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

SN/T 5276-2020

**Determination of multiple allergens in export foods Liquid
chromatography-mass spectrometry/mass spectrometry**

出口食品中多种过敏原的测定 液相色谱—质谱/质谱法

(English Translation)

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Foreword

This standard was drafted in accordance with the rules given in GB/T 1.1-2009.

1 Scope

This standard specifies the liquid chromatography-mass spectrometry/mass spectrometry detection of allergen proteins in milk, eggs, soybeans, peanuts and nuts in processed foods.

Method of measurement.

This standard applies to milk, eggs, soybeans, peanuts, hazelnuts, almonds and other foods such as biscuits, bread, beverages, and breakfast cereals.

Determination and confirmation of walnut allergen protein by liquid chromatography-mass spectrometry/mass spectrometry.

2 Normative references

The following documents are indispensable for the application of this document. For dated reference documents, only the dated version applies to this article. For undated reference documents, the latest version (including all amendments) is applicable to this document.

GB/T 6682 Analytical laboratory water specifications and test methods

3 Principles of the method

The allergen protein in the sample is extracted and purified, reduced by dithiothreitol (DDT), alkylated with iodoacetamide (IAA), and alkali Trypsin digestion, liquid chromatography-mass spectrometry/mass spectrometry detection. Establish a linear relationship between the response intensity of characteristic peptides and the content of allergens, Quantification by external standard method.

4 Reagents and materials

Unless otherwise specified, the reagents used are all analytically pure, and the water is first-grade water that meets the requirements of GB/T 6682.

4.1 Reagents

4.1.1

Ammonium bicarbonate (NH_4HCO_3).

4.1.2

Dithiothreitol ($\text{C}_4\text{H}_{10}\text{O}_2\text{S}_2$, DTT).

4.1.3

Iodoacetamide ($\text{ICH}_2\text{CONH}_2$, IAA).

4.1.4

Calcium chloride (CaCl_2).

4.1.5

Acetic acid (CH_3COOH). chromatographically pure.

4.1.6

Formic acid (HCOOH). chromatographically pure.

4.1.7

Acetonitrile (CH_3CN). chromatographically pure.

4.1.8

Alkaline trypsin. activity greater than 10 000 BAEE per mg protein.

4.1.9

Tris ($\text{C}_4\text{H}_{11}\text{NO}_3$, Tris).

4.1.10

Hydrochloric acid (HCl).

4.1.11

n-hexane (C_6H_{14}). chromatographically pure.

4.2 Reagent preparation

4.2.1

Ammonium bicarbonate solution (500 mmol/L). Weigh 3.95 g of ammonium bicarbonate (4.1.1), dissolve in water and dilute to 100 mL.

4.2.2

Dithiothreitol solution (500 mmol/L). Weigh 0.771 g of dithiothreitol (4.1.2) and use ammonium bicarbonate solution (4.2.1) After dissolving, dilute to 10 mL. It can be stored at 4 °C for one month.

4.2.3

Iodoacetamide solution (500 mmol/L). Weigh 0.925 g of iodoacetamide (4.1.3) and use ammonium bicarbonate solution (4.2.1) After dissolving, dilute to 10 mL. Store at 4 °C in the dark and refrigerate for one month.

4.2.4

Calcium chloride solution (100 mmol/L). Weigh 0.111 g of calcium chloride (4.1.4), dissolve in water and dilute to 10 mL.

4.2.5

Acetic acid solution (1%, volume ratio). Pipette 0.1 mL of acetic acid (4.1.5), dilute with water and dilute to 10 mL.

4.2.6

Bovine trypsin solution (500 µg/mL). Weigh 5 mg alkaline bovine trypsin (4.1.8) and dissolve it with acetic acid solution (4.2.5) After solution, make the volume to 10 mL. The solution can be stored at -20 °C for 6 months.

4.2.7

Aqueous solution of formic acid (0.1%, volume ratio). accurately pipette 1.0 mL of formic acid (4.1.6), and dilute to 1 000 mL with water.

4.2.8

Acetonitrile solution of formic acid (0.1%, volume ratio). accurately pipette 1.0 mL of formic acid (4.1.6), and dilute to volume with acetonitrile (4.1.7) 1 000 mL.

4.2.9

Tris-HCl solution (40 mmol/L). Weigh 2.42 g of Tris (tris (trishydroxymethylaminomethane)), dissolve it in 800 mL of water, and use Adjust the pH to 8.0 ± 0.10 with 10% hydrochloric acid, and dilute to 1000 mL with water.

4.3 Standard product

4.3.1

NIST SRM 8445.spray-dried whole egg for allergen detection (spray-dried whole egg for allergen detection).

4.3.2

NIST SRM 2387.peanut butter.

4.3.3

NIST SRM 1549a. whole milk powder.

4.3.4

NIST SRM 3234.soy flour.

(Or standard reference materials from other sources are also acceptable.)

4.4 Preparation of standard solution

4.4.1

NIST SRM 8445, SRM 2387, SRM 1549a, SRM 3234 protein extract standard stock solution. accurately weighed separately Take 1 g of NIST SRM 8445, SRM 2387, SRM 1549a, SRM 3234, add Tris-HCl (4.2.9) solution, and dilute to 20 mL. Shake uniformly to completely dissolve the sample, and place it in a water bath at 60 °C for heating and shaking for 3 hours. The extract is centrifuged at a high speed of 10 000 r/min After 5 minutes, the supernatant was too low protein adsorption 0.45 μm cellulose acetate filter membrane, the Bradford method was used to determine the concentration of total protein in the extract.

Temperature, the extract is stored in a refrigerator at -20 °C.

4.4.2

Because it is difficult to purchase standard materials, 1 g of the roasted hazelnuts, walnuts, and almonds were accurately weighed in the experiment, and Tris-HCl was added.

(4.2.9) Solution, dilute to 20 mL. Shake uniformly to completely dissolve the sample, and