Original: Japanese

Analytical Method for Demeton-*S*-methyl and Oxydemeton-methyl (Agricultural Products)

1. Analytes

Demeton-*S*-methyl

Oxydemeton-methyl (synonym: demeton-*S*-methyl sulfoxide)

2. Application

Agricultural 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.

Thiourea: Thiourea (special grade)

Graphitized carbon black/ethylenediamine-*N*-propylsilanized silica gel layered cartridge (500 mg/500 mg): A polypropylene tube of 12-13 mm in inside diameter packed with 500 mg of graphitized carbon black in an upper layer and 500 mg of ethylenediamine-*N*-propylsilanized silica gel in a lower layer, or a cartridge equivalent to the specified one in separation capability.

Reference standard of demeton-*S*-methyl: Contains not less than 98% of demeton-*S*-methyl.

Reference standard of oxydemeton-methyl: Contains not less than 98% of oxydemeton-methyl.

5. Procedure

1) Extraction

i) Grains, legumes and tea leaves

For grains and legumes, weigh 10.0 g of sample. For tea leaves, weigh 5.00 g of sample. Add 20 mL of 0.2 w/v% Thiourea solution, and let stand for 30 minutes.

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 with suction. Combine the resulting filtrates, and add acetone to make exactly 200 mL.

Take 20 mL of the solution accurately, and concentrate to 5 mL or less at below 40℃. Dissolve 1 g of sodium chloride, and transfer to a porous diatomaceous earth cartridge (for 5 mL volume). Let stand for about 10 minutes, and elute with 40 mL of ethyl acetate. Concentrate the eluate at below 40℃, and remove the solvent. Add 20 mL of *n*-hexane to the residue, and extract with shaking twice with 20 mL each of acetonitrile saturated with *n*-hexane. Combine the eluates, concentrate at below 40℃, and remove the solvent. Dissolve the residue in 2 mL of acetonitrile/toluene (3:1, v/v).

ii) Fruits and vegetables

Weigh sample accurately, add equal amount in weight ratio of 0.2 w/v% thiourea,

homogenize, and take an amount equivalent to 20.0 g of the sample.

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 with suction. Combine the filtrates, and add acetone to make exactly 200 mL.

Take 20 mL of the solution accurately, and concentrate to 5 mL or less at below 40℃. Dissolve 1 g of sodium chloride, and transfer to a porous diatomaceous earth cartridge (for 5 mL volume). Let stand for about 10 minutes, and elute with 40 mL of ethyl acetate. Concentrate the eluate at below 40℃, and remove the solvent. Dissolve the residue in 2 mL of acetonitrile/toluene (3:1, v/v).

2) Clean-up

Add 10 mL of acetonitrile/ toluene (3:1, v/v) to a graphitized carbon black/ ethylenediamine-*N*-propylsilanized silica gel layered cartridge (500 mg/500 mg), and discard the effluents. Transfer the solution obtained in **1)** to the cartridge, add 12 mL of acetonitrile/ toluene (3:1, v/v), and collect the total eluate including the transferred solutions. Concentrate the eluate at below 40℃, and remove the solvent. Dissolve the residue in water/ methanol (4:1, v/v) to make exactly 2 mL for grains and legumes, 1 mL for tea leaves, and 4 mL for fruits and vegetables, and use these solutions as the test solutions.

6. Calibration curve

Prepare standard solutions by dissolving demeton-*S*-methyl and oxydemeton-methyl in methanol respectively, and prepare several diluted solutions at appropriate concentration range using water/methanol (4:1, v/v). Inject each standard solution to LC-MS/MS, and make calibration curves by peak-height or peak-area method. When the test solution is prepared following the above procedure, the sample containing 0.01 mg/kg of demeton-*S*-methyl or oxydemeton-methyl gives the test solution of 0.005 mg/L in concentration.

7. Quantification

Inject the test solution to LC-MS/MS, and calculate the concentration of demeton-*S*-methyl or oxydemeton-methyl from the calibration curves made in **6**.

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

Column: Octadecylsilanized silica gel, 2.0 mm in inside diameter, 150 mm in length and 3 μm in particle diameter

Column temperature: 40°C

Mobile phase: Linear gradient from 5 mmol/L ammonium acetate solution and 5 mmol/L ammonium acetate/methanol solution (4:1, v/v) to (1:99, v/v) for 10 min, and hold for 10 min.

Ionization mode: ESI (+)

Major monitoring ions (*m/z*): Demeton-*S*-methyl: Precursor ion 231, product ions 89, 61

Oxydemeton-methyl: Precursor ion 247, product ions 169, 109

Injection volume: 3 µL

Expected retention time: Demeton-*S*-methyl: 8 min

Oxydemeton-methyl: 5 min

10. Limit of quantification

Demeton-*S*-methyl: 0.01 mg/kg

Oxydemeton-methyl: 0.01 mg/kg

11. Explanatory note

1) Outline of the analytical method

The method consists of extracting demeton-*S*-methyl or oxydemeton-methyl from sample using acetone with thiourea, transferring into ethyl acetate using a porous diatomaceous earth cartridge, defatting by acetonitrile/ hexane partitioning, purifying with a graphitized carbon black/ ethylenediamine-*N*-propylsilanized silica gel layered cartridge, quantifying and confirming using LC-MS/MS.

2) Notes

i) When 1 g of sodium chloride is dissolved into the eluate after concentration, sodium chloride should be fully saturated, for example, using an ultrasonic washing machine. The amount of sodium chloride may be reduced, if necessary, but ensure to add a sufficient amount to saturation.

ii) Control the flow rate at 5 mL/min or lower when a porous diatomaceous earth cartridge is used, so that water may not be eluted. A stop valve may be attached to the outlet port of the cartridge to adjust the flow rate. It may be desirable to use such as Teflon valve to avoid contamination.

iii) The process of acetonitrile/hexane partitioning may be added for fruits and vegetables, if necessary.

iv) After transferring into a porous diatomaceous earth cartridge, the container may be washed with 5 mL of ethyl acetate twice, then washed again with 30 mL of ethyl acetate. If an adhering substance can be found at washing, add an appropriate amount of anhydrous sodium sulfate and wash using an ultrasonic washing machine, as necessary. An adhering substance may be found inside the container during the concentration depending on a sample, and anhydrous sodium sulfate is used to disperse such a substance.

v) Demeton-*S*-methyl may be converted to oxydemeton-methyl or further to demeton-*S*-methylsulfone. To confirm that the conversion does not occur, conduct a spike and recovery test, using a reference standard of demeton-*S*-methyl, as necessary.

vi) When the analytical method for demeton-*S*-methyl, oxydemeton-methyl and demeton-*S*-methylsulfone using LC-MS/MS was developed, the following monitoring ions were used:

- Demeton-*S*-methyl

for quantification (*m/z*): precursor ion 231, product ion 89

for confirmation (*m/z*): precursor ion 231, product ion 61

- Oxydemeton-methyl

  for quantification (*m/z*): precursor ion 247, product ion 169

  for confirmation (*m/z*): precursor ion 247, product ion 109

- Demeton-*S*-methylsulfone

  for quantification (*m/z*): precursor ion 263, product ion 169

  for confirmation (*m/z*): precursor ion 263, product ion 109

vii) Food items considered when developing the analytical method: brown rice, soya bean, potato, spinach, cabbage, apple, orange and tea leaf.

12. References

Ueno, E., et.al., Analysis of demeton-*S*-methyl, oxydemeton-methyl and demeton-*S*-methylsulfone in agricultural products by LC-MS, Shokuhin Eiseigaku Zasshi, 50, 64-69, 2009

13. Type

C