GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5009.85-2016

National food safety standard - Determination of vitamin B_2 in foods

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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 B_2 content", GB/T 7629-2008" Determination of vitamin B_2 in cereals "and GB 5413.12-2010" National food safety standard-Determination of vitamin B_2 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 B_2 in foods

1 Scope

This standard specifies the determination of vitamin B_2 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 B_2 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.

3.1 Reagents

3.1.1 Hydrochloric acid (HCl).

3.1.2 Glacial acetic acid (CH_3COOH).

3.1.3 Sodium hydroxide (NaOH).

3.1.4 Sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$).

3.1.5 Methanol (CH_3OH): Chromatographically pure.

3.1.6 Papain: active unit ≥ 10 U/mg.

3.1.7 Taka-amylase: active units ≥ 100 U/mg, or similar performance.

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.

10.2.6 Eluent: acetone-glacial acetic acid-water (5+2+9, volume ratio).

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.

10.2.8 Hydrogen peroxide solution (3%): PIPETTE 10 mL of 30% hydrogen peroxide, USE water to dilute it to 100 mL.

10.2.9 Sodium dithionite solution (200 g/L): Accurately WEIGH 20 g of sodium dithionite, USE water to dissolve it and dilute it to 100 mL. This solution is prepared before use and stored in an ice-water bath, valid for 4h.

10.3 Standard products

Vitamin B_2 ($C_{17}H_{20}N_4O_6$, CAS number: 83-88-5): Purity≥98%.

10.4 Standard solution preparation

10.4.1 Vitamin B_2 standard stock solution (100 μg/mL): PLACE the vitamin B_2 standard in a vacuum desiccator or dryer with phosphorus pentoxide; after drying for 24h, accurately WEIGH 10 mg (accurate to 0.1 mg) Vitamin B_2 standard; ADD 2 mL of hydrochloric acid solution (1+1) to dissolve it in ultrasound; immediately USE water to transfer it and dilute it to 100 mL. After mixing it uniformly, TRANSFER it into a brown glass container; STORE it in a 4°C refrigerator with shelf life of 2 months. The standard stock solution requires concentration correction before use. The method of correction sees Appendix A.

10.4.2 Vitamin B_2 standard intermediate solution (10 μg/mL): Accurately PIPETTE 10.00 mL of Vitamin B_2 standard stock solution; USE water to dilute it to 100 mL. STORE it in a 4°C refrigerator with shelf life of 1 months.

10.4.3 Vitamin B_2 standard solution (1 μg/mL): Accurately PIPETTE 10 mL of vitamin B_2 standard intermediate solution; USE water to dilute it to 100 mL. This solution is equivalent to 1.00 μg of vitamin B_2 per ml. Store it in a 4°C refrigerator with shelf life of 1 week.

11 Instrument and equipment

11.1 Fluorescence Spectrophotometer.

11.2 Balance: sensitivities are 1 mg and 0.01 mg.

According to riboflavin content in test sample TAKE out a certain volume of sample extract (containing about 1 μg ~ 10 μg of vitamin B_2) and vitamin B_2 standard solution; PLACE it respectively into 20 mL graduated test tube with cover; ADD water to 15 mL. ADD 0.5 mL of glacial acetic acid in each tube; MIX it uniformly. ADD 0.5 mL of 30 g/L potassium permanganate solution; after shaking it up, PLACE it for 2 min to get rid of impurities through oxidation. ADD dropwise 3% hydrogen peroxide solution until the color of potassium permanganate fades away. SHAKE out the tube vigorously to allow excess oxygen to escape.

12.3 Adsorption and elution of vitamin B_2

12.3.1 Vitamin B_2 adsorption column

Use wet method to fill approximately 1 g of silica-magnesium adsorbent into the column, accounting for 1/2 ~ 2/3 of the column length (about 5 cm) (use a small group of absorbent cotton pads to absorb the lower end of the adsorption column). Do not generate bubbles in the column; adjust flow rate to about 60 drops/min.

Note: USE equivalent commercial column.

12.3.2 Column and elution

After all the oxidized sample solution and standard solution pass through the adsorption column, USE about 20 mL of hot water to rinse the impurities in sample solution. USE 5 mL of eluant to elute vitamin B_2 of the sample into 10mL volumetric flask; then USE 3 mL ~ 4 mL of water to wash adsorption column; the eluant is combined into the volumetric flask; USE water to dilute it to the mark; WAIT for determination after mixing it uniformly.

12.4 Preparation of standard curve

Respectively, accurately pipette the standard solution of vitamin B_2 0.3 mL, 0.6 mL, 0.9 mL, 1.25 mL, 2.5 mL, 5.0 mL, 10.0 mL, 20.0 mL (equivalent to 0.3 μg, 0.6 μg, 0.9 μg, 1.25 μg, 2.5 μg, 5.0 μg, 10.0 μg, 20.0 μg Vitamin B_2) or pipette the solution that the single point standard is similar to the sample content according to the operation in 12.2 and 12.3.

12.5 Determination of sample solution

When excitation wavelength is 440 nm and emission wavelength is 525 nm, measure the fluorescence value of sample tube and standard tube. After measuring the fluorescence value of sample tube and standard tube, add 0.1 mL of 20% sodium dithionite solution in the remaining liquid of (about 5 mL ~ 7 mL) each tube and immediately mix it uniformly to measure the fluorescence value of each tube within 20s for their own blank value.

Appendix A

Concentration calibration method of vitamin B_2 standard solution

A.1 Preparation of standard calibration solution

Accurately PIPETTE 1.00 mL of vitamin B_2 standard stock solution; ADD 1.30 mL of 0.1 mol/L of sodium acetate solution; USE water to dilute it to 10 mL as standard test solution.

A.2 Preparation of control solution

Accurately PIPETTE 1.00 mL of 0.012 mol/L hydrochloric acid solution; ADD 1.30mL of 0.1mol/L of sodium acetate solution; USE water to dilute it to 10 mL as a control solution.

A.3 Determination of absorption value

PLACE 1cm cuvette at a wavelength of 444 nm; REGARD the control solution as blank control; DETERMINE absorbance value of standard calibration solution.

A.4 Concentration calculation of standard solution

The mass concentration of standard stock solution is calculated in accordance with the equation (A.1):

$$\rho = \frac{A_{444} \times 10^4 \times 10}{328} \qquad \cdots\cdots (A.1)$$

Where:

ρ - The mass concentration of standard stock solution, in micrograms per milliliter (μg/mL);

A_{444} - Absorbance value of standard test solution at a wavelength of 444 nm;

10^4 - Conversion factor that converts the concentration unit of 1% standard solution into the concentration unit of test solution (μg/mL);

10 - Dilution factor of standard stock solution;

328 - Percentile absorption coefficient $E^{1\%}_{1cm}$ for vitamin B_2 at a wavelength of 444 nm; that is, at a wavelength of 444 nm, with 1cm thickness of liquid

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