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Determination of residues of nitrofuran metabolites in foodstuffs of animal origin -- HPLC-MS/MS method

动物源性食品中硝基呋喃类药物代谢物残留量检测方法

高效液相色谱／串联质谱法

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1 Scope

This Standard specifies the HPLC-MS/MS method for determination of residues for nitrofuran metabolites: 3-amino-2-oxazolidinone (AOZ), 5-morpholinomethyl-3-amino-2-oxazolidinone (AMOZ), 1-amino-hydantoin (AHD) and semi-carbazide (SEM), in foodstuffs of animal origin.

This Standard is applicable to the qualitative confirmation and quantitative determination of residues for nitrofuran metabolites: 3-amino-2-oxazolidinone, 5-morpholinomethyl-3-amino-2-oxazolidinone, 1-amino-hydantoin and semi-carbazide, in muscle, offal, fish, shrimp, eggs, milk, honey and casings.

2 Normative references

The provisions in following documents become the provisions of this Standard through reference in this Standard. For dated references, the subsequent amendments (excluding corrigendum) or revisions do not apply to this Standard, however, parties who reach an agreement based on this Standard are encouraged to study if the latest versions of these documents are applicable. For undated references, the latest edition of the referenced document applies.

GB/T 6682, *Water for analytical laboratory use -- Specification and test methods* (GB/T 6682-1992, neq ISO 3696:1987)

3 Principle

The sample is hydrolyzed by hydrochloric acid. O-nitrobenzaldehyde is derivatized overnight. After adjusting the pH value to 7.4, use ethyl acetate to extract and n-hexane to purify. Analytes are qualitatively detected by HPLC-MS/MS. Use stable isotope internal standard method for quantitative determination.

4 Reagents and materials

Unless otherwise specified, all reagents are analytically pure, and the water is grade one water specified in GB/T 6682.

4.1 Methanol: HPLC level.

4.2 Acetonitrile: HPLC level.

4.3 Ethyl acetate: HPLC level.

4.4 N-hexane: HPLC level.

4.5 Concentrated hydrochloric acid.

4.6 Sodium hydroxide.

4.7 Formic acid: HPLC level.

4.8 O-nitrobenzaldehyde.

4.9 Potassium phosphate trihydrate.

4.10 Ammonium acetate.

4.11 0.2 mol/L hydrochloric acid solution: Accurately measure 17 mL of concentrated hydrochloric acid (4.5). Use water to set the volume constant to 1 L.

4.12 2.0 mol/L sodium hydroxide solution: Accurately weigh 80 g of sodium hydroxide (4.6). Use water to dissolve and set the volume constant to 1 L.

4.13 0.1 mol/L o-nitrobenzaldehyde solution: Accurately weigh 1.5 g of o-nitrobenzaldehyde (4.8). Use methanol to dissolve and set the volume constant to 100 mL.

4.14 0.3 mol/L potassium phosphate solution: Accurately weigh 79.893 g of potassium phosphate trihydrate (4.9). Use water to dissolve and set the volume constant to 1 L.

4.15 Acetonitrile saturated n-hexane: Measure 80 mL of n-hexane into a 100 mL separating funnel. After adding an appropriate amount of acetonitrile, shake vigorously. After the distribution is balanced, discard the acetonitrile layer to obtain.

4.16 0.1% formic acid aqueous solution (containing 0.0005 mol/L ammonium acetate): Accurately measure 1 mL of formic acid (4.7) and 0.0386 g of ammonium acetate (4.10) into a 1 L volumetric flask. Use water to set the volume constant to 1 L.

4.17 Standard material: 3-amino-2-oxazolidinone, 5-morpholinomethyl-3-amino-2-oxazolidinone, 1-amino-hydantoin and semi-carbazide. The purity is ≥ 99%.

4.18 Internal standard substances: Internal standard substance of 3-amino-2-oxazolidinone, D_4-AOZ; internal standard substance of 5-morpholinomethyl-3-amino-2-oxazolidinone, D_5-AMOZ; internal standard substance of 1-amino-hydantoin, ^{13}C-AHD; and internal standard substance of semi-carbazide, $^{13}C^{15}N$-SEM. The purity is ≥ 99%.

5.3 Analytical balance: resolution is 0.0001 g, 0.01 g.

5.4 Homogenizer: 10000 r/min.

5.5 Oscillator.

5.6 Constant temperature box.

5.7 pH meter: measurement accuracy is ±0.02pH unit.

5.8 Centrifuge: 10000 r/min.

5.9 Nitrogen blowing instrument.

5.10 Vortex mixer.

5.11 Volumetric flasks: 1 L, 100 mL, 10 mL.

5.12 Stoppered plastic centrifuge tube: 50 mL.

5.13 Graduated test tube: 10 mL.

5.14 Pipette guns: 5 mL, 1 mL, 100 μL.

6 Specimen preparation and storage

6.1 Muscle, viscera, fish and shrimp

Take about 500 g of representative sample from the original sample. Use the tissue masher to thoroughly mash and mix. Divide into two equal parts. Respectively put them into clean containers as samples. Seal. Indicate the mark. Store the specimens in a freezer at -18°C from light.

6.2 Casing

Take about 100 g of representative sample from the original sample. Use scissors to cut into squares with side length < 5 mm. Divide into two equal parts after mixing. Respectively put them into clean containers as samples. Seal. Indicate the mark. Store the specimens in a freezer at -18°C from light.

6.3 Egg

Take about 500 g of representative sample from the original sample. After shelling, use the tissue masher to stir and mix thoroughly. Divide into two equal parts. Respectively put them into clean containers as samples. Seal. Indicate the mark. Store the specimens in a refrigerator at 4°C from light.

6.4 Milk and honey

Take about 500 g of representative sample from the original sample. use the tissue masher to stir and mix thoroughly. Divide into two equal parts. Respectively put them into clean containers as samples. Seal. Indicate the mark. Store the specimens in a refrigerator at 4°C from light.

NOTE: During the operation of sample preparation, the contamination of the sample or the change of the residue content shall be prevented.

7 Sample processing

7.1 Hydrolysis and derivatization

7.1.1 Muscle, viscera, fish, shrimp and casing

Weigh about 2 g of specimen (accurate to 0.01 g) into a 50 mL plastic centrifuge tube. Add 10 mL of methanol-water mixed solution (1+1, volume ratio). After oscillating for 10 min, centrifuge at 4000 r/min for 5 min. Discard the liquid. Add 10 mL of 0.2 mol/L hydrochloric acid to the residue. Use the homogenizer to homogenize at 10000 r/min for 1 min. Add 100 μL of mixed internal standard solution (4.24) and 100 μL of o-nitrobenzaldehyde solution (4.13) in turn. Vortex for 30 s. Then oscillate for 30 min. Place in a 37°C incubator overnight (16 h) to react.

7.1.2 Egg, milk and honey

Weigh about 2 g of specimen (accurate to 0.01 g) into a 50 mL plastic centrifuge tube. Add 10 mL ~ 20 mL of 0.2 mol/L hydrochloric acid (subject to the complete infiltration of the sample). Use the homogenizer to homogenize at 10000 r/min for 1 min. Add 100 μL of mixed internal standard solution (4.24) and 100 μL of o-nitrobenzaldehyde solution (4.13) in sequence. Vortex for 30 s. Then oscillate for 30 min. Place in a 37°C incubator overnight (16 h) to react.

7.2 Extraction and purification

Take out the sample. Cool to room temperature. Add 1 mL ~ 2 mL of 0.3 mol/L potassium phosphate (1 mL of hydrochloric acid solution plus 0.1 mL of potassium phosphate solution). After adjusting the pH to 7.4 (± 0.2) with 2.0 mol/L sodium hydroxide, add 10 mL ~ 20 mL of ethyl acetate (the volume of ethyl acetate added is the same as the volume of hydrochloric acid solution). Oscillate and extract for 10 min. Centrifuge at 10000 r/min for 10 min. Collect the ethyl acetate layer. Use 10 mL ~ 20 mL of ethyl acetate to extract the residue one more time. Combine the ethyl acetate layers. The collected solution is blown dry with N_2 at 40°C. The residue is dissolved in 1 mL of 0.1% formic acid aqueous solution (4.16). Then use 3 mL of acetonitrile-saturated n-hexane (4.15) for two liquid-liquid partitions to remove fat. After the lower aqueous phase passed through a 0.20 μm microporous membrane, take 10 μL for instrumental measurement.

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