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Determination of multi-residues of chloramphenicol in animal-original food

动物源性食品中氯霉素类药物残留量测定

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Determination of multi-residues of chloramphenicol in animal-original food

1 Scope

This Standard specifies the methods of gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry/mass spectrometry to determine the multi-residues of chloramphenicol in animal-original food.

This Standard is applicable to the qualitative confirmation and quantitative determination of chloramphenicol, florfenicol and thiamphenicol residues in aquatic products, livestock and poultry products and livestock and poultry by-products.

2 Gas chromatography-mass spectrometry

2.1 Principle

The sample is extracted by ethyl acetate. It is distributed and purified by 4% sodium chloride solution and n-hexane solution. After purification by Florisil column, take toluene as the reaction medium. Use N,O bis(trimethylsilyl)trifluoroacetamide-trimethylchlorosilane (BSTFA+TMCS, 99+1) to silanize at 70°C. Use gas chromatography/negative chemical ionization source mass spectrometry to determine. Use internal standard working curve method for quantification.

2.2 Reagents and materials

Unless otherwise stated, it shall only use the confirmed analytically-pure reagents and secondary deionized water or water of equivalent purity in the analysis.

2.2.1 Methanol: Chromatographically pure.

2.2.2 Toluene: Pesticide residue level.

2.2.3 N-hexane: Pesticide residue level.

2.2.4 Ethyl acetate.

2.2.5 Ether.

2.2.6 Sodium chloride.

2.2.7 Chloramphenicol (CAP), florfenicol (FF), thiamphenicol (TAP) standard material:

2.4 Determination steps

2.4.1 Extraction

Weigh 10g (accurate to 0.01g) of the crushed tissue sample into a 50mL stoppered centrifuge tube. Add 1.0mL of internal standard solution (2.2.11) and 30mL of ethyl acetate. Shake for 30min. Centrifuge at 4000r/min for 2min. Transfer the supernatant to a round bottom flask. Use 30mL of ethyl acetate to extract the residue one more time. Combine the extracts. Conduct rotary evaporation at 35 ℃ to 1mL ~ 2mL, for purification.

2.4.2 Purification

2.4.2.1 Liquid-liquid extraction

Extract the concentrate (2.4.1). Add 1mL of methanol to dissolve. Use 20mL of sodium chloride solution (2.2.9) and 20mL of n-hexane to conduct liquid-liquid extraction. Discard the n-hexane layer. Use 40mL of ethyl acetate to extract the aqueous phase twice. Combine the ethyl acetate phases in a heart-shaped flask. Rotate and evaporate to near dryness at 35°C. Use nitrogen to dry slowly.

2.4.2.2 Florey silica column purification

Use 5mL of methanol, 5mL of methanol-diethyl ether (3+7) solution and 5mL of diethyl ether successively to rinse Flory silica column, for future use. Use 5.0mL of ether to dissolve the residue (2.4.2.1) and load the sample. Use 5.0mL of ether to rinse the Florisil column. Use 5.0mL of methanol-diethyl ether solution (3+7) to elute. Use nitrogen to slowly blow dry the nitrogen, for silanization.

2.4.3 Silanization

Use 0.2mL of toluene to dissolve the purified specimen (2.4.2.2). Add 0.1mL of silanization reagent (2.2.13) to mix. Derivatize at 70°C for 60min. Use nitrogen to blow dry slowly. Use 1.0mL of n-hexane to set volume constant, for determination.

2.4.4 Determination

2.4.4.1 Gas chromatography-mass spectrometry conditions

a) Chromatographic column: DB-5MS capillary column, 30m×0.25mm (inner diameter)×0.25μm, or its equivalent;

b) Column temperature: at 50°C for 1min; 25°C/min to 280°C; hold for 5 min;

c) Inlet temperature: 250°C;

d) Injection method: splitless injection; splitless time is 0.75min;

e) Carrier gas: high-purity helium; purity ≥99.999%;

2.7 Recovery rate and precision

See Annex B.

3 Liquid chromatography-mass spectrometry/mass spectrometry

3.1 Principle

For residues of chloramphenicol, thiamphenicol and florfenicol in different animal-original food, use acetonitrile, ethyl acetate-ether or ethyl acetate to respectively extract. The extract is purified with a solid phase extraction cartridge. Use liquid chromatography-mass spectrometry/mass spectrometer to determine. Chloramphenicol is quantified by internal standard method. Thiamphenicol and florfenicol are quantified by external standard method.

3.2 Reagents and materials

Unless otherwise stated, it shall only use the confirmed analytically-pure reagents and secondary deionized water or water of equivalent purity in the analysis.

3.2.1 Methanol: liquid chromatography level.

3.2.2 Acetonitrile: liquid chromatography level.

3.2.3 Acetone: liquid chromatography level.

3.2.4 n-propanol: liquid chromatography level.

3.2.5 n-hexane: liquid chromatography level.

3.2.6 Ethyl acetate: liquid chromatography level.

3.2.7 Diethyl ether.

3.2.8 Sodium acetate.

3.2.9 Ammonium acetate.

3.2.10 β-glucuronidase: about 40,000 activity units.

3.2.11 Acetonitrile saturated n-hexane: Take 200mL of n-hexane (3.2.5) into a 250mL separatory funnel. Add a small amount of acetonitrile (3.2.2). Shake vigorously. After standing for stratification, discard the lower acetonitrile layer.

3.2.12 Acetone-n-hexane (1+9): Mix acetone (3.2.3) and n-hexane (3.2.5) well in a volume ratio of 1:9.

3.2.13 Acetone-n-hexane (6+4): Mix acetone (3.2.3) and n-hexane (3.2.5) well in a volume ratio of 6:4.

3.2.14 Ethyl acetate-diethyl ether (75+25): Mix 75mL of ethyl acetate (3.2.6) and 25mL of ether (3.2.7) solution well.

3.2.15 Sodium acetate buffer (0.1mol/L): Weigh 13.6g of sodium acetate (3.2.8) into a 1000mL volumetric flask. Add 980mL of water to dissolve and mix well. Use acetic acid to adjust the pH to 5.0. Set the volume to the scale and mix well.

3.2.16 Ammonium acetate solution (10mmol/L): Weigh 0.77g of ammonium acetate (3.2.9) into a 1000mL volumetric flask. Use water to set the volume to the scale. Mix well.

3.2.17 Chloramphenicol, thiamphenicol and florfenicol standard substances: purity is ≥99.0%.

3.2.18 Chloramphenicol deuterated internal standard (chloramphenicol-D_5) substance: purity is ≥99.0%.

3.2.19 Standard stock solution: Accurately and respectively weigh an appropriate amount of chloramphenicol, thiamphenicol and florfenicol standard substances (3.2.17) (accurate to 0.1mg). Use acetonitrile to prepare 500μg/mL standard stock solution (it can be used for 6 months when stored in the dark at 4°C).

3.2.20 Standard intermediate solutions of chloramphenicol, thiamphenicol and florfenicol: Accurately and respectively pipette appropriate amounts of standard stock solutions of chloramphenicol, thiamphenicol and florfenicol (3.2.19). Use acetonitrile to dilute to 50μg/mL standard intermediate solution of chloramphenicol, thiamphenicol and florfenicol (it can be used for 3 months when stored in the dark at 4°C).

3.2.21 Mixed standard working solution of chloramphenicol, thiamphenicol and florfenicol: Accurately and respectively pipette an appropriate amount of standard intermediate solutions of chloramphenicol, thiamphenicol and florfenicol (3.2.20). Use mobile phase to dilute to a suitable mixed standard working solution (prepare when it is required).

3.2.22 Chloramphenicol deuterated internal standard (chloramphenicol-D_5) stock solution: Accurately weigh an appropriate amount of chloramphenicol-D_5 standard substance (3.2.18) (accurate to 0.1mg). Use acetonitrile to prepare 100μg/mL standard stock solution (it can be used for 12 months when stored in the dark at 4°C).

3.2.23 Chloramphenicol deuterated internal standard (chloramphenicol-D_5) intermediate solution: Accurately pipette an appropriate amount of chloramphenicol-D_5 stock solution (3.2.22). Use acetonitrile to prepare 1μg/mL internal standard intermediate solution (it can be used for 6 months when stored in the dark at 4°C).

The specimen shall be stored at -20°C.

3.5 Determination steps

3.5.1 Extraction

3.5.1.1 Animal tissues (except liver and kidney) and aquatic products

Weigh 5g of specimen (accurate to 0.01g). Place it in a 50mL centrifuge tube. Add 100μL of chloramphenicol deuterated internal standard (chloramphenicol-D_5) working solution (3.2.24) and 30mL of acetonitrile. Homogenate. Centrifuge for 5min. Transfer the supernatant to a 250mL separatory funnel. Add 15mL of acetonitrile-saturated n-hexane (3.2.11). Shake for 5min. Let it still for layering. Transfer the acetonitrile layer to a 100 mL brown heart-shaped bottle. Add 30mL of acetonitrile to the residue. Shake for 3min. Centrifuge for 5min. Transfer the supernatant to the same separatory funnel. Shake for 5min. Let it still for layering. Transfer the acetonitrile layer to the same brown heart-shaped bottle. Add 5mL of n-propanol to the heart-shaped bottle. Evaporate to dryness in a water bath at 40°C. Blow dry with nitrogen. Add 5mL of acetone-n-hexane (3.2.12) to dissolve the residue.

3.5.1.2 Animal liver and kidney tissue

Weigh 5g of specimen (accurate to 0.01g). Place in a 50mL centrifuge tube. Add 30mL of sodium acetate buffer (3.2.15). Homogenize for 2min. Add 300μL of β-glucuronidase (3.2.10). Incubate overnight at 37°C. Add 100μL of chloramphenicol deuterated internal standard (chloramphenicol-D_5) working solution (3.2.24) and 20mL of ethyl acetate-diethyl ether (3.2.14) to the digested sample. Shake for 2min. Centrifuge for 5min. Take the upper organic layer into a heart-shaped bottle. Make it nearly dry by rotary evaporation in a water bath at 40°C. Blow dry with nitrogen. Add 5mL of acetone-n-hexane (3.2.12) to dissolve the residue.

3.5.1.3 Honey

Weigh 5g of honey specimen (accurate to 0.01g). Place in a 50mL centrifuge tube. Add 100μL of chloramphenicol deuterated internal standard (chloramphenicol-D_5) working solution (3.2.24), 5mL of water. Mix well. Add 20mL of ethyl acetate. Shake for 2min. Centrifuge for 5min. Pipette the organic layer into a 100mL brown heart-shaped bottle. Add 20mL of ethyl acetate to the centrifuge tube. Shake for 2min. Centrifuge for 5min. Combine the organic layers in a brown heart-shaped bottle. Conduct rotary evaporation to dryness in a 40°C water bath. Use 3mL of water to dissolve the residue. Mix well.

3.5.2 Purification

3.5.2.1 Animal tissue and aquatic products

Use 5mL of acetone-n-hexane (3.2.12) to rinse the LC-Si silica gel cartridge. Discard the eluent. Transfer the residue solution (3.5.1.1, 3.5.1.2) to the solid phase extraction

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