



**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB 5009.85-2016

**National food safety standard - Determination of vitamin B2 in
foods**

食品安全国家标准 食品中维生素B2的测定

(English Translation)

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Foreword

This standard replaces GB/T 5009.85-2003 "Determination of riboflavin in foods", GB/T 9695.28-2008 "Meat and meat products-Determination of vitamin B2 content", GB/T 7629-2008 "Determination of vitamin B2 in cereals" and GB 5413.12-2010 "National food safety standard-Determination of vitamin B2 in foods for infants and young children, milk and milk products".

As compared with GB/T 5009.85-2003, the main changes of this standard are as follows.

- The standard name is changed to "National food safety standard-Determination of vitamin B2 in foods";
- Add high performance liquid chromatography;
- Delete microbiological method.

National food safety standard -

Determination of vitamin B2 in foods

1 Scope

This standard specifies the determination of vitamin B2 in foods.

The first method of this standard is high performance liquid chromatography and the second method is fluorescence spectrophotometry, which apply to the determination of vitamin B2 in various type of foods.

Method I. High performance liquid chromatography

2 Principles

The sample is hydrolyzed in dilute hydrochloric acid at constant temperature, adjusted to pH 6.0-6.5; use papain and taka-amylase to carry out enzymolysis. After filtered through constant-volume, the filter liquor is separated through reversed phase column and detected by HPLC fluorescence detector. Use external standard method to quantify.

3 Reagents and materials

Unless otherwise indicated, the reagents used in this method are analytical pure, the water is the grade-1 water specified in GB/T 6682.

3.2.1 Hydrochloric acid solution (0.1 mol/L). PIPETTE 9 mL of hydrochloric acid; USE water to dilute it to 1000mL.

3.2.4 Sodium acetate solution (0.1 mol/L). Accurately WEIGH 13.60 g of sodium acetate trihydrate; ADD 900 mL of water to dissolve it; USE water to dilute it to

3.2.5 Sodium acetate solution (0.05 mol/L). Accurately WEIGH 6.80 g of sodium acetate trihydrate; ADD 900 mL of water to dissolve it; ADJUST the pH to 4.0 ~

3.2.6 Mixed enzyme solution. Accurately WEIGH 2.345 g of papain and 1.175g of taka-amylase; ADD water to dissolve it; DILUTE it to 50 mL. Prepare it before use.

3.3 Standards

Vitamin B2 (C₁₇H₂₀N₄O₆, CAS number. 83-88-5). Purity ≥ 98%.

3.4.1 Vitamin B2 standard stock solution (100 µg/mL). PLACE the vitamin B2

standard in a vacuum desiccator or dryer with phosphorus pentoxide; after drying for 24h, accurately WEIGH 10 mg (accurate to 0.1 mg) Vitamin B2 standard; ADD 2 mL of hydrochloric acid solution (1+1) to dissolve it in ultrasound;

3.4.2 Vitamin B2 standard intermediate solution (2.00 µg/mL). Accurately

PIPETTE 2.00 mL of Vitamin B2 standard stock solution; USE water to dilute it to 100 mL. Prepare before use.

4.1 High performance liquid chromatograph. equipped with fluorescence detector.

5.1 Sample preparation

TAKE out 500 g of sample; USE tissue-mash-machine to smash it uniformly; respectively PLACE it into clean brown grinding mouth bottle; MAKE a mark after sealing; STORE it for spare-use in dark place.

5.3 Drawing of standard curve

LEAD the standard series working solution into high performance liquid chromatograph respectively; MEASURE the corresponding peak area. With the concentration of standard working solution as abscissa AND the peak area as vertical axis, DRAW a standard curve.

5.4 Determination of sample solution

LEAD the sample solution into high performance liquid chromatograph to obtain the corresponding peak area; OBTAIN the vitamin B2 concentration of the solution under test according to the standard curve.

6 Analysis results expression

The Vitamin B2 content in the sample is calculated in accordance with the equation (1).

7 Precision

The absolute difference between two independent determinations obtained under repeatability conditions shall not exceed 10% of the arithmetic mean.

8 Others

When the sampling weight is 10.00 g, the method detection limit is 0.02 mg/100g and the quantitative limit is 0.05 mg/100g.

9 Principles

Exposed under the light at a wavelength of 440 nm ~ 500 nm, vitamin B2 sends out yellowish green fluorescence. In dilute solution, its fluorescence intensity is proportional to the concentration of vitamin B2. Measure its fluorescence intensity at a wavelength of 525 nm.

10 Reagents and materials

Unless otherwise indicated, the reagent used in this method is analytical pure, the water is the grade-1 water specified in GB/T 6682 regulations.

10.2.1 Hydrochloric acid solution (0.1 mol/L). PIPETTE 9 mL of hydrochloric acid; USE water to dilute it to 1000 mL.

10.2.5 Mixed enzyme solution. Accurately WEIGH 2.345 g of papain and 1.175 g of taka-amylase; ADD water to dissolve it; USE water to dilute it to 50 mL. Prepare it before use.

10.2.7 Potassium permanganate solution (30 g/L). Accurately WEIGH 3 g of potassium permanganate; USE water to dissolve it and dilute it to 100 mL.