

Analytical Method for BHC, γ -BHC, DDT, Aldrin and Dieldrin, Ethalfluralin, Etridiazole, Endrin, Quintozene, Chlordane, Dicofol, Tecnazene, Tetradifon, Tefluthrin, Trifluralin, Halfenprox, Fenpropathrin, Hexachlorobenzene, Heptachlor, Benfluralin and Methoxychlor (Agricultural Products)

1. Analytes

Compositional substances of agricultural chemicals	Analytes
BHC (sum of α -BHC, β -BHC, γ -BHC and δ -BHC)	α -BHC, β -BHC, γ -BHC, δ -BHC
γ -BHC (lindane)	γ -BHC (lindane)
DDT (including DDD and DDE)	<i>pp'</i> -DDD, <i>pp'</i> -DDE, <i>pp'</i> -DDT, <i>op'</i> -DDT
Aldrin and dieldrin (sum of both compounds)	Aldrin, Dieldrin
Ethalfluralin	Ethalfluralin
Etridiazole	Etridiazole
Endrin	Endrin
Quintozene	Quintozene
Chlordane	<i>trans</i> -Chlordane, <i>cis</i> -Chlordane
Dicofol	Dicofol
Tecnazene	Tecnazene
Tetradifon	Tetradifon
Tefluthrin	Tefluthrin
Trifluralin	Trifluralin
Halfenprox	Halfenprox
Fenpropathrin	Fenpropathrin
Hexachlorobenzene	Hexachlorobenzene
Heptachlor	Heptachlor, Heptachlorepoxyde
Benfluralin	Benfluralin
Methoxychlor	Methoxychlor

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.

Reference standard of α -BHC: Contains not less than 99% of α -BHC. Melting point of the standard is 157–159°C.

Reference standard of β -BHC: Contains not less than 98% of β -BHC. Melting point of the standard is 308–310°C.

Reference standard of γ -BHC: Contains not less than 99% of γ -BHC. Melting point of the standard is 112–114°C.

Reference standard of δ -BHC: Contains not less than 95% of δ -BHC. Melting point of the standard is 137–140°C.

Reference standard of *pp'*-DDD: Contains not less than 98% of *pp'*-DDD. Melting point of the standard is 108–110°C.

Reference standard of *pp'*-DDE: Contains not less than 99% of *pp'*-DDE. Melting point of the standard is 88–90°C.

Reference standard of *op'*-DDT: Contains not less than 98% of *op'*-DDT. Melting point of the standard is 73–75°C.

Reference standard of *pp'*-DDT: Contains not less than 99% of *pp'*-DDT. Melting point of the standard is 108–110°C.

Reference standard of aldrin: Contains not less than 97% of aldrin. Melting point of the standard is 102–104°C.

Reference standard of ethalfluralin: Contains not less than 98% of ethalfluralin. Melting point of the standard is 55–56°C.

Reference standard of etridiazole: Contains not less than 98% of etridiazole. Melting point of the standard is 20°C.

Reference standard of quintozene: Contains not less than 98% of quintozene. Melting point of the standard is 143–144°C.

Reference standard of *trans*-chlordane: Contains not less than 98% of *trans*-chlordane. Melting point of the standard is 104–105°C.

Reference standard of *cis*-chlordane: Contains not less than 98% of *cis*-chlordane. Melting point of the standard is 106–107°C.

Reference standard of dicofol: Contains not less than 95% of dicofol. Melting point of the standard is 73–76°C.

Reference standard of dieldrin: Contains not less than 98% of dieldrin. Melting point of the standard is 177–179°C.

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

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

Reference standard of tefluthrin: Contains not less than 98% of tefluthrin. Melting point of the standard is 44–45°C.

Reference standard of trifluralin: Contains not less than 98% of trifluralin. Melting point of the standard is 46–50°C.

Reference standard of halfenprox: Contains not less than 99% of halfenprox. Melting point of the standard is 291°C.

Reference standard of fenpropathrin: Contains not less than 99% of fenpropathrin. Melting point of the standard is 45–50°C.

Reference standard of hexachlorobenzene: Contains not less than 98% of hexachlorobenzene. Melting point of the standard is 226°C.

Reference standard of heptachlor: Contains not less than 98% of heptachlor. Melting point of the standard is 95–96°C.

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

Reference standard of benfluralin: Contains not less than 98% of benfluralin. Melting point of the standard is 65–67°C.

Reference standard of methoxychlor: Contains not less than 98% of methoxychlor. Melting point of the standard is 89°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 water and let stand for 2 hours.

Add 100 mL of acetone to the sample, homogenize for 3 minutes, filter through a filter paper, covered with 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, 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 *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 the 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 concentrate *n*-hexane at below 40°C.

Add 20 mL of *n*-hexane to the residue, and transfer to a 100 mL separating funnel. Add 40 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. Dissolve the residue in *n*-hexane to make exactly 5 mL.

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 to the sample, homogenize for 3 minutes, and filter through a filter paper covered with 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 *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 10 mL.

iii) Tea leaves except for powdered tea

a) Analysis of BHC, DDT, aldrin and dieldrin, endrin, dicofol, tetradifon, trifluralin, halfenprox and fenprothrin

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, and 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, and wash the residue on the filter paper with the washing. Transfer the washing to the separating funnel.

Add 30 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 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 *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.

- b) Analysis of quintozone, chlordane, tecnazene, tefluthrin, hexachlorobenzene and heptachlor

Grind sample, and 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 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, elute with 200 mL of diethyl ether/*n*-hexane (3:17), collect the eluate to a vacuum rotary evaporator flask, and remove diethyl ether and *n*-hexane at below 40°C. Dissolve the residue in *n*-hexane to make exactly 2 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 under both measurement conditions. Note that dicofol should be tested under the measurement condition 1 describe below.

Measurement condition 1

Column: Silicate glass capillary 0.25 mm in inside diameter, 10–30 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 (5 min)

Injection port temperature: 230°C

Detector temperature: 300°C

Carrier gas and flow rate: Helium. Adjust the flow rate to elute aldrin at about 10 min.

Measurement condition 2

Column: Silicate glass capillary 0.25 mm in inside diameter, 10–30 m in length coated with 14% cyanopropylphenyl-methyl silicone for gas chromatography 0.25 µm in film thickness

Column temperature: 80°C (2 min) - 30°C/min heating - 190°C (0 min) - 3.6°C/min heating - 250°C (8 min).

Injection port temperature: 230°C

Detector temperature: 300°C

Carrier gas and flow rate: Helium. Adjust the flow rate to elute aldrin at about 10 min.

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 with peak-height or peak-area method.

6. Limit of quantification

γ-BHC (lindane): 0.01 mg/kg

Aldrin: 0.005 mg/kg

Ethalfluralin: 0.01 mg/kg

Etridiazole: 0.01 mg/kg

Endrin: 0.005 mg/kg

Quintozene: 0.01 mg/kg

Chlordane: 0.01 mg/kg

Dieldrin: 0.005 mg/kg

Tecnazene: 0.01 mg/kg

Tetradifon: 0.01 mg/kg

Tefluthrin: 0.01 mg/kg

Hexachlorobenzene: 0.01 mg/kg

Heptachlor: 0.01 mg/kg

Benfluralin: 0.01 mg/kg

Methoxychlor: 0.01 mg/kg

Trifluralin: 0.005 mg/kg

Halfenprox: 0.02 mg/kg

Fenpropathrin: 0.01 mg/kg

7. Explanatory note

- 1) Quantify α -BHC, β -BHC, γ -BHC and δ -BHC individually, and regard the sum of the results as the analytical result of BHC.
- 2) Quantify *pp'*-DDD, *pp'*-DDE, *pp'*-DDT and *op'*-DDT individually, and regard the sum of the results as the analytical result of DDT.
- 3) Quantify aldrin and dieldrin individually, and regard the sum of the results as the analytical result of aldrin and dieldrin.
- 4) Quantify *trans*-chlordane and *cis*-chlordane individually, and regard the sum of the results as the analytical result of chlordane.
- 5) Quantify heptachlor and heptachlorepoxyde individually, and regard the sum of the results as the analytical result of heptachlor.
- 6) The limits of quantification are the values expected for grains, legumes, nuts and seeds, fruits, vegetables and herbs. The limits of quantification for tea leaves are about twice, and those of powdered tea and hops are about four times as large as those of grains, legumes, nuts and seeds, 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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