GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

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National Food Safety Standard -

Determination of Citrinin in Foods

食品安全国家标准

食品中桔青霉素的测定

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China Food and Drug Administration.

Table of Contents

National Food Safety Standard - Determination of Citrinin in Foods

1 Scope

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

Method 1 of this standard is applicable to the determination of citrinin in rice, corn, pepper and red yeast products; method 2 is applicable to the determination of citrinin in rice, barley, oats and wheat.

Method 1 -- Immunoaffinity column purification - high performance liquid chromatography

2 Principle

For the citrinin in the sample, use methanol-water to extract; filter and dilute the extract; use the immunoaffinity column to purify; use the liquid chromatography and the fluorescence detector to determine the content of citrinin; use the external standard method to quantify.

3 Reagents and materials

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

3.1 Reagents

3.1.1 Methanol (CH_3OH): chromatographic pure.

3.1.2 Acetonitrile (CH_3CH): chromatographic pure.

3.1.3 Phosphoric acid (H_3PO_4): chromatographic pure.

3.1.4 Glacial acetic acid ($C_2H_4O_2$): chromatographic pure.

3.1.5 Sodium hydroxide (NaOH).

3.1.6 Tween-20 ($C_{58}H_{114}O_{26}$).

3.4.2 Standard intermediate solution: accurately transfer 1.0 mL of the citrinin standard stock solution in a 10 mL volumetric flask; use methanol to fix-volume; the concentration is 10 μg/mL; store at 4°C.

3.4.3 Matrix standard working solution: according to the need, take an appropriate amount of standard intermediate solution; use blank sample extract to prepare matrix standard working solutions of different concentrations. Prepare when needed.

3.5 Materials

3.5.1 Citrinin immunoaffinity column: column volume of 3 mL, maximum column capacity of 20 ng, or equivalent column.

3.5.2 Glass-fiber filter paper: diameter of 11 cm; aperture of 1.5 μm.

4 Instruments and apparatuses

4.1 High performance liquid chromatography, with fluorescence detector.

4.2 Analytical balance: sensitivity of 0.000 1 g and 0.01 g.

4.3 High-speed homogenizer: ≥12 000 r/min.

4.4 Mixer.

5 Analysis steps

5.1 Extraction

5.1.1 Rice, corn, pepper

Weigh 10.0 g (accurate to 0.1 g) of the fully pulverized homogeneous sample into a 150 mL stoppered conical flask; add 50 mL of methanol-water (70+30) extract; use high-speed homogenizer to extract for 2 min at high speed; filter the extract; transfer 1.0 mL of the filtrate to another clean container; add 49 mL of 10 mmol/L phosphoric acid solution (pH 7.5) to dilute and mix; use a glass-fiber filter paper to filter the to-be-purified immunoaffinity column.

5.1.2 Red yeast and its products

Weigh 1.0 g (accurate to 0.1 g) of the fully pulverized homogeneous sample into a 50 mL stoppered conical flask; add 20 mL of methanol-water (70+30) extract; use high-speed homogenizer to extract for 2 min at high speed; filter the extract; transfer 1.0 mL of the filtrate to another clean container; add 39 mL

Prepare matrix standard working solutions of 6 concentrations, namely 0.0 ng/mL, 1.0 ng/mL, 2.0 ng/mL, 5.0 ng/mL, 10.0 ng/mL, and 20.0 ng/mL. Under the optimal working conditions of the instrument, use the matrix standard working solution for injection respectively; take the chromatographic peak area of the corresponding citrinin as the ordinate, and the concentration of citrinin in the matrix standard working solution as the abscissa to draw the standard curve.

See Figure A.1 and Figure A.2 for chromatograms of the citrinin standard.

5.4.3 Determination of sample solution

Inject the sample solution into the high performance liquid chromatography to determine the corresponding peak area. Obtain the concentration of citrinin in the sample solution from the standard curve.

6 Description of the analysis result

Calculate the content of citrinin in the sample according to Formula (1):

$$X = \frac{\rho \times V \times f}{m} \qquad \cdots\cdots (1)$$

Where:

X -- the content of citrinin in the sample, in micrograms per kilogram (μg/kg);

ρ -- the concentration of citrinin in the sample solution, in micrograms per liter (μg/L);

V -- constant volume, in milliliters (mL);

f -- the dilution factor of the sample solution;

m -- the sample amount that is represented by the sample solution, in grams (g).

The calculation result needs to be deducted from the blank value. The calculation result shall keep two significant figures.

7 Precision

The absolute difference of two independent test results under repeatability cannot exceed 10% of the arithmetic mean value.

10.3 Standard

Citrinin ($C_{13}H_{14}O_5$, CAS No.: 518-75-2), purity ≥ 99%. or the standard substance that is certified by the state and awarded with the standard substance certificate.

10.4 Preparation of standard solution

10.4.1 Citrinin standard stock solution: weigh an appropriate amount of citrinin standard substance; use acetonitrile to dissolve and fix-volume to 1.0 mg/mL; store at 0°C ~ 4°C.

10.4.2 Citrinin standard working solution: use the mobile phase to dilute the standard stock solution into standard working solution of 25 ng/mL, 50 ng/mL, 100 ng/mL, 500 ng/mL, 1000 ng/mL as needed.

10.5 Materials

10.5.1 C_{18} solid phase extraction column: packing of 500 mg, column volume of 3 mL, or equivalent column.

10.5.2 Glass-fiber filter paper: diameter of 11 cm; aperture of 1.5 μm.

11 Instruments and apparatuses

11.1 High performance liquid chromatography, with fluorescence detector.

11.2 Oscillator.

11.3 Centrifuge: ≥ 6 500 r/min.

11.4 Vacuum solid-phase extraction device.

11.5 Nitrogen-blowing instrument.

11.6 Analytical balance: sensitivity of 0.000 1 g and 0.01 g.

12 Analysis steps

12.1 Extraction

Take 500 g of sample; use a pulverizer to pulverize; pass through an 830 μm round sieve; mix well; divide into 2 portions; place in a clean container; seal and store. Weigh approximately 5 g (accurate to 0.01 g) of the sample into a 50 mL centrifuge tube; add 10 mL of extraction solvent (10.2.1); extract for 30 min on the shaker. Centrifugate at 3 500 r/min for 4 min; transfer the supernatant to another centrifuge tube. Add another 5 mL of extraction solvent (10.2.1) to the residue; repeat the above operation; combine the supernatant. Add water to the

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