Analytical Method for Alachlor, Isoprocarb, Kresoxim-methyl, Diethofencarb, Thenylchlor, Tebufenpyrad, Paclobutrazol, Bitertanol, Pyriproxyfen, Pyriminobac-methyl, Fenarimol, Butachlor, Flutolanil, Pretilachlor, Metolachlor, Mefenacet, Mepronil and Lenacil (Agricultural Products)

1. Analytes

Compositional substances of agricultural chemicals	Analytes
Alachlor	Alachlor
Isoprocarb	Isoprocarb
Kresoxim-methyl	Kresoxim-methyl
Diethofencarb	Diethofencarb
Thenylchlor	Thenylchlor
Tebufenpyrad	Tebufenpyrad
Paclobutrazol	Paclobutrazol
Bitertanol	Bitertanol
Pyriproxyfen	Pyriproxyfen
Pyriminobac-methyl	(*E*)-Pyriminobac-methyl, (*Z*)-Pyriminobac-methyl
Fenarimol	Fenarimol
Butachlor	Butachlor
Flutolanil	Flutolanil
Pretilachlor	Pretilachlor
Metolachlor	Metolachlor
Mefenacet	Mefenacet
Mepronil	Mepronil
Lenacil	Lenacil

2. Instruments

Gas chromatograph-flame thermionic detector (GC-FTD) or gas chromatograph-nitrogen phosphorous 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 alachlor: Contains not less than 98% of alachlor. Melting point of the standard is 39–42°C.

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

the standard is 88–93°C.

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

Reference standard of diethofencarb: Contains not less than 98% of diethofencarb. Melting point of the standard is 146–147°C.

Reference standard of thenylchlor: Contains not less than 99% of thenylchlor. Melting point of the standard is 75°C.

Reference standard of tebufenpyrad: Contains not less than 98% of tebufenpyrad. Melting point of the standard is 61–62°C.

Reference standard of paclobutrazol: Contains not less than 97% of paclobutrazol. Melting point of the standard is 165–166°C.

Reference standard of bitertanol: Contains not less than 99% of bitertanol. Melting point of the standard is 110–120°C.

Reference standard of pyriproxyfen: Contains not less than 99% of pyriproxyfen. Melting point of the standard is 45–47°C.

Reference standard of (*E*)-pyriminobac-methyl: Contains not less than 99% of (*E*)-pyriminobac-methyl. Melting point of the standard is 109–110°C.

Reference standard of (*Z*)-pyriminobac-methyl: Contains not less than 99% of (*Z*)-pyriminobac-methyl. Melting point of the standard is 71–72°C.

Reference standard of fenarimol: Contains not less than 99% of fenarimol. Melting point of the standard is 117–119°C.

Reference standard of butachlor: Contains not less than 98% of butachlor. Boiling point of the standard is 156°C (reduced pressure: 0.0067 kPa).

Reference standard of flutolanil: Contains not less than 99% of flutolanil. Melting point of the standard is 104–105°C.

Reference standard of pretilachlor: Contains not less than 99% of pretilachlor.

Reference standard of metolachlor: Contains not less than 97% of metolachlor. Boiling point of the standard is 100°C (reduced pressure: 0.00013 kPa).

Reference standard of mefenacet: Contains not less than 99% of mefenacet. Melting point of the standard is 134–135°C.

Reference standard of mepronil: Contains not less than 99% of mepronil. Melting point of the standard is 94°C.

Reference standard of lenacil: Contains not less than 99% of lenacil. Melting point of the standard is 135°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 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 ethyl acetate and transfer the washing to the separating funnel. Shake the separating funnel vigorously for 5 minutes with a shaker, let stand, and transfer the ethyl acetate layer to a 300 mL conical flask. Add 50 mL of ethyl acetate to the aqueous layer, treat as described above, and combine the ethyl acetate layer in the conical flask. Add an appropriate quantity of anhydrous sodium sulfate to the ethyl acetate 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 ethyl acetate, 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 ethyl acetate 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 it 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 2 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 for 2 hours. 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, 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 ethyl acetate, and transfer the washing to the separating funnel. Shake the separating funnel vigorously for 5 minutes with a shaker, let stand, and transfer the ethyl acetate layer to a 300 mL conical flask. Add 50 mL of ethyl acetate to the aqueous layer, treat as described above, and combine the ethyl acetate layer in the conical flask. Add an appropriate quantity of anhydrous sodium sulfate to the ethyl acetate 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 ethyl acetate, 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 ethyl acetate at below 40°C. Add 10 mL of *n*-hexane to the residue, and remove *n*-hexane at below 40°C. Dissolve the residue in 2 mL of *n*-hexane.

iii) Tea leaves except for powdered tea

a) Analysis of tebufenpyrad

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 2 mL of saturated lead acetate solution to the conical flask, 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 water, and wash the residue on the filter paper with the washing. Transfer the washing to the separating funnel.

Add 25 g of sodium chloride and 100 mL of ethyl acetate to the separating funnel, shake vigorously for 5 minutes with a shaker, let stand, and transfer the ethyl acetate layer to a 300 mL conical flask. Add 100 mL of ethyl acetate to the aqueous layer, treat as described above, and combine the ethyl acetate layer in the conical flask. Add an appropriate quantity of anhydrous sodium sulfate to the ethyl acetate 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 ethyl acetate, 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 ethyl acetate at below 40°C. Add 10 mL of *n*-hexane to the residue, and remove *n*-hexane below 40°C. Dissolve the residue in 2 mL of *n*-hexane.

b) Analysis of kresoxim-methyl, bitertanol, pyriproxyfen and fenarimol

For tea leaves except for powdered tea, treat following the procedure for powdered tea

described in ii).

2) Clean-up

Place 5 g of synthesized 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 the solution obtained in 1) to the column, add 50 mL of diethyl ether/*n*-hexane (1:99, v/v), and discard the effluent. Elute with 50 mL of acetone/*n*-hexane (3:7, v/v), collect the eluate to a vacuum rotary evaporator flask, and remove acetone, diethyl ether and *n*-hexane at below 40°C. Dissolve the residue in acetone to make exactly 5 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 5% phenyl-methyl silicone for gas chromatography 0.25 μm in film thickness

Column temperature: 160°C (1 min) - 10°C/min heating - 190°C (1 min) - 2°C/min heating - 210°C (2 min) - 5°C/min heating - 240°C (1 min) - 10°C/min heating - 260°C (6 min)

Injection port temperature: 210°C

Detector temperature: 210°C

Carrier gas and flow rate: Helium. Optimize the flow rates of air and hydrogen.

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 using the measurement conditions described in 1). The results shall agree with those obtained using the reference standards. When necessary, quantify by peak-height or peak-area method.

6. Limit of quantification

Alachlor: 0.005 mg/kg

Isoprocarb: 0.1 mg/kg

Kresoxim-methyl: 0.01 mg/kg

Diethofencarb: 0.01 mg/kg

Thenylchlor: 0.01 mg/kg

Tebufenpyrad: 0.01 mg/kg

Paclobutrazol: 0.005 mg/kg

Bitertanol: 0.01 mg/kg

Pyriproxyfen: 0.01 mg/kg

Pyriminobac-methyl: 0.01 mg/kg

Fenarimol: 0.02 mg/kg

Butachlor: 0.05 mg/kg

Flutolanil: 0.025 mg/kg

Pretilachlor: 0.01 mg/kg

Metolachlor: 0.005 mg/kg

Mefenacet: 0.01 mg/kg

Mepronil: 0.01 mg/kg

Lenacil: 0.05 mg/kg

7. Explanatory note

1) Quantify (*E*)-pyriminobac-methyl and (*Z*)-pyriminobac-methyl individually, and regard the sum of the results as the analytical result of pyriminobac-methyl.

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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