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Water quality — Determination of short-chain polychlorinated alkanes (SCCP) in water — Method using gas chromatography-mass spectrometry (GC-MS) and negative-ion chemical ionization (NCI)

Qualité de l'eau — Détermination des alcanes polychlorés à chaîne courte (SCCP) dans l'eau — Méthode par chromatographie gazeuse-spectrométrie de masse (CG-SM) avec ionisation chimique négative (ICN)

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

This second edition cancels and replaces the first edition (ISO 12010:2012), which has been technically revised. The main changes compared to the previous edition are:

- the m/z values (mass/charge ratios) for quantification and identification;
- the calibration mixtures;
- the clean up procedure by gel chromatography;
- reduced interferences.

Introduction

The user should be aware that particular problems might require the specifications of additional marginal conditions.

This document achieves synergetic effects in the practical laboratory work. The following points partially allow a combination of water and sediment analysis:

1) same mass combination as for sediment analysis (see ISO 18635[2]);
2) same calibration mixtures as for sediment analysis (see ISO 18635);
3) same GPC-clean up as for sediment analysis (see ISO 18635).

Water quality — Determination of short-chain polychlorinated alkanes (SCCP) in water — Method using gas chromatography-mass spectrometry (GC-MS) and negative-ion chemical ionization (NCI)

WARNING — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices.

IMPORTANT — It is absolutely essential that tests conducted in accordance to this document be carried out by suitably qualified staff.

1 Scope

This document specifies a method for the quantitative determination of the sum of short-chain polychlorinated *n*-alkanes also known as short-chain polychlorinated paraffins (SCCPs) in the carbon bond range n-C_{10} to n-C_{13} inclusive, in mixtures with chlorine mass fractions ("contents") between 50 % and 67 %, including approximately 6 000 of approximately 8 000 congeners.

This method is applicable to the determination of the sum of SCCPs in unfiltered surface water, ground water, drinking water and waste water using gas chromatography-mass spectrometry with electron capture negative ionization (GC-ECNI-MS).

Depending on the capability of the GC-ECNI-MS instrument, the concentration range of the method is from 0,1 µg/l or lower to 10 µg/l. Depending on the waste water matrix, the lowest detectable concentration is estimated to be > 0,1 µg/l. The data of the interlaboratory trial concerning this method are given in Annex I.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 5667-1, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes and sampling techniques*

ISO 5667-3, *Water quality — Sampling — Part 3: Preservation and handling of water samples*

ISO 8466-1, *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 1: Statistical evaluation of the linear calibration function*

ISO/TS 13530, *Water quality — Guidance on analytical quality control for chemical and physicochemical water analysis*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at https://www.iso.org/obp
- IEC Electropedia: available at http://www.electropedia.org/

4 Principle

Determination of the sum of SCCPs in the carbon bond range n-C_{10} to n-C_{13} inclusive, in technical and environmental transposed mixtures with chlorine mass fractions (“contents”) between 50 % and 67 % (for example a mean of approximately 4 to 10 chlorine atoms per molecule) and independent of the C-number distribution pattern of the congeners. No recognition of the chlorine content is necessary.

The analysed sum of SCCPs includes the variety of SCCPs with their differing chlorine content and C-number distribution patterns as found in technical mixtures as well as compositions in the environment (References [5] to [9]).

SCCPs in whole water samples are fortified with an internal standard and extracted using liquid-liquid extraction with an organic solvent. The sample enrichment procedure is followed by a clean-up procedure to eliminate interfering compounds. Gas chromatography (GC) is undertaken using a short capillary column within a short retention time range. The detection of selected mass fragments is carried out using mass spectrometry (MS) in selected ion-monitoring mode using electron capture negative ionization mode (ECNI). The mass fragments and the compositions of the calibration solutions used in this document are essential for the analysis of the sum of SCCPs (see References [3] and [4]).

The selected ion chromatogram is integrated over the full retention time range of the SCCPs. The quantification of the sum of SCCPs is carried out after establishing a calibration by a multiple linear regression. The calibration requires the specified three differently composed standard mixtures fortified with an internal standard.

These standard mixtures mimick different mixtures found in the environment. In this method, only the multiple linear regression quantification with these specific mixtures enables the quantification of the variety of observed mixtures of SCCP in the environment and in technical compositions, described in Clause 1 and in References [3] and [4]. It is not possible to use only one reference mixture for that complex task.

The method allows for a quantification of the sum of SCCPs expected to be within an expanded measurement uncertainty of less than 50 %.

5 Interferences

Non-specific matrix interferences, as well as interferences from other environmental situations, are dealt with using the given clean-up procedure. A further reduction of matrix effects can be achieved by reducing the mass spectrometric resolution power to, for example, 0,4 u, which is often possible with a quadrupole mass spectrometer. The exact m/z values are 374,958 8; 410,916 9; 422,935 5; 448,810 6 (see Reference [8]).

Applying the entire procedure using the clean up procedure given in 9.3, a selection of chlorinated pollutants has been tested and found not to cause interferences below the concentrations given in Table 1.

Table 1 — Highest concentration level which causes no interferences higher than the limit of quantification of 0,1 µg/l

Potential interfering compounds	Highest concentration level which causes no interferences higher than the limit of quantification of 0,1 µg/l
Aroclor 1262[a]	1,25 µg/l
Aroclor 1242[a]	10 µg/l
Aroclor 1221[a]	10 µg/l

[a] Aroclor 1262, Aroclor 1242, Aroclor 1221, Halowax 1014 and Halowax 1051 are examples of suitable products available commercially. These examples are given for the convenience of users of this document and do not constitute an endorsement by ISO of these products.

Table 1 *(continued)*

Potential interfering compounds	Highest concentration level which causes no interferences higher than the limit of quantification of 0,1 µg/l
Campheclor (toxaphene)	1,75 µg/l
Halowax 1014[a]	10 µg/l
Halowax 1051[a]	0,4 µg/l
MCCP (medium-chain chlorinated *n*-alkanes C_{14}-C_{17}) 42 % chlorine	10 µg/l
MCCP (medium-chain chlorinated *n*-alkanes C_{14}-C_{17}) 52 % chlorine	6 µg/l
MCCP (medium-chain chlorinated *n*-alkanes C_{14}-C_{17}) 57 % chlorine	10 µg/l

[a] Aroclor 1262, Aroclor 1242, Aroclor 1221, Halowax 1014 and Halowax 1051 are examples of suitable products available commercially. These examples are given for the convenience of users of this document and do not constitute an endorsement by ISO of these products.

6 Reagents and standards

Use solvents and reagents of sufficient purity, i.e. with negligibly low concentrations of SCCPs, e.g. lower than the limit of detection of the method. Check blanks regularly over the entire procedure to ensure they are suitable and establish proper analytical control.

6.1 Solvents for extraction and preparation of stock solutions

The solvent for extraction is *n*-heptane. Other non-polar solvents, e.g. *n*-hexane (C_6H_{14}), cyclohexane (C_6H_{12}), can be used if the extraction efficiency is comparable with those of *n*-heptane.

Use 2,2,4-trimethylpentane (C_8H_{18}, isooctane) for conditioning of the glass bottles (7.1).

For preparation of the stock solution and dilutions of the internal standard, use propanone (acetone), C_3H_6O.

For conditioning of the clean-up columns, use mixtures of *n*-heptane and propanone (acetone).

For the first elution step of the filtrated suspended matter, use methanol (CH_3OH).

6.2 Reference SCCP stock solutions

Use commercially available solutions, such as in cyclohexane or *n*-hexane, of the single mixtures of SCCP congeners with defined carbon chain length and with different defined chlorine contents (see Table 2, first two columns). Alternatively, use commercially available ready mixed solutions with the same composition.

Mixtures of synthetic solutions were used to simulate environmentally occurring SCCPs or technical products of SCCPs. For example, the synthetic mixed calibration stock solution “Lake Ontario water” is mixed to resemble a Lake Ontario water as reported in Reference [6]. Its characteristic is a relatively high content of C_{10} to C_{12}, especially C_{12} and a low chlorine content as partly reported in water samples too. The synthetic mixed calibration stock solution “Perch” simulates a C-number distribution found in a perch (see Reference [7]). The standard mixture “Sediment Drevnice” simulates a natural mixture reported about a sediment of the river Drevnice (see Reference [8]) with a high content of C_{13} and a higher chlorine content.

The compositions of the calibration mixtures as well as of the independent quality assurance solutions are mandatory to achieve the quantification of the variety of SCCP-mixtures.

Prepare the solutions “Lake Ontario water”, ”Perch”, and “Sediment Drevnice” according to Table 2.

Table 2 — Reference substances stock solutions

Commercially available standard solutions			**Reference substances stock solutions in accordance with 6.2** (Synthetic mixed standard solutions, which resemble environmental mixtures composition, ng/ml)		
n-alkane chain length	Chlorine content % of the individual C-number mixtures as certified	Mean number of chlorines in the molecules (calculated)	“Lake Ontario water” Chlorine content[a] 50,2 %	“Perch” Chlorine content[a] 60,6 %	“Sediment Drevnice” Chlorine content[a] 65,0 %
C_{10}	50,18	3,97	1 000		
C_{10}	55,00	4,79	1 000		
C_{10}	60,09	5,86		500	
C_{10}	65,02	7,16		1 100	280
C_{11}	45,50	3,63	1 000		
C_{11}	50,21	4,37	1 000		
C_{11}	55,20	5,31		600	
C_{11}	60,53	6,55		1 000	500
C_{11}	65,25	7,94		3 000	660
C_{12}	45,32	3,93	2 000		
C_{12}	50,18	4,76	2 000	800	
C_{12}	55,00	5,74	2 000	2 000	
C_{12}	65,08	8,59		900	1 000
C_{12}	69,98	10,62			830
C_{13}	59,98	7,56		100	730
C_{13}	65,18	9,34			6 000
Sum of SCCP (ng/ml)			**10 000**	**10 000**	**10 000**

[a] The chlorine content of the mixtures is calculated as the weighted mean.

Store the prepared solutions in a refrigerator at 2 °C to 8 °C. Avoiding losses of the solvent by evaporation, solutions can be used for five years.

Use as well commercially available solutions, e.g. in cyclohexane or *n*-hexane, of the reference substances stock solutions (see Table 2, last three columns) of SCCP. See Reference [8].

An example is DRE-ZS22102105HP[1]. See Reference [8].

6.3 Internal standard stock solutions from individual congeners

Use commercially available individual congener standard solutions and prepare a stock solution in propanone (acetone) (6.1) at a concentration of, for example, 1 µg/ml.

Individual SCCP congeners with chlorine contents of between 50 % and 67 % are suitable as internal standards, for example:

- 1,1,1,3,10,11-hexachloroundecane, with e.g. 0,1 µg/ml;
- 1,1,1,3,11,13,13,13-octachlorotridecane, with e.g. 0,1 µg/ml;
- 1,2,5,5,6,9,10-heptachlorodecane, with e.g. 0,01 µg/ml.

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1) DRE-ZS22102105HP is an example of a suitable product available commercially. These examples are given for the convenience of users of this document and do not constitute an endorsement by ISO of these products.

NOTE 1 The different individual SCCP congeners used as internal standard substances contribute in environmental samples to the sum of SCCPs. Nevertheless, the contribution is approximately <1 %, which means that the enhancement of the measurement uncertainty is negligible.

NOTE 2 Different individual SCCP congeners can produce different response factors, hence it can be necessary for different concentrations to be used.

If validated, other individual SCCP congeners can be used as the internal standard if the congener shows the same properties over the entire analytical process as the SCCPs being determined.

The solutions can be stored in a refrigerator at 2 °C to 8 °C.

6.4 Calibration solutions

Use the standard mixtures according to Table 2. Prepare a minimum of nine calibration solutions (see Table 3) with concentrations according to the detection capability of the mass spectrometer. Combine and dilute the solutions (6.2) and the internal standard solution (6.3) with *n*-heptane to produce solutions for the calibration range.

Table 3 — Calibration solutions

Sum of SCCPs, µg/ml	**“Lake Ontario water”** µg/ml	**“Perch”** µg/ml	**“Sediment Drevnice”** µg/ml	**Internal standard** e.g. 1,1,1,3,11,13,13,13-octa-chlorotridecane µg/ml
0,15	0,15			0,1
0,15		0,15		0,1
0,15			0,15	0,1
0,3	0,3			0,1
0,3		0,3		0,1
0,3			0,3	0,1
0,6	0,6			0,1
0,6		0,6		0,1
0,6			0,6	0,1

The solutions may be stored in a refrigerator at 2 °C to 8 °C at least for six months. Check the concentration of the calibration solutions against an independently prepared standard prior to use.

Quality control check solutions shall be prepared to check the calibration independently. To do so, use the mixtures as given in Annex A.

6.5 Extraction auxiliary and clean-up materials

6.5.1 Copper powder, grain size < 63 µm. Copper powder is used in the clean-up procedure to remove sulfur and sulfur-containing matrix components.

6.5.2 Hydrochloric acid, 2 mol/l, used for copper activation in the clean-up column.

6.5.3 Aluminium oxide, Al_2O_3, neutral, high activity (10 % water).

6.5.4 Glass wool.

6.6 Operating gases, for GC-MS, of high purity and in accordance with the manufacturer’s specifications.

6.7 Nitrogen, N_2, purity ≥ 99,996 % volume fraction, for concentrating the solutions.

6.8 Sodium sulfate, anhydrous, Na_2SO_4, powdered.

6.9 Test solution for check of linearity of the internal standards.

Prepare solutions of the internal standard used at concentrations of 0,1 µg/ml, 0,5 µg/ml, and 1 µg/ml.

7 Apparatus

Glassware and equipment which may come into contact with water samples or their extracts should be free from interfering compounds.

Clean all glassware by rinsing with propanone (acetone) (6.1).

7.1 Flat-bottomed glass bottles, conical shoulder, 1 000 ml capacity, for collecting water samples, preferably with glass stoppers.

The sample bottle shall enable direct extraction of the sample to be undertaken.

Before use, condition it by rinsing the dry sample bottle with, for example, 2 ml of isooctane (6.1). Then, invert it and allow the solvent to drain and evaporate from it.

7.2 Evaporation device, e.g. rotary evaporator, or nitrogen evaporating system.

7.3 Separator, for example micro-separator in accordance with ISO 6468[1], separation funnel or other suitable device for phase separation.

7.4 Vials, compatible with the GC-autosampler (e.g. with a capacity of 1,5 ml).

7.5 Chromatographic column, internal diameter (ID) 10 mm (empty) for clean-up.

7.6 Gas chromatograph, temperature-programmable, with all required accessories, including gases, capillary column, split/splitless injector and mass spectrometer detector with negative-ion chemical ionization option and appropriate reactant gas (CH_4).

7.7 Volumetric flasks, 1 ml, 2 ml, 10 ml and 25 ml.

7.8 Disposable glass Pasteur pipettes, e.g. 150 mm or 250 mm.

7.9 Syringes, 2 µl, 5 µl, 10 µl and 50 µl.

7.10 Analytical column

Fused silica column with medium or non-polar low bleed separating phase (see Annex C for examples); e.g. ID < 0,25 mm, length 15 m and film thickness 0,1 µm.

7.11 Glass fibre filter, binderless, fine porosity (<0,45 µm particle retention).

7.12 Vacuum filtration device, volume 1 l.

7.13 Shaking device or magnetic stirrer device (with a magnetic stir bar).

7.14 GPC clean-up system (with modular design).

7.14.1 Pump, sampling injector, sample rack; fraction collector.

7.14.2 GPC-Column: Shodex CLNpakPAE 800 AC®[2], Maximum pore size 40 nm, column size 80 mm (inner diameter) × 300 mm (length).

8 Sampling and sample pretreatment

Take samples as specified in ISO 5667-1 and ISO 5667-3. To collect water samples (1 l per sample), use conditioned glass bottles (7.1). Do not fill the sample bottle completely (e.g. fill to the shoulder) in order to allow the addition of the extracting solvent.

Samples are extracted without filtering the sample and suspended solids are not removed prior to analysis.

Weigh, to the nearest gram, the sample bottle with its contents and cap, and record the mass for subsequent use. Thoroughly shake the bottle to homogenize the water sample. Add the internal standard solution (6.3) to achieve a concentration of, for example, 0,1 µg/l in the water sample. Record the mass, in micrograms, of internal standard added. Shake the bottle thoroughly.

9 Procedure

9.1 Extraction with liquid-liquid extraction

Add 10 ml of extraction solvent, *n*-heptane (6.1), to the bottle and shake it or stir (7.13) thoroughly for about 2 h to carry out the extraction directly in the sample bottle. Allow the phases to separate and use the separator (7.3) to collect the organic extract in a separate tube. If an emulsion forms, break it by centrifuging and/or by adding sodium sulfate (6.8) to the tube. Discard the remaining water to waste. Transfer the solvent from the tube to the evaporating device (7.2) or, using a gentle stream of nitrogen (6.7), carefully evaporate the solvent (at a temperature of 40 °C) to about 1 ml. Weigh, to the nearest gram, the empty sample bottle and cap. Calculate the volume of water extracted and the concentration of internal standard in the water.

Proceed as in 9.3.

9.2 Extraction with higher content of suspended matter

If the content of suspended matter is higher than approximately 200 mg/l, filter the sample through a glass fibre filter (7.11) and collect the filtrate in the bottle (7.1).

Weigh, to the nearest gram, the empty sample bottle and cap. Calculate the volume of water extracted and the concentration of internal standard in the water.

Add 10 ml of methanol to the filter (without vacuum) separately to extract the suspended matter. Allow to soak for 5 min, then use vacuum to add methanol to the sample filtrate collected before.

Add 10 ml *n*-heptane (without vacuum) to the filter and allow to soak for another 5 min, then use weak vacuum to add *n*-heptane also to the sample filtrate collected before.

Shake or stir (7.13) the mixture thoroughly for about 2 h to carry out the extraction directly in the sample bottle. Allow the phases to separate and use the separator (7.3) to collect the organic extract in a separate tube. If an emulsion forms, break it by centrifuging and/or by adding sodium sulfate (6.8) to the tube. Discard the remaining water to waste. Transfer the solvent from the tube to the evaporating

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[2] Shodex CLNpakPAE 800 AC is an example of a suitable product available commercially. This example is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

device (7.2) or, using a gentle stream of nitrogen (6.7), carefully evaporate the solvent (at a temperature of 40 °C) to about 1 ml.

9.3 Extract clean-up

Often, matrix contents can be cleaned by procedure b) only.

A two-step clean-up procedure [a) and b)] for high matrix content shall be carried out.

Begin the cleanup procedure using the extract concentrated to approximately 1 ml in *n*-heptane:

a) column chromatographic clean-up with 2 g activated copper powder and (6.5.1) 2 g Al_2O_3, neutral, high activity (6.5.3);

b) gel chromatographic clean-up (7.14). with Shodex CLNpakPAE 800 AC®[3], 8,0 mm × 300 mm and 0,5 ml/min propanone (acetone) as the eluent.

Fill the copper powder in a glass column with a glass wool plug. Activate the copper by adding 10 ml of 2 mol/l hydrochloric acid (6.5.2). Allow all of the hydrochloric acid (6.5.2) to soak into the copper powder before washing the column, first with 25 ml of water and subsequently with 20 ml of acetone to remove acid and water from the column. Then, a stopcock may be attached to the bottom of the column for controlling the elution progress. After the solvent level has reached the upper level of the copper powder, wash the copper layer with 3 × 2 ml *n*-heptane. Then, 2 g of aluminium oxide (10 % water) and about 10 ml of *n*-heptane are added for obtaining a copper/aluminium oxide - sandwich column. The wet column is used for applying the sample extract. The SCCPs are eluted by 10 ml of a mixture of *n*-heptane/acetone (98:2) which is concentrated to approx. 1,2 ml. It shall be noted that the column shall never run dry.

This concentrate is used for the subsequently following GPC clean-up. Inject e.g 1 ml of the concentrate and elute in a fraction between 12 ml and 13,5 ml. This fraction is concentrated again to, for example, 1 ml, dried by sodium sulfate (6.8) and transferred into a sample vial for injection into the GC-MS.

When using a new column for the GPC clean-up, verify the eluent volume for complete elution of the analytes of interest by analysing an appropriate standard solution and/or spiked sample extract. Recoveries of SCCPs should be > 50 % and no interfering peak should appear in the gas chromatogram. If necessary, GPC conditions need to be modified to meet these requirements.

NOTE 1 Alternative clean-up procedures, an extended column chromatographic clean-up (see Annex F) and a modified gel chromatographic clean-up (see Annex G) can be used. The interferences quantified in Clause 4 of this document apply only to the conditions described in this clause.

NOTE 2 Due to the physico-chemical properties of SCCP's results in water higher than the LOD (limit of detection) are very rare. The absence of SCCP can be proved by testing without the described clean up steps only after drying the extract. If the peak areas of the specific mass ions are below the peak areas of the LOD, a result < LOD can be reported.

9.4 Measurement and integration of the chromatogram

Optimize the operating conditions of the GC-ECNI-MS system, e.g. according to the manufacturer's instructions. Examples of the gas chromatographic conditions are given in Annex C.

Prior to analysis, verify the performance of the GC-ECNI-MS system by analysis of calibration standards. Use as a minimum the calibration solutions "Lake Ontario water" and "Sediment Drevnice" to optimize the GC-ECNI-MS system.

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[3] Shodex CLNpakPAE 800 AC® is an example of a suitable product available commercially. This example is given only as information for the convenience of users of this document and does not constitute an endorsement by ISO of this product.