Analytical Method for Cafenstrole, Difenoconazole, Cyproconazole, Simetryn, Thifluzamide, Tetraconazole, Tebuconazole, Triadimenol, Fludioxonil, Propiconazole, Hexaconazole and Penconazole (Agricultural Products)

1. Analytes

Compositional substances of agricultural chemicals	Analytes
Cafenstrole	Cafenstrole
Difenoconazole	Difenoconazole
Cyproconazole	Cyproconazole
Simetryn	Simetryn
Thifluzamide	Thifluzamide
Tetraconazole	Tetraconazole
Tebuconazole	Tebuconazole
Triadimenol	Triadimenol
Fludioxonil	Fludioxonil
Propiconazole	Propiconazole
Hexaconazole	Hexaconazole
Penconazole	Penconazole

2. Instruments

Gas chromatograph-flame thermionic detector (GC-FTD)

Gas chromatograph-nitrogen phosphorus detector (GC-NPD)

Gas chromatograph-mass spectrometer (GC-MS)

3. Reagents

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

Reference standard of cafenstrole: Contains not less than 99% of cafenstrole. Melting point of the standard is 116°C.

Reference standard of difenoconazole: Contains not less than 97% of difenoconazole. Melting point of the standard is 76°C.

Reference standard of cyproconazole: Contains not less than 98% of cyproconazole. Melting point of the standard is 103-105°C.

Reference standard of simetryn: Contains not less than 98% of simetryn. Melting point of the standard is 82-83°C.

Reference standard of thifluzamide: Contains not less than 99% of thifluzamide. Melting point of the standard is 178-179°C.

Reference standard of tetraconazole: Contains not less than 97% of tetraconazole. Decomposition point of the standard is 240°C.

Reference standard of tebuconazole: Contains not less than 99% of tebuconazole. Melting point of the standard is not less than 102-103°C.

Reference standard of triadimenol: Contains not less than 99% of triadimenol. Melting point of the standard is 133-138°C.

Reference standard of fludioxonil: Contains not less than 99% of fludioxonil. Melting point of the standard is 199-200°C.

Reference standard of propiconazole: Contains not less than 97% of propiconazole. Melting point of the standard is 180°C (with reduced pressure of 0.013 kPa).

Reference standard of hexaconazole: Contains not less than 97% of hexaconazole. Melting point of the standard is 110-112°C.

Reference standard of penconazole: Contains not less than 99% of penconazole. Melting point of the standard is 57-61°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, 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 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 layers 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 5 mL of *n*-hexane.

ii) Fruits, vegetables, herbs, powdered tea 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 equivalent to 20.0 g.

For powdered tea, weigh 5.00 g of sample, add 20 mL of water, and let stand 2 hours.

For hops, weigh 5.00 g of sample after grind, add 20 mL of water, and let stand 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 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 layers 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 5 mL of *n*-hexane.

iii) Tea leaves except powdered tea

a) Analytical method for difenoconazole, tetraconazole and propiconazole

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

Add 100 g of sodium chloride and 100 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 100 mL of *n*-hexane to the aqueous layer, treat as described above, and combine the *n*-hexane layers 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 5 mL of *n*-hexane.

b) Analytical method for tebuconazole, triadimenol, hexaconazole and penconazole

For grind sample, treat following the procedure for powdered tea described in **ii)**.

2) Clean-up

Place 10 g of synthetic magnesium silicate for column chromatography suspended in *n*-hexane, and then 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 the solution obtained in 1) to the column, add 100 mL of acetone/*n*-hexane (1:19, v/v), and discard the effluent. Elute with 100 mL of acetone/*n*-hexane (3:7, v/v), transfer the eluate to a vacuum rotary evaporator flask, and remove acetone and *n*-hexane at below 40°C. Dissolve the residue in acetone to make exactly 4 mL, and use this solution as the test solution.

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 phenyl-methyl silicone for gas chromatography 0.25 μm in film thickness

Column temperature: 100°C (1 min) - 30°C/min heating - 250°C - 6°C/min heating - 300°C (2 min)

Injection port temperature: 250°C

Injection method: Splitless

Detector temperature: 280°C

Carrier gas and flow rate: Helium. Adjust the flow rate to elute cafenstrole at about 12 minutes. Optimize the flow rate of air and hydrogen.

2) Quantification

i) Quantify using peak-height or peak-area method, on the basis of the result obtained using the measurement conditions described in **1)**.

3) Confirmation

i) Perform gas chromatography-mass spectrometry using the measurement conditions described in **1)**. The result shall agree with that obtained using the reference standard. When necessary, quantify using peak-height or peak-area method.

6. Limit of quantification

Cafenstrole: 0.01 mg/kg

Difenoconazole: 0.01 mg/kg

Cyproconazole: 0.005 mg/kg

Simetryn: 0.01 mg/kg

Thifluzamide: 0.01 mg/kg

Tetraconazole: 0.02 mg/kg

Tebuconazole: 0.005 mg/kg

Triadimenol: 0.01 mg/kg

Fludioxonil: 0.005 mg/kg

Propiconazole: 0.01 mg/kg

Hexaconazole: 0.01 mg/kg

Penconazole: 0.01 mg/kg

7. Explanatory note

1) Difenoconazole and Propiconazole are detected with two peaks in qualification, quantification and confirmation respectively. The calibration curves should be made by sum of areas of the two peaks.

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 of tea leaves and hops are about four times as large as those of fruits, vegetables and herbs. When maximum residue limit 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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