INTERNATIONAL STANDARD

ISO 6468

First edition
1996-12-15

Water quality — Determination of certain organochlorine insecticides, polychlorinated biphenyls and chlorobenzenes — Gas chromatographic method after liquid-liquid extraction

Qualité de l'eau — Dosage de certains insecticides organochlorés, des polychlorobiphényles et des chlorobenzènes — Méthode par chromatographie en phase gazeuse après extraction liquide-liquide

Reference number
ISO 6468:1996(E)

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 6468 was prepared by Technical Committee ISO/TC 147, *Water quality,* Subcommittee SC 2, *Physical, chemical, biochemical methods.*

Annex A forms an integral part of this International Standard. Annexes B to H are for information only.

International Organization for Standardization
Case Postale 56 • CH-1211 Genève 20 • Switzerland

Printed in Switzerland

Water quality — Determination of certain organochlorine insecticides, polychlorinated biphenyls and chlorobenzenes — Gas chromatographic method after liquid-liquid extraction

WARNING AND SAFETY PRECAUTIONS — This method makes use of flammable and toxic organic solvents. Observe the safety regulations in effect.

The electron-capture detector (ECD) contains radionuclides. Adequate safety precautions and legal requirements must be observed.

The halogenated hydrocarbons and chloropesticides, used for the preparation of the calibration standards are toxic. Therefore, the safety regulations pertaining must be strictly observed.

1 Scope

This International Standard describes a method for determining certain organochlorine insecticides, polychlorinated biphenyls (PCBs) and chlorobenzenes (except the mono- and dichlorobenzenes) in drinking waters, ground waters, surface waters and waste waters.

The method is applicable to samples containing up to 0,05 g/l of suspended solids. In the presence of organic matter, suspended matter and colloids, interferences are more numerous and consequently the detection limits are higher.

The method described in this International Standard only gives information on specific PCB compounds but no information on the level of total PCBs.

According to the types of compounds to be detected and the source of the water, the detection limits given in table 1 are applicable for the method described in this International Standard, with waters of low organic contents.

Given the very low concentrations normally present in the waters, the problem of contamination is extremely important. The lower the level measured, the more precautions have to be observed; below concentrations of 10 ng/l, special care is necessary.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on the International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 5667-1:1980, *Water quality — Sampling — Part 1: Guidance on the design on sampling programmes.*

ISO 5667-2:1991, *Water quality — Sampling — Part 2: Guidance on sampling techniques.*

3 Principle

Liquid-liquid extraction of organochlorine insecticides, chlorobenzenes and PCBs by an extraction solvent. After the concentration of the components with low volatility and after any clean-up steps which may be necessary, the sample extracts are analysed by gas chromatography, using an electron-capture detector.

Table 1 — Detection limits

Acronyms	Chemical names (IUPAC)	Detection limits
Organochlorine insecticides:		
HCH	1, 2, 3, 4, 5, 6-hexachlorocyclohexane, five stereoisomers: alpha-HCH, beta-HCH, gamma-HCH, delta-HCH, epsilon-HCH	1 ng/l to 10 ng/l depending on the compound
Lindane		
o,p′-DDE	1,1-dichloro-2-(2-chlorophenyl 1)-2-(4-chlorophenyl)ethylene	
p,p′-DDE	1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene	
o,p′-TDE	1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane (= *o,p′*-DDD)	
p,p′-TDE	1,1-dichloro-2, 2-bis(4-chlorophenyl)ethane (= *p,p′*-DDD)	
o,p′-DDT	1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane	
p,p′-DDT	1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane	
Methoxychlor	1,1,1-trichloro-2,2-bis(4-methoxyphenyl)ethane	
Aldrin	(1*R*, 4*S*, 4a*S*, 5*S*, 8*R*, 8a*R*)-1, 2, 3, 4, 10, 10-hexachloro-1, 4, 4a, 5, 8, 8a-hexahydro-1, 4 : 5,8-dimethanonaphthalene	
Dieldrin	(1*R*, 4*S*, 4a*S*, 5*R*, 6*R*, 7*S*, 8*S*, 8a*R*)-1, 2, 3, 4, 10, 10-hexachloro-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-6, 7-epoxy-1, 4 : 5,8-dimethanonaphthalene	
Endrin	(1*R*, 4*S*, 4a*S*, 5*S*, 6*S*, 7*R*, 8*R*, 8a*R*)-1, 2, 3, 4, 10, 10-hexachloro-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-6, 7-epoxy-1, 4 : 5, 8-dimethanonaphthalene	
Heptachlor[1]	1, 4, 5, 6, 7, 8, 8-heptachloro-3a, 4, 7, 7a-tetrahydro-4, 7-methanoindene[1]	
Heptachlor-epoxide	1, 4, 5, 6, 7, 8, 8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7 -methanoindane	
Endosulfan[1) 2)]	1, 4, 5, 6, 7, 7, 7-hexachloro-8, 9, 10-trinorborn-5-en-2, 3-ylene-dimethyl-enesulfite: alpha-Endosulfan, beta-Endosulfan	
Chlorobenzenes:		1 ng/l to 10 ng/l depending on the compound
TrCB	trichlorobenzene	
TeCB	tetrachlorobenzene	
PeCB	pentachlorobenzene	
HCB	hexachlorobenzene	
PCNB (Quintozene)	pentachloronitrobenzene	
Polychlorinated biphenyls:		1 ng/l to 50 ng/l depending on the compound
PCB 28	2,4,4′-trichlorobiphenyl	
PCB 52	2,2′,5,5′-tetrachlorobiphenyl	
PCB 101	2,2′,4,5,5′-pentachlorobiphenyl	
PCB 138	2,2′,3,4,4′,5′-hexachlorobiphenyl	
PCB 153	2,2′,4,4′,5,5′-hexachlorobiphenyl	
PCB 180	2,2′,3,4,4′,5,5′-heptachlorobiphenyl	
PCB 194	2,2′,3,3′,4,4′,5,5′-octachlorobiphenyl	

1) The analysis of α and β - endosulfan as well as heptachlor requires special care due to its low stability.

2) The name "endosulfan" is not acceptable for use in Italy, as it is in conflict with a trade mark registered there.

Any substance capable of producing a response on the electron-capture detector, at a retention time indistinguishable from any compound of interest, will interfere. In practice, many potentially interfering substances will be removed during the extraction and clean-up procedures.

NOTE 1 In general, the use of two capillary columns of different polarity is sufficient for the organochlorine compounds analysed according to this International Standard. The results so calculated should be considered as the maximum concentrations, possibly still influenced by coeluting substances. It is possible that there will be cases where a more definite identification is required.

4 Reagents and materials

All reagents shall be sufficiently pure to not give rise to significant interfering peaks in the gas chromatograms of the blanks. The purity of reagents used in the procedure shall be checked by blank determinations (7.6).

NOTE 2 Commercial "pesticide grade" solvents are available. The use of these products is recommended only after verifying their quality. The quality of a solvent is checked by evaporation of about 200 ml down to 1 ml and analysis of the concentrate to determine the compounds subsequently analysed. The solvent should be considered acceptable if it does not give any detectable interfering peaks in the chromatogram for the substance of interest.

4.1 Water purified, for example, using ion-exchange or carbon-column adsorption.

4.2 Extraction solvent.

Hexane, petroleum ether or heptane are suitable.

NOTE 3 Any other solvents meeting the requirements of 8.3 (recovery rate ⩾ 60 %) may be used.

4.3 Sodium sulfate (Na_2SO_4), anhydrous.

Heat a portion of about 250 ml to 300 ml of sodium sulfate powder at 500 °C ± 20 °C for 4 h ± 30 min, cool to about 200 °C in a muffle furnace and then to ambient temperature in a desiccator containing magnesium perchlorate or an equivalent alternative.

4.4 Decane ($C_{10}H_{22}$) or **dodecane** ($C_{12}H_{26}$), or any keeper which is not detected by the electron-capture detector.

4.5 Dry alumina.

Heat a batch of inert alumina, containing particles of size 50 µm to 200 µm and of maximum mass 500 g, at 500 °C ± 20 °C for 4 h ± 30 min on a silica dish in a muffle furnace. Cool to about 200 °C in the furnace and then to ambient temperature in a desiccator. Store in a sealed glass container.

4.6 Deactivated alumina.

Weigh a portion of dry alumina (4.5) into a sealable all-glass container and add 7 % ± 0,2 % (*m*/*m*) of water (4.1). Seal and agitate for at least 2 h to ensure uniformity. Store in a sealed glass container.

Once the seal has been broken, storage time is normally about one week. After the maximum storage time, reprocess batches as described in 4.5 and this subclause.

4.7 Alumina/silver nitrate.

Dissolve 0,75 g ± 0,01 g of silver nitrate in 0,75 ml ± 0,01 ml of water (4.1) using a microburette. Add 4,0 ml ± 0,2 ml of acetone followed by 10 g ± 0,2 g of deactivated alumina (4.6). Mix thoroughly by shaking in an open-topped conical flask, protected from light. Allow the acetone to evaporate at room temperature and prevent condensation, for example by warming with the hand.

Store in the dark and use within 4 h after preparation.

4.8 Silica gel, of particle size 63 µm to 200 µm, heated at 500 °C ± 30 °C in batches not larger than 500 g, for about 14 h. Cool to about 200 °C in the furnace and then to ambient temperature in a sealed flask which is placed in a desiccator without desiccant. Use this material within one week. Deactivate the silica gel by weighing a suitable quantity of silica and adding 3 % (*m*/*m*) of water (4.1). Agitate for at least 2 h to ensure uniformity and store in a sealed glass container.

The deactivated silica gel shall be used within 24 h.

4.9 Toluene.

4.10 Diethylether, free from peroxides.

4.11 Anti-bumping granules, washed with acetone.

4.12 Standard stock solutions.

Pure or certified standards of organochlorine insecticides, chlorobenzenes, and PCBs shall be used for the preparation of standard stock solutions.

NOTE 4 Suitable solvents for the preparation of standard stock solutions are acetone, pentane, hexane, dimethylbenzene or isooctane.

The containers containing the solutions shall be marked or weighed so that any evaporation losses of the solvent may be recognized. The solutions shall be stored in volumetric flasks with ground-glass stoppers at a temperature of 4 °C in the dark. Prior to use, they shall be brought to ambient temperature and the level of solvent shall be adjusted, if necessary.

NOTE 5 A convenient concentration of standard stock solution is obtained by weighing 50 mg of each determinand and dissolving it in 100 ml of the solvent.

The solution is stable for about 1 year.

4.13 Intermediate standard solutions.

Prepare intermediate standard solutions by a suitable dilution of the stock solution (4.12) with the extraction solvent (4.2).

A typical value is 10 µg/ml.

Store the intermediate standard solutions at about 4 °C in the dark. These solutions are stable for six months.

4.14 Working standard solutions.

Prepare at least five different concentrations by suitable dilutions of the intermediate standard solutions (4.13) with the extraction solvent (4.2).

Suitable concentrations are in the nanograms per millilitre range.

Store the solutions at about 4 °C in the dark. These solutions are stable for at least one month.

4.15 Cotton wool or glass wool, washed with extraction solvent.

4.16 Water-miscible solvent.

NOTE 6 Acetone, methanol or dimethylformamide may be used.

5 Apparatus

5.1 Gas chromatograph, with an electron-capture detector (ECD) and suitable for use with capillary columns. This shall be operated in accordance with the manufacturer's instructions. On-column or glass-lined injection systems can be used. The oven shall be suitable for isothermal and temperature-programmable operation.

5.2 Capillary columns, glass or fused-silica capillaries, with an inside diameter of less than 0,4 mm and a length of 25 m to 60 m, coated with stationary phases capable of separating the compounds of interest.

Annex B provides examples of gas chromatographic conditions (tables B.1, B.2 and B.3) and the corresponding gas chromatograms (figures B.1 and B.2).

5.3 Separating funnels, of nominal capacities 1 litre to 5 litres, with a glass tap washed by hexane or a polytetrafluoroethylene (PTFE) tap.

5.4 High-speed stirrer and magnetic stirring bar, washed with hexane and coated with polytetrafluoroethylene (PTFE).

5.5 Microseparator, see example in figure C.1.

5.6 Kuderna-Danish evaporator, see example in figure D.1.

5.7 Snyder microcolumn.

5.8 Rotary evaporator or any suitable system of evaporation.

5.9 Column for drying the extract, filled with 5 g to 7 g of sodium sulfate (4.3) giving a height of about 7 cm to 10 cm. For example, the dimensions are 10 mm internal diameter and 250 mm length (see figure E.1).

5.10 Column for the alumina-alumina/silver nitrate clean-up, for example, the dimensions are 10 mm internal diameter and 250 mm length (see figure E.1).

5.11 Macrocolumn for the silica gel clean-up, for example, the dimensions are 19 mm internal diameter and 400 mm length (see figure E.1).

5.12 Microcolumn for the silica gel clean-up, for the dimensions see figure F.1.

5.13 Microlitre syringes.

5.14 Miscellaneous glassware.

Laboratory glassware shall be cleaned using a cleaning agent (laboratory detergent) followed, for example, by either a treatment with chromium(VI)/sulfuric acid mixture, or peroxodisulfate/sulfuric acid mixture and subsequently washed by hexane or heated for at least 12 h at 200 °C, except for the calibrated glassware.

The efficiency of the treatment shall be experimentally checked at random by blank determinations to ensure that no interfering contamination has occurred.

6 Sampling and sample preparation

Take samples according to ISO 5667-1 and ISO 5667-2.

Collect the water samples in brown glass bottles cleaned as described in 5.14 (do not use plastics bottles) with ground-glass stoppers or with screw caps with PTFE liners, of nominal capacity 1 litre to 5 litres. Fill the bottles to 80 % to 90 %.

On sample collection, ensure that no interfering substances enter the water sample, and no losses of the determinands occur. This is especially important when using plastics tubing with the sampling apparatus. If necessary, it shall be proved by control tests that no losses by adsorption occur. Glass and stainless steel devices shall preferably be used.

Check the pH. If necessary, correct the pH immediately after collection in order to be in the range pH 5 to 7,5.

If endosulfan is to be determined, take a separate sample and keep it under acidic conditions (pH 2) until extraction.

Do not place samples in close proximity to the concentrated insecticide or PCB or chlorobenzene solutions. Store in the dark at a temperature of approximately 4 °C prior to extraction.

Ensure that all samples are extracted as soon as possible (preferably within 24 h) to avoid decomposition of the compounds after sampling.

Halogenated hydrocarbons of low volatility and organochlorine insecticides are relatively stable if transferred into an organic solvent. Therefore, it is permissible to store the dried solvent extracts in a refrigerator at 4 °C for up to two months. Evaporation of the solvent can still occur even under refrigeration. Extracts shall not be allowed to go to dryness and the volume of solvent shall be restored to the original amount before starting analysis.

7 Procedure

7.1 Sample pretreatment

Sample pretreatment is not normally necessary.

If the sample container is filled up to the ground-glass joint, shake and pour off 30 ml to 100 ml of the sample in order to obtain sufficient free volume for the subsequent addition of the solvent.

Measure the volume of the water to be extracted by weighing the bottle before extraction and after emptying.

7.2 Extraction and separation

Use either of these two procedures for extraction and separation:

— extraction in the sample container and separation in a separating funnel (7.2.1);

— extraction in the sample container with a magnetic stirrer or a high-speed stirrer and separation by a microseparator (7.2.2).

NOTE 7 Depending on the method used, varying recoveries and reproducibilities may be obtained. The yields of the selected method should be checked by the laboratory (8.3).

It is recommended to perform the extraction in the sample container. Usually, a sample volume of about 1 litre is used.

7.2.1 Extraction by shaking the sampling bottle and separation in a separating funnel

Add 30 ml of the extraction solvent (4.2) to the sample (7.1) and shake for at least 10 min.

Transfer to a separating funnel of suitable capacity (5.3) and allow the phases to separate.

Run the lower aqueous phase back into the sample container. Repeat the extraction twice with 20 ml to 30 ml of the extraction solvent (4.2).

Dry the extract using one of the following procedures:

— Pass the extract through a drying column (5.9) containing anhydrous sodium sulfate (4.3), previously washed with the solvent (4.2) and collect the eluate in the evaporating vessel.

NOTE 8 It is advisable to wash the column with a further 10 ml to 20 ml portion of the solvent (4.2) to obtain a better recovery. Collect the washings in the evaporating vessel.

Or

— Add anhydrous sodium sulfate (4.3) to the flask. Shake for 1 min. Leave for 5 min and decant the extract into the concentration apparatus. The sodium sulfate is washed with a further 10 ml to 20 ml of solvent (4.2) and the washings added to the evaporating vessel.

Or

— Freeze the extract at – 18 °C for 2 h. The solvent extract is decanted from the ice and transferred to the evaporating vessel. The ice is washed with a further 10 ml of solvent (4.2) and the washings are added to the evaporating vessel.

7.2.2 Extraction with a magnetic or a high-speed stirrer and separation in a microseparator

Add 20 ml to 30 ml of the extraction solvent (4.2) to the sample (7.1).

With a magnetic stirrer and a stirring bar (5.4), stir for at least 10 min, at a speed of at least 1 000 r/min (the solvent needs to be dispersed finely in the water) keeping the sample covered, and then allow the phases to separate. Alternatively, if a high-speed stirrer (5.4) is used, stir for 2 min while keeping the sample covered at a temperature of 4 °C and allow the phases to separate.

Assemble the microseparator (5.5); pour purified water (4.1) into the funnel until the surface of the organic phase rises sufficiently for the extract to be withdrawn with a pipette.

Dry the extract as described in 7.2.1.

7.3 Concentration of the extract

Concentrate the combined dried extracts from either 7.2.1 or 7.2.2 by either of the procedures described in 7.3.1 or 7.3.2 or by any other suitable system (5.8). Ensure that no significant losses of the more volatile determinands of interest occur.

7.3.1 Concentration using a Kuderna-Danish evaporator

Good detection limits can be obtained by evaporating the sample extract to a small volume with the Kuderna-Danish evaporator (5.6) and a Snyder microcolumn (5.7) as follows.

Collect the dried extract in a Kuderna-Danish evaporator.

Add two anti-bumping granules (4.11) and evaporate to 5 ml ± 1 ml on a steam bath. Further concentrate the extract to less than 1 ml using a Snyder microcolumn or a gentle stream of clean inert gas (e.g. nitrogen) with a tube placed in a warm water bath (not exceeding 40 °C).

NOTE 9 No further precautions are necessary if the extract is evaporated with this apparatus to a final volume of not less than 0,5 ml. If a smaller final volume is required, it is recommended to use a keeper (4.4) in order to avoid significant losses. Decane or dodecane may be used as keepers because they are not detected by the electron-capture detector. 0,1 ml of a solution containing 20 g/l of decane or dodecane in hexane are added to the extract to be concentrated.

7.3.2 Concentration using a rotary evaporator

Concentrate the extract in a tapered flask, or preferably, in a tapered flask with an ampoule extension on a rotary evaporator (5.8) to not less than 0,6 ml at a constant vacuum of greater than 340 mbar. A Kuderna-Danish evaporation flask (5.6) is mounted between the evaporating vessel and the rotary evaporator.

Place the evaporating vessel with the solvent extract in an unheated water bath or, for higher boiling extractants, in a water bath at a temperature not exceeding 50 °C. When the concentration is finished, quantitatively transfer the extract into a 1 ml measuring flask. Carefully rinse the walls of the evaporating vessel with a small volume of solvent (4.2). Transfer the rinsings to the measuring flask and fill up to volume with the solvent.

7.4 Gas chromatography

For extracts of samples from clean waters, perform gas chromatographic analysis at this stage without further clean-up.

If the analysis has to be performed with a purification step, proceed to 7.5.

Set up the gas chromatograph (5.1), fitted with an electron-capture detector and equipped with a suitable column (5.2) according to the instructions of the manufacturer, and ensure it is in a stable condition.

Inject the extract (usually between 1 µl and 10 µl but the same volume as that used for calibration) into the gas chromatograph and run a chromatogram.

Compare the gas chromatogram obtained to those of the standard solutions (see clause 8).

Evaluate the gas chromatogram qualitatively and quantitatively (see clause 9).

The requirements applicable to the extent of the measurements, and the calibration, evaluation and calculation techniques to be used, are described in clause 8. The gas chromatogram obtained is checked for overlapping occurring at the locations of the retention times of the determinands of interest. If interfering peaks are present, one of the purification methods described in 7.5 shall be applied. Otherwise, identify and quantify according to clause 9.

7.5 Clean-up and separation

Applying the procedure described in 7.2 may lead to coextraction of relatively polar and/or other undesired substances, which are likely to interfere by the appearance of unknown peaks overlapping the pesticide peaks.

NOTE 10 Treatment by column chromatography may help to eliminate some of the substances. However, this method cannot be considered as an absolute system.

Use one or both of the following procedures:

— clean-up on an alumina-alumina/silver nitrate column, for purification to remove polar compounds (7.5.1);

— clean-up on a silica gel column, for separation of PCB from most insecticides (7.5.2).

NOTE 11 The quality of each batch of columns should be checked with standard solutions.

7.5.1 Clean-up on alumina-alumina/silver nitrate column

Carry out the purification on an alumina-alumina/silver nitrate column as described in 7.5.1.1 and 7.5.1.2. If interference persists, the additional procedure described in annex A may be carried out.

NOTE 12 Some compounds, for example endosulfan, may be retained on the column.

7.5.1.1 Preparation of the column

Place 15 ml ± 1 ml of the extraction solvent (4.2) in the column (5.10), then add 1,0 g ± 0,2 g of alumina/silver nitrate (4.7) and allow to settle while tapping gently. Then add 2,0 g ± 0,2 g of alumina (4.6) and again allow to settle while tapping gently. Add a sufficient amount of sodium sulfate (4.3) to produce a 5 mm layer on top of the column. Prepare the column immediately before use.

7.5.1.2 Purification

Prepare an alumina-alumina/silver nitrate column as described in 7.5.1.1. Run off the surplus of the extraction solvent (4.2). When the solvent level reaches the top of the column, add the concentrated sample extract (see 7.3). Wash the sample vessel with 2 ml ± 0,5 ml of extraction solvent and add the washings to the column. Elute the column with 30 ml ± 1 ml of extraction solvent. Collect and concentrate the extract as described in 7.3 and then perform the gas chromatographic analysis according to 7.4.

During addition to the column, do not allow the meniscus of the solvent (4.2) to fall below the surface of the alumina. If the alumina/silver nitrate column blackens along its entire length, prepare a fresh column (see 7.5.1.1) and repeat the purification. If total blackening is a common occurrence, larger columns may be used but additional solvent will be required for elution.

7.5.2 Clean-up on silica gel

7.5.2.1 Preparation of the column

Choose a chromatography column (5.12) as shown in figure F.1 in annex F. [Initially without the solvent reservoir (figure E.2) attached.] Plug the column temporarily with a rubber cap at the lower end, and fill it with extraction solvent (4.2).

Insert a plug of glass wool (4.15) close to the lower end.

Suspend 1 g of silica gel (4.8) in the extraction solvent (4.2) in a small beaker.

Transfer the suspension to the chromatography column with the aid of a pipette.

Let the silica gel settle down during constant vibration of the column, to produce a dense layer. Otherwise, the sodium sulfate which is placed onto the silica gel will move into the silica gel layer.

Remove the rubber cap.

Carry out the following steps, including the steps described in 7.5.2.2, without interuption as soon as the column starts dripping continuously.

Place 0,2 g of sodium sulfate (4.3) onto the layer of silica gel. Attach the solvent reservoir to the column and rinse the system with 5 ml of solvent (4.2).

Once again, remove the solvent reservoir as soon as the level of solvent has moved down to the column section of the apparatus and follow the steps described in 7.5.2.2 immediately.

NOTE 13 Alternatively, dry packed and/or commercially available disposable columns may be used, if they are found to be equally suitable.

7.5.2.2 Clean-up and separation

Add 100 µl of the sample extract onto the column with the aid of a 100 µl syringe, just before the meniscus of the solvent has reached the sodium sulfate layer.

NOTES

14 The flow rate should be about 1 to 2 drops per second.

15 Depending on the concentration of organochlorine compounds in the sample, it is recommended that at least 1/10 of the whole sample extract be taken for the clean-up. This means that the sample extract has to be concentrated to a volume of 1 ml or less by the methods described in 7.3, prior to the clean-up.

Attach the solvent reservoir again (see 7.5.2.1) and add 5 ml of extraction solvent (4.2).

For the acceleration of the chromatography process, connect a pressurized inert gas supply (e.g. nitrogen) at a pressure of about 25 mbar.

Collect the first fraction in a graduated Kuderna-Danish vessel. When the meniscus of the solvent has reached the sodium sulfate layer, add additional solvent. After disconnecting from the pressurized gas supply, repeat the steps in the following order:

— second fraction: 2,5 ml of solvent (4.2);

— third fraction: 2,5 ml of solvent;

— fourth fraction: 8 ml of solvent;

— fifth fraction: 8 ml of solvent/toluene (4.9) (95:5) (*V/V*);

— sixth fraction: 16 ml of solvent/toluene (90:10) (*V/V*);

— seventh fraction: 8 ml of solvent/diethylether (4.10) (99,5:0.5) (*V/V*).

Before concentrating, combine the fractions as appropriate.

Concentrate the fractions collected as described in 7.3 and then perform a gas chromatographic analysis according to 7.4.

NOTE 16 Table G.1 in annex G gives a typical example covering the elution sequence of 27 compounds and of their recoveries with the macrocolumn for the silica gel clean-up (5.11), including a subsequent concentration with the rotary evaporator procedure.

7.6 Blank determination

Carry out the complete procedure (pretreatment, extraction, concentration, clean-up, gas chromatographic analysis) using a sample of pure water (4.1).

If the blank value is unreasonably high, i.e. greater than 10 % of the lowest value for any of the compounds of interest, carry out a step-by-step examination of the procedure and eliminate the cause.

8 Calibration

Initially, it is necessary to determine the recovery using the following methods.

a) Calibration by direct injection of solvent standard solutions (8.1).

This gives information on the linear working range of the detector, retention times and relative responses of the determinands.

b) Calibration of the overall procedure (8.2) using water samples (preferably of the same type as those being analysed), which are spiked and extracted and, if necessary, cleaned-up.

The data obtained from a) are compared with those from b) in order to calculate the recovery (8.3) of each determinand.

Carry out the daily recalibration (8.4) with solvent standard solutions according to a) or with spiked water extracts according to b).

Table 2 gives an explanation of the subscripts used in the equations and in the explanations of symbols after the equations.

Table 2 — Explanation of the subscripts used in the symbols

Index	Meaning
i	Identity of the determinand
e	Measured value in calibration
g	Entire procedure

8.1 Calibration by external standard, not using the overall procedure

Inject volumes in the range of 1 µl to 10 µl of the working standard solutions (4.14) into the gas chromatograph.

Measure the gas chromatographic signals for each substance (peak heights or peak areas or area integration units, respectively) and calculate the concentrations.

For a graphic presentation of the calibration curve, plot the respective measured values, y_{ie}, on the ordinate against the respective mass concentrations, ρ_{ie}, of the substance i (e.g. in the solvent) on the abscissa.

The injection volume used for calibration and for the measurement of the sample solutions shall be kept constant.

The series of measured values thus obtained shall be used to establish the linear regression function as follows:

$$y_{ie} = m_i \cdot \rho_{ie} + b_i \qquad \dots (1)$$

where

y_{ie} is the dependent variable: measured response of the substance i, dependent on ρ_{ie} (its unit depends on the evaluation, e.g. area value);

ρ_{ie} is the independent variable: mass concentration of the substance i (external standard) in the calibration solution, in nanograms per microlitre;

m_i is the slope of the calibration curve of the substance i (its unit depends on the evaluation, e.g. area value × µl/ng);

b_i is the intercept of the calibration curve on the ordinate (its unit depends on the evaluation, e.g. area value). As a rule, the intercept is very small. If large intercepts occur, omit the highest concentration(s) of the standard(s) and recalculate the linear regression function. This should reduce the value of the intercept. If not, the gas chromatographic system and the evaluation system should be checked.

8.2 Calibration of the overall procedure using an external standard

For each compound, a separate calibration graph (via the overall procedure) shall be established, consisting of at least five points. It is permissible to examine several compounds in one calibration experiment.

To calibrate the entire procedure, prepare aqueous solutions by spiking water (4.1) with the compounds to be determined in an individual concentration range within the linear dynamic range of the detector, as follows.

8.2.1 Preparation of the spiked aqueous standard solutions

To a 100 ml graduated flask, containing about 90 ml of water-miscible solvent (4.16) using a microlitre syringe (5.13), add defined quantities of the standard stock solutions (4.12) of each determinand, under the surface of the solvent.

Immediately dilute to volume with the water-miscible solvent (4.16).

Stopper the flask with its ground-glass stopper and cautiously shake the solution.

Calculate the respective concentration of each substance added.

The stock solution prepared in this way can be stored at a temperature of about 4 °C in the dark for several weeks. Prior to use, equilibrate at room temperature for at least 15 min.

Prepare at least five spiked aqueous standard solutions covering (depending on the compounds) the range 1 ng/l to 200 ng/l, by adding different volumes of this stock solution to water (4.1).

For blank measurements, to one bottle of water (4.1), add the same quantity of solvent as that used for the preparation of the spiked aqueous standard solutions.

Use the quantities such that the volume added is as small as possible (< 1 ml/l of water), in order to minimize any effect on the partition equilibrium.

Prepare the spiked aqueous standard solutions on the day of use.

8.2.2 Calibration curve

Extract and concentrate these spiked aqueous standard solutions as described in 7.2 and 7.3.

Inject the extract of the blank into the gas chromatograph, and then the calibration solutions with concentrations ρ_{ieg} in ascending order. Measure the peak values y_{ieg} of the calibration samples.

Calculate a regression function for each substance using the pairs of values y_{ieg} and ρ_{ieg}:

$$y_{ieg} = m_{ig} \cdot \rho_{ieg} + b_{ig} \qquad \dots (2)$$

where

y_{ieg} is the dependent variable: measured response of the substance i during calibration, dependent on ρ_{ieg}, (its unit depends on the evaluation, e.g. area value);

ρ_{ieg} is the independent variable: mass concentration of the substance i in the calibration solution, expressed in micrograms per litre;

m_{ig} is the slope of the calibration curve of the substance i, often referred to as f_i (its unit depends on the evaluation, e.g. area value × l/µg);

b_{ig} is the intercept of the calibration curve on the ordinate (its unit depends on the evaluation, e.g. area value).

Plot the reference functions in a diagram with the ordinate as the specific measured signals of the sub-