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National Food Safety Standard -

Determination of Zearalenone in Cereals

食品安全国家标准

食品中玉米赤霉烯酮的测定

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National Food Safety Standard -

Determination of Zearalenone in Cereals

1 Scope

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

The method 1 of this Standard is applicable to the determination of zearalenone in food and food products, alcohol, soy sauce, vinegar, sauce and sauce products, soybean, rapeseed and edible vegetable oil; method 2 is applicable to the determination of zearalenone in soybean, rapeseed and edible vegetable oil; method 3 is applicable to the determination of zearalenone in beef, pork, beef liver, milk and egg.

Method 1 -- Liquid Chromatography

2 Principle

Use acetonitrile solution to extract zearalenone in the sample; after it is purified by the immunoaffinity column, use high-performance liquid chromatography fluorescence detector to measure; use 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-1 water 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 Sodium chloride (NaCl).

3.1.4 Potassium chloride (KCl).

3.1.5 Disodium hydrogen phosphate (Na_2HPO_4).

3.1.6 Monopotassium phosphate (KH_2PO_4).

glass-fiber filter paper to filter it until the filtrate is clarified; reserve the filtrate for later use.

5.1.4 Alcohol

Take 20.0 g of degassed alcohol sample (alcohols which contain carbon dioxide, before using, shall be placed in a refrigerator at 4°C for 30 min, and filtered or ultrasonically degassed) or other alcohol samples which do not contain carbon dioxide in a 50 mL volumetric flask; use acetonitrile to fix-volume to the scale; shake well. Pipette 10.0 mL of the filtrate; add 40 mL of water; dilute and mix; use a glass-fiber filter paper to filter it until the filtrate is clarified; reserve the filtrate for later use.

5.2 Purification

5.2.1 Food and food products

Connect the immunoaffinity column to a glass syringe; accurately transfer 10 mL (equivalent to 0.8 g of samples) of the filtrate in 5.1; inject into the glass syringe. Connect the air pressure pump to the glass syringe; adjust the pressure, so that the solution passes through the immunoaffinity column at a flow rate of 1 drop/s ~ 2 drops/s, until part of the air enters the affinity column. Use 5 mL of water to rinse the column for one time, at a flow rate of 1 drop/s ~ 2 drops/s, until part of the air enters the affinity column; discard all the effluent. Accurately add 1.5 mL of methanol to elute at a flow rate of about 1 drop/s. Collect the eluate in a glass test tube; use nitrogen to dry at 55°C or lower; then, use 1.0 mL of the mobile phase to dissolve the residue, which is for liquid chromatography determination.

5.2.2 Soy sauce, vinegar, sauce and sauce products, alcohol

Connect the immunoaffinity column to a glass syringe; accurately transfer 10.0 mL of the filtrate in 5.1.2 or 5.1.4; inject into the glass syringe. Connect the air pressure pump to the glass syringe; adjust the pressure, so that the solution slowly passes through the immunoaffinity column at a flow rate of 1 drop/s ~ 2 drops/s, until part of the air enters the affinity column. Successively use 10 mL of PBS washing buffer and 10 mL of water to rinse the immunoaffinity column, at a flow rate of 1 drop/s ~ 2 drops/s, until the air enters the affinity column; discard all the effluent. Accurately add 1.0 mL of methanol to elute at a flow rate of about 1 drop/s. Collect the eluate in a glass test tube; use nitrogen to dry at 55°C or lower; then, use 1.0 mL of the mobile phase to dissolve the residue, which is for liquid chromatography determination.

5.2.3 Soybean, rapeseed, edible vegetable oil

Connect the immunoaffinity column to a glass syringe; accurately transfer 10 mL of filtrate in 5.1.3; inject into the glass syringe. Connect the air pressure

Method 2 -- Fluorescence spectrophotometry

9 Principle

Use acetonitrile solution to extract zearalenone in the sample; purify through the immunoaffinity column; then, add aluminum chloride solution for derivation. Use a fluorometer to determine the eluent.

10 Reagents and materials

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

10.1 Reagents

10.1.1 Acetonitrile (CH_3CH): chromatographic pure.

10.1.2 Methanol (CH_3OH): chromatographic pure.

10.1.3 Sodium chloride (NaCl).

10.1.4 Disodium hydrogen phosphate (Na_2HPO_4).

10.1.5 Monopotassium phosphate (KH_2PO_4).

10.1.6 Potassium chloride (KCl).

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

10.1.8 Hydrochloric acid (HCl).

10.1.9 Sulfuric acid (H_2SO_4).

10.1.10 Aluminum chloride ($AlC_{l3}\cdot 10H_2O$).

10.1.11 Quinine sulfate ($C_{20}H_{24}N_2O_2\cdot H_2SO_4\cdot 2H_2O$).

10.2 Preparation of reagents

10.2.1 Extract: acetonitrile-water (9+1).

10.2.2 PBS/ Tween-20 buffer: weigh 8.0 g of sodium chloride, 1.2 g of disodium hydrogen phosphate, 0.2 g of monopotassium phosphate, 0.2 g of potassium chloride in 900 mL of water; use hydrochloric acid to adjust the pH to 7.0; add 1 mL of Tween-20; use water to dilute to 1 L.

12.2 Purification

Connect the immunoaffinity column to a glass syringe; accurately transfer 10 mL of filtrate; inject into the glass syringe. Connect the air pressure pump to the glass syringe; adjust the pressure, so that the solution slowly passes through the immunoaffinity column at a flow rate of 1 drop/s ~ 2 drops/s, until part of the air enters the affinity column. Successively use 10 mL of PBS/ Tween-20 buffer solution and 10 mL of water to rinse the immunoaffinity column, at a flow rate of 1 drop/s ~ 2 drops/s, until the air enters the affinity column; discard all the effluent. Accurately add 1.0 mL of methanol to elute at a flow rate of about 1 drop/s. Collect the eluate in a glass test tube; use nitrogen to dry at 55°C or lower; then, use 1.0 mL of the mobile phase to dissolve the residue, which is for determination.

12.3 Blank test

Do not weigh the sample; do a blank test according to the steps of 12.1 and 12.2. It shall be confirmed that it does not contain any substance that interferes with the to-be-test component.

12.4 Determination

12.4.1 Determination conditions

Excitation wavelength of 360 nm; emission wavelength of 450 nm.

12.4.2 Fluorescence photometer calibration

Use the 0.05 mol/L sulfuric acid solution as a blank for zero setting; use the fluorescence photometer calibration solution for calibration.

12.4.3 Sample solution determination

Add 1.0 mL of aluminum chloride-derived solution to the sample solution that is obtained in 12.2; immediately place it in a fluorescence photometer to read the concentration of zearalenone.

13 Description of the analysis result

Calculate the content of zearalenone in the sample according to Formula (2):

$$X = \frac{\rho \times V \times 1\,000}{m \times 1\,000} \times f \qquad \cdots\cdots(2)$$

Where:

17.1 Reagents

17.1.1 Methanol (CH_3OH): chromatographic pure.

17.1.2 Acetonitrile (CH_3CH): chromatographic pure.

17.1.3 Absolute diethyl ether ($C_4H_{10}O$).

17.1.4 Trichloromethane ($CHCl_3$).

17.1.5 Sodium hydroxide (NaOH).

17.1.6 Sodium acetate trihydrate ($CH_3COONa·3H_2O$).

17.1.7 Phosphoric acid (H_3PO_4): purity greater than 85%.

17.1.8 Glacial acetic acid (CH_3COOH): chromatographic pure.

17.1.9 β-glucuronide.

17.1.10 Sulfatase.

17.2 Preparation of reagents

17.2.1 Sodium hydroxide solution (0.5 mol/L): weigh 20 g of sodium hydroxide; use water to dissolve and fix-volume to 1 L.

17.2.2 Sodium acetate buffer solution (0.05 mol/L): weigh 6.8 g of sodium acetate trihydrate; use 900 mL of water to dissolve; use glacial acetic acid to adjust the pH to 4.8; fix-volume to 1 L.

17.2.3 Phosphoric acid-water solution: (1+4).

17.2.4 Methanol-water solution: (1+1).

17.2.5 β-glucuronide/ sulfate complex enzyme: 96 000 U/mL β-glucuronide, 390 U/mL sulfatase.

17.3 Standard

Zearalenone ($C_{18}H_{22}O_5$, CAS No.: 17924-92-4), purity ≥ 98.0%. or the standard substance that is certified by the state and awarded with the standard substance certificate.

17.4 Preparation of standard solution

17.4.1 Standard stock solution: accurately weigh an appropriate amount of standard (accurate to 0.000 1 g); use acetonitrile to dissolve it and prepare it a

Note: During the sample preparation, sample contamination or changes in residue content shall be prevented.

19.1.1 Muscles and internal organs

Take 500 g of sample; use a tissue masher to thoroughly mash and mix; divide into two portions equally, which are respectively placed in clean containers as the sample; seal. Store in the dark below -18°C.

19.1.2 Milk

Take 500 g of sample; fully mix; divide into two portions equally, which are respectively placed in clean containers as the sample; seal. Store at 0°C ~ 4°C in the dark.

19.1.3 Egg

Take 500 g of sample; remove the shell; use a tissue masher to thoroughly mix; divide into two portions equally, which are respectively placed in clean containers as the sample; seal. Store at 0°C ~ 4°C in the dark.

19.2 Hydrolysis

Weigh 5.0 g of sample (accurate to 0.1g) in a 50 mL stoppered centrifuge tube; add 10 mL of 0.05 mol/L sodium acetate buffer solution and 0.025 mL of β-glucuronide/ sulfate complex enzyme; vortex and mix; vibrate at a 37°C water bath for 12 h.

19.3 Extraction

After hydrolysis, add 15 mL of anhydrous diethyl ether; shake and extract for 5 min; then, centrifuge at 4 000 r/min for 2 min; transfer the supernatant to a concentrated bottle; then, use 15 mL of anhydrous diethyl ether to repeatedly extract; combine the supernatant; rotate and concentrate at 40°C or below to nearly dry. Add 1 mL of trichloromethane to dissolve the residue; sonicate for 2 min to help solubilization; transfer to a 10 mL centrifuge tube; then, use 3 mL of 0.5 mol/L sodium hydroxide solution to rinse the concentrated flask; then, transfer to the same centrifuge tube; vortex to mix; centrifuge at 4 000 r/min for 2 min; absorb the upper sodium hydroxide solution. Then, use 3 mL of 0.5 mol/L sodium hydroxide to rinse repeatedly and to extract once; combine the sodium hydroxide extract; add 1 mL of phosphoric acid-water solution; mix well for later purification.

19.4 Purification

Before using, successively use 5 mL of methanol and 5 mL of water to pre-rinse the solid phase extraction column.

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