



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

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National food safety standard
**Determination of vitamin B₆ in foods for infants and
young children, milk and milk products**

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Determination of vitamin 6 in foods for infants and young children, milk and milk products

1. Range:

The standard specifies a method for the detection of vitamin 6 in foods for infants and young children, milk and milk products.

.The standard applies a method for the detection of vitamin 6 in foods for infants and young children, milk and milk products

2. Referenced normative documents

References application is necessary to this standard. All indication date of references for only indication date version, for undated references, the latest version (include all modification) is also applies.

3. Principle

After extracted with hot water, etc. sample is separated by C-18 chromatogram column and determinate with fluorescence detector and measured Vitamin B6 (pyridoxine, pyridoxal, and pyridoxamine) content by external standard method.

4. Reagent and material

All reagent, if no special specification, refers to analytic reagent; All experiment water, refers to 2level water.

4.1 Amylase: enzymatic activity ≥ 1.5 U/mg

4.2 Octane sulfonate: GR

4.3 Glacial acetic acid: GR

4.4 methanol: chromatography

4.5 Triethylamine: chromatography

4.6 HCL solution: Conc. 5.0mol/L,0.1mol/L

4.7 NaOH solution: Conc. 5.0mol/L,0.1mol/L。

4.8 Standard Solution

4.8.1 Vitamin B6 (pyridoxine, pyridoxal, pyridoxamine) stock standard solution (1mg/ml) :

Each weigh about 0.05g (Accurately to 0.0001g) pyridoxine, pyridoxal, pyridoxamine standard substance to 50mL volumetric flask, dissolved with water, and add water to the mark.

Accurately weigh about 0.05g (accurate to 0.0001g) pyridoxine, pyridoxal, pyridoxamine standard substance to each 50mL volumetric flask, dissolved with water, and add water to the mark.

4.8.2 Vitamin B6 (pyridoxine, pyridoxal, pyridoxamine) mixing middle standard solution (20 μ g/ml): each pipette 1ml pyridoxine, pyridoxal, pyridoxamine stock standard solution(4.8.1) to 50ml volumetric flask, and add water to the mark.

4.8.3 Vitamin B6 (pyridoxine, pyridoxal, and pyridoxamine) mixing standard test solution: each pipette 4.8.2 Vitamin B6 mixing middle standard solution (4.8.2) 0.0ml, 1.0ml, 2.0ml, 3.0ml, 5.0ml, to 100ml volumetric flask, adds water to the mark. A serie of standard Conc: each 0.00 μ g/ml,0.2 μ g/ml,0.40 μ g/ml,0.60 μ g/ml,1.00 μ g/ml. Fresh preparation.

5. Instrument and Equipment

5.1 Ultrasound oscillator

5.2 HPLC chromatography: equipped with fluorescence detector

5.3 Analytical balance: accurate to 0.1mg

5.4 PH meter

5.5 oven 30°C~80°C

6. Analysis procedure

6.1 Sample Pretreatment

6.1.1 amyllum sample

weigh about 5.0g mixing solid samples (accurate to 0.0001g) , add about 25ml 45°C~50f°C water, or weigh about 20.0g mixing liquid samples (accurate to 0.0001g), to a 150mL conical flask, add about 0.5g α-amylase, then fill nitrogen to the conical flask and seal it, placed it into 50°C~60°C oven for 30min, then cool it to room temperature.

6.1.2 No amyllum sample

weigh about 5.0g mixing solid samples (accurate to 0.0001g) , add about 25ml 45°C~50f°C water, or weigh about 20.0g mixing liquid samples (accurate to 0.0001g), to a 150mL conical flask, mixing and dissolved thoroughly, stand 5 min~10min, then cool it to room temperature.

6.2 Sample solution preparation

6.2.1 Slowly adjust PH of it to 1.7 ± 0.1 with hydrochloric acid solution (4.6), let it stand for 1min, then adjust pH of it to 4.5 ± 0.1 with sodium hydroxide (4.7)

6.2.2 Transfer the sample solution into a 50mL volumetric flask; wash the conical flask with distilled water repeatedly and combine the washing liquid to the 50mL volumetric flask then add water to the mark.

6.2.3 Place above-mentioned conical flask to ultrasonator (5.1) for shaking 10min

6.2.4 To take the triangle funnel with filter paper, above another 50ml flask, pour sample solution into filter paper, filter the solution through filter paper. Again filter the solution through 0.45μm membrane filter and collect the filtrate as injection sample.

6.3 Reference Chromatography Parameter

Column: C18 150*4.6mm, 5um, or or equivalent

Mobile phase: 50ml methanol (4.4), 2.0g Octane sulfonate (4.2), 2.5ml Triethylamine, add water dissolved to mark 1000m,l adjust pH of the mobile phase to 3.0 ± 0.1 with Glacial acetic acid (4.3) and filter it through 0.45μm membrane filter.

Flowrate: 1.00 ml/min

Fluorescence detector: excitation wavelength: 293nm, emission wavelength: 395nm.

Inject volume: 10ul

6.4 Quantitative analysis

6.4.1 Drawing standard curve:

Inject a certain volume of mixing standard test solution (4.8.3) to HPLC to get area (or height) of peak of compound, to the peak area or height for the vertical, to standard test solution Conc. for the abscissa, to draw standard curve

6.4.2 Samples test:

Inject a certain volume of samples solution (4.8.3) to HPLC to get area (or height) of peak of compound, according to standard curve to calculate Vitamin B6 each compound Conc. ci in sample solution.

7 Calculations and Expression

7.1 calculate the content of compound in sample

Calculate the contents of compound as formula (1):

$$X_i = \frac{c_i \times V \times 100}{m} \dots\dots\dots (1)$$

In this formula,

X_i —the content of compound in sample, the unit is milligram per hundred grams ($\mu\text{g}/100\text{g}$)

m —Sample weight, the unit is gram (g) ;

c_i —the concentration of Vitamin B6 in sample solution, the unit is microgram per milliliter ($\mu\text{g}/\text{ml}$);

V —the volume of sample solution, the unit is milliliter (ml).

7.2 calculate the total content of Vitamin B6

Calculate the content of total Vitamin B6 as formula (2):

$$X = X_{\text{py}} + X_{\text{px}} \times 1.012 + X_{\text{pax}} \times 1.006 \dots\dots\dots (2)$$

In this formula,

X — The content of Vitamin 6 in sample, the unit is $\mu\text{g}/100\text{g}$

X_{py} —The content of pyridoxine in sample, the unit is $\mu\text{g}/100\text{g}$

X_{px} —The content of pyridoxal in sample, the unit is $\mu\text{g}/100\text{g}$

X_{pax} — The content of pyridoxamine in sample, the unit is $\mu\text{g}/100\text{g}$

1.012—a conversion factor of 1.012 is used to convert pyridoxal to pyridoxine.

1.006---a conversion factor of 1.012 is used to convert pyridoxamine to pyridoxine.

Note: The content of Vitamin 6 in sample expressed by pyridoxine.

The result is the arithmetical mean of two independent tests, and reserved three decimal digits.

8 Precision

Absolute difference of two independent test results should not exceed 10% of arithmetical mean under the repeated test condition.

9. Other

The test method LOD: pyridoxine 1.5 $\mu\text{g}/100\text{g}$, pyridoxal 1.3 $\mu\text{g}/100\text{g}$, pyridoxamine 1.6 $\mu\text{g}/100\text{g}$.

Appendix A

(Informative Annex)

Liquid chromatogram of B₆ standard solution

A.1 Liquid chromatogram of B₆ standard solution

