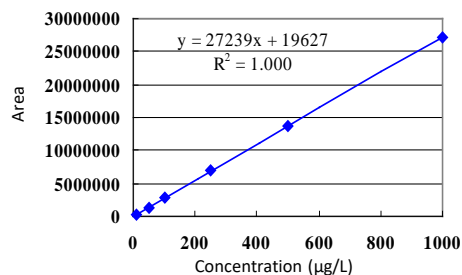
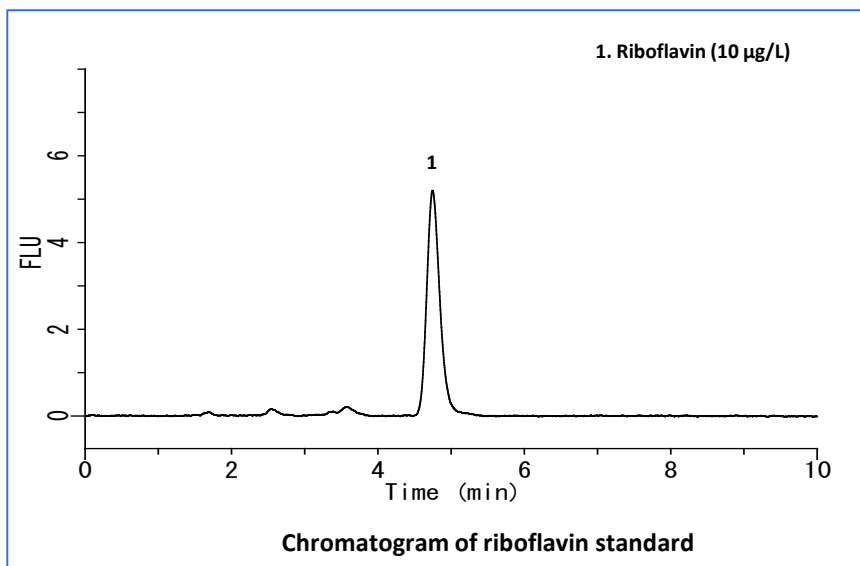


1. Analysis of total vitamin B2

There are riboflavin and riboflavin phosphate esters as components with vitamin B2 activity in food. Here, in accordance with the Guidelines Food Hygiene Inspection, we introduce a method to convert a component having B2 activity into riboflavin by enzymatic degradation and measure it as riboflavin..



HPLC conditions

Column : Inertsil ODS-3
(5 µm, 150 x 4.6 mm I.D.)

Temperature : 40 °C

Detector : FL Ex 445 nm Em 530 nm

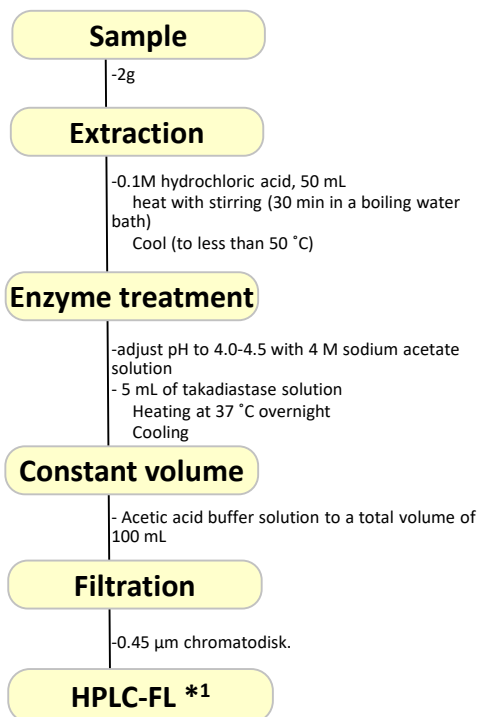
Injection volume : 20 µL

Eluent : A) CH₃OH B) Acetic Acid Buffer
A/B = 35/65, v/v

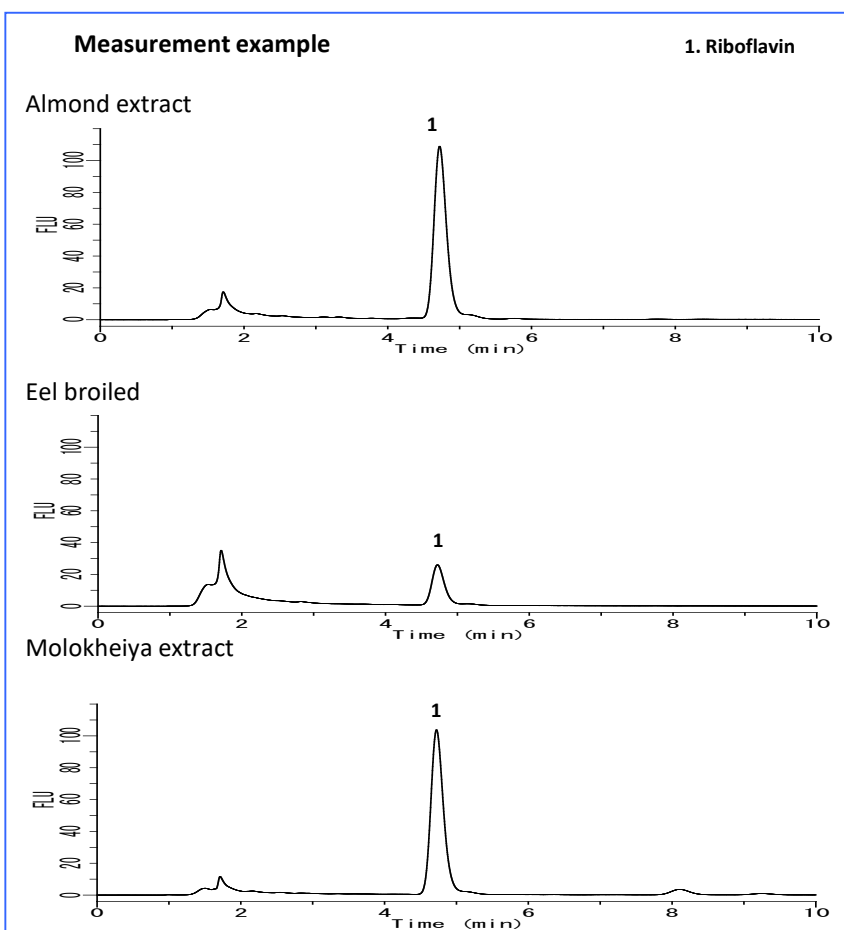
Flow rate : 1.0 mL/min

Acetic Acid Buffer :
Mix 40 mL of 4 M sodium acetate solution and 20 mL of 50% acetic acid solution. Dissolve in 2 L of ultrapure water (pH 4.5)

Example of pretreatment



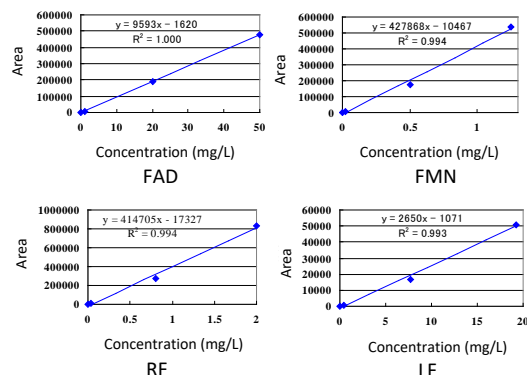
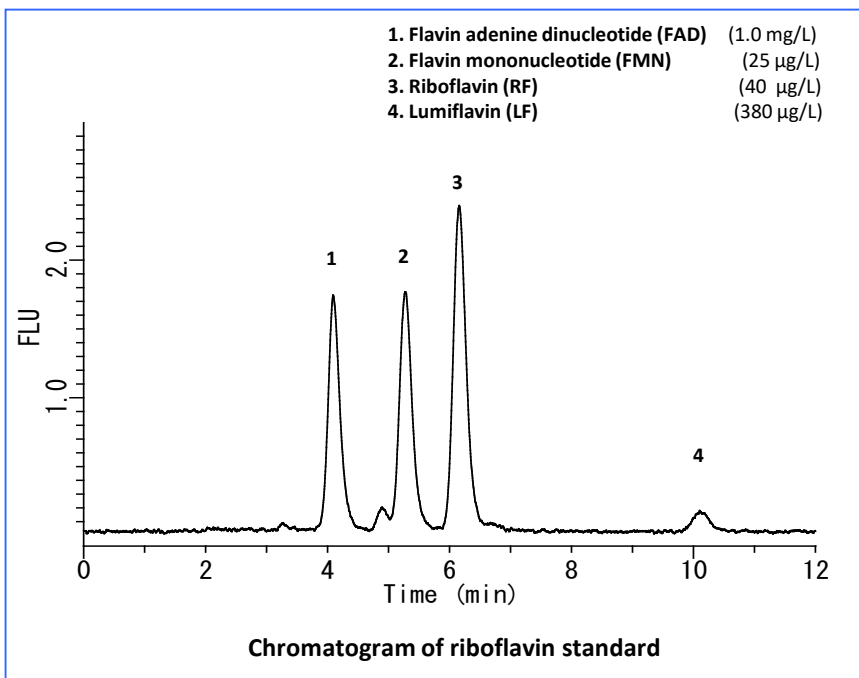
*1 Because vitamin B2 is present at trace levels in common foods, Sensitive analysis with a fluorescence detector is required.



2. Simultaneous Analysis of Riboflavin and Riboflavin Phosphate Esters

Riboflavin (RF) binds to phosphate and ATP in cells to form flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). Photodegradation also results in lumiflavin (LF).

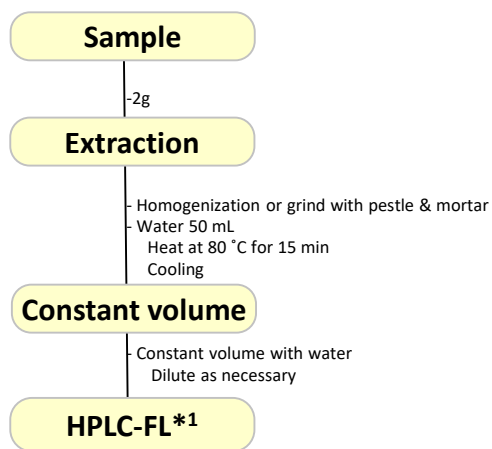
Here, a simultaneous analysis of the four vitamin B2 components (including riboflavin) was made.



HPLC conditions

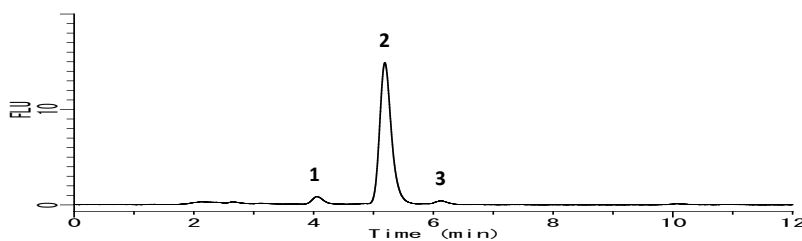
Column : Inertsil ODS-3 (5 µm, 150 x 4.6 mm I.D.)
Temperature : 40 °C
Detector : FL Ex 445 nm Em 530 nm
Injection volume : 20 µL
Eluent : A) CH₃OH B) 0.01M NaH₂PO₄ (pH 5.5) A/B = 35/65, v/v
Flow rate : 0.8 mL/min
 (Reference: New Food Analytical Procedure P. 392 ISBN 4-7712-9604-9)

Example of pretreatment

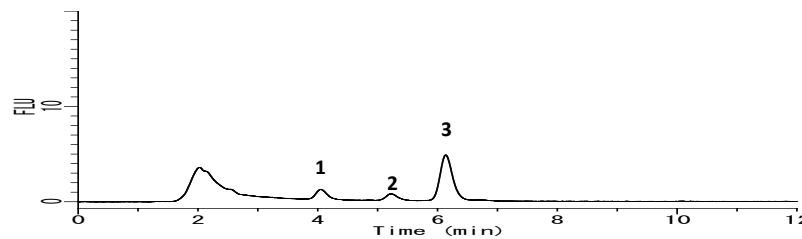


Measurement example

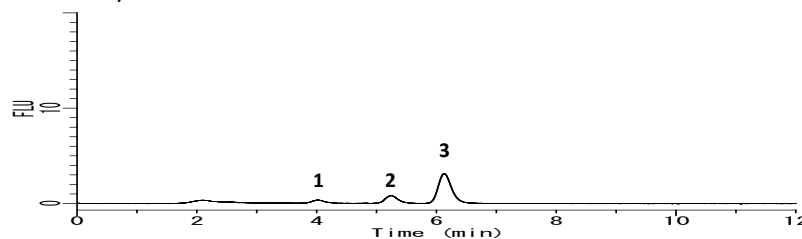
Almond extract



Baker's baker's extract



Molokheiya extract



1. FAD
2. FMN
3. RF

*1 Because vitamin B2 is present at trace levels in common foods, Sensitive analysis with a fluorescence detector is required.

3. Analysis of riboflavin butyrate

Riboflavin tetrabutrate that is Riboflavin Butyrate is a fat-soluble vitamin B2 and is added to supplements and nutritional drinks to improve cholesterol.

In this section Riboflavin tetrabutrate was analyzed according to the reference method in the Guidelines Food Hygiene Inspection.

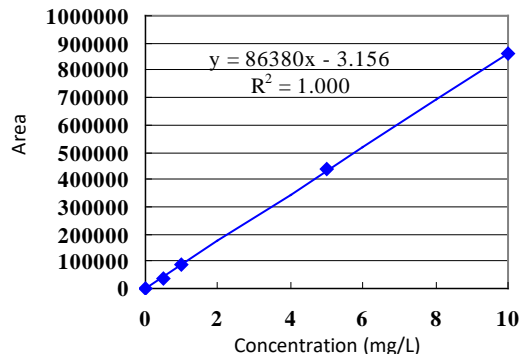
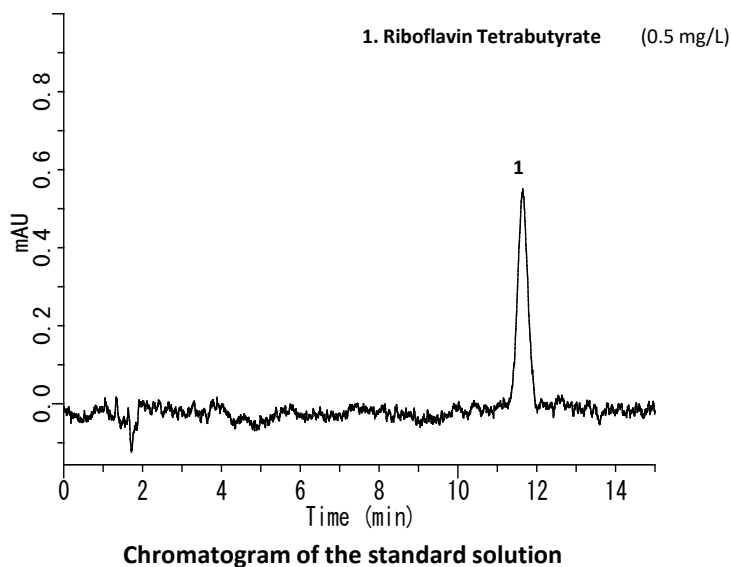
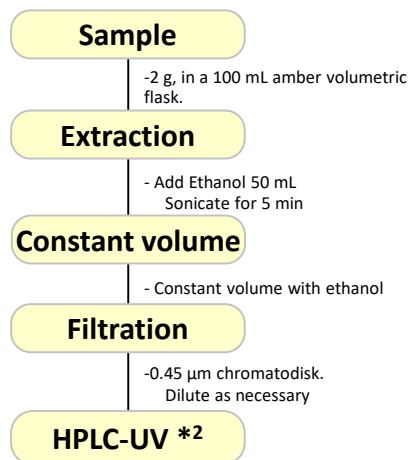


Fig. Riboflavin tetrabutrate calibration curve

HPLC conditions

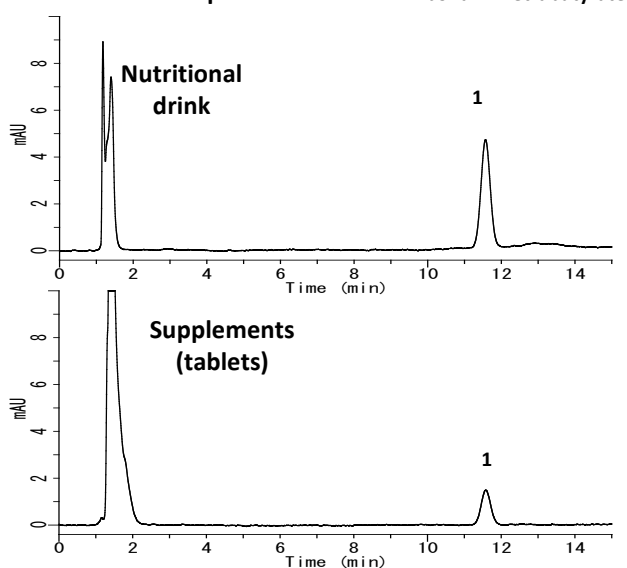
Column	:Inertsil ODS-3 (5 μ m, 150 x 4.6 mm I.D.)
Temperature	:40 $^{\circ}$ C
Detector	:UV 445 nm
Injection volume	:20 μ L
Eluent	: A) CH ₃ CN B) H ₂ O A/B = 3/2, v/v
Flow rate	: 1.0 mL/min

Example of pretreatment



*2 Riboflavin butyrate is often added in high concentrations to supplements, Assay with UV detector is possible.

Measurement example



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