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

GB 5413.11-2010

National food safety standard

Determination of vitamin B_1 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 5413.11-1997, *Milk powder and formula foods for infant and young children — Determination of Vitamin B_1 content.*

Compared with GB 5413.11-1997, the following modifications have been carried out in this present National Standard:

— The name of standard has been modified as *Determination of Vitamin B_1 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.

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

— In the calculation, it is specified that the content of Vitamin B_1 in sample should be measured based on thiamine.

— Liquid chromatograms of standard substance and test sample in Annex A have been supplemented.

Annex A of this present National Standard is informative annex.

This present National Standard was proposed by and is under the jurisdiction of Ministry of Health, the People's Republic of China.

The original editions replaced by this present National Standard include:

— GB/T 5413 -1985 and GB/T 5413.11 - 1997.

National food safety standard
Determination of vitamin B_1 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_1 in foods for infants and young children, milk ad milk products.

This present National Standard is applicable to the determination of Vitamin B_1 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 derived with basic potassium ferricyanide. After extraction by normal butyl alcohol or isobutyl alcohol, it is separated through C_{18} reversed phase chromatographic column. Then measure it with fluorescence detector (E_x：375 nm, E_m：435 nm), and the content of Vitamin B_1 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 Normal butyl alcohol or isobutyl alcohol.

4.2 Potassium ferricyanide.

4.3 Sodium hydroxide.

4.4 Condensed hydrochloric acid.

4.5 Sodium acetate trihydrate.

4.6 Glacial acetic acid.

4.7 Methanol: chromatographically pure.

4.8 Vitamin B_1 standard substance: thiamine hydrochloride or thiamine nitrate, Purity ≥ 99%.

4.9 Potassium ferricyanide solution（20 g/L）: Weigh 2 grams of potassium ferricyanide (4.2), and dissolve and dilute with water to 100 mL. Prepare before use.

4.10 100 g/L Sodium hydroxide solution: Weigh 25 grams of sodium hydroxide (4.3), and dissolve and dilute with water to 250 ml.

4.11 Basic potassium ferricyanide solution: Mix 5 mL of the potassium ferricyanide solution (4.9)

with 200 ml of the sodium hydroxide solution (4.10). Prepare before use.

4.12 hydrochloric acid solution（0.1 mol/L）: Dissolve 9 mL of the condensed hydrochloric acid (4.4) with 1000 ml of water.

4.13 hydrochloric acid solution（0.01 mol/L）: Draw 50 mL of the 0.1 mol/L hydrochloric acid solution (4.12), and dilute to 500 mL with water.

4.14 sodium acetate solution（0.05 mol/L）: Weigh 6.80 g of the sodium acetate trihydrate (4.5), add 900 mL of water to dissolve it, and adjust the pH value to 4.0 ~ 5.0 with glacial acetic acid (4.6); dilute to 1000 mL. Filter it through a 0.45 μm microporous membrane.

4.15 sodium acetate solution（2.0 mol/L）: Weigh 27.22 grams of sodium acetate trihydrate (4.5), dissolve it with water and dilute to 100 mL.

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

4.17 Standard solutions

4.17.1 Vitamin B_1 standard stock solution（500 μg/mL）:

Weigh 50mg of standard substance (accuracy of 0.1mg) of Vitamin B_1 (4.8) accurately. Dissolve it with the 0.01 mol/L hydrochloric acid (4.13), and dilute to 100 mL. Store it in 0 ℃～4 ℃refrigerator, and the storage period should be 3 months.

4.17.2 Vitamin B_1 standard intermediate solution.

Pipette 2.00 mL of the standard stock solution (4.17.1) accurately, and dilute it with water to 100 mL. The concentration of Vitamin B_1 in this solution is 10 μg/mL. Prepare before use.

4.17.3 Vitamin B_1 standard working solution

Pipet 0, 0.50, 1.00, 2.00, 5.00, and 10.00 mL of the standard intermediate solution of Vitamin B_1 (4.17.2) respectively, and dilute them to 100 mL with water separately. The concentrations of this series of standard working solutions of Vitamin B_1 are 0, 0.05, 0.10, 0.20, 0.5, 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 Centrifuge: rotation speed ≥ 4000 rpm.

5.4 pH meter: with accuracy of 0.01.

5.5 Tissue grinder(adjustable within the speed rate of 0-12000 r/min)

5.6 0.45 μm micropore organic filter membrane.

5.7 Balance: with the sensitivity of 0.001g and 0.0001g.

6. Analytical steps

6.1 Pretreatment of sample

6.1.1 Extraction of test sample solution:

Weigh 5~ 10 grams（accuracy of 0.01g） of sample (in which Vitamin B_1 content is over 5μg; when necessary, the sample can be ground in the grinder) accurately in a 100 mL conical flask, and then add 60 mL of the 0.1 mol/L hydrochloric acid solution (4.12). Shake to mix well. Seal it with cotton lid and kraft paper, and then transfer it into an autoclave; keep it at 121□ for 30 minutes. Take it out after it gets to below 40 □. Shake slightly for a few times. Adjust the pH value to about 4.0 with the 2.0 mol/L sodium acetate solution (4.15), add 2.0 mL of the mixed enzyme solution (4.16) (of which the amount can be adjusted to some extent according to enzyme activities). Shake it to mix well. Keep it in a incubator at 37□ overnight. Transfer the enzymolysis solution to a 100 mL volumetric flask, and dilute to volume with water. Filter it through quantitative filter paper, and store the filtrate before use.

6.1.2 Derivation of test sample solution:

Pipette 10.00 ml of the filtrate mentioned above to a 25 mL cuvette with lid. Add 5 ml of the basic potassium ferricyanide solution (4.11). Mix well. Add 10.00 mL of normal butyl alcohol or isobutyl alcohol (4.1); shake vigorously and place still for about 10min. Let stand for mixing. Draw the normal butyl alcohol or isobutyl alcohol phase (top layer), centrifuge at 4000 ~ 6000 rpm for 5 minutes, and take the supernatant for injection through an organic micropore filter membrane (5.6).

Take 10.00 mL of the standard working solution (4.17.3), and carry out derivation with the test sample solution simultaneously.

Note1: at ambient temperature, the derivative product is stable within 4 hours.

Note2: the operating process of 6.1.1 and 6.1.2 should be carried out under environments avoiding of bright light.

6.2 Determination

6.2.1 Reference condition for chromatography

Chromatographic column: C_{18} reversed phase chromatographic column (dp 5μm, 250 mm×4.6 mm) or other columns with equivalent performance.

Mobile phase: 0.05 mol/L sodium acetate solution (4.14)-methanol (4.7) = 65+35.

Flow rate: 1.00 mL/min.

Detection wavelength: excitation wavelength: 375 nm; emission wavelength: 435 nm.

Injection volume: 20μL.

6.2.2 Drawing of standard curve.

Determine the derivatives of serial standard working solutions of Vitamin B_1 (4.17.3) under the condition recommended above, record the peak area, the chromatographic maps see Annex A. 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 chromatographic conditions, and consult the standard curve for corresponding concentration.

7. Expression of result

The content of vitamin B_1 in the test sample should be calculated according to formula (1):

$$X = \frac{c \times V \times 100}{m \times 1000} \quad \text{.....(1)}$$

in which:

X is the content of Vitamin B_1 in test sample (as thiamine), 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

When the sample size is 10.00 g, the quantitative limit under this standard is 0.05 mg/100g.

Annex A

(Informative annex)

Liquid chromatogram of standard vitamin B_1 solution

A.1 Liquid chromatogram of standard vitamin B_1 solution

Liquid chromatogram of vitamin B_1 standard solution sees Fig.A.1.

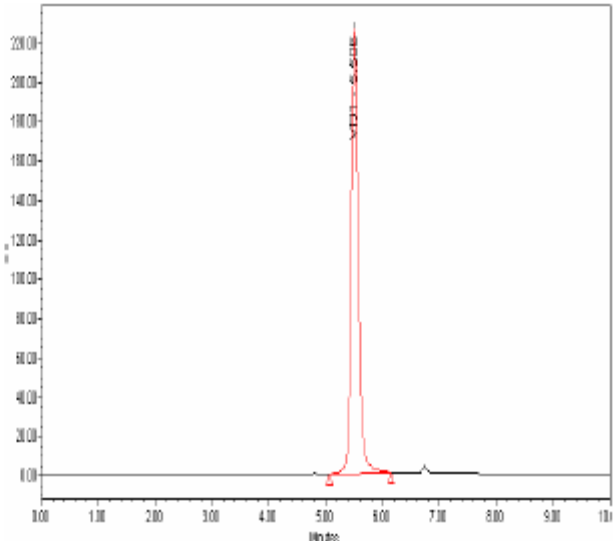

Figure A.1 Liquid chromatogram of vitamin B_1 standard solution