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National Food Safety Standard -

Determination of Sterigmatocystin in Food

食品安全国家标准 食品中杂色曲霉素的测定

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National Food Safety Standard - Determination of Sterigmatocystin in Food

1 Scope

This Standard specifies the methods for the determination of sterigmatocystin: liquid chromatography - tandem mass spectrometry and high-performance liquid chromatography.

This Standard is applicable to the determination of sterigmatocystin in rice, corn, wheat, soy and peanut.

Method I -- Liquid Chromatography - Tandem Mass Spectrometry

2 Principle

Use acetonitrile - aqueous solution to extract sterigmatocystin in the sample. Through vortex, ultrasound and centrifugation, take the supernatant for dilution. Through solid phase extraction column or immunoaffinity column, purify and concentrate it. Use methanol - aqueous solution to reach a constant volume. Use microporous membrane to filter it. Use liquid chromatography for separation. Use electrospray ion source for ionization. Use multiple reactive ion monitoring for detection. Use isotope internal standard method to quantify it.

3 Reagents and Materials

Unless it is otherwise stipulated, all reagents used in this Method shall be analytically pure. Water shall be Grade-1 water stipulated in GB/T 6682.

3.1 Reagents

3.1.1 Acetonitrile (CH_3CN): chromatographic purity.

3.1.2 Methanol (CH_3OH): chromatographic purity.

3.1.3 Sodium chloride (NaCl).

3.4.3 Isotope internal standard working solution (1.0 μg/mL): accurately transfer-take 0.40 mL of sterigmatocystin isotope internal standard (25 μg/mL), then, place it into a 10 mL volumetric flask. Use methanol to dilute to a constant volume. Store it at -20 °C. The storage life is 3 months.

3.4.4 Standard series working solution: accurately transfer-take a proper amount of the standard working solution, then, place it into a 5 mL volumetric flask. Add 50 μL of 1.0 μg/mL isotope internal standard working solution. Use methanol - aqueous solution (70 + 30) to reach to a constant scale (series standard solutions, in which, sterigmatocystin concentration is: 1 ng/mL, 2 ng/mL, 5 ng/mL, 10 ng/mL, 20 ng/mL, 30 ng/mL, 40 ng/mL and 50 ng/mL). Prepare it right before usage.

3.5 Materials

3.5.1 Immunoaffinity column: column capacity ≥ 600 ng (please refer to B.2 for the method of validating column capacity).

3.5.2 Solid phase extraction column: N-vinyl pyrrolidone and divinylbenzene copolymer packed column (200 mg/6 mL), or equivalent. Before usage, respectively use 5 mL of methanol and 5 mL of water to activate it.

3.5.3 Microporous membrane: 0.22 μm.

4 Instruments and Equipment

4.1 Liquid chromatograph - tandem mass spectrometer: equipped with electrospray ion source.

4.2 High-speed pulverizer.

4.3 Vortex mixer.

4.4 Ultrasonic generator.

4.5 Balance: division value: 0.01 g and 0.00001 g.

4.6 Centrifuge: rotating speed ≥ 6,000 r/min.

4.7 Solid phase extraction device (equipped with vacuum pump).

4.8 Nitrogen blower.

4.9 Test sieve: aperture 1 mm ~ 2 mm.

5 Analytical Procedures

5.1 Sample Preparation

eluent into the scale test tube. Use vacuum pump to drain the affinity column. At 60 °C, use nitrogen to slowly blow the eluent, till it becomes dry. Use methanol - aqueous solution (70 + 30) to dilute to a constant volume of 1.0 mL. Conduct vortex for 30 s to dissolve the residue. Use 0.22 μm membrane to filter it. Gather the filtrate in an injection bottle to prepare for sample injection. In accordance with the same method of operation, conduct blank test.

NOTE: in accordance with the practical situation in the laboratory, one of the above-mentioned purification methods may be selected.

5.4 Reference Conditions of Instruments

5.4.1 Chromatographic reference conditions

a) Liquid phase chromatographic column: C_{18} column, column length: 100 mm; internal diameter: 2.1 mm; particle size: 1.8 μm, or equivalent chromatographic column;

b) Mobile phase: Phase-A: water; Phase-B: methanol;

c) Gradient elution conditions: 70% B (0 min ~ 5 min); 100% B (5 min ~ 8 min); 70% B (8 min ~ 12 min);

d) Flow rate: 0.2 mL/min;

e) Chromatographic column temperature: 40 °C;

f) Injection volume: 10 μL.

5.4.2 Mass spectrometry reference conditions

a) Mode of detection: multi-ion reaction monitoring (MRM);

b) Please refer to Table A.1 for mass spectrometry conditions and ion selection parameters;

c) Please refer to Figure A.1 ~ Figure A.2 for sub-ion scan;

d) Please refer to Figure A.3 for liquid chromatography - mass spectrometry.

5.5 Draw a Standard Curve

In accordance with the sequence from low concentration to high concentration, inject the standard series working solution into the liquid chromatograph - tandem mass spectrometer; measure the peak area of corresponding chromatographic peak. Take the concentration of sterigmatocystin in the standard series working solution as the x-coordinate. Take the ratio of the peak area of sterigmatocystin chromatographic peak and the peak area of isotope internal standard chromatographic peak as the y-

X---content of sterigmatocystin in the sample, expressed in (μg/kg);

ρ---concentration of sterigmatocystin in the sample solution, obtained through the standard curve, expressed in (ng/mL);

V---final constant volume, expressed in (mL);

m---weighing mass of the sample, expressed in (g);

f---dilution factor (f = 10);

The calculation result shall retain 3 significant figures.

7 Precision

The absolute difference of two independent determination results obtained under repeatability condition shall not exceed 20% of the arithmetic mean value.

8 Others

When 5 g of rice, corn and wheat sample is weighed and taken, the detection limit is 0.6 μg/kg; the quantitation limit is 2 μg/kg. When 2 g of soy and peanut sample is weighed and taken, the detection limit is 1.5 μg/kg; the quantitation limit is 5 μg/kg.

Method II -- Liquid Chromatography

9 Principle

Use acetonitrile - aqueous solution to extract sterigmatocystin in the sample. Through homogenization, vortex, ultrasound and centrifugation, take the supernatant; use phosphate buffer solution to dilute it. Use immunoaffinity column for purification and elution. Use nitrogen to blow it to dryness and concentrate it. Use the mobile phase to reach a constant volume. Use microporous membrane to filter it. Use liquid chromatography to separate UV detector for detection. Use external standard method to quantify it.

10 Reagents and Solutions

Unless it is otherwise stipulated, all reagents used in this Method shall be analytically pure. Water shall be Grade-1 water stipulated in GB/T 6682.

the standard working solution, then, place it into a 5 mL volumetric flask. Use acetonitrile - aqueous solution (50 + 50) to reach to a constant scale (series standard solutions, in which, sterigmatocystin concentration is: 5 ng/mL, 10 ng/mL, 25 ng/mL, 50 ng/mL, 75 ng/mL and 100 ng/mL). Prepare it right before usage.

10.5 Materials

10.5.1 Immunoaffinity column: column capacity ≥ 600 ng (please refer to B.2 for the method of validating column capacity).

10.5.2 Microporous membrane: 0.22 μm.

11 Instruments and Equipment

11.1 Liquid chromatograph: equipped with UV detector.

11.2 High-speed pulverizer.

11.3 Vortex mixer.

11.4 Ultrasonic generator.

11.5 Balance: division value: 0.01 g and 0.00001 g.

11.6 Centrifuge: rotating speed ≥ 6,000 r/min.

11.7 Solid phase extraction device (equipped with vacuum pump).

11.8 Nitrogen blower.

11.9 Test sieve: aperture 1 mm ~ 2 mm.

12 Analytical Procedures

The operation of immunoaffinity column manufactured by different manufacturers might be slightly different in sample injection, rinsing and elution. The operation shall comply with operation instructions provided by manufacturers.

12.1 Sample Preparation

Use high-speed pulverizer to grind the sample. Then, use a test sieve (aperture 1 mm ~ 2 mm) to sieve the sample. After the sample is well-mixed, take 100 g of the sample for tests.

12.2 Sample Extraction

Weigh-take 5 g (accurate to 0.01 g) of homogeneous sample, then, place it into a 50

measure the peak area of corresponding chromatographic peak. Take the concentration of sterigmatocystin in the standard series working solution as the x-coordinate. Take the peak area of sterigmatocystin chromatographic peak as the y-coordinate. Thus, draw a standard curve.

12.6 Determination of Sample Solution

Inject sample solution into the liquid chromatograph for determination; measure the peak area of corresponding chromatographic peak. In accordance with the standard curve, obtain the concentration of sterigmatocystin in the sample solution.

13 Expression of Analytical Result

The content of sterigmatocystin in the sample shall be calculated in accordance with Formula (2):

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

Where,

X---content of sterigmatocystin in the sample, expressed in (μg/kg);

ρ---concentration of sterigmatocystin, calculated in accordance with the chromatographic peak area of sterigmatocystin in the sample, expressed in (ng/mL);

V---final constant volume, expressed in (mL);

m---weighing mass of the sample, expressed in (g);

f---dilution factor (f = 10).

The calculation result shall retain 3 significant figures.

14 Precision

The absolute difference of two independent determination results obtained under repeatability condition shall not exceed 20% of the arithmetic mean value.

15 Others

When 5 g of rice, corn, wheat, soy and peanut sample is weighed and taken, the detection limit is 6 μg/kg; the quantitation limit is 20 μg/kg.

18 Instruments and Equipment

18.1 Small-sized grinder.

18.2 Sample sieve (sieve aperture size: 0.850 mm and 2 mm).

18.3 Electric oscillator.

18.4 All-glass concentrator.

18.5 Glass plate: 10 cm × 10 cm; 10 cm × 18.5 cm.

18.6 Developing tank: internal length: 10 cm; width: 4.5 cm; height: 17 cm. Internal length: 11.5 cm; width: 60 cm; height: 19 m.

18.7 Glass sprayer.

18.8 Air pump or oil pump.

19 Analytical Procedures

19.1 Extraction

Rice, corn, wheat, soy and peanut: weigh-take 20.00 g of rice, corn, wheat and soy grinded sample which is sieved through 0.85 mm sieve mesh (peanut grinded sample shall be sieved through 2 mm sieve mesh). Place the sample into a conical flask with a stopper. Add 80 mL of methanol - sodium chloride solution (90 + 10). Conduct oscillation for 30 min, then, filter it. Gather 40 mL of the sample solution (in terms of soy and peanut sample, take 20 mL of the sample solution, then, add 20 mL of extracting solution); transfer it into a 250 mL separating funnel. Add 25 mL of sodium chloride solution (the volume ratio of methanol and water shall be 55 + 45) and 25 mL of petroleum ether. Shake it for 2 min, then, place it still for stratification. Place the upper layer of petroleum ether solution into a conical flask; transfer the lower layer of solution into the previous separating funnel. Then, use 25 mL of petroleum ether to extract once. In the end, combine the respectively obtained upper layer of petroleum ether solution; add 25 mL of methanol - sodium chloride solution (55 + 45) [in terms of soy and peanut sample, add 25 mL of methanol - sodium chloride solution (70 + 30); shake it for 30 s]. Combine the lower layer into the previous methanol aqueous layer. Repeatedly use methanol - sodium chloride solution (55 + 45) to extract twice [in terms of soy and peanut sample, repeatedly use methanol - sodium chloride solution (70 + 30) to extract once], so as to extract sterigmatocystin in that layer. After combining the lower layer of solution, add 30 mL of trichloromethane (in terms of soy and peanut sample, except from adding trichloromethane, add 13 mL of sodium chloride solution; the volume ratio of methanol and water shall be 55 + 45); shake it for 2 min, then, place it still. When the upper layer of turbid liquid becomes partially pellucid, use quantitative

expand to around 9 cm; take it out to evaporate it.

19.2.1.3.2 Vertical expansion: developing solvent is 15 mL of benzene - methanol - glacial acetic acid (90 + 8 + 2 or 92.5 + 6 + 1.5). Place the side that is closer to the standard point and the sample solution point into the slot, then, expand to around 9 cm; take it out to evaporate it.

19.2.1.4 Fluorescence developing

On a thin-layer plate, spray aluminum trichloride - ethanol solution (200 g/L); heat it up at 80 °C for 10 min. Then, immediately observe the result under ultraviolet light (wavelength 365 nm). After the thin-layer plate cools down, thinly spray for the second time (no need for heating), then, directly observe the result.

19.2.1.5 Observe and evaluate the result

Observe under ultraviolet light. If the second point of the second plate displays the minimum detection amount in corresponding area of the standard point, but in the same position on the first plate, fluorescent point does not emerge, then, the content of sterigmatocystin in the sample is 5 μg/kg (in terms of soy and peanut sample, the content of sterigmatocystin is 20 μg/kg). If the emerging fluorescence intensity is equivalent to the fluorescence intensity of the minimum detection amount of the standard point, and this florescence point overlaps with the standard point of the second plate of sample solution, then, the content of sterigmatocystin in the sample is 5 μg/kg (in terms of soy and peanut sample, the content of sterigmatocystin is 20 μg/kg). If the emerging fluorescence intensity is stronger than the minimum detection amount of the standard point, then, in accordance with the estimated fluorescence intensity, decrease the microliter drops being added, or, dilute the sample solution, then, add drops of different microliters, till the fluorescence intensity of the sample solution point is consistent with the fluorescence intensity of the minimum detection amount. After spraying aluminum trichloride for the first time and the second time, respectively observe and evaluate the result; the two results shall be consistent. If the result is positive, place the thin-layer plate in the dark for 10 min, then, observe once. If the sample is still positive, conduct further confirmatory test. Namely, on a baseline, which is at a distance of 3 cm from the lower end of the thin-layer plate (10 cm × 18.5 cm), dropwise add 1 point of 10 μL of the standard service solution (0.4 μg/mL) and 3 points of sample solution, 16 μL per point. On 1 point of the sample solution, dropwise add 10 μL of the standard service solution (0.4 μg/mL); on the other point, dropwise add 10 μL of the standard service solution (1 μg/mL). On each point, add a small drop of trifluoroacetate; place it in the dark to react for 10 min; use hot wind to blow it for 5 min, so that the temperature of the thin-layer plate is not higher than 40 °C. Use glacial acetic acid - benzene (10 + 90) to expand it for 1 ~ 2 times, till the derivative of sterigmatocystin is separated from impurities. During the expansion, keep it away from light. The subsequent fluorescence developing step is the same as 19.2.1.4. In the end, observe the plate under ultraviolet light: if the sample solution is positive, derivative which overlaps with the sterigmatocystin standard shall be generated. The minimum

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