

National standard of People's Republic of China

GB 5413.22—2010

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National food safety standard

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

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Issued by the Ministry of Health of People's Republic of China

## Preface

This standard is in substitution of GB/T 5413.22-1997 《Determination of phosphorus in formula and milk powder for infants and young children》。The Compared with GB 5413.22-1997, main changes in this standard are as follows:

——digestion of three strong acids ‘nitric acid-sulphuric acid-perchloric acid’ is changed into the digestion of nitric acid-perchloric acid.

This standard substitutes all previous standards as follows:

——GB 5413-1985、GB/T 5413.22-1997。

## National standard on food safety

# Determination of phosphorus in foods for infants and young children, milk and milk products

### 1 .Scope

This standard specifies the method for the determination of phosphorus in foods and dairy products for infants and young children.

This standard applies to the determination of phosphorus in foods and dairy products for infants and young children.

### 2. Normative cited reference

The reference document of this standard is essential to the application. For dated reference documents, only the date of the release note applies to this standard. For undated reference documents, the latest versions (including all amendments) apply to this standard.

### 3. Principle

Samples are subjected to acid oxidation to make phosphorus react with vanadium-ammonium molybdate and form yellow complex compound in nitric acid solution. Spectrophotometer is used to determine the absorbance at the wavelength of 440 nm with an aim to quantitatively analyze the content of phosphorus.

### 4. Reagents and materials

Unless specified, all reagents used in this method are analytical reagents, and water is the 3<sup>rd</sup> grade water specified by GB/T6682.

**4.1** Nitric acid: guaranteed reagent

**4.2** Vanadium-ammonium molybdate [  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ ]: guaranteed reagent

**4.3** Phenol ( $\text{C}_6\text{H}_6\text{O}$ )

**4.4** Sodium metavanadate ( $\text{NH}_4\text{VO}_3$ )

**4.5** Sodium hydrate ( $\text{NaOH}$ )

**4.6** Standard solution of potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ )

**4.7** 2, 6-dinitrophenol or 2, 4-dinitrophenol [ $\text{C}_6\text{H}_3\text{OH}(\text{NO}_2)_2$ ]

**4.8** Sulphuric acid: guaranteed reagent

**4.9** Vanadium-ammonium molybdate reagents:

Solution A: Dissolve 25g vanadium-ammonium molybdate  $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}]$  in 400mL water.

Solution B: Dissolve 1.25g sodium metavanadate ( $\text{NH}_4\text{VO}_3$ ) in 300mL boiling water, after cooling add 250mL nitric acid.

Slowly add solution A into solution B and stir fully and continuously;

Dilute the mixture of solution A to solution B to 1L with water and store in brown bottle.

**4.10** 6mol/L NaOH solution: weigh 240g NaOH and dissolve in 1000mL water.

**4.11** 0.1 mol/L NaOH solution: weigh 4g NaOH and dissolve in 1000mL water.

**4.12** 0.2 mol/L  $\text{HNO}_3$  solution: draw 12.5mL  $\text{HNO}_3$ , dilute to 1000mL water.

**4.13** 50  $\mu\text{g}/\text{mL}$  standard stock solution of phosphorus: weigh 0.2197g standardized solution of  $\text{KH}_2\text{PO}_4$  (see at 4.6) which is oven dried to constant weight at  $105^\circ\text{C} \pm 1^\circ\text{C}$ , dissolve in 400mL water, add 8mL  $\text{H}_2\text{SO}_4$  (see at 4.8), and dilute it into 1L. The solution can be stored for long term.

**4.14** 2 g/L Dinitrophenol indicator: weigh 0.2g 2, 6- or 2, 4-  $[\text{C}_6\text{H}_3\text{OH}(\text{NO}_2)_2]$  (see at 4.7) and dissolve in 100mL water.

## **5. Equipments and devices:**

**5.1** Analytic balance: with 0.1 mg sensibility.

**5.2** Electric hot plate.

**5.3** Spectrophotometer.

## **6 Analytical procedure**

### **6.1 Sample processing**

Weigh 0.5g solid sample or 2.5 g (accurate to 0.1 mg) liquid sample, put it into a 125mL Erlenmeyer flask. Add 10mL  $\text{HNO}_3$  (see at 4.1), and heat it on the electric hot plate (see at 5.2). After fully reacted, take the flask off the electric hot plate and wait until it is cooled off. Add 10mL  $\text{HClO}_3$  (see at 4.2), and put the flask back on the electric hot plate to heat.

If the solution turns black, take it off and add 5mL  $\text{HNO}_3$  (see at 4.1) and continue heating till the solution turns into colorless or amber and with white smoke. When there is 3-5mL left in the flask, cool it off and transfer into a 50mL volumetric flask and meter volume.

The blank experiment should be carried out at the same time.

### **6.2 The preparation of the standard curve**

**6.2.1** Take 0mL, 2.5mL, 5mL, 7.5mL, 10mL and 15mL standard stock solution of phosphorus (see at 4.13) to a 50mL volumetric flask respectively.

**6.2.2** Add 10.00mL vanadium-ammonium molybdate reagent (see at 4.9) and meter to 50mL with water. The concentrations of this series are 0 $\mu\text{g}/\text{mL}$ , 2.5 $\mu\text{g}/\text{mL}$ , 5 $\mu\text{g}/\text{mL}$ ,

7.5 $\mu$ g/mL, 10 $\mu$ g/mL and 15 $\mu$ g/mL respectively.

After coloration 25-30 °C for 15 min, determine the absorbance at the wavelength of 440 nm using 1cm colorimetric cylinders.

The standard curve is plotted with the absorbance as Y-axis and the concentration of phosphorus as X-axis.

### 6.3 Sample measurement

Take 10mL sample solution (see at 6.1) to a 50mL volumetric flask, add some water and two drops of dinitrophenol indicator (see at 4.14). First add NaOH (see at 4.10) to adjust the solution to yellow, add HNO<sub>3</sub> (see at 4.12) till the solution becomes colorless, then add NaOH (see at 4.11) to adjust it into amber.

The following steps are the same as 6.2.2.

Blank solution is used for zero adjustment.

The phosphorus concentration of the sample solution is obtained according to the standard curve.

### 7. Result calculation and representation

The content of phosphorus in the sample is calculated according to formula (1).

$$X = \frac{c \times V \times V_2}{m \times V_1 \times 1000} \times 100 \dots\dots\dots (1)$$

In this formula:

X— content of phosphorus in the sample, mg/100 g

c—the concentration of phosphorus that is obtained from the standard curve, $\mu$ g/mL;

V—the metered volume after the sample elixation, mL;

V<sub>1</sub>—The volume of the sample solution that is taken, mL;

V<sub>2</sub>—The metered volume of the coloration solution, mL;

m—the mass of the sample, g.

The calculated result is represented by the arithmetic mean from two independent results of determination, and should display in 3 significant figures.

### 8. Precision

The absolute difference between the two independent determination results that are obtained under repeated conditions should not exceed 5% of the arithmetic mean.

### 9. Others

The detection limit of this standard is 2 mg/100g.