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National Food Safety Standard - Determination of B-group and G-group Aflatoxins in Foods

食品安全国家标准

食品中黄曲霉素 B 族和 G 族的测定

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Method I: Isotope dilution liquid chromatography-tandem mass spectrometry

2 Principle

The aflatoxin B_1, aflatoxin B_2, aflatoxin G_1, aflatoxin G_2 in the specimen are extracted by acetonitrile-water solution or methanol-water solution. After the extract is diluted by the phosphate buffer solution which contains 1% Triton X-100 (or Tween-20) (if necessary, it is initially purified by the aflatoxin solid-phase purification column), through purification and enrichment by immunoaffinity column, the purification liquid is concentrated, constant-volume, filtered, separated by liquid chromatography, tested by tandem mass spectrometry, then subjected to quantification by isotope internal standard method.

3 Reagents and materials

Unless otherwise stated, the reagents used in this method are of analytical grade, the water is the grade I water as specified in GB/T 6682.

3.1 Reagents

3.1.1 Acetonitrile (CH_3CN): Chromatographically pure.

3.1.2 Methanol (CH_3OH): Chromatographically pure.

3.1.3 Ammonium acetate (CH_3COONH_4): Chromatographically pure.

3.1.4 Sodium chloride (NaCl).

3.1.5 Disodium hydrogen phosphate (Na_2HPO_4).

3.1.6 Potassium dihydrogen phosphate (KH_2PO_4).

3.1.7 Potassium chloride (KCl).

3.1.8 Hydrochloric acid (HCl).

3.1.9 Triton X-100 [$C_{14}H_{22}O(C_2H_4O)_n$] (or Tween-20, $C_{58}H_{114}O_{26}$).

3.2 Preparation of reagents

3.2.1 Ammonium acetate solution (5 mmol/L): WEIGH 0.39 g of ammonium acetate; DISSOLVE it in water; USE water to dilute it to 1000 mL; MIX it uniformly.

of exposure to severe poisons.

5.1 Preparation of sample

5.1.1 Liquid samples (vegetable oil, soy sauce, vinegar, etc.)

The sampling amount shall be greater than 1 L. For packaging samples such as bags and bottles, it shall collect at least 3 packages (same batch or number). All liquid samples shall be mixed by a homogenizer in a container, any 100 g (mL) of sample is taken for testing.

5.1.2 Solid samples (cereals and their products, nuts and seeds, cereal supplements for infants, etc.)

The sampling amount shall be greater than 1 kg. It shall be crushed by high-speed pulverizer and sieved to make the particle size less than the 2 mm aperture test sieve. It is evenly mixed and then reduced to 100 g, stored in the sample bottle, sealed for preservation, to prepare for testing.

5.1.3 Semi-fluid (fermented bean curd, fermented soybean, etc.)

The sampling amount shall be greater than 1 kg (L). For packaging samples such as bags and bottles, it shall collect at least 3 packages (same batch or number). Then it is crushed and mixed uniformly by the tissue crusher, stored in sample bottle, sealed for preservation, to prepare for testing.

5.2 Extraction of sample

5.2.1 Liquid sample

5.2.1.1 Vegetable oils

WEIGH 5 g of specimen (accurate to 0.01 g) in a 50 mL centrifuge tube; ADD 100 μL of isotope internal standard working solution (3.4.3); OSCILLATE to mix it uniformly; LET it be standing for 30 min. ADD 20 mL of acetonitrile-water solution (84 + 16) or methanol-water solution (70 + 30); VORTEX to mix it uniformly; PLACE it in the ultrasonic/vortex oscillator or shaker to oscillate it for 20 min (or homogenized by homogenizer for 3 min). CENTRIFUGE it at 6000 r/min for 10 min, TAKE the supernatant to prepare for use.

5.2.1.2 Soy sauce, vinegar

WEIGH 5 g of specimen (accurate to 0.01 g) into a 50 mL centrifuge tube; ADD 125 μL of isotope internal standard working solution; OSCILLATE to mix it; LET it be standing for 30 min. USE acetonitrile or methanol to make its volume reach to 25 mL (accurate to 0.1 mL); VORTEX to mix it uniformly; PLACE it in the ultrasonic/vortex oscillator or shaker to oscillate it for 20 min (or homogenized by homogenizer for 3 min). CENTRIFUGE it at 6000 r/min for 10 min (or

RESTORE the immunoaffinity column which is preserved at low temperature to room temperature.

5.3.1.3 Purification of specimen

After the original liquid in the immunoaffinity column is exhausted, PIPETTE the above sample solution into a 50 mL injection syringe tube; ADJUST the dropping speed, to make the sample solution drop stably at the speed of 1 mL/min ~ 3 mL/min. After the sample solution drops completely, ADD 2 x 10 mL of water into the injection syringe tube; RINSE the immunoaffinity column at a steady flow rate. After the water dropping is finished, USE a vacuum pump to empty the immunoaffinity column. REMOVE it from the vacuum system; PLACE a 10 mL graduated test tube in the lower part of the immunoaffinity column; TAKE off the 50 mL injection syringe tube; ADD 2 × 1 mL methanol to elute the immunoaffinity column; CONTROL the dropping speed at 1 mL/min ~ 3 mL/min; then USE the vacuum pump to empty the immunoaffinity column; COLLECT all the eluate into the test tube. At 50 °C, USE nitrogen to slowly blow the eluate to almost dry; ADD 1.0 mL of initial mobile phase; VORTEX it for 30 s to dissolve the residue; USE the 0.22 μm membrane to filter it; COLLECT the filtrate in the sample bottle to prepare for sample injection.

5.3.2 Simultaneous use of aflatoxin solid-phase purification column and immunoaffinity column (for complex substrates such as Chinese red pepper, black pepper and chili pepper)

5.3.2.1 Purification of purification column

PIPETTE an appropriate amount of supernatant; FOLLOW the operation instruction of purification column to purify it; COLLECT all the purified solution.

5.3.2.2 Purification of immunoaffinity column

USE a graduated pipette to accurately pipette 4 mL of the above purified solution; ADD 46 mL of PBS which contains 1% Trition X-100 (or Tween-20) [when extracted by methanol-water solution, ADD 23 mL of PBS which contains 1% Trition X-100 (or Tween-20)], MIX it uniformly. PROCESS it in accordance with 5.3.1.2 and 5.3.1.3.

Note: Fully-automatic (online) or semi-automatic (offline) solid-phase extraction instruments may be used after optimizing the operating parameters.

5.4 Reference conditions for liquid chromatography

The reference conditions for liquid chromatography are listed below:

a) Mobile phase: phase A: 5 mmol/L ammonium acetate solution; phase B: acetonitrile-methanol solution (50 + 50);

12 Analytical procedures

12.1 Preparation of sample

12.1.1 Liquid samples (vegetable oil, soy sauce, vinegar, etc.)

The sampling amount shall be greater than 1 L. For packaging samples such as bags and bottles, it shall collect at least 3 packages (same batch or number). All liquid samples shall be mixed by a homogenizer in a container, any 100 g (mL) of sample is taken for testing.

12.1.2 Solid samples (cereals and their products, nuts and seeds, cereal supplements for infants, etc.)

The sampling amount shall be greater than 1 kg. It shall be crushed by high-speed pulverizer and sieved to make the particle size less than the 2 mm aperture test sieve. It is evenly mixed and then reduced to 100 g, stored in the sample bottle, sealed for preservation, to prepare for testing.

12.1.3 Semi-fluid (fermented bean curd, fermented soybean, etc.)

The sampling amount shall be greater than 1 kg (L). For packaging samples such as bags and bottles, it shall collect at least 3 packages (same batch or number). Then it is crushed and mixed uniformly by the tissue crusher, stored in sample bottle, sealed for preservation, to prepare for testing.

12.2 Extraction of sample

12.2.1 Liquid sample

12.2.1.1 Vegetable oils

WEIGH 5 g of specimen (accurate to 0.01 g) in a 50 mL centrifuge tube; ADD 20 mL of acetonitrile-water solution (84 + 16) or methanol-water solution (70 + 30); VORTEX to mix it uniformly; PLACE it in the ultrasonic/vortex oscillator or shaker to oscillate it for 20 min (or homogenized by homogenizer for 3 min). CENTRIFUGE it at 6000 r/min for 10 min, TAKE the supernatant to prepare for use.

12.2.1.2 Soy sauce, vinegar

WEIGH 5 g of specimen (accurate to 0.01 g) into a 50 mL centrifuge tube; USE acetonitrile or methanol to make its volume reach to 25 mL (accurate to 0.1 mL); VORTEX to mix it uniformly; PLACE it in the ultrasonic/vortex oscillator or shaker to oscillate it for 20 min (or homogenized by homogenizer for 3 min). CENTRIFUGE it at 6000 r/min for 10 min (or otherwise homogenized and then filtered by glass-fiber filter paper), TAKE the supernatant to prepare for use.

The reference conditions for chromatography are listed below:

a) Mobile phase: phase A: water; phase B: acetonitrile-methanol solution (50 + 50);

b) Gradient elution: 24% B (0 min ~ 6 min), 35% B (8.0 min ~ 10.0 min), 100% B (10.2 min ~ 11.2 min), 24% B (11.5 min ~ 13.0 min);

c) Chromatography column: C_{18} column (column's length 150 mm or 250 mm, column's inner diameter 4.6 mm; packing's particle size 5.0 μm), or equivalent;

d) Flow rate: 1.0 mL/min;

e) Column temperature: 40 °C;

f) Injection volume: 50 μL.

g) Detection wavelength: Excitation wavelength 360 nm; Emission wavelength 440 nm;

h) Liquid chromatogram: See Figure D.1.

12.6 Determination of sample

12.6.1 Production of standard curve

The series standard working solution is subjected to sample injection testing in the order from low to high concentration. USE the peak area as the ordinate; USE the concentration as the abscissa to make plotting, to obtain the standard curve regression equation.

12.6.2 Determination of sample solution

The response value of the compound to be tested in the sample solution to be tested shall be within the linear range of the standard curve. The sample whose concentration exceeds the linear range shall be subjected to sample injection analysis again.

12.6.3 Blank test

DO not weigh the specimen; PERFORM the blank test in accordance with the steps of 12.2, 12.3 and 12.4. It shall be confirmed that it does not contain substances that interfere with the component to be tested.

13 Expression of analytical results

The residual amount of AFT B_1, AFT B_2, AFT G_1 and AFT G_2 in the specimen is

17.1.11 Electrochemical derivatization reagents: potassium bromide (KBr), concentrated nitric acid (HNO_3).

17.2 Preparation of reagent

17.2.1 Acetonitrile-water solution (84 + 16): TAKE 840 mL of acetonitrile; ADD 160 mL of water.

17.2.2 Methanol-water solution (70 + 30): TAKE 700 mL of methanol; ADD 300 mL of water.

17.2.3 Acetonitrile-water solution (50 + 50): TAKE 500 mL of acetonitrile; ADD 500 mL of water.

17.2.4 Acetonitrile-water solution (10 + 90): TAKE 100 mL of acetonitrile; ADD 900 mL of water.

17.2.5 Acetonitrile-methanol solution (50 + 50): TAKE 500 mL of acetonitrile; ADD 500 mL of methanol.

17.2.6 Phosphate buffer solution (hereinafter referred to as PBS): WEIGH 8.00 g of sodium chloride, 1.20 g of disodium hydrogen phosphate (or 2.92 g of disodium hydrogen phosphate dodecahydrate), 0.20 g of potassium dihydrogen phosphate, 0.20 g of potassium chloride; USE 900 mL of water to dissolve it; USE hydrochloric acid to adjust the pH to 7.4; USE water to make its volume reach to 1000 mL.

17.2.7 The PBS which contains 1% Triton X-100 (or Tween-20): TAKE 10 mL of Triton X-100; USE PBS to dilute it to 1000 mL.

17.2.8 The 0.05% iodine solution: WEIGH 0.1g of iodine; USE 20 mL of methanol to dissolve it; ADD water to make its volume reach to 200 mL; USE the 0.45 μm filter membrane to filter it; PREPARE it before use (only used for iodine post-column derivatization).

17.2.9 The 5 mg/L pyridine tribromide aqueous solution: WEIGH 5 mg of pyridine tribromide; DISSOLVE it into 1 L of water; USE the 0.45 μm filter membrane to filter it; PREPARE it before use (only used for bromine post-column derivatization).

17.3 Standard substance

17.3.1 AFT B_1 standard substance ($C_{17}H_{12}O_6$, CAS: 1162-65-8): Purity ≥ 98%, or other standard substance which is nationally certified and awarded with the standard substance certificate.

17.3.2 AFT B_2 standard substance ($C_{17}H_{14}O_6$, CAS: 7220-81-7): Purity ≥ 98%, or other standard substance which is nationally certified and awarded with the

18.5 Balance: Sensitivity 0.01 g and 0.00001 g.

18.6 Vortex mixer.

18.7 High-speed homogenizer: speed 6500 r/min ~ 24000 r/min.

18.8 Centrifuge: Speed ≥ 6000 r/min.

18.9 Glass-fiber filter paper: Fast, high-load, which retains the 1.6 μm particles in the liquid.

18.10 Solid-phase extraction device (equipped with vacuum pump).

18.11 Nitrogen-blowing instrument.

18.12 Liquid chromatograph: Equipped with a fluorescence detector (with a flow cell of general volume or large volume).

Note: When it is equipped with a flow cell of large volume, it does not require the post-column derivative of any model or any methods.

18.13 Liquid chromatogram column.

18.14 Photochemical post-column derivative device (for photochemical post-column derivatization).

18.15 Solvent post-column derivative device (for iodine or bromine reagent derivatization).

18.16 Electrochemical post-column derivative device (for electrochemical post-column derivatization).

18.17 Immunoaffinity column: Capacity of AFT B_1 column ≥ 200 ng, recovery rate of AFT B_1 column ≥ 80%, cross-reaction rate of AFT G_2 ≥ 80% (see Appendix B for verification method).

Note: Quality verification is required for each batch of immunoaffinity columns before use.

18.18 Aflatoxin solid-phase purification column or functionally equivalent solid-phase extraction column (hereinafter referred to as purification column): It is used for the determination of samples of complex matrix.

18.19 Disposable microporous filter: Equipped with 0.22 μm microporous membrane (before use, the selected membrane shall be tested by standard solution to confirm that there is no adsorption).

18.20 Sieve: 1 mm ~ 2 mm aperture test sieve.

TAKE off the 50 mL injection syringe tube; ADD 2 × 1 mL methanol to elute the immunoaffinity column; CONTROL the dropping speed at 1 mL/min ~ 3 mL/min; then USE the vacuum pump to empty the immunoaffinity column; COLLECT all the eluate into the test tube. At 50 °C, USE nitrogen to slowly blow the eluate to almost dry; USE the initial mobile phase to make its volume reach to 1.0 mL; VORTEX it for 30 s to dissolve the residue; USE the 0.22 μm membrane to filter it; COLLECT the filtrate in the sample bottle to prepare for sample injection.

19.3.2 Simultaneous use of aflatoxin solid-phase purification column and immunoaffinity column (for complex substrates such as Chinese red pepper, black pepper and chili pepper)

19.3.2.1 Purification of purification column

PIPETTE an appropriate amount of supernatant; FOLLOW the operation instruction of purification column to purify it; COLLECT all the purified solution.

19.3.2.2 Purification of immunoaffinity column

USE a graduated pipette to accurately pipette 4 mL of the above purified solution; ADD 46 mL of PBS which contains 1% Trition X-100 (or Tween-20) (such amount may be halved when extracted by methanol-water solution); MIX it uniformly. PROCESS it in accordance with 19.4.1.3.

Note: Fully automatic (online) or semi-automatic (offline) solid-phase extraction instruments may be used after optimizing operating parameters.

19.4 Reference conditions for liquid chromatography

19.4.1 Non-derivatization method (direct testing with large flow cell)

The reference conditions for the liquid chromatography are listed below:

a) Mobile phase: phase A, water; phase B, acetonitrile-methanol (50 + 50);

b) Equal-gradient elution conditions: A, 65%; B, 35%;

c) Chromatography column: C_{18} column (column's length 100 mm, column's inner diameter 2.1 mm, packing's particle size 1.7 μm), or equivalent;

d) Flow rate: 0.3 mL/min;

e) Column temperature: 40 °C;

f) Injection volume: 10 μL;

g) Excitation wavelength: 365 nm; emission wavelength: 436 nm (AFT B_1, AFT B_2), 463 nm (AFT G_1, AFT G_2);

injection solution in accordance with the internal standard method in the standard curve, in nanograms per milliliter (ng/mL);

V_1 - The volume of specimen extract (based on the volume of the added extract for plant oil, solid, semi-solid; based on the total constant volume for soy sauce, vinegar), in milliliter (mL);

V_3 - The final constant volume of the sample after subjected to purification and elution by immunoaffinity column, in milliliters (mL);

V_2 - The volume of the sample taken for the immunoaffinity column, in milliliters (mL);

1000 - Conversion factor;

m - The weighing amount of specimen, in grams (g).

The calculation result retains three significant digits.

21 Precision

The absolute difference between two independent determinations obtained under repeatability conditions shall not exceed 20% of the arithmetic mean.

22 Others

When 5 g of sample is weighed, for the post-column photochemical derivatization, post-column bromine derivatization, post-column iodine derivatization, post-column electrochemical derivatization, the detection limit of AFT B_1 is 0.03 μg/kg, the detection limit of AFT B_2 is 0.01 μg/kg, the detection limit of AFT G_1 is 0.03 μg/kg, the detection limit of AFT G_2 is 0.01 μg/kg; for the non-derivatization, the detection limit of AFT B_1 is 0.02 μg/kg, the detection limit of AFT B_2 is 0.003 μg/kg, the detection limit of AFT G_1 is 0.02 μg/kg, the detection limit of AFT G_2 is 0.003 μg/kg;

For the post-column photochemical derivatization, post-column bromine derivatization, post-column iodine derivatization, post-column electrochemical derivatization: the limit of quantification of AFT B_1 is 0.1 μg/kg, the limit of quantification of AFT B_2 is 0.03 μg/kg, the limit of quantification of AFT G_1 is 0.1 μg/kg, the limit of quantification of AFT G_2 is 0.03 μg/kg; for the non-derivatization: the limit of quantification of AFT B_1 is 0.05 μg/kg, the limit of quantification of AFT B_2 is 0.01 μg/kg, the limit of quantification of AFT G is 0.05 μg/kg, the limit of quantification of AFT G_2 is 0.01 μg/kg.

25.8 Instruments required for the kit.

26 Analytical procedures

26.1 Preparation of sample

26.1.1 Liquid samples (fat and seasoning)

TAKE 100 g of the sample to be tested; SHAKE it uniformly; WEIGH 5.0 g of sample into a 50 mL centrifuge tube; ADD the extract required for the kit; FOLLOW the methods as described in the operation instruction on the kit to perform testing.

26.1.2 Solid samples (cereals, nuts and special dietary foods)

WEIGH at least 100 g of the sample; USE a grinder to pulverize it; after pulverization, MAKE the sample pass through the 1 mm ~ 2 mm aperture test sieve. TAKE 5.0 g of the sample in a 50 mL centrifuge tube; ADD the extract required for the kit; FOLLOW the methods as described in the operation instruction on the kit to perform testing.

26.2 Testing of sample

The specimen (liquid) to be tested is quantitatively tested in accordance with the operation procedure as described in the enzyme-linked immunosorbent assay kit.

27 Expression of analytical results

27.1 Drawing of standard working curve for quantitative testing of enzyme-linked immunosorbent assay kit

In accordance with the calculation method or computer software provided in the operation instruction on the kit, DRAW the standard working curve in accordance with the relationship between the concentration of the standard substance and the change of absorbance.

27.2 Calculation of liquid concentration to be tested

In accordance with the calculation method or computer software provided in the operation instruction on the kit, the absorbance of the liquid to be tested is substituted into the formula as obtained in 27.1, to calculate the concentration of the liquid to be tested (ρ).

27.3 Calculation of results

Method V - Thin-layer chromatography

30 Principle

After the sample is extracted, concentrated, and thin-layer separated, the aflatoxin B_1 produces blue-violet fluorescence under ultraviolet light (wavelength 365 nm), the content is determined based on the minimum detected amount of fluorescence as displayed on the thin-layer.

31 Reagents and materials

Unless otherwise stated, the reagents used in this method are of analytical grade and the water is the grade I water as specified in GB/T 6682.

31.1 Reagents

31.1.1 Methanol (CH_3OH).

31.1.2 n-Hexane (C_6H_{14}).

31.1.3 Petroleum ether (boiling range 30 °C ~ 60 °C or 60 °C ~ 90 °C).

31.1.4 Chloroform ($CHCl_3$).

31.1.5 Benzene (C_6H_6).

31.1.6 Acetonitrile (CH_3CN).

31.1.7 Anhydrous ether (C_2H_6O).

31.1.8 Acetone (C_3H_6O).

Note: The above reagents shall be subjected to one reagent blank test before the test. If it does not interfere with the determination, it can be used. Otherwise, it needs to be re-steamed one by one.

31.1.9 Silica gel G: for thin-layer chromatography.

31.1.10 Trifluoroacetic acid (CF_3COOH).

31.1.11 Anhydrous sodium sulfate (Na_2SO_4).

31.1.12 Sodium chloride (NaCl).

31.2 Preparation of reagent

solution stock solution in a 10 mL volumetric flask; ADD the benzene-acetonitrile mixed solution to the mark; MIX it uniformly. Each millimeter of this solution is equivalent to 1.0 μg of AFT B_1. PIPETTE 1.0 mL of this dilution into a 5 mL volumetric flask; ADD the benzene-acetonitrile mixed solution to dilute it to the mark. Each millimeter of this solution is equivalent to 0.2 μg of AFT B_1. Then TAKE another 1.0 mL of AFT B_1 standard sputum (0.2 μg/mL) into a 5 mL volumetric flask; ADD the benzene-acetonitrile mixed solution to dilute it to the mark. Each millimeter of this solution is equivalent to 0.04 μg of AFT B_1.

32 Instruments and equipment

32.1 Round-hole sieve: 2.0 mm aperture sieve.

32.2 Small-sized pulverizer.

32.3 Electric oscillator.

32.4 Full-glass concentrator.

32.5 Glass plate: 5 cm × 20 cm.

32.6 Thin-layer plate applicator.

Note: Commercially available thin-layer plate which is suitable for testing of aflatoxin testing may be used.

32.7 Developing tank: length 25 cm, width 6 cm, height 4 cm.

32.8 Ultra-violet lamp: 100 W ~ 125 W, equipped with a 365 nm optical filter.

32.9 Microinjector or haemochrome pipette.

33 Analytical steps

Caution: The entire operation shall be carried out in darkroom conditions.

33.1 Extraction of sample

33.1.1 Corn, rice, wheat, flour, dried potato, beans, peanuts, peanut butter, etc.

33.1.1.1 Method A: WEIGH 20.00 g of crushed and sieved specimen (flour, peanut butter do not need to be crushed); PLACE it in a 250 mL stoppered conical flask; ADD 30 mL of n-hexane or petroleum ether as well as 100 mL of aqueous methanol solution; APPLY a layer of water onto the stopper; COVER it tightly to avoid leakage. OSCILLATE it for 30 min; LET it be standing for a while; USE the folded fast filter paper to filter it into a separatory funnel. After

33.1.3 Soy sauce, vinegar

WEIGH 10.00 g of the specimen in a small beaker. To prevent emulsification during the extraction, ADD 0.4 g of sodium chloride; TRANSFER it into a separatory funnel; USE 15 mL of chloroform to rinse the beaker for several times; COLLECT the rinsing solution into the separatory funnel. PERFORM the subsequent steps from "SHAKE it for 2 min; LET it be standing for delamination ....." in accordance with 33.1.1.1. Finally ADD 2.5 mL of benzene-acetonitrile mixed solution. Each millimeter of this solution is equivalent to 4 g of specimen.

Or WEIGH 10.00 g of specimen; PLACE it in a separatory funnel; then ADD 12 mL of methanol (because the volume of soy sauce is used instead of water, the volume ratio of methanol to water is still about 55:45); USE 20 mL of chloroform to extract it. PERFORM the subsequent steps from "SHAKE it for 2 min; LET it be standing for delamination ....." in accordance with 33.1.1.1. Finally ADD 2.5 mL of benzene-acetonitrile mixed solution. Each millimeter of this solution is equivalent to 4 g of specimen.

33.1.4 Dry sauces (including fermented soybean, fermented bean curd products)

WEIGH 20.00 g of the uniformly ground specimen; PLACE it in a 250 mL stoppered conical flask; ADD 20 mL of n-hexane or petroleum ether as well as 50 mL of aqueous methanol solution. SHAKE it for 30 min; LET it be standing for a while; USE the folded fast qualitative filter paper to filter it. After the filtrate is standing for delamination, TAKE 24 mL of methanol water layer (equivalent to 8 g of specimen, including 8 g of dry sauce itself which contains about 4 mL of water); PLACE it in a separatory funnel; ADD 20 mL of chloroform. PERFORM the subsequent steps from "SHAKE it for 2 min; LET it be standing for delamination ....." in accordance with 33.1.1.1. Finally ADD 2 mL of benzene-acetonitrile mixed solution. Each millimeter of this solution is equivalent to 4 g of specimen.

33.2 Determination

33.2.1 One-way developing method

33.2.1.1 Preparation of thin-layer plates

WEIGH about 3 g of silica gel G; ADD water equivalent to 2 times ~ 3 times of silica gel; GRIND it hard for 1 min ~ 2 min until it is into a paste; immediately POUR it into the applicator; COAT it into three 5 cm × 20 cm thin-layer plates which have a thickness about 0.25 mm. After drying it in the air for about 15 min; ACTIVATE it at 100 °C for 2 h; TAKE it out; PRESERVE it in a desiccator. Generally, it can be preserved for 2 d ~ 3 d. If it is placed for a long time, it can be used after reactivation.

derivative is about 0.1. DROP two points to the left of the thin-layer plate sequentially.

First point-drop: 10 μL of 0.04 μg/mL AFT B_1 standard working solution.

Second point-drop: 20 μL of sample solution. ADD a small drop of trifluoroacetic acid to the above two points, respectively; after reaction for 5 min, USE a hair dryer to blow hot air against it for 2 min; LET the temperature of the hot air which is blown to the thin-layer plate is not more than 40 °C; then ADD the following two points onto the thin-layer plate.

Third point-drop: 10 μL of 0.04 μg / mL AFT B_1 standard working solution.

Fourth point-drop: 20 μL of sample solution.

MAKE it develop again (same as 16.2.1.3); under ultraviolet light, OBSERVE whether the sample solution produces the same derivative as the AFT B_1 standard point. The third and fourth points on which the trifluoroacetic acid is not added can be used as the derivative blank control for the sample solution and the standard.

33.2.1.5 Quantification of dilution

If the fluorescence intensity of the AFT B_1 fluorescent spot in the sample solution is consistent with the fluorescence intensity of the lowest detected amount of the AFT B_1 standard point (0.0004 μg), the AFT B_1 content in the specimen is 5 μg/kg. If the fluorescence intensity in the sample solution is more intense than the minimum detected amount, then based on its intensity estimate, reduce the microliter number or otherwise add different microliter number after diluting the sample solution, until the fluorescence intensity of the sample solution point is consistent with that of the minimum detected amount. The dropping pattern is as follows:

First point-drop: 10 μL of AFT B_1 standard working solution (0.04 μg/mL)

Second point-drop: ADD 10 μL of sample solution dropwise in accordance with the situation.

Third point-drop: ADD 15 μL of sample solution dropwise in accordance with the situation.

Fourth point-drop: ADD 20 μL of sample solution dropwise in accordance with the situation.

33.2.1.6 Calculation of results

The content of AFT B_1 in the specimen is calculated in accordance with formula (5):

another 10 μL of the AFT B_1 standard use solution (0.04 μg/mL) to the sample solution point on the second plate; ADD another 10 μL of 0.2 μg/mL AFT B_1 standard use solution to the sample solution point on the third plate.

33.2.2.1.2 Development

33.2.2.1.2.1 Transverse development: Along the long side of the developing tank, PLACE a glass bracket; ADD 10 mL of anhydrous ethanol; PLACE the long side of the thin-layer plate to which sample is applied and which is close to the standard point int the developing tank to develop it; after developing to the plate's end, TAKE it out to evaporate it dry. Or otherwise REPEAT development again for 1 ~ 2 times if required, based on actual conditions.

33.2.2.1.2.2 Longitudinal deployment: USE the acetone-chloroform (8 + 92) to develop the thin-layer plate which is evaporated dry to 10 cm ~ 12 cm. The ratio of acetone to chloroform is self-regulating in accordance with different conditions.

33.2.2.1.3 Observation and assessment results

Under the ultraviolet light, OBSERVE the first plate and the second plate; if the minimum detected amount occurs at the second point of the second plate in the position corresponding to the AFT B_1 standard point, whilst the fluorescent point does not occur at the same positions on the first plate and the second plate, the content of AFT B_1 in the specimen is below 5 μg/kg.

If the fluorescent points occur at the same position of the first plate and the second plate, COMPARE the first plate with the third plate, to check whether the fluorescent point at the same position of the second point of the third plate and the second point of the first point overlaps with the AFT B_1 standard point; if they overlap, PERFORM the confirmation test. In the specific determination, the first, second and third plates can be subjected to confirmation test at the same time or in sequence. If it is subjected to confirmation test in sequence, when the first plate is negative, the third plate can be exempted of such test. If the first plate is positive, the second plate can be exempted of such test, TEST the third plate directly.

33.2.2.1.4 Confirmation test

TAKE another two thin-layer plates; ADD 10 μL of AFT B_1 standard use solution (0.04 μg/mL) and 1 small drop of trifluoroacetic acid to the fourth and fifth plates at a distance 0.8 cm ~ 1 cm from the left edge. At the position 2.8 cm ~ 3 cm from the left edge, ADD 20 μL of sample solution and 1 small drop of trifluoroacetic acid dropwise to the fourth plate; ADD 20 μL of the sample solution, 10 μL of the AFT B_1 standard use solution (0.04 μg/mL) and 1 small drop of trifluoroacetic acid dropwise onto the fifth plate. After reacting for 5 minutes, USE hair dry to blow hot air against it for 2 min; LET the temperature

following confirmation test.

33.2.2.2.4 Confirmation test

TAKE another two plates; at 0.8 cm ~ 1 cm from the left edge; ADD 20 μL of sample solution and 1 drop of trifluoroacetic acid onto the fourth plate; ADD 20 μL of sample solution, 10 μL of 0.04 μg/mL AFT B_1 standard use solution, 1 drop of trifluoroacetic acid onto the fifth plate. The derivative and developing method are the same as 33.2.2.1. OBSERVE the above two plates under an ultraviolet lamp, to determine whether the sample solution point produces a derivative which overlaps with the AFT B_1 standard point. When observed, it may use the first plate as the derivative blank plate of the sample solution. After the above confirmation test, if it is positive, PERFORM the quantification of dilution; if the content of AFT B_1 is low, it requires no dilution or otherwise the dilution factor is small, but the impurity fluorescence still has serious interference, it may be based on the intensity of the fluorescence of the aflatoxin B_1 in the sample solution, it is directly quantified by the use of two-way developing method.

33.2.2.2.5 Calculation of results

Same as 33.2.1.6.

34 Precision

TAKE two sets for each specimen to perform parallel determination; USE the arithmetic mean as the analytical result.

The relative difference of the analytical results shall be not more than 60%.

35 Others

The minimum detected amount of aflatoxin B_1 on the thin-layer plate is 0.0004 μg, the limit of detection is 5 μg/kg.

Appendix B

Method of verification of immunoaffinity column

B.1 Verification of column capacity

In 30 mL of 1% Triton X-100 (or Tween-20)-PBS, ADD 600 ng of AFT B_1 standard stock solution; MIX it uniformly. Respectively TAKE 3 immunoaffinity columns from the same batch; the sample loading amount of each column is 10 mL. After loading, washing, eluting, COLLECT the eluate; USE nitrogen to blow it dry to 1 mL; USE the initial mobile phase to make its volume reach to 10 mL; USE liquid chromatography to separate and determine the content of AFT B_1.

Judgement of result: If the result is AFT B_1 ≥ 160 ng, it is judged as a usable commodity.

B.2 Verification of column's recovery rate

In 30 mL of 1% Triton X-100 (or Tween-20)-PBS, ADD 600 ng of AFT B_1 standard stock solution; MIX it uniformly. Respectively TAKE 3 immunoaffinity columns from the same batch; the sample loading amount of each column is 10 mL. After loading, washing, eluting, COLLECT the eluate; USE nitrogen to blow it dry to 1 mL; USE the initial mobile phase to make its volume reach to 10 mL; USE liquid chromatography to separate and determine the content of AFT B_1.

Judgement of result: If the result is AFT B_1 ≥ 160 ng, that is, the recovery rate ≥ 80%, it is judged as a usable commodity.

B.3 Verification of cross-reaction rate

In 30 mL of 1% Triton X-100 (or Tween-20)-PBS, ADD 300 ng of AFT G_2 standard stock solution; MIX it uniformly. Respectively TAKE 3 immunoaffinity columns from the same batch; the sample loading amount of each column is 10 mL. After loading, washing, eluting, COLLECT the eluate; USE nitrogen to blow it dry to 1 mL; USE the initial mobile phase to make its volume reach to 10 mL; USE liquid chromatography to separate and determine the content of AFT G_2.

Judgement of result: If the result is AFT G_2 ≥ 80 ng, it can be judged as the commodity that can be used for simultaneous determination of AFT B_1, AFT B_2, AFT G_1, and AFT G_2.

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