Analytical Method for EPN, Anilofos, Isazophos, Iprobenfos, Ethion, Edifenphos, Ethoprophos, Etrimfos, Cadusafos, Quinalphos, Chlorpyrifos, Chlorpyrifos-methyl, Chlorfenvinphos, Cyanophos, Disulfoton, Dimethylvinphos, Dimethoate, Sulprofos, Diazinon, Thiometon, Tetrachlorvinphos, Terbufos, Triazophos, Tribuphos, Tolclofos-methyl, Parathion, Parathion-methyl, Piperophos, Pyraclofos, Pyrazophos, Pyridaphenthion, Pirimiphos-methyl, Fenamiphos, Fenitrothion, Fensulfothion, Fenthion, Phenthoate, Butamifos, Prothiofos, Propaphos, Profenofos, Bromophos, Bensulide, Phoxim, Phosalone, Fosthiazate, Phosphamidon, Phosmet, Phorate, Malathion, Mecarbam, Methacrifos, Methidathion and Mevinphos (Agricultural Products)

1. Analytes

Compositional substances of agricultural chemicals	Analytes
EPN	EPN
Anilofos	Anilofos
Isazophos	Isazophos
Iprobenfos	Iprobenfos
Ethion	Ethion
Edifenphos	Edifenphos
Ethoprophos	Ethoprophos
Etrimfos	Etrimfos
Cadusafos	Cadusafos
Quinalphos	Quinalphos
Chlorpyrifos	Chlorpyrifos
Chlorpyrifos-methyl	Chlorpyrifos-methyl
Chlorfenvinphos	(*E*)-Chlorfenvinphos, (*Z*)-Chlorfenvinphos
Cyanophos	Cyanophos
Disulfoton	Disulfoton, Disulfoton-sulfon
Dimethylvinphos	(*E*)-Dimethylvinphos, (*Z*)-Dimethylvinphos
Dimethoate	Dimethoate
Sulprofos	Sulprofos
Diazinon	Diazinon
Thiometon	Thiometon
Tetrachlorvinphos	(*Z*)-Tetrachlorvinphos
Terbufos	Terbufos

Triazophos	Triazophos
Tribuphos	Tribuphos
Tolclofos-methyl	Tolclofos-methyl
Parathion	Parathion
Parathion-methyl	Parathion-methyl
Piperophos	Piperophos
Pyraclofos	Pyraclofos
Pyrazophos	Pyrazophos
Pyridaphenthion	Pyridaphenthion
Pirimiphos-methyl	Pirimiphos-methyl
Fenamiphos	Fenamiphos
Fenitrothion	Fenitrothion
Fensulfothion	Fensulfothion
Fenthion	Fenthion
Phenthoate	Phenthoate
Butamifos	Butamifos
Prothiofos	Prothiofos
Propaphos	Propaphos
Profenofos	Profenofos
Bromophos	Bromophos
Bensulide	Bensulide
Phoxim	Phoxim
Phosalone	Phosalone
Fosthiazate	Fosthiazate
Phosphamidon	(*E*)-Phosphamidon, (*Z*)-Phosphamidon
Phosmet	Phosmet
Phorate	Phorate
Malathion	Malathion
Mecarbam	Mecarbam
Methacrifos	Methacrifos
Methidathion	Methidathion
Mevinphos	(*E*)-Mevinphos, (*Z*)-Mevinphos

2. Instruments

Gas chromatograph-flame thermionic detector (GC-FTD), Gas chromatograph-flame photometric detector (with interference filter for phosphorus, wavelength 526 nm) (GC-FPD(P)) or Gas chromatograph-nitrogen phosphorous 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.

Reference standard of EPN: Contains not less than 98% of EPN. Melting point of the standard is 36°C.

Reference standard of anilofos: Contains not less than 98% of anilofos. Melting point of the standard is 50–53°C.

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

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

Reference standard of ethion: Contains not less than 98% of ethion. Melting point of the standard is −15 – −12°C.

Reference standard of edifenphos: Contains not less than 97% of edifenphos. Boiling point of the standard is 154°C (reduced pressure: 0.0013 kPa).

Reference standard of ethoprophos: Contains not less than 98% of ethoprophos. Boiling point of the standard is 86–89°C (reduced pressure: 0.027 kPa).

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

Reference standard of cadusafos: Contains not less than 95% of cadusafos. Boiling point of the standard is 112–114°C (reduced pressure: 0.11 kPa).

Reference standard of quinalphos: Contains not less than 96% of quinalphos. Melting point of the standard is 31–32°C.

Reference standard of chlorpyrifos: Contains not less than 99% of chlorpyrifos. Melting point of the standard is 41–43°C.

Reference standard of chlorpyrifos-methyl: Contains not less than 98% of chlorpyrifos-methyl. Melting point of the standard is 45–47°C.

Reference standard of (*E*)-chlorfenvinphos: Contains not less than 97% of (*E*)-chlorfenvinphos. Boiling point of the standard is 168–170°C (reduced pressure: 0.067 kPa).

Reference standard of (*Z*)-chlorfenvinphos: Contains not less than 97% of (*Z*)-chlorfenvinphos. Boiling point of the standard is 132–134°C (reduced pressure: 0.0040 kPa).

Reference standard of cyanophos: Contains not less than 98% of cyanophos. Melting point of the standard is 14–15°C.

Reference standard of disulfoton: Contains not less than 98% of disulfoton. Melting point of the standard is lower than −25°C.

Reference standard of disulfoton-sulfon: Contains not less than 98% of disulfoton-sulfon.

Reference standard of (*E*)-dimethylvinphos: Contains not less than 95% of

(*E*)-dimethylvinphos.

Reference standard of (*Z*)-dimethylvinphos: Contains not less than 99% of (*Z*)-dimethylvinphos. Melting point of the standard is 69–70°C.

Reference standard of dimethoate: Contains not less than 97% of dimethoate. Melting point of the standard is 49–51°C.

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

Reference standard of diazinon: Contains not less than 98% of diazinon. Boiling point of the standard is 83–84°C (reduced pressure: 0.00027 kPa).

Reference standard of thiometon: Contains not less than 92% of thiometon. Boiling point of the standard is 100°C (reduced pressure: 0.013 kPa).

Reference standard of tetrachlorvinphos: Contains not less than 98% of (*Z*)-tetrachlorvinphos. Melting point of the standard is 94–97°C.

Reference standard of terbufos: Contains not less than 97% of terbufos. Boiling point of the standard is 64°C (reduced pressure: 0.0013 kPa).

Reference standard of triazophos: Contains not less than 98% of triazophos. Melting point of the standard is 0–5°C.

Reference standard of tribuphos: Contains not less than 98% of tribuphos. Melting point of the standard is not higher than −25°C.

Reference standard of tolclofos-methyl: Contains not less than 99% of tolclofos-methyl. Melting point of the standard is 78–80°C.

Reference standard of parathion: Contains not less than 97% of parathion. Boiling point of the standard is 375°C.

Reference standard of parathion-methyl: Contains not less than 98% of parathion-methyl. Melting point of the standard is 35–36°C.

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

Reference standard of pyraclofos: Contains not less than 99% of pyraclofos. Boiling point of the standard is 164°C (reduced pressure: 0.0013 kPa).

Reference standard of pyrazophos: Contains not less than 98% of pyrazophos. Melting point of the standard is 51–52°C.

Reference standard of pyridaphenthion: Contains not less than 98% of pyridaphenthion. Melting point of the standard is 54–56°C.

Reference standard of pirimiphos-methyl: Contains not less than 98% of pirimiphos-methyl.

Reference standard of fenamiphos: Contains not less than 98% of fenamiphos. Melting point of the standard is 49°C.

Reference standard of fenitrothion: Contains not less than 98% of fenitrothion. Boiling point of the standard is 140–141°C (reduced pressure: 0.013 kPa).

Reference standard of fensulfothion: Contains not less than 98% of fensulfothion. Boiling point of the standard is 138–141°C (reduced pressure: 0.0013 kPa).

Reference standard of fenthion: Contains not less than 98% of fenthion. Boiling point of the standard is 87°C (reduced pressure: 0.0013 kPa).

Reference standard of phenthoate: Contains not less than 98% of phenthoate. Decomposition point of the standard is 202–204°C.

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

Reference standard of prothiofos: Contains not less than 98% of prothiofos. Boiling point of the standard is 125–128°C (reduced pressure: 1.7 kPa).

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

Reference standard of profenofos: Contains not less than 99% of profenofos.

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

Reference standard of bensulide: Contains not less than 98% of bensulide. Melting point of the standard is 34°C.

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

Reference standard of phosalone: Contains not less than 98% of phosalone. Melting point of the standard is 46–48°C.

Reference standard of fosthiazate: Contains not less than 98% of (*R*,*S*-)fosthiazate. Boiling point of the standard is 198°C (reduced pressure: 0.067 kPa).

Reference standard of phosphamidon: Mixture of (*E*)-phosphamidon and (*Z*)-phosphamidon, containing not less than 98% of phosphamidon.

Reference standard of phosmet: Contains not less than 98% of phosmet. Melting point of the standard is 70–73°C.

Reference standard of phorate: Contains not less than 98% of phorate. Melting point of the standard is not higher than −15°C.

Reference standard of malathion: Contains not less than 98% of malathion. Boiling point of the standard is 156–157°C (reduced pressure: 0.093 kPa).

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

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

Reference standard of methidathion: Contains not less than 98% of methidathion. Melting point of the standard is 39–40°C.

Reference standard of mevinphos: Mixture of (*E*)-mevinphos and (*Z*)-mevinphos, containing not less than 98% of mevinphos.

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 sample, 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 remove acetone at below 40°C.

Transfer the concentrated filtrate to a 300 mL separating funnel containing 100 mL of saturated sodium chloride solution. Wash the vacuum rotary evaporator flask with 100 mL of ethyl acetate/*n*-hexane (1:4, v/v), 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/*n*-hexane layer to a 300 mL conical flask. Add 50 mL of ethyl acetate/*n*-hexane (1:4, v/v) to the aqueous layer, treat as described above, and combine the ethyl acetate/*n*-hexane layer in the conical flask. Add an appropriate quantity of anhydrous sodium sulfate to the ethyl acetate/*n*-hexane 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 *n*-hexane, 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, and remove ethyl acetate and *n*-hexane at below 40°C.

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 vacuum rotary evaporator flask. Add 30 mL of acetonitrile saturated with *n*-hexane to the *n*-hexane layer, treat as described above twice, combine the acetonitrile layers in the vacuum rotary evaporator flask, and remove acetonitrile at below 40°C. Dissolve the residue in 5 mL of acetone/*n*-hexane (1:1, v/v).

ii) Fruits, vegetables, herbs, powdered tea 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 powered tea, weigh 5.00 g of sample, add 20 mL of water and let stand for 2 hours.

For hops, grind sample. Weigh 5.00 g of the sample, 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, and combine the filtrate in the vacuum rotary evaporator flask, and remove acetone at below 40°C.

Transfer the concentrated filtrate to a 300 mL separating funnel containing 100 mL of saturated sodium chloride solution. Wash the vacuum rotary evaporator flask with 100 mL of ethyl acetate/*n*-hexane (1:4, v/v), 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/*n*-hexane layer to a 300 mL conical flask. Add 50 mL of ethyl acetate/*n*-hexane (1:4, v/v) to the aqueous layer, treat as described above, and combine the ethyl acetate/*n*-hexane layer in the conical flask. Add an appropriate quantity of anhydrous sodium sulfate to the ethyl acetate/*n*-hexane 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 *n*-hexane, 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, and remove ethyl acetate and *n*-hexane at below 40°C. Dissolve the residue in 5 mL of acetone/*n*-hexane (1:1, v/v).

iii) Tea leaves except for powdered tea

a) Analysis of ethion, chlorpyrifos, dimethoate, diazinon, parathion, parathion-methyl, pyraclofos, pirimiphos-methyl, fenitrothion, phenthoate, prothiofos, profenofos, phosalone and methidathion

Immerse 9.00 g of sample in 540 mL of water at 100°C, let stand for 5 minutes at room temperature, filter, cool, and transfer 360 mL of the filtrate to a 500 mL conical flask. Add 5 mL of saturated lead acetate solution to the filtrate, and let stand for 1 hour at room temperature. 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. Wash the conical flask with 50 mL of acetone, and wash the residue on the filter paper with the washing. Transfer the washing to the separating funnel.

Add 100 g of sodium chloride and 100 mL of ethyl acetate/*n*-hexane (1:4, v/v) to the separating funnel, shake the separating funnel vigorously for 5 minutes with a shaker, let stand, and transfer the ethyl acetate/*n*-hexane layer to a 300 mL conical flask. Add 100 mL of ethyl acetate/ *n*-hexane (1:4, v/v) to the aqueous layer, treat as described above, and combine the ethyl acetate/*n*-hexane layer in the conical flask. Add an appropriate quantity of anhydrous sodium sulfate to the ethyl acetate/*n*-hexane 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 *n*-hexane, 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, and remove ethyl acetate and

n-hexane at below 40°C. Dissolve the residue in 5 mL of acetone/*n*-hexane (1:1, v/v).

b) Analysis of ethoprophos, quinalphos, chlorpyrifos-methyl, disulfoton, terbufos, triazophos, pyrazophos, fenamiphos, bensulide, phoxim, phosphamidon, phosmet, phorate, malathion, mecarbam and methacrifos

Grind sample, and treat following the procedure for powdered tea described in ii).

2) Clean-up

Place 5 g of silica gel for column chromatography (63–200 μm in particle diameter) suspended in acetone/*n*-hexane (1:1, v/v) and then about 5 g of anhydrous sodium sulfate in a chromatographic tube of 15 mm in inside diameter and 300 mm in length, let flow out acetone/*n*-hexane (1:1, v/v) to the extent that only a small quantity of acetone/*n*-hexane (1:1, v/v) remains on the top of the column. Transfer the solution obtained in 1) to the column, elute with 100 mL of acetone/*n*-hexane (1:1, v/v), collect the eluate to a vacuum rotary evaporator flask, and remove acetone and *n*-hexane at below 40°C. Dissolve the residue in acetone to make exactly 5 mL, and use this solution as the test solution.

5. Measurement

1) Qualification

i) Analysis of pesticide except phoxim

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

Measurement condition 1

Column: Silicate glass capillary 0.53 mm in inside diameter, 10–30 m in length coated with methyl silicone for gas chromatography 1.5 μm in film thickness

Column temperature: 80°C (1 min) - 8°C/min heating - 250°C (5 min)

Injection port temperature: 230°C

Detector temperature: 280°C

Carrier gas and flow rate: Helium. Adjust the flow rate to elute chlorpyrifos at about 14 min. Optimize the flow rate of air and hydrogen.

Measurement condition 2

Column: Silicate glass capillary 0.32 mm in inside diameter, 10–30 m in length coated with 50% trifluoropropyl-methyl silicone for gas chromatograghy 0.25 μm in film thickness

Column temperature: 70°C (1 min) - 25°C/min heating - 125°C (0 min) - 10°C/min heating - 235°C (12 min)

Injection port temperature: 230°C

Detector temperature: 280°C

Carrier gas and flow rate: Helium. Adjust the flow rate to elute chlorpyrifos at about 12 min. Optimize the flow rate of air and hydrogen.

ii) Analysis of phoxim

Perform test under the measurement conditions described below. The result shall agree with the result obtained from the reference standard.

Measurement conditions

Column: Silicate glass capillary 0.53 mm in inside diameter, 10 m in length coated with methyl silicone for gas chromatography 1.5 μm in film thickness

Column temperature: 50°C (1 min) - 30°C/min heating - 150°C (10 min) - 30°C/min heating - 250°C (2 min)

Injection temperature: 150°C

Detector temperature: 250°C

Carrier gas and flow rate: Helium. Adjust the flow rate to elute phoxim at about 9 min. Optimize the flow rate of air and hydrogen.

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) or the measurement conditions described below. The results shall agree with those obtained using the reference standards. When necessary, quantify with peak-height or peak-area method.

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 (10 min)

Injection port temperature: 250°C

Carrier gas: Helium

Ionization mode (ionization energy): EI (70 eV)

6. Limit of quantification

EPN: 0.02 mg/kg

Anilofos: 0.025 mg/kg

Isazophos: 0.01 mg/kg

Iprobenfos: 0.01 mg/kg

Ethion: 0.01 mg/kg

Edifenphos: 0.02 mg/kg
Ethoprophos: 0.005 mg/kg
Etrimfos: 0.01 mg/kg
Cadusafos: 0.01 mg/kg
Quinalphos: 0.01 mg/kg
Chlorpyrifos: 0.01 mg/kg
Chlorpyrifos-methyl: 0.01 mg/kg
Chlorfenvinphos: 0.02 mg/kg
Cyanophos: 0.01 mg/kg
Disulfoton: 0.01 mg/kg
Dimethylvinphos: 0.04 mg/kg
Dimethoate: 0.02 mg/kg
Sulprofos: 0.01 mg/kg
Diazinon: 0.01 mg/kg
Thiometon: 0.01 mg/kg
Tetrachlorvinphos: 0.01 mg/kg
Terbufos: 0.005 mg/kg
Triazophos: 0.05 mg/kg
Tribuphos: 0.01 mg/kg
Tolclofos-methyl: 0.02 mg/kg
Parathion: 0.01 mg/kg
Parathion-methyl: 0.01 mg/kg
Piperophos: 0.01 mg/kg
Pyraclofos: 0.05 mg/kg
Pyrazophos: 0.01 mg/kg
Pyridaphenthion: 0.03 mg/kg
Pirimiphos-methyl: 0.01 mg/kg
Fenamiphos: 0.01 mg/kg
Fenitrothion: 0.01 mg/kg
Fensulfothion: 0.02 mg/kg
Fenthion: 0.01 mg/kg
Phenthoate: 0.01 mg/kg
Butamifos: 0.01 mg/kg
Prothiofos: 0.01 mg/kg
Propaphos: 0.01 mg/kg
Profenofos: 0.01 mg/kg

Bromophos: 0.01 mg/kg

Bensulide: 0.03 mg/kg

Phoxim: 0.02 mg/kg

Phosalone: 0.02 mg/kg

Fosthiazate: 0.02 mg/kg

Phosphamidon: 0.01 mg/kg

Phosmet: 0.01 mg/kg

Phorate: 0.01 mg/kg

Malathion: 0.01 mg/kg

Mecarbam: 0.01 mg/kg

Methacrifos: 0.01 mg/kg

Methidathion: 0.01 mg/kg

Mevinphos: 0.01 mg/kg

7. Explanatory note

1) Quantify (*E*)-chlorfenvinphos and (*Z*)-chlorfenvinphos individually, and regard the sum of the results as the analytical result of chlorfenvinphos.

   Quantify (*E*)-dimethylvinphos and (*Z*)-dimethylvinphos individually, and regard the sum of the results as the analytical result of dimethylvinphos.

   Quantify disulfoton and disulfoton-sulfon individually, and regard the sum of the result of disulfoton-sulfon multiplied by 0.895 and the result of disulfoton as the analytical result of disulfoton.

2) When qualification and quantification with the flame photometric detector is interfered by various organic sulfur compounds contained in onion, garlic, and so on, application of a flame thermionic detector or nitrogen phosphorous detector reduces interference considerably.

3) The limits of quantification are the values expected for fruits, vegetables and herbs. The limits of quantification for grains, legumes, nuts and seeds are about twice, and those of tea leaves and hops are four times as large as those of fruits, vegetables and herbs. When maximum residue limit of the sample is lower than the limit of quantification, concentrate the test solution, increase the injection volume to gas chromatograph, or use alternative methods for quantification.

8. References

None

9. Type

A