



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

GB 5009.248-2016

National food safety standard - Determination of lutein in foods

食品安全国家标准 食品中叶黄素的测定

(English Translation)

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Foreword

This Standard replaces GB/T 23209-2008 "Determination of lutein in milk powder - HPLC-UV method".

As compared with GB/T 23209-2008, the main changes of this Standard are as follows.

- CHANGE the standard's name to "National Food Safety Standard - Determination of lutein in foods";
- EXPAND the scope of application; ADD the liquid chromatography for the determination of lutein in frozen drinks, rice and flour products, bakery products, jams, jellies, and beverages;
- MODIFY the "Principles" of the former standard. The former "USE acetone as a solvent to extract" is changed to "USE diethyl ether-n-hexane-cyclohexane solvent system to extract";
- ADD the points worthy of notice for the operation process;
- ADD a saponification step for samples with high fat content including milk powder; and PROVIDE different pretreatment methods to accommodate

various sample matrix analysis needs;

- ADD the correction requirement for the concentration of lutein standard solution;
- For the phenomenon that lutein may be isomerized during the test operation, ADD the qualitative and quantitative requirements for cis-lutein produced due to isomerization.

National Food Safety Standard -
Determination of lutein in foods

1 Scope

This Standard specifies the liquid chromatography for the determination of lutein in foods.

This Standard applies to liquid chromatography determination of lutein in infant formula, dairy products, frozen drinks, rice and flour products, bakery products, jams, jellies, and beverages.

2 Principles

After foods with high fat content (fat content of not less than 3% on a dry basis) are saponified with potassium hydroxide solution at room temperature to free lutein, then USE diethyl ether-n-hexane-cyclohexane (40+40+20, volume ratio) to extract; USE liquid chromatography to separate; USE UV-detector or diode array detector to detect; and USE external standard method for quantification. Lutein in the samples of the other foods is directly extracted by diethyl ether-n-hexane-cyclohexane (40+40+20, volume ratio). After the extract is purified by a neutral alumina solid phase extraction cartridge, USE liquid chromatography to separate; USE UV-detector or diode array detector to detect; and USE external standard method for quantification.

3 Reagents and materials

Unless otherwise stated, the reagents used in this method are of analytically pure; the water is Grade I water specified in GB/T 6682.

3.2 Preparation of reagents

3.2.1 10% potassium hydroxide solution. WEIGH 10 g of potassium hydroxide (3.1.7); ADD water to dissolve and dilute to 100 mL.

3.2.2 20% potassium hydroxide solution. WEIGH 20 g of potassium hydroxide (3.1.7); ADD water to dissolve and dilute to 100 mL.

3.2.3 Extraction solvent. WEIGH 1 g of BHT (3.1.6); USE 200 mL of cyclohexane (3.1.1) to dissolve; ADD 400 mL of diethyl ether (3.1.2) and 400 mL of n-hexane (3.1.3), and MIX well.

3.3 Standard

Lutein (CAS number. 127-40-2), with a purity of not less than 98.0%.

0.01 mg) of lutein (3.1.3); USE 0.1% BHT ethanol solution (3.2.4) to dissolve and dilute to 100 mL. This standard stock solution can be stored for six months by filling nitrogen and avoiding light in a refrigerator of -20 °C or below.

25 mL brown volumetric flasks; USE 0.1% BHT ethanol solution (3.2.4) to dilute

to volume; and OBTAIN a series of standard working solutions having concentrations of 0.100 µg/mL, 0.200 µg/mL, 0.400 µg/mL, 0.800 µg/mL, and

5 Analytical procedures

Note: Because lutein is sensitive to light, unless otherwise stated, all test operations shall be performed in a yellow or red light source environment with no UV light below 500 nm.

5.1 Sample preparation

A certain number of samples are pulverized, homogenized, and divided as required, and stored in a sample bottle. The prepared sample shall be filled with nitrogen and sealed; and stored in a refrigerator at -20 °C or below.

5.2.1 Foods with high fat content (such as infant formula, milk powder, ice cream, bakery nuts, etc.)

5.3.1 Chromatographic column. C30 column, 5 μ m, 250 mmx4.6 mm (inner diameter) or equivalent;

5.3.3 Mobile phase. methanol/water (88+12, volume ratio, containing 0.1% BHT)-methyl tert-butyl ether (containing 0.1% BHT), gradient elution. 0 min~18 min, methanol/water is changed from 100% to 10%; 18.1 min, methanol/water is changed from 10% to 100%, retained for 10 min;

5.4 Making of standard curve

The series of standard working solutions are separately injected into the liquid chromatograph, to determine the corresponding peak area. USE the concentration of standard working solution as the abscissa. USE the peak area as the ordinate. The standard curve is drawn.

5.5 Determination of sample solution

The response value of lutein in the sample solution to be determined shall be within the linear response range of the instrument, otherwise it shall be diluted or concentrated appropriately.

6 Analysis results expression

The content of lutein in the sample shall be calculated according to formula (1).

7 Precision

The absolute difference between the two independent determination results,