LT143
GL Sciences Inc.

HPLC Analysis of Vitamin B1, B2

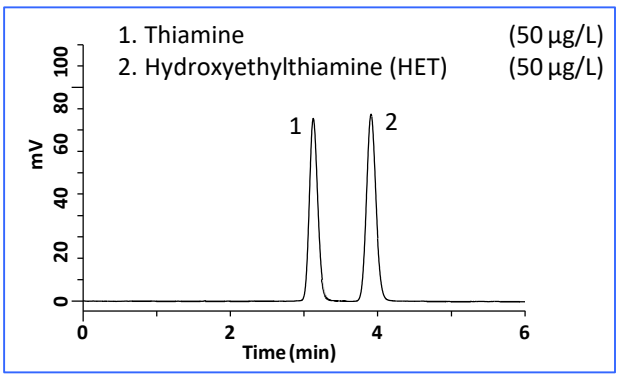
Takadiastase (Aspergillus oryzae carboxyl proteinase) has been used for hydrolyses of thiamin phosphate ester and riboflavin phosphate ester to analyse thiamin and riboflavin, respectively by HPLC.

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) CH_3OH

B) 10 mM NaH_2PO_4, 150 mM $NaClO_4$ (pH 2.2) A/B = 1/9, v/v (Mixed by a gradient mixer)

Flow Rate: 1.0 mL/min

Col. Temp.: 40 ℃

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

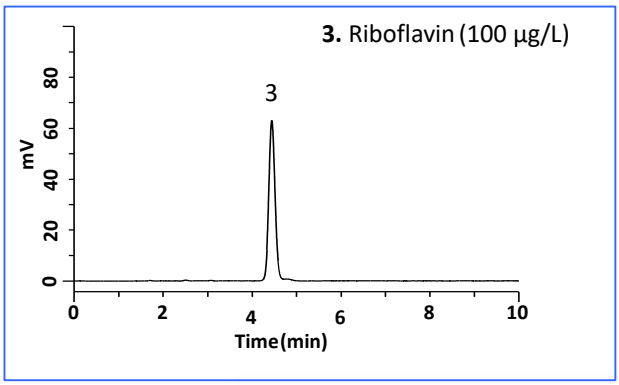
Inj. Vol. : 20 µL

Reaction Reagent: 0.05 w/v % $C_6FeK_3N_6$, 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) CH_3OH

B) 40 mL of 4 M CH_3COONa, 20 mL of 50 % CH_3COOH, 1940 mL of H_2O (pH 4.5)

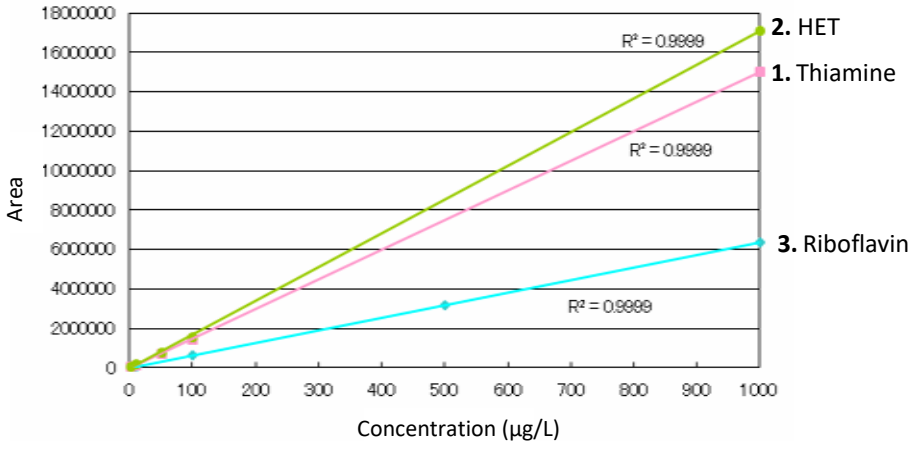
A/B = 35/65, v/v (Mixed by a gradient mixer)

Flow Rate : 1.0 mL/min

Col. Temp. : 40 ℃

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

Inj. Vol. : 20 µL



Calibration Curve

GL Sciences

Sample Preparation Method Example

Solid Phase Extraction

Sample
- 2 g

Extraction
- 50 mL of 0.1 M HCl
- Heating and Mixing (30 min)
- Cooling (below 50 ℃)

Hydrolysis (Enzyme-treated)
- Adjust pH 4.0 – 4.5 with 4 M CH_3COONa
- 1 mL of 1 % Acid phosphatase
- Heating (37 ℃, 12 hours)
- Cooling

Dilute to Volume
- 100 mL with Eluent B for V.B2 Analysis

Filtration
- Centrifugation (3000 rpm, 10 min)※1
- 0.45 µm Chromato Disk※1

Extract

HPLC-FL
Vitamin B2 Analysis

InertSep SCX-2 500 mg/6 mL

Conditioning
- 5 mL of CH_3OH
- 5 mL of H_2O
- 5 mL of Eluent B for V.B Analysis

Applying Extract

10 mL of Extract

Wash
- 5 mL of H_2O
- 5 mL of CH_3OH/H_2O = 70/30, v/v※1

Elution

10 mL of CH_3OH/25 % KCl, 0.1 M HCl = 2/8, v/v
8 mL of 25 % KCl, 0.1 M HCl

Dilute to Volume

20 mL with Eluent A/B = 1/9, v/v for V.B1 Analysis
Dilution with Eluent A/B = 1/9, v/v for V.B1 Analysis *1

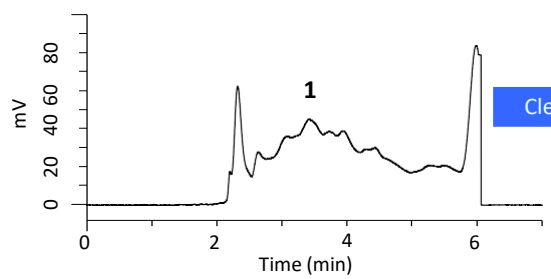
Post column derivatization HPLC-FL
Vitamin B1 Analysis

*1 Please do these steps as necessary.

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

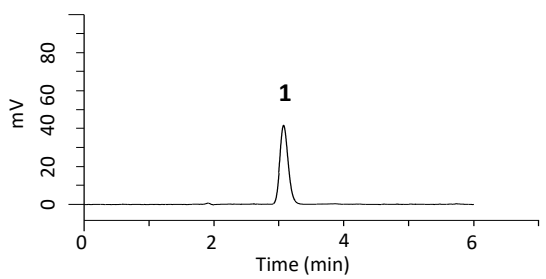
1. Thiamine

No Sample preparation



Clean Up

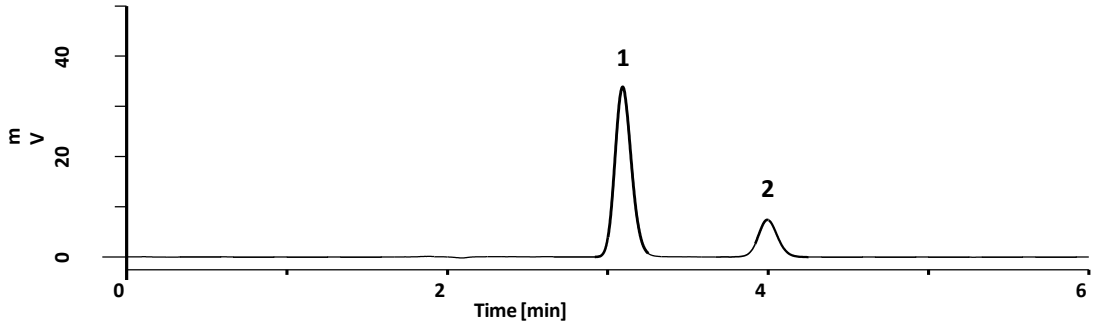
With Sample preparation



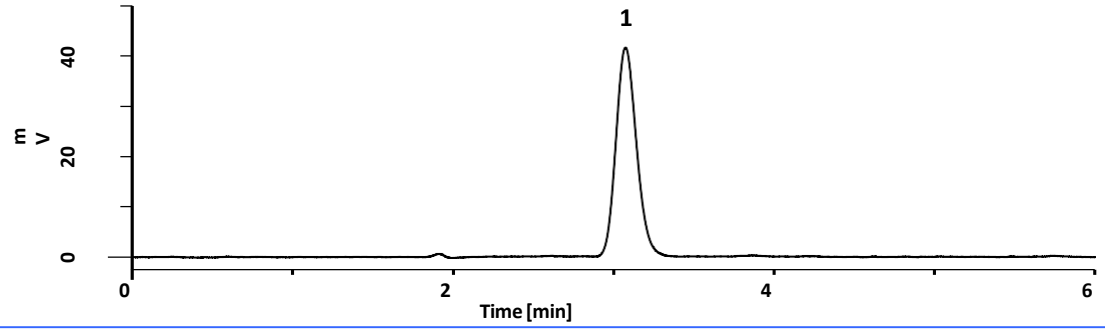
Vitamin B1 in foods

1. Thiamine
2. Hydroxyethylthiamine (HET)

An extract of beef



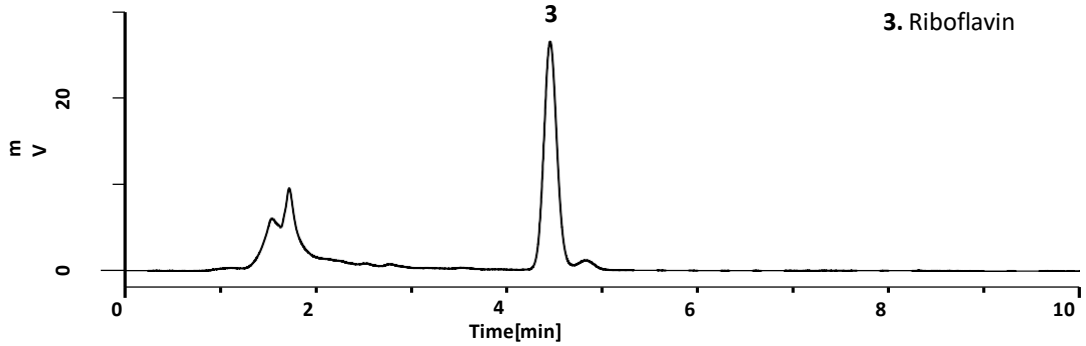
A commercial instant noodle



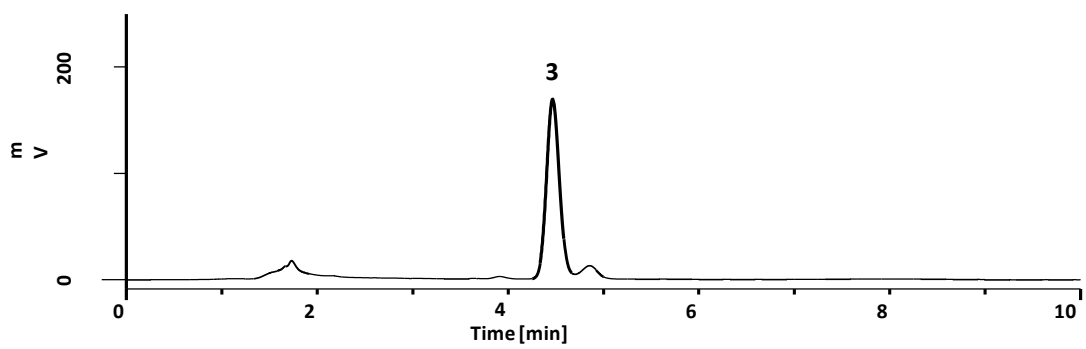
Vitamin B2 in foods

An extract of beef

3. Riboflavin

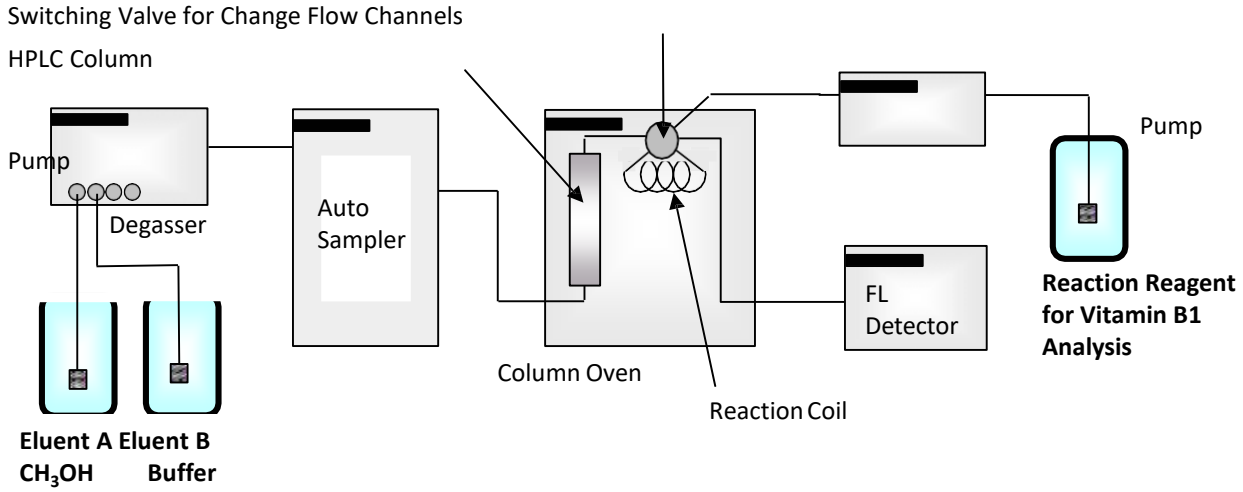


A commercial instant noodle



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



HPLC Column

Inertsil ODS-3 5 µm, 150 × 4.6 mm I.D.
Cat. No. 5020-01731

Syringe Filter

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

SPE Column

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

GL Sciences, Inc. Japan
22-1 Nishishinjuku 6-Chome
Shinjuku-ku, Tokyo,
163-1130, Japan
Phone: +81-3-5323-6620
Fax: +81-3-5323-6621
Email: world@gls.co.jp
Web: www.glsciences.com

GL Sciences B.V.
De Sleutel 9
5652 AS Eindhoven
The Netherlands
Phone: +31 (0)40 254 95 31
Email: info@glsciences.eu
Web: www.glsciences.eu

GL Sciences (ShangHai) Ltd.
Tower B, Room 2003,
Far East International Plaza,
NO,317 Xianxia Road,
Changning District.
Shanghai, China P.C. 200032
Phone: +86 (0)21-6278-2272
Email: contact@glsciences.com.cn
Web: www.glsciences.com.cn

GL Sciences, Inc. USA
4733 Torrance Blvd. Suite 255
Torrance, CA 90503
Phone: 310-265-4424
Fax: 310-265-4425
Email: info@glsciencesinc.com
Web: www.glsciencesinc.com

International Distributors
Visit our Website at:
https://www.glsciences.com/company/distributor.html