Original: Japanese
Provisional translation

Analytical Method for Fenthion (Animal and Fishery Products)

1. Analytes

Fenthion
Fenthion sulfoxide (hereinafter referred to as metabolite B)
Fenthion sulfone (hereinafter referred to as metabolite C)
Fenthion oxon (hereinafter referred to as metabolite D)
Fenthion-oxon-sulfoxide (hereinafter referred to as metabolite E)
Fenthion-oxon-sulfone (hereinafter referred to as metabolite F)

2. Application

Animal and fishery products

3. Instrument

Liquid chromatograph-tandem mass spectrometer (LC-MS/MS)

4. Reagents

Use the reagents listed in Section 3 of the General Rules, except the following.
Reference standard of fenthion: Contains not less than 97% of fenthion.
Reference standard of metabolite B: Contains not less than 98% of metabolite B.
Reference standard of metabolite C: Contains not less than 98% of metabolite C.
Reference standard of metabolite D: Contains not less than 98% of metabolite D.
Reference standard of metabolite E: Contains not less than 98% of metabolite E.
Reference standard of metabolite F: Contains not less than 98% of metabolite F.

5. Procedure

1) Extraction

i) Except for honey

Add 100 mL of acetone to 10.0 g of sample, homogenize, and filter with suction. Add 50 mL of acetone to the residue on the filter paper, homogenize, and filter with suction as described above. Combine the resulting filtrates and add acetone to make exactly 200 mL. Take exactly a 2 mL aliquot of the solution, add 30 mL of *n*-hexane, and extract with shaking twice with 30 mL each of acetonitrile saturated with *n*-hexane.

ii) Honey

Dissolve 10.0 g of sample in 20 mL of water. Add 100 mL of acetone to the sample, homogenize, and filter with suction. Add 10 mL of water and 50 mL of acetone to the residue on the filter paper, homogenize, and filter with suction as described above. Combine the resulting filtrates and add acetone to make exactly 200 mL. Take exactly a 2 mL aliquot of the solution, add 30 mL of *n*-hexane, and extract with shaking twice

with 30 mL each of acetonitrile saturated with *n*-hexane.

2) Clean-up

Inject 10 mL of acetonitrile saturated with *n*-hexane into a graphite carbon/aminopropylsilanized silica gel layered cartridge (500 mg/500 mg), and discard the effluent. Transfer the acetonitrile layer obtained in 1) to the cartridge, concentrate the eluate at below 40°C, and remove the solvent. Dissolve the residue in 2 mL of acetone.

3) Oxidation reaction

Add 1 mL of 5 w/v% potassium permanganate solution to the solution obtained in 2), shake gently to mix, let stand for 10 min at room temperature, concentrate to approximately 1 mL at below 40°C, and add 3 mL of water.

4) Clean-up

Inject the solution obtained in 3) into a porous diatomaceous earth cartridge (for holding 5 mL), let stand for 5 min at room temperature, add 40 mL of ethyl acetate and *n*-hexane (1:1) mixed solution, concentrate the eluate at below 40°C, and remove the solvent. Dissolve the residue in 2 mL of water and methanol (1:1) mixed solution and use this solution as the test solution.

6. Calibration curve

Dissolve each reference standard of metabolite C and metabolite F in acetone respectively to make 500 mg/L, and use these solutions as stock standard solutions. Prepare standard solutions of several concentrations by mixing each stock standard solution appropriately and diluting with a mixed solution of water and methanol (1:1). Inject each solution into LC-MS/MS respectively, and make calibration curves by peak-height or peak-area method. When the test solution is prepared following the above procedure, the concentration of metabolite C and metabolite F in the test solution corresponding to 0.01 mg/kg in the sample is 0.0005 mg/L.

7. Quantification

Inject the test solution into LC-MS/MS and calculate each concentration of metabolite C and metabolite F from the calibration curve prepared in **6**. Use the following equation to calculate the concentration of fenthion including its metabolites.

Concentration (ppm) of fenthion (including its metabolites) = A × 0.8969 + B × 0.9459

A: Concentration (ppm) of metabolite C

B: Concentration (ppm) of metabolite F

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

Column: Octadecylsilanized silica gel, 2.1 mm inside diameter, 150 mm in length and 5 μm in particle diameter.

Column temperature: 40°C

Mobile phase: Linear gradient from a mixed solution of 2 mmol/L ammonium acetate solution

and methanol (7:3) to (3:7) in 10 min and hold at (3:7) for 5 min.

Ionization mode: ESI (+)

Major monitoring ions (*m/z*)

Metabolite C: Precursor ion 311, product ions 125, 109

Metabolite F: Precursor ion 295, product ions 217, 104

Injection volume: 5 µL

Expected retention time

Metabolite C: 11 min

Metabolite F: 6 min

10. Limit of quantification

0.01 mg/kg for each analyte

11. Explanatory note

1) Outline of analytical method

The method consists of extracting fenthion and its metabolites from sample with acetone, defatting by liquid-liquid partitioning using acetonitrile and hexane, cleaning up with a graphite carbon/aminopropylsilanized silica gel layered cartridge, oxidizing with potassium permanganate to metabolite C or metabolite F, cleaning up with a porous diatomaceous earth cartridge, and quantifying and confirming using LC-MS/MS.

2) Notes

i) If acetone remains during clean-up using a porous diatomaceous earth cartridge, potassium permanganate will not be removed sufficiently, resulting in a purple liquid being eluted. Therefore, ensure that acetone is thoroughly removed during concentration.

ii) When the analytical method for metabolite C and metabolite F using LC-MS/MS was developed, the following monitoring ions were used:

Metabolite C

for quantitative ions (*m/z*): precursor ion 311, product ion 125

for qualitative ions (*m/z*): precursor ion 311, product ion 109

Metabolite F

for quantitative ions (*m/z*): precursor ion 295, product ion 217

for qualitative ions (*m*/*z*): precursor ion 295, product ion 104

iii) Food items used to develop the analytical method: cattle (muscle, fat, liver and milk), chicken (muscle and egg), honey, eel, salmon and *Corbicula* (freshwater clam).

12. Reference

None

13. Type

C