GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5413.12-2010

National Food Safety Standard

Determination of Vitamin B_2 in Foods for Infants and Young Children, Milk and Milk Products

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Foreword

This Standard replaces GB/T 5413.12-1997 *Milk Powder and Formula Foods for Infant and Young Children - Determination of Vitamin B_2 Content.*

Compared with GB/T 5413.12-1997, this Standard mainly has the following changes:

--- Modify the name of Standard into *Determination of Vitamin B_2 in Foods for Infants and Young Children, Milk and Milk Products*.

--- Delete the method- fluorescence spectrophotometry.

--- Modify the structure of original standard.

--- In the mixed enzyme solution, ADD acid phosphatase, so as to resolve the sample determination that riboflavin phosphate is added as the enhancer of Vitamin B_2.

--- In the sample treatment method, ADD the notice of “Avoid direct irradiation of strong light”.

--- External standard quantification adopts multiple points standard curve method.

--- In the result calculation, CLARIFY that the content of Vitamin B_2 in sample is measured based on riboflavin.

--- ADD Appendix A – Liquid Chromatograms of Standard Solution.

This Standard’s Appendix A is informative.

The historical editions replaced by this Standard are as follows:

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

National Food Safety Standard

Determination of Vitamin B_2 in Foods for Infants and Young Children, Milk and Milk Products

1 Scope

This Standard specifies the method for determination of Vitamin B_2 in foods for infants and young children, milk and milk products.

This Standard is applicable to the determination of Vitamin B_2 in foods for infants and young children, milk and milk products.

2 Normative References

The following documents are essential to the application of this document. For the dated documents, only the versions with the dates indicated are applicable to this document; for the undated documents, only the latest version (including all the amendments) are applicable to this standard.

3 Principle

In hydrochloric acid, sample is thermostat-hydrolysis and enzymolysis; then it is separated through C18 reversed phase chromatographic column. Use fluorescence detector (Ex: 462 nm; Em: 522 nm) to detect, and quantify with external standard method.

4 Reagents and Materials

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

4.1 Hydrochloric acid.

4.2 Sodium acetate trihydrate.

4.3 Glacial acetic acid.

5.2 Autoclave.

5.3 pH meter: Precision is 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 Treatment of sample

Weigh 5~10 g of sample (accurate to 0.01 g) (if necessary, the sample can be ground in the grinder; the sample contains more than 5μg of Vitamin B_2) in a 100 mL conical flask; then add 60 mL of 0.1 mol/L hydrochloric acid solution (4.7). Shake to mix well. Seal it with cotton stopper and Kraft paper; then transfer it into an autoclave; keep it at 121°C for 30 minutes. Take it out after it is cooled to below 40°C. Shake slightly for a few times. Use 2.0 mol/L sodium acetate solution (4.10) to adjust the pH value to about 4.0; add 2.0 ml of mixed enzyme solution (4.11). Shake it to mix well. Keep it in an incubator at 37°C for overnight. Transfer the enzymolysis solution to a 100 mL volumetric flask; use water to fix the volume to mark. Filter it through quantitative filter paper; take the filtrate then filter through 0.45μm membrane (5.5); store the filtrate for later use.

Note: During the operation, irradiation of strong light shall be avoided.

6.2 Determination

6.2.1 Reference chromatography conditions

Chromatographic column: C18 reversed phase chromatographic column (particle diameter 5μm, 250mm×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.

Detection wavelength: excitation wavelength: 462 nm; emission wavelength: 522 nm.

Injection volume: 20μL.

6.2.2 Drawing of standard curve

Sequentially conduct the chromatography determination to the series of standard working solutions of Vitamin B_2 (4.12.3) (see Figure A.1 of Appendix A for the standard

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