Original: Japanese

Provisional Translation

Analytical Method for Nitenpyram (Agricultural Products)

1. Analytes

Nitenpyram

CPMA〔2-[*N*-(6-chloro-3-pyridylmethyl)-*N*-ethyl] amino-2-methylimino acetic acid〕

CPMF〔*N*-(6-chloro-3-pyridylmethyl)-*N*-ethyl-*N*'-methylformamidine〕

2. Instrument

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

Gas chromatograph-flame thermionic detector (GC-FTD)

Gas chromatograph-nitrogen phosphorus detector (GC-NPD)

Gas chromatograph-mass spectrometer (GC-MS)

3. Reagents

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

Styrene-divinylbenzene copolymer adsorbent: Wash styrene-divinylbenzene copolymer (non-polar, 150-250 μm in particle size, 30 nm in mean pore diameter) thoroughly with acetone, and reserve it in acetone.

4. Reference standards

Reference standard of nitenpyram: Contains not less than 99% of nitenpyram. Melting point of the standard is 83ºC.

Reference standard of *N*-(6-chloro-3-pyridylmethyl)- *N*-ethylformamide (CPF): Contains not less than 97% of CPF.

5. Procedure

1) Nitenpyram

i) Extraction

a) Grains

Grind sample to pass through a standard sieve (420 μm), weigh 10.0 g of the sample, and 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 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 20 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 dichloromethane, and transfer the washing to the separating funnel. Shake the separating funnel vigorously for 5 minutes with a shaker, let stand, and transfer the dichloromethane layer to a 300 mL conical flask. Add 50 mL of dichloromethane to the aqueous layer, treat as

described above, and combine the *n*-hexane layer in the conical flask. Repeat this step one more time. Add an appropriate quantity of anhydrous sodium sulfate to the dichloromethane layer, let stand for 15 minutes with occasional shaking.

b) Fruits and vegetables

Homogenize about 1 kg of sample, and then weigh 20.0 g of the sample. Add 100 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 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 20 mL at below 40°C. Transfer the solution of the vacuum rotary evaporator flask to a porous diatomaceous earth cartridge (to hold 20 mL of solution), let stand for 15 minutes, add 50 mL of *n*-hexane, and discard the effluent. Add 50 mL of diethyl ether/*n*-hexane (1:1, v/v) to the cartridge, and discard the effluent. Elute with 50 mL of dichloromethane, and collect the effluent to a 100 mL conical flask.

c) Powdered tea

Weigh 5.00 g of the sample, add 100 mL of 0.05 mol/L hydrochloric acid, let stand for 2 hours, and shake vigorously for 30 minutes with a shaker. Add 10 g of diatomaceous earth, shake it, and filter through a filter paper, covered with a 1-cm-thick layer of diatomaceous earth, with suction into a 300 mL conical flask. Wash the residue on the filter paper with 50 mL of 0.05 mol/L hydrochloric acid, transfer the washing to the conical flask, and add 0.05 mol/L hydrochloric acid to make exactly 200 mL. Place 20 mL of styrene-divinylbenzene copolymer suspended in acetone in a chromatographic tube of 15 mm in inside diameter and 300 mm in length, and let flow out acetone to the extent that only a small quantity of acetone remains on the top of the column. Add 50 mL of acetone to the cartridge, and discard the effluent. Add 200 mL of water to the cartridge, and discard the effluent. Add the solution to the cartridge, let flow out 5 mL for 1 minute, and discard the effluent. Add 50 mL of 0.05 mol/L hydrochloric acid to the cartridge, and discard the effluent. Add 50 mL of water, let flow out 5 mL for 1 minute, and discard the effluent. Add 100 mL of acetone to the cartridge, let flow out 5 mL for 1 minute, and collect the effluent to a 100 mL conical flask. Insert absorbent cotton in the upper part of the benzenesulfonylpropylsilylated silica gel cartridge (500 mg), add 5 mL of acetone to the cartridge, and discard the effluent. Add the solution described above to the cartridge, let flow out 3 mL for 1 minute, and discard the effluent. Add 20 mL of water to the cartridge, and discard the effluent. Add 10 mL of 0.5 mol/L ammonia water to the cartridge, collect the effluent to a 50 mL conical flask, and add 0.3 mL of acetic acid. Add the solution described above to porous diatomaceous earth cartridge (to hold 20 mL of solution), let stand for 15 minutes, add 50 mL of *n*-hexane to the cartridge, and discard the effluent. Add 50 mL of diethyl ether/*n*-hexane (1:1, v/v) to the cartridge, and discard the effluent. Add 50 mL of dichloromethane to the cartridge, collect the effluent to a 100 mL conical flask.

d) 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, and add 8 mL of 2 mol/L hydrochloric acid. Place 20 mL of styrene-divinylbenzene copolymer suspended in acetone in a chromatographic tube of 15 mm in inside diameter and 300 mm in length, and let flow out acetone to the extent that only a small quantity of acetone remains on the top of the column. Add 50 mL of acetone to the cartridge, and discard the effluent. Add 200 mL of water to the cartridge, and discard the effluent. Add the solution to the cartridge, and let flow out 5 mL for 1 minute, and discard the effluent. Add 50 mL of 0.05 mol/L hydrochloric acid to the cartridge, and discard the effluent. Add 50 mL of water, let flow out 5 mL for 1 minute, and discard the effluent. Add 100 mL of acetone to the cartridge, let flow out 5 mL for 1 minute, and collect the effluent to a 100 mL conical flask. Insert absorbent cotton in the upper part of the benzenesulfonic-propylsilanized silica gel cartridge (500 mg), add 5 mL of acetone to the cartridge, and discard the effluent. Add the solution described above to the cartridge, let flow out 3 mL for 1 minute, and discard the effluent. Add 20 mL of water to the cartridge, and discard the effluent. Add 10 mL of 0.5 mol/L ammonia water to the cartridge, collect the effluent to a 50 mL conical flask, and add 0.3 mL of acetic acid. Add the solution described above to porous diatomaceous earth cartridge (to hold 20 mL of solution), let stand for 15 minutes, add 50 mL of *n*-hexane to the cartridge, and discard the effluent. Add 50 mL of diethyl ether/*n*-hexane (1:1, v/v) to the cartridge, and discard the effluent. Add 50 mL of dichloromethane to the cartridge, collect the effluent to a 100 mL conical flask.

ii) Clean-up

Place 10 g of silica gel for column chromatography (63-200 μm in particle size) suspended in dichloromethane, 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 dichloromethane to the extent that only a small quantity of dichloromethane remains on the top of the column.

Add the solution obtained in i) to the cartridge, elute with 50 mL of acetone/ dichloromethane (1:1, v/v), and discard the effluent. Add 50 mL of acetone, collect the effluent to a vacuum rotary evaporator flask, and remove acetone at below 40°C. Dissolve the residue in methanol/0.05 mol/L sodium dihydrogen phosphate (3:17, v/v) to make exactly 5 mL, and use this solution as the test solution.

2) CPMA〔2-[*N*-(6-chloro-3-pyridylmethyl)-*N*-ethyl] amino-2-methylimino acetic acid〕
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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. Stand the filtrate in water bath at 50°C for 90 minutes, and concentrate to about 10 mL at below 40°C. Add 0.2 mL of trimethylamine, and stand the concentrated filtrate in water bath at 50°C for 30 minutes. 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 diethyl ether, and transfer the washing to the separating funnel. Shake the separating funnel vigorously for 5 minutes with a shaker, let stand, and transfer the diethyl ether layer to a 300 mL conical flask. Add 50 mL of diethyl ether to the aqueous layer, treat as described above, and combine the diethyl ether layer in the conical flask. Add an appropriate quantity of anhydrous sodium sulfate to the conical flask, let stand for 15 minutes with occasional shaking.

b) Fruits and vegetables

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. Add 100 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 50 mL of acetone, homogenize for 3 minutes, treat as described above, and combine the filtrate in the vacuum rotary evaporator flask. Stand the filtrate in water bath at 50°C for 90 minutes, and concentrate to about 10 mL at below 40°C. Add 0.2 mL of trimethylamine, and stand the concentrated filtrate in water bath at 50°C for 30 minutes. Add the solution to a porous diatomaceous earth cartridge (to hold 20 mL of solution), let stand for 15 minutes, add 50 mL of *n*-hexane to the cartridge, and discard the effluent. Add 50 mL of diethyl ether, and collect the effluent to a 100 mL conical flask.

c) Powdered tea

Weigh 5.00 g of the sample, add 100 mL of 0.05 mol/L hydrochloric acid, let stand for 2 hours, and shake vigorously for 30 minutes with a shaker. Add 10 g of diatomaceous earth, shake it, and filter through a filter paper, covered with a 1-cm-thick layer of diatomaceous earth, with suction into a 300 mL conical flask. Wash the residue on the filter paper with 50 mL of 0.05 mol/L hydrochloric acid, transfer the washing to the conical flask, and add 0.05 mol/L hydrochloric acid to make exactly 200 mL. Place 20 mL of styrene-divinylbenzene copolymer suspended in acetone in a chromatographic tube of 15 mm in inside diameter and 300 mm in length, and let flow out acetone to the extent that only a small quantity of acetone remains on the top of the column. Add 50 mL of acetone to the cartridge, and discard the effluent. Add 200 mL of water to the cartridge, and discard the effluent. Add the solution to the cartridge, let flow out 5 mL for 1 minute, and discard the effluent. Add 50 mL of 0.05 mol/L hydrochloric acid to the cartridge, and discard the effluent. Add 50 mL of water, let flow out 5 mL for 1 minute, and discard the effluent. Add 100 mL of acetone to the cartridge, let flow out 5 mL for 1 minute, collect the effluent to a vacuum rotary evaporator flask. Stand the filtrate in water

bath at 50℃ for 90 minutes, and concentrate to about 10 mL at below 40°C. Add 0.2 mL of trimethylamine, and stand the concentrated filtrate in water bath at 50℃ for 30 minutes. Add the solution to a porous diatomaceous earth cartridge (to hold 20 mL of solution), let stand for 15 minutes, add 50 mL of *n*-hexane to the cartridge, and discard the effluent. Add 50 mL of diethyl ether, and collect the effluent to a 100 mL conical flask.

d) Tea leaves, except powdered tea

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ii) Clean-up

Place 10 g of silica gel for column chromatography (63-200 μm in particle size) suspended in diethyl ether, 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 diethyl ether to the extent that only a small quantity of diethyl ether remains on the top of the column. Add the solution obtained in i) to the cartridge, elute with 50 mL of acetone/*n*-hexane (1:4, v/v), and discard the effluent. Add 50 mL of acetone, collect the effluent to a vacuum rotary evaporator flask, and remove acetone at below 40°C. Dissolve the residue in acetone to make exactly 5mL, and use this solution as the test solution.

6. Measurement

1) Qualification

i) Nitenpyram

Perform the test with high performance liquid chromatograph-ultraviolet pectrophotometric detector (HPLC-UV) under the measurement conditions described below. The result shall agree with that obtained using the reference standard.

Measurement conditions

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

Column: A stainless tube of 4.6 mm in inner diameter, 150 mm in length.

Column temperature: 40ºC

Detector: Operate with an absorption wavelength of 270 nm.

Mobile phase: Use methanol/0.05 mol/L potassium dihydrogen phosphate solution (3:17, v/v). Adjust the flow rate to elute nitenpyram at about 9 minutes.

ii) CPMA〔2-[*N*-(6-chloro-3-pyridylmethyl)-*N*-ethyl] amino-2-methylimino acetic acid〕
CPMF〔*N*-(6-chloro-3-pyridylmethyl)-*N*-ethyl-*N*'-methylformamidine〕

Perform the test with gas chromatograph-flame thermionic detector (GC-FTD) or gas chromatograph-nitrogen phosphorus detector (GC-NPD) under the measurement conditions described below. The result shall agree with that obtained using the reference standard of CPF.

Measurement conditions

Column: Silicate glass capillary 0.25 mm in inside diameter, 30 m in length coated with 50 % phenyl-methyl silicone for gas-chromatography 0.25 μm in film thickness.

Column temperature: 50ºC (2 min) - 10ºC/min heating - 250ºC (10 min)

Inlet temperature: 270ºC

Carrier gas and flow rate: Helium. Adjust the flow rate to elute CPF at about 22 minutes. Optimize the flow rate of air and hydrogen.

2) Quantification

Quantify using peak-height or peak-area method, for nitenpyram and each of CPF derived from CPMA and CPMF on the basis of the result obtained using the measurement conditions described in 1), calculate the concentration of nitenpyram and CPF, and use the following equation to calculate the concentration of nitenpyram including CPMA and CPMF.

Concentration of Concentration of nitenpyram (including CPMA and CPMF) (ppm) =

$A + B \times 1.36$

A: Concentration of nitenpyram (ppm)

B: Concentration of CPF (ppm)

3) Confirmation

For the test solution of CPMA and CPMF, 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 using peak-height or peak-area method.

7. Limit of quantification

0.05 mg/kg for rice, 0.025 mg/kg for fruits and vegetables, 0.1 mg/kg for tea

8. Explanatory note

Nitenpyram, CPMF as metabolite of nitenpyram and CPF derived from CPMF are quantified respectively, calculate the concentration of nitenpyram, and for CPF, converted to the concentration of nitenpyram by multiplying by a factor, and regard the sum of the results as the analytical result of nitenpyram.

9. Reference

None

10. Type

A