National standard for Food Safety of the P. R. China

GB 5413.12-2010

National food safety standard

Determination of vitamin B₂ in foods for infants and young children, milk and milk products

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Issued by Ministry of Health, the People’s Republic of China
Foreword

This present National Standard replaces GB/T 5413.12—1997, Milk powder and formula foods for infant and young children — Determination of Vitamin B<sub>2</sub> content.

Compared with GB/T 5413.12—1997, the following modifications have been carried out in this present National Standard:

— The name of standard has been modified as Determination of Vitamin B<sub>2</sub> in foods for infants and young children, raw milk and dairy products

— The first method in the original edition, the fluorescence spectrophotometry, has been cancelled.

— The structure of original edition has been modified.

— In the mixed enzyme solution, acid phosphatase is supplemented, in order to add riboflavin phosphate as the Vitamin B<sub>2</sub> fortifier for sample determination.

— A notice of avoiding direct irradiation of strong light is supplemented.

— Multiple points standard curve method is used for the external standard quantification.

— In the calculation, it is specified that the content of Vitamin B<sub>2</sub> in sample should be measured based on riboflavin.

— The liquid chromatograms of standard solution of Annex A have been supplemented.

Annex A of this present National Standard is informative annex.

The original editions replaced by this present National Standard include:

National food safety standard

Determination of vitamin B<sub>2</sub> in foods for infants and young children, milk and milk products

1. Scope

This present National Standard specifies the method for determination of Vitamin B<sub>2</sub> in foods for infants and young children, milk and milk products.

This present National Standard is applicable to the determination of Vitamin B<sub>2</sub> in foods for infants and young children, milk and milk products.

2. Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this present standard. As for the dated references, all the amendments or revisions after them except the corrigenda are not applicable to this present standard. As for the references that are not dated, their most recent editions are applicable to this present national standard.

3. Principle

After pyrohydrolysis in diluted hydrochloric acid and enzymolysis, sample solution is separated through C<sub>18</sub> reversed phase chromatographic column. Then measure it with fluorescence detector (E<sub>x</sub>: 462 nm; E<sub>m</sub>: 522 nm), and the content of Vitamin B<sub>2</sub> can be determined with external standard method.

4. Reagents and Materials

Unless otherwise specified, purity of all reagents used in this present method is analytically pure, and that of water used in the test is the first-graded specified in GB/T 6682.

4.1 Hydrochloric acid.

4.2 Sodium acetate trihydrate

4.3 Glacial acetic acid.

4.4 Methanol: Chromatographically pure.

4.5 Standard substance of Vitamin B<sub>2</sub> (Riboflavin): Purity ≥ 99%.

4.6 hydrochloric acid solution(1+1): Pipette 100mL of hydrochloric acid (4.1), and slowly put in 100mL of water, mix them evenly.

4.7 0.1 mol/L hydrochloric acid solution: Dissolve 9 mL of the condensed hydrochloric acid (4.1) with 1000 mL of water.

4.8 Hydrochloric acid solution (0.01 mol/L): Draw 50 mL of the 0.1 mol/L hydrochloric acid solution (4.7), and dilute to 500 mL with water.

4.9 Sodium acetate solution (0.05 mol/L): Weigh 6.80g of sodium acetate trihydrate (4.2), add 900 mL of water to dissolve it, and adjust the pH value to 4.0 ~ 5.0 with glacial acetic acid; dilute to 1000 mL. Filter it through a 0.45 μm microporous membrane.

4.10 sodium acetate solution (2.0 mol/L): Weigh 27.22 g of sodium acetate trihydrate (4.2),
dissolve it with water and dilute to 100 mL.

4.11 Mixed enzyme solution: Weigh 2.345 g of papain (activity unit ≥ 600 U/g), 1.175 g of amylase (activity unit ≥ 4000 U/g), and 1.000 g of acid phosphatase (activity unit ≥ 4000 U/g); dilute with water to 50 mL. Prepare before use.

4.12 Standard solution

4.12.1 Vitamin B₉ standard stock solution(250μg/mL):
Weigh 25 mg of standard substance of Vitamin B₉ (4.5) accurately (accuracy to 0.1mg). Add 2 mL of the hydrochloric acid (4.6); after ultrasonic dissolution, transfer it with water immediately, and dilute to 100 mL. Store it in brown glass container at 0°C - 4°C in refrigerator, and the storage period should not be over 3 months.

4.12.2 Vitamin B₉ standard intermediate solution.
Pipet 4.00 mL of the standard stock solution (4.12.1) accurately, and dilute it with water to 100 mL. The concentration of Vitamin B₉ in this solution is 10 μg/mL. Prepare before use.

4.12.3 Vitamin B₉ standard working solution: Pipet 0.00, 0.50, 1.00, 2.00, 5.00, and 10.00 mL of the standard intermediate solution of Vitamin B₉ (4.12.2) respectively, and dilute them to 100 mL with water separately. The concentrations of this series of standard working solutions of Vitamin B₉ are 0.00, 0.05, 0.10, 0.20, 0.50, and 1.00 μg/mL respectively. Prepare before use.

5. Apparatus

5.1 High performance liquid chromatograph: with fluorescence detector.

5.2 Autoclave.

5.3 pH meter: Precision 0.01

5.4 Tissue grinder.

5.5 0.45 μm micropore aqueous phase filter membrane.

5.6 Balance: sensitivity of 1 mg and 0.1 mg.

6. Analytical steps

6.1 Pretreatment of sample
Weigh 5 ~ 10 grams of sample (in which Vitamin B₉ content is over 5 μg; when necessary, the sample can be ground in the grinder) accurately (accuracy of 0.01g) in a 100 mL conical flask, and then add 60 mL of the 0.1 mol/L hydrochloric acid solution (4.7). Shake to mix well. Seal it with cotton lid and kraft paper, and then transfer it into an autoclave; keep it at 121°C for 30 minutes. Take it out after it gets to below 40°C. Shake slightly for a few times. Adjust the pH value to about 4.0 with the 2.0 mol/L sodium acetate solution (4.10), add 2.0 ml of the mixed enzyme solution (4.11). Shake it to mix well. Keep it in a incubator at 37°C overnight. Transfer the enzymolysis solution to a 100 mL volumetric flask, and dilute to volume with water. Filter it through quantitative filter paper first, and then 0.45 μm membrane (5.5); store the filtrate before use.
Note: During the operation, irradiation of strong light should be avoided.

6.2 Determination

6.2.1 Reference condition for chromatography

Chromatographic column: C₁₈ reversed phase chromatographic column (dp 5μm, 250 mm×4.6 mm) or other columns with the same performance.

Mobile phase: 0.05 mol/L sodium acetate solution (4.9) — methanol (4.4) = 65+35

Flow rate: 1.0 mL/min.


Injection volume: 20μL.

6.2.2 Drawing of standard curve.

Determine the series of standard working solutions of Vitamin B₂ (4.12.3) under the condition recommended above (see the fig.A.1 of Annex A of standard sample chromatogram maps), record the peak area. Draw the standard curve, in which y-axis is the peak area, and x-axis is the concentration.

6.2.3 Determination of test sample solution

Determine the derivatives of test sample solution under the condition recommended above, and consult the standard curve for corresponding concentration.

6.2.4 Blank test

Operate as the steps mentioned above, except that the test sample is not added in.

7. Expression of results

The content of vitamin B₂ in the test sample should be calculated according to formula (1):

\[ X = \frac{c \times V \times 100}{m \times 1000} \] .......................... (1)

in which:

\( X \) is the content of Vitamin B₂ in test sample (as riboflavin), mg/100g;

\( c \) is concentration of injected sample solution, μg/mL;

\( V \) is the constant volume of the pretreated sample solution, mL;

\( m \) is mass of sample, g.

The calculation result should be expressed as the arithmetic mean of two individual determinations under repeated condition, and should be accurate to three decimal places.

8. Precision

The absolute difference of results of two individual determinations under repeated condition should not be over 10 % of the arithmetic mean.

9. Others

The quantitative limit under this standard: 0.05 mg/100 g when the sample size is 10.00 g.
Annex A

(Informative annex)

Liquid chromatogram of standard vitamin B$_2$ solution

A.1 Liquid chromatogram of standard vitamin B$_2$ solution

Liquid chromatogram of vitamin B$_2$ standard solution sees Fig.A.1.

Figure A.1 Liquid chromatogram of vitamin B$_2$ standard solution