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NATIONAL STANDARD OF
THE PEOPLE'S REPUBLIC OF CHINA

GB 5416.40-2016

**National Standard of Food Safety -
Determination of Nucleotide in Foods and Milk
Products for Infants and Young Children**

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Determination of Nucleotide in Foods and Milk Products for Infants and Young Children

1 Scope

This Standard specifies the method for the determination of free nucleotide by liquid chromatography.

This Standard applies to the determination of total amount of free nucleotides (including cytosine nucleotides, uracil nucleotides, hypoxanthine nucleotides, guanine nucleotides, adenine nucleotides) in infants and young children.

2 Principle

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

3 Reagents and material

Unless otherwise stated, the reagents used in this method are of pure analytical grade. The water is the grade three water specified in GB/T 6682. The water used by liquid chromatography mobile phase is the grade one water specified in GB/T 6682.

3.1 Reagents

3.1.1 Amylase: Enzyme activity ≥ 1.5 U/mg

3.1.2 Glacial acetic acid (CH_3COOH)

3.1.3 Tetrabutylammonium bisulfate [$(\text{C}_4\text{H}_9)_4\text{NHSO}_4$]

3.1.4 Methanol (CH_3OH)

3.1.5 Potassium hydrogen phosphate (K_2HPO_4)

3.1.6 Potassium dihydrogen phosphate (KH_2PO_4)

3.1.7 Phosphoric acid (H_3PO_4)

5 Analysis steps

5.1 Sample preparation

5.1.1 Sample pre-treatment

5.1.1.1 Sample containing starch

WEIGH about 5 g of well-mixed solid sample (to the nearest of 0.1 mg) into a 100 mL conical flask. ADD about 0.2 g of amylase. ADD 20 mL of hot water (30°C ~ 40°C) to completely dissolve the sample. OR WEIGH about 20 g of well-mixed solid sample (to the nearest of 1 mg) into a 100 mL conical flask. ADD about 0.2 g of amylase and well SHAKE. ENZYMOLYSE in a 37°C±2°C incubator for 30 min. TAKE it OUT for cooling to room temperature.

5.1.1.2 Starch-free sample

WEIGH about 5 g of well-mixed solid sample (to the nearest of 0.1 mg) into a 100 mL conical flask. ADD 20 mL of hot water (50°C ~ 60°C) to completely dissolve the sample. COOL to room temperature. OR WEIGH about 20 g of well-mixed solid sample (to the nearest of 1 mg) into a 100 mL conical flask.

5.1.2 Preparation of test solution

USE acid solution to adjust pH of sample solution to 4.1. MOVE it to a 50 mL measuring flask. SET volume. USE filter paper to filter. USE 0.45 µm micro-membrane to filter the obtained filtrate for use.

5.2 Reference chromatographic conditions

5.2.1 Mobile phase: phosphate buffer + methanol = 1000 + 40

5.2.2 Chromatographic column: C18-T reversed-phase column (250 mm × 4.6 mm, 5 µm) or equivalent column

5.2.3 Flow rate: 1 mL/min

5.2.4 Wavelength: 254 nm

5.2.5 Column temperature: 25°C

5.2.6 Injection volume: 10 µL

NOTE If necessary, use phosphoric acid or dipotassium hydrogen phosphate solution to appropriately adjust the pH of the mobile phase to the best.

5.3 Standard curve drawing

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