Analytical Method for Chlorantraniliprole (Agricultural Products)

1. Analyte
   Chlorantraniliprole

2. Instrument
   Liquid chromatograph-mass spectrometer (LC-MS)

3. Reagents
   Use the reagents listed in Section 3 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$_2$HPO$_4$) and 30.2 g of potassium dihydrogen phosphate (KH$_2$PO$_4$), 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 chlorantraniliprole: Contains not less than 98% of chlorantraniliprole.

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 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 a 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, concentrate the solution and remove the solvent. Dissolve the residue in methanol to make exactly 4 mL for grains, legumes, nuts and seeds, 8 mL solution 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 chlorantraniliprole 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 chlorantraniliprole 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.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>A (%)</th>
<th>B (%)</th>
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<tbody>
<tr>
<td>0</td>
<td>85</td>
<td>15</td>
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Ionization mode: ESI (−)
Major monitoring ions (m/z): 484, 482, 480
Expected retention time: 15 min

9. Limit of quantification
0.01 mg/kg

10. Explanatory note
1) Outline of analytical method
The method consists of extraction of chlorantraniliprole from sample with acetonitrile, removal of water by salting out, clean-up with an octadecylsilylanaized silica gel cartridge for grains, legumes, nuts and seeds only, clean-up with a graphitized carbon black/aminopropylsilylanaized 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 chlorantraniliprole using LC-MS (ESI (−)) was developed, the following monitoring ions were used:
   for quantification (m/z): 482
   for confirmation (m/z): 484, 480
iii) When LC-MS/MS (ESI(+) is used, the major monitoring ions are as follows:
   precursor ion (m/z): 484
   product ion (m/z): 452, 285

11. References
MHLW Director Notice (Syoku-An No.1003001 October 3, 2006) “Multi-residue Method I for Agricultural Chemicals by LC-MS (Agricultural Products)”.

12. Type
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