



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

中华人民共和国国家标准

GB 5413.12-2010

National food safety standard

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

食品安全国家标准

婴幼儿食品和乳品中维生素 B₂ 的测定

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Issued by the Ministry of Health of P.R 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 B₂ 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 B₂ 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 B₂ 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 B₂ 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:

- GB 5413 -1985 and GB/T 5413.12 -1997.

National food safety standard

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

1. Scope

This present National Standard specifies the method for determination of Vitamin B₂ in foods for infants and young children, milk and milk products.

This present National Standard is applicable to the determination of Vitamin B₂ 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 C₁₈ reversed phase chromatographic column. Then measure it with fluorescence detector (E_x: 462 nm; E_m: 522 nm), and the content of Vitamin B₂ 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 B₂ (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),



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