PICKERING LABORATORIES

Analysis of Vitamin B1 in Foods and Dietary Supplements by HPLC With Post-Column Derivatization and Fluorescence Detection

Thiamine (vitamin B1) plays important role in many cellular processes and its deficiency can quickly lead to serious health problems. Since humans and animals can't synthesize vitamin B1, they must obtain a sufficient amount through their diet. The requirements of nutritional labeling have led to increased demand for methods to analyze vitamin B1 in different matrices.

This application note describes a sensitive and accurate HPLC method capable of measuring Thiamine and its biologically active phosphorylated derivatives in foods and dietary supplements. Thiamine and its derivatives are separated on reversed phase column and converted using post-column derivatization into highly fluorescent compounds. To determine total vitamin B1 content in foods, an enzymatic reaction with Taka-diastase was employed to convert all Thiamine esters to free Thiamine.

Method

Sample Preparation

- *Food Samples:* To 5 g of sample add 60 mL of 0.1 N HCl, blend at high speed for 2-3 min and heat the mixture at 100 °C for 1 h. Cool the mixture to room temperature and adjust pH to 4.0 – 4.5 using 2.5 M Sodium Acetate solution. Add 200 mg of Taka-diastase, shake well and incubate for 18 h at 45 °C. After enzymatic hydrolysis is complete add 2 mL of 50% Trichloro-acetic acid solution in water and heat at 100 °C for 15 min to precipitate proteins. Adjust pH to 2.6 – 2.8 with Sodium Acetate and bring the volume to 100 mL with DI water. Filter through 0.45 um filter.
- *Dietary Supplements:* Mix the contents of at least 10 capsules. Take 250 mg portion and dissolve in 100 mL of DI water acidified to pH 2.6-2.8 with 0.1 N HCl. Dilute the solution further with acidified water to fit the calibration curve as needed. Filter through 0.45 um filter.

Standards Preparation

- *Standard solutions:* Prepare standard solutions of Thiamine and its derivatives in water acidified to pH 2.6 – 2.8 with HCl. Make fresh daily.
- *Calibration Range:* Thiamine: 0.1 – 25 ug/mL, R^2=0.999; Thiamine Pyrophosaphate: 0.02 – 5 ug/mL, R^2=0.999; Thiamine Mono-phosphate: 0.02 – 5 ug/mL, R^2=0.999.

Analytical Conditions

Analytical Column: ThermoHypersil, Aquasil C18

Flow Rate: 1 mL/min

Column Temperature: 40 °C

Mobile Phase: 10% Acetonitrile - 90% Phosphate Buffer (6 g/L of Phosphoric Acid adjusted to pH 5.9 with NaOH)

Injection Volume: 10 uL

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Reactor Volume: 0.5 mL

Reactor Temperature: 55 °C

Reagent: 40 g/L NaOH, 600 mg/L Potassium Ferricyanide in water

Reagent Flow Rate: 0.5 mL/min

Detection: FLD, Ex 375 nm, Em 430 nm

Table 1. Thiamine Analysis in Foods

Sample	Thiamine Found in the Sample	RSD%, N=4	Thiamine Spike Concentration	Recoveries	RSD%, N=4
Whole Yellow Peas	13 ug/g	1.5	50 ug/g	92%	7.8
Cereal	21.5 ug/g	3.6	50 ug/g	102%	2.8
Pork Sausages	5.0 ug/g	2-2	50 ug/g	108%	1.6

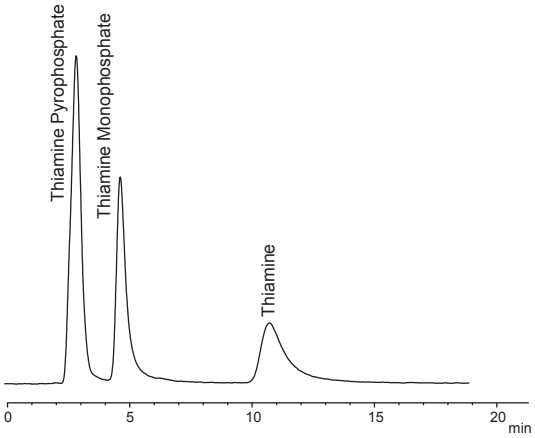

Fig 1. *Chromatogram of 1 ug/mL calibration standard of Thiamine and its phosphorylated derivatives*

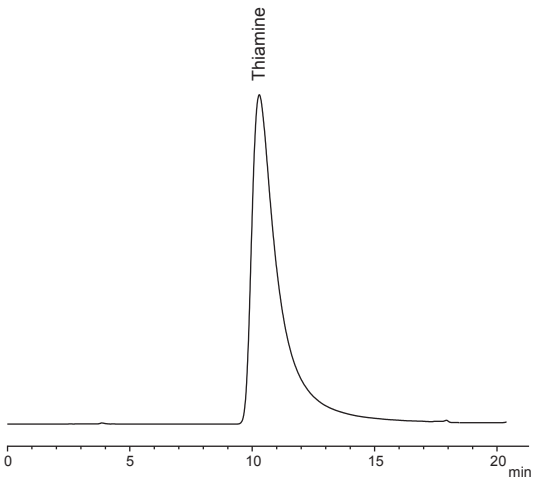

Fig 2. *Chromatogram of Vitamin B Complex supplement*

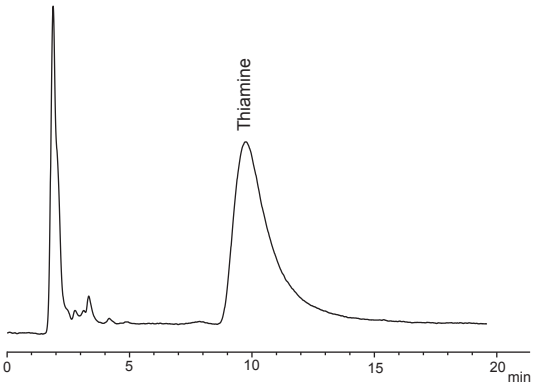

Fig 3. *Chromatogram of cereal sample*

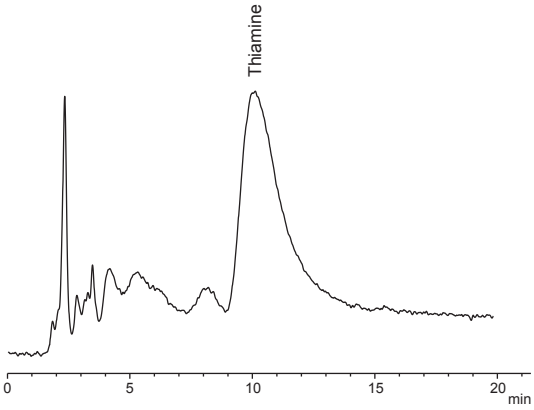

Fig 4. *Chromatogram of pork sausage sample*

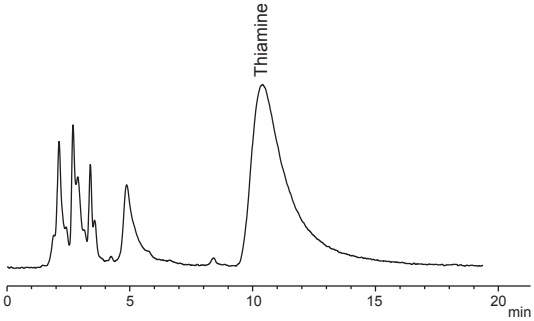

Fig 5. *Chromatogram of whole yellow peas sample*