Analytical Method for Imicyafos (Agricultural Products)

1. Analyte

Imicyafos

2. Instrument

Liquid chromatograph-mass spectrometer (LC-MS)

3. Reagents

Use the reagents listed in Chapter I of the General Rules, except the following.

0.5 mol/L phosphate buffer (pH 7.0): Weigh 52.7 g of dipotassium hydrogen phosphate (K_2HPO_4) and 30.2 g of potassium dihydrogen phosphate (KH_2PO_4), dissolve in about 500 mL of water, adjust pH to 7.0 with 1 mol/L sodium hydroxide solution or 1 mol/L hydrochloric acid, and add water to make exactly 1 L.

Reference standard of imicyafos: Contains not less than 98% of imicyafos.

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 30 minutes.

Add 50 mL of acetonitrile, homogenize, and filter with suction. Add 20 mL of acetonitrile to the residue on the filter paper, homogenize, and filter with suction. Combine the resulting filtrates, and add acetonitrile to make exactly 100 mL.

Take a 20 mL aliquot of the extract, add 10 g of sodium chloride and 20 mL of 0.5 mol/L phosphate buffer (pH 7.0), and shake for 10 minutes. Let stand, and discard the separated aqueous layer.

Add 10 mL of acetonitrile to octadecylsilanized silica gel cartridge (1,000 mg), and discard the effluent. Transfer the acetonitrile layer described above to the cartridge, and elute with 2 mL of acetonitrile. Combine the eluates, and dehydrate with anhydrous sodium sulfate. Filter out anhydrous sodium sulfate, concentrate the filtrate at below 40°C and remove the solvent. Dissolve the residue in 2 mL of acetonitrile/toluene (3:1, v/v).

ii) Fruits, vegetables, herbs, tea leaves and hops

For fruits, vegetables and herbs, weigh 20.0 g of sample. For tea leaves and hops, weigh 5.00 g of sample, add 20 mL of water and let stand for 30 minutes.

Add 50 mL of acetonitrile, homogenize, and filter with suction. Add 20 mL of acetonitrile to the residue on the filter paper, homogenize, and filter with suction. Combine the resulting filtrates, and add acetonitrile to make exactly 100 mL.

Take a 20 mL aliquot of the extract, add 10 g of sodium chloride and 20 mL of 0.5 mol/L

phosphate buffer (pH 7.0), and shake. Let stand, and discard the separated aqueous layer. Dehydrate the acetonitrile layer with anhydrous sodium sulfate. Filter out anhydrous sodium sulfate, concentrate the filtrate at below 40°C 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 graphitized carbon black/aminopropylsilanized silica gel layered cartridge (500 mg/500 mg), and discard the effluent. Transfer the solution obtained in 1) to the cartridge, elute with 20 mL of acetonitrile/toluene (3:1, v/v), and concentrate the combined eluates to less than 1 mL at below 40°C. Add 10 mL of acetone to the concentrated solution, and concentrate to less than 1 mL at below 40°C. Add 5 mL of acetone again, concentrated the solution and remove the solvent. Dissolve the residue in methanol to make exactly 4 mL for grains, legumes, nuts and seeds, 8 mL for fruits, vegetables and herbs, 2 mL for tea leaves and hops, and use this solution as the test solution.

5. Calibration curve

Prepare 0.005–0.1 mg/L imicyafos standard solutions (methanol). 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 imicyafos from the calibration curve made in **5**.

7. Confirmation

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

8. Measurement conditions

Example

Column: Octadecylsilanized silica gel, 2.1 mm in inside diameter, 150 mm in length and 3.5 μm in particle diameter

Column temperature: 40°C

Mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: 5 mmol/L ammonium acetate solution

Mobile phase B: 5 mmol/L ammonium acetate /methanol solution

Time (min)	A (%)	B (%)
0	85	15
1	60	40
3.5	60	40
6	50	50
8	45	55
17.5	5	95
30	5	95
30	85	15

Ionization mode: ESI (+)

Major monitoring ions (*m/z*): 306, 305

Expected retention time: 14 min

9. Limit of quantification

0.01 mg/kg

10. Explanatory note

1) Outline of analytical method

The method consists of extraction of imicyafos from sample with acetonitrile, removal of water by salting out, clean-up with an octadecylsilanized silica gel cartridge for grains, legumes, nuts and seeds only, clean-up with a graphitized carbon black/aminopropylsilanized silica gel layered cartridge for all samples, quantification and confirmation using LC-MS.

2) Notes

i) This analytical method is based on “Multi-residue Method I for Agricultural Chemicals by LC-MS (Agricultural Products)”.

ii) When the analytical method for imicyafos using LC-MS was developed, the following ions were used:

for quantification (*m/z*): 305

for confirmation (*m/z*): 306

iii) When LC-MS/MS is used, the major monitoring ions are as follows:

precursor ion (*m/z*): 305

product ion (*m/z*): 235, 201

11. References

MHLW Director Notice (Syoku-An No.1129002 November 29, 2005) “Analytical Methods for Residual Compositional Substances of Agricultural Chemicals, Feed Additives, and Veterinary Drugs in Food (Partial Amendment)”, “Multi-residue Method I for Agricultural Chemicals by LC-MS (Agricultural Products)”

12. Type

C