

<Brief Note>

Purity of standard solutions of thiamine and its phosphate esters for HPLC analysis

Hiroshi Ihara¹⁾, Naotaka Hashizume¹⁾, Akiko Hirano²⁾,
Linlin Wang²⁾ and Mitsumasa Okada²⁾

Summary Analytical results for thiamine and its phosphate esters are dependent on the purity of the standard solutions prepared from these materials. Our sources of reagents were Wako Pure Chemical Industries, Ltd., Osaka, Japan, and Sigma Chemical Co., St. Louis, MO, USA. The purity of thiamine and its phosphate esters in 0.1 mol/L HCl were based on the published absorptivities of these materials at 248 nm. Thiamine from Wako gave 100.9% purity, and the same from Sigma gave 96.8% purity. Thiamine monophosphate (anhydrate and dihydrate) from Sigma and thiamine triphosphate from Wako all showed > 82% purity. Thiamine diphosphate from Wako was > 86.0% pure, and the same from Sigma was > 81.5% pure, $p < 0.05$.

Key words: Vitamin B₁, Thiamine, Thiamine phosphate ester, Absorptivity.

1. Introduction

Thiamine (vitamin B₁) is an essential vitamin in the common pathway of oxidation of carbohydrate and pentose phosphate pathway and acts as a coenzyme for the reaction of pyruvate dehydrogenase (EC, 1.2.4.1), α -ketoglutaric dehydrogenase (EC, 1.2.4.2) and transketolase (EC, 2.2.1.1). We described an assay for thiamine and its phosphate esters, i.e.; thiamine monophosphate (TMP), thiamine diphosphate (TDP), and thiamine triphosphate (TTP) in whole blood

specimens by high-performance liquid chromatography (HPLC)^{1,2)}. In the HPLC analysis, we measured thiamine and its phosphate esters using materials purchased from Wako and Sigma. No purification attempts were made by us based on our belief that these materials had purities of ca. 98% to 100%. Pentinen³⁾ was the source of our absorptivity data for thiamine, TMP, TDP and TTP. After finding some unexpected analytical results, we decided to check the purity of solutions of these standard materials from Wako and Sigma.

1) Department of Laboratory Medicine, Ohashi Hospital, Toho University School of Medicine, Tokyo, Japan;

2) Department of Biomolecular Science, Faculty of Science, Toho University

Correspondence to: Hiroshi Ihara, Department of Laboratory Medicine, Ohashi Hospital, Toho University

School of Medicine, 2-17-6 Ohashi, Meguro, Tokyo 153-8515, Japan.

(Phone: +81-3-3468-1251, Fax: +81-3-3468-1906)

E-mail: ihara@med.toho-u.ac.jp

Received for Publication February 5, 2002

Accepted for Publication April 3, 2002

2. Materials and methods

Materials

We obtained ampoules of thiamine (thiamine chloride hydrochloride; 500 mg/L in 0.001 mol/L HCl, Wako cat. no. 609-02011; $C_{12}H_{17}ClN_4OS \cdot Cl$); we diluted this material with 0.1 mol/L HCl to give a thiamine concentration of 10 mg/L. A vial of thiamine powder (cat. no. T4625) was purchased from Sigma, and 10.0 mg were dissolved in 0.1 mol/L HCl to 10 mg/L solution. We used vials of powders for the following reagents to prepare 10 mg/L solutions in 0.1 mol/L HCl: TMP (thiamine monophosphate chloride, Sigma no. T8637, $C_{12}H_{18}ClN_4O_4PS$); TMP dihydrate (Sigma no. also T8637, $C_{12}H_{18}ClN_4O_4PS \cdot 2H_2O$); TDP (thiamine diphosphate chloride, Wako no. 032-11651 and Sigma no. T8754, $C_{12}H_{19}ClN_4O_7P_2S$); and TTP (thiamine triphosphate, Wako no. 209-10841, $C_{12}H_{19}N_4O_{10}P_3S$). For TMP dihydrate, we prepared solutions of 10.9 mg of TMP dihydrate in 0.1 mol/L HCl. This concentration was corrected for the additional water content in TMP dihydrate. We weighed all chemicals on an analytical balance from a newly opened vial, and they were not correct for moisture. Wako does not sell TMP, and Sigma does not sell TTP.

Methods

We measured the absorbancies at 248 nm of each of the 10.0-mg/L standard solutions of thiamine, TMP,

TMP dihydrate, TDP, and TTP. The photometric accuracy in the UV region of the Hitachi U-3010 spectrophotometer was checked and calibrated with $K_2Cr_2O_7$ solutions. Penttinen³⁾ reported that a 1% solution of thiamine, TMP, TDP and TTP in 0.1 mol/L HCl had extinction coefficients (248 nm, 1.0 cm light path, all means of six values) of 434 ± 6 , 422 ± 7 , 379 ± 6 and 370 ± 10 , respectively. This author used thiamine, TMP and TDP purchased from Sigma and carried out a three-fold purification by crystallization. He prepared TTP, and also crystallized it three times to remove inorganic phosphate. We compared the absorbancies determined by us with Penttinen's values.

3. Results and discussion

Table 1 shows the absorbancies measured at 248 nm and the expected (theoretical) values of each 10.0-mg/L standard solution of thiamine, TMP, TMP dihydrate, TDP, and TTP. Although solutions of thiamine from Wako or Sigma showed absorbancies of > 96% of the expected value, thiamine from Wako showed significantly greater purity than that from Sigma ($p < 0.05$). Solutions of TMP (Sigma), TMP dihydrate (Sigma), TDP (Wako and Sigma) and TTP (Wako) showed 15-20% lower absorbancies than their expected values. In addition, the purity of TDP from Wako showed significantly higher values than that from Sigma ($p < 0.05$).

Impurities in thiamine were described earlier;

Table 1 Estimates of purity of thiamine and its phosphate esters by comparison of absorbancies at 248 nm of standard solutions with their expected (theoretical) values

Solution in 0.1 mol/L hydrochloride	Manufacturer	Spectrophotometric absorbance at 248 nm		
		Measured	Theoretical	Purity (%)
Thiamine (10.0 mg/L)	Wako	0.438 ± 0.007 (7)	0.434	100.9 ± 1.6
Thiamine (10.0 mg/L)	Sigma	0.420 ± 0.015 (8)	0.434	96.8 ± 3.4
TMP (10.0 mg/L)	Sigma	0.346 ± 0.031 (6)	0.422	82.0 ± 7.3
TMP dihydrate (10.9 mg/L)	Sigma	0.357 ± 0.009 (6)	0.422	84.6 ± 2.1
TDP (10.0 mg/L)	Wako	0.326 ± 0.012 (6)	0.379	86.0 ± 3.2
TDP (10.0 mg/L)	Sigma	0.309 ± 0.006 (6)	0.379	81.5 ± 1.5
TTP (10.0 mg/L)	Wako	0.309 ± 0.010 (6)	0.370	83.5 ± 2.7

Values were mean \pm SD (number of analyses).

Purities were estimated from absorbance measurements at 248 nm of solutions of known concentrations and the absorptivities of the compounds.

from Hoffmann-La Roche⁴⁾, TMP (Sigma)⁵⁾, TDP (Merck AG, Darmstadt)⁴⁾, and TDP (Sigma)⁵⁾. There are no widely accepted data in the literature on the purity of each standard material nor of their expected absorptivities. Penttinen³⁾ reported on the assay of thiochrome (Thc), ThcMP, ThcDP and ThcTP by fluorometry. The general unavailability of fluorometers in clinical laboratories limits this approach. Eitenmiller et al.,⁶⁾ also reported the absorptivity of thiamine at 246, 234 or 364 nm. Unfortunately, they did not report the ultraviolet absorptivities for TMP, TDP and TTP. The fluorescence of standard solutions of Thc formed by different oxidizing reagents (potassium ferricyanide, cyanogen bromide or mercuric chloride) were not the same⁷⁾. Calibration based on UV absorbance appears to be suitable for routine testing in the clinical laboratory.

TMP, TDP and TTP, from either Wako or Sigma exhibited purities of 80-85% based on their absorptivities. These lower purity values include minor but unavoidable errors in weighing as well as errors in the preparation of the solutions in 0.1 mol/L HCl. Solutions of TMP, TDP and TTP stored at 4°C were stable for about 24 hours, after which there is a slow degradation to thiamine, and standards must be freshly prepared⁸⁾. The occurrence of an HPLC absorbance peak due to thiamine phosphate esters indicates degradation of the analytes, and the standard solutions should be discarded⁹⁾.

We conclude that the purity of the standard solutions of thiamine and its phosphate esters should be confirmed by measurements of the absorbance at 248 nm. We recommend that HPLC of the compounds be carried out before they are used as standards owing to the lability of thiamine phosphate esters in dilute HCl solutions and the formation of thiamine compounds that absorb at 248 nm.

Acknowledgments

We express our appreciation to Dr. John A. Lott, Professor of Pathology, Ohio State University Medical Center, Columbus, OH, for his helpful suggestions on this manuscript.

References

- 1) Ishii K, Sarai K, Sanemori H, Kawasaki T: Analysis of thiamine and its phosphate esters by high-performance liquid chromatography. *Anal. Biochem.*, 97: 191-195, 1979
- 2) Tallaksen CME, Bohmer T, Bell H: Concomitant determination of thiamin and its phosphate esters in human blood and serum by high-performance liquid chromatography. *J. Chromatogr.*, 564: 127-136, 1991
- 3) Penttinen HK: Differences in thiochrome fluorescence produced by thiamine and its mono-, di-, and triphosphate esters. *Acta. Chem. Scand., Ser. B*, 30: 659-663, 1976
- 4) Schrijver J, Speek AJ, Klosse JA, van Rijn HJM, Schreurs WHP: A reliable semiautomated method for the determination of total thiamine in whole blood by the thiochrome method with high-performance liquid chromatography. *Ann. Clin. Biochem.*, 19: 52-56, 1982
- 5) Defibaugh PW: Evaluation of selected enzymes for thiamine determination. *J. Assoc. Off. Anal. Chem.*, 70: 514-517, 1987
- 6) Eitenmiller RR, Landen, WO Jr.: Thiamin. In: *Vitamin Analysis for the Health and Food Sciences*, 271-297, CRC Press, London, 1999
- 7) Lewin LM, Wei R: Microassay of thiamine and its phosphate esters after separation by paper chromatography. *Anal. Biochem.*, 16: 29-35, 1966
- 8) Kawasaki T, Egi Y: Thiamine. In: *De Leenheer AP, Lambert WE, Van Bocxlaer JF, eds., Modern Chromatographic Analysis of Vitamins*, 3rd Edition, Revised and Expanded, 365-389, Marcel Dekker, Inc., New York, 2000
- 9) Ihara H, Hashizume N, Hirano A, Okada M: A simplified HPLC method for thiamine and its phosphate esters in whole blood. *J. Anal. Bio-Sc.*, 24: 235-238, 2001