Original: Japanese

Provisional Translation

Analytical Method for Spiromesifen (Animal and Fishery Products)

1. Analytes

Spiromesifen

4-Hydroxy-3-mesityl-1-oxaspiro[4.4]nona-3-en-2-one (hereafter referred to as the enol compound)

2. Instruments

Liquid chromatograph-mass spectrometer (LC-MS)

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

3. Reagents

Use the reagents listed in Section 3 of the General Rules, except the following.

Reference standard of spiromesifen: Contains not less than 98% of spiromesifen. Melting point of the standard is 96–101°C.

Reference standard of the enol compound: Contains not less than 99% of the enol compound. Melting point of the standard is 256–258°C.

4. Procedure

1) Extraction

Add 100 mL of acetonitrile/formic acid/water (80:1:20, v/v/v) and 10 mL of *n*-hexane to 5.00 g of sample, homogenize, and centrifuge at 3,000 rpm for 5 minutes. Collect the acetonitrile/formic acid/water layer. Add 50 mL of acetonitrile/formic acid/water (80:1:20, v/v/v) to the *n*-hexane layer and the residue, homogenize, and centrifuge as described above. Combine the acetonitrile/formic acid/water layers and add acetonitrile/formic acid/water (80:1:20, v/v/v) to make exactly 200 mL. Take a 20 mL aliquot of the solution, and concentrate to about 4 mL at below 40°C.

2) Clean-up

Add 10 mL each of acetonitrile and water to an octadecylsilanized silica gel cartridge (1,000 mg) sequentially, and discard the effluent. Add 10 mL of water to the solution obtained in 1), and transfer to the cartridge. Wash the container with 10 mL of acetonitrile/0.01% formic acid (2:3, v/v), add the washing to the cartridge, and discard the effluent. Elute with 10 mL of acetonitrile/0.01% formic acid (9:1, v/v), add acetonitrile/0.01% formic acid (9:1, v/v) to the eluate to make exactly 10 mL, and use this solution as the test solution.

5. Calibration curve

Prepare 0.0005–0.01 mg/L spiromesifen and 0.00025–0.005 mg/L the enol compound standard solutions (acetonitrile/0.01% formic acid (9:1, v/v)). Inject 10 µL of each standard solution to LC-MS or 4µL to LC-MS/MS, and make calibration curves by peak-height or peak-area method.

6. Quantification

Inject 10 µL of the test solution to LC-MS or 4µL to LC-MS/MS, and calculate the concentration of spiromesifen and the enol compound from the calibration curves made in **5**. Use the following equation to calculate the concentration of spiromesifen including that of the enol compound.

Concentration (ppm) of spiromesifen (including that of the enol compound)

= A + B × 1.36

A: Concentration (ppm) of spiromesifen

B: Concentration (ppm) of the enol compound

7. Confirmation

Confirm using LC-MS or LC-MS/MS.

8. Measurement conditions

Column: Octadecylsilanized silica gel, 2.0 mm in inside diameter, 150 mm in length and 5 µm in particle diameter

Column temperature: 40°C

Mobile phase: Linear gradient from acetonitrile/0.01% formic acid (1:1, v/v) to (9:1, v/v) in 4 min and hold for 11 min

Ionization mode: Spiromesifen ESI (+), the enol compound ESI (-)

Major monitoring ion (*m/z*):

1) LC-MS

Spiromesifen: 273, the enol compound: 271

2) LC-MS/MS

Spiromesifen: Precursor ion 273, product ion 91, 67

The enol compound: Precursor ion 271, product ion 209, 159

Injection volume:

1) LC-MS 10 µL

2) LC-MS/MS 4µL

Expected retention time: Spiromesifen 9 min, the enol compound 5 min

9. Limit of quantification

0.01 mg/kg for each analyte (The concentration of the enol compound is calculated as spiromesifen)

10. Explanatory note

1) Outline of analytical method

The method consists of the extraction of spiromesifen and the enol compound with acetonitrile/formic acid/water (80:1:20, v/v/v) and *n*-hexane, discarding of the *n*-hexane layer, clean-up of the acetonitrile/formic acid/water (80:1:20, v/v/v) layer with an octadecylsilanized silica gel cartridge, quantification and confirmation using LC-MS or LC-MS/MS.

Spiromesifen and the enol compound are quantified individually. The concentration of the enol compound is converted to the concentration of spiromesifen by multiplying by the conversion factor, and the sum of the concentration of spiromesifen and the enol compound is

regarded as the analytical result of spiromesifen.

2) Notes

i) Extraction is performed under acidic conditions with formic acid to prevent the transformation of spiromesifen to the corresponding enol compound.

ii) Clean-up with graphitized carbon black cartridge (250 mg) should be performed if clean-up is insufficient.

Outline of the procedure is as follows. After washing the octadecylsilanized silica gel cartridge with 10 mL of acetonitrile/0.01% formic acid (2:3, v/v) and discarding of the effluent, add 5 mL of acetonitrile/0.01% formic acid (9:1, v/v) to a graphitized carbon black cartridge and discard the effluent. Connect the graphitized carbon black cartridge under the octadecylsilanized silica gel cartridge, and elute with 10 mL of acetonitrile/0.01% formic acid (9:1, v/v). Add acetonitrile/0.01% formic acid (9:1, v/v) to the eluate to make exactly 10 mL, and use this solution as the test solution.

11. References

None

12. Type

C