

Introduction to the Analysis of Vitamin B2 by HPLC

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..

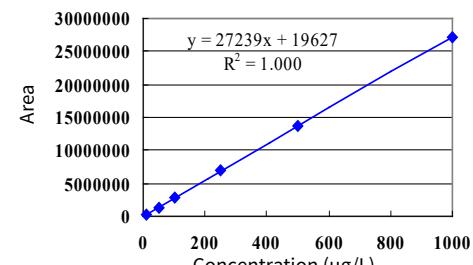
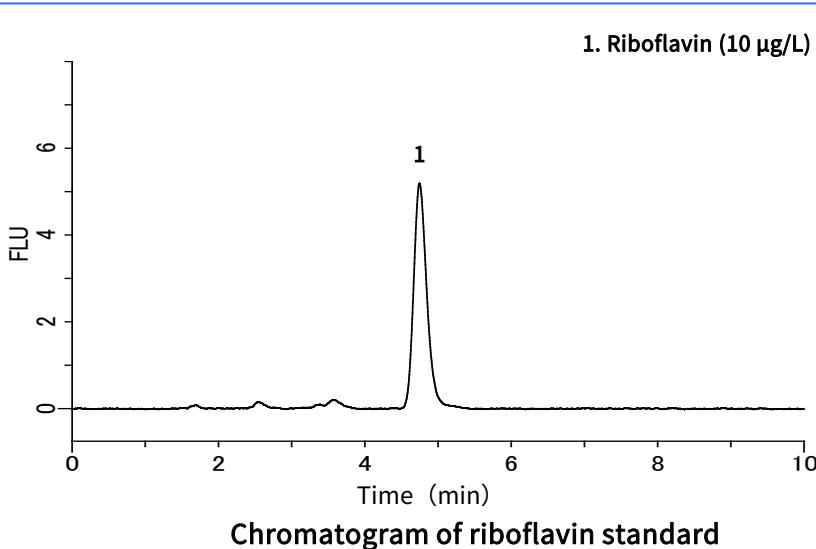


Fig. Riboflavin calibration curve

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) CH ₃ OH 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

Sample

-2g

Extraction

-0.1M hydrochloric acid, 50 mL
heat with stirring (30 min in a boiling
water bath)
Cool (to less than 50 °C)

Enzyme treatment

-adjust pH to 4.0-4.5 with 4 M sodium
acetate solution
- 5 mL of takadiastase solution
Heating at 37 °C overnight
Cooling

Constant volume

- Acetic acid buffer solution to a total
volume of 100 mL

Filtration

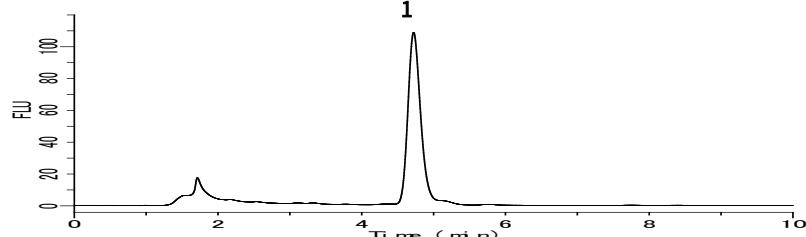
-0.45 µm chromatodisk.

HPLC-FL *1

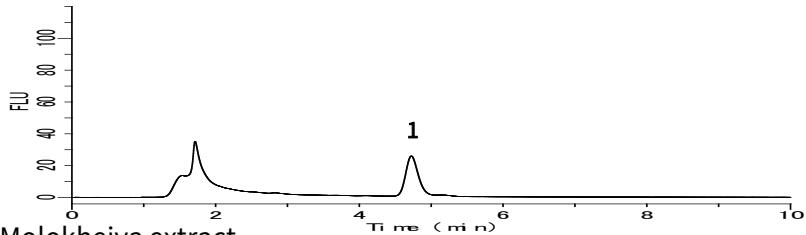
Measurement example

1. Riboflavin

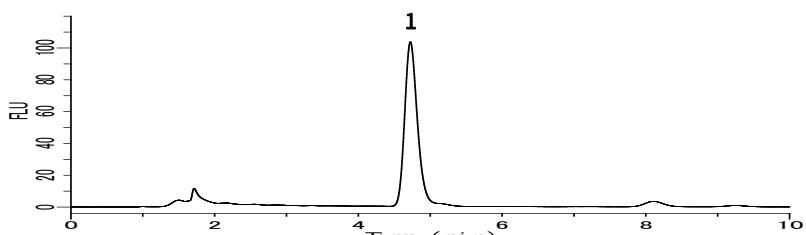
Almond extract



Eel broiled



Molokheiya extract



*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.

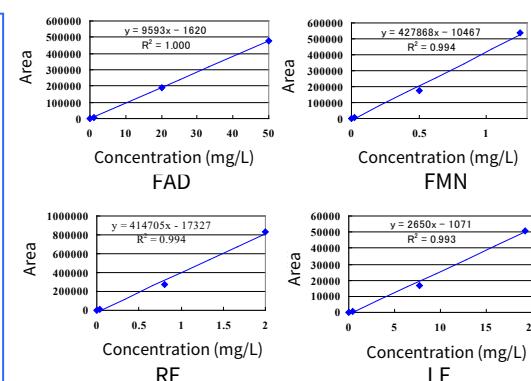
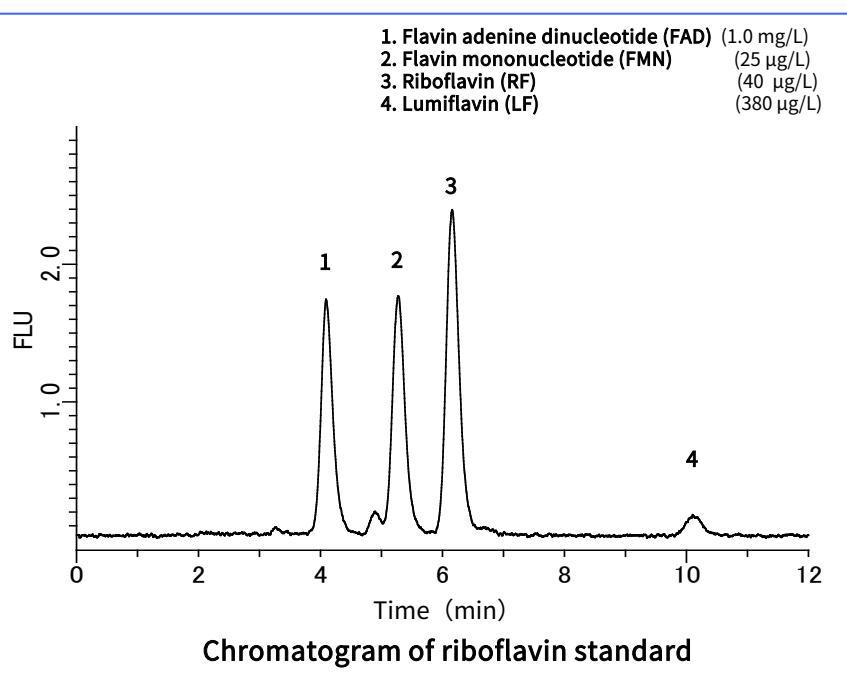
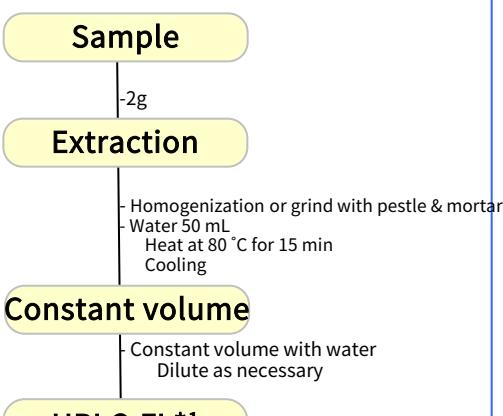


Fig. Calibration curves

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) CH ₃ OH B) 0.01M NaH ₂ PO ₄ (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)

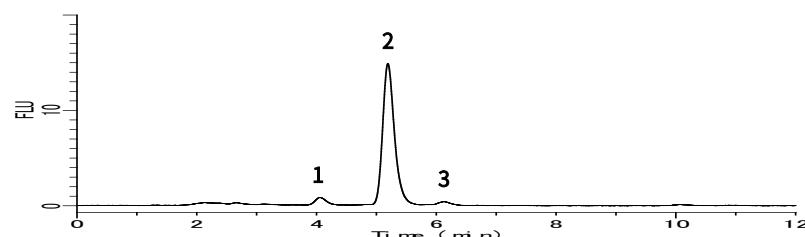
Example of pretreatment



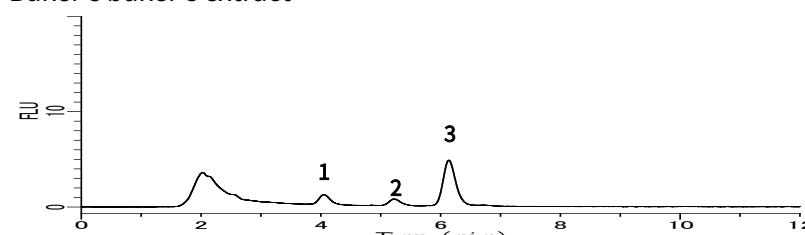
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Measurement example

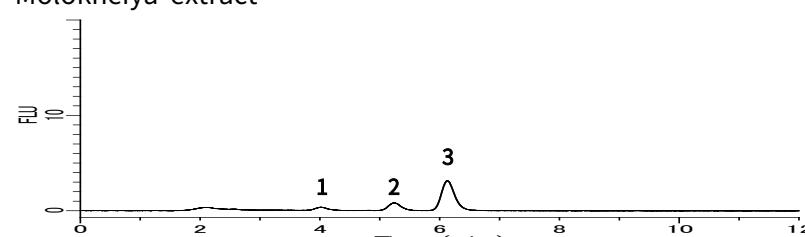
Almond extract



Baker's baker's extract



Molokheiya extract



1. FAD
2. FMN
3. RF

3. Analysis of riboflavin butyrate

Riboflavin tetrabutyrate 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 tetrabutyrate was analyzed according to the reference method in the Guidelines Food Hygiene Inspection.

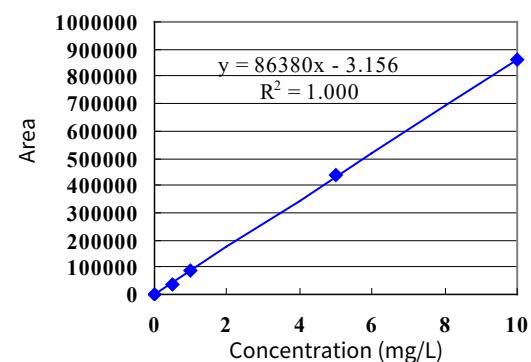
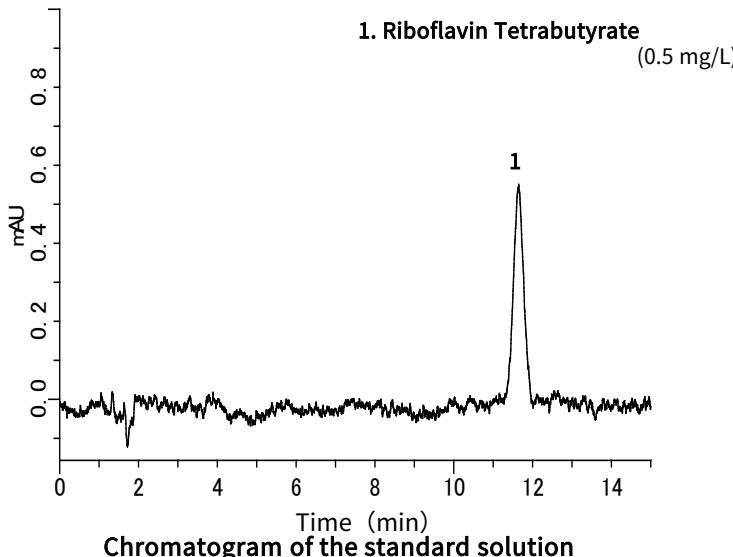


Fig. Riboflavin tetrabutyrate calibration curve

HPLC conditions

Column	:Inertsil ODS-3 (5 µm, 150 x 4.6 mm I.D.)
Temperature	:40 °C
Detector	:UV 445 nm
Injection volume	:20 µL
Eluent	:A) CH ₃ CN B) H ₂ O A/B = 3/2, v/v
Flow rate	:1.0 mL/min

Example of pretreatment

Sample

-2 g. in a 100 mL amber volumetric flask.

Extraction

- Add Ethanol 50 mL
Sonicate for 5 min

Constant volume

- Constant volume with ethanol

Filtration

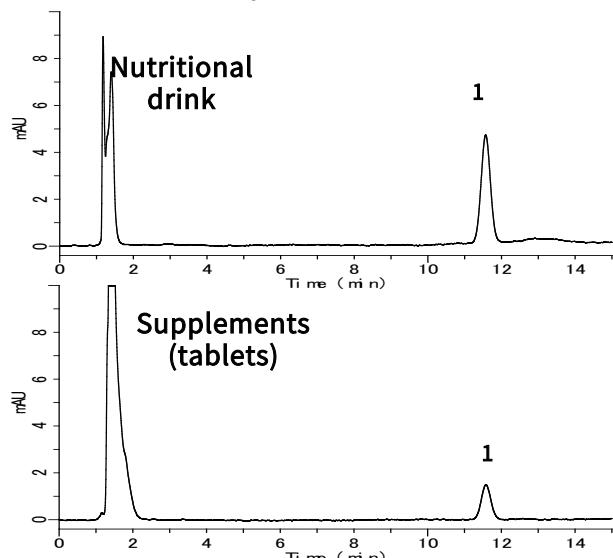
-0.45 µm chromatodisk.
Dilute as necessary

HPLC-UV *2

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

Measurement example

1. Riboflavin Tetrabutyrate



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