Original: Japanese
Provisional translation

Analytical Method for Imidacloprid (Animal and Fishery Products)

1. Analytes

Imidacloprid

Metabolites containing the 6-chloropyridyl moiety (including compounds converted to 6-chloronicotinic acid by oxidation reaction).

2. Application

Animal and fishery 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 imidacloprid: Contains not less than 98% of imidacloprid.

Reference standard of 6-chloronicotinic acid: Contains not less than 98% of 6-chloronicotinic acid.

5. Procedure

1) Extraction

Add 50 mL of *n*-hexane and 100 mL of water and methanol (1:3) mixed solution to 10.0 g (5.00 g for fat) of sample, homogenize, and centrifuge at 3,000 rpm for 5 min. Collect the methanol layer, add 50 mL of water and methanol (1:3) mixed solution to the *n*-hexane layer and residue, homogenize, centrifuge as described above, combine with the resulting water and methanol (1:3) mixed layer, and add water and methanol (1:3) mixed solution to make exactly 200 mL. Take exactly a 5 mL (2 mL for honey and 10 mL for fat) aliquot of the solution and concentrate to approximately 1 mL at below 40°C.

2) Oxidation

Add 5 mL of 32 w/v% sodium hydroxide solution and 50 mL of 5 w/v% potassium permanganate solution to the solution obtained in 1), attach an air-condenser, heat in an oil bath at 120°C for 15 min and oxidize imidacloprid and metabolites containing a 6-chloropyridyl group to 6-chloronicotinic acid. After heating under reflux, while cooling with cold water, add 50 mL of water, 50 mL of 10 v/v% sulfuric acid and 5 g of sodium hydrogensulfite to complete the oxidation reaction, and extract with shaking twice with 100 mL and 50 mL each of ethyl acetate. Combine the extracts, dehydrate the extract with anhydrous sodium sulfate, filter out anhydrous sodium sulfate, concentrate at below 40°C, and remove the solvent. Dissolve the residue in 0.1 vol% acetic acid and methanol (9:1) mixed solution to make exactly 2.5 mL (1 mL for honey) and use this solution as the test

solution.

6. Calibration curve

Dissolve the reference standard of 6-chloronicotinic acid in acetone to make a standard stock solution. Dilute the stock standard solution with 0.1 vol% acetic acid and methanol (9:1) mixed solution and prepare standard solutions of several concentrations. Inject each solution into 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 concentration of 6-chloronicotinic acid in the test solution corresponding to 0.01 mg/kg (equivalent to imidacloprid) in the sample is 0.001 mg/L (equivalent to imidacloprid).

7. Quantification

Inject the test solution into LC-MS/MS and calculate the concentration of 6-chloronicotinic acid from the calibration curve prepared in **6**. The concentration of imidacloprid (including metabolites containing the 6-chloropyridyl moiety) is calculated using the following equation.

Concentration of imidacloprid (including metabolites containing the 6-chloropyridyl moiety) (ppm) = A × 1.623

A: concentration (ppm) of 6-chloronicotinic acid

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

Column: Octadecylsilanized silica gel 2.1 mm inside diameter, 250 mm in length and 3 μm in particle diameter

Column temperature: 40°C

Mobile phase: Linear gradient from a mixed solution of acetonitrile and 0.1 vol% acetic acid (1:9) to (9:1) in 10 min and hold at (9:1) for 5 min.

Ionization mode: ESI (+)

Major monitoring ions (*m/z*): Precursor ion 158, product ions 122, 78

Injection volume: 10 μL

Expected retention time: 9 min

10. Limit of quantification

0.01 mg/kg (equivalent to imidacloprid)

11. Explanatory note

1) Outline of analytical method

The method consists of extracting imidacloprid and metabolites containing the 6-chloropyridyl moiety from sample using water and methanol (1:3) mixed solution in the presence of *n*-hexane, heating under reflux with potassium permanganate solution under alkaline conditions, oxidizing imidacloprid and metabolites containing the 6-chloropyridyl moiety to 6-chloronicotinic acid, extracting with ethyl acetate and quantifying and confirming using LC-MS/MS. In this method, 6-chloronicotinic acid is quantified, and the

concentration of 6-chloronicotinic acid is converted to the concentration of imidacloprid (including metabolites containing the 6-chloropyridyl moiety) by multiplying by the conversion factor to be used as the analytical result of imidacloprid.

2) Notes

i) For the oxidation reaction, confirm that the oxidation reaction has progressed sufficiently using the reference standard of imidacloprid. Pay attention to the reaction time since 6-chloronicotinic acid decreases with prolonged heating under reflux.

ii) When the analytical method for 6-chloronicotinic acid using LC-MS/MS was developed, the following monitoring ions were used:

for quantitative ions (*m/z*): precursor ion 158, product ion 122

for qualitative ions (*m/z*): precursor ion 158, product ion 78

iii) Food items used to develop the analytical method: cattle (muscle, fat, liver and milk), chicken (muscle and egg), honey, eel, salmon and *Corbicula* (freshwater clam).

12. Reference

None

13. Type

C