Original: Japanese

Provisional Translation

Analytical Method for 2,4-D, 2,4-DB and Cloprop (Animal and Fishery Products)

1. Analytes

2,4-D

2,4-DB

Cloprop

2. Applicable foods

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.

Graphitized carbon black/ethylenediamine-*N*-propylsilanized silica gel layered cartridge (500 mg/500 mg): A polyethylene tube of 12–13 mm in inside diameter packed with 500 mg of graphite carbon in the upper layer and 500 mg of ethylenediamine-*N*-propylsilanized silica gel in the lower layer, or a cartridge equivalent to the specified one in separation capability.

Reference standard of 2,4-D: Contains not less than 98% of 2,4-D

Reference standard of 2,4-DB: Contains not less than 98% of 2,4-DB

Reference standard of cloprop: Contains not less than 98% of cloprop

5. Procedure

1) Extraction

i) Muscle, liver, kidney, milk, egg, and fish/shellfish

Add 5 mL of 4 mol/L hydrochloric acid and 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. Transfer exactly 10 mL of the resulting solution, add 100 mL of 10% (w/v) sodium chloride solution and extract with shaking twice with 100 mL mixture of ethyl acetate and *n*-hexane (1:1) and then with 50 mL mixture of ethyl acetate and *n*-hexane (1:1). Combine the mixture of ethyl acetate and *n*-hexane (1:1) extracts, dehydrate with anhydrous sodium sulfate, and remove the anhydrous sodium sulfate by filtration. Concentrate the filtrate at below 40°C and remove the solvent. To the residue, add 30 mL of *n*-hexane and extract three times by shaking with 30 mL mixture of acetonitrile and water (99:1) each time. Combine the mixture extracts, and concentrate the filtrate at below 40°C, and remove the solvent.

ii) Fat

Add 5 mL of 4 mol/L hydrochloric acid and 100 mL of acetone to 5.0 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. Transfer exactly 20 mL of the resulting solution and add 100 mL of 10% (w/v) sodium chloride solution and extract with shaking twice with 100 mL mixture of ethyl acetate and *n*-hexane (1:1) and then with 50 mL mixture of ethyl acetate and *n*-hexane (1:1). Combine the mixture of ethyl acetate and *n*-hexane (1:1) extracts, dehydrate with anhydrous sodium sulfate, and remove the anhydrous sodium sulfate by filtration. Concentrate the filtrate at below 40°C and remove the solvent. To the residue, add 30 mL of *n*-hexane and extract with shaking three times with 30 mL mixture of acetonitrile and water (99:1) each time. Combine the mixture extracts, and concentrate the filtrate at below 40°C, and remove the solvent.

iii) Honey

Dissolve 10.0 of honey in 20 mL of water. Add 5 mL of 4 mol/L hydrochloric acid and 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.

Transfer exactly 10 mL of the resulting solution, add 100 mL of 10% (w/v) sodium chloride solution, and extract with shaking twice with 100 mL mixture of ethyl acetate and *n*-hexane (1:1) and then with 50 mL mixture of ethyl acetate and *n*-hexane (1:1). Combine the mixture of ethyl acetate and *n*-hexane (1:1) extracts, dehydrate with anhydrous sodium sulfate, and remove the anhydrous sodium sulfate by filtration. Concentrate the filtrate at below 40°C, and remove the solvent.

2) Hydrolysis

Dissolve the residue obtained in 1) in 2 mL of methanol, add 1 mL of a 1.5 mol/L sodium hydroxide solution. Attach a reflux condenser to the flask, heat at 80°C for 30 minutes in a water bath, and allowed to cool. Add 1.5 mol/L hydrochloric acid to adjust pH 7.5-8.0, and add 16 mL of 0.1 w/v% sodium hydrogen carbonate solution.

3) Clean-up

i) Octadecylsilanized silica gel column chromatography

Add 10 mL each of methanol and water to an octadecylsilanized silica gel cartridge (1,000 mg) sequentially, and discard the effluents. Transfer the solution obtained in 2) to the cartridge, and discard the effluent. Elute with 20 mL of 0.1 w/v% sodium hydrogen carbonate/methanol (1:1, v/v), add 5 mL of 4 mol/L hydrochloric acid to the eluate, and adjust pH 1.0 or less. Add 100 mL of 10 w/v% sodium chloride solution to the resulting solution, and extract with shaking twice with 50 mL ether. Combine the extracts, dehydrate with sodium sulfate (anhydrous), and filter out the sodium sulfate (anhydrous), concentrate the filtrate at below 40ºC, and remove the solvent. Dissolve the residue in 3 mL of acetonitrile/toluene (3:1, v/v).

ii) Graphitized carbonblack/ethylenediamine-*N*-propylsilanized silica gel layered column

chromatography

Add 10 mL of acetonitrile/toluene (3:1, v/v) to a graphitized carbonblack/ethylenediamine-*N*-propylsilanized silica gel layered cartridge (500 mg/500 mg), and discard the effluent. Transfer the solution obtained in i), add 7 mL of acetonitrile/toluene (3:1, v/v), and discard the effluent. Elute with 30 mL of acetonitrile/formic acid/toluene (75:1:25, v/v/v), concentrated the eluate at below 40ºC, and remove the solvent. Dissolve the residue in methanol to make exactly 1 mL, and use this solution as the test solution.

6. Calibration curve

Prepare 2,4-D, 2,4-DB and cloprop standard solution (methanol) 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 2,4-D, 2,4-DB and cloprop give the test solution of 0.005 mg/L in concentration.

7. Quantification

Inject the test solution to LC-MS/MS and calculate the concentration of 2,4-D, 2,4-DB and cloprop from the calibration curve made in 6.

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

Example

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 5 mmol/L ammonium acetate solution/5 mmol/L ammonium acetate-methanol solution (7:3, v/v) to (1:9, v/v) in 20 min.

Ionization mode: ESI (–)

Major monitoring ions (*m/z*)

2,4-D: Precursor ion 219, product ion 161
Precursor ion 221, product ion 163

2,4-DB: Precursor ion 247, product ion 161
Precursor ion 249, product ion 163

Cloprop: Precursor ion 199, product ion 127
Precursor ion 201, product ion 129

Injection volume: 5 μL

Expected retention time

2,4-D: 9 min

2,4-DB: 13 min

Cloprop: 8 min

10. Limit of quantification

2,4-D: 0.01 mg/kg

2,4-DB: 0.01 mg/kg

Cloprop: 0.01 mg/kg

11. Explanatory note

1) Outline of analytical method

The method consists of extraction of 2,4-D, 2,4-DB and cloprop from sample with acetone under acidic condition of hydrochloric acid, transferring into ethyl acetate/n-hexane. Defatting by acetonitrile/hexane partitioning for high fat foods. Hydrolyze, clean-up with octadecylsilanized silica gel cartridge and ethylenediamine-*N*-propylsilanized silica gel cartridge, and quantification and confirmation using LC-MS/MS. 2,4,5-T can be analyzed simultaneously.

2) Notes

i) 2,4-D: including the compounds hydrolysable, 2,4-D-sodium, 2,4-D-dimethylamine, 2,4-D-ethyl, 2,4-D-isopropyl, 2,4-D-butoxyethyl, and 2,4-D-alkanolamine

2,4-DB: including the compounds hydrolysable, 2,4-DB-sodium, 2,4-DB-potassium, 2,4-DB butyl, 2,4-DB-isooctyl, and 2,4-DB-dimethylamine

ii) Since 2,4-D, 2,4-DB and cloprop are soluble in water under the basic condition, keep acidic in the extraction and transferring steps.

iii) Analyze appropriate change of linear gradient program on the LC-MS/MS conditions, when interfering components cause the enhancement or suppression of sensitivity in the measurement by LC-MS/MS.

iv) When the analytical method for 2,4-D, 2,4-DB and cloprop using LC-MS/MS was developed, the following monitoring ions were used.

2,4-D:

for quantification (m/z): precursor ion 219, product ion 161

for confirmation (m/z): precursor ion 221, product ion 163

2,4-DB:

for quantification (m/z): precursor ion 247, product ion 161

for confirmation (m/z): precursor ion 249, product ion 163

Cloprop:

for quantification (m/z): precursor ion 199, product ion 127

for confirmation (m/z): precursor ion 201, product ion 129

v) The foods examined in the development of the analytical method: Beef muscle, beef fat, beef liver, chicken muscle, chicken egg, cow’s milk, eel, salmon, corbicula, honey

11. Reference

MHLW Director Notice, Syoku-An No.0315001, analytical method for 2,4-D, 2,4-DB and cloprop (Agricultural Products), (March 15, 2006)

MHLW Notification No. 199, analytical Method for 2, 4, 5-T

12. Type

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