Original: Japanese

Provisional Translation

Analytical Method for Ethoxyquin (Animal and Fishery Products)

1. Analyte

Ethoxyquin

2. Instrument

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 ethoxyquin: Contains not less than 96% of ethoxyquin.

4. Procedure

1) Extraction

i) Except for honey

Add 20 mL of 10 w/v% sodium carbonate solution and 100 mL of 50 mg/L dibutylhydroxy-toluene (BHT)-acetone solution to 10.0 g (5.00 g for fat) of sample, homogenize, and filter with suction. Add 50 mL of 50 mg/L BHT-acetone solution to the residue on the filter paper, homogenize, and filter with suction. Combine the resulting filtrates, and add 50 mg/L BHT-acetone solution to make exactly 200 mL. Take 10 mL of the solution and add 10 mL of water.

ii) Honey

Add 20 mL of water to 10.0 g of sample and mix well. Add 20 mL of 10 w/v% sodium carbonate solution and 100 mL of 50 mg/L BHT-acetone solution, homogenize, and filter with suction. Add 50 mL of 50 mg/L BHT-acetone solution to the residue on the filter paper, homogenize, and filter with suction. Combine the resulting filtrates, and add 50 mg/L BHT-acetone solution to make exactly 200 mL. Take 10 mL of the solution and add 10 mL of water.

2) Clean-up

i) Except for fat

Add 5 mL of acetonitrile and 10 mL of water to an octadecylsilanized silica gel cartridge (1,000 mg) sequentially, and discard the effluent. Transfer the solution obtained in 1) to the cartridge, add 10 mL of acetonitrile/water (3:7, v/v), and discard the effluent. Elute with 10 mL of acetonitrile, add acetonitrile to the eluate to make exactly 10 mL, and use this solution as the test solution.

ii) Fat

Add 5 mL of acetonitrile and 10 mL of water to an octadecylsilanized silica gel cartridge (1,000 mg) sequentially, and discard the effluent. Transfer the solution obtained in 1) to the cartridge, add 10 mL of acetonitrile/water (3:7, v/v), and discard the effluent. Elute with 10 mL

of acetonitrile, concentrate the eluate to about 2 mL at below 40 ºC, add acetonitrile to make exactly 5 mL, and use this solution as the test solution.

5. Calibration curve

Prepare 0.0005–0.01 mg/L ethoxyquin standard solutions (50 mg/L BHT-acetone solution) of several concentrations. Inject 5 µL of each standard solution to LC-MS/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/MS and calculate the concentration of ethoxyquin from the calibration curve made in **5**.

7. Confirmation

Confirm using 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 2 mmol/L ammonium acetate solution/methanol (9:11, v/v) to (1:19, v/v) in 10 min and hold for 5 min.

Ionization mode: ESI (+)

Major monitoring ions (*m/z*): precursor ion 218, product ion 174, 148

Expected retention time: 11 min

9. Limit of quantification

0.01 mg/kg

10. Explanatory note

1) Outline of analytical method

The method consists of extraction of ethoxyquin from sample with acetone under basic condition, clean-up with an octadecylsilanized silica gel cartridge, quantification and confirmation using LC-MS/MS.

2) Notes

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

for quantification (*m/z*): precursor ion 218, product ion 148

for confirmation (*m/z*): precursor ion 218, product ion 174

ii) Because ethoxyquin is liable to degrade during the extraction step, BHT is added to prevent the degradation. If the recovery is not stable despite the addition of BHT, it can be improved by cooling with ice during the extraction step.

iii) Because ethoxyquin is lost in the step of concentration to dryness, the method does not include a concentration-to-dryness step. Concentration under reduced pressure is applicable, if not to

dryness.

iv) For fat, take precautions not to concentrate the eluate to dryness after the clean-up step. The concentration procedure can be omitted, and prepare the test solution by adding acetonitrile to the eluate to make exactly 10 mL. In this case, the injection volume should be 10 μL.

11. References

Sasaki, *et al.* Performance Study of Analytical Method for Ethoxyquin in Fruits. *Food Hyg. Saf. Sci.* **43**, 366-370 (2002)

12. Type

C