



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

GB 5009.185-2016

**National food safety standard - Determination of Patulin in
Foods**

食品安全国家标准 食品中展青霉素的测定

(English Translation)

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1 Scope

This Standard specifies methods for the determination of patulin in foods.

Method 1 of this Standard is isotope dilution - liquid chromatography tandem mass spectrometry, which applies the determination of patulin content in fruits and products of apple and hawthorn, fruit and vegetable juices and alcoholic foods.

Method 2 of this Standard is high performance liquid chromatography, which applies the determination of patulin content in products of apple, its fruit and vegetable juices and alcoholic foods.

Method 1 -- Isotope dilution - liquid chromatography - tandem mass spectrometry

2 Principle

The patulin in the sample (turbid juice, semi-fluid and solid samples are enzymatically treated by pectinase) is subjected by solvent extraction, purified and concentrated through patulin solid-phase purification column or mixed anion-exchange column, ionized by electrospray ionization source, detected by multiple reaction monitoring, and quantified by internal standard method.

3 Reagents and materials

Unless otherwise specified, all the reagents in this method are analytical reagents, the water is grade-1 water that is specified by GB/T 6682.

3.1 Reagents

3.1.1

Acetonitrile (CH_3CN)

Chromatographic pure.

3.1.2

Methanol (CH_3OH)

Chromatographic pure.

3.1.3

Acetic acid (CH_3COOH)

Chromatographic pure.

4 Instruments and apparatuses

4.1

Liquid chromatography - mass spectrometer

With electrospray ion source.

4.2

Homogenizer.

4.3

High-speed pulverizer.

4.4

Tissue stamping machine.

4.5

Vortex oscillator.

4.6

pH meter

measurement accuracy ± 0.02 .

4.7

Balance

The sensitivity is 0.01 g and 0.000 01 g.

4.8 50 mL PVC centrifuge tube with a plug.

4.9

Centrifuge

Speed $\geq 6\,000$ r/min.

4.10 Patulin solid-phase purification column (hereinafter referred to as purification

column): mixed filler purification column MycosepTM228 or the equivalent.

4.11 Mixed anion exchange column: a solid-phase extraction column that uses N-

Vinyl-2-pyrrolidone-divinylbenzene copolymer matrix- $\text{CH}_2\text{N}(\text{CH}_3)_2\text{C}_4\text{H}_9^+$ as the filler, or the equivalent. Before using, respectively use 6 mL of methanol and 6 mL of water to pre-elute and keep the column moist.

4.12 100 mL pear-shaped flask.

4.13 Solid-phase extraction device.

4.14 Rotary evaporator.

4.15 Nitrogen-blowing instrument.

5.1.1 Liquid sample (apple juice, hawthorn juice, etc.)

Pour the sample into a homogenizer and mix; take any 100 g (or mL) of the sample for testing.

Alcohol samples need be degassed by ultrasound for 1 h or be stored at low temperature of 4°C overnight.

residue; use a 0.22 μm filter to filter it; collect the filtrate in the sample bottle for sample injection. Use the same operation method to do a blank test.

5.2.2.1 Sample extraction

5.2.2.1.1

Liquid sample

Weigh 4 g of sample (accurate to 0.01 g) to a 50 mL centrifuge tube; add 250 μ L of isotope internal standard working solution; add 21 mL of acetonitrile; mix well;

centrifuge at 6 000 r/min for 5 min; place aside for purification.

5.2.2.1.2

Solid, semi-fluid sample

Weigh 1 g of sample (accurate to 0.01g) in a 50 mL centrifuge tube; add 100 μ L of isotope internal standard working solution; mix well and let stand for a while; then, add

10 mL of water and 150 μ L of pectinase solution to mix; leave it at room temperature overnight; add 10.0 mL of ethyl acetate; eddy and mix for 5 min; centrifuge at 6 000 r/min for 5 min; transfer the ethyl acetate layer to a pear-shaped flask. Then, use 10.0 mL of ethyl acetate to extract for one more time; combine the two ethyl acetate extracts;

use a rotary evaporator to concentrate it to dryness in the water bath at 40°C; use 2.0 mL of acetic acid solution to dissolve the residue; add 8 mL of acetonitrile; mix well for later purification.

5.2.2.2 Purification

Operate according to the instruction of the purification column which is used; use a purification column to purify the extract; discard the first 1 mL of the purification solution, and collect the subsequent portion.

Use a centrifuge tube to accurately absorb 5.0 mL of purification solution; add 20 μ L of acetic acid; use nitrogen to slowly blow it to near dryness at 40°C; use acetic acid solution to fix-volume to 1 mL; roll for 30 s to dissolve the residue; use a 0.22 μ m filter to filter it; collect the filtrate in the sample bottle for sample injection. Use the same operation method to do a blank test.

Note: The sample extraction and purification part which is mention above includes mixed anion exchange column purification and purification column purification method, which can be selected according to the actual situation.

5.3.1 Chromatography reference conditions

a) Chromatographic column: T3 chromatographic column with a column length of 100 mm, an inner diameter of 2.1 mm, a particle size of 1.8 μ m, or columns of equivalent