



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

GB 5413.40-2016

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

食品安全国家标准 婴幼儿食品和乳品中核苷酸的测定

(English Translation)

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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.

3 Reagents and material

Unless otherwise stated, the reagents used in this method are of pure analytical grade.

3.2 Preparation of reagents

3.2.1

Acetic acid solution (100 mL/L). PIPET 10 mL of glacial acetic acid; ADD water to a volume of 100 mL.

3.4 Preparation of standard solution

Nucleotide standard mixture (should be configured that day). respectively

WEIGH 10 mg of nucleotide standard products CMP, AMP, UMP, 5 mg of GMP and IMP (to the nearest of 0.1 mg); USE ultra-pure water to dissolve into the same 100 mL volumetric

flask; USE water to set the volume to 100 mL.

4.1 High performance liquid chromatograph. equipped with UV detector or diode array detector

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.

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

5.3 Standard curve drawing

Respectively PIPET 2 mL, 4 mL, 6 mL, 8 mL, 10 mL of standard mixed solution.

ADD ultra-pure water to set the volume to 50 mL to prepare nucleotide series of standard working fluid, as shown in Table 1.

5.4 Determination of sample solution

Inject the prepared sample solution into the high performance liquid chromatography. Determine the corresponding peak area or peak height.

6.1 Results calculation

Calculate the content of the free nucleotides in the sample X_i (CMP, UMP, GMP, IMP, AMP) according to equation (1).

6.2 Results representation

The arithmetic results are expressed as the arithmetic mean of two independent determinations obtained under repeatability conditions. The results shall be reserved for two digits after the decimal point.

7 Precision

The absolute difference between the two independent determinations obtained under repeatability shall not exceed 10% of the arithmetic mean.

8 Others

The high performance liquid chromatography ultraviolet standard and the sample nucleotide chromatogram are shown in Annex A.

Annex A

Nucleotide standard product and sample high performance liquid chromatography

A.1 Nucleotide standard chromatogram

The nucleotide standard chromatogram is shown as Figure A.1.

A.2 Sample chromatogram

The sample chromatogram is shown as Figure A.2.

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