Original: Japanese

Provisional Translation

Analytical Method for Benthiavalicarb-isopropyl (Agricultural Products)

1. Analyte

Benthiavalicarb-isopropyl

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 benthiavalicarb-isopropyl: Contains not less than 98% of benthiavalicarb-isopropyl. Melting point of the standard is 152°C.

4. Procedure

1) Extraction

i) Grains, legumes, nuts and seeds

Add 20 mL of water to 10.0 g of sample and let stand for 2 hours. Add 100 mL of acetone, homogenize, and filter with suction. Add 50 mL of acetone to the residue on the filter paper, homogenize, and filter as described above. Combine the resulting filtrates, and add acetone to make exactly 200 mL. Take a 20 mL aliquot of the extract, and concentrate to about 1 mL at below 40°C.

ii) Fruits and vegetables

Add 100 mL of acetone to 20.0 g of sample, homogenize, and filter with suction. Add 50 mL of acetone to the residue on the filter paper, homogenize, and filter as described above. Combine the resulting filtrates, and add acetone to make exactly 200 mL. Take a 10 mL aliquot of the extract, and concentrate to about 1 mL at below 40°C.

iii) Tea leaves

Add 20 mL of water to 5.00 g of sample and let stand for 2 hours. Add 100 mL of acetone, homogenize, and filter with suction. Add 50 mL of acetone to the residue on the filter paper, homogenize, and filter as described above. Combine the resulting filtrates, and add acetone to make exactly 200 mL. Take a 40 mL aliquot of the extract, and concentrate to about 1 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/water (3:7, v/v), add the washing to the cartridge, and discard the effluent. Elute with 10 mL of acetonitrile/water (1:1, v/v), add acetonitrile/water (1:1, v/v) to the eluate to make exactly 10 mL, and use this solution as

the test solution.

5. Calibration curve

Prepare 0.001–0.02 mg/L benthiavalicarb-isopropyl standard solutions (acetonitrile/water (1:1, v/v)). Inject 5 µL of each standard solution to LC-MS, and make a calibration curve by peak-height or peak-area method.

6. Quantification

Inject 5 µL of the test solution to LC-MS, and calculate the concentration of benthiavalicarb-isopropyl from the calibration curve made in **5**.

7. Confirmation

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

8. Measurement conditions

1) LC-MS

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

Column temperature: 40°C

Mobile phase: Acetonitrile/2 mmol/L ammonium acetate solution (9:11, v/v)

Ionization mode: ESI (+) or ESI(−)

Major monitoring ions (*m/z*): 382 (+) or 380 (−)

Injection volume: 5 µL

Expected retention time: 10 min

2) LC-MS/MS

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

Column temperature: 40°C

Mobile phase: Acetonitrile/2 mmol/L ammonium acetate solution (1:1, v/v)

Ionization mode: ESI (+)

Major monitoring ions (*m/z*): Precursor ion 382, product ion 180, 72

Injection volume: 1 µL

Expected retention time: 7 min

9. Limit of quantification

0.01 mg/kg

10. Explanatory note

1) Outline of analytical method

The method consists of extraction of benthiavalicarb-isopropyl from sample with acetone, clean-up with an octadecylsilanized silica gel cartridge, quantification using LC-MS, and confirmation using LC-MS or LC-MS/MS.

2) Notes

i) If clean-up is not sufficient, clean-up with a graphitized carbon black cartridge before using the octadecylsilanized silica gel cartridge is recommended.

Outline of the procedure: Wash a graphitized carbon black cartridge with acetone, transfer 10 mL (20 mL for grains, 40 mL for tea leaves) of the extract to the cartridge, and then elute with 10 mL of acetone. Concentrate the total eluate, add 10 mL of water, and transfer to the octadecylsilanized silica gel cartridge.

ii) For LC-MS/MS analysis, use product ions (*m/z*) of 180 for quantification and 72 for confirmation.

11. References

None

12. Type

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