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NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5009.285-2022

National food safety standard - Determination of Vitamin B12 in foods

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Foreword

This Standard replaces GB 5413.14-2010 National food safety standard -

Determination of vitamin B12 in foods for infants and young children, milk and milk

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products.

Compared with GB 5413.14-2010, the major changes of this Standard are as follows.

-- Add Method I Liquid chromatography;

-- Add Method II Liquid chromatography - mass spectrometry;

-- Remove normative references;

-- Modify the original microbiological method to method III.

National food safety standard - Determination of Vitamin B12 in foods

1 Scope

This Standard specifies the determination method of vitamin B12 in foods.

Method I of liquid chromatography applies to the determination of vitamin B12 in foods for infants and young children, milk and milk products, meat and meat products.

Method II of liquid chromatography - mass spectrometry applies to the determination of vitamin B12 in foods for infants and young children, milk and milk products, meat and meat products, ready-to-eat cereals, baked foods, jelly, and beverages.

Method III of microbiological method applies to the determination of vitamin B12 in foods for infants and young children, milk and milk products.

Method I - Liquid chromatography

2 Principle

After the sample is enzymatically hydrolyzed, use potassium cyanide (or sodium cyanide) solution to convert cobalamin isomers (hydroxocobalamin, methylcobalamin and 5-deoxyadenosylcobalamin, etc.) to cyanocobalamin. After the sample solution is purified and concentrated by the immunoaffinity column, use the reversed-phase liquid chromatography column for separation, the ultraviolet detector for detection, and the external standard method for quantification.

3 Reagents and materials

Unless otherwise specified, all the reagents are analytical reagents, and the water is

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grade-1 water which is specified by GB/T 6682.

3.3 Standard

Vitamin B12 (cyanocobalamin) standard substance (C63H88CoN14O14P, CAS number. 68-19-9). purity >=99%, or standard substance certified by the nation and granted a certificate of reference material.

5 Analysis steps

Note. Avoid ultraviolet light during the operation, and operate as far away from light as possible.

5.1.1 Sample preparation

Crush and grind the solid sample, or use a meat grinder to make it into chyme; homogenize and mix. Shake and mix the liquid sample before testing.

5.1.2 Sample extraction

Weigh 5 g ~ 10 g of the sample (accurate to 0.01 g) into a 150 mL conical flask with stopper; add 30 mL of sodium acetate buffer, 0.2 g of pepsin, 0.05 g of amylase and 2 mL of potassium cyanide (or sodium cyanide) solution in turn; mix well. Put the sample solution into a water bath constant temperature oscillator; at 37 °C, carry out enzymatic hydrolysis for 30 minutes (for meat samples, carry out enzymatic hydrolysis for 10 h ~

5.1.3 Purification

Connect the immunoaffinity column to the solid-phase extraction device. After discarding the buffer in the immunoaffinity column, pipette an appropriate amount of the above filtrate (containing 10 ng ~ 500 ng of vitamin B12) to pass through the column; adjust the column passing speed to 2 mL/min ~ 3 mL/min. After the sample solution has completely passed through the column, use 10 mL of water to rinse the immunoaffinity column at a steady flow rate and drain. Place a 10 mL glass test tube under the immunoaffinity column; use 3 mL of methanol to elute in three times; collect all the eluates; use nitrogen flow to blow slowly below 60 °C until nearly dry; use 0.04% trifluoroacetic acid solution to fix the volume to 1.0 mL; vortex for 30 s to dissolve the

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residue; filter through a 0.22 μm filter; test.

5.2.1 Chromatographic column. C18 column (column length 150 mm, column inner

diameter 4.6 mm, packing particle size 2.5 μm), or equivalent.

5.2.3 Gradient elution. 0 min ~ 6.0 min, 90% A; 6.0 min ~ 8.5 min, 90% ~ 0% A; 8.5 min ~ 14.0 min, 90% A.

5.3 Preparation of the standard curve

Inject the standard series solutions into the liquid chromatograph from low concentration to high concentration in turn; measure the corresponding chromatographic peak areas.

5.4 Sample determination

Inject the to-be-tested sample solution into the liquid chromatograph, to obtain the peak area of vitamin B12 in the to-be-tested solution; measure the concentration of vitamin B12 in the test solution according to the standard curve. The response value of vitamin B12 in the to-be-tested sample solution shall be within the linear range of the standard curve. If it exceeds the linear range, the solution on the machine shall be diluted, or the sampling amount shall be adjusted and the analysis shall be carried out again after processing according to the sample analysis steps.

5.5 Blank test

Do not weigh the sample; operate according to the sample analysis steps. Substances that interfere with the to-be-measured components shall not be contained.

6 Description of the analysis results

The content of vitamin B12 (calculated as cyanocobalamin) in the sample is calculated according to Formula (1).

7 Precision

The absolute difference of two independent test results obtained under repeatability

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