Analytical Method for Propham
(Targeted to Agricultural, Animal and Fishery Products)

The target compound to be determined is propham.

1. Instrument

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

2. Reagents

Use the reagents listed in Section C ***Reagent/Test Solution, Etc.***, Part II ***Food Additives***, except the following.

Reagents designated as “special grade” in this section must meet the requirements for “special grade” specified in the Japan Industrial Standards for the reagents.

Acetonitrile: Use a reagent not containing any substance that may interfere with the analysis of the target compound.

Acetone: Use a reagent not containing any substance that may interfere with the analysis of the target compound.

Ethylenediamine-*N*-propylsilanized silica gel cartridge (500 mg): A polyethylene tube of 8-9 mm in inside diameter packed with 500 mg of ethylenediamine-*N*-propylsilanized silica gel, or a cartridge equivalent to the specified one in separation capability.

Octadecylsilanized silica gel cartridge (1,000 mg): A polyethylene tube of 12-13 mm in inside diameter packed with 1,000 mg of octadecylsilanized silica gel, or a cartridge equivalent to the specified one in separation capability.

Ammonium formate: Ammonium formate (special grade)

Diethylene glycol: Contains not less than 98% of diethylene glycol.

n-Hexane: Use a reagent not containing any substance that may interfere with the analysis of the target compound.

Water: Use water suitable for chemical analysis, including distilled water, purified water, or pure water. If it contains any substance that may interfere with the analysis of the target compound, wash with a solvent such as *n*-hexane before use.

Methanol: Use a reagent not containing any substance that may interfere with the analysis of the target compound.

3. Reference standard

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

4. Procedure

a. 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 100 mL of acetone, 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, and add acetone to make exactly 200 mL. Take exactly a 20 mL aliquot of the solution, and concentrate to about 3 mL at below 40ºC. Add 100 mL of 10 w/v% sodium chloride solution, and extract with shaking twice with 100 mL and 50 mL of *n*-hexane. Combine the extracts, dehydrate with anhydrous sodium sulfate, filter out the anhydrous sodium sulfate, and add 0.2 mL of 2 vol% diethylene glycol-acetone solution. Concentrate the filtrate at below 40ºC, and remove the solvent. Add 30 mL of *n*-hexane to the residue, and extract with shaking twice with 30 mL each of acetonitrile saturated with *n*-hexane. Combine the extracts, concentrate at below 40ºC, and remove the solvent. Dissolve the residue in 4 mL of acetone, and add 16 mL of water.

ii. Fruits and vegetables

Add 100 mL of acetone to 20.0 g of sample, 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, and add acetone to make exactly 200 mL. Take exactly a 10 mL aliquot of the solution, concentrate to about 2 mL at below 40ºC. Add 100 mL of 10 w/v% sodium chloride solution, and extract with shaking twice with 100 mL and 50 mL of *n*-hexane. Combine the extracts, dehydrate with anhydrous sodium sulfate, filter out the anhydrous sodium sulfate, and add 0.2 mL of 2 vol% diethylene glycol-acetone solution. Concentrate the filtrate at below 40ºC, and remove the solvent. Dissolve the residue in 4 mL of acetone, and add 16 mL of water.

iii. Tea and hops

Add 20 mL of water to 5.00 g of sample, and let stand for 30 minutes. Add 100 mL of acetone, 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, and add acetone to make exactly 200 mL. Take exactly a 40 mL aliquot of the solution, concentrate to about 6 mL at below 40ºC. Add 100 mL of 10 w/v% sodium chloride solution, and extract with shaking twice with 100 mL and 50 mL of *n*-hexane.

Combine the extracts, dehydrate with anhydrous sodium sulfate, filter out the anhydrous sodium sulfate, and add 0.2 mL of 2 vol% diethylene glycol-acetone solution. Concentrate the filtrate at below 40ºC, and remove the solvent. Dissolve the residue in 4 mL of acetone, and add 16 mL of water.

iv. Muscle, fat, liver, kidney, milk, egg and fish/shellfish

Add 100 mL of acetone to 10.0 g of sample, 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, and add acetone to make exactly 200 mL. Take exactly a 20 mL aliquot of the solution, concentrate to about 3 mL at below 40ºC. Add 100 mL of 10 w/v% sodium chloride solution, and extract with shaking twice with 100 mL and 50 mL of *n*-hexane. Combine the extracts, dehydrate with anhydrous sodium sulfate, filter out the anhydrous sodium sulfate, and add 0.2 mL of 2 vol% diethylene glycol-acetone solution. Concentrate the filtrate at below 40ºC, and remove the solvent.

Add 30 mL of *n*-hexane to the residue, and extract with shaking twice with 30 mL each of acetonitrile saturated with *n*-hexane. Combine the extracts, concentrate at below 40ºC, and remove the solvent. Dissolve the residue in 4 mL of acetone, and add 16 mL of water.

v. Honey

Dissolve the 10.0 g of sample with 20 mL of water. Add 100 mL of acetone, 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, and add acetone to make exactly 200 mL. Take exactly a 20 mL aliquot of the solution, concentrate to about 3 mL at below 40ºC. Add 100 mL of 10 w/v% sodium chloride solution, and extract with shaking twice with 100 mL and 50 mL of *n*-hexane. Combine the extracts, dehydrate with anhydrous sodium sulfate, filter out the anhydrous sodium sulfate, and add 0.2 mL of 2 vol% diethylene glycol-acetone solution. Concentrate the filtrate at below 40ºC, and remove the solvent. Dissolve the residue in 4 mL of acetone, and add 16 mL of water.

b. Clean-up

Add 10 mL each of acetonitrile and acetone/water (1:4, v/v) to an octadecylsilanized silica gel cartridge (1,000 mg) sequentially, and discard the effluents. Add 10 mL of acetonitrile/water (7:3, v/v) to an ethylenediamine-*N*-propylsilanized silica gel cartridge (500 mg), and discard the effluents. Transfer the solution obtained in “a. Extraction” to

the octadecylsilanized silica gel cartridge, add 10 mL of acetonitrile/water (1:4, v/v), and discard the effluents. Connect the ethylenediamine-*N*-propylsilanized silica gel cartridge to the bottom of the octadecylsilanized silica gel cartridge, elute with 10 mL of acetonitrile/water (7:3, v/v), collect the eluate. Add acetonitrile/water (7:3, v/v) to make exactly 10 mL, and use this solution as the test solution.

5. Measurement

a. Calibration curve

Prepare propham standard solutions (acetonitrile/water (7:3, v/v)) of several concentrations. Inject each standard solution to LC-MS/MS, and make a calibration curve 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 propham gives the test solution of 0.001 mg/L in concentration.

b. Quantification

Inject the test solution to LC-MS/MS, and calculate the concentration of propham from the calibration curve made in “a. Calibration curve”.

c. Confirmation

Confirm using LC-MS/MS.

d. Measurement conditions

Column: Octadecylsilanized silica gel, 2.1 mm in inside diameter, 150 mm in length, 3 μm in particle diameter

Column temperature: 40°C

Mobile phase: Linear gradient from 2 mmol/L ammonium formate/methanol (1:1, v/v) to (1:9, v/v) in 10 min

Ionization mode: ESI (+)

Major monitoring ions (*m/z*): Precursor ion 180, product ion 138, 120

Injection volume: 5 μL

Expected retention time: 8 min

6. Limit of quantification

0.01 mg/kg