Original: Japanese
Provisional translation

Analytical Method for Abamectin (Agricultural Products)

1. Analytes

Avermectin B_{1a}

Avermectin B_{1b}

8,9-Z-avermectin B_{1a}

2. Applicable food

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.

Reference standard of avermectin B_{1a}: Contains not less than 95% of avermectin B_{1a}

Reference standard of avermectin B_{1b}: Contains not less than 95% of avermectin B_{1b}

Reference standard of 8,9-Z-avermectin B_{1a}: Contains not less than 95% of 8,9-Z-avermectin B_{1a}

5. Procedure

1) Extraction

i) Grains, legumes, nuts and seeds

Add 20 mL of water to 10.0 g of the sample and let stand for 30 min. 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 exactly a 20 mL aliquot of the solution, concentrate at below 40℃, and remove acetone. Add 50 mL of 10 w/v% sodium chloride solution and extract with shaking twice with 50 mL each of ethyl acetate. Combine the extracts, dehydrate the extracts with anhydrous sodium sulfate, and filter out the anhydrous sodium sulfate. Concentrate the filtrates at below 40°C and remove the solvent. Add 30 mL of *n*-hexane to the residue and extract with shaking 3 times with 30 mL each of acetonitrile saturated with *n*-hexane. Combine the extracts, concentrate at below 40°C, and remove the solvent. Dissolve the residue in 2 mL of acetonitrile and toluene (3:1, v/v).

ii) Fruits and vegetables

Add 100 mL of acetone to 20.0 g of the 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 exactly a 10 mL aliquot of the solution, concentrate at below 40℃, and remove acetone. Add 50 mL of 10 w/v% sodium chloride solution and extract with shaking twice with 50 mL each of ethyl acetate. Combine the extracts, dehydrate the extracts with anhydrous sodium sulfate, and

filter out anhydrous sodium sulfate. Concentrate the filtrates at below 40°C and remove the solvent. Dissolve the residue in 2 mL of acetonitrile and toluene (3:1, v/v).

iii) Tea leaves

Add 20 mL of water to 5.00 g of the sample and let stand for 30 min. 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 exactly a 10 mL aliquot of the solution, concentrate at below 40℃, and remove acetone. Add 50 mL of 10 w/v% sodium chloride solution and extract with shaking twice with 50 mL each of ethyl acetate. Combine the extracts, dehydrate the extracts with anhydrous sodium sulfate, and filter out anhydrous sodium sulfate. Concentrate the filtrates at below 40°C and remove the solvent. Dissolve the residue in 2 mL of acetonitrile. Inject 10 mL of acetonitrile into an octadecylsilanized silica gel cartridge (1,000 mg) and discard the effluent. Transfer the acetonitrile solution described above to the cartridge and add another 20 mL of acetonitrile. Collect the total eluate including the transferred solutions, concentrate at below 40°C, and remove the solvent. Dissolve the residue in 2 mL of acetonitrile and toluene (3:1, v/v).

2) Clean-up

Inject 10 mL of acetonitrile and toluene (3:1, v/v) into a graphitized carbon/aminopropylsilanized silica gel layered cartridge (500 mg/500 mg) and discard the effluent. Transfer the solution obtained in 1) to the cartridge and add 25 mL of acetonitrile and toluene (3:1, v/v). Collect the total eluate including the transferred solutions, concentrate at below 40℃, and remove the solvent. Dissolve the residue in acetonitrile to make exactly 1 mL and use this solution as the test solution.

6. Calibration curve

Dissolve each reference standard in acetonitrile respectively to prepare stock standard solutions. Mix these stock standard solutions appropriately, dilute with acetonitrile, and prepare standard solutions of several concentrations. Inject each standard solution into LC-MS/MS and make a calibration curve by peak-height or peak-area method. When the test solution is prepared following the above procedure, for grains, legumes, nuts, seeds, fruits and vegetables, the concentration of avermectin B_{1a}, avermectin B_{1b} and 8,9-*Z*-avermectin B_{1a} in the test solution corresponding to 0.005 mg/kg in the sample results in 0.005 mg/L for each analyte. For tea leaves, the concentration of avermectin B_{1a}, avermectin B_{1b} and 8,9-*Z*-avermectin B_{1a} in the test solution corresponding to 0.02 mg/kg in the sample results in 0.005 mg/L for each analyte.

7. Quantification

Inject the test solution into LC-MS/MS and calculate the concentration of avermectin B_{1a}, avermectin B_{1b} and 8,9-*Z*-avermectin B_{1a} from the calibration curve made in 6.

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

Column: Octadecylsilanized silica gel: 2.1 mm inside diameter, 150 mm in length, 3 μm in particle diameter

Column temperature: 40°C

Mobile phase: Initially, 5 mmoL/L ammonium acetate solution and 5 mmoL/L ammonium acetate-acetonitrile solution (1:9, v/v) for 1 min, followed by a linear gradient to (1:19, v/v) in 7 min, and hold for 2 min.

Ionization mode: ESI (+)

Major monitoring ions (*m/z*)

Avermectin B_{1a}: Precursor ion 891, product ions 567, 305, 145

Avermectin B_{1b}: Precursor ion 877, product ions 553, 291, 145

8,9-Z-avermectin B_{1a}: Precursor ion 891, product ions 567, 305, 145

Injection volume: 5 μL

Expected retention time

Avermectin B_{1a}: 5 min

Avermectin B_{1b}: 4 min

8,9-Z-avermectin B_{1a}: 6 min

10 Limit of quantification

Grains, legumes, nuts, fruits and vegetables: 0.005 mg/kg for each analyte

Tea leaves: 0.02 mg/kg for each analyte

11, Explanatory note

1) Outline of analytical method

The method consists of extraction of avermectin B_{1a}, avermectin B_{1b} and 8,9-*Z*-avermectin B_{1a} from the sample with acetone, transfer into ethyl acetate for re-dissolution, then, for grains, legumes and nuts, defatting by acetonitrile/hexane partitioning (for tea leaves, clean-up with an octadecylsilanized silica gel cartridge), clean-up with a graphitized carbon/aminopropylsilanized silica gel layered cartridge, and quantification and confirmation using LC-MS/MS. In the method, avermectin B_{1a}, avermectin B_{1b} and 8,9-*Z*-avermectin B_{1a} are quantified individually, and the sum of the concentrations of avermectin B_{1a}, avermectin B_{1b} and 8,9-*Z*-avermectin B_{1a} is regarded as the analytical result of abamectin.

2) Notes

i) Some standard solutions that are commercially available may contain each other's analytes to be analyzed. Therefore, use a reference standard purified as a single substance when preparing a mixed standard solution.

ii) It is recommended that the test solution be diluted, or the measurement conditions be reviewed when a food matrix influences the measurement.

iii) When the analytical methods for avermectin B_{1a}, avermectin B_{1b} and 8,9-*Z*-avermectin B_{1a} using LC-MS/MS were developed, the following monitoring ions were used:

Avermectin B_{1a}

for quantitative ions (*m*/*z*): precursor ion 891, product ion 567

for qualitative ions (*m*/*z*): precursor ion 891, product ion 305

Avermectin B_{1b}

for quantitative ions (*m*/*z*): precursor ion 877, product ion 553

for qualitative ions (*m*/*z*): precursor ion 877, product ion 291

8,9-Z-avermectin B_{1a}

for quantitative ions (*m*/*z*): precursor ion 891, product ion 567

for qualitative ions (*m*/*z*): precursor ion 891, product ion 305

iv) The foods examined in the development of the analytical method: brown rice, soybeans, spinach, welsh onion, potatoes, oranges, apples and tea leaves

12. Reference

None

13. Type

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