Original: Japanese

Provisional Translation

# Analytical Method for Acetamiprid (Animal and Fishery Products)

## 1. Analytes

Acetamiprid

Metabolite IM-2-1 ($N^1$-[(6-Chloro-3-pyridyl) methyl]-$N^2$-Cyanoacetamidine) (hereafter referred to as IM-2-1)

## 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 acetamiprid: Contains not less than 98% of acetamiprid. Melting point of the standard is 98.9°C.

Reference standard of IM-2-1: Contains not less than 98% of IM-2-1.

## 4. Procedure

1) Extraction

i) Muscle, fat, liver, kidney, fish and shellfish

Add 100 mL of acetone to 10.0 g of sample (5.00 g for fat), 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, concentrate to about 20 mL at below 40ºC. Add 100 mL of 10 w/v% sodium chloride solution, shake with 50 mL of *n*-hexane, and discard the *n*-hexane layer. Extract the remaining water layer with shaking twice with 100 mL and 50 mL of ethyl acetate. Dehydrate the extract with anhydrous sodium sulfate, filter out the anhydrous sodium sulfate, and add ethyl acetate to the filtrate to make exactly 200 mL. Take a 4 mL (an 8 mL for fat) aliquot of the solution, concentrate at below 40°C, and remove the solvent. Dissolve the residue in 5 mL of acetone/*n*-hexane (3:17, v/v).

ii) Milk, egg and honey

Add 100 mL of acetone (20 mL of water and 100 mL of acetone for honey) to 10.0 g of sample, homogenize, and centrifuge at 3,000 rpm for 5 minutes. Collect the acetone/water layer, add 50 mL of acetone (20 mL of water and 50 mL of acetone for honey) to the residue, homogenize, and centrifuge as described above. Combine the resulting acetone/water layers, and concentrate to about 20 mL (about 50 mL for honey) at below 40ºC. Add 100 mL of 10 w/v% sodium chloride solution, shake with 50 mL of *n*-hexane, and discard the *n*-hexane layer. Extract the remaining water layer with shaking twice with 100 mL and 50 mL of ethyl acetate. Dehydrate the extract with anhydrous sodium sulfate, filter out the anhydrous

sodium sulfate, and add ethyl acetate to the filtrate to make exactly 200 mL. Take a 4 mL aliquot of the solution, concentrate at below 40°C, and remove the solvent. Dissolve the residue in 5 mL of acetone/*n*-hexane (3:17, v/v).

2) Clean-up

i) Synthetic magnesium silicate column chromatography

Add 5 mL each of acetone and *n*-hexane to a synthetic magnesium silicate cartridge (910 mg) sequentially, and discard the effluent. Transfer the solution obtained in 1) to the cartridge, add 15 mL of acetone/*n*-hexane (3:17, v/v), and discard the effluent. Add 10 mL of acetone/*n*-hexane (1:4, v/v), and discard the effluent. Elute with 20 mL of acetone/*n*-hexane (2:3, v/v), concentrate the eluate at below 40°C, and remove the solvent. Dissolve the residue in 5 mL of water/methanol (2:3, v/v).

ii) Graphitized carbon black column chromatography

Add 5 mL each of methanol and water to a graphitized carbon black cartridge (250 mg) sequentially, and discard the effluent. Transfer the solution obtained in i) to the cartridge, add 5 mL of water/methanol (2:3, v/v), and discard the effluent. Elute with 10 mL of methanol, concentrate the eluate at below 40°C, and remove the solvent. Dissolve the residue in acetonitrile/water (1:4, v/v) to make exactly 4 mL, and use this solution as the test solution.

**5. Calibration curve**

Dissolve reference standard of acetamiprid and IM-2-1 in acetonitrile to make 500 mg/L respectively, and use these solutions as stock standard solution. Mix these stock standard solutions appropriately, dilute the stock standard solution with acetonitrile/water (1:4, v/v) and prepare solutions of several concentrations. Inject each standard solution to LC-MS or 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 each analyte (The concentration of IM-2-1 is calculated as acetamiprid.) gives the test solution of 0.0005 mg/L in concentration.

**6. Quantification**

Inject the test solution to LC-MS or LC-MS/MS, and calculate the concentration of acetamiprid and IM-2-1 from the calibration curves made in **5**. Use the following equation to calculate the concentration of acetamiprid including IM-2-1.

Concentration (ppm) of acetamiprid (including IM-2-1) = A + B × 1.067

A: Concentration (ppm) of acetamiprid

B: Concentration (ppm) of IM-2-1

**7. Confirmation**

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

**8. Measurement conditions**

Example

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 vol% formic acid (1:4, v/v) to (4:1, v/v) in 10 min and hold (9:1, v/v) for 5 min.

Ionization mode:

Acetamiprid: ESI (+)

IM-2-1: ESI (+)

Major monitoring ions (*m/z*):

1) LC-MS

Acetamiprid: 223

IM-2-1: 209

2) LC-MS/MS

Acetamiprid: precursor ion 223, product ion 126

precursor ion 225, product ion 128

IM-2-1: precursor ion 209, product ion 126

precursor ion 211, product ion 128

Injection volume: 4 μL

Expected retention time: Acetamiprid 8 min, IM-2-1 7 min

**9. Limit of quantification**

0.01 mg/kg for each analyte (The concentration of IM-2-1 is calculated as acetamiprid.)

**10. Explanatory note**

1) Outline of analytical method

The method consists of extraction of acetamiprid and IM-2-1 from sample with acetone, defatting by *n*-hexane, transferring of the extract into ethyl acetate, clean-up with a synthetic magnesium silicate cartridge and a graphitized carbon black cartridge, and quantification and confirmation using LC-MS or LC-MS/MS. Acetamiprid and IM-2-1 are quantified individually. For the concentration of acetamiprid including IM-2-1, the concentration of IM-2-1 is converted to the concentration of acetamiprid by multiplying by the conversion factor, and the sum of the concentration of acetamiprid and IM-2-1 is regarded as the analytical result of acetamiprid.

2) Notes

i) Be careful for bumping of the extract under the vacuum concentration step. For some samples, clean-up step using a graphitized carbon black cartridge can be omitted.

ii) When the analytical method for acetamiprid and IM-2-1 using LC-MS/MS was developed, the following monitoring ions were used:

Acetamiprid

for quantification (*m/z*): precursor ion 223, product ion 126

for confirmation (*m/z*): precursor ion 225, product ion 128

IM-2-1

for quantification (*m/z*): precursor ion 209, product ion 126

for confirmation (*m/z*): precursor ion 211, product ion 128

**11. References**

None

**12. Type**

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