure solutions and injections at 246 nm.9 The method was later used for the assay of thiamine HCl in multivitamin preparations after separation on a Zeokerb 226 column, elution with dilute HCl solution and assay at 246 nm.10 Several spectrometric methods based on colorimetric reactions were later developed including those with iodobismuthic acid, diazotized norsulphazole, diazotized ethyl-4-aminobenzoate, diazotized 6-aminothymol, 2-(4-aminobenzene sulphonamido) pyridine, 4-aminoacetophenone, bromothymol, 4-acetylphenyldiazonium chloride.11 Further developments in the spectrometric and other methods of analysis of thiamine have been reviewed by Elifson12, Song et.al13, and Eitenmiller14. Some other spectrometric methods are described below. A simple and sensitive spectrometric method for the assay of thiamine in pharmaceutical preparations is based on treatment with leucocrystal violet and measurement of absorbance at the absorption maximum.15 The simultaneous multi-component spectrometric determination of thiamine and other B vitamins has been carried out by using double divisor ration spectra derivative- zero crossing method in the presence of strongly overlapping signals without any chemical separation.16 A new method for the simultaneous determination of thiamine along with other B vitamins involves total absorbance measurements and their processing by partial least square regression to obtain the individual concentrations.17 The binary mixtures of thiamine and pyridoxine in a vitamin combination have been determined by UV-visible spectrometry using three newly developed genetic algorithms based multivariate calibration methods.18 Multivariate calibration methods based on Partial least squares regression (PLS) have been applied for the determination of thiamine in vitamin mixtures by UV-visible spectrometry.16 A method of simultaneous determination of thiamine and pyridoxine by a UV-spectrometric flow-through sensing device has been developed. The sensor is based on the retention of the vitamins on a cationic exchanger gel placed in the detection zone in to a quartz flow cell.19 A method of micro determination of thiamine in B vitamins has been carried out by solid phase UV spectrometry. Thiamine is strongly sorbed on Sephadez CMC-25 ion exchanger at pH 4.8 and its absorbance is measured directly in the solid phase at 247 nm.20

Flourimetric Methods

The earlier fluorimetric method is based on alkaline

potassium ferricyanide oxidation of thiamine to thiochrome in alkaline medium. An excess ferricyanide may destroy the thiochrome so the oxidation reaction should be performed quantitatively. The standard solution contains 0.2 µg/ml of thiamine HCl. After the addition of the oxidizing agent, the mixture is shaken for 90 minutes and extracted with isobutanol for measurement of its fluorescence.21 The early developments in florimetric methods have been reviewed by Elifson12 .

A flow injection fluorimetric method for the determination of thiamine has been reported. Thiamine is oxidized with potassium hexacyanoferrate on an anionic exchange resin and the fluorescence of thiochrome is measured. The method is suggested for the assay of thiamine in pharmaceutical products. The RSD value of the method is 1.8% at the concentrations of 1-4 ppm.22 A simple fluorimetric method has been reported for the determination of thiamine in food. The method has been found to be reproducible and shows good recovery without any interference.23 Another flow injection fluorimetric method for the determination of thiamine and copper has been proposed where thiamine is oxidized to thiochrome with the help of copper II. The method is applicable to the pharmaceutical formulations. Linear calibration graphs were obtained at the concentrations of 0.30-6.02 µg ml-1.24

For the analysis of thiamine and ascorbic acid in the pharmaceutical products, a fluorimetric method has been proposed. Thiamine is oxidized to thiochrome by mercury II. The calibration graphs are linear in the concentration range 2-100 µg ml-1. The flow injection analysis has been carried out showing a linear relation between concentration and flouresence (?ex = 356nm, ?em = 440nm).25 An official method for foods containing thiamine diphosphate is based on acid digestion, enzymatic hydrolysis, purification of thiamine by cation exchange column chromatography, oxidation to thiochrome, extraction in isobutanol and flourensece measurement.26 Several other methods for the determination of thiamine in pharmaceutical products have been reported.27-32 HPLC based assays for the thiamine in foods and biological materials involve fluorescence detection. The pre-column conversion of thiamine to thiochrome gives better resolution, but may affect the working life of the column due to the use of caustic mobile phase.33,34

Chromatographic Methods

Chromatographic methods have been utilized widely for the determination of vitamin B1 in pharmaceutical preparations. Some of the analytical parameters of these methods are reported in Table 1.

High performance liquid chromatography (HPLC) methods are now the most popular and widely applied methods for the determination of vitamins including thiamine HCl. A simple, fast and reliable HPLC method for the simultaneous, routine determination of thiamine and riboflavin in mushrooms has been developed. It involves an extraction procedure followed by chromatographic separation on a reversed-phase Spherisorb ODS column using methanol-water as mobile phase gradient.35 A simple and sensitive HPLC method has been proposed for the detection of thiamine in microalgae. The extraction of thiamine and its derivatives is carried out in acid solution followed by separation and the flouresence detection by liquid chromatography having minimum detection limit (15 fmol) of thiamine. 36 A HPLC method has been used for the analysis of thiamine in pasteurized milk, UHT sterilized milk and yogurt. Reversed phase was used with C18 column, connected to a fluorescence detector. The thiamine content in yogurt ranged from 0.355-0.04 to 0.404-0.02 mg/l. The linearity, reproducibility and recovery of the method was satisfactory.37 Free thiamine present in the food can be determined by HPLC with post column derivitization. Here HCl and sodium acetate can be used for extraction of the vitamin and methanol as a mobile phase.38 Thiamine in capsules has been determined by HPLC with detection at 240 nm on

a Sino Chrom ODS column using acetonitrile: water: triethylamine: phosphoric acid (15:85:0.3:0.3) as the mobile phase.39 The determination of thiamine in B vitamin syrups by ion pair HPLC has been reported.40

A comparison of spectrophotometric and HPLC methods has been carried out for the assay of thiamine HCl and pyridoxine HCl in a vitamin combination. Both methods have shown good accuracy, precision and recovery of the vitamins and the results are in agreement with in the experimental error.41 A simplified method for the determination of thiamine in meat products is proposed. The vitamin is extracted with 0.1M HCl followed by an enzymatic hydrolysis and determination by HPLC.42 Various studies has been done regarding the assay of thiamine in whole blood43 and its phosphate esters44, 45 , in urine and erythrocytes 46 and in pharmaceutical products.40 Flow Injection Turbidimetric Method

A simple flow injection method has been proposed for the assay of thiamine. It is based on the precipitation of thiamine with silicotungstic acid in acidic solution to form thiamine silicotungstate. The absorbance of this salt is measured at 420 nm. The calibration graph is linear in the vitamin concentration range of 5.0 x 10-5 to 3.0 x 10-4 M with a detection limit of 1.0 x 10-5 M. The RSD of 10 measurements of 1.0-2.5 x 10-5M thiamine are less than 1%. The method has been applied to the assay of thiamine in pharmaceutical formulations and biological fluids.47

Light Scattering Methods

A linear relationship has been found between the concentration of thiamine (0.02-0.04 µg/ml) and the resonance Reyleign scattering (RRS). The method has been applied for the detection of thiamine in the presence of excess iodide and cobalt nanoparticles.48 A novel assay of thiamine based on its enhancement of total internal reflected resonance light scattering signals of sodium dodecylbenzene sulfonate at the water/tetrachloromethane interface has been reported. The enhanced TIR-RLS intensity at 375.0 nm is proportional to the concentration of thiamine in the range 0.12–850 ng ml-1 and its limit of detection (3s) is 120 pg ml-1. The results of analysis of artificial samples are in agreement with the specified values, and those ones for Vitamin B1 tablets and injection solutions are identical with those obtained according to the method of the Chinese Pharmacopoeia.49 The evaluation of turbidimetric and nephelometric techniques for analytical determination of thiamine in pharmaceutical formulations has been made by employing a lab-made portable microcontrolled turbidimeter and nephelometer. The Limits of detection (LOD) of 2.6 × 10-6 and 7.5 × 10-6 mol L-1 were acquired for these procedures. 50

Microbiological Methods

An easy microbiological method has been proposed for the assay of thiamine in foods and biological fluids utilizing 96-well microtitre plates and an automatic plate reader. Thiamine is extracted for 20 minutes by acid digestion using acetate buffer (pH 4.5) at 110 ^{o}C. Results of the method were compared with HPLC and the traditional microbiological methods. The values of the results were higher than those of HPLC and the method reduces the reagent cost and use of serum volume and also increases the number of analysis.51

Another sensitive, cheap and reliable microbiological method has been proposed for the assay of thiamine in serum and red cells developing chloramphenicol resistant strain of Lactobacillus fermenti as the test organism. Chloramphenicol suppresses the bacterial and yeast contamination which helps to perform the assay without carrying out an aseptic procedure. The method is sensitive to 3.0 nmol/l of thiamine and shows good recovery. The results were compared with the established colorimetric method and for the red cells were found to be higher than those of colorimetric methods.52

Table 1: Analytical parameters for HPLC methods of thiamine assay.

Material	Technique	Column	Mobile phase	Flow rate ml/min^{-1}	Detection (nm)	Conc. range $\mu g/ml^{-1}$	Reference
Thiamine in multi-vitamin mixture	HPLC and corona-charged aerosol detection	Lichrosorb RP-C 18	0.05 M ammonium acetate: methanol 90:10 (v/v)	0.5	-	0.17-0.62 mg l^{-1}	53
Thiamine in B vitamin mixture	HPLC	Phenomenex C18	-	0.9	266	-	54
Thiamine in multi vitamin mixture	HPLC	Zorbax SB- Aq 1C 18	0.0125 M hexane -1-sulfonic acid Na salt in 0.1 % o-phosphoric acid pH 2.4-2.5 and acetonitrile	1	210, 230, 254	-	55
Thiamine, B_2 and B_6	HPLC	Inertsil ODS-3	0.05 mol/l KH_2PO_4 (pH 6) - methanol	-	265	-	56
Thiamine, B_6 and B_{12}	HPLC with coulometric electrochemical and UV detection	Supelco LC 18	0.05 M phosphate buffer – 10% methanol and 0.018 M trimethylamine pH 3.55	1	-	-	57
Thiamine and riboflavin	HPLC with fluorescence detection	-	72% 0.005 M NH_4OAc and 28% methanol	1.450	-	-	58
Thiamine in multi vitamin mixture	HPLC ion-pair	Hypersil C 18	Methanol-aqueous 0.5% acetic acid solution (18:82, v/v; containing 2.5 mM Na hexanesulfonate, pH 2.8)	1.2	280	5-90	59
Thiamine, B_2, ASA, caffeine, codeine and paracetamol	Reversed phase LC	Nucleosil C 18	Water (5 min) and acetonitrile – water (75+25, v/v; 9 min, H 2.1 with phosphoric acid)	-	285	500-1000 mg/l	60
Thiamine and riboflavin	LC	C 18	72% 0.005 M NH_4OAc (pH 5.0) and 28% methanol	-	370-435	-	61
Thiamine in B vitamin mixture	HPLC	Phenomenex C18	-	0.5	-	-	54
Thiamine, B_2, B_3, B_6 and sorbic acid	HPLC	RP C18	0.1% methanolic hexane sulphonic acid Na salt and 0.01 M phosphate buffer containing 0.1% hexane sulphonic acid Na salt pH 2.7	-	220, 288	-	62
B-complex vitamins	Capillary zone electrophoresis (CZE)	Silica capillary	20 mM tetra borate buffer pH 9.2	-	214	-	63
Thiamine and multi vitamin mixture	HPLC	Bondapak C 18	Methanol-water (27:73, v/v), pH 3 using glacial acetic acid	0.35	250-295	20-90	64
Thiamine and ascorbic acid	HPLC Continuous flow solid- phase spectroscopic sensor	Sephadex SP C-25	0.1 5M sodium acetate/ acetic acid and 0.18 M citric acid/K_2HPO_4	-	250	0.5-15	66

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