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

GB/T 5413.13-1997

Replacing GB 5413.13-1997

**National food safety standard -- Determination of vitamin B6 in
foods for infants and young children, milk and milk products
(English Translation)**

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Foreword

This standard replaces GB/T 5413.13-1997 "Determination of infant formula and milk powder vitamin B6".

This standard compared with GB/T 5413.13-1997, the main changes are as follows.

--- standard name to "infant food and dairy vitamin B6 determination";

--- improve the calculation of vitamin B6 content.

Appendix A to this standard is an informative appendix.

This standard replaced the previous version of the standard release.

--- GB 5413-1985, GB/T 5413.13-1997.

National standards for food safety Determination of Vitamin B6 in Infant Food and Dairy

1 Scope

This standard specifies the determination of vitamin B6 in infant food and dairy products.

This standard applies to the determination of vitamin B6 in infant food and dairy products.

2 Normative reference documents

The documents cited in this standard are indispensable for the application of this standard. Any reference to the date of the document, only the date of the edition this applies to this standard. For undated references, the latest edition (including all amendments) applies to this standard.

3 Principle

After the sample was hot water extraction and other pretreatment, the C18 column separation, fluorescence detector detection, external standard quantitative determination of vitamin B6 (pyridox Alcohol, pyridoxal, pyridoxamine).

4 Reagents and materials

Unless otherwise specified, the reagents used in this method are of analytical grade and water is a primary water specified in GB/T 6682.

4.1

Amylase. Enzyme activity $\geq 1.5\text{U/mg}$.

4.2

sodium octane sulfonate. excellent grade pure.

4.3

glacial acetic acid. excellent grade pure.

4.4

Methanol. Chromatography Pure.

4.5

Triethylamine. Chromatographic pure.

4.6

hydrochloric acid solution (5.0mol/L, 0.1mol/L).

4.7

sodium hydroxide solution (5.0 mol/L, 0.1 mol/L).

4.8

standard solution.

4.8.1

Vitamin B6 (pyridoxine, pyridoxal, pyridoxamine) standard stock solution (1mg/mL). Weigh 0.05g (accurate to 0.0001g) Pyridoxal,, pyridoxamine standards, were placed in 50mL volumetric flask, dissolved with water to determine the volume.

4.8.2

Vitamin B6 (pyridoxine, pyridoxal, pyridoxamine) mixed standard intermediate (20µg/mL). respectively, accurate absorption of pyridoxine, pyridox Aldehyde, pyridoxamine standard stock solution (4.8.1) 1mL to 50mL volumetric flask, with water volume.

4.8.3

Vitamin B6 (pyridoxine, pyridoxal, pyridoxamine) mixed standard series of liquid. respectively, accurate absorption of vitamin B6 mixed standard (4.8.2) in 0.0 mL, 1.0 mL, 2.0 mL, 3.0 mL, 5.0 mL to 100 mL volumetric flask and fixed in water. The standard series Concentrations were 0.00 µg/mL, 0.20 µg/mL, 0.40 µg/mL, 0.60 µg/mL, 1.00 µg/mL, respectively. Pro preparation before use.

5 Instruments and equipment

5.1

Ultrasonic Oscillator.

5.2

High Performance Liquid Chromatograph with Fluorescence Detector.

5.3

balance. sense of 0.1mg.

5.4

pH meter. accuracy of 0.01.

5.5

incubator. 30 °C ~ 80 °C.

6.1 Sample pretreatment

6.1.1

Starch-containing sample. Weigh about 5.0 g (accurate to 0.0001 g) of mixed homogeneous solid sample, add about 25 mL of 45 ° C ~ 50 ° C or about 20.0 g (exactly to 0.0001 g) in a mixed homogeneous liquid sample in a 150 mL Erlenmeyer flask. Add about 0.5g Amylase

(4.1), mix well and then fill the conical flask with nitrogen, cover the stopper, set 50 °C ~ 60 °C incubator about 30min. Remove and cool to Room temperature.

6.1.2

Sample without starch. Weigh about 5.0 g (accurate to 0.0001 g) of mixed homogeneous solid sample, add about 25 mL of 45 ° C ~ 50 ° C or about 20.0 g (exactly to 0.0001 g) in a mixed homogeneous liquid sample in a 150 mL Erlenmeyer flask. Mix and full Dissolved, standing for 5min ~ 10min, cooling to room temperature.

6.2 Preparation of test solution

6.2.1

Adjust the pH of the sample solution to 1.7 ± 0.1 with hydrochloric acid solution (4.6) for about 1 min. And then with sodium hydroxide solution (4.7) Adjust the pH of the sample solution to 4.5 ± 0.1 .

6.2.2

Transfer the sample solution to a 50 mL volumetric flask and rinse the conical flask with water. Wash the lotion in a 50 mL volumetric flask and set the volume with water to 50mL.

6.2.3

The above-mentioned volumetric flask into the ultrasonic oscillator, ultrasonic oscillation about 10min.

6.2.4

Another 50mL Erjiao bottle, the top into the triangle funnel and filter paper, the ultrasonic oscillation of the sample solution into which the natural filter.

The filtrate was further filtered through a 0.45 µm microporous membrane and collected with a test tube.

6.3 Reference chromatographic conditions

Column. C18 column (particle size 5µm, 150mm x 4.6mm) or equivalent column.

Mobile phase. methanol (4.4) 50mL, octane sulfonate (4.2) 2.0g, triethylamine (4.5) 2.5mL, dissolved in water and fixed to After 1000 mL, the pH was adjusted to 3.0 ± 0.1 with glacial acetic acid (4.3), and then filtered through a 0.45 µm microporous membrane.

Flow rate. 1.0 mL/min.

Detection wavelength. excitation wavelength 293nm, emission wavelength 395nm.

Injection volume. 10 µL.