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**Testing methods for pesticides
in industrial water and waste water**

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chromatography

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Foreword

This translation has been made based on the original Japanese Industrial Standard revised by the Minister of Economy, Trade and Industry through deliberations at the Japanese Industrial Standards Committee, as the result of proposal for revision of Japanese Industrial Standard submitted by the Japan Industrial Water Association (JIWA)/the Japanese Standards Association (JSA) with the draft being attached, based on the provision of Article 12 Clause 1 of the Industrial Standardization Law. Consequently **JIS K 0128 : 1994** is replaced with this Standard.

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Testing methods for pesticides in industrial water and waste water

Introduction This Japanese Industrial Standard was established in 1994 on pesticides in industrial water and waste water. In consideration of demands following advance of analytical technique, change of social situation and the like, this Standard has been revised.

1 Scope This Standard specifies the testing methods for pesticides shown in Attached Table 1 among those likely to be contained in industrial water and waste water.

Remarks 1 Attached Table 2 shows the outline of each testing method stated in this Standard.

2 Attached Table 3 shows the structural formula of each pesticide in Attached Table 1.

2 Normative references The standards given in Attached Table 4 contain provisions which, through reference in this Standard, constitute provisions of this Standard. The most recent editions of the standards (including amendments) shall apply.

3 General matters The general matters shall be as follows.

- a) **General rule** General rule in chemical analyses shall comply with **JIS K 0050**.
- b) **Definitions** For the purposes of this Standard, the definitions in **JIS K 0101**, **JIS K 0102**, **JIS K 0211**, and **JIS K 0215** apply.
- c) **Gas chromatography** General matters in GC shall comply with **JIS K 0114**.
- d) **Gas chromatography-mass spectrometry (GC/MS)** General matters in GC/MS shall comply with **JIS K 0123**.
- e) **High-performance liquid chromatography (HPLC)** General matters in HPLC shall comply with **JIS K 0124**.
- f) **Name for items** Names for items in this Standard are expressed in the internal common names shown in Attached Table 1. They conform to **ISO**-registered pesticide names except for **EPN** which is used inland. Some names conform to trade-registered pesticide names. Further, some trivial names are used as compound names.

Compound name used in "Chemical Abstracts Index Guide 9th, 10th" is appended to the internal common name in parentheses in each item.
- g) **Determination range** Determination range shown in each testing method shall be expressed in the units of mass (ng) of the target pesticide contained in the solvent to be injected.
- h) **Repeatability** Repeatability shown in each testing method shall be expressed in the coefficient of variation (%) obtained from the tests repeated in the determination range⁽¹⁾.

Note (1) Coefficient of variation (%) = $\frac{\sigma}{\bar{x}} \times 100$

where, σ : standard deviation
 $\bar{x}$: averaged value

- i) **Water** A1 to A4 water specified in **JIS K 0557**. For preparation of reagents and blank tests, A4 (or A3) water apply, provided that confirmation of applicability is carried out by blank test for each testing method before use.
- j) **Reagents**
 - 1) Reagents shall, when the product name is specified, be the one given in **JIS**, or the one which has no hindrance for test when there is no specification in **JIS**.
 - 2) The units of g/L or mg/L for mass concentration of reagent in solution and the units of mol/L or mmol/L for molar concentration of reagent (as anhydride) in solution are used as a rule.
 Concentration of reference solution is expressed in the mass per ml (mg/ml, µg/ml, or ng/ml).
 - 3) When the reagent is liquid, the concentration of reagent is expressed in the mixing ratio [reagent (a+b)] of reagent and water (or other liquid). This means that a ml of reagent is mixed with b ml of water (or other liquid). However, it is sufficient to express the name of the reagent only for the solution of the reagent given in Table 3 of **JIS K 0050** without dilution.
 - 4) The concentration shown in parentheses behind the solution name of reagent is the approximate concentration except for standard solution. For instance, sodium hydroxide solution (0.1 mol/L) means sodium hydroxide solution with approximate 0.1 mol/L concentration.
 When the concentration is given in the front of solution name, it is the exact value. Generally, this value is expressed in figures without fraction and a factor shall be obtained separately.
 - 5) The names of reagents are based on the Nomenclature of Inorganic and Organic Chemistry and Nomenclature in the International Union of Pure and Applied Chemistry (IUPAC), and then it has been coordinated as far as possible with the Nomenclature of Compounds authorized by the Chemical Society of Japan, and with Nomenclature of reagents by **JIS**.
 - 6) For preparation of standard solution, concentration-known pesticides are used, and they are described as standard products, in this text. Since similar compounds are often contained therein as impurities, the ones without any hindrance for the test shall be used.
 - 7) The reagents, waste liquid and the like shall be handled with care sufficiently according to relevant laws, regulations and the like.
- k) **Glassware** Unless otherwise specified, glassware shall be those specified in **JIS R 3503** and **JIS R 3505**. In cases where special implements are required, they are exemplified in each item with a figure or explanation.

When heating procedures are accompanied, the borosilicate glass-1 specified in **JIS R 3503** shall be employed.

Desiccant used in a desiccator should be, unless otherwise specified, silica gel⁽²⁾.

Glassware to be used for the test shall be washed with water before use, and further washed with acetone of 300 or over concentration quality specified in **JIS K 8040**.

Note ⁽²⁾ Employ the silica gel type A1 for packaging specified in **JIS Z 0701**.

- l) **Working curve (gas chromatography, gas chromatography-mass spectrometry, and high-performance liquid chromatography)** When preparing working curve, divide the determination range shown in testing method into 4 to 6 steps, then take respective volumes of standard solution to meet each step, and prepare it to fall in the determination range.

The working curve shall be newly prepared in each test, and when the same item is successively tested on a lot of samples, it is necessary to occasionally reconfirm the indicated value using standard solution in the midst of test.

- m) **Notes and remarks** Notes and remarks in this Standard are put with serial numbers in every item.

Remarks : Symbols used for notes are classified in ⁽¹⁾ and ^(*). The latter is positioned in Remarks.

4 Sample

4.1 Sampling Sampling shall be as follows.

- a) **Reagents** The following reagents shall be used.

- 1) **Hydrochloric acid** That specified in **JIS K 8180**.
- 2) **Sodium hydroxide solution (100 g/L)** It is prepared from sodium hydroxide specified in **JIS K 8576**.
- 3) **Acetone** Acetone of 300 or over concentration quality specified in **JIS K 8040**⁽¹⁾.

Note ⁽¹⁾ No hindrance for the test shall be confirmed before the test. After unsealing, it shall be stored at a place without any contamination.

- b) **Implements** Implements shall be as follows.

- 1) **Container for sample** 200-ml to 1 000-ml glass bottle with a ground stopper, which has been washed with water and then washed with acetone, followed by drying.

- c) **Sampling** Sampling shall be carried out as follows.

- 1) **Sampling of surface layer water** Take a sample according to 4.1.1 or 4.1.2 of **JIS K 0094**, transfer it into a sample container to fill and tightly seal.
- 2) **Sampling water at each depth** Take a sample according to 4.1.4 of **JIS K 0094**, transfer it into a sample container, fill and tightly seal.

- 3) **Sampling from piping arrangement** Take a sample according to 4.3 of JIS K 0094, transfer it into a sample container, fill and tightly seal.

Remarks : When residual chlorine is found, put sample gently into a glass bottle with a ground stopper without making bubbles, and add L(+)-ascorbic acid solution (40 g/L) prepared from that specified in JIS K 9502 at the rate of 0.1 ml to 1 mg of residual chlorine, and stopper it closely after filling it with sample so that no bubble remains.

4.2 Handling of sample Because some pesticides are easily decomposed, carry out the test immediately after sampling. If immediate test is impossible, preserve it in dark place at 0 °C to 10 °C, and carry out the test as soon as possible.

5 Expression of results In the expression of results, employed test method shall be clearly mentioned.

6 Pretreatment of sample Since the preparatory operation before carrying out the test operation on each item is almost common irrespective of the kind of pesticides, operations capable of being applied commonly to many pesticides are collectively specified as follows. However, in the case where it is specified in each item, that description shall apply.

6.1 Solvent extraction method After extracting a sample by dichloromethane under coexistence of sodium chloride, a specific amount is obtained by dehydration and concentration. Further, in the laboratory wherein volatile organic compound is tested, care shall be taken for contamination in the laboratory due to dichloromethane. Further, since dichloromethane is poisonous substance, care shall be taken for its handling.

a) **Reagents** The following reagents shall be used.

- 1) **Sodium chloride** Sodium chloride (heated at 600 °C for about 1 h and allowed to cool in a desiccator) specified in JIS K 8150.
- 2) **Sodium sulfate** That specified in JIS K 8987.
- 3) **Dichloromethane** That of 300 or over concentration quality specified in JIS K 8117⁽¹⁾.
- 4) **Hexane** That of 300 or over concentration quality specified in JIS K 8825⁽¹⁾.

Note ⁽¹⁾ As described in Note ⁽¹⁾ of 4.

b) **Implement and apparatus** The implements shall be as follows.

- 1) **Separatory funnel** That of 200-ml to 1 000-ml capacity
- 2) **Egg-apple type flask** A 100-ml to 500-ml flask capable of being connected to a concentrator through a ground connector.
- 3) **Erlenmeyer flask** That of 300-ml to 500-ml suitable capacity
- 4) **Shaker**
- 5) **Concentrator**⁽²⁾ Rotary evaporator or Kuderna-Danish type concentrator

Note ⁽²⁾ A part coming in contact with sample solution in concentration shall be preliminarily washed with water and acetone.

c) **Operation** The operation shall be carried out as follows.

- 1) Take an appropriate amount (100 ml to 1 000 ml) of the sample⁽³⁾ sampled in 4.1 c) into a separatory funnel, add 5 g of sodium chloride and 10 ml⁽⁴⁾ of dichloromethane per 100 ml of the solution, mix by shaking for about 10 min by using a shaker, and allow it to stand.
- 2) Transfer the aqueous layer into another separatory funnel. Transfer dichloromethane into an Erlenmeyer flask, wash the separatory funnel with a small amount of dichloromethane, and combine washings into the Erlenmeyer flask. Add 10 ml of dichloromethane per 100 ml of the solution to the aqueous layer in the separatory funnel, mix by shaking again for about 10 min by using a shaker, and allow it to stand. Combine the dichloromethane layer to the Erlenmeyer flask.
- 3) Add about 3 g⁽⁵⁾ of sodium sulfate per 20 ml of dichloromethane solution, and lightly mix by shaking. After allowing to stand for about 10 min⁽⁶⁾, filter by using filter paper of class 5A (or class 5B)⁽⁷⁾, and receive the filtrate into an egg-apple type flask⁽⁸⁾. Wash the Erlenmeyer flask two or three times by using a small amount of dichloromethane, further wash sodium sulfate on the filter paper by the washings, and combine the washings into the dichloromethane solution.
- 4) Use a concentrator⁽⁹⁾, and concentrate the dichloromethane solution to about 5 ml on a water bath at about 40 °C⁽⁹⁾.
- 5) Add about 50 ml of hexane to the concentration solution, carry out the operation of 4), and make a constant amount of 5 ml⁽¹⁰⁾.
- 6) As a blank test, take the same amount of water as that of the sample into a separatory funnel, add 5 g of sodium chloride and 10 ml of dichloromethane per 100 ml of the liquid amount, mix by shaking for about 10 min by using a shaker, and allow it to stand. Successively carry out the operations of 2) to 5).

Notes ⁽³⁾ When acidity or alkalinity of the sample is strong, neutralize by using sodium hydroxide solution (40 g/L) or hydrochloric acid (1+11).

⁽⁴⁾ When emulsion is generated and the separation is difficult, increase the amount of dichloromethane.

⁽⁵⁾ When the amount of solvent is 200 ml, about 30 g of sodium sulfate is sufficient. When emulsion is generated, further an excess of sodium sulfate is to be added.

⁽⁶⁾ In concentration using Kuderna-Danish type concentrator, when dehydration is insufficient, sometimes bumping is generated. In order to obtain sufficient dehydration, 2 or more hours are required.

For another method for dehydration, there is a method wherein extraction solvent is reserved in a dark place at -20 °C to freeze water content. This method is preferably used when the concentrating operation of 4) is not carried out successively.

- (7) The filter paper is washed with the solvent to be used for extraction before use.
- (8) When Kuderna-Danish type concentrator is used, instead of the egg-apple type flask, a 500-ml concentration flask with a concentration pipe is used.
- (9) When Kuderna-Danish type concentrator is used, a reduced pressure system is not adopted, and heating is carried out at 75 °C or below under the atmospheric pressure to concentrate. After finishing concentration, detach Snyder's column from the apparatus with attached to the concentration part, wash with a small amount of hexane running from the upper part of Snyder's column and cool with attached to the concentration part.
- (10) When measuring operation is not carried out successively, the concentration liquid is reserved in a dark place at 0 °C to 10 °C.

6.2 Solid phase extraction method Allow the solid phase column or the solid phase disk filled with base material of silica gel series or polymer gel series to adsorb pesticides in a sample using a pressurizing method or a reduced pressure method, and elute pesticides into a test tube with a ground stopper with acetone to make a specific amount. When column chromatographic separation is carried out, add hexane to the acetone solution made a specific amount. After concentration, make a specific amount.

a) **Reagents** The following reagents shall be used.

- 1) **Acetone** As described in 4.1 a) 3)(¹).
- 2) **Nitrogen** High purity nitrogen class 2 specified in JIS K 1107.

b) **Implements** The implements shall be as follows.

- 1) **Test tube with a ground stopper** That of an appropriate capacity such as 5 ml to 20 ml.
- 2) **Solid phase column** A cartridge of 5 mm to 10 mm inner diameter which is 30 mm to 100 mm in length. As a column packing, styrene divinylbenzene copolymer (polystyrene gel) or silica gel combined chemically with an octadecyl group, or that having the same performance as this is used. Before use, it is washed with about 10 ml of acetone and about 10 ml of water.

Remarks 1 As the solid phase for analysis of pesticides, there is a disk type on the market and it may be used. A speed of passing water and elution solvent in this case is preliminarily confirmed.

Informative reference 1 As a solid phase column or a solid phase disk, the following exists.

Sep-Pak PS-2, OasisTM HLB cartridge, empore disk SDB-RPS and the like.

c) **Operation** The operation is carried out as follows.

- 1) Pass an appropriate amount (generally 200 ml) of the sample(¹¹) sampled in 4.1 c) at 10 ml/min to 20 ml/min(¹⁶) of flow rate through a solid phase column(¹²) (¹³) by a pressurizing method(¹⁴) or a reduced pressure method(¹⁵).

- 2) Allow 10 ml of water⁽¹⁷⁾ to flow through the solid phase column. After washing, remove water content by suction or the like for about 30 min⁽¹⁸⁾.
- 3) Gently pass⁽¹⁹⁾ about 3 ml⁽¹⁷⁾ of acetone from the upper end of the solid phase column, and elute the target pesticides. As the receiver of the eluted solution, a test tube with a ground stopper shall be used.
- 4) Gently blow nitrogen onto the eluted solution to make a specific amount of 1 ml⁽¹⁰⁾ ⁽²⁰⁾ ⁽²¹⁾.
- 5) Pass 200 ml of water as a blank test, through the solid phase column⁽¹²⁾ ⁽¹³⁾ at 10 ml/min to 20 ml/min⁽¹⁶⁾ of flow rate by the pressurizing method⁽¹⁴⁾ or the reduced pressure method⁽¹⁵⁾. Successively carry out 2) to 4) operations.
- 6) In the case of carrying out the chromatographic separation of **6.3**, add about 50 ml of hexane successively from the operation of 4), concentrate to 6 ml to 7 ml by using a concentrator⁽⁸⁾ ⁽⁹⁾, and gently blow nitrogen onto the concentration liquid to make a specific amount of 1 ml⁽²¹⁾.

For a blank test in this case, carry out the operations of 2) to 4) on 200 ml of water, successively add about 50 ml of hexane, concentrate to 6 ml to 7 ml by using a concentrator⁽⁸⁾ ⁽⁹⁾, and gently blow nitrogen on the concentration liquid to make a specific amount of 1 ml⁽¹⁰⁾ ⁽²¹⁾.

Notes ⁽¹¹⁾ Filter a sample, which has much suspension material, by using glass fibre filter paper (pore diameter not more than 1 µm). Elute the target pesticides adsorbed to the suspension material on the filter paper with acetone or acetonitrile [that of 300 or over concentration quality specified in **JIS K 8039**]. Combine the eluted liquid into the eluted liquid of 3).

Confirm no influence of the glass fibre filter paper to be used on the test by carrying out a series of operations beforehand.

- ⁽¹²⁾ Preliminarily confirm the procedure of preparation of the solid phase column, recovery and the like, and use.
- ⁽¹³⁾ The holding rate in a solid phase is sometimes relative to pH depending on the target pesticide or the packing material.

As in the case of oxine-copper, when styrene divinyl benzene copolymer is used as a packing material, the pH is to be set to 3.5. In the case of using silica gel to which an octadecyl group (ODS) is chemically bonded as the packing, there is no necessity for adjusting pH. In the case of using polystyrene based gel and polymer gel of polymethacrylate, it is required to adjust the pH to 3.5 for some products.

Though hydrochloric acid specified in **JIS K 8180** diluted to an appropriate concentration is used for adjusting the pH, nitric acid specified in **JIS K 8541** diluted to an appropriate concentration is preferably used for some packing materials.

- ⁽¹⁴⁾ The pressurizing method is a method for sending the sample under pressurized state into the solid phase column.

The apparatus consists of an adjusting part and a pump part, and the adjusting part controls water-passing time and water-passing speed. As the pump part, there are a system for using a syringe and a system for using peristaltic pump.

For the syringe, a tube made of tetrafluoroethylene resin is used. For the peristaltic pump, a tube for pumping suitable for a solvent to be used shall be used.

- (15) The reduced pressure method means a method for sending a sample under reduced pressure state on the bottom side of the solid phase column.

The apparatus consists of a piping group for suction and a vacuum pump.

The reduced pressure method is difficult to control a flow rate constant due to dispersion of liquid-passing resistance of the solid phase, suspension material in the sample and the like. However, there is effective in the case where many samples can be treated at once by a simple apparatus and the like.

- (16) When the amount of passing water is too much, the holding rate lessens for some target pesticides. The flow rate of passing water should not exceed 20 ml/min. A width of adsorption band to the solid phase column becomes minimum at about 1-ml/min flow rate, however, since an adsorption speed is very quick in the case of pesticides, sufficient reproducibility and recovery can be obtained even at 10 ml/min to 20 ml/min, and there are merits such as shortening the treating time. In the case of a sample with high concentration of organic material, when the flow rate is decreased (5 ml/min or lower), reproducibility and the recovery rate may be improved. The flow rate shall be kept constant.
- (17) For the amount to be used for washing and elution after adsorption, previously confirm that it has a suitable amount for eluting the target pesticides.
- (18) This removal of water is the operation for making concentration of the eluted liquid easy and lessening the mixing of water content into the solution to be injected into a capillary gas chromatograph. After passing the sample with water through the solid phase column, suction using an aspirator or the like shall be carried out in order to remove the water content as much as possible.
- (19) The flow rate is controlled to a degree that liquid drops of the eluted liquid from the column are not continuous.
- (20) In the case of using the disk form, add about 50 ml of hexane successively from the operation of 4). After dehydration, concentrate using a concentrator, gently blow nitrogen onto the concentration liquid to make 1 ml of a specific amount.
- (21) In the operation for blowing nitrogen, adjust the flow rate of nitrogen to a degree that moving of the surface of concentration liquid can be barely seen, and care shall be taken not to scatter the concentration liquid. Further, when dried to be solid, the target pesticides sometimes

are also vaporized by blowing the nitrogen, and therefore care shall be taken.

6.3 Chromatographic separation After carrying out the procedures in **6.1** or **6.2**, carry out column chromatographic separation wherein an active magnesium silicate is used as the packing material of a column.

When there is no obstacle substance, this procedure may be omitted.

Column chromatograph should be selected according to the contents of the obstacle substance.

a) **Reagents** The following reagents shall be used.

- 1) **Acetone** As described in **4.1 a) 3)**.
- 2) **Diethyl ether** That of 300 or over concentration quality⁽¹⁾ specified in **JIS K 8357**.
- 3) **Hexane** As described in **6.1 a) 4)**.
- 4) **Diethyl ether—hexane eluent (7+13)** Prepare it from diethyl ether of **2)** and hexane of **3)**.
- 5) **Nitrogen** As described in **6.2 a) 2)**.

b) **Implements** The implements shall be as follows.

- 1) **Egg-apple type flask** As described in **6.1 b) 2)**.
- 2) **Chromatograph tube** The following conditions shall be satisfied. An example is given in Fig. 1.
 - 2.1) **Tube for column** Glass tube with cock of about 1 cm inner diameter and about 30 cm in length.
 - 2.2) **Column packing** Active magnesium silicate⁽²²⁾ for column chromatograph (particle diameter, 75 to 150 µm. Heat at 130 °C for about 16 h, then allow to cool in a desiccator). That not generating a peak in the retention time of the target pesticides.

Informative reference 2 Active magnesium silicate is sold on the market as the name of Florisil and the like.

- 2.3) **Preparation of chromatograph tube** Make about 8 g of column packing slurry with hexane, pour into the tube for column⁽²³⁾ not to contain a bubble, and laminate about 5 g of sodium sulfate [**6.1 a) 2)**] thereon.

Unit : cm

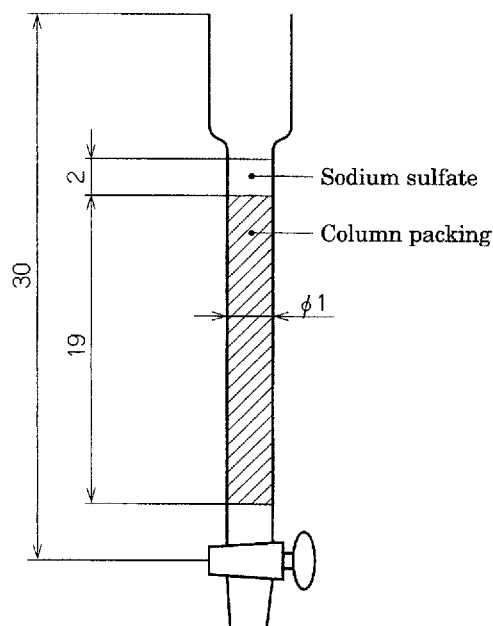


Fig. 1 An example of chromatograph tube

3) **Concentrator** As described in 6.1 b) 5).

Notes ⁽²²⁾ For the measurement of dichlorvos, this column packing can not be used. In this case, the silica gel for residual pesticides in Remarks 2 is used.

⁽²³⁾ In order to fill the column packing uniformly into the tube for column, after pouring in, it is preferred to vibrate the tube in the length and breath direction.

Remarks 2 Silica gel for residual pesticides [(particle diameter, 150 μm to 250 μm . Heat at 130 $^{\circ}\text{C}$ for about 16 h, then allow to cool in a desiccator.) That not generating a peak in the retention time of target pesticides.] can be used as the column packing.

c) **Operation** The operations shall be as follows .

- 1) Pour 1 ml taken from the hexane solution made a specific amount in 6.1 c) 5) or total amount of the hexane solution made a specific amount in 6.2 c) 6) into a chromatograph tube.
- 2) Allow 100 ml of hexane to flow down from the upper part of the chromatograph tube, and discard the hexane effluent.
- 3) Allow successively 100 ml of diethyl ether-hexane eluent (7+13)⁽²⁴⁾ to flow down at a flow rate of about 1 ml/min from the upper part of the chromatograph tube to eluate the target pesticides⁽²⁵⁾, and receive the eluate by an egg-apple type flask⁽⁸⁾.
- 4) Further, allow 100 ml of acetone to flow down at a flow rate of about 1 ml/min from the upper part of the chromatograph tube to elute the target pesticides⁽²⁵⁾, and receive the eluate by a 100 ml to 200 ml egg-apple type flask⁽⁸⁾.

- 5) Concentrate those eluate to about 10 ml on a water bath at about 40 °C⁽⁹⁾ by using a concentrator⁽⁹⁾. After adding about 100 ml of hexane to each egg-apple type flask, concentrate again the organic solvent layer to 2 ml to 5 ml by using the concentrator⁽⁹⁾, remove the receiver, and blow on gently nitrogen to make 1 ml of a specific amount⁽¹⁰⁾ ⁽²¹⁾.
- 6) As a blank test, pour 1 ml from the hexane solution made a specific amount (5 ml) in **6.1 c) 5)** or total amount of the hexane solution made a specific amount in **6.2 c) 6)** into the chromatograph tube, and successively carry out the operations of **2)**, **3)** and **5)** or **2)**, **4)** and **5)**.

Notes ⁽²⁴⁾ As a substitute for diethyl ether–hexane eluent (7+13), acetone–hexane eluent (1+19) may be used. The operation in this case is as described in Remarks 4.

⁽²⁵⁾ A fraction wherein the target component (the target pesticide) is eluted shall be confirmed beforehand.

Remarks 3 In the case where silica gel for residual pesticides in Remarks 2 is used as a column packing, the following shall apply.

In the case where silica gel is used as column packing

- 1) Pour 1 ml taken from the hexane solution made a specific amount in **6.1 c) 5)** or total amount of the hexane solution made a specific amount in **6.2 c) 6)** into a chromatograph tube.
- 2) Allow 80 ml of hexane to flow down from the upper part of the chromatograph tube, and discard the hexane effluent.
- 3) Carry out the operations of **c) 3)** to **5)**.
- 4) As a blank test, carry out the operation of **c) 6)**.
- 4 The operation in the case of using acetone–hexane eluent (1+19) is used, shall be as follows.
 - 1) Carry out the operations of **c) 1)** to **3)** in **6.1**.
 - 2) Use a concentrator^(*)1), concentrate dichloromethane solution on a water bath at about 40 °C^(*)1) to 1 ml to 2 ml, remove the receiver, gently blow nitrogen, and vaporize the dichloromethane^(*)2) ^(*)3).
 - 3) After dissolving contents of the receiver with a small amount of hexane, transfer it into the 10-ml volumetric flask, further wash the receiver sufficiently 2 and 3 times with 2 ml to 3 ml of hexane, transfer washings into the 10-ml volumetric flask, and add hexane to the mark line.
 - 4) Pour total amount of the contents of the 10-ml flask from the upper part of the chromatograph tube^(*)4). Further, sufficiently wash the flask 2 or 3 times with 2 ml to 3 ml of hexane, and pour the washings into the chromatograph tube.
 - 5) Successively elute the target pesticides^(*)5) by allowing 50 ml of acetone–hexane eluent (1+19) [prepared using acetone of **6.2 a) 1)** and hexane of **6.1 a) 4)**.] to flow down, and receive the eluate by an egg-apple type flask^(*)6).

- 6) Concentrate it to 1 ml to 2 ml on a water bath at about 40 °C by using the concentrator^{(*)1}, detach the receiver, gently blow on nitrogen, vaporize the eluate, and add correctly 1 ml of acetone^{(*)7}.
- 7) As a blank test, take the same amount of water as that of the sample into a separatory funnel, and carry out the operations of 1) to 6).

Notes ^{(*)1} As described in Note ⁽⁹⁾.

^{(*)2} Since chromatographic separation is influenced when dichloromethane remains, vaporize dichloromethane. However, when nitrogen is blown on even after the dichloromethane was vaporized, the target pesticides are sometimes vaporized, and care shall be taken therefor.

^{(*)3} As described in Note ⁽²¹⁾.

^{(*)4} As described in **b) 2)** of **6.3**.

^{(*)5} As described in Note ⁽²⁵⁾.

^{(*)6} As described in Note ⁽⁸⁾.

^{(*)7} As described in Note ⁽¹⁰⁾.

- 5 The extraction method wherein the organic solvent of ethyl acetate-hexane mixture is used, should be applicable according to the target pesticides. The extracting operation and the like are as follows.

Solvent extraction method using mixed organic solvent After extracting a sample by the mixed solution of ethyl acetate-hexane under coexistence with sodium chloride, dehydrate and make a specific amount by concentration.

a) **Reagents** The following reagents shall be used.

- 1) **Sodium chloride** As described in **6.1 a) 1)**.
- 2) **Sodium sulfate** As described in **6.1 a) 2)**.
- 3) **Acetone** As described in **4.1 a) 3)**.
- 4) **Ethyl acetate** That of 300 or over concentration quality specified in **JIS K 8110^{(*)1}**.
- 5) **Hexane** As described in **6.1 a) 4)**.
- 6) **Acetone-hexane eluent (1+19)** As described in **5)** of Remarks 4.
- 7) **Ethyl acetate-hexane mixed solution (1+1)** Prepare from ethyl acetate of 4) and hexane of 5).
- 8) **Nitrogen** As described in **6.2 a) 2)**.

Note ^{(*)1} As described in Note ⁽¹⁾.

b) **Implements** The implements shall be as follows.

- 1) **Separatory funnel** As described in **6.1 b) 1)**.
- 2) **Egg-apple type flask** As described in **6.1 b) 2)**.

- 3) **Erlenmeyer flask** As described in 6.1 b) 3).
- 4) **Chromatograph tube** That satisfying the following conditions.
 - 4.1) **Tube for column** A glass tube with a cock of about 1.5 cm inner diameter and about 30 cm length.
 - 4.2) **Column packing** As described in 6.3 b) 2.2).
 - 4.3) **Preparation of chromatograph tube** Make about 5 g of column packing slurry by hexane, pour it into the tube for column so that no bubble is contained^{(*)2}, and laminate about 4 g of sodium sulfate on its upper part.
- 5) **Shaker**
- 6) **Concentrator** As described in 6.1 b) 5).

Note ^{(*)2} As described in Note ⁽²³⁾.
- c) **Operation** The operation shall be carried out as follows.
 - 1) Take a proper amount (200 ml to 400 ml) of the sample^{(*)3} according to 4.1 c) into a separatory funnel, add 5 g of sodium chloride and 10 ml^{(*)4} of ethyl acetate–hexane mixed solution (1+1) per 100 ml of the solution, mix by shaking for about 10 min by using a shaker, and allow it to stand.
 - 2) Transfer the aqueous layer into another separatory funnel. Transfer organic solvent into an Erlenmeyer flask, wash the separatory funnel with a small amount of ethyl acetate–hexane mixed solution (1+1), and combine washings into the Erlenmeyer flask. Add 10 ml of ethyl acetate–hexane mixed solution (1+1) per 100 ml of the solution to the aqueous layer of the separatory funnel, mix by shaking for about 10 min by using a shaker again, and allow it to stand. Combine the organic solvent layer into the Erlenmeyer flask.
 - 3) Add about 5 g^{(*)5} of sodium sulfate per 20 ml of organic solvent, and lightly mix by shaking. After allowing to stand for about 10 min^{(*)6}, filter using class 5A (or class 5B)^{(*)7} of filter paper, and receive the filtrate in an egg-apple type flask^{(*)8}. Wash the Erlenmeyer flask 2 or 3 times with a small amount of ethyl acetate–hexane mixed solution (1+1), further wash sodium sulfate on the filter paper with the washings, and combine the washings to the organic solvent.
 - 4) Concentrate the organic solvent to 1 ml to 2 ml on a water bath at about 40 °C^{(*)9} by using a concentrator^{(*)9}, detach the receiver, gently blow on nitrogen, and vaporize organic solvent^{(*)10} ^{(*)11}.
 - 5) After dissolving the contents with a small amount of hexane, transfer it into the volumetric flask, further wash the receiver sufficiently 2 or 3 times by 2 ml to 3 ml of hexane, transfer washings also into the volumetric flask, and add hexane to the mark^{(*)12}.

- 6) Pour 5 ml of hexane solution into a chromatograph tube from 10 ml thereof.
- 7) Elute the target pesticides^{(*)13} by allowing 50 ml of acetone–hexane eluent solution (1+19) to flow down at about 1 ml/min of flow rate from the upper part of the chromatograph tube, and receive the eluate in an egg-apple type flask^{(*)8}.
- 8) Concentrate the eluate to 1 ml to 2 ml on a water bath at about 40 °C^{(*)9} by using a concentrator^{(*)9}, detach the receiver, blow on gently nitrogen, vaporize the eluate, and correctly add 4 ml of acetone^{(*)10} ^{(*)11}.
- 9) As a blank test, put the same amount of water as that of the sample into a separatory funnel, add 5 g of sodium chloride and 10 ml^{(*)4} of ethyl acetate–hexane mixed solution (1+1) per 100 ml of the solution, mix by shaking for about 10 min by using a shaker, and allow it to stand. Successively carry out the operations of 2) to 8).

Notes ^{(*)3} As described in Note ⁽³⁾.

^{(*)4} As described in Note ⁽⁴⁾.

^{(*)5} As described in Note ⁽⁵⁾.

^{(*)6} As described in Note ⁽⁶⁾.

^{(*)7} As described in Note ⁽⁷⁾.

^{(*)8} As described in Note ⁽⁸⁾.

^{(*)9} As described in Note ⁽⁹⁾.

^{(*)10} Since the target pesticides may be vaporized when nitrogen is blown on even after finishing vaporization of organic solvent, care shall be taken therefore.

^{(*)11} As described in Note ⁽²¹⁾.

^{(*)12} As described in Note ⁽¹⁰⁾.

^{(*)13} As described in Note ⁽²⁵⁾.

7 Multicomponent simultaneous measurement This is a method to measure simultaneously the pesticides in the sample in which multicomponents are contained, and gas chromatography-mass spectrometry, gas chromatography and high performance liquid chromatography shall apply.

Remarks 1 This test method may be applicable to the cases where sample does not bear any colour and turbidity and not contain any disturbances for the test, and where the operation of 6.3 can be omitted.

7.1 Gas chromatography-mass spectrometry After concentrating pesticides in a sample according to 6.1 or 6.2 for pretreatment, inject a specific amount thereof into a gas chromatograph-mass spectrometer, and determine by using a selected ion monitoring method (SIM) or mass chromatography (MC) as a detection method for target pesticides. Target pesticides, its determination range, and repeatability (in coefficient of variation) are as given in Table 1 (Each of them varies according to the apparatus and measuring conditions.).

Table 1 Target pesticides, determination range and repeatability

Number	Target pesticides	Determination range (ng)		Repeatability (%)	
		Solvent extraction method	Solid phase extraction method	Solvent extraction method	Solid phase extraction method
1	EPN ($C_{14}H_{14}NO_4PS$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
2	isoxathion ($C_{13}H_{16}NO_4PS$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
3	isofenphos ($C_{15}H_{24}NO_4PS$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
4	isoprothiolane ($C_{12}H_{18}O_4S_2$)	0.1 to 2	0.1 to 2	20 to 30	20 to 30
5	iprodione ($C_{13}H_{13}Cl_2N_3O_3$)	0.2 to 4	0.2 to 4	20 to 30	20 to 30
6	iprobenfos [IBP] ($C_{13}H_{21}O_3PS$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
7	esprocarb ($C_{15}H_{23}NOS$)	0.4 to 8	0.4 to 8	10 to 20	10 to 20
8	edifenphos [EDDP] ($C_{14}H_{15}O_2PS_2$)	0.4 to 8	0.4 to 8	10 to 20	10 to 20
9	etridiazole ($C_5H_5Cl_3N_2OS$)	0.3 to 6	0.3 to 6	10 to 20	10 to 20
10	carbaryl [NAC] ($C_{12}H_{11}NO_2$)	0.4 to 8	0.4 to 8	10 to 20	10 to 20
11	captan ($C_9H_8Cl_3NO_2S$)	0.1 to 2	0.1 to 2	20 to 30	20 to 30
12	chlornitrofen [CNP] ($C_{12}H_6Cl_3NO_3$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
13	chlorpyrifos ($C_9H_{11}Cl_3NO_3PS$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
14	chlorothalonil [TPN] ($C_8Cl_4N_2$)	0.1 to 2	0.1 to 2	20 to 30	20 to 30
15	chloroneb ($C_8H_8Cl_2O_2$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
16	dichlofenthion [ECP] ($C_{10}H_{13}Cl_2O_3PS$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
17	dichlorvos [DDVP] ($C_4H_7Cl_2O_4P$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
18	dithiopyr ($C_{15}H_{16}F_6NO_2S_2$)	0.4 to 8	0.4 to 8	10 to 20	10 to 20
19	simazine [CAT] ($C_7H_{12}ClN_5$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
20	simetryn ($C_8H_{15}N_5S$)	0.05 to 1	0.05 to 1	10 to 20	10 to 20

Table 1 (concluded)

Number	Target pesticides	Determination range (ng)		Repeatability (%)	
		Solvent extraction method	Solid phase extraction method	Solvent extraction method	Solid phase extraction method
21	diazinon (C ₁₂ H ₂₁ N ₂ O ₃ PS)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
22	thiobencarb [benthicarb] (C ₁₂ H ₁₆ ClNOS)	0.05 to 1	0.05 to 1	10 to 20	10 to 20
23	terbucarb [MBPMC] (C ₁₇ H ₂₇ NO ₂)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
24	triclopyrbutoxyethyl ester (C ₁₃ H ₁₆ Cl ₃ NO ₄) ⁽¹⁾	0.4 to 8	0.4 to 8	10 to 20	10 to 20
25	trichlorfon [DEP] (C ₄ H ₈ Cl ₃ O ₄ P)	0.5 to 10	0.5 to 10	20 to 30	20 to 30
26	tricyclazole (C ₉ H ₇ N ₃ S)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
27	tolclophos-methyl (C ₉ H ₁₁ Cl ₂ O ₃ PS)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
28	napropamide (C ₁₇ H ₂₁ NO ₂)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
29	pyridaphenthion (C ₁₄ H ₁₇ N ₂ O ₄ PS)	0.2 to 4	0.2 to 4	20 to 30	20 to 30
30	pyributicarb (C ₁₈ H ₂₂ N ₂ O ₂ S)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
31	fenitrothion [MEP] (C ₉ H ₁₂ NO ₃ PS)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
32	fenobucarb [BPMC] (C ₁₂ H ₁₇ NO ₂)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
33	ftalide (C ₈ H ₂ Cl ₄ O ₂)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
34	butamifos (C ₁₃ H ₂₁ N ₂ O ₄ PS)	0.1 to 2	0.1 to 2	20 to 30	20 to 30
35	buprofezin (C ₁₆ H ₂₃ N ₃ OS)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
36	flutolanil (C ₁₇ H ₁₆ F ₃ NO ₂)	0.1 to 2	0.1 to 2	20 to 30	20 to 30
37	pretilachlor (C ₁₇ H ₂₆ ClNO ₂)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
38	propyzamide (C ₁₂ H ₁₁ Cl ₂ NO)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
39	probenazole (C ₁₀ H ₉ NO ₃ S)	0.4 to 8	0.4 to 8	10 to 20	10 to 20
40	bromobutide (C ₁₅ H ₂₂ BrNO) ⁽²⁾	0.1 to 2	0.1 to 2	10 to 20	10 to 20
41	pencycuron (C ₁₉ H ₂₁ ClN ₂ O)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
42	bensulide [SAP] (C ₁₄ H ₂₄ NO ₄ PS ₃)	0.5 to 10	0.5 to 10	10 to 20	10 to 20
43	pendimethalin (C ₁₃ H ₁₉ N ₃ O ₄)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
44	benfluralin (C ₁₃ H ₁₆ F ₃ N ₃ O ₄)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
45	malathion (C ₁₀ H ₁₉ O ₆ PS ₂)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
46	metalaxyl (C ₁₅ H ₂₁ NO ₄)	0.4 to 8	0.4 to 8	10 to 20	10 to 20
47	methyldymron (C ₁₇ H ₂₀ N ₂ O)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
48	mefenacet (C ₁₆ H ₁₄ N ₂ O ₂ S)	0.4 to 8	0.4 to 8	10 to 20	10 to 20
49	mepronil (C ₁₇ H ₁₉ NO ₂)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
50	molinate (C ₉ H ₁₇ NOS)	0.4 to 8	0.4 to 8	10 to 20	10 to 20

Notes ⁽¹⁾ For determination of triclopyr, triclopyrbutoxyethyl ester is determined by a gas chromatography-mass spectrometry in **38.1**, and the obtained value is multiplied by factor 0.719 2. Separately, triclopyr (free) is determined by **7.3** or **38.1**. The total of both values is obtained as the amount of triclopyr.

⁽²⁾ For determination of bromobutide, add the amount (ng) of bromobutide to the amount of debrominated bromobutide (ng) multiplied by factor 1.338 in order to converting to bromobutide, which are obtained by using a gas chromatography-mass spectrometry, and calculate the concentration by the formula of **d) 4)**.

a) **Reagents** The following reagents shall be used.

- 1) **Sodium chloride** As described in **6.1 a) 1)**.
- 2) **Sodium sulfate** As described in **6.1 a) 2)**.
- 3) **Acetone** As described in **4.1 a) 3)**.
- 4) **Dichloromethane** As described in **6.1 a) 3)**.
- 5) **Hexane** As described in **6.1 a) 4)**.
- 6) **Target pesticide standard solution (0.2 mg/ml to 5 mg/ml)** The sampling amount of standard product used for preparation of the standard solution of target pesticide and a solvent used for dissolution are given in Table 2⁽³⁾. Preparation of the standard solutions of those target pesticides shall be as follows.

Dissolve a specific amount [the sampling amount is given in the concentration (A) in Table 2.] of the standard product of a target pesticide in a small amount of acetone or hexane, transfer each solution into a 100-ml volumetric flask, and add acetone or hexane to the marked line⁽⁴⁾.

- 7) **Mixed standard solution (5 µg/ml to 50 µg/ml)** Take a specific amount (the sampling amount is given in the column of mixed standard solution in Table 2) of target pesticide standard solutions (0.2 mg/ml to 5 mg/ml) individually prepared in **6)** into a 100-ml volumetric flask, and add hexane to the mark⁽⁵⁾. When reserved, place it in a dark place at -20 °C.
- 8) **Mixed standard solution (0.5 µg/ml to 5 µg/ml)** Take 1 ml of mixed standard solution (5 µg/ml to 50 µg/ml) prepared in **7)** into a 10-ml volumetric flask, and add hexane to the mark⁽⁵⁾. Prepare in use.
- 9) **Nitrogen** As described in **6.2 a) 2)**.

Notes ⁽³⁾ For isoxathion, chlornitrofen, chlorothalonil, dichlorvos, fenobucarb and propyzamide, dissolve a specific amount of each standard product in a small amount of acetone, transfer into a 100-ml flask, and add hexane to the mark.

⁽⁴⁾ In the case where standard solution is reserved for a long period of time, immediately put the prepared standard solution into an ampul, seal it by fusion and place in a dark place at -20 °C.

⁽⁵⁾ For preparation of mixed standard solution when the pretreatment operation of **6.2** is carried out, use acetone in place of hexane.

b) **Implements and apparatus** Implements and apparatus shall be as follows.

- 1) **Separatory funnel** As described in 6.1 b) 1).
- 2) **Egg-apple type flask** As described in 6.1 b) 2).
- 3) **Erlenmeyer flask** As described in 6.1 b) 3).
- 4) **Test tube with a ground stopper** As described in 6.2 b) 1).
- 5) **Solid phase column** As described in 6.2 b) 2).
- 6) **Microsyringe** An appropriate one of 1 μ l to 10 μ l. An automatic injector may be used.
- 7) **Gas chromatograph-mass spectrometer** That satisfying the following conditions⁽⁶⁾.
 - 7.1) **Tube for capillary column** That made of stainless steel, quartz glass or borosilicate glass of 0.2 mm to 0.7 mm inner diameter and 10 m to 60 m in length, or that equal in quality thereto.
 - 7.2) **Capillary column** That wherein the inner wall of a tube for capillary column is coated by 0.1 μ m to 1.0 μ m thickness of methyl silicone series stationary phase liquid⁽⁷⁾, or that having separation performance equal in quality thereto.

Informative reference 1 For the capillary column used for this test, there are DB-1, CBP-1, CP-SIL-5CB, NB-1, SPB-1, Ultra-1 and the like wherein 100 % methyl silicone is used as a stationary phase liquid.
 - 7.3) **Detector** That capable of carrying out selected ion monitoring method (SIM) or mass chromatography (MC).
 - 7.4) **Carrier gas** Use helium (99.999 9 vol% or over) by adjusting the linear velocity within a range of 20 cm/s to 40 cm/s.
 - 7.5) **Sample introducing method and sample introducing part temperature** To the sample introducing method, splitless injection method (undivided introducing system) or cold on-column injection method (total amount introducing system) applies. Sample introducing part temperature is 220 °C to 280 °C for splitless injection method and 50 °C to 100 °C for cold-on column injection method.
 - 7.6) **GC/MS connection part⁽⁸⁾ temperature** 200 °C to 280 °C
 - 7.7) **Ion source temperature** 200 °C to 250 °C
 - 7.8) **Electron accelerating voltage** 20 V to 70 V
 - 7.9) **Temperature programming** 50 °C to 280 °C (programming of 2 °C/min to 30 °C/min)
- 8) **Shaker**
- 9) **Concentrator** As described in 6.1 b) 5).

Notes ⁽⁶⁾ Those conditions are different according to apparatus, and measuring conditions.

- (7) As methyl silicone series stationary phase liquid, 100 % methyl silicone, 5 % phenyl-95 % methyl silicone, 50 % phenyl-50 % methyl silicone or that having the same separating performance equal in quality thereto is used.
- (8) The GC/MS connection part is sometimes called a separator part according to an apparatus.

Remarks 1 As the sensitivity of gas chromatograph mass spectrometer, inject 1 µl of mixed standard solution (0.05 µg/ml to 0.5 µg/ml) [prepared from 1 ml of the mixed standard solution of a) 8) (0.5 µg/ml to 5 µg/ml) diluted to 10 ml.] into the gas chromatograph-mass spectrometer, and adjust the sensitivity of the detector so that the amount (0.05 ng to 0.5 ng) of target pesticides can be confirmed.

Table 2 Target pesticide standard solution and mixed standard solution

Number	Target pesticides	Concentration (A) 0.2 mg/ml to 5 mg/ml		Mixed standard solution	
		Solvent	Sampling amount of standard material (g/100 ml)	Sampled amount (ml/100 ml)	Concentration (µg/ml)
1	EPN (C ₁₄ H ₁₄ NO ₄ PS)	hexane	0.100	1	10
2	isoxathion (C ₁₃ H ₁₆ NO ₄ PS)	Note (3)	0.100	1	10
3	isofenphos (C ₁₅ H ₂₄ NO ₄ PS)	acetone	0.100	1	10
4	isoprothiolane (C ₁₂ H ₁₈ O ₄ S ₂)	hexane	0.100	1	10
5	iprodione (C ₁₃ H ₁₃ Cl ₂ N ₃ O ₃)	acetone	0.200	1	20
6	iprobefos [IBP] (C ₁₃ H ₂₁ O ₃ PS)	hexane	0.100	1	10
7	esprocarb (C ₁₅ H ₂₃ NOS)	acetone	0.400	1	40
8	edifenphos [EDDP] (C ₁₄ H ₁₅ O ₂ PS ₂)	acetone	0.400	1	40
9	etridiazole (C ₆ H ₅ Cl ₃ N ₂ OS)	acetone	0.300	1	30
10	carbaryl [NAC] (C ₁₂ H ₁₁ NO ₂)	acetone	0.400	1	40
11	captan (C ₉ H ₈ Cl ₃ NO ₂ S)	acetone	0.100	1	10
12	chlornitrofen [CNP] (C ₁₂ H ₆ Cl ₃ NO ₃)	Note (3)	0.100	1	10
13	chlorpyrifos (C ₉ H ₁₁ Cl ₃ NO ₃ PS)	acetone	0.100	1	10
14	chlorothalonil [TPN] (C ₈ Cl ₄ N ₂)	Note (3)	0.100	1	10
15	chloroneb (C ₈ H ₈ Cl ₂ O ₂)	acetone	0.100	1	10
16	dichlofenthion [ECP] (C ₁₀ H ₁₃ Cl ₂ O ₃ PS)	acetone	0.100	1	10
17	dichlorvos [DDVP] (C ₄ H ₇ Cl ₂ O ₄ P)	Note (3)	0.100	1	10
18	dithiopyr (C ₁₅ H ₁₆ F ₅ NO ₂ S ₂)	acetone	0.400	1	10
19	simazine [CAT] (C ₇ H ₁₂ ClN ₅)	acetone	0.020	2.5	5
20	simetryn (C ₈ H ₁₅ N ₅ S)	acetone	0.100	1	10
21	diazinon (C ₁₂ H ₂₁ N ₂ O ₃ PS)	hexane	0.100	1	10

Table 2 (concluded)

Number	Target pesticides	Concentration (A) 0.2 mg/ml to 5 mg/ml		Mixed standard solution	
		Solvent	Sampling amount of standard material (g/100 ml)	Sampled amount (ml/100 ml)	Concentration (µg/ml)
22	thiobencarb [benthiocarb] (C ₁₂ H ₁₆ ClNOS)	hexane	0.050	1	5
23	terbucarb [MBPMC] (C ₁₇ H ₂₇ NO ₂)	acetone	0.100	1	10
24	triclopyrbutoxyethyl ester (C ₁₃ H ₁₆ Cl ₃ NO ₄)	acetone	0.400	1	40
25	trichlorfon [DEP] (C ₄ H ₈ Cl ₃ O ₄ P)	acetone	0.500	1	50
26	tricyclazole (C ₉ H ₇ N ₃ S)	acetone	0.100	1	10
27	tolclophos-methyl (C ₉ H ₁₁ Cl ₂ O ₃ PS)	acetone	0.100	1	10
28	napropamide (C ₁₇ H ₂₁ NO ₂)	acetone	0.100	1	10
29	pyridaphenthion (C ₁₄ H ₁₇ N ₂ O ₄ PS)	acetone	0.200	1	20
30	pyributicarb (C ₁₈ H ₂₂ N ₂ O ₂ S)	acetone	0.100	1	10
31	fenitrothion [MEP] (C ₉ H ₁₂ NO ₃ PS)	hexane	0.100	1	10
32	fenobucarb [BPMC] (C ₁₂ H ₁₇ NO ₂)	Note (3)	0.100	1	10
33	ftalide (C ₈ H ₂ Cl ₄ O ₂)	acetone	0.100	1	10
34	butamifos (C ₁₃ H ₂₁ N ₂ O ₄ PS)	acetone	0.100	1	10
35	buprofezin (C ₁₆ H ₂₃ N ₃ OS)	acetone	0.100	1	10
36	flutolanil (C ₁₇ H ₁₆ F ₃ NO ₂)	acetone	0.100	1	10
37	pretilachlor (C ₁₇ H ₂₆ ClNO ₂)	acetone	0.100	1	10
38	propyzamide (C ₁₂ H ₁₁ Cl ₂ NO)	Note (3)	0.100	1	10
39	probenazole (C ₁₀ H ₉ NO ₃ S)	acetone	0.400	1	40
40	bromobutide (C ₁₅ H ₂₂ BrNO)	acetone	0.050	1	5
	debrominated bromobutide (C ₁₅ H ₂₃ NO)	acetone	0.050	1	5
41	pencycuron (C ₁₉ H ₂₁ ClN ₂ O)	acetone	0.100	1	10
42	bensulide [SAP] (C ₁₄ H ₂₄ NO ₄ PS ₃)	acetone	0.500	1	50
43	pendimethalin (C ₁₃ H ₁₉ N ₃ O ₄)	acetone	0.100	1	10
44	benfluralin (C ₁₃ H ₁₆ F ₃ N ₃ O ₄)	acetone	0.100	1	10
45	malathion (C ₁₀ H ₁₉ O ₆ PS ₂)	acetone	0.100	1	10
46	metalaxyl (C ₁₅ H ₂₁ NO ₄)	acetone	0.400	1	40
47	methyldymron (C ₁₇ H ₂₀ N ₂ O)	acetone	0.100	1	10
48	mefenacet (C ₁₆ H ₁₄ N ₂ O ₂ S)	acetone	0.400	1	40
49	mepronil (C ₁₇ H ₁₉ NO ₂)	acetone	0.100	1	10
50	molinate (C ₉ H ₁₇ NOS)	acetone	0.400	1	40

c) **Preparatory operation** Preparatory operation shall be carried out as follows.

1) Carry out the operation of **6.1** or **6.2**.

d) **Operation** The operation shall be carried out as follows.

- 1) Sample 1 µl of mixed standard solution (0.5 µg/ml to 5 µg/ml) of **a) 8**) by a microsyringe⁽⁹⁾, inject into a gas chromatograph by a splitless injection method or a cold on-column injection method, set⁽¹⁰⁾ a fragment ion (m/z) particular to the target pesticides, record its mass fragmentgram by selected ion monitoring method or mass chromatography, and confirm the peak position corresponding to retention time of the target pesticides.
- 2) Sample 1 µl of the concentration liquid obtained in **c)** by a microsyringe⁽⁹⁾, record the mass fragmentgram by carrying out the operation of **1)**, confirm the retention time of **1)**, and read an indication value⁽¹¹⁾ for the peak of a position corresponding to the retention time.
- 3) As a blank test, carry out the operation of **1)** and **2)** for the concentration liquid of the blank test obtained in **c)**, and in the case where a peak is detected at the position corresponding to the retention time of the target pesticides and the indication value⁽¹¹⁾ is $\geq 1/3$ of the indication value for determination lower limit value, carry out the operation again from the preparatory operation.
- 4) Obtain the amount of target pesticides from the working curve, and calculate the concentration (µg/L) of target pesticides in the sample according to the following formula.

$$N = a \times 10^{-3} \times \frac{v_1}{C \times 10^{-3}} \times \frac{1\,000}{V}$$

where, N : concentration of target pesticides (µg/L)

a : amount of target pesticides obtained from the working curve (ng)

C : injection amount (µl)

V : sample (ml)

v_1 : the amount of hexane in **6.1 c) 5)** or the amount of acetone in **6.2 c) 4)** (ml)

10^{-3} : coefficient for converting ng to µg, and µl to ml

Working curve Sample stepwise 0.1 ml to 2 ml of the mixed standard solution (5 µg/ml to 50 µg/ml) in **a) 7)** into the 10-ml volumetric flask, and add hexane⁽⁵⁾ to the mark. Take a specific amount [the same amount as the sample (for example, 1 µl)] of each solution by a microsyringe, and prepare the relation curve between the amount (ng) of target pesticide and the indication value⁽¹¹⁾ by carrying out the operations of **1)** and **2)**. Carry out preparation of the working curve in measuring the sample.

Notes ⁽⁹⁾ Use the same one as that in preparing the working curve.

⁽¹⁰⁾ It is preferable to refer to Table 3 in order to set a particular fragment ion.

⁽¹¹⁾ Peak height or peak area

Remarks 2 In the case where confirmation of analyzed pesticides is required, concentrate a sample, and take a mass spectrum of the target pesticides. When the concentration of target pesticides in the sample is low, and the mass spectrum can not be obtained, set a plurality of fragment ions, and obtain the ion intensity ratio of the chromatogram obtained by measuring. Judge by comparing the mass spectrum or peak strength ratio obtained by measuring the standard product of target pesticides in the same way as that.

3 The determination may be carried out by the method using internal standard substance such as naphthalene-d₈ (m/z 136), phenanthrene-d₁₀ (m/z 188), fluoranthene-d₁₀ (m/z 212), chrysene-d₁₀ (m/z 240) and the like.

Table 3 An example of fragment ion in selected ion monitoring method

Number	Target pesticides	Fragment ion (m/z) ⁽¹²⁾ ⁽¹³⁾				
		157	169	185	141	—
1	EPN (C ₁₄ H ₁₄ NO ₄ PS)	157	169	185	141	—
2	isoxathion (C ₁₃ H ₁₆ NO ₄ PS)	105	177	77	313	—
3	isofenphos (C ₁₅ H ₂₄ NO ₄ PS)	213	121	255	185	—
4	isoprothiolane (C ₁₂ H ₁₈ O ₄ S ₂)	118	162	189	290	204
5	iprodione (C ₁₃ H ₁₃ Cl ₂ N ₃ O ₃)	314	316	187	189	—
6	iprobenfos [IBP] (C ₁₃ H ₂₁ O ₃ PS)	91	204	123	—	—
7	esprocarb (C ₁₅ H ₂₃ NOS)	91	222	71	162	—
8	edifenphos [EDDP] (C ₁₄ H ₁₅ O ₂ PS ₂)	109	173	310	201	—
9	etridiazole (C ₅ H ₅ Cl ₃ N ₂ OS)	211	183	213	185	140
10	carbaryl [NAC] (C ₁₂ H ₁₁ NO ₂)	144	115	—	—	—
11	captan (C ₉ H ₈ Cl ₃ NO ₂ S)	79	149	264	—	—
12	chlornitrofen [CNP] (C ₁₂ H ₆ Cl ₃ NO ₃)	317	319	236	287	289
13	chlorpyrifos (C ₉ H ₁₁ Cl ₃ NO ₃ PS)	197	199	314	316	258
14	chlorothalonil [TPN] (C ₈ Cl ₄ N ₂)	266	264	268	—	—
15	chloroneb (C ₆ H ₅ Cl ₂ O ₂)	191	193	206	208	141
16	dichlofenthion [ECP] (C ₁₀ H ₁₃ Cl ₂ O ₃ PS)	279	223	97	251	162
17	dichlorvos [DDVP] (C ₄ H ₇ Cl ₂ O ₄ P)	109	185	79	—	—
18	dithiopyr (C ₁₅ H ₁₆ F ₅ NO ₂ S ₂)	354	306	—	—	—
19	simazine [CAT] (C ₇ H ₁₂ ClN ₅)	201	186	173	158	—
20	simetryn (C ₈ H ₁₅ N ₅ S)	213	170	155	—	—
21	diazinon (C ₁₂ H ₂₁ N ₂ O ₃ PS)	179	137	304	152	199
22	thiobencarb [benthicarb] (C ₁₂ H ₁₆ ClNOS)	100	72	125	257	—
23	terbucarb [MBPMC] (C ₁₇ H ₂₇ NO ₂)	205	220	145	—	—

Table 3 (concluded)

Num- ber	Target pesticides	Fragment ion (m/z) ⁽¹²⁾ ⁽¹³⁾				
24	triclopyrbutoxyethyl ester (C ₁₃ H ₁₆ Cl ₃ NO ₄)	57	85	210	212	182
25	trichlorfon [DEP] (C ₄ H ₈ Cl ₃ O ₄ P)	109	79	145	139	—
26	tricyclazole (C ₉ H ₇ N ₃ S)	189	162	161	—	—
27	tolclophos-methyl (C ₉ H ₁₁ Cl ₂ O ₃ PS)	265	267	125	—	—
28	napropamide (C ₁₇ H ₂₁ NO ₂)	72	128	271	100	115
29	pyridaphenthion (C ₁₄ H ₁₇ N ₂ O ₄ PS)	340	199	188	97	77
30	pyributicarb (C ₁₈ H ₂₂ N ₂ O ₂ S)	165	108	181	—	—
31	fenitrothion [MEP] (C ₉ H ₁₂ NO ₅ PS)	277	125	109	260	79
32	fenobucarb [BPMC] (C ₁₂ H ₁₇ NO ₂)	121	150	91	—	—
33	ftalide (C ₈ H ₂ Cl ₄ O ₂)	243	241	245	272	270
34	butamifos (C ₁₃ H ₂₁ N ₂ O ₄ PS)	286	200	232	202	258
35	buprofezin (C ₁₆ H ₂₃ N ₃ OS)	105	172	106	175	305
36	flutolanil (C ₁₇ H ₁₆ F ₃ NO ₂)	173	281	145	—	—
37	pretilachlor (C ₁₇ H ₂₆ ClNO ₂)	162	238	176	202	262
38	propyzamide (C ₁₂ H ₁₁ Cl ₂ NO)	173	175	255	145	254
39	probenazole (C ₁₀ H ₉ NO ₃ S)	130	103	159	104	132
40	bromobutide (C ₁₅ H ₂₂ BrNO)	119	120	118	232	91
	debrominated bromobutide (C ₁₅ H ₂₃ NO)	119	233	177	—	—
41	pencycuron (C ₁₉ H ₂₁ ClN ₂ O)	125	180	127	209	182
42	bensulide [SAP] (C ₁₄ H ₂₄ NO ₄ PS ₃)	77	131	141	—	—
43	pendimethalin (C ₁₃ H ₁₉ N ₃ O ₄)	252	281	162	—	—
44	benfluralin (C ₁₃ H ₁₆ F ₃ N ₃ O ₄)	292	264	276	—	—
45	malathion (C ₁₀ H ₁₉ O ₆ PS ₂)	173	125	127	93	158
46	metalaxyl (C ₁₅ H ₂₁ NO ₄)	206	249	220	234	—
47	methyldymron (C ₁₇ H ₂₀ N ₂ O)	107	119	91	—	—
48	mefenacet (C ₁₆ H ₁₄ N ₂ O ₂ S)	192	120	136	—	—
49	mepronil (C ₁₇ H ₁₉ NO ₂)	119	269	91	—	—
50	molinate (C ₉ H ₁₇ NOS)	126	55	187	—	—

Notes ⁽¹²⁾ The target pesticides are successively displayed in the order from that having larger fragment ion intensity.

⁽¹³⁾ Avoid the fragment ion disturbing measurement of a sample. Further, use of those of ≤ 100 fragment ion should be preferably avoided.

7.2 Gas chromatography Gas chromatography using respectively a flame thermionic detector (FTD), a flame photometric detector (FPD) and an electron capture detector (ECD) applies.

7.2.1 Gas chromatography using flame thermionic detector (FTD) After concentrating pesticides in a sample by applying 6.1 or 6.2 to pretreatment, a specific amount thereof is determined by a method using a flame thermionic detector. Target pesticides, its determination range and repeatability (by coefficient of variation) are as given in Table 4. (They vary according to the apparatus and measuring conditions.)

Table 4 Target pesticides, determination range and repeatability (FTD)

Number	Target pesticides	Determination range (ng)		Repeatability (%)	
		Solvent extraction method	Stationary phase extraction method	Solvent extraction method	Stationary phase extraction method
1	EPN ($C_{14}H_{14}NO_4PS$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
2	isoxathion ($C_{13}H_{16}NO_4PS$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
3	isofenphos ($C_{15}H_{24}NO_4PS$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
5	iprodione ($C_{13}H_{13}Cl_2N_3O_3$)	0.2 to 4	0.2 to 4	20 to 30	20 to 30
6	iprobefos [IBP] ($C_{13}H_{21}O_3PS$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
13	chlorpyrifos ($C_9H_{11}Cl_3NO_3PS$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
14	chlorothalonil [TPN] ($C_8Cl_4N_2$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
17	dichlorvos [DDVP] ($C_4H_7Cl_2O_4P$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
19	simazine [CAT] ($C_7H_{12}ClN_5$)	0.05 to 1	0.05 to 1	10 to 20	10 to 20
21	diazinon ($C_{12}H_{21}N_2O_3PS$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
22	thiobencarb [benthicarb] ($C_{12}H_{16}ClNOS$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
23	terbucarb [MBPMC] ($C_{17}H_{27}NO_2$)	0.4 to 8	0.4 to 8	10 to 20	10 to 20
27	tolclophos-methyl ($C_9H_{11}Cl_2O_3PS$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
28	napropamide ($C_{17}H_{21}NO_2$)	0.4 to 8	0.4 to 8	10 to 20	10 to 20
29	pyridaphenthion ($C_{14}H_{17}N_2O_4PS$)	0.2 to 4	0.2 to 4	10 to 20	10 to 20
30	pyributicarb ($C_{18}H_{22}N_2O_2S$)	0.05 to 1	0.05 to 1	10 to 20	10 to 20
31	fenitrothion [MEP] ($C_9H_{12}NO_5PS$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
32	fenobucarb [BPMC] ($C_{12}H_{17}NO_2$)	0.6 to 12	0.6 to 12	10 to 20	10 to 20
34	butamifos ($C_{13}H_{21}N_2O_4PS$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
36	flutolanil ($C_{17}H_{16}F_3NO_2$)	0.2 to 4	0.2 to 4	10 to 20	10 to 20
38	propyzamide ($C_{12}H_{11}Cl_2NO$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
41	penycuron ($C_{19}H_{21}ClN_2O$)	0.4 to 8	0.4 to 8	10 to 20	10 to 20
42	bensulide [SAP] ($C_{14}H_{24}NO_4PS_3$)	0.5 to 10	0.5 to 10	10 to 20	10 to 20
43	pendimethalin ($C_{13}H_{19}N_3O_4$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20

Table 4 (concluded)

Number	Target pesticides	Determination range (ng)		Repeatability (%)	
		Solvent extraction method	Stationary phase extraction method	Solvent extraction method	Stationary phase extraction method
47	methyldymron ($C_{17}H_{20}N_2O$)	1.0 to 20	1.0 to 20	10 to 20	10 to 20
49	mepronil ($C_{17}H_{19}NO_2$)	1.0 to 20	1.0 to 20	10 to 20	10 to 20

a) **Reagents** The following reagents shall be used.

- 1) **Sodium chloride** As described in 6.1 a) 1).
- 2) **Sodium sulfate** As described in 6.1 a) 2).
- 3) **Acetone** As described in 4.1 a) 3).
- 4) **Dichloromethane** As described in 6.1 a) 3).
- 5) **Hexane** As described in 6.1 a) 4).
- 6) **Target pesticide standard solution (0.5 mg/ml to 6 mg/ml)** The sampling amount of the standard product used for preparing the standard solution of target pesticide and the solvent used for dissolution are given in Table 5⁽¹⁴⁾. Preparation of those target pesticide standard solutions shall be as follows.

Dissolve a specific amount [the sampling amount shall be shown in the column of concentration (A) in Table 2.] of target pesticide standard product with a small amount of acetone or hexane per each target pesticide, transfer respectively into a 100-ml volumetric flask, and add acetone or hexane to each mark⁽⁴⁾.

- 7) **Mixed standard solution (5 µg/ml to 100 µg/ml)** For the target pesticide standard solution (0.5 mg/ml to 6 mg/ml) prepared individually in 6), each specific amount (sampling amount shall be shown in the column of mixed standard solution in Table 2.) is sampled into a 100-ml volumetric flask, and hexane is added to the mark⁽⁵⁾. When reserved, it is placed in a dark place at -20 °C.
- 8) **Mixed standard solution (0.5 µg/ml to 10 µg/ml)** Sample 1 ml of the mixed standard solution (5 µg/ml to 100 µg/ml) prepared in 7) into the 10-ml volumetric flask, and add hexane to the mark⁽⁵⁾. Prepare in use.
- 9) **Nitrogen** As described in 6.2 a) 2).

Note ⁽¹⁴⁾ Isoxathion, chlorothalonil, dichlorvos, fenobucarb and propyzamide are as described in Note ⁽³⁾.

Table 5 Target pesticide standard solution and mixed standard solution (FTD)

Number	Target pesticides	Concentration (A) 0.5 mg/ml to 6 mg/ml		Mixed standard solution	
		Solvent	Sampling amount of standard material (g/100 ml)	Sampling amount (ml/100 ml)	Concentration (µg/ml)
1	EPN (C ₁₄ H ₁₄ NO ₄ PS)	hexane	0.100	1	10
2	isoxathion (C ₁₃ H ₁₆ NO ₄ PS)	Note ⁽¹⁴⁾	0.100	1	10
3	isofenphos (C ₁₅ H ₂₄ NO ₄ PS)	acetone	0.100	1	10
5	iprodione (C ₁₃ H ₁₃ Cl ₂ N ₃ O ₃)	acetone	0.200	1	20
6	iprobenfos [IBP] (C ₁₃ H ₂₁ O ₃ PS)	hexane	0.100	1	10
13	chlorpyrifos (C ₉ H ₁₁ Cl ₃ NO ₃ PS)	acetone	0.100	1	10
14	chlorothalonil [TPN] (C ₈ Cl ₄ N ₂)	Note ⁽¹⁴⁾	0.100	1	10
17	dichlorvos [DDVP] (C ₄ H ₇ Cl ₂ O ₄ P)	Note ⁽¹⁴⁾	0.100	1	10
19	simazine [CAT] (C ₇ H ₁₂ ClN ₅)	acetone	0.020	2.5	5
21	diazinon (C ₁₂ H ₂₁ N ₂ O ₃ PS)	hexane	0.100	1	10
22	thiobencarb [benthiocarb] (C ₁₂ H ₁₆ ClNOS)	hexane	0.100	1	10
23	terbucarb [MBPMC] (C ₁₇ H ₂₇ NO ₂)	acetone	0.400	1	40
27	tolclophos-methyl (C ₉ H ₁₁ Cl ₂ O ₃ PS)	acetone	0.100	1	10
28	napropamide (C ₁₇ H ₂₁ NO ₂)	acetone	0.400	1	40
29	pyridaphenthion (C ₁₄ H ₁₇ N ₂ O ₄ PS)	acetone	0.200	1	20
30	pyributicarb (C ₁₈ H ₂₂ N ₂ O ₂ S)	acetone	0.050	1	5
31	fenitrothion [MEP] (C ₉ H ₁₂ NO ₅ PS)	hexane	0.100	1	10
32	fenobucarb [BPMC] (C ₁₂ H ₁₇ NO ₂)	Note ⁽¹⁴⁾	0.600	1	60
34	butamifos (C ₁₃ H ₂₁ N ₂ O ₄ PS)	acetone	0.100	1	10
36	flutolanil (C ₁₇ H ₁₆ F ₃ NO ₂)	acetone	0.200	1	20
38	propyzamide (C ₁₂ H ₁₁ Cl ₂ NO)	Note ⁽¹⁴⁾	0.100	1	10
41	pencycuron (C ₁₉ H ₂₁ ClN ₂ O)	acetone	0.400	1	40
42	bensulide [SAP] (C ₁₄ H ₂₄ NO ₄ PS ₃)	acetone	0.500	1	50
43	pendimethalin (C ₁₃ H ₁₉ N ₃ O ₄)	acetone	0.100	1	10
47	methyldymron (C ₁₇ H ₂₀ N ₂ O)	acetone	0.200	5	100
49	mepronil (C ₁₇ H ₁₉ NO ₂)	acetone	0.200	5	100

b) **Implements and apparatus** Implements and apparatus shall be as follows.

1) **Separatory funnel** As described in 6.1 b) 1).

- 2) **Egg-apple type flask** As described in 6.1 b) 2).
- 3) **Erlenmeyer flask** As described in 6.1 b) 3).
- 4) **Test tube with a ground stopper** As described in 6.2 b) 1).
- 5) **Solid phase column** As described in 6.2 b) 2).
- 6) **Microsyringe** As described in 7.1 b) 6).
- 7) **Gas chromatograph** That satisfying the following conditions.
 - 7.1) **Tube for capillary column** As described in 7.1 b) 7.1).
 - 7.2) **Capillary column** As described in 7.1 b) 7.2).
 - 7.3) **Detector** Flame thermionic detector⁽¹⁵⁾.
 - 7.4) **Carrier gas** Helium ($\geq 99.999\ 9$ vol%) is used, and the linear velocity is adjusted within a range of 20 cm/s to 40 cm/s to be used. As addition gas⁽¹⁶⁾, the same gas as carrier gas is connected to the outlet of the column, and the flow rate is adjusted to 30 ml/min to 60 ml/min.
 - 7.5) **Sample introducing method and sample introducing part temperature** As described in 7.1 b) 7.5).
 - 7.6) **Fuel gas and supporting gas** As the fuel gas, hydrogen class 4 specified in JIS K 0512, and as the supporting gas refined air are used. As the flow rate, the fuel gas is set at 2 ml/min to 10 ml/min and the supporting gas is at 100 ml/min to 180 ml/min.
 - 7.7) **Column chamber** It is kept at 50 °C to 60 °C for 2 min, and temperature is raised at 2 °C/min to 20 °C/min within the range of 50 (or 60) °C to 260 °C⁽¹⁷⁾.
 - 7.8) **Detector chamber temperature** 250 °C to 280 °C
- 8) **Shaker**
- 9) **Concentrator** As described in 6.1 b) 5).

Notes ⁽¹⁵⁾ A high sensitive nitrogen phosphorus detector (NPD) capable of detecting in high sensitivity nitrogen and phosphorus may be used.

⁽¹⁶⁾ In the case where a packed capillary column of ≥ 0.53 mm inner diameter is used, it is not necessary to use addition gas.

⁽¹⁷⁾ The raised temperature is the column chamber temperature in the case of using hexane as final solvent. The temperature regulation of the column chamber in the case of using acetone as the final solvent is as follows.

In the case of using acetone as the final solvent Keep at 40 °C to 50 °C for 2 min, and a temperature is raised at 2 °C/min to 20 °C/min within the range of 40 (or 50) °C to 280 °C.

Remarks 4 As the sensitivity of a gas chromatograph, adjust the sensitivity of the detector so that the amount (0.05 ng to 1 ng) of target pesticides can be recognized by injecting 1 ml of mixed standard solution (0.05 µg/ml to 1 µg/ml) [1 ml of mixed standard solution of a) 8) (0.5 µg/ml to 10 µg/ml) diluted to 10 ml.].

- 5 In the case where the operation of **6.3** is carried out successively after the preparatory operation of **c)**, a packed column can be used as the separator column of the gas chromatograph. Conditions for the gas chromatograph in this case shall be as follows.

Gas chromatograph Satisfying the following conditions.

- 1) **Tube for column** A glass tube of 2 mm to 3 mm inner diameter and 1 000 mm to 1 500 mm length.
- 2) **Column packing** Wash fire brick^{(*)1} (150 µm to 250 µm particle size) by hydrochloric acid (6 mol/L) by heating and reflux for about 2 h. After washing by water until washings become neutral, dry. Then, after treating by methylsilazane and impregnate it with silicone based stationary phase liquid of about 5 %.
- 3) **Detector** Flame thermionic detector^{(*)2}.
- 4) **Carrier gas** Use helium ($\geq 99.999\ 9$ vol%), and use by adjusting a flow rate at 40 ml/min to 50 ml/min.
- 5) **Fuel gas and supporting gas** As described in **b) 7.6)**.
- 6) **Sample vaporization room temperature** 250 °C to 300 °C
- 7) **Column chamber temperature^{(*)3}** 180 °C to 220 °C
- 8) **Detector chamber temperature** 250 °C to 280 °C

Notes ^{(*)1} Brick of 1 100 °C in refractoriness having diatomaceous earth as main component.

^{(*)2} As described in Note ⁽¹⁵⁾.

^{(*)3} Since column chamber temperature sometimes changes according to the pesticides, the column chamber temperature shall be set according to the target pesticides.

Informative reference 2 For column packing on the market, there are, as fire brick, Chromosorb W, Chromosorb G or that having the same performance equivalent in quality thereto as a carrier, which is impregnated with DC-200, OV-1, OV-17 and the like as silicone based stationary phase liquid.

- c) **Preparatory operation** The preparatory operation shall be carried out as follows.

- 1) Carry out the operations of **6.1** or **6.2**.

- d) **Operation** Carry out the operation as follows.

- 1) Sample 1 µl of mixed standard solution (0.5 µg/ml to 10 µg/ml) of **a) 8)** by a microsyringe⁽⁹⁾, inject it into gas chromatograph by splitless injection method or cold on-column injection method, and confirm the position of a peak corresponding to retention time particular to the target pesticides.
- 2) Sample 1 µl of the concentration liquid obtained in **c)** by a microsyringe⁽⁹⁾, carry out the operation of **1)**, record a gas chromatogram, confirm that the retention time conforms to the retention time of **1)**, and read the indication value⁽¹¹⁾ on the peak of the position corresponding to the retention time.

- 3) As a blank test, carry out the operations of 1) and 2) for the concentration liquid of the blank test obtained in c), detect a peak at the position corresponding to the retention time of the target pesticides, and when the indication value⁽¹¹⁾ is $\geq 1/3$ of the indication value of the determination lower limit value, carry out the operation again from the preparatory operation.
- 4) Obtain the amount of target pesticides from the working curve, and calculate the concentration ($\mu\text{g/L}$) of target pesticides in the sample by the formula of 7.1 d) 4).

Working curve Sample stepwise 0.1 ml to 2 ml of mixed standard solution (5 $\mu\text{g/ml}$ to 100 $\mu\text{g/ml}$) of a) 7) into a 10-ml volumetric flask, and add hexane⁽⁵⁾ to the mark. Take a specific amount [the same amount as that of the sample (for example, 1 μl)] of each solution by a microsyringe, carry out the operations of 1) and 2), and prepare the relation curve between the amount (ng) of target pesticides and the indication value⁽¹¹⁾. Carry out preparation of the working curve at measurement of the sample.

Remarks 6 As described in Remarks 2.

7.2.2 Gas chromatography using flame photometric detector (FPD) After concentrating pesticides in a sample by applying 6.1 or 6.2 to the pretreatment, determine its specific amount by a method using a flame photometric detector. Target pesticides, its determination range and repeatability (by coefficient of variation) are as given in Table 6. (Each of them varies according to the apparatus and the measuring conditions.)

Table 6 Target pesticides and determination range and repeatability (FPD)

Number	Target pesticides	Determination range (ng)		Repeatability (%)	
		Solvent extraction method	Stationary phase extraction method	Solvent extraction method	Stationary phase extraction method
1	EPN ($\text{C}_{14}\text{H}_{14}\text{NO}_4\text{PS}$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
2	isoxathion ($\text{C}_{13}\text{H}_{16}\text{NO}_4\text{PS}$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
3	isofenphos ($\text{C}_{15}\text{H}_{24}\text{NO}_4\text{PS}$)	0.05 to 1	0.05 to 1	10 to 20	10 to 20
6	iprobenfos [IBP] ($\text{C}_{13}\text{H}_{21}\text{O}_3\text{PS}$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
13	chlorpyrifos ($\text{C}_9\text{H}_{11}\text{Cl}_3\text{NO}_3\text{PS}$)	0.05 to 1	0.05 to 1	10 to 20	10 to 20
17	dichlorvos [DDVP] ($\text{C}_4\text{H}_7\text{Cl}_2\text{O}_4\text{P}$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
21	diazinon ($\text{C}_{12}\text{H}_{21}\text{N}_2\text{O}_3\text{PS}$)	0.05 to 1	0.05 to 1	10 to 20	10 to 20
25	trichlorfon [DEP] ($\text{C}_4\text{H}_8\text{Cl}_3\text{O}_4\text{P}$)	1.0 to 20	1.0 to 20	10 to 20	10 to 20
27	tolclophos-methyl ($\text{C}_9\text{H}_{11}\text{Cl}_2\text{O}_3\text{PS}$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
29	pyridaphenthion ($\text{C}_{14}\text{H}_{17}\text{N}_2\text{O}_4\text{PS}$)	0.2 to 4	0.2 to 4	10 to 20	10 to 20
31	fenitrothion [MEP] ($\text{C}_9\text{H}_{12}\text{NO}_5\text{PS}$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
34	butamifos ($\text{C}_{13}\text{H}_{21}\text{N}_2\text{O}_4\text{PS}$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20

a) **Reagents** The following reagents shall be used.

- 1) **Sodium chloride** As described in 6.1 a) 1).
- 2) **Sodium sulfate** As described in 6.1 a) 2).
- 3) **Acetone** As described in 4.1 a) 3).
- 4) **Dichloromethane** As described in 6.1 a) 3).
- 5) **Hexane** As described in 6.1 a) 4).
- 6) **Target pesticide standard solution (0.5 mg/ml to 2 mg/ml)** The sampling amount of the standard product used for preparation of the standard solution of target pesticide and the solvent used for dissolution are given in Table 7⁽¹⁸⁾. Preparation of those target pesticides standard solution shall be as follows.

Dissolve a specific amount [the sampling amount shall be shown in concentration (A) in Table 7.] of target pesticide standard product per each target pesticide in a small amount of acetone or hexane, transfer respectively into a 100-ml volumetric flask, and add acetone or hexane to the mark⁽⁴⁾.

- 7) **Mixed standard solution (5 µg/ml to 100 µg/ml)** For target pesticide standard solution (1 mg/ml to 2 mg/ml) prepared individually in 6), sample a specific amount (the sampling amount shall be shown in the mix standard solution in Table 7.) of each into a 100-ml volumetric flask, and add hexane to the mark⁽⁵⁾. When reserved, place it in a dark place at -20 °C.
- 8) **Mixed standard solution (0.5 µg/ml to 10 µg/ml)** Sample 1 ml of mixed standard solution (5 µg/ml to 100 µg/ml) prepared in 7) into the 10-ml volumetric flask, and add hexane to the mark⁽⁵⁾. Prepare in use.
- 9) **Nitrogen** As described in 6.2 a) 2).

Note ⁽¹⁸⁾ Isoxathion and dichlorvos are as described in Note ⁽³⁾.

Table 7 Target pesticide standard solution and mixed standard solution (FPD)

Number	Target pesticides	Concentration (A) 0.5 mg/ml to 2 mg/ml		Mixed standard solution	
		Solvent	Sampling amount of standard material (g/100 ml)	Sampling amount (ml/100 ml)	Concentration (µg/ml)
1	EPN (C ₁₄ H ₁₄ NO ₄ PS)	hexane	0.100	1	10
2	isoxathion (C ₁₃ H ₁₆ NO ₄ PS)	Note ⁽¹⁸⁾	0.100	1	10
3	isofenphos (C ₁₅ H ₂₄ NO ₄ PS)	acetone	0.050	1	5
6	iprobefos [IBP] (C ₁₃ H ₂₁ O ₃ PS)	hexane	0.100	1	10
13	chlorpyrifos (C ₉ H ₁₁ Cl ₃ NO ₃ PS)	acetone	0.050	1	5
17	dichlorvos [DDVP] (C ₄ H ₇ Cl ₂ O ₄ P)	Note ⁽¹⁸⁾	0.100	1	10
21	diazinon (C ₁₂ H ₂₁ N ₂ O ₃ PS)	hexane	0.050	1	5
25	trichlorfon [DEP] (C ₄ H ₈ Cl ₃ O ₄ P)	acetone	0.200	5	100
27	tolclophos-methyl (C ₉ H ₁₁ Cl ₂ O ₃ PS)	acetone	0.100	1	10
29	pyridaphenthion (C ₁₄ H ₁₇ N ₂ O ₄ PS)	acetone	0.200	1	20
31	fenitrothion [MEP] (C ₉ H ₁₂ NO ₅ PS)	hexane	0.100	1	10
34	butamifos (C ₁₃ H ₂₁ N ₂ O ₄ PS)	acetone	0.100	1	10

b) **Implements and apparatus** Implements and apparatus shall be as follows.

- 1) **Separatory funnel** As described in 6.1 b) 1).
- 2) **Egg-apple type flask** As described in 6.1 b) 2).
- 3) **Erlenmeyer flask** As described in 6.1 b) 3).
- 4) **Test tube with a ground stopper** As described in 6.2 b) 1).
- 5) **Solid phase column** As described in 6.2 b) 2).
- 6) **Microsyringe** As described in 7.1 b) 6).
- 7) **Gas chromatograph** That satisfying the following conditions.
 - 7.1) **Tube for capillary column** As described in 7.1 b) 7.1).
 - 7.2) **Capillary column** As described in 7.1 b) 7.2).
 - 7.3) **Detector** Flame photometric detector. As interference filter a phosphorus filter is used, and measuring wavelength is 526 nm.
 - 7.4) **Carrier gas** As described in 7.2.1 b) 7.4).
 - 7.5) **Sample introducing method and sample introducing part temperature** As described in 7.1 b) 7.5).

- 7.6) **Fuel gas and supporting gas** As described in 7.2.1 b) 7.6). However, the fuel gas is set at a flow rate of 50 ml/min to 100 ml/min, and the supporting gas is set at 80 ml/min to 150 ml/min.
- 7.7) **Column chamber temperature** As described in 7.2.1 b) 7.7).
- 7.8) **Detector chamber temperature** As described in 7.2.1 b) 7.8).
- 8) **Shaker**
- 9) **Concentrator** As described in 6.1 b) 5).

Remarks 7 As described in Remarks 4.

- 8 When column chromatograph separation is carried out successively from the preparatory operation of c), a packed column may be used as the separation column of gas chromatograph. Though conditions of the gas chromatograph in this case are as described in Remarks 5, conditions for detector, fuel gas and supporting gas are as follows.

- 1) **Detector** As described in b) 7.3).
- 2) **Fuel gas and supporting gas** As described in b) 7.6).

- c) **Preparatory operation** The preparatory operation shall be carried out as follows.

- 1) Carry out the operation of 6.1 or 6.2.

- d) **Operation** Carry out the operation as follows.

- 1) Sample 1 µl of mixed standard solution (0.5 µg/ml to 10 µg/ml) of a) 8) by microsyringe⁽⁹⁾, inject into gas chromatograph by a splitless injection method or a cold on-column injection method, and confirm preliminarily the position of a peak corresponding to the retention time particular to the target pesticides.
- 2) Sample 1 µl of concentration liquid obtained in c) by a microsyringe⁽⁹⁾, and carry out the operation of 7.2.1 d) 2).
- 3) As a blank test, carry out the operation of 7.2.1 d) 3) for the concentration liquid of the blank test obtained in c).
- 4) Obtain the amount of target pesticides from the working curve, and calculate the concentration (mg/L) of the target pesticides in the sample according to the formula of 7.1 d) 4).

Working curve Take stepwise 0.1 ml to 2 ml of mixed standard solution (5 µg/ml to 100 µg/ml) of a) 7) into a volumetric flask of 10 ml, and add hexane⁽⁵⁾ to the mark. Take a specific amount [the same amount as that of the sample (for example, 1 µl).] of each solution by a microsyringe, and prepare the relation curve between the amount (ng) of target pesticides and the indication value⁽¹¹⁾ by carrying out the operations of 1) and 2). Carry out the preparation of the working curve in measuring the sample.

Remarks 9 As described in Remarks 2.

7.2.3 Gas chromatography using electron capture detector (ECD) After concentrating pesticides in a sample by applying 6.1 or 6.2 to the pretreatment, determine its specific amount by a method using an electron capture detector. Target pesticides, determination range and precision of analysis in the partial different conditions (by coefficient of variation) are as given in Table 8.

Table 8 Target pesticides, determination range and repeatability (ECD)

Number	Target pesticides	Determination range (ng)		Repeatability (%)	
		Solvent extraction method	Stationary phase extraction method	Solvent extraction method	Stationary phase extraction method
1	EPN ($C_{14}H_{14}NO_4PS$)	0.02 to 0.4	0.02 to 0.4	10 to 20	10 to 20
2	isoxathion ($C_{13}H_{16}NO_4PS$)	0.05 to 1	0.05 to 1	10 to 20	10 to 20
4	isoprothiolane ($C_{12}H_{18}O_4S_2$)	0.02 to 0.4	0.02 to 0.4	10 to 20	10 to 20
9	etridiazole ($C_5H_5Cl_3N_2OS$)	0.02 to 0.4	0.02 to 0.4	20 to 30	20 to 30
11	captan ($C_9H_8Cl_3NO_2S$)	0.05 to 1	0.05 to 1	10 to 20	10 to 20
12	chlornitrofen [CNP] ($C_{12}H_6Cl_3NO_3$)	0.02 to 0.4	0.02 to 0.4	10 to 20	10 to 20
14	chlorothalonil [TPN] ($C_8Cl_4N_2$)	0.02 to 0.4	0.02 to 0.4	20 to 30	20 to 30
15	chloroneb ($C_8H_8Cl_2O_2$)	0.1 to 2	0.05 to 1	10 to 20	10 to 20
17	dichlorvos [DDVP] ($C_4H_7Cl_2O_4P$)	0.02 to 0.4	0.02 to 0.4	10 to 20	10 to 20
21	diazinon ($C_{12}H_{21}N_2O_3PS$)	0.02 to 0.4	0.02 to 0.4	10 to 20	10 to 20
22	thiobencarb [benthiocarb] ($C_{12}H_{16}ClNOS$)	0.1 to 2	0.05 to 1	10 to 20	10 to 20
31	fenitrothion [MEP] ($C_9H_{12}NO_5PS$)	0.02 to 0.4	0.02 to 0.4	10 to 20	10 to 20
38	propyzamide ($C_{12}H_{11}Cl_2NO$)	0.1 to 2	0.1 to 2	10 to 20	10 to 20
44	benfluralin ($C_{13}H_{16}F_3N_3O_4$)	0.02 to 0.4	0.02 to 0.4	10 to 20	10 to 20

a) **Reagents** The following reagents shall be used.

- 1) **Sodium chloride** As described in 6.1 a) 1).
- 2) **Sodium sulfate** As described in 6.1 a) 2).
- 3) **Acetone** As described in 4.1 a) 3).
- 4) **Dichloromethane** As described in 6.1 a) 3).
- 5) **Hexane** As described in 6.1 a) 4).
- 6) **Target pesticide standard solution (0.2 mg/ml to 1 mg/ml)** The sampling amount of standard product used for preparing the standard solution of target pesticide and the solvent used for dissolution are given in Table 9⁽¹⁹⁾. The preparation of those target pesticides are as follows.

Dissolve a specific amount [the sampling amount is shown in concentration (A) in Table 9.] of target pesticide standard product in a small amount of acetone or hexane per each target pesticide, respectively transfer into a 100-ml volumetric flask, and add acetone or hexane to the mark⁽⁴⁾.

- 7) **Mixed standard solution (2 µg/ml to 10 µg/ml)** Sample a specific amount (the sampling amount is shown in the mixed standard solution in Table 9.) of each of the standard solution (0.2 mg/ml to 1 mg/ml) of the target pesticides prepared individually in 6) into a 100-ml volumetric flask, and add hexane to the mark⁽⁵⁾. When reserved, place it in a dark place at -20 °C.
- 8) **Mixed standard solution (0.2 µg/ml to 1 µg/ml)** Sample 1 ml of mixed standard solution (2 µg/ml to 10 µg/ml) prepared in 7) into the 10-ml volumetric flask, and add hexane to the mark⁽⁵⁾. Prepare in use.
- 9) **Nitrogen** As described in 6.2 a) 2).

Note ⁽¹⁹⁾ Isoxathion, chlornitrofen, chlorothalonil, dichlorvos and propyzamide are as described in Note ⁽³⁾.

Table 9 Target pesticide standard solution and mixed standard solution (ECD)

Number	Target pesticides	Concentration (A) 0.2 mg/ml to 1 mg/ml		Mixed standard solution	
		Solvent	Sampling amount of standard material (g/100 ml)	Sampling amount (ml/100 ml)	Concentration (µg/ml)
1	EPN (C ₁₄ H ₁₄ NO ₄ PS)	hexane	0.020	1	2
2	isoxathion (C ₁₃ H ₁₆ NO ₄ PS)	Note ⁽¹⁹⁾	0.050	1	5
4	isoprothiolane (C ₁₂ H ₁₈ O ₄ S ₂)	hexane	0.020	1	2
9	etridiazole (C ₅ H ₅ Cl ₃ N ₂ OS)	acetone	0.020	1	2
11	captan (C ₉ H ₈ Cl ₃ NO ₂ S)	acetone	0.050	1	5
12	chlornitrofen [CNP] (C ₁₂ H ₆ Cl ₃ NO ₃)	Note ⁽¹⁹⁾	0.020	1	2
14	chlorothalonil [TPN] (C ₈ Cl ₄ N ₂)	Note ⁽¹⁹⁾	0.020	1	2
15	chloroneb (C ₈ H ₈ Cl ₂ O ₂)	acetone	0.100	1	10
17	dichlorvos [DDVP] (C ₄ H ₇ Cl ₂ O ₄ P)	Note ⁽¹⁹⁾	0.020	1	2
21	diazinon (C ₁₂ H ₂₁ N ₂ O ₃ PS)	hexane	0.020	1	2
22	thiobencarb [benthiocarb] (C ₁₂ H ₁₆ ClNOS)	acetone	0.100	1	10
31	fenitrothion [MEP] (C ₉ H ₁₂ NO ₅ PS)	hexane	0.020	1	2
38	propyzamide (C ₁₂ H ₁₁ Cl ₂ NO)	Note ⁽¹⁹⁾	0.100	1	10
44	benfluralin (C ₁₃ H ₁₆ F ₃ N ₃ O ₄)	acetone	0.020	1	2

b) **Implements and apparatus** Implements and apparatus shall be as follows.

- 1) **Separatory funnel** As described in 6.1 b) 1).
- 2) **Egg-apple type flask** As described in 6.1 b) 2).
- 3) **Erlenmeyer flask** As described in 6.1 b) 3).
- 4) **Test tube with a ground stopper** As described in 6.2 b) 1).

- 5) **Solid phase column** As described in 6.2 b) 2).
- 6) **Microsyringe** As described in 7.1 b) 6).
- 7) **Gas chromatograph** That satisfying the following conditions.
 - 7.1) **Tube for capillary column** As described in 7.1 b) 7.1).
 - 7.2) **Capillary column** As described in 7.1 b) 7.2).
 - 7.3) **Detector** An electron capture detector.
 - 7.4) **Carrier gas** As described in 7.2.1 b) 7.4). However, connect highly pure nitrogen grade 1 specified in **JIS K 1107** as addition gas⁽²⁰⁾ to the column outlet, and adjust at a flow rate of 30 ml/min to 60 ml/min.
 - 7.5) **Sample introducing method and sample introducing part temperature** As described in 7.1 b) 7.5).
 - 7.6) **Column chamber temperature** As described in 7.2.1 b) 7.7).
 - 7.7) **Detector chamber temperature** As described in 7.2.1 b) 7.8).
- 8) **Shaker**
- 9) **Concentrator** As described in 6.1 b) 5).

Note ⁽²⁰⁾ As described in Note ⁽¹⁶⁾.

Further, as a load gas, argon mixed gas containing methane of 5 vol% may be used.

Remarks 10 As the sensitivity of gas chromatograph, inject 1 µl of mixed standard solution (0.02 µg/ml to 0.1 µg/ml) [prepared from 1 ml of mixed standard solution (0.2 µg/ml to 1 µg/ml) of a) 8) diluted to 10 ml] into a gas chromatograph, and adjust the sensitivity of the detector so that the amount (0.02 ng to 0.1 ng) of the target pesticide can be confirmed.

11 When the column chromatograph separation of 6.3 is carried out successively from the preparatory operation of c), as the separation column of gas chromatograph, a packed column can be used. Though conditions of gas chromatograph in this case are as described in Remarks 5, conditions for the detector and the carrier gas shall be as follows.

- 1) **Detector** As described in b) 7.3).
- 2) **Carrier gas** As described in b) 7.4).
- 3) **Sample vaporization room temperature** 250 °C to 300 °C
- 4) **Column chamber temperature** 200 °C to 240 °C
- 5) **Detector chamber temperature** 250 °C to 300 °C

c) **Preparatory operation** The preparatory operation shall be carried out as follows.

- 1) Carry out the operation of 6.1 or 6.2.

d) **Operation** Carry out the operation as follows.

- 1) Sample 1 µl of mixed standard solution (0.2 µg/ml to 1 µg/ml) of **a) 8)** by a microsyringe⁽⁹⁾, inject into a gas chromatograph by a splitless injection method or a cold on-column injection method, and preliminarily confirm the position of a peak corresponding to retention time particular to the target pesticide.
- 2) Sample 1 µl of the concentration liquid obtained in **c)** by a microsyringe⁽⁹⁾, and carry out the operation of **7.2.1 d) 2)**.
- 3) As a blank test, carry out the operation of **7.2.1 d) 3)** for the concentrated solution of the blank test obtained in **c)**.
- 4) Obtain the amount of the target pesticide from the working curve, and calculate the concentration (µg/L) of the target pesticide in the sample according to the formula of **7.1 d) 4)**.

Working curve Take stepwise 0.1 ml to 2 ml of mixed standard solution (2 µg/ml to 10 µg/ml) of **a) 7)** into a 10-ml volumetric flask, and add hexane⁽⁵⁾ to the mark. Take a specific amount [the same amount as that of the sample (for example, 1 µl).] of each solution by a microsyringe, and prepare the relation curve between the amount (ng) of target pesticide and the indication value⁽¹¹⁾ by carrying out the operations of **1)** and **2)**. Carry out preparation of the working curve in measuring the sample.

Remarks 12 As described in Remarks 2.

7.3 High performance liquid chromatography Adjust pH to 3.5 by adding hydrochloric acid to a sample, pour it into a solid phase column wherein styrene vinyl benzene copolymer is packed, develop by acetonitrile, and add 2, 2'-oxybisethanol (diethylene glycol) to the eluate. After concentration, add acetonitrile–water mixed solution (7+13) to make a specific amount. Inject a specific amount thereof into high performance liquid chromatograph (HPLC), and determine by measuring an absorbance by using an absorption spectrophotometric detector. Target pesticides, their determination range, and precision of analysis in the partial different conditions (by coefficient of variation) are as given in Table 10 (Each of them varies according to the apparatus and measuring conditions.).

Table 10 Target pesticides, determination range and repeatability (HPLC)

Number	Target pesticides	Determination range (ng)	Repeatability (%)
1	asulam ($C_8H_{10}N_2O_4S$)	4 to 80	10 to 20
2	oxine-copper ($C_{18}H_{12}CuN_2O_2$)	20 to 200	10 to 20
3	thiram ($C_6H_{12}N_2S_4$)	2 to 40	10 to 20
4	mecoprop [MCP] ($C_{10}H_{11}ClO_3$)	20 to 200	10 to 20
5	triclopyr (free) ($C_7H_4Cl_3NO_3$) ⁽²¹⁾	5 to 100	10 to 20

Note ⁽²¹⁾ Measure for determining triclopyr of **7.1.1.**

a) **Reagents** The following reagents shall be used.

- 1) **Hydrochloric acid (2 mol/L)** Prepare from hydrochloric acid specified in **JIS K 8180**.
- 2) **Acetonitrile** That of ≥ 300 concentration quality specified in **JIS K 8039**⁽²²⁾.
- 3) **Acetone** As described in 4.1 a) 3).
- 4) **Chloroform** That of ≥ 300 concentration quality specified in **JIS K 8322**⁽²²⁾.
- 5) **2, 2'-Oxybisethanol (diethylene glycol)**⁽²²⁾ ⁽²³⁾
- 6) **Hexane** As described in 6.1 a) 4).
- 7) **2, 2'-Oxybisethanol-acetone solution (1+49)** Prepare from 2, 2'-oxybisethanol in 5) and acetone in 3).
- 8) **Acetonitrile-water mixed solution (7+13)** Prepare from acetonitrile in 2) and water.
- 9) **Target pesticide standard solution (0.2 mg/ml to 1 mg/ml)** A sampling amount of standard product used for preparation of the standard solution of target pesticide and a solvent used for dissolution are shown in Table 11. Preparation of those target pesticide standard solutions shall be as follows.

Dissolve a specific amount [the sampling amount is shown in concentration (A) in Table 11.] of target pesticide standard product in a small amount of acetonitrile or chloroform per each target pesticide, transfer respectively into a 100-ml volumetric flask, and add respectively acetonitrile or chloroform to the mark⁽⁴⁾.

- 10) **Mixed standard solution (5 µg/ml to 50 µg/ml)** Sample a specific amount (the sampling amount is shown in the mixed standard solution in Table 11.) of each into a 100-ml volumetric flask for target pesticide standard solution (0.2 mg/ml to 1 mg/ml) prepared individually in 9), and add acetonitrile to the mark. When reserved, place it in a dark place at -20°C .
- 11) **Mixed standard solution (0.5 µg/ml to 5 µg/ml)** Sample 1 ml of mixed standard solution (5 µg/ml to 50 µg/ml) of 10) into the 10-ml volumetric flask, and add acetonitrile to the mark. Prepare in use.

Notes ⁽²²⁾ As described in Note ⁽¹⁾ in 4.

- ⁽²³⁾ Concentrate 300 ml of it to about 1 ml. A peak shall not generated in the retention time corresponding to target pesticide in d) for its 40 µl.

Table 11 Target pesticide standard solution and mixed standard solution (HPLC)

Number	Target pesticides	Concentration (A) 0.2 mg/ml to 1 mg/ml		Mixed standard solution	
		Solvent	Sampling amount of standard material (g/100 ml)	Sampling amount (ml/100 ml)	Concentration (µg/ml)
1	asulam ($C_8H_{10}N_2O_4S$)	acetonitrile	0.100	1	10
2	oxine-copper ($C_{18}H_{12}CuN_2O_2$)	chloroform	0.020	25	50
3	thiram ($C_6H_{12}N_2S_4$)	chloroform	0.100	0.5	5
4	mecoprop [MCPP] ($C_{10}H_{11}ClO_3$)	acetonitrile	0.100	5	50
5	triclopyr (free) ($C_7H_4Cl_3NO_3$)	acetonitrile	0.050	2.5	12.5

b) **Implements and apparatus** Implements and apparatus shall be as follows.

- 1) **Egg-apple type flask** It is interchangeable ground joint and can be connected to the concentrator.
- 2) **Erlenmeyer flask** As described in 6.1 b) 3).
- 3) **Test tube with a ground stopper** As described in 6.2 b) 1).
- 4) **Solid phase column** As described in 6.2 b) 2).
- 5) **Microsyringe** Suitable one of $\leq 100 \mu\text{l}$. Or an automatic injector may be used.
- 6) **High performance liquid chromatography** That satisfying the following conditions.
 - 6.1) **Separation tube** Stainless steel-made one of 2 mm to 6 mm inner diameter and 15 cm to 30 cm length.
 - 6.2) **Column packing** That in which octadecyl group (ODS) is chemically bonded to polyurethane based neutral pole gel or silica gel or that having separation performance equivalent thereto.
 - 6.3) **Eluent** Preparation of eluent shall be as follows.
 - 6.3.1) **Phosphate buffer solution (pH3.3)** After adding about 950 ml of water to 10 ml of phosphoric acid (1 mol/L) (dissolve 69 ml of phosphoric acid specified in **JIS K 9005** in water to make 1 L.), adjust pH to 3.3 by adding sodium hydroxide solution (10 mol/L) (prepared using sodium hydroxide specified in **JIS K 8576**.), and add water to make 1 L.
 - 6.3.2) **Eluent** Acetonitrile of a) 2) is mixed with phosphate buffer solution (pH3.3) by 7 : 13 volume ratio.
 - 6.4) **Flow rate** About 1 ml/min
 - 6.5) **Detector** An absorption spectrophotometric detector capable of measuring 230 nm to 280 nm. Measuring wavelength of each pesticide is as follows.

279 nm for asulam, 240 nm for oxine-copper and 230 nm for thiram, mecoprop and triclopyr.

6.6) **Column chamber temperature** 40 °C to 45 °C

7) **Shaker**

8) **Concentrator** As described in 6.1 b) 5).

Remarks 13 For the sensitivity of high performance liquid chromatograph, inject 40 µl of mixed standard solution (0.05 µg/ml to 0.5 µg/ml) [1 ml of mixed standard solution (0.5 µg/ml to 5 µg/ml) of a) 11) diluted to 10 ml.] into the high performance liquid chromatograph, and adjust the sensitivity of a detector so that the amount (2 ng to 20 ng) of target pesticide can be confirmed.

14 As described in Remarks 1 of 6.

c) **Preparatory operation** The preparatory operation is carried out as follows.

- 1) Sample 250 ml of the sample⁽²⁴⁾ sampled in 4.1 c) into an Erlenmeyer flask, and adjust pH to 3.5 by adding hydrochloric acid (2 mol/L).
- 2) Pass this solution through a solid phase column at a flow rate of 10 ml/min to 20 ml/min⁽²⁷⁾ by a pressure method⁽²⁵⁾ or a reduced pressure method⁽²⁶⁾.
- 3) Allow 10 ml⁽²⁸⁾ of water to flow through the solid phase column. After washing the column, continue suction for about 10 min, and remove water content by separation⁽²⁹⁾.
- 4) Gently pass⁽³⁰⁾ about 5 ml of acetonitrile⁽²⁸⁾ from the upper end of the solid phase column, and elute the target pesticide. Receive the eluate by an egg-apple type flask.
- 5) Add 0.5 ml of 2, 2'-oxybisethanol-acetone solution (1+49) to the egg-apple type flask.
- 6) Use a concentrator⁽³¹⁾ and remove the solvent by distillation at ≤ 40 °C.
- 7) Dissolve the residue by dropping acetonitrile-water mixed solution (7+13), and obtain a specific amount of 2 ml in the acetonitrile-water mixed solution (7+13)⁽³²⁾.
- 8) As a blank test, carry out the operations of 2) to 7) for 250 ml of water.

Notes ⁽²⁴⁾ As described in Note ⁽¹¹⁾ of 6.

⁽²⁵⁾ As described in Note ⁽¹⁴⁾ of 6.

⁽²⁶⁾ As described in Note ⁽¹⁵⁾ of 6.

⁽²⁷⁾ As described in Note ⁽¹⁶⁾ of 6.

⁽²⁸⁾ As described in Note ⁽¹⁷⁾ of 6.

⁽²⁹⁾ As described in Note ⁽¹⁸⁾ of 6.

⁽³⁰⁾ As described in Note ⁽¹⁹⁾ of 6.

⁽³¹⁾ As described in Note ⁽⁹⁾ of 6.

⁽³²⁾ As described in Note ⁽¹⁰⁾ of 6.

d) **Operation** The operation is carried out as follows.

- 1) Sample 40 µl of mixed standard solution (0.5 µg/ml to 5 µg/ml) of **a(9), inject into a high performance liquid chromatograph and preliminarily confirm the position of a peak corresponding to the retention time of target pesticide.**
- 2) Sample 40 µl of acetonitrile–water mixed solution (7+13) obtained in **c**) 7) by a microsyringe⁽⁹⁾, record a chromatogram by carrying out the operation of 1), confirm conformance with the retention time of 1), and read the indication value⁽¹¹⁾ on the position of a peak corresponding to the retention time.
- 3) As a blank test, carry out the operation of 2) on the acetonitrile–water mixed solution (7+13) obtained in **c**) 8), and when a peak is detected at the position corresponding to the retention time of the target pesticide and the indication value⁽¹¹⁾ is $\geq 1/3$ of the indication value of the determination lower limit value, carry out the operation again from the preparatory operation.
- 4) Obtain the amount of target pesticide from the working curve, and calculate the concentration (µg/L) of target pesticide in the sample from the following formula.

$$N = a \times 10^{-3} \times \frac{v_1}{C \times 10^{-3}} \times \frac{1\,000}{V}$$

where, N : concentration of target pesticide (µg/L)
 a : amount of target pesticide obtained from working curve (ng)
 C : injection amount (µl)
 V : sample (ml)
 v_1 : amount of acetonitrile–water mixed solution of **c**) 6) (7+13) (ml)
 10^{-3} : coefficient for converting ng to µg and µl to ml

Working curve Take stepwise 0.1 ml to 2 ml of mixed standard solution (5 µg/ml to 50 µg/ml) of **a**) 10) into a 10-ml volumetric flask, and add acetonitrile–water mixed solution (7+13) to the mark. Take a specific amount [the same amount as that of a sample (for example, 40 µl).] of each solution by a microsyringe, carry out the operations of 1) and 2), and prepare the relation curve between the amount (ng) of target pesticide and the indication value⁽¹¹⁾. Carry out the preparation of working curve in measuring the sample.

Remarks 15 As described in Remarks 2. When the judgement is difficult even by gas chromatography-mass spectrometry, it is preferable to use high performance liquid chromatography-mass spectrometry.

8 1, 3-Dichloropropene (C₃H₄Cl₂) As described in **JIS K 0125**.

9 EPN ($C_{14}H_{14}NO_4PS$) Apply gas chromatography-mass spectrometry and gas chromatography to determination of EPN [phenylphosphonothioic acid *O*-ethyl *O*-(4-nitrophenyl) ester].

9.1 Gas chromatography-mass spectrometry Carry out extraction operation of a sample by 6.1 or 6.2, successively carry out the operation of 6.3 and concentrate. Inject its specific amount into a gas chromatograph-mass spectrometer and determine by using selected ion monitoring method or mass chromatography as detection method for target pesticide.

Determination range: $C_{14}H_{14}NO_4PS$ 0.1 ng to 2 ng

Repeatability: 10 % to 20 % by coefficient of variation (varies according to the apparatus and measuring conditions)

a) **Reagents** The following reagents shall be used.

- 1) **Sodium chloride** As described in 6.1 a) 1).
- 2) **Sodium sulfate** As described in 6.1 a) 2).
- 3) **Acetone** As described in 4.1 a) 3).
- 4) **Dichloromethane** As described in 6.1 a) 3).
- 5) **Hexane** As described in 6.1 a) 4).
- 6) **Diethyl ether-hexane eluent (7+13)** As described in 6.3 a) 4).
- 7) **EPN standard solution (1 mg $C_{14}H_{14}NO_4PS$ /ml)** Take 0.100 g of standard product of EPN, dissolve in a small amount of hexane, transfer into a volumetric flask of 100 ml, and add hexane to the mark⁽¹⁾.
- 8) **EPN standard solution (10 $\mu gC_{14}H_{14}NO_4PS$ /ml)** Take 1 ml of EPN standard solution (1 mg $C_{14}H_{14}NO_4PS$ /ml) in a volumetric flask of 100 ml, and add hexane⁽²⁾ to the mark. When reserved, place it in a dark place at $-20\text{ }^{\circ}C$.
- 9) **EPN standard solution (1 $\mu gC_{14}H_{14}NO_4PS$ /ml)** Take 1 ml of EPN standard solution (10 $\mu gC_{14}H_{14}NO_4PS$ /ml) into a volumetric flask of 10 ml, and add hexane⁽²⁾ to the mark. Prepare in use.
- 10) **Nitrogen** As described in 6.2 a) 2).

Notes (1) As described in Note (4) of 7.

(2) For preparation of mixed standard solution in c), in the case of applying the procedure of 6.2 and omitting the operation of 6.3, use acetone instead of hexane.

b) **Implements and apparatus** Implements and apparatus shall be as follows.

- 1) **Separatory funnel** As described in 6.1 b) 1).
- 2) **Egg-apple type flask** As described in 6.1 b) 2).
- 3) **Erlenmeyer flask** As described in 6.1 b) 3).
- 4) **Test tube with a ground stopper** As described in 6.2 b) 1).
- 5) **Solid phase column** As described in 6.2 b) 2).