Analytical Method for Acrinathrin, Cyhalothrin, Cyfluthrin, Cypermethrin, Deltamethrin and Tralomethrin, Bifenthrin, Pyrethrins, Fenvalerate, Flucythrinate, Fluvalinate and Permethrin (Agricultural Products)

1. Analytes

Compositional substances of agricultural chemicals	Analytes
Acrinathrin	Acrinathrin
Cyhalothrin	Cyhalothrin
Cyfluthrin	Cyfluthrin
Cypermethrin	Cypermethrin
Deltamethrin and tralomethrin	Deltamethrin, Tralomethrin
Bifenthrin	Bifenthrin
Pyrethrins	Pyrethrin I, Pyrethrin II
Fenvalerate	Fenvalerate
Flucythrinate	Flucythrinate
Fluvalinate	Fluvalinate
Permethrin	Permethrin

2. Instruments

Gas chromatograph-electron capture detector (GC-ECD)

Gas chromatograph-mass spectrometer (GC-MS)

3. Reagents

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

Coagulating solution: Dissolve 2 g of sodium chloride and 4 mL of phosphoric acid in water to make exactly 400 mL.

Reference standard of acrinathrin: Contains not less than 98% of acrinathrin. Melting point of the standard is 81–83°C.

Reference standard of cyhalothrin: Contains not less than 97% of cyhalothrin. Boiling point of the standard is 187–190°C (reduced pressure: 0.027 kPa).

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

Reference standard of cypermethrin: Contains not less than 96% of cypermethrin. Melting point of the standard is 60–80°C.

Reference standard of deltamethrin: Contains not less than 98% of deltamethrin. Melting point of the standard is 98–101°C.

Reference standard of bifenthrin: Contains not less than 99% of bifenthrin. Melting point of

the standard is 69–71°C.

Reference standard of pyrethrins: Contains 11–12% of pyrethrin I and 13–15% of pyrethrin II.

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

Reference standard of flucythrinate: Contains not less than 98% of flucythrinate. Boiling point of the standard is 108°C (reduced pressure: 0.047 kPa).

Reference standard of fluvalinate: Contains not less than 92% of fluvalinate.

Reference standard of permethrin: Contains not less than 97% of permethrin. Melting point of the standard is 34–39°C.

4. Procedure

1) Extraction

i) Grains, legumes, nuts and seeds

Grind sample to pass through a standard sieve (420 μm). Weigh 10.0 g of sample, and add 20 mL of water and let stand for 2 hours.

Add 100 mL of acetone to the sample, homogenize for 3 minutes, and filter through a filter paper, covered with a 1-cm-thick layer of diatomaceous earth, with suction into a vacuum rotary evaporator flask. Collect the residue on the filter paper, add 50 mL of acetone, homogenize for 3 minutes, treat as described above, and combine the filtrate in the vacuum rotary evaporator flask, and concentrate to about 30 mL at below 40°C.

Transfer the concentrated filtrate to a 300 mL separating funnel containing 100 mL of 10% sodium chloride solution. Wash the vacuum rotary evaporator flask with 100 mL of *n*-hexane, and transfer the washing to the separating funnel. Shake the separating funnel vigorously for 5 minutes with a shaker, let stand, and transfer the *n*-hexane layer to a 300 mL conical flask. Add 50 mL of *n*-hexane to the aqueous layer, treat as described above, and combine the *n*-hexane layer in the conical flask. Add an appropriate quantity of anhydrous sodium sulfate to the *n*-hexane layer, let stand for 15 minutes with occasional shaking, and filter into a vacuum rotary evaporator flask. Wash the conical flask with 20 mL of *n*-hexane, and wash the residue on the filter paper with the washing. Repeat this step two times. Combine the washings in the vacuum rotary evaporator flask, and remove *n*-hexane at below 40°C.

Add 30 mL of *n*-hexane to the residue, and transfer to a 100 mL separating funnel. Add 30 mL of acetonitrile saturated with *n*-hexane to the separating funnel, shake vigorously for 5 minutes with a shaker, let stand, and transfer the acetonitrile layer to a vacuum rotary evaporator flask. Add 30 mL of acetonitrile saturated with *n*-hexane to the *n*-hexane layer, treat as described above twice, combine the acetonitrile layers in the vacuum rotary evaporator flask, and remove acetonitrile at below 40°C. Dissolve the

residue in *n*-hexane to make exactly 5 mL.

ii) Fruits, vegetables, herbs and hops

For fruits, vegetables and herbs, weigh about 1 kg of sample accurately, add an appropriate quantity of water (if necessary), homogenize and then take the sample of equivalent to 20.0 g.

For hops, grind sample. Weigh 5.00 g of the sample, add 20 mL of water and let stand for 2 hours.

Add 100 mL of acetone to the sample, homogenize for 3 minutes, and filter through a filter paper, covered with a 1-cm-thick layer of diatomaceous earth, with suction into a vacuum rotary evaporator flask. Collect the residue on the filter paper, add 50 mL of acetone, homogenize for 3 minutes, treat as described above, combine the filtrate in the vacuum rotary evaporator flask, and concentrate to about 30 mL at below 40°C.

Transfer the concentrated filtrate to a 300 mL separating funnel containing 100 mL of 10% sodium chloride solution. Wash the vacuum rotary evaporator flask with 100 mL of *n*-hexane, and transfer the washing to the separating funnel. Shake the separating funnel vigorously for 5 minutes with a shaker, let stand, and transfer the *n*-hexane layer to a 300 mL conical flask. Add 50 mL of *n*-hexane to the aqueous layer, treat as described above, and combine the *n*-hexane layer in the conical flask. Add an appropriate quantity of anhydrous sodium sulfate to the *n*-hexane layer, let stand for 15 minutes with occasional shaking, and filter into a vacuum rotary evaporator flask. Wash the conical flask with 20 mL of *n*-hexane, and wash the residue on the filter paper with the washing. Repeat this step two times. Combine the washings in the vacuum rotary evaporator flask, and remove *n*-hexane at below 40°C. Dissolve the residue in *n*-hexane to make exactly 5 mL.

iii) Powdered tea

Weigh 5.00 g of sample, add 20 mL of water and let stand for 2 hours. Add 100 mL of acetone to the sample, homogenize for 3 minutes, and filter through a filter paper, covered with a 1-cm-thick layer of diatomaceous earth, with suction into a vacuum rotary evaporator flask. Collect the residue on the filter paper, add 50 mL of acetone, homogenize for 3 minutes, treat as described above, and combine the filtrate in the vacuum rotary evaporator flask, and concentrate to about 30 mL at below 40°C.

Transfer the concentrated filtrate to a 300 mL separating funnel containing 100 mL of 10% sodium chloride solution. Wash the vacuum rotary evaporator flask with 100 mL of *n*-hexane, and transfer the washing to the separating funnel. Shake the separating funnel vigorously for 5 minutes with a shaker, let stand, and transfer the *n*-hexane layer to a 300 mL conical flask. Add 50 mL of *n*-hexane to the aqueous layer, treat as described above,

and combine the *n*-hexane layer in the conical flask. Add an appropriate quantity of anhydrous sodium sulfate to the *n*-hexane layer, let stand for 15 minutes with occasional shaking, and filter into a vacuum rotary evaporator flask. Wash the conical flask with 20 mL of *n*-hexane, and wash the residue on the filter paper with the washing. Repeat this step two times. Combine the washings in the vacuum rotary evaporator flask, and remove *n*-hexane at below 40°C.

Dissolve the residue in 50 mL of acetone, add 50 mL of coagulating solution, shake gently, and let stand for 5 minutes. Add 2 g of diatomaceous earth, shake gently, filter through a filter paper, covered with a 1-cm-thick layer of diatomaceous earth, with suction, and transfer the filtrate to a 300 mL separating funnel. Wash the vacuum rotary evaporator flask with 25 mL of acetone/coagulating solution (1:1, v/v), and wash the residue on the filter paper with the washing. Repeat this step two times. Combine the washings in the separating funnel. Add 10 g of sodium chloride and 50 mL of *n*-hexane to the separating funnel, shake vigorously for 5 minutes with a shaker, let stand, and transfer the *n*-hexane layer to a 300 mL conical flask. Add 50 mL of *n*-hexane to the aqueous layer, treat as described above, and combine the *n*-hexane layer in the conical flask. Add an appropriate quantity of anhydrous sodium sulfate to the *n*-hexane layer, let stand for 15 minutes with occasional shaking, and filter into a vacuum rotary evaporator flask. Wash the conical flask with 20 mL of *n*-hexane, and wash the residue on the filter paper with the washing. Repeat this step two times. Combine the washings in the vacuum rotary evaporator flask, and remove *n*-hexane at below 40°C. Dissolve the residue in *n*-hexane to make exactly 5 mL.

iv) Tea leaves except for powdered tea

Immerse 9.00 g of sample in 540 mL of water at 100°C, let stand for 5 minutes at room temperature, filter, cool, and transfer 360 mL of the filtrate to a 500 mL conical flask. Add 100 mL of acetone and 2 mL of saturated lead acetate solution, let stand for 1 hour at room temperature, filter through a filter paper, covered with a 1-cm-thick layer of diatomaceous earth, with suction, and transfer the filtrate to a 1,000 mL separating funnel. Wash the conical flask with 50 mL of acetone/water (1:1, v/v), and wash the residue on the filter paper with the washing. Transfer the washing to the separating funnel.

Add 20 g of sodium chloride and 100 mL of *n*-hexane to the separating funnel, shake the separating funnel vigorously for 5 minutes with a shaker, let stand, and transfer the *n*-hexane layer to a 300 mL conical flask. Add 100 mL of *n*-hexane to the aqueous layer, treat as described above, and combine the *n*-hexane layer in the conical flask. Add an appropriate quantity of anhydrous sodium sulfate to the *n*-hexane layer, let stand for 15

minutes with occasional shaking, and filter into a vacuum rotary evaporator flask. Wash the conical flask with 20 mL of *n*-hexane, and wash the residue on the filter paper with the washing. Repeat this step two times. Combine the washings in the vacuum rotary evaporator flask, and remove *n*-hexane at below 40°C. Dissolve the residue in *n*-hexane to make exactly 5 mL.

2) Clean-up

Place 5 g of synthetic magnesium silicate for column chromatography suspended in *n*-hexane, and then about 5 g of anhydrous sodium sulfate in a chromatographic tube of 15 mm in inside diameter and 300 mm in length, and let flow out *n*-hexane to the extent that only a small quantity of *n*-hexane remains on the top of the column. Transfer 2 mL of the solution obtained in 1) to the column, add 50 mL of *n*-hexane, and discard the effluent. Elute with 150 mL of diethyl ether/*n*-hexane (1:3, v/v), and collect the eluate in a vacuum rotary evaporator flask (I). Then, elute with 100 mL of diethyl ether/*n*-hexane (3:2, v/v), and collect the eluate in a vacuum rotary evaporator flask (II). Remove diethyl ether and *n*-hexane in both vacuum rotary evaporator flasks at below 40°C. Dissolve the residue in the vacuum rotary evaporator flask (I) in *n*-hexane to make exactly 2 mL, and use this solution as the test solution for acrinathrin, cyhalothrin, cyfluthrin, cypermethrin, deltamethrin and tralomethrin, bifenthrin, fenvalerate, flucythrinate, fluvalinate and permethrin. Dissolve the residue in the vacuum rotary evaporator flask (II) in *n*-hexane to make exactly 2 mL, and use this solution as the test solution for pyrethrins.

5. Measurement

1) Qualification

Perform the test under the measurement conditions described below. The results shall agree with those obtained using the reference standards.

Measurement conditions

Column: Silicate glass capillary 0.25 mm in inside diameter, 30 m in length coated with methyl silicone for gas chromatography 0.25 μm in film thickness

Column temperature: 50°C (1 min) - 25°C/min heating - 175°C (0 min) - 10°C/min heating - 300°C (4 min)

Injection port temperature: 280°C

Injection mode: Splitless

Detector temperature: 320°C

Carrier gas and flow rate: Helium. Adjust the flow rate to elute deltamethrin at about 19 min. Optimize the flow rate of nitrogen (make-up gas)

2) Quantification

Quantify using peak-height or peak-area method, on the basis of the results obtained using

the measurement conditions described in 1).

3) Confirmation

Perform gas chromatography-mass spectrometry under the measurement condition described in 1). The results shall agree with those obtained using the reference standards. When necessary, quantify using peak-height or peak-area method.

6. Limits of quantification

Acrinathrin: 0.01 mg/kg

Cyhalothrin: 0.02 mg/kg

Cyfluthrin: 0.05 mg/kg

Cypermethrin: 0.01 mg/kg

Deltamethrin: 0.01 mg/kg

Bifenthrin: 0.01 mg/kg

Pyrethrins: 0.2 mg/kg

Fenvalerate: 0.005 mg/kg

Flucythrinate: 0.005 mg/kg

Fluvalinate: 0.01 mg/kg

Permethrin: 0.02 mg/kg

7 Explanatory note

1) Because tralomethrin is transformed to deltamethrin at the injection port of a gas chromatograph, quantify deltamethrin and tralomethrin with the use of deltamethrin as the reference standard, and compare the results with the maximum residue limits for deltamethrin and tralomethrin.

Regard the sum of the results of pyrethrin I and pyrethrin II as the analytical result of pyrethrins.

2) The limits of quantification are the values expected for fruits, vegetables and herbs. The limits of quantification for grains, legumes, nuts and seeds are about twice, and those for tea leaves and hops are four times as large as those of fruits, vegetables and herbs. When maximum residue limits of the sample is lower than the limit of quantification, concentrate the test solution, increase the injection volume to gas chromatograph, or use alternative methods for quantification.

8. References

None

9. Type

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