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Method for determination of tetra-through
octachlorodibenzo-*p*-dioxins, tetra-through
octachlorodibenzofurans and dioxin-like
polychlorinatedbiphenyls in industrial
water and waste water

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Foreword

This Japanese Industrial Standard has been revised by the Minister of Economy, Trade and Industry, through deliberations at the Japanese Industrial Standards Committee in accordance with the Industrial Standardization Act. This edition replaces the previous edition (**JIS K 0312**:2008), which has been technically revised.

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Method for determination of tetra-through octachlorodibenzo-*p*-dioxins, tetra-through octachlorodibenzofurans and dioxin-like polychlorinated biphenyls in industrial water and waste water

Introduction

This Japanese Industrial Standard was established in 1999 as “Method for determination of tetra-through octachlorodibenzo-*p*-dioxins, tetra-through octachlorodibenzofurans and coplanar polychlorobiphenyls in industrial water and waste water”, and has gone through three revisions including this one. This Standard has been prepared based on **ISO 18073**:2004, Edition 1, and **ISO 17858**:2007, Edition 1, with some modifications of the technical contents.

The vertical lines on both sides and dotted underlines indicate changes from the corresponding International Standards. A list of modifications with the explanations is given in Annex JE. Annex JA to Annex JD are unique to **JIS** and not given in the corresponding International Standards.

1 Scope

This Standard specifies a method for the determination of tetra-through octachlorodibenzo-*p*-dioxins, tetra-through octachlorodibenzofurans and dioxin-like PCBs in industrial water and waste water using a gas chromatograph/mass spectrometer (hereafter referred to as GC-MS). The GC-MS employed in this Standard shall be the double-focusing mass spectrometer (hereafter referred to as MS) where the capillary column of a gas chromatograph (hereafter referred to as GC) is used and its resolution is 10 000 or more.

NOTE The International Standards corresponding to this Standard and the symbol of degree of correspondence are as follows.

ISO 18073:2004 *Water quality—Determination of tetra- to octa-chlorinated dioxins and furans—Method using isotope dilution HRGC/HRMS*

ISO 17858:2007 *Water quality—Determination of dioxin-like polychlorinated biphenyls—Method using gas chromatography/mass spectrometry (overall evaluation: MOD)*

In addition, symbols which denote the degree of correspondence in the contents between the relevant International Standards and **JIS** are IDT (identical), MOD (modified), and NEQ (not equivalent) according to **ISO/IEC Guide 21-1**.

WARNING Since dioxins are very toxic, inhalation, accidental ingestion, direct contact to skin, etc. shall be avoided as much as possible, and ventilation of pretreatment room and analysis room and control of waste liquid and waste shall be sufficiently carried out. Since the health of the measurer may be damaged by inhalation or accidental ingestion

of other drugs, solvents, etc., the handling shall be carried out as carefully as possible, and attention shall be paid to sufficient ventilation in the laboratory.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this Standard. The most recent editions of the standards (including amendments) indicated below shall be applied.

- JIS K 0094 *Sampling methods for industrial water and industrial wastewater*
- JIS K 0114 *General rules for gas chromatography*
- JIS K 0123 *General rules for gas chromatography/mass spectrometry*
- JIS K 0211 *Technical terms for analytical chemistry (General part)*
- JIS K 0215 *Technical terms for analytical chemistry (Analytical instrument part)*
- JIS K 0216 *Technical terms for analytical chemistry (Environmental part)*
- JIS K 0557 *Water used for industrial water and wastewater analysis*
- JIS K 1107 *Nitrogen*
- JIS K 8040 *Acetone for pesticide residue and polychlorinated biphenyl tests (Reagent)*
- JIS K 8117 *Dichloromethane for pesticide residue and polychlorinated biphenyl analysis (Reagent)*
- JIS K 8180 *Hydrochloric acid (Reagent)*
- JIS K 8550 *Silver nitrate (Reagent)*
- JIS K 8574 *Potassium hydroxide (Reagent)*
- JIS K 8637 *Sodium thiosulfate pentahydrate (Reagent)*
- JIS K 8680 *Toluene (Reagent)*
- JIS K 8825 *Hexane for pesticide residue and polychlorinated biphenyl tests (Reagent)*
- JIS K 8891 *Methanol (Reagent)*
- JIS K 8951 *Sulfuric acid (Reagent)*
- JIS K 8987 *Sodium sulfate (Reagent)*
- JIS K 9502 *L(+)-Ascorbic acid (Reagent)*
- JIS K 9702 *Dimethyl sulfoxide (Reagent)*
- JIS K 9703 *2,2,4-Trimethylpentane (Reagent)*
- JIS R 3503 *Glass apparatus for chemical analysis*
- JIS R 3505 *Volumetric glassware*
- JIS Z 8401 *Rounding of numbers*

3 Terms, definitions and abbreviations

3.1 Terms and definitions

For the purpose of this Standard, the terms and definitions given in **JIS K 0094**, **JIS K 0114**, **JIS K 0123**, **JIS K 0211**, **JIS K 0215** and **JIS K 0216**, and the following apply.

3.1.1

dioxins

generic term of tetra-through octachlorodibenzo-*p*-dioxins (hereafter referred to as PCDDs), tetra-through octachlorodibenzofurans (hereafter referred to as PCDFs) and dioxin-like PCBs (hereafter referred to as DL-PCBs)

NOTE This definition differs from that specified in **JIS K 0216:2014**.

3.1.2

isomer

each compound whose number of chlorines substituent is the same, but their positions are different

3.1.3

homologue

a group of compounds whose number of chlorines substituent is the same, but their positions are different

3.1.4

isomers substituted by chlorine at 2,3,7,8-positions

total 17 types of isomers substituted by chlorine at 2,3,7,8-positions, being composed of 7 types of PCDDs, and 10 types of PCDFs, as given in Table 1

Table 1 Isomers substituted by chlorine at 2,3,7,8-positions

	Isomers substituted by chlorine at 2,3,7,8-positions	Abbreviation
PCDDs	2,3,7,8-Tetrachlorodibenzo- <i>p</i> -dioxin	2,3,7,8-TeCDD
	1,2,3,7,8-Pentachlorodibenzo- <i>p</i> -dioxin	1,2,3,7,8-PeCDD
	1,2,3,4,7,8-Hexachlorodibenzo- <i>p</i> -dioxin	1,2,3,4,7,8-HxCDD
	1,2,3,6,7,8-Hexachlorodibenzo- <i>p</i> -dioxin	1,2,3,6,7,8-HxCDD
	1,2,3,7,8,9-Hexachlorodibenzo- <i>p</i> -dioxin	1,2,3,7,8,9-HxCDD
	1,2,3,4,6,7,8-Heptachlorodibenzo- <i>p</i> -dioxin	1,2,3,4,6,7,8-HpCDD
	1,2,3,4,6,7,8,9-Octachlorodibenzo- <i>p</i> -dioxin	1,2,3,4,6,7,8,9-OCDD
PCDFs	2,3,7,8-Tetrachlorodibenzofuran	2,3,7,8-TeCDF
	1,2,3,7,8-Pentachlorodibenzofuran	1,2,3,7,8-PeCDF
	2,3,4,7,8-Pentachlorodibenzofuran	2,3,4,7,8-PeCDF
	1,2,3,4,7,8-Hexachlorodibenzofuran	1,2,3,4,7,8-HxCDF
	1,2,3,6,7,8-Hexachlorodibenzofuran	1,2,3,6,7,8-HxCDF
	1,2,3,7,8,9-Hexachlorodibenzofuran	1,2,3,7,8,9-HxCDF
	2,3,4,6,7,8-Hexachlorodibenzofuran	2,3,4,6,7,8-HxCDF
	1,2,3,4,6,7,8-Heptachlorodibenzofuran	1,2,3,4,6,7,8-HpCDF
	1,2,3,4,7,8,9-Heptachlorodibenzofuran	1,2,3,4,7,8,9-HpCDF
	1,2,3,4,6,7,8,9-Octachlorodibenzofuran	1,2,3,4,6,7,8,9-OCDF

3.1.5

DL-PCBs

of PCBs, compounds where ortho positions (2,2',6 and 6') are not substituted by chlorine (hereafter referred to as non-ortho compounds) and compounds having one substituted chlorine at the ortho position (hereafter referred to as mono-ortho compounds), which are given in Table 2

Table 2 DL-PCBs

	DL-PCBs	Abbreviation
Non-ortho compounds	3,3',4,4'-Tetrachlorobiphenyl	3,3',4,4'-TeCB (#77)
	3,4,4',5-Tetrachlorobiphenyl	3,4,4',5-TeCB (#81)
	3,3',4,4',5-Pentachlorobiphenyl	3,3',4,4',5-PeCB (#126)
	3,3',4,4',5,5'-Hexachlorobiphenyl	3,3',4,4',5,5'-HxCB (#169)
Mono-ortho compounds	2,3,3',4,4'-Pentachlorobiphenyl	2,3,3',4,4'-PeCB (#105)
	2,3,4,4',5-Pentachlorobiphenyl	2,3,4,4',5-PeCB (#114)
	2,3',4,4',5-Pentachlorobiphenyl	2,3',4,4',5-PeCB (#118)
	2',3,4,4',5-Pentachlorobiphenyl	2',3,4,4',5-PeCB (#123)
	2,3,3',4,4',5-Hexachlorobiphenyl	2,3,3',4,4',5-HxCB (#156)
	2,3,3',4,4',5'-Hexachlorobiphenyl	2,3,3',4,4',5'-HxCB (#157)
	2,3',4,4',5,5'-Hexachlorobiphenyl	2,3',4,4',5,5'-HxCB (#167)
	2,3,3',4,4',5,5'-Heptachlorobiphenyl	2,3,3',4,4',5,5'-HpCB (#189)
NOTE Figures in parentheses indicate IUPAC No.		

3.2 Abbreviations

Abbreviations shall be as given in Table 3.

Table 3 Abbreviations

Abbreviation	Name
TeCDD	tetrachlorodibenzo- <i>p</i> -dioxin
PeCDD	pentachlorodibenzo- <i>p</i> -dioxin
HxCDD	hexachlorodibenzo- <i>p</i> -dioxin
HpCDD	heptachlorodibenzo- <i>p</i> -dioxin
OCDD	octachlorodibenzo- <i>p</i> -dioxin
TeCDF	tetrachlorodibenzofuran
PeCDF	pentachlorodibenzofuran
HxCDF	hexachlorodibenzofuran
HpCDF	heptachlorodibenzofuran
OCDF	octachlorodibenzofuran
TeCB	tetrachlorobiphenyl
PeCB	pentachlorobiphenyl
HxCB	hexachlorobiphenyl
HpCB	heptachlorobiphenyl
DeCB	decachlorobiphenyl
DMSO	dimethyl sulfoxide
IUPAC	International Union of Pure and Applied Chemistry
NOTE The abbreviation of chemical compound may be expressed with "s" which means multiple.	

4 Outline of determination method

After the dioxins in industrial water and waste water are extracted, they shall be cleaned up, then identified and determined using a GC-MS. The flow diagram of the analysis is shown in Figure 1.

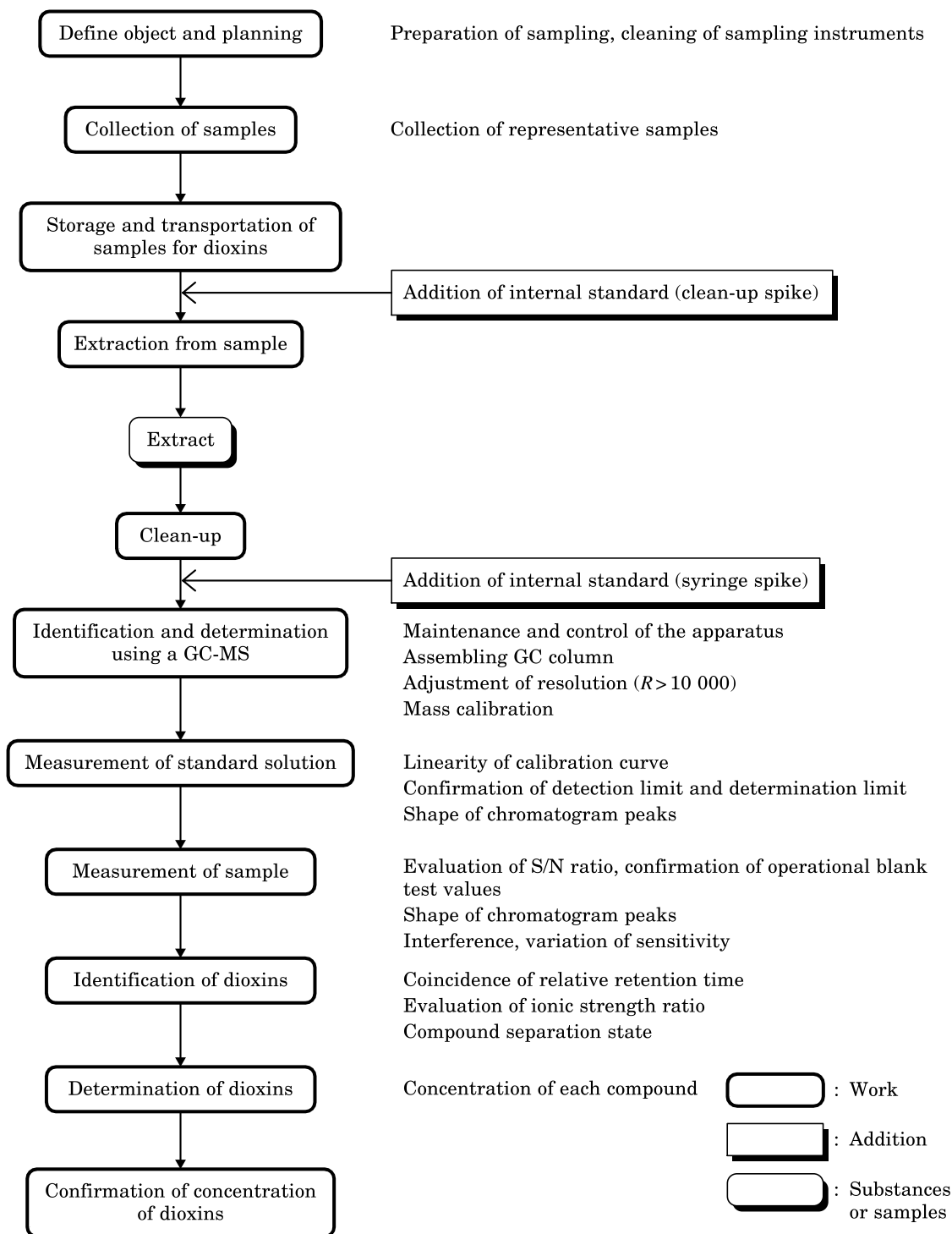


Figure 1 Flow diagram of the determination of dioxins in industrial water and waste water

5 Sample

5.1 Selection of sampling time and sampling point

The sampling time and the sampling point of samples shall be selected in accordance with **JIS K 0094** so that the representativeness of samples is secured.

5.2 Sampling

5.2.1 Instrument

The instrument shall be as follows.

5.2.1.1 Sampling bottle Unless otherwise specified, a sampling bottle made of glass shall be used and shall be thoroughly washed with methanol or acetone, and toluene or dichloromethane prior to use. Care shall be taken so that the solvents used for washing do not remain in the bottles. Plugs shall be e.g. screw caps which can form a tight seal. Do not use plugs made of rubber or cork. No hindrance to measurement shall be confirmed by means of a blank test, etc.

5.2.1.2 Water sampler The water sampler used shall be e.g. a glass bottle or a stainless steel bottle which does not allow the substance to be determined to be adsorbed on the inside wall.

5.2.2 Amount of sample collected

The amount of sample collected shall be determined according to the following procedures.

- Determine the minimum concentration to be evaluated from the purpose of measurement.
- Unless otherwise specified, set the limit of detection in sample at not more than 1/30 of the concentration determined in **a**).
- Calculate the minimum amount of sample required for measurement according to Formula (1).

$$V = DL \times \frac{y}{x} \times \frac{V_E}{V'_E} \times \frac{1}{S_{DL}} \dots\dots\dots (1)$$

where, V : minimum amount of sample required for measurement (L)

DL : limit of detection for determination method (pg)

y : liquid amount of test sample (μl)

x : injection amount to GC-MS (μl)

V_E : amount of extract (ml)

V'_E : aliquot amount of extract (ml)

S_{DL} : limit of detection for sample being set (pg/L)

- Take the amount of not less than the minimum amount V of sample calculated as the amount of sample collected.

5.2.3 Sampling method

The sampling procedure shall be in accordance with **JIS K 0094**. However, the container shall not be washed with the sample water. Place the samples collected into sampling bottles leaving a sufficient space around the sample, and seal tightly. When the residual chlorine exists in sample water, add 7.0 mg/L of sodium thiosulfate pentahydrate specified in **JIS K 8637** per 1 mg/L of residual chlorine or 20 mg/L of L(+)-ascorbic acid specified in **JIS K 9502** per 1 mg/L of residual chlorine, and mix

thoroughly. Under certain circumstances, samples may be collected by using the large volume collecting apparatus which collects dioxins by passing sample water at the measurement point (see Annex JA).

Considering the possibility of restarting the procedure from the extraction process, reserve samples should also be collected.

5.3 Record of sampling

The following information shall be recorded at the time of sampling.

- a) Sample name and sample number
- b) Name of sampling site and sampling position
- c) Sampling date and time
- d) Weather at the time of sampling and on the previous day
- e) Sampler's name
- f) Condition of the sampling site (matters which are deemed to affect the water quality of the sample, e.g. a simplified diagram of the sampling sites)
- g) Air and water temperature at the time of sampling
- h) River flow rate or volume of effluent
- i) Other useful information such as appearance (e.g. colour and turbidity of the samples), odour of the samples, and if necessary, transparency, pH, residual chlorine, and photo of sampling site.

5.4 Handling of samples

The samples shall be shielded from light during transportation and measured immediately. If the measurement cannot be carried out immediately, samples shall be stored in a dark place at 0 °C to 10 °C and measured as soon as possible.

6 Pretreatment of the sample

6.1 Outline of the pretreatment of the sample

After the addition of the internal standard for clean-up spike to the sample collected, the extractions shall be carried out either by first filtering and then extracting the filtrate and the filtration residue separately, or by collecting in the solid phase in the sample and then filtering to extract only the filtrate residue. After the extraction, an aliquot of the solution shall be taken as required. The refining operation shall be carried out by the operation of sulfuric acid treatment—silica gel column chromatograph, the operation of multilayer silica gel column chromatograph, or combination of these. Then, the refining operation shall be carried out by any one of the operation of alumina column chromatograph, the operation of high performance liquid chromatograph, the operation of activated carbon column chromatograph, or combination of these. When the sample contains large amount of oil such as mineral oil, the treatment operation using the difference in partition coefficients between DMSO and hexane (hereafter referred to as DMSO treatment operation) may be added to the refining operation as necessary. After these refining operations are carried out, the samples are analysed by gas chromatography mass spectrometry. The summary of refining operations is given in Table 4.

Figure 2 shows an example of flow diagram from the pretreatment of a sample to the measurement.

Table 4 Summary of refining operations

Name of operation	Main effect
Operation of sulfuric acid treatment—silica gel column chromatograph	Decomposition-removal of the greater part of matrix Removal of coloured substance, polynuclear aromatic hydrocarbon, high polar substance
Operation of multilayer silica gel column chromatograph	Removal of phenols, acidic substance, lipid, protein, sulfur compound, aliphatic hydrocarbons, high polar substance, coloured substance, polynuclear aromatic hydrocarbon
Operation of alumina column chromatograph	Removal of low polar substance, organic chlorine compound
Operation of high performance liquid chromatograph	Separation-refining of PCDDs, PCDFs, DL-PCBs
Operation of activated carbon column chromatograph	Separation-refining of PCDDs, PCDFs, DL-PCBs
Operation of DMSO treatment	Removal of low polar substance such as aliphatic hydrocarbon

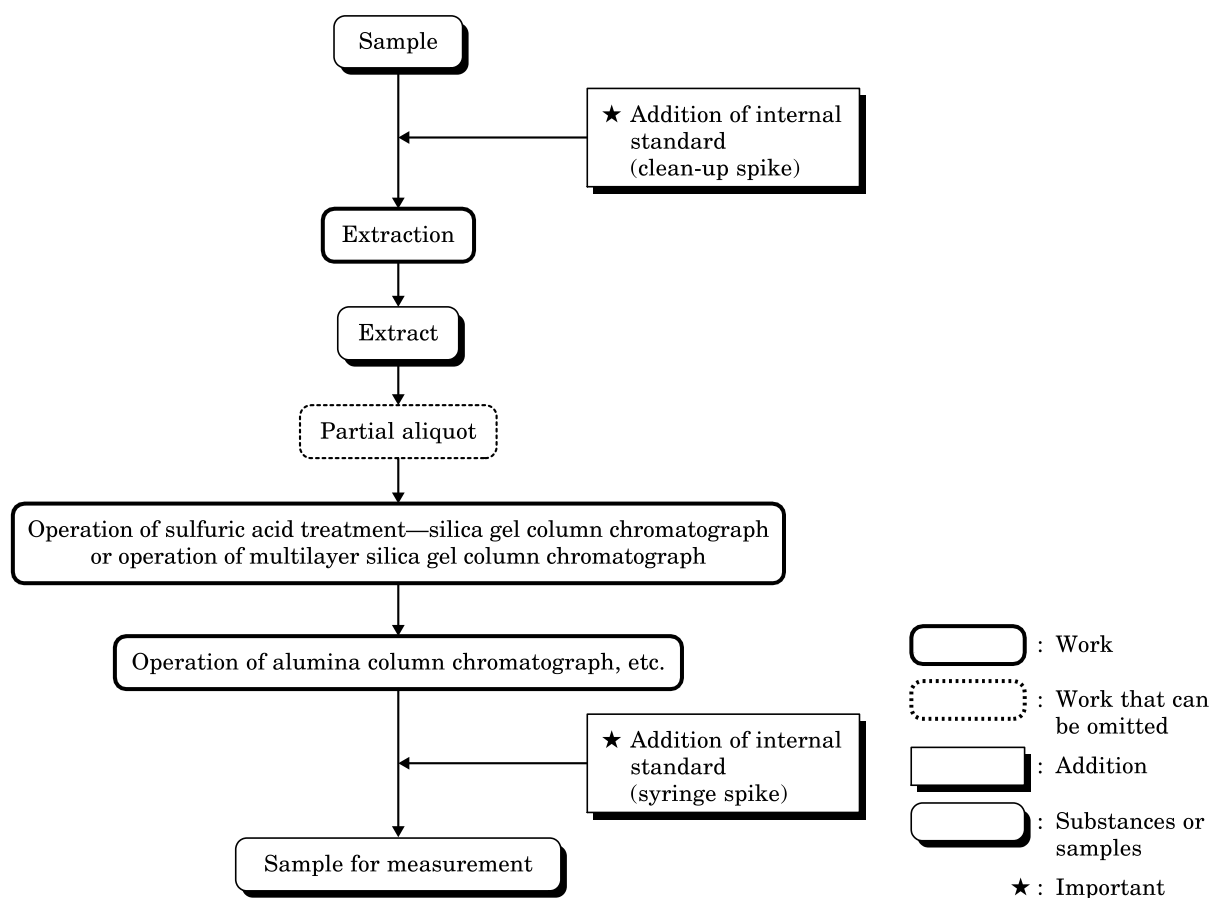


Figure 2 An example of flow diagram from the pretreatment of a sample to the measurement

Other refining operations than those listed above may be used if it is confirmed that all of the following conditions are met. For this confirmation, use samples taken from 5 or more different sampling points and repeat measurements 5 times or more for each sample; thus, the data of 25 points or more in total shall be used for the sample medium to be applied.

- a) The recovery of target dioxins is not less than 90 %.
- b) The sample solution obtained by means of the refining operation specified in this Standard and the sample solution obtained by means of a new operation method to be adopted are measured by using a GC-MS of low resolution such as quadruple pole type according to the total ion detection method in the range of 50 to 450 in measurement mass number under the GC condition in **7.3.1 a)**. When the obtained each chromatogram is compared, no difference exists in refining effect, or the effect equivalent to that resulting from the refining operation of this Standard can be obtained.
- c) When the measurement is carried out in accordance with **7.3.3** for the sample solution obtained by means of a new operation method to be adopted, no variation is found in response of the monitor ion of the standard sample for correction in the neighbourhood where the peak due to analyte appears.

6.2 Reagents and materials

Reagents and materials used for pretreatment of the sample shall be as follows. Blank test and the like shall be performed to confirm that these reagents and materials do not affect the measurement.

6.2.1 Water, of A4 (or A3) specified in **JIS K 0557**.

6.2.2 Methanol, as specified in **JIS K 8891**, or equivalent grade.

6.2.3 Acetone, as specified in **JIS K 8040**, or equivalent grade.

6.2.4 Toluene, as specified in **JIS K 8680**, or equivalent grade.

6.2.5 Dichloromethane, as specified in **JIS K 8117**, or equivalent grade.

6.2.6 Hexane, as specified in **JIS K 8825**, or equivalent grade.

6.2.7 Dimethyl sulfoxide, as specified in **JIS K 9702**, or equivalent grade.

NOTE For dimethyl sulfoxide specified in **JIS K 9702**, DMSO is used as an abbreviation in this Standard (see Table 3).

6.2.8 Decane, having a quality that does not affect the measurement.

6.2.9 Nonane, having a quality that does not affect the measurement.

6.2.10 2,2,4-trimethylpentane, as specified in **JIS K 9703**, or equivalent grade.

6.2.11 Sodium sulfate, as specified in **JIS K 8987**, or equivalent grade.

6.2.12 Hydrochloric acid, of guaranteed grade as specified in **JIS K 8180**, or equivalent grade.

6.2.13 Sulfuric acid, as specified in **JIS K 8951**, or equivalent grade.

6.2.14 Potassium hydroxide, as specified in **JIS K 8574**, or equivalent grade.

6.2.15 Silver nitrate, as specified in **JIS K 8550**, or equivalent grade.

6.2.16 Hexane washing water, prepared by rinsing thoroughly water in **6.2.1** with hexane in **6.2.6**.

6.2.17 Internal standard for clean-up spike, which is the internal standard added to check the results of the entire pretreatment operations from extraction through clean up and to be used as a reference for the determination of dioxins (see Table 5 and Annex JB). The solution to be used shall contain appropriate type and concentration of dioxins labelled with ^{13}C or ^{37}Cl for all carbon and chlorine atoms. The internal standard other than that used for the internal standard for syringe spike in **6.2.18** shall be used. It shall be used by diluting with acetone in **6.2.3** prior to use.

For the internal standard for clean-up spike of PCDDs and PCDFs, at least one type of isomers substituted by chlorine at 2,3,7,8 positions shall be selected for each chlorine number. For PCBs, all types of non-ortho DL-PCBs, and one type of mono-ortho DL-PCBs or the other PCBs shall be selected for each chlorinated compound. If possible, all of the compounds with a non-zero toxicity equivalent factor (hereafter referred to as TEF) should be selected.

Since some internal standards may interfere with measurements depending on the measurement conditions of GC-MS, the internal standards shall be thoroughly examined and confirmed prior to use.

6.2.18 Internal standard for syringe spike, which is the internal standard added to check the injection of test sample solution into the GC-MS (see Table 5 and Annex JB). The solution to be used shall contain appropriate type of PCDDs, PCDFs and PCBs labelled with ^{13}C or ^{37}Cl for all carbon and chlorine atoms and shall be diluted to the appropriate concentration using the same solvent as test sample solution such as nonane in **6.2.9**. The internal standard other than that used for the internal standard for clean-up spike in **6.2.17** shall be used.

6.2.19 Silica gel Place the silica gel (grain size 63 μm to 212 μm) for the column chromatograph in a beaker to wash with methanol in **6.2.2** and thoroughly vaporize the methanol. Transfer the silica gel into an evaporating dish or a beaker so that it forms a layer of 10 mm or less. After heating for approximately 18 h at 130 $^{\circ}\text{C}$, allow to cool for approximately 30 min in a desiccator. After preparation, place it in an airtight reagent bottle and store in a desiccator.

6.2.20 Silica gel impregnated with potassium hydroxide (2 % mass fraction) After adding 40 ml of potassium hydroxide solution (50 g/L) prepared with potassium hydroxide in **6.2.14** to 100 g of silica gel in **6.2.19**, dehydrate it under the reduced pressure at approximately 50 $^{\circ}\text{C}$ in a rotary evaporator. After removing almost all the moisture, raise the temperature to the range between 50 $^{\circ}\text{C}$ and 80 $^{\circ}\text{C}$, continue the dehydration under the reduced pressure at this temperature for approximately 1 h to pulverize the silica gel. After preparation, place it in an airtight reagent bottle and store in a desiccator.

6.2.21 Silica gel impregnated with sulfuric acid (22 % mass fraction) After adding 28.2 g of sulfuric acid in **6.2.13** to 100 g of silica gel in **6.2.19**, shake the mixture sufficiently to pulverize it. After preparation, place it in an airtight reagent bottle and store in a desiccator.

6.2.22 Silica gel impregnated with sulfuric acid (44 % mass fraction) After adding 78.6 g of sulfuric acid in **6.2.13** to 100 g of silica gel in **6.2.19**, shake the mixture sufficiently to pulverize it. After preparation, place it in an airtight reagent bottle and store in a desiccator.

6.2.23 Silica gel impregnated with silver nitrate (10 % mass fraction) After adding 28 ml of silver nitrate solution (400 g/L) prepared with silver nitrate in **6.2.15** to 100 g of silica gel in **6.2.19**, remove the moisture thoroughly in a rotary evaporator. After preparation, place it in an airtight coloured bottle and store in a desiccator.

The bottle shall be shaded from light during preparation and storage as much as possible.

6.2.24 Alumina Pre-activated alumina for use in column chromatograph (basic, Activity grade I), if available, may be used as it is. If activation is necessary, place alumina in a beaker so that it forms a layer of 10 mm or less and heat at 130 °C for approximately 18 h, or place alumina in a Petri dish so that it forms a layer of approximately 5 mm and heat at 500 °C for approximately 8 h. Allow to cool for approximately 30 min in a desiccator and store in an airtight reagent bottle. After activation, the alumina shall be used as soon as possible.

NOTE The activity of alumina changes significantly depending on the manufacturing lot or storage period after opening. For alumina with lowered activity, 1,3,6,8-TeCDD, 1,3,6,8-TeCDF, etc. may be eluted into the first fraction, and OCDD, OCDF, etc. may not be eluted into the second fraction with the specified volume of hexane solution containing dichloromethane (50 % volume fraction).

6.2.25 Carbon column for high performance liquid chromatograph Porous graphitized carbon column for high performance liquid chromatograph, or that having the equivalent separation performance to this.

NOTE An example of graphitized carbon column for high performance liquid chromatograph is Hypercarb® (4.6 mm in inside diameter, 100 mm in length) (Thermo Fisher Scientific K.K.). This information is provided for the convenience of users of this Standard and is not a recommendation of this product.

6.2.26 Activated carbon column packing Silica gel impregnated or dispersed with activated carbon or that having the equivalent separation performance to this.

NOTE Examples of activated carbon column packing are activated carbon impregnated silica gel (FUJIFILM Wako Pure Chemical Corporation) and activated carbon dispersed silica gel (KANTO CHEMICAL CO., INC.). This information is provided for the convenience of users of this Standard and is not a recommendation of these products.

6.2.27 Glass fiber filter, having a pore size of approximately 0.5 µm and containing no organic binder. The filter shall be used in a Büchner funnel.

NOTE In some articles on the market, the pore size is also indicated as retained particle size, particle retention capacity, etc.

6.2.28 Solid phase for extraction Disk-shaped, column-shaped or cartridge-shaped solid phase with silica gel to which octadecyl groups (ODS) are chemically bonded or one having an equal extraction property. All the following conditions shall be satisfied.

- a) The internal standard for clean-up spike in **6.2.17** is added to water of A3 specified in **JIS K 0557** or tap water to prepare the sample. When 20 L of the sample is passed, the recovery of the internal standard added is not less than 90 %. The recovery is confirmed by the mean value obtained by carrying out the procedure at least twice with each concentration in neighbourhood of the minimum limit of determination and of 10 times the minimum limit of determination.
- b) When 100 L of tap water is passed, the loss of dioxins is not found. After the internal substance for clean-up spike in **6.2.17** is added to the solid phase, 100 L of tap water is passed and the recovery of the internal standard added is obtained. The recovery is in the range between 70 % and 130 %.

6.2.29 Dispersion-type solid phase adsorbent—flocclulants A mixture of dispersion-type solid phase adsorbents and inorganic flocclulants which are dispersed in the sample or that having equivalent extraction performance. When the measurement is performed with the sample prepared by adding the internal standard for clean-up spike in **6.2.17** to water of A3 specified in **JIS K 0557** or tap water, the recovery of the internal standard added is not less than 90 %. The recovery is confirmed by the mean value obtained by carrying out the procedure at least twice with each concentration in neighbourhood of the minimum limit of determination and of 10 times the minimum limit of determination.

NOTE An example of dispersion-type solid phase adsorbent—flocclulants is Dioflock® (MIURA CO., LTD.). This information is provided for the convenience of users of this Standard and is not a recommendation of this product.

6.2.30 Nitrogen, of Grade 2 specified in **JIS K 1107**, or equivalent grade.

Table 5 Examples of internal standards for dioxins

		Internal standard
PCDDs	TeCDD	$^{13}\text{C}_{12}$ -1,2,3,4-TeCDD
		$^{13}\text{C}_{12}$ -2,3,7,8-TeCDD
		$^{37}\text{Cl}_4$ -2,3,7,8-TeCDD
	PeCDD	$^{13}\text{C}_{12}$ -1,2,3,7,8-PeCDD
	HxCDD	$^{13}\text{C}_{12}$ -1,2,3,4,7,8-HxCDD
		$^{13}\text{C}_{12}$ -1,2,3,6,7,8-HxCDD
		$^{13}\text{C}_{12}$ -1,2,3,7,8,9-HxCDD
	HpCDD	$^{13}\text{C}_{12}$ -1,2,3,4,6,7,8-HpCDD
	OCDD	$^{13}\text{C}_{12}$ -1,2,3,4,6,7,8,9-OCDD
PCDFs	TeCDF	$^{13}\text{C}_{12}$ -2,3,7,8-TeCDF
		$^{13}\text{C}_{12}$ -1,2,7,8-TeCDF
	PeCDF	$^{13}\text{C}_{12}$ -1,2,3,7,8-PeCDF
		$^{13}\text{C}_{12}$ -2,3,4,7,8-PeCDF
	HxCDF	$^{13}\text{C}_{12}$ -1,2,3,4,7,8-HxCDF
		$^{13}\text{C}_{12}$ -1,2,3,6,7,8-HxCDF
		$^{13}\text{C}_{12}$ -1,2,3,7,8,9-HxCDF
		$^{13}\text{C}_{12}$ -2,3,4,6,7,8-HxCDF
	HpCDF	$^{13}\text{C}_{12}$ -1,2,3,4,6,7,8-HpCDF
		$^{13}\text{C}_{12}$ -1,2,3,4,7,8,9-HpCDF
	OCDF	$^{13}\text{C}_{12}$ -1,2,3,4,6,7,8,9-OCDF
PCBs	TeCB	$^{13}\text{C}_{12}$ -2,3',4',5'-TeCB (#70)
		$^{13}\text{C}_{12}$ -3,3',4,4'-TeCB (#77) ^{a)}
		$^{13}\text{C}_{12}$ -3,4,4',5'-TeCB (#81) ^{a)}
	PeCB	$^{13}\text{C}_{12}$ -2,3,3',4,4'-PeCB (#105) ^{a)}
		$^{13}\text{C}_{12}$ -2,3,4,4',5'-PeCB (#114) ^{a)}
		$^{13}\text{C}_{12}$ -2,3',4,4',5'-PeCB (#118) ^{a)}
		$^{13}\text{C}_{12}$ -2',3,4,4',5'-PeCB (#123) ^{a)}
		$^{13}\text{C}_{12}$ -3,3',4,4',5'-PeCB (#126) ^{a)}
	HxCB	$^{13}\text{C}_{12}$ -2,3,3',4,4',5'-HxCB (#156) ^{a)}
		$^{13}\text{C}_{12}$ -2,3,3',4,4',5'-HxCB (#157) ^{a)}
		$^{13}\text{C}_{12}$ -2,3',4,4',5,5'-HxCB (#167) ^{a)}
		$^{13}\text{C}_{12}$ -3,3',4,4',5,5'-HxCB (#169) ^{a)}
	HpCB	$^{13}\text{C}_{12}$ -2,2',3,3',4,4',5'-HpCB (#170)
		$^{13}\text{C}_{12}$ -2,2',3,4,4',5,5'-HpCB (#180)
		$^{13}\text{C}_{12}$ -2,3,3',4,4',5,5'-HpCB (#189) ^{a)}

NOTE Figures in parentheses indicate IUPAC No.
Note ^{a)} It indicates DL-PCB.

6.3 Instrument and apparatus

The instrument and apparatus used for pretreatment of the sample shall be washed sufficiently with methanol or acetone, and toluene or dichloromethane. Blank tests shall be conducted to confirm that they will not affect the measurement.

6.3.1 Glassware, as specified in **JIS R 3503** and **JIS R 3505**. Those with cocks made of fluorocarbon resin may be used.

6.3.2 Solid phase extraction apparatus, consisting of a disk-shaped solid phase, a funnel, a support screen, a gasket, a base, a clamp, a rubber plug, a suction bottle and a suction pump (see Figure 3).

6.3.3 Soxhlet extractor, as specified in **JIS R 3503**, or equivalent property. No sealant grease shall be used in the coupling section.

6.3.4 Concentrator Kuderna-Danish (KD) concentrator or a rotary evaporator. No sealant grease shall be used in the coupling section.

6.3.5 Column-chromatograph tube, made of glass or of equivalent material having an inside diameter of 10 mm or 15 mm and a length of 300 mm, free from the adsorption and/or mixing of dioxins or the elution of interfering substances.

6.3.6 Büchner funnel

6.3.7 High performance liquid chromatograph, equipped with a passage changeable valve.

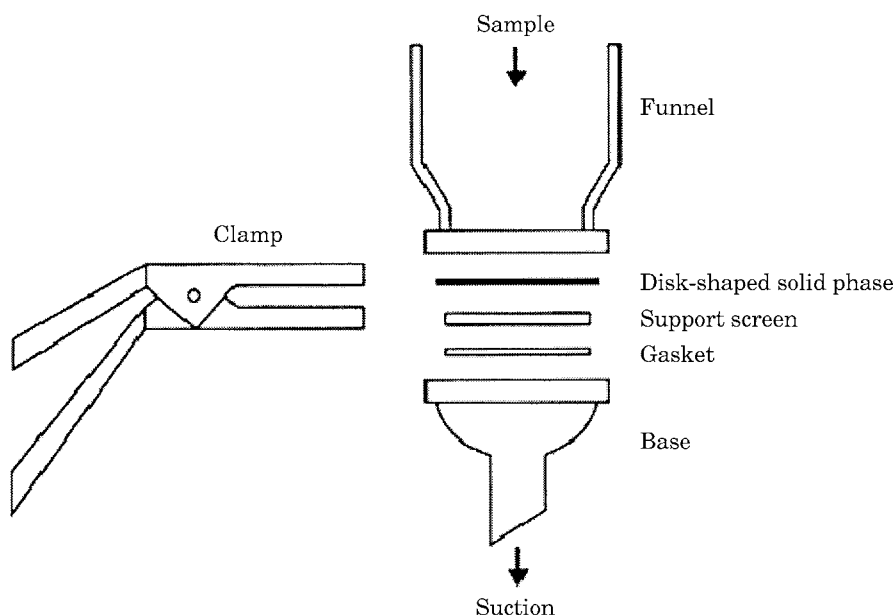


Figure 3 An example of solid phase extraction apparatus

6.4 Pretreatment operations

6.4.1 Record of sample amount

The total amount of the sample in the sampling bottle shall be used for measurement. The amount of the sample shall be obtained by subtracting the mass of the empty bottle from the mass of the bottle containing the sample. Alternatively, the water line of the sample shall be marked on the sampling bottle at the time of sampling, and when measurement is finished, water is added up to the mark and the water volume shall be recorded as the volume of the sample.

6.4.2 Addition of internal standard (clean-up spike)

A specific amount of the internal standard for clean-up spike shall be added to the samples before extraction. Amount to be added is generally 0.1 ng to 2 ng for TeCDDs to HpCDDs and for TeCDFs to HpCDFs, 0.2 ng to 4 ng for OCDD and OCDF, and 0.1 ng to 2 ng for DL-PCBs. When the concentration of dioxins in the sample is extremely high, and the total concentration is expected to exceed the dynamic range by the addition of the normal volume of internal standard, the internal standard may be added in an amount exceeding the range. If the sample has been collected in more than one sampling bottle, the internal standard shall be added to each container so that the concentration is almost uniform, and the total amount added shall be recorded.

6.4.3 Extraction from sample

6.4.3.1 General

For the extraction from the sample to which the internal standard has been added, considering the amount of sample, the amount of coexisting organic substances, etc., it shall be carried out either by first filtering and then extracting the filtrate and the filtration residue separately, or by collecting in the solid phase in the sample and then filtering to extract only the filtration residue.

6.4.3.2 Method for filtering first

The method for filtering first shall be as follows.

- a) **Filtration of sample** The sample to which the internal standard has been added is subjected to suction filtration through a glass fiber filter to separate it into the filtration residue and the filtrate. The sample containing a lot of suspended matters and liable to clog the filter may be filtered by the multistage method with filter paper having a large pore size and then filtered with a glass fiber filter with a pore size of approximately 0.5 μm .
- b) **Extraction from filtrate** The extraction from filtrate shall be carried out by either of the following.
 - 1) **Solid phase extraction method** The filtrate obtained in a) is passed through the selected solid phase for extraction and then the moisture is sufficiently removed. After the moisture is removed, the solid phase is subjected to Soxhlet extraction and the extraction into solvent shall be performed. For the sample with which the amount of passing liquid causing adsorption breakthrough has not been confirmed, the amount of liquid passing through one solid phase for extraction shall be not more than 5 L.

The detailed extraction operation shall be in accordance with the recommended method of the selected solid phase for extraction.
 - 2) **Liquid—liquid extraction method** Pour filtrate obtained in a) into a separating funnel. Add toluene or dichloromethane in the ratio of 100 ml per litre of filtrate. Extract by shaking at approximately 5 cm in amplitude, 100 or more r.p.m. for approximately 20 min. Carry out the extraction three times. Mix the extract and dehydrate with sodium sulfate.

When the extraction efficiency is confirmed to be not less than 90 %, other liquid—liquid extraction methods than this operation method, such as the mixing-up extraction method, may be used.

- c) **Extraction from filtration residue** Remove the moisture sufficiently from the filtration residue obtained in a), and then extract by means of Soxhlet extraction or the extraction method equivalent to this. The judgement of the equivalence to Soxhlet extraction method shall be carried out by either of the following methods.

Whether conditions are satisfied shall be confirmed using nine data in total by preparing at least three samples and repeating three measurements for each sample.

- A certified reference material such as soil is extracted by a new extraction method to be adopted and measured, and the difference between the measurement result and certified value is within ± 2 times the interlaboratory standard deviation.

NOTE In the case of items for which the certified value is not described in the certified reference material, the difference from the indicative value may be applied.

- A sample such as soil is extracted by the Soxhlet extraction method and measured. At the same time, the sample is extracted by a new extraction method to be adopted and measured. The average of both measurement results is determined, and the measurement result obtained by the new extraction method to be adopted is within ± 15 % of the average.

Carry out Soxhlet extraction using toluene for 16 h or more (about 100 revolutions). In the Soxhlet extraction, since the moisture remaining in the sample may cause lower extraction efficiency, moisture shall be removed using a suitable method before extraction. Attention shall be given to photolysis during extraction because extraction requires a long time.

In the Soxhlet extraction, the method using a Soxhlet/Dean-Stark extractor may be used (see **EPA Method 1613** and others).

- d) **Preparation of extract** Combine the extract obtained in b) and c). Wash the inside wall of the sampling bottle with toluene or dichloromethane and dehydrate the washings with sodium sulfate. Combine this with the extract obtained above. Concentrate with a concentrator and add toluene to dilute to certain volume. This is used as the extract.

6.4.3.3 Method for filtration after collection in solid phase

The method for filtration after collection in solid phase shall be as follows. The detailed operation shall be in accordance with the recommended method of the selected dispersion-type solid phase adsorbent—flocculants.

- a) **Collection in solid phase** In the dispersion-type solid phase adsorbent—flocculation method, adjust pH of the sample to which the internal standard is added in the range where flocculation occurs. Add the dispersion-type solid phase adsorbent—flocculants, and stir sufficiently. Then allow to stand until the flocculates precipitate.

For the sample containing a lot of suspended matters and liable to clog the filter, the following procedures may be performed: add the internal standard and filter by the multistage method with filter paper having a large pore size; then adjust pH of the filtrate in the range which causes flocculation and add the dispersion-type solid phase adsorbent—flocclulants. At this time, extract filtration residues simultaneously with the operation in c).

- b) **Filtration of sample** Filter the sample collected in the solid phase in a) in the same way as 6.4.3.2 a).
- c) **Extraction from filtration residues** Carry out extraction from filtration residues in the same way as 6.4.3.2 c).
- d) **Preparation of extract** Wash the inside wall of the sampling bottle with toluene or dichloromethane and dehydrate the washings with sodium sulfate. Combine this with the extract obtained in c). Concentrate with a concentrator and add toluene to dilute to certain volume. This is used as the extract.

6.4.4 Elution conditions in the operation of column chromatograph

Since the elution conditions for dioxins in the operation of column chromatograph in 6.4.6 and 6.4.7 differ according to the type or lot of the column packing to be used, they shall be confirmed by a fraction test beforehand by using the sample solution containing all dioxins such as extract of fly ash. The fraction test shall be carried out for at least every lot of column packing.

6.4.5 Concentration of extract

Take a suitable aliquot of the extract obtained in 6.4.3 and concentrate it to approximately 5 ml with a concentrator. Then, remove toluene by nitrogen gas flow to make approximately 100 µl of concentrated solution. Add 2 ml to 3 ml of hexane and remove solvent by nitrogen gas flow to make approximately 100 µl of concentrated solution. Repeat this operation one more time. When the operation in 6.4.6 a) is carried out, the transfer operation to hexane using a concentrator may be performed.

To avoid the loss of substances to be analysed due to the concentration with nitrogen gas flow, care shall be taken not to scatter the solution by adjusting nitrogen gas flow to the level where motion on the surface of the solution is slightly visible. The solution shall not be evaporated to dryness.

Since reanalysis may be required, part of the extract should be stored.

NOTE In the concentration with nitrogen gas flow, spraying nitrogen enough to form a large vortex in the solution or over-drying of the solution may cause loss of the substances to be analysed.

6.4.6 Operation of sulfuric acid treatment—silica gel column chromatograph or operation of multilayer silica gel column chromatograph

The refining operation by the operation of sulfuric acid treatment—silica gel column chromatograph or the operation of multilayer silica gel column chromatograph shall be as follows.

After the operations described in a) 1) to 2) below, the solution may be refined by the operation described in b).

WARNING When adding the sulfuric acid, care shall be taken regarding bumping of the solvents resulting from reactions between the sulfuric acid and organic substances. Start with several millilitres, and then add gradually depending on the degree of colouration. Be sure to wear gloves, a mask, and other protective gear.

- a) **Operation of sulfuric acid treatment—silica gel column chromatograph** The operation of sulfuric acid treatment—silica gel column chromatograph shall be as follows.

The procedure as shown herein is a standard, and if the effect of refining can be sufficiently obtained, this procedure may not necessarily be followed.

- 1) Transfer the concentrated solution obtained in **6.4.5** into a separating funnel (300 ml) while washing with 50 ml to 150 ml of hexane, add 5 ml of sulfuric acid, shake to mix gently, allow to stand and remove the layer of sulfuric acid. Repeat this operation 3 or 4 times until the colour of sulfuric acid layer becomes light.
- 2) Repeat the washing of the hexane layer with 50 ml of hexane washing water until the washings become almost neutral. After dehydrating with sodium sulfate, concentrate it to approximately 2 ml with a concentrator.

When desulfurization is required, carry out silver nitrate treatment or copper chip treatment after sulfuric acid treatment. Specifically, silver nitrate impregnated silica gel or a copper chip (copper wire treated with hydrochloric acid and cut into small pieces) should be packed in a column and the sample solution should be passed through the column.

- 3) Put a plug of quartz glass wool into the bottom of the column-chromatograph tube. Wash inside of the tube with 10 ml of hexane and leave some hexane up to the upper part of the quartz glass wool. Weigh out 3 g of silica gel in a beaker containing 10 ml of hexane, remove foam by stirring gently with a glass rod and pour it into a column-chromatograph tube. After running down hexane and stabilizing the silica gel layer, form a layer of sodium sulfate of approximately 10 mm in thickness on the silica gel layer and wash down sodium sulfate that adheres to the tube wall with several millilitres of hexane.
 - 4) After running down 50 ml of hexane into a silica gel column, bring down the water level to the upper surface of sodium sulfate layer, transfer the solution prepared in **2)** into the column gently, and wash it several times with 1 ml of hexane. Bring down the water level to the upper surface of sodium sulfate layer. Run down 150 ml of hexane at the rate of approximately 2.5 ml/min (approximately one drop per second).
 - 5) Concentrate the eluate to approximately 2 ml with a concentrator.
- b) **Operation of multilayer silica gel column chromatograph** The operation of multilayer silica gel column chromatograph shall be according to the following procedures.

The procedure as shown herein is a standard, and if the effect of refining can be sufficiently obtained, this procedure may not necessarily be followed.

- 1) Put a plug of quartz glass wool into the bottom of the column-chromatograph tube (inside diameter 15 mm) and pack in sequence, 0.9 g of silica gel, 3 g of silica gel impregnated with potassium hydroxide (2 % mass fraction), 0.9 g of silica gel, 4.5 g of silica gel impregnated with sulfuric acid (44 % mass fraction), 6 g of silica gel impregnated with sulfuric acid (22 % mass fraction), 0.9 g of silica gel, 3 g of silica gel impregnated with silver nitrate (10 % mass fraction) and 6 g of sodium sulfate into the tube (see Figure 4).

Since the same effect as sulfuric acid treatment can be obtained by treatment using only sulfuric acid silica gel, for some samples, the column of silica gel impregnated with sulfuric acid (22 % mass fraction) may be employed instead of the multilayer silica gel column.

NOTE Silica gel impregnated with silver nitrate (10 % mass fraction) is effective for the treatment of the samples of especially high sulfur concentration.

- 2) After running down 50 ml of hexane, lower the water level to the upper surface of sodium sulfate layer.
- 3) Transfer gently the concentrated solution obtained in **6.4.5** into the column and lower the water level to the upper surface of the sodium sulfate layer.
- 4) Wash the container of the concentrated solution with 1 ml of hexane, and put the washings into the column while rinsing its inside wall. Repeat this washing process 2 or 3 times.
- 5) Run down 120 ml of hexane at the rate of approximately 2.5 ml/min (approximately one drop per second).
- 6) Concentrate the eluate to approximately 2 ml with a concentrator. When the packed layers become dark in colour, repeat the operations in **1)** to **6)**.

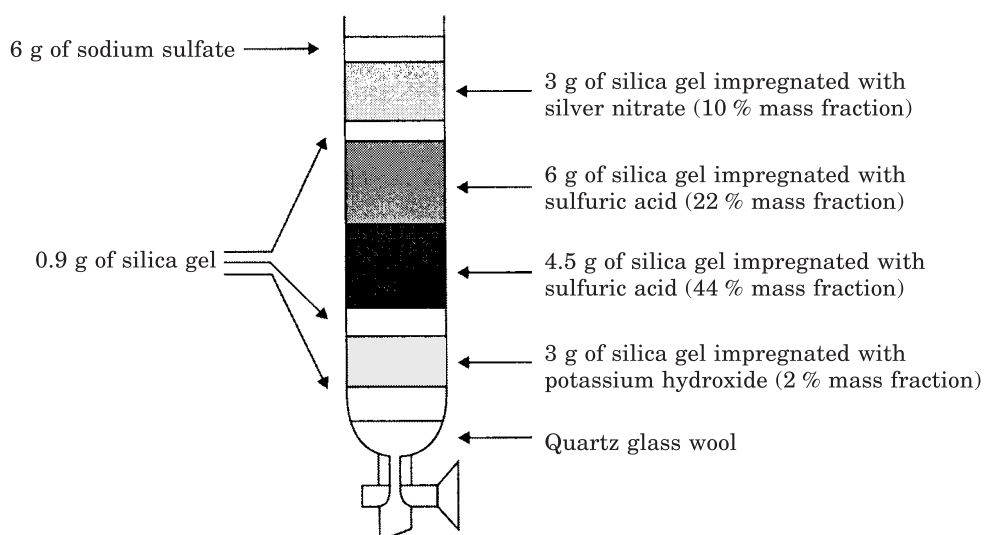


Figure 4 An example of multilayer silica gel column

6.4.7 Other refining operations

The concentrated solution obtained in 6.4.6 shall be refined by means of either operation as shown in the following or combination of these, and the concentrated solution for measuring PCDDs and PCDFs and for measuring DL-PCBs shall be prepared.

The procedure as shown herein is a standard, and if the effect of refining can be sufficiently obtained, this procedure may not necessarily be followed.

a) **Operation of alumina column chromatograph** When the operation of alumina column chromatograph is carried out, the concentrated solution shall be divided for the measurement of PCDDs and PCDFs and the measurement of DL-PCBs. Each operation procedure shall be as follows. The operation may be carried out without dividing the concentrated solution depending on the operating conditions of identification and determination. In such a case, the following procedure may not apply.

1) **Operation of alumina column chromatograph for the measurement of PCDDs and PCDFs** The operation of alumina column chromatograph for the measurement of PCDDs and PCDFs shall be according to the following procedures.

1.1) Put a plug of quartz glass wool into the bottom of the column-chromatograph tube. Wash inside of the tube with 10 ml of hexane and leave some hexane up to the upper part of the quartz glass wool. Weigh out 10 g of alumina in a beaker containing 10 ml of hexane, remove foam by stirring gently with a glass rod and pour it into a column-chromatograph tube. After running down hexane and stabilizing the alumina layer, form a layer of sodium sulfate of approximately 10 mm in thickness on the alumina layer and wash down sodium sulfate that adheres to the tube wall with several millilitres of hexane. After running down 50 ml of hexane, lower the water level to the upper surface of sodium sulfate layer.

1.2) After dividing the concentrated solution into two parts accurately, concentrate one part of them to approximately 0.5 ml by means of concentration with nitrogen gas flow in 6.4.5, transfer it gently into the column of 1.1), wash it several times with 1 ml of hexane, and lower the water level to the upper surface of sodium sulfate layer. Run down 100 ml of hexane solution containing dichloromethane (2 % volume fraction) at the rate of approximately 2.5 ml/min (approximately one drop per second) and obtain the first fraction. This fraction shall be stored until the measurement is finished.

1.3) Run down 150 ml of hexane solution containing dichloromethane (50 % volume fraction) at the rate of approximately 2.5 ml/min (approximately one drop per second) and obtain the second fraction. This fraction contains PCDDs and PCDFs.

1.4) Concentrate the second fraction to approximately 2 ml with a concentrator.

2) **Operation of alumina column chromatograph for the measurement of DL-PCBs** The operation of alumina column chromatograph for the measurement of DL-PCBs shall be according to the following procedures.

2.1) Put a plug of quartz glass wool into the bottom of the column-chromatograph tube. Wash inside of the tube with 10 ml of hexane and leave some hexane up

to the upper part of the quartz glass wool. Weigh out 10 g of alumina in a beaker containing 10 ml of hexane, remove foam by stirring gently with a glass rod and pour it into a column-chromatograph tube. After running down hexane and stabilizing the alumina layer, form a layer of sodium sulfate of approximately 10 mm in thickness on the alumina layer and wash down sodium sulfate that adheres to the tube wall with several millilitres of hexane. After running down 50 ml of hexane, lower the water level to the upper surface of sodium sulfate layer.

- 2.2) After dividing the concentrated solution into two parts accurately, concentrate one part of them to approximately 0.5 ml by means of concentration with nitrogen gas flow in **6.4.5**, and transfer it gently into the column-chromatograph tube of **2.1**, wash it several times with 1 ml of hexane, and lower the water level to the upper surface of sodium sulfate layer. Run down 40 ml of hexane at the rate of approximately 2.5 ml/min (approximately one drop per second) and elute chain hydrocarbons, etc.
- 2.3) Run down 120 ml of hexane solution containing dichloromethane (5 % volume fraction) at the rate of approximately 2.5 ml/min (approximately one drop per second) and obtain the first fraction. This fraction contains DL-PCBs.
- 2.4) Run down 160 ml of hexane solution containing dichloromethane (50 % volume fraction) at the rate of approximately 2.5 ml/min (approximately one drop per second) and obtain the second fraction. This fraction shall be stored until the measurement is finished.
- 2.5) Concentrate the first fraction to approximately 2 ml with a concentrator.

- b) **Operation of high performance liquid chromatograph** The operation of high performance liquid chromatograph shall be in accordance with the following procedures.

As the operating conditions shown hereafter differ slightly depending on the apparatus, column, etc. to be used, differences shall be confirmed beforehand by the fraction test using e.g. the extract of fly ash.

- 1) Install the carbon column in a high performance liquid chromatograph so that the direction of the flow through a mobile phase is changeable. Set the flow rate of eluent to 2 ml/min. Connect an absorbance detector and prepare it so that an aliquot of eluate can be taken from an exit of the detector.
- 2) Run the toluene eluent toward the normal direction and wash the column thoroughly. Change the eluent to hexane and replace the toluene contained in the passage of the column and the apparatus with hexane. Whether toluene has been replaced by hexane should be judged by the change of the indicated value in the detector.
- 3) Concentrate further the concentrated solution to approximately 100 µl by means of concentration with nitrogen gas flow in **6.4.5**. Inject this solution into the high performance liquid chromatograph. Run the hexane for 4 min as the eluent. Take a 8 ml aliquot of the eluate as the first fraction. This fraction contains PCBs except for DL-PCBs.