Original: Japanese
Provisional translation

Analytical Method for Carboxin (Agricultural Products)

1. Analytes

Carboxin

5,6-dihydro-3-carboxanilide-2-methyl-1,4-oxathiine-4-oxide (hereinafter referred to as carboxin sulfoxide)

2. Application

Grains, legumes, nuts, seeds and vegetables

3. Instrument

Liquid chromatograph-tandem mass spectrometer (LC-MS/MS)

4. Reagents

Use the reagents listed in Section 3 of the General Rules, except for the following.

0.5 mol/L phosphate buffer solution (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 approximately 500 mL of water, adjust pH to 7.0 using 1 mol/L sodium hydroxide or 1 mol/L hydrochloric acid, and add water to make 1 L.

Thiourea: thiourea (special grade)

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

Reference standard of carboxin sulfoxide: Contains not less than 98% of carboxin sulfoxide.

5. Procedure

1) Extraction

i) Grains, legumes, nuts and seeds

Add 20 mL of 5 w/v% thiourea solution to 10.0 g of sample and let stand for 30 min. Add 100 mL of acetonitrile to the sample, homogenize, and filter with suction. Add 50 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 200 mL.

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

Inject 10 mL of acetonitrile into an octadecylsilanized silica gel cartridge (1,000 mg) and discard the effluent. Transfer the acetonitrile layer described above to the cartridge, add another 6 mL of acetonitrile, collect the total eluate, concentrate at below 40°C, and

remove the solvent. Dissolve the residue in 2 mL of acetonitrile and toluene (3:1) mixed solution.

ii) Vegetables

Weigh the sample accurately, add an equal amount of 5 w/v% thiourea solution by weight, homogenize, and take the sample equivalent to 20.0 g. Add 100 mL of acetonitrile, homogenize, and filter with suction. Add 50 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 200 mL.

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

Inject 10 mL of acetonitrile into an octadecylsilanized silica gel cartridge (1,000 mg) and discard the effluent. Transfer the acetonitrile layer described above to the cartridge, add another 6 mL of acetonitrile, collect the total eluate, concentrate at below 40°C, and remove the solvent. Dissolve the residue in 2 mL of acetonitrile and toluene (3:1) mixed solution.

2) Clean-up

Inject 10 mL of acetonitrile and toluene (3:1) mixed solution into a graphite carbon/aminopropylsilanized silica gel layered cartridge (500 mg/500 mg) and discard the effluent. Transfer the solution obtained in 1) to the cartridge, add another 15 mL of acetonitrile and toluene (3:1) mixed solution, and remove the solvent in the total eluate at below 40°C. Dissolve the residue in methanol to make exactly 5 mL for grains, legumes, nuts and seeds or exactly 10 mL for vegetables, and use these solutions as the test solution.

6. Calibration curve

Prepare stock standard solutions by dissolving each reference standard of carboxin and carboxin sulfoxide in acetone, respectively. Mix each stock standard solution appropriately, dilute with methanol, 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 carboxin and carboxin sulfoxide in the test solution corresponding to 0.005 mg/kg (Carboxin sulfoxide is calculated as carboxin.) in the sample is 0.001 mg/L (Carboxin sulfoxide is calculated as carboxin.).

7. Quantification

Inject the test solutions into LC-MS/MS, and calculate the concentration of carboxin and carboxin sulfoxide from the calibration curves prepared in **6**. Use the following equation to calculate the concentration of carboxin including carboxin sulfoxide.

Concentration (ppm) of carboxin (including carboxin sulfoxide) = A + B × 0.9363

A: Concentration (ppm) of carboxin

B: Concentration (ppm) of carboxin sulfoxide

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

Column: Octadecylsilanized silica gel, 2.1 mm inside diameter, 150 mm in length and 5 μm in particle diameter

Column temperature: 40°C

Mobile phase: Linear gradient from 2 mmol/L ammonium formate solution and a mixed solution of 2 mmol/L ammonium formate and methanol (7:3) to (1:4) in 20 min.

Ionization mode: ESI (+)

Major monitoring ions (*m/z*)

Carboxin: precursor ion 236, product ions 143, 87

Carboxin sulfoxide: precursor ion 252, product ions 159, 131

Injection volume: 2 μL

Expected retention time

Carboxin: 17 min

Carboxin sulfoxide: 10 min

10. Limit of quantification

Carboxin: 0.005 mg/kg

Carboxin sulfoxide: 0.005 mg/kg (equivalent to carboxin)

11. Explanatory note

1) Outline of analytical method

The method consists of extracting carboxin and carboxin sulfoxide from sample with acetonitrile in the presence of thiourea, dehydrating by salting out, cleaning up with an octadecylsilanized silica gel cartridge and a graphite carbon/aminopropylsilanized silica gel layered cartridge, and quantifying and confirming with LC-MS/MS.

2) Notes

i) When the analytical method for carboxin and carboxin sulfoxide using LC-MS/MS was developed, the following monitoring ions were used:

Carboxin

for quantitative ions *(m/z)*: precursor ion 236, product ion 143

for qualitative ions (*m/z*): precursor ion 236, product ion 87

Carboxin sulfoxide

for quantitative ions *(m/z)*: precursor ion 252, product ion 159

for qualitative ions (*m/z*): precursor ion 252, product ion 131

ii) At the time of developing the analytical method, no conversion of carboxin and carboxin sulfoxide to carboxin sulfone (oxycarboxin) was observed during the analysis. However, it should be confirmed that there is no conversion to carboxin sulfone when necessary.

iii) Food items used to develop the analytical method: wheat, green soybean, Azuki bean, onion, and kidney beans (immature).

12. References

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

Sasaki, K., *et al*. Performance Study of Analytical Method for Ethoxyquin in Fruit. *Shokuhin Eiseigaku Zasshi*, **43**, 366–370 (2002)

13. Type

C