Original: Japanese
Provisional translation

Analytical Method for Iprodione (Agricultural Products)

1. Analyte

Iprodione

2. Application

Agricultural Products

3. Instrument

High performance liquid chromatograph-ultraviolet spectrophotometric detector (HPLC-UV)

4. Reagents

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

Reference standard of iprodione: Contains not less than 95% of iprodione.

5. Procedure

1) Extraction

i) Grains, legumes, nuts and seeds

Grind the sample to pass through a standard sieve (420 μm) and weigh 20.0 g of the sample.

Add 100 mL of acetonitrile to the sample, let stand for 1 hr, pulverize for 3 min, filter through a filter paper, covered with a 1-cm-thick layer of diatomaceous earth, with suction, and transfer the filtrate to a 500 mL separating funnel (I). Then, collect the residue on the filter paper, add 100 mL of acetonitrile, pulverize for 3 min, filter through a filter paper, covered with a 1-cm-thick layer of diatomaceous earth, with suction, and combine the filtrate in the separating funnel (I). Add 50 mL of *n*-hexane saturated with acetonitrile to the separating funnel and shake it vigorously for 5 min with a shaker, let stand, transfer the acetonitrile layer to a vacuum rotary evaporator flask, and concentrate to approximately 30 mL at below 40°C.

Transfer the solution to a 500 mL separating funnel (II) containing 100 mL each of 10% sodium chloride solution and ethyl acetate, shake it vigorously for 5 min with a shaker, let stand, and transfer the ethyl acetate layer to a 500 mL separating funnel (III). Add 100 mL of ethyl acetate to the aqueous layer, treat as described above, and combine the ethyl acetate layer in the separating funnel (III). Add 50 mL of 10% sodium chloride solution to the separating funnel (III), shake vigorously for 5 min

with a shaker, let stand, and transfer the ethyl acetate layer to a 500 mL conical flask. Add an appropriate amount of anhydrous sodium sulfate to the conical flask, let stand for 15 min with occasional shaking, and filter into a vacuum rotary evaporator flask. Then, wash the conical flask above with 50 mL of ethyl acetate, use the washing to wash the residue on the filter paper, and repeat the process twice. Combine both washings in the vacuum rotary evaporator flask, and remove ethyl acetate at below 40°C. Dissolve the residue in 5 mL of *n*-hexane.

ii) Fruits, vegetables and powdered tea

For fruits and vegetables, weigh approximately 1 kg of sample accurately, weigh and add an appropriate amount of water as needed, homogenize, and take the sample equivalent to 20.0 g.

For powdered tea, weigh 10.0 g of sample.

Add 100 mL of acetone to the sample, pulverize for 3 min, 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, pulverize for 3 min, treat as described above, combine the filtrate in the vacuum rotary evaporator flask, and concentrate to approximately 50 mL at below 40°C.

Add 5 mol/L sodium hydroxide solution to the solution to adjust pH to 6–7, and transfer to a 500 mL separating funnel (I) containing 100 mL each of 10% sodium chloride solution and ethyl acetate. Shake the separating funnel vigorously for 5 min with a shaker, let stand, and transfer the ethyl acetate layer to a 500 mL separating funnel (II). Add 100 mL of ethyl acetate to the aqueous layer, treat as described above, and combine the ethyl acetate layer in the separating funnel (II).

Add 50 mL of 10% sodium chloride solution to the separating funnel (II), shake vigorously for 5 min with a shaker, let stand, and transfer the ethyl acetate layer to a 500 mL conical flask. Add an appropriate amount of anhydrous sodium sulfate to the conical flask, let stand for 15 min with occasional shaking, and filter into a vacuum rotary evaporator flask. Then, wash the conical flask with 50 mL of ethyl acetate, use the washing to wash the residue on the filter paper, and repeat the process twice. Combine both washings in the vacuum rotary evaporator flask, and remove ethyl acetate at below 40°C. Dissolve the residue in 5 mL of *n*-hexane.

iii) Tea leaves except powdered tea

Immerse 10.0 g of the sample in 600 mL of water at 100ºC, let stand for 5 min at room temperature, filter, cool and transfer 300 mL of the filtrate to a 500 mL conical flask (I). Add 100 mL of acetone and 2 mL of saturated lead acetate solution to the

conical flask, shake gently to mix for 15 min, 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. Then, wash the conical flask (I) with 50 mL of acetone and water (1:1) mixed solution, and use the washing to wash the residue on the filter paper. Combine the washings in the separating funnel above.

Add 20 g of sodium chloride and 100 mL of *n*-hexane to the separating funnel, shake vigorously for 5 min with a shaker, let stand, and transfer the *n*-hexane layer to a 500 mL separating funnel. Add 100 mL of *n*-hexane to the aqueous layer, treat as described above, and combine the *n*-hexane layer in the separating funnel above.

Add 50 mL of 10% sodium chloride solution to the separating funnel, shake for 5 min with a shaker, let stand, and transfer the *n*-hexane layer to a 500 mL conical flask (II). Add an appropriate amount of anhydrous sodium sulfate to the conical flask, let stand for 15 min with occasional shaking, and filter into a vacuum rotary evaporator flask. Then, wash the conical flask (II) with 50 mL of *n*-hexane, use the washing to wash the residue on the filter paper, and repeat the process twice. Combine both washings in the vacuum rotary evaporator flask, and remove *n*-hexane at below 40°C. Dissolve the residue in 5 mL of *n*-hexane.

2) Clean-up

In a chromatographic tube of 20 mm in inside diameter and 300 mm in length, place 10 g of synthetic magnesium silicate for column chromatography suspended in *n*-hexane, followed by approximately 8 g of anhydrous sodium sulfate on top, and allow *n*-hexane to flow out until a small amount remains at the top of the column. Inject the solution obtained in 1) Extraction to the column, add 100 mL of ether and *n*-hexane (1:1) mixed solution, and discard the effluent. Then, add 100 mL of acetone and *n*-hexane (3:17) mixed solution, transfer the eluate to a vacuum rotary evaporator flask, and remove acetone and *n*-hexane at below 40°C. Dissolve the residue in acetonitrile to make exactly 5 mL, and use this solution as the test solution.

6. Measurement

1) Qualification

Perform the test under the measurement conditions described below. The result should agree with that obtained using the reference standard.

Measurement conditions

(Example)

Column Packing: Octadecylsilanized silica gel (5 μm in particle size)

Column: A stainless tube of 4.5 mm in inside diameter and 150 mm in length.

Column temperature: 25°C

Detector: Operate at 230 nm wavelength.

Mobile phase: Use a mixed solution of acetonitrile and water (3:2). Adjust the flow rate to elute iprodione in about 9 min.

2) Quantification

Quantify iprodione using peak-height or peak-area method based on the test results obtained under the same measurement conditions as in 1) Qualification, and calculate the concentration of iprodione.

7. Limit of quantification

0.05 mg/kg

8. Reference

None

9. Type

A