Original: Japanese

Provisional translation

Analytical Method for Triflumizole (Agricultural Products)

1. Analytes

Triflumizole

Triflumizole Metabolite (4-chloro-α,α,α-trifluoro-*N*-(1-amino-2-propoxyethylidene)-*o*-toluidine)

2. Instrument

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

3. Reagents

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

Synthetic magnesium silicate (Florisil) for column chromatography: Heat Florisil (150-250 μm in particle size) at 130°C for 12 hours or longer, and allow to cool in a desiccator. Then, add 5% of water relative to this.

0.01 mol/L carbonate buffer solution: Add water to 0.8 mL of 0.2 mol/L sodium carbonate solution and 9.2 mL of 0.2 mol/L sodium hydrogen carbonate solution to make exactly 200 mL.

Reference standard solution of triflumizole and 4-chloro-α,α,α-trifluoro-*N*-(1-amino-2-propoxy-ethylidene)-*o*-toluidine: Dissolve 10 mg each of triflumizole and 4-chloro-α,α,α-trifluoro-*N*-(1-amino-2-propoxyethylidene)-*o*-toluidine in acetonitrile to make exactly 100 mL.

0.05 mol/L phosphate buffer solution: Add water to 55.4 mL of 0.5 mol/L monopotassium phosphate solution and 48.2 mL of 0.5 mol/L disodium phosphate solution to make exactly 1,000 mL (pH 6.8).

4. Reference standard

Reference standard of triflumizole: Contains not less than 99% of triflumizole. Melting point of the standard is 63.5°C

Reference standard of 4-chloro-α,α,α-trifluoro-*N*-(1-amino-2-propoxyethylidene)-*o*-toluidine: Contains not less than 98% of 4-chloro-α,α,α-trifluoro-*N*-(1-amino-2-propoxyethylidene)-*o*-toluidine

5. Procedure

a) Extraction

i) Grains, nuts and seeds

Grind sample to pass through a standard sieve (420 μm), weigh 20.0 g of sample, add 40 mL of water, and let stand for 2 hours.

Add 100 mL of methanol to the sample, shake vigorously for 30 minutes with a shaker, and let stand. 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 of separating funnel. Collect the residue on the filter paper, add 50 mL of methanol, shake vigorously for 30 minutes with a shaker, treat as described above, and combine the filtrates in the 500 mL of separating funnel described above.

Add 200 mL of 5% sodium chloride solution and 100 mL of dichloromethane (special grade) to the separating funnel, shake vigorously for 5 minutes with a shaker, let stand, and transfer the dichloromethane layer to a 300 mL conical flask. Add 100 mL of dichloromethane (special grade) to the aqueous layer, treat as described above, and combine the dichloromethane layers in the conical flask above. Add an appropriate quantity of anhydrous sodium sulfate to the conical flask, let stand for 15 minutes with occasional shaking, and filter into a vacuum rotary evaporator flask. Wash the conical flask with 20 mL of dichloromethane (special grade), and wash the residue on the filter paper with the washing. Combine the washings in the vacuum rotary evaporator flask, and remove dichloromethane at below 40°C. Dissolve the residue in 5 mL of acetone/*n*-hexane (1:19, v/v).

ii) Fruits, vegetables and powdered tea

For fruits and vegetables, weigh about 1 kg of sample accurately, add an appropriate quantity of water (if necessary), homogenize, take the sample equivalent to 20.0 g, and then add 20 mL of 0.05 mol/L phosphate buffer solution.

For powdered tea, weigh 20.0 g of sample, and add 20 mL of 0.05 mol/L phosphate buffer solution. Add 100 mL of methanol to the sample, shake vigorously for 30 minutes with a shaker, and let stand. 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 of separating funnel. Collect the residue on the filter paper, add 50 mL of methanol, shake vigorously for 30 minutes with a shaker, treat as described above, and combine the filtrates in the 500 mL of separating funnel described above. Measure the pH of this solution, and if acidic, adjust it to around pH 7 using a 0.1 mol/L sodium hydroxide solution.

Add 200 mL of 5% sodium chloride solution and 100 mL of dichloromethane (special grade) to the solution, shake vigorously for 5 minutes with a shaker, let stand, and transfer the dichloromethane layer to a 300 mL conical flask. Add 100 mL of dichloromethane (special grade) to the aqueous layer, treat as described above, and combine the dichloromethane layers in the conical flask described above. Add an appropriate quantity of anhydrous sodium sulfate to the conical flask, let stand for 15 minutes with occasional shaking, and filter into a vacuum rotary evaporator flask. Then, wash the conical flask with 20 mL of dichloromethane (special grade), and wash the residue on the filter paper with the washing. Combine the washings in the vacuum rotary evaporator flask, and remove dichloromethane at below 40°C. Dissolve the residue in 5 mL of acetone/*n*-hexane (1:19, v/v).

iii) Tea leaves except powdered tea

Immerse 4.00 g of sample in 240 mL of water at 100ºC, let stand for 5 minutes at room temperature, and filter. Add 5 mL each of acetonitrile and water to an octadecylsilanized silica gel mini column (360 mg), and discard the effluent. Transfer 30 mL of the filtrate described above to this column, add 10 mL of water and 10 mL of acetonitrile/water (3:7, v/v) sequentially, and discard the effluents. Then, add 5 mL of acetonitrile/water (4:1, v/v) to the column, collect the effluent, and use this solution as the test solution. (No operation by “b) Clean-up” is required.)

b) Clean-up

Place 10 g of synthetic magnesium silicate for column chromatography suspended in acetone/*n*-hexane (1:19, v/v) and 5 g of anhydrous sodium sulfate in a chromatographic tube of 15 mm in inside diameter and 300 mm in length sequentially, and let flow out acetone/*n*-hexane (1:19, v/v) to the extent that only a small quantity of acetone/*n*-hexane (1:19, v/v) remains on the top of the column. Transfer the solution obtained in a) to the column, add 50 mL of acetone/*n*-hexane (1:19, v/v), and discard the effluent. Add 100 mL of acetone/*n*-hexane (1:9, v/v), and take 40 mL of the first effluent to a 200 mL conical flask (I) and 60 mL of the next effluent to a 200 mL conical flask (II). Transfer each effluent to vacuum rotary evaporator flasks respectively, and remove acetone and *n*-hexane at below 40°C. Dissolve each residue in acetonitrile respectively to make exactly 10 mL, and use the former as the test solution of 4-chloro-α,α,α-trifluoro-*N*-(1-amino-2-propoxyethylidene)-*o*-toluidine and the latter as the test solution of triflumizole.

Test 4-chloro-α,α,α-trifluoro-*N*-(1-amino-2-propoxyethylidene)-*o*-toluidine test solution according to "6. Measurement, a) Qualification", and if the test is difficult due to impurities in the test solution, perform the following operation.

Dissolve the residue of the effluent obtained in the conical flask (I) in 100 mL of dichloromethane (special grade), and transfer to the 300 mL separating funnel (I). Add 50 mL of 1 mol/L hydrochloric acid, shake well, let stand, and transfer the aqueous layer to the 300 mL separating funnel (II). Add 50 mL of 1 mol/L hydrochloric acid to the dichloromethane layer, treat as described above, and combine the aqueous layer with the separating funnel (II). Add 11 mL of 10 mol/L sodium hydroxide solution to the separating funnel (II), add 50 mL of dichloromethane (special grade), shake well, let stand, and transfer the dichloromethane layer to a 200 mL conical flask. Add 50 mL of dichloromethane (special grade) to the aqueous layer, treat as described above, and combine the dichloromethane layer with the conical flask above. Add an appropriate quantity of anhydrous sodium sulfate to the conical flask, let stand for 15 minutes with occasional shaking, and filter into a vacuum rotary evaporator flask. Then, wash the conical flask with a small amount of dichloromethane (special grade), combine the washings in the vacuum rotary evaporator flask above, and remove dichloromethane at below 40°C. Dissolve the residue in acetonitrile to make exactly 10 mL, and use this solution as the test solution of 4-chloro-α,α,α-trifluoro-*N*-(1-amino-2-propoxyethylidene)-*o*-toluidine.

6. Measurement

a) Qualification

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

Measurement conditions

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

Chromatographic column: A stainless column of 2-5 mm in inside diameter and 200-500 mm in length

Column temperature: 40°C

Detector: Operate with a wavelength of 238 nm.

Mobile phase: Use acetonitrile/0.01 mmol/L carbonate buffer/water (7:1:2, v/v/v) solution added 4% of phosphoric acid to adjust the pH 9.0. For test of triflumizole, adjust the flow rate to elute triflumizole at about 10 minutes. For the test of 4-chloro-α,α,α-trifluoro-*N*-(1-amino-2-propoxyethylidene)-*o*-toluidine, adjust the flow rate to elute 4-chloro-α,α,α-trifluoro-*N*-(1-amino-2-propoxyethylidene)-*o*-toluidine at about 6 minutes.

b) Quantification

Quantify using peak-height or peak-area method, for triflumizole and 4-chloro-α,α,α-trifluoro-*N*-(1-amino-2-propoxyethylidene)-*o*-toluidine on the basis of the result obtained using the measurement conditions described in a), calculate the concentration of triflumizole and 4-chloro-α,α,α-trifluoro-*N*-(1-amino-2-propoxyethylidene)-*o*-toluidine, and use the following equation to calculate the concentration of triflumizole including 4-chloro-α,α,α-trifluoro-*N*-(1-amino-2-propoxyethylidene)-*o*-toluidine.

Concentration of triflumizole (including 4-chloro-α,α,α-trifluoro-*N*-(1-amino-2-propoxyethylidene)-*o*-toluidine) (ppm) = A + B×1.17

A: Concentration of triflumizole (ppm)

B: Concentration of 4-chloro-α,α,α-trifluoro-*N*-(1-amino-2-propoxyethylidene)-*o*-toluidine (ppm)

7. Limit of quantification

0.05 mg/kg

8. Explanatory note

For triflumizole, triflumizole and 4-chloro-α,α,α-trifluoro-*N*-(1-amino-2-propoxyethylidene)-*o*-toluidine as triflumizole metabolite are quantified respectively, and for 4-chloro-α,α,α-trifluoro-*N*-(1-amino-2-propoxyethylidene)-*o*-toluidine, converted to the concentration of triflumizole by multiplying by a factor, and the sum of the concentrations of triflumizole and 4-chloro-α,α,α-trifluoro-*N*-(1-amino-2-propoxyethylidene)-*o*-toluidine is regarded as the analytical result of triflumizole.

9. Reference

None

10. Type

A