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

1. Analytes

Compositional substances of agricultural chemicals	Analytes
2,4-D	2,4-D, 2,4-D sodium salt, 2,4-D dimethylamine salt, 2,4-D ethyl, 2,4-D isopropyl, 2,4-D butoxyethyl and 2,4-D alcanolamine salt
2,4-DB	2,4-DB, 2,4-DB sodium salt, 2,4-DB butyl, 2,4-DB dimethylammonium salt and 2,4-DB isooctyl
Cloprop	Cloprop

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.

Butyl esterification reagent: Dissolve 10 g of boron trifluoride diethyl ether complex in 25 mL of *n*-butanol.

Reference standard of 2,4-D: Contains not less than 99% of 2,4-D. Melting point of the standard is 138°C.

Reference standard of 2,4-DB: Contains not less than 98% of 2,4-DB. Melting point of the standard is 117–119°C.

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

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 and 5 mL of 4 mol/L hydrochloric acid 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, concentrate to about 1 mL at below 40°C, and evaporate to dryness at room temperature under a stream of nitrogen.

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 200 mL separating funnel. Add 30 mL of acetonitrile saturated with *n*-hexane to the *n*-hexane layer, treat as described above twice, and combine the acetonitrile layers in the 200 mL separating funnel. Add 50 mL of *n*-hexane saturated with acetonitrile to the 200 mL separating funnel, shake gently, and let stand. Transfer the acetonitrile layer to a vacuum rotary evaporator flask, concentrate to about 1 mL at below 40°C, and evaporate to dryness at room temperature under a stream of nitrogen.

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 equivalent to 20.0 g.

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

Add 100 mL of acetone and 5 mL of 4 mol/L hydrochloric acid to the sample, homogenize for 3 minutes, and filter through a filter paper, covered with a 1-cm-thick layer 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, concentrate to about 1 mL at below 40°C, and evaporate to dryness at room temperature under a stream of nitrogen.

2) Hydrolysis

Dissolve the residue obtained in 1) in 20 mL of methanol, transfer to a 100 mL recovery flask, and add 10 mL of 1.5 mol/L sodium hydroxide solution. Connect a reflux condenser to the recovery flask, and heat in an 80°C water bath for 30 minutes, and allow to cool. Transfer the reaction mixture to a vacuum rotary evaporator flask, and remove most of methanol at below 40°C. Filter the resultant solution through a glass filter (G3) with suction, and transfer the filtrate to a 300 mL separating funnel (I). Wash the residue on the glass filter with a small amount of acetone and water, and transfer the washing to the separating funnel (I). Add 50 mL of diethyl ether and 100 mL of 10% sodium chloride solution, shake vigorously for 5 minutes with a shaker, let stand, and transfer the aqueous layer to a 300 mL separating funnel (II). Add 4 mol/L hydrochloric acid to the aqueous layer to adjust pH lower than 1, add 50 mL of ethyl acetate, shake 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. Transfer the washing to the vacuum rotary evaporator flask, and concentrate to about 1 mL under 40°C.

3) Butyl esterification

Transfer the solution obtained in 2) to a 20 mL recovery flask, evaporate to dryness at room temperature under a stream of nitrogen, and add 1 mL of butyl esterification reagent. Connect a reflux condenser to the recovery flask, and heat in a 90°C water bath for 30 minutes, and allow to cool. Transfer the reaction mixture to a 200 mL separating funnel containing 50 mL of 10% sodium chloride solution and 50 mL of *n*-hexane, shake the separating funnel vigorously for 5 minutes with a shaker, let stand, and transfer the *n*-hexane layer to a 200 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 10 mL of *n*-hexane, and wash the residue on the filter paper with the washing. Transfer the washing to the vacuum rotary evaporator flask, and concentrate to about 2 mL under 40°C.

4) 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 inside diameter and 300 mm 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 3) to the column, elute with 150 mL of diethyl ether/*n*-hexane (3:17, v/v). Collect the eluate to a vacuum rotary evaporator flask, concentrate to about 1 mL at below 40°C, and evaporate to dryness under a stream of nitrogen. 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 treated following **4** 3).

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: 50°C (1 min) - 25°C/min heating - 125°C (0 min) - 10°C/min heating - 300°C (5 min)

Injection port temperature: 260°C

Detector temperature: 300°C

Carrier gas: Nitrogen or helium

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 treated following **4** 3). When necessary, quantify by peak-height or peak-area method.

6. Limit of quantification

2,4-D: 0.005 mg/kg (0.01 mg/kg for grains)

2,4-DB and cloprop: 0.01 mg/kg (GC-MS)

7. Explanatory note

1) This analytical method was developed by modifying the notified "Analytical Method for 2,4-D" to analyze 2,4-D, 2,4-DB and cloprop simultaneously. The points of modifications are (i) omission of the washing step with 50 mL of diethyl ether/*n*-hexane (1:19, v/v) after transferring sample solution to a synthetic magnesium silicate column and (ii) change in volume of the final test solution to 2 mL.
2) Because 2,4-D dissolves in water under a basic condition, extraction should be carried out under an acidic condition. Ethyl acetate should be removed before butyl esterification.
3) The sensitivity of cloprop with GC-ECD is low, and thus use of GC-MS is recommended for qualification and quantification. Major monitoring ions (*m/z*) are 231 for 2,4-DB, and 256 for cloprop.

8. References

None

9. Type

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