Analytical Method for Aldicarb and Aldoxycarb, Ethiofencarb, Oxamyl, Carbaryl, Pirimicarb, Fenobucarb and Bendiocarb (Agricultural Products)

1. Analytes

Compositional substances of agricultural chemicals	Analytes
Aldicarb and aldoxycarb	Aldicarb, Aldicarb-sulfoxide, Aldicarb-sulfone
Ethiofencarb	Ethiofencarb
Oxamyl	Oxamyl
Carbaryl	Carbaryl
Pirimicarb	Pirimicarb
Fenobucarb	Fenobucarb
Bendiocarb	Bendiocarb

2. Instruments

High-performance liquid chromatograph-postcolumn fluorescence detector (HPLC-FL)

Liquid chromatograph-mass spectrometer (LC-MS)

3. Reagents

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

Fluorescence solution: Add 0.05 mol/L of sodium borate solution to 10 mg of o-Phthalaldehyde and 5μL of 2-Mercaptoethanol to make exactly 100 mL.

Phosphate buffer solution: Dissolve 1.75 g of sodium hydroxide and 11.7 g of monosodium phosphate in about 800 mL of water, and add water to make exactly 1000 mL.

4. Reference standard

Reference standard of aldicarb: Contains not less than 99% of aldicarb. Melting point of the standard is 98-100°C.

Reference standard of aldicarb-sulfoxide: Contains not less than 96% of aldicarb-sulfoxide. Melting point of the standard is 100-104°C.

Reference standard of aldicarb-sulfone: Contains not less than 98% of aldicarb-sulfone. Melting point of the standard is 132-142°C.

Reference standard of ethiofencarb: Contains not less than 99% of ethiofencarb. Melting point of the standard is 33-34°C.

Reference standard of oxamyl: Contains not less than 99% of oxamyl. Melting point of the standard is 100-102°C.

Reference standard of carbaryl: Contains not less than 99% of carbaryl. Melting point of the standard is 138-140°C.

Reference standard of pirimicarb: Contains not less than 99% of pirimicarb. Melting point of the standard is 90-91°C.

Reference standard of fenobucarb: Contains not less than 98% of fenobucarb. Melting point of the standard is 32°C.

Reference standard of bendiocarb: Contains not less than 99% of bendiocarb. Melting point of the standard is 129-130°C.

5. Procedure

1) Extraction

i) Grains, legumes, fruits, vegetables, nuts, seeds, powdered tea and hop

For grains, legumes, nuts and seeds, weigh 20.0 g of sample, add 100 mL of water, and let stand for 2 hours.

For fruits and vegetables, take sample equivalent to 20.0 g.

For powdered tea and hop, weigh 20.0 g of sample.

Add 200 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 100 mL of acetone, treat as described above, combine the filtrate in the vacuum rotary evaporator flask, and concentrate to about 20 mL at 40°C or below.

Transfer to a 500 mL separating funnel containing 200 mL of 5% sodium chloride solution and 100 mL of dichloromethane (guaranteed reagent), shake the separating funnel vigorously for 5 minutes with a shaker, let stand, and transfer the dichloromethane layer to a 500 mL conical flask. Add 100 mL of dichloromethane (guaranteed reagent) to the aqueous layer, treat as described above, and combine the dichloromethane layer in the conical flask. Add an appropriate quantity of anhydrous sodium sulfate, let stand for 15 minutes with occasional shaking, and filter into a vacuum rotary evaporator flask. Wash the conical flask with 50 mL of dichloromethane (guaranteed reagent), and wash the residue on the filter paper with the washing. Repeat this step two more times. Combine the washings in the vacuum rotary evaporator flask, concentrate to about 1 mL at 40°C or below, and air-dry at room temperature.

Dissolve the residue in 25 mL of *n*-hexane and 30 mL of acetonitrile saturated with *n*-hexane, and transfer to a 100 mL separating funnel. Shake vigorously for 10 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 layer to the separating funnel. Add 50 mL of *n*-hexane saturated with acetonitrile, shake vigorously for 5 minutes with a shaker, let stand, transfer the acetonitrile layer to a vacuum rotary evaporator flask, concentrate to about 1 mL at 40°C or below, and air-dry at room temperature. Dissolve the residue in methanol, and make exactly 2 mL.

ii) Tea leaves except powdered tea

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 4 mL of saturated lead acetate solution to the filtrate, shake for 10 seconds, 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 conical flask with 50 mL of acetone, wash the residue on the filter paper with the washing, and combine the washing in the separating funnel. Add 100 mL of ether and 100 g of sodium chloride in the separate funnel, sake vigorously for 5 minutes with a shaker, let stand, and transfer the ether layer to a 300 mL conical flask. Add 100 mL of ether to the aqueous layer, treat as described above one more time, and combine the ether layer in the conical flask. Add an appropriate quantity of anhydrous sodium sulfate to the ether layer, let stand for 15 minutes with occasional shaking, and filter into a vacuum rotary evaporator flask. Wash the conical flask with 30 mL of ether, and wash the residue on the filter paper with the washing. Repeat this step two more times. Combine the washings in the vacuum rotary evaporator flask, concentrate to about 1 mL at 40°C or below, and air-dry at room temperature. Dissolve the residue in methanol, make exactly 2 mL.

2) Clean-up

i) Weigh 0.3 mL of the solution obtained in **1)**, add 3 mL of dilute hydrochloric acid, shake gently, and filter through a membrane filter with pore openings of 0.45 μm. Use this solution as the test solution.

6. Measurement

1) Qualification

i) Aldicarb, aldicarb-sulfoxide, aldicarb-sulfone, ethiofencarb, oxamyl, carbaryl, fenobucarb and bendiocarb

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

Measurement conditions

Column: Octadecylsilylated silica gel (particle diameter 5μm), 3.9 mm in inside diameter, 150 m in length

Column temperature: 40°C

Detector: excitation wavelength 339 nm, fluorescence wavelength 445 nm

Mobile phase: A: tetrahydrofuran, B: water, C: methanol. Adjust the flow rate to elute aldicarb at about 12 min.

Linear gradient: Initially a mixture of water/methanol (22:3, v/v) for 0.1 min, followed by a linear gradient from mobile phase A/mobile phase B (1:9, v/v) to (3:7, v/v) for 19.9 min, a mixture of tetrahydrofuran/water (3:7, v/v) for 10 min, and a mixture of water/methanol (22:3, v/v) for 10 min.

Hydrolysis reactor vessel: Inject 0.05 mol/L of sodium hydroxide solution into a mobile phase. Maintain a constant injection.

Hydrolysis reactor vessel temperature: 80°C

Fluorescence reactor vessel: Inject a fluorescence solution into a mobile phase. Maintain a constant injection.

ii) Pirimicarb

Perform the test under the measurement conditions described below. Use the test solution obtained in **1)** of **5**. The results shall agree with that obtained using the reference standards.

Measurement conditions

Column: Octadecylsilylated silica gel (particle diameter 5μm), 4.0-4.6 mm in inside diameter, 250 m in length

Detector: excitation wavelength 312 nm, fluorescence wavelength 382 nm

Mobile phase: Use a mixture of water/methanol/phosphate buffer solution (1:7:2, v/v/v). Adjust the flow rate to elute pirimicarb at about 5 min.

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)**.

For aldicarb and aldoxycarb, quantify respective aldicarb, aldicarb-sulfoxide and aldicarb-sulfone using peak-height or peak-area method, on the basis of the result obtained using the measurement conditions described in **1)**, estimate concentrations of aldicarb, aldicarb-sulfoxide and aldicarb-sulfone, and estimate concentration of aldicarb including aldicarb-sulfone and aldicarb sulfone using the following equation:

concentration of aldicarb (including aldicarb-sulfoxide and aldicarb-sulfone) ppm=A+B×0.9224+C×0.8560

A: concentration of aldicarb (ppm)

B: concentration of aldicarb-sulfoxide (ppm)

C: concentration of aldicarb-sulfone (ppm)

3) Confirmation

Perform liquid chromatography-mass spectrometry using the measurement conditions described below. Use the test solution obtained in **1)** of **5**. The result shall agree with that obtained using the reference standard. When necessary, quantify using peak-height or peak-area method.

Measurement conditions

Column: Octadecylsilylated silica gel (particle diameter 3-5μm), 2.0-4.6 mm in inside diameter, 75-150 m in length

Column temperature: 50°C

Mobile phase: A: mixture of water/methanol (9:1, v/v), B: mixture of water/methanol (1:9, v/v).

Linear gradient: Initially A:B (9:1, v/v) for 0.1 min, followed by a linear gradient from A:B (1:9, v/v) to (1:3, v/v) for 24.9 min, a linear gradient from A:B (1:3, v/v) to (0:1, v/v) for 5 min, and then A:B (9:1, v/v) for 5 min.

Ionization mode: ESI (+)

Major monitoring ion (*m/z*)

Aldicarb: 213, 116

Aldicarb-sulfoxide: 207, 132

Aldicarb-sulfone: 223

Ethiofencarb: 226

Oxamyl: 237

Carbaryl: 202, 145

Pirimicarb: 239

Fenobucarb: 208

Bendiocarb: 224

7. Limit of quantification

Aldicarb: 0.005 mg/kg

Aldicarb-sulfoxide: 0.005 mg/kg

Aldicarb-sulfone: 0.005 mg/kg

Ethiofencarb: 0.005 mg/kg

Oxamyl: 0.005 mg/kg

Carbaryl: 0.01 mg/kg

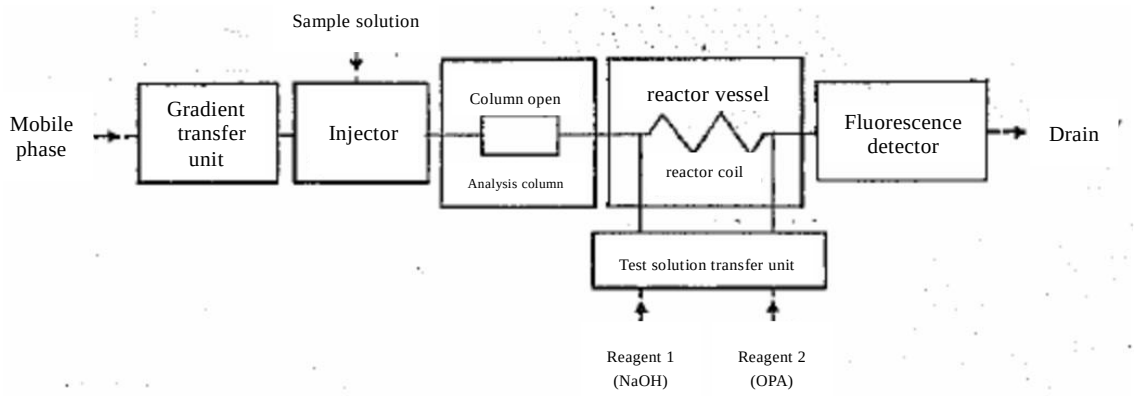
Pirimicarb: 0.005 mg/kg

Fenobucarb: 0.01 mg/kg

Bendiocarb: 0.005 mg/kg

8. Explanatory note

1) The structure in the high-performance liquid chromatograph-postcolumn fluorescence detector is as follows:



2) Add about 5 g of sodium hydrogen carbonate when pirimicarb is extracted simultaneously from highly acidic foods, such as citrus fruits.

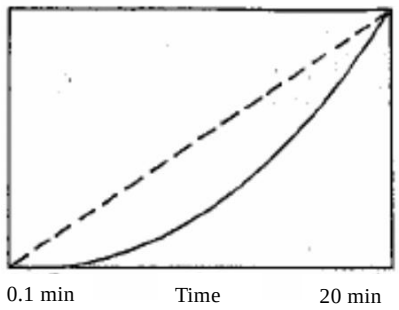
3) A sample containing little or no fat can be measured without the process of acetonitrile/hexane partition.

4) A membrane filter may capture analytes. Make sure that the analytes can be surely collected by use of the membrane filter.

5) The measurement conditions can be varied depending on types of instruments or cartridges.

Distinguish cautiously aldicarb-sulfoxide and aldicarb-sulfone from other components such as oxamyl since aldicarb-sulfoxide and aldicarb-sulfone can be eluted immediately.

6) Refer to the curve in the graph shown below for the linear gradient in **1)** of **6**.



7) One of major ions of aldicarb, *m/z* 213, is [M+Na] in **3)** of **6**.

8) Use a 250 mg graphitized carbon black cartridge, a 500 mg ethylenediamine-*N*-propylsilanized silica gel cartridge and a 500 mg trimethyl aminopropylsilanized silica gel cartridge to clean up a sample that includes preventive components.

Measurement: Inject 30 mL of acetone and 20 mL of *n*-hexane to respective cartridges of a 250 mg graphitized carbon black cartridge, a 500 mg ethylenediamine-*N*-propylsilanized silica gel cartridge, and a 500 mg trimethyl aminopropylsilanized silica gel cartridge. Discard effluent, and connect these cartridges from the upper side to the lower side in order of the 250 mg graphitized carbon black cartridge, the 500 mg ethylenediamine-*N*-propylsilanized silica gel cartridge and the 500 mg trimethyl aminopropylsilanized silica gel cartridge. Weigh 0.5 mL of the sample extract, remove methanol in a nitrogen gas stream, dissolve in 0.5 mL of acetone/*n*-hexane (1:4, v/v) mixture, and inject into the connected cartridge described above. Inject 20 mL of acetone/*n*-hexane mixture (1;4, v/v) mixture, pass at a flow rate of 0.5 mL/min, and take the eluate. Remove the 250 mL graphitized carbon black cartridge and the 500 mL ethylenediamine-*N*-propylsilanized silica gel cartridge, followed by inject 10 mL of acetone/*n*-hexane (3:7, v/v) mixture in the 500 mL trimethyl aminopropylsilanized silica gel cartridge. Combine the eluates, concentrate at 40℃ or below, and remove the solvent. Dissolve the residue in 0.5 mL of methanol.

9) When an extract obtained in **1)** of **5** with some types of measurement instruments or from some types of food samples, and followed by clean-up with the cartridges described in **8)** as necessary, the extract can be analyzed directly by an LC/MS. Make sure if this option is available for each case before measurements in order to avoid any influence of food compositions.

10) Aldicarb-sulphone is an identical compound of aldoxycarb.

9. References

Nagayama, *et al*., Journal of the Food Hygienics Society of Japan, 35, 470 (1994)

Kobayashi, *et al*., Journal of the Food Hygienics Society of Japan, 43, 133 (2002)

10. Type

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