

HPLC Analysis of Vitamin B1, B2

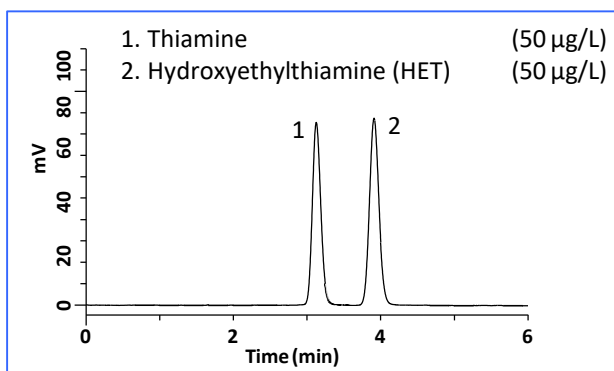
Takadiastase (*Aspergillus oryzae* carboxyl proteinase) has been used for hydrolyses of thiamin phosphate ester and riboflavin phosphate ester to analyse thiamin and riboflavin, respectively by HPLC.

However, it is difficult to get commercial Takadiastase stable nowadays.

So in this technical note, we introduce a new sample preparation method for thiamin and riboflavin analyses using acid phosphatase hydrolysis instead of Takadiastase hydrolysis.

Standard Solution (Vitamin B1)

Separated thiamine by ODS column was derivatized with potassium ferricyanide under alkaline condition. Fluorescent compound thiochrome was produced and detected by fluorescence detector.



HPLC Conditions (Vitamin B1)

Column: Inertsil ODS-3 5 mm, 150 × 4.6 mm I.D.

Eluent : A) CH₃OH

B) 10 mM NaH₂PO₄, 150 mM NaClO₄ (pH 2.2) A/B = 1/9, v/v (Mixed by a gradient mixer)

Flow Rate: 1.0 mL/min

Col. Temp.: 40 °C

Detection : FL Ex. 375 nm, Em. 440 nm

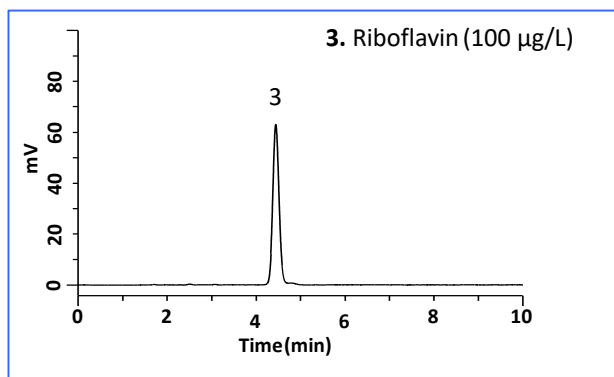
Inj. Vol. : 20 µL

Reaction Reagent: 0.05 w/v % C₆FeK₃N₆, 15 w/v % NaOH

Flow Rate of Reaction Reagent: 0.4 mL/min

Reaction Coil: 5000 × 0.33 mm I.D. (PTFE tube)

Standard Solution (Vitamin B2)



HPLC Conditions (Vitamin B2)

Column : Inertsil ODS-3 5 µm, 150 × 4.6 mm I.D.

Eluent : A) CH₃OH

B) 40 mL of 4 M CH₃COONa, 20 mL of 50 % CH₃COOH, 1940 mL of H₂O (pH 4.5)

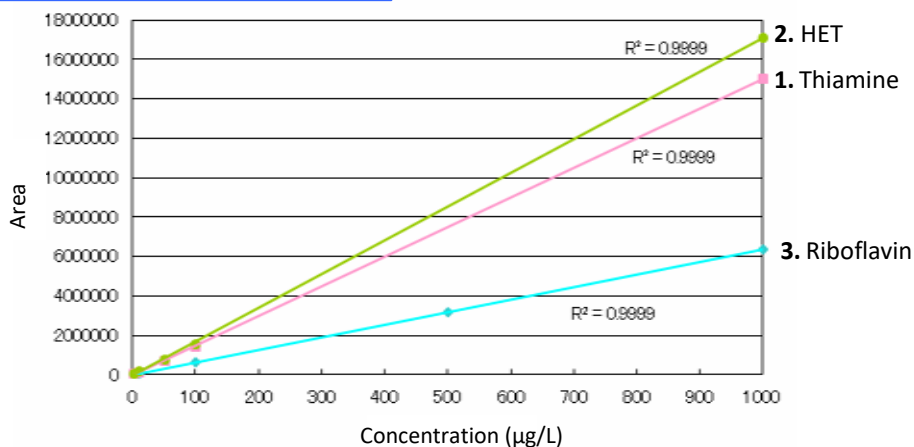
A/B = 35/65, v/v (Mixed by a gradient mixer)

Flow Rate : 1.0 mL/min

Col. Temp. : 40 °C

Detection : FL Ex. 445 nm, Em. 530 nm

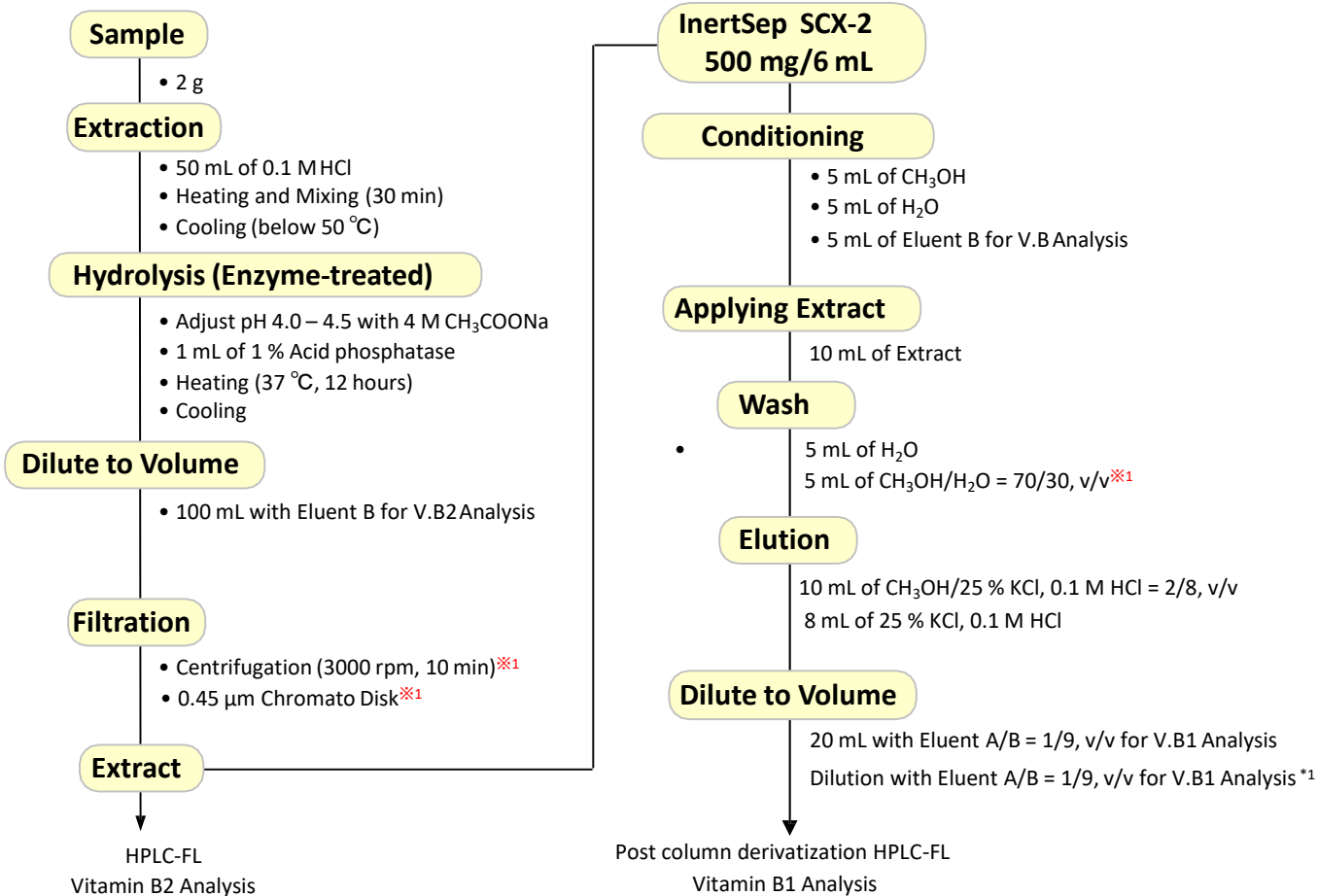
Inj. Vol. : 20 µL



Calibration Curve

Sample Preparation Method Example

Solid Phase Extraction

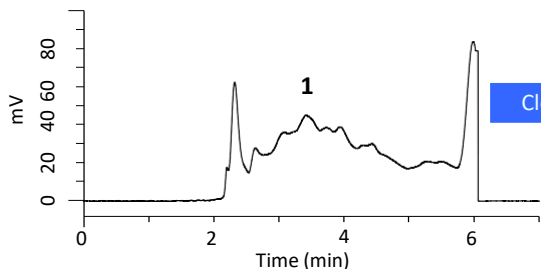


*1 Please do these steps as necessary.

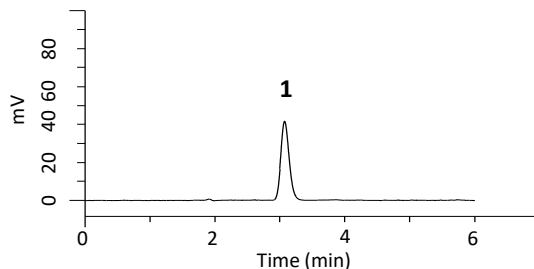
An Effect of Solid Phase Extraction (a case of commercial instant noodle)

1. Thiamine

No Sample preparation



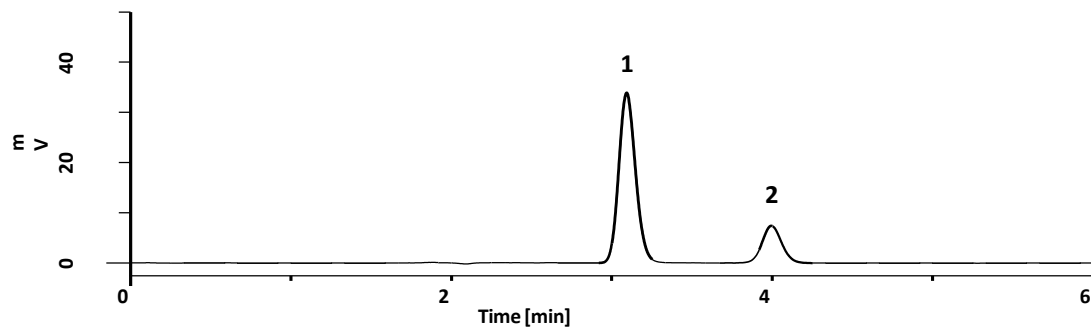
With Sample preparation



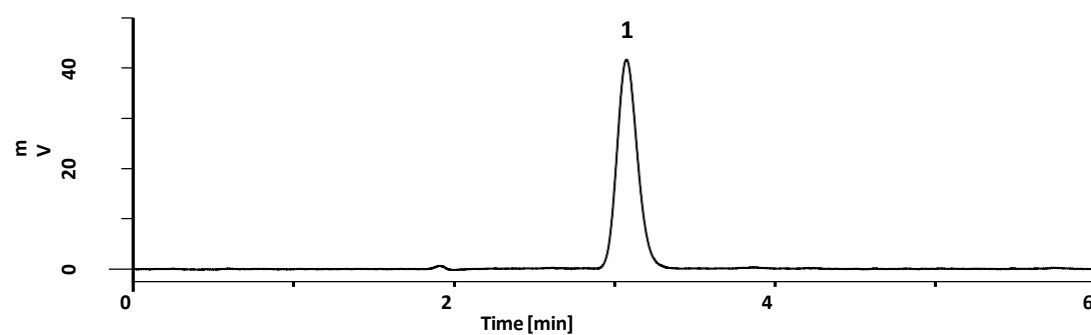
Vitamin B1 in foods

1. Thiamine
2. Hydroxyethylthiamine (HET)

An extract of beef



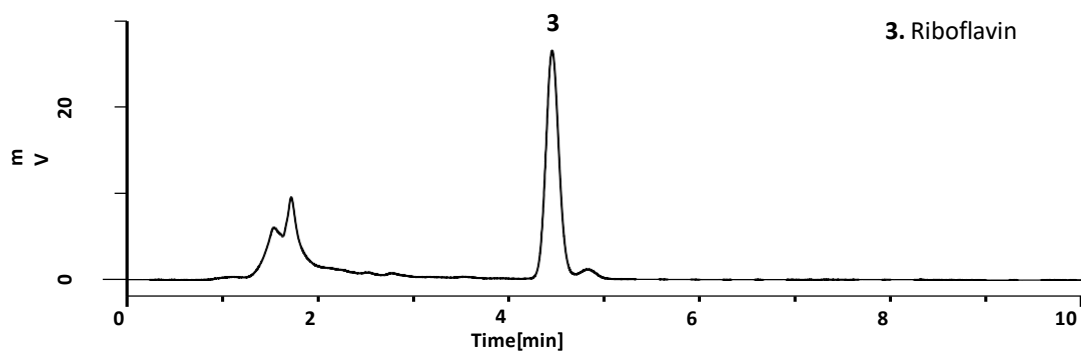
A commercial instant noodle



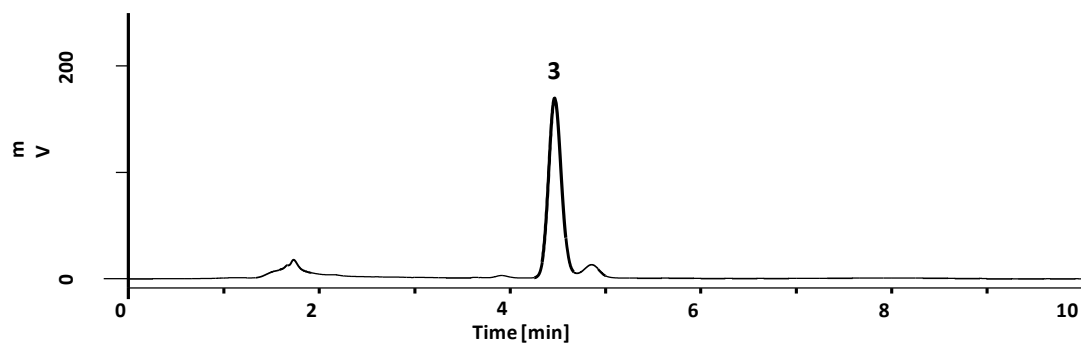
Vitamin B2 in foods

An extract of beef

3. Riboflavin



A commercial instant noodle

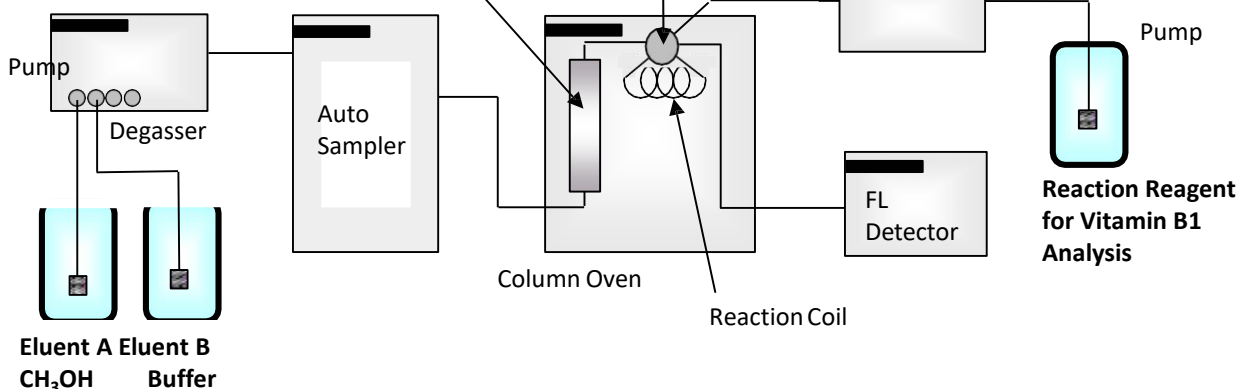


Post column derivatization HPLC system was used for Vitamin B1 analysis. On the other hand, Vitamin B2 is natural fluorescence compound and was analyzed by normal HPLC system. In this time, HPLC system in which switching valve was mounted like below.

Schematic Diagram

Switching Valve for Change Flow Channels

HPLC Column



HPLC Column

Inertsil ODS-3 5 μ m, 150 \times 4.6 mm I.D.
Cat. No. 5020-01731

SPE Column

InertSep SCX-2 500 mg/6 mL
Cat. No. 5010-61724

Syringe Filter

GL Chromato Disk Sample Filters 25A
PORE SIZE 0.45 μ m
Cat. No. 5040-28512

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