INTERNATIONAL STANDARD

ISO 12010

Second edition
2019-03

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)

Reference number
ISO 12010:2019(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

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.

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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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.

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

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.

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.

Check the GC-ECNI-MS system performance regularly, for example between every 10 to 20 samples, by independently prepared calibration solutions (see Table 3) with a concentration of e.g. 1 µg/ml sum of SCCP.

The measurement is performed in the selected ion mode with four selected mass ion fragments (mass to charge values, *m/z*), i.e. *m/z* 375, *m/z* 411, and *m/z* 423, *m/z* 449. The peak areas of the two masses, *m/z* 375 and *m/z* 423, are used in the multiple linear regression calculation of the sum of SCCP's. The peak areas of *m/z* 411 and *m/z* 449 are used for an additional identification. This specific selection has been carried out by data analysis to analyse the sum of SCCPs of a large variety of environmentally occurring SCCP-mixtures. For an explanation of this selection, see Reference [4].

The integration of the different *m/z* values should be carried out within different retention time ranges that are established from calibration solutions. Retention time ranges of chromatograms in Annex D are given in Table 4 as an example.

Table 4 — Typical retention time ranges

m/z value	Approximate retention time range min	Approximate retention time range of the response maximum[a] min
375	6,6 to 9,0	7,1 to 8,2
411	7,0 to 8,8	7,4 to 8,2
423	7,0 to 9,0	7,5 to 8,4
449	7,0 to 8,7	7,5 to 8,2

[a] This represents the major portion of the SCCPs for the mass ion fragment monitored and is represented by an unresolved complex mixture of peaks.

An example for integration of a real sample is given in Annex H.

Use selected ion mode measurements for detecting the internal standard. Integrate the response of the internal standard as a single peak with the following *m/z* values (see Table 5).

Table 5 — *m/z* values of internal standard

Internal standard substance	*m/z* for quantification	*m/z* optional
1,1,1,3,10,11-Hexachloroundecane	364	362
1,1,1,3,11,13,13,13-Octachlorotridecane	460	458
1,2,5,5,6,9,10-Heptachlorodecane	348	346

Examples with chromatograms of the mixtures are given in Annex D.

9.5 Calibration

9.5.1 General

Short-chain polychlorinated *n*-alkanes with 50 % to 67 % chlorine content are mixtures containing approximately 6 000 congeners. SCCP compounds of different chlorine contents exhibit different response factors in ECNI-MS. Interferences occur in the mass spectra because individual compounds cannot be separated by GC.

Using multiple linear regression techniques quantification can be carried out to a large extent independent of chlorine content. See Annex B and Reference [4].

While modern mass spectrometric software frequently does not enable multiple linear regression techniques to be carried out, commercial software that does is available. See also the ready to use Excel sheet accessible through the following link: http://standards.iso.org/iso/12010/ed-2/en

9.5.2 Basic calibration

Analyse the calibration solutions (6.4) and integrate the responses as described in 9.4. Calibration is carried out by multiple linear regression using Formula (1):

$$\rho_{\sum SCCPscal} = b_0 + b_1 \frac{A_1}{A_{IS}} + b_2 \frac{A_2}{A_{IS}} \quad (1)$$

or in case of calibration only with *m/z* 411, using Formula (2):

$$\rho_{\sum SCCPscal} = b_0 + b_1 \frac{A_1}{A_{IS}} \quad (2)$$

where

$\rho_{\Sigma SCCPscal}$ is the target concentration of the sum of SCCPs in the calibration solution, in micrograms per millilitre, µg/ml;

b_0 is the regression intercept, in micrograms per millilitre, µg/ml; use it if significant, otherwise it is may be set to zero.

b_1, b_2 are the regression coefficients, in micrograms per millilitre, µg/ml;

A_1, A_2 are the peak areas of the analyte, e.g. *m/z* 375, *m/z* 423;

A_{IS} is the peak area of the internal standard, e.g. *m/z* 460.

The regression coefficients determined are used for quantification of unknown concentrations in samples. Typical regression coefficients are given in Table 6. A graphical presentation of the three-dimensional calibration area is given in B.3.

The graphical presentation of calculated against target sum concentrations of SCCPs is a suitable means for two-dimensional graphical assessment of the goodness of fit. A typical example is given in Annex E, using 9 calibration solutions in Table 3.

Table 6 — Typical regression coefficients for the sum of SCCP with 50 % to 67 % chlorine content based on internal standardization

	Regression coefficient with associated standard deviation (in brackets) for the mass ion fragment combination *m/z* 375 and *m/z* 423	**Regression coefficient with associated standard deviation (in brackets) for the mass ion fragment combination** *m/z* 411 and m/z 449	**Regression coefficient with associated standard deviation (in brackets) for the mass ion fragment** *m/z* 411
Concentration range 0,15 µg/ml to 0,6 µg/ml, see Table 3	b_0	b_0	b_0
	0,024 3; $s(b_0)$ = 0,010 2	0,079 3; $s(b_0)$ = 0,060 1 Not significant	0,097 5 $s(b_0)$ = 0,062 3 Not significant
	b_1 (*m/z* 375)	b_1(*m/z* 411)	b_1(*m/z* 411)
	0,117 7; $s(b_1)$ = 0,003 39	0,205 7; $s(b_1)$ = 0,053 79	0,141 9; $s(b_1)$ = 0,029 34
	b_2(*m/z* 423)	b_2(*m/z* 449)	

Table 6 *(continued)*

	Regression coefficient with associated standard deviation (in brackets) for the mass ion fragment combination *m/z* 375 and *m/z* 423	Regression coefficient with associated standard deviation (in brackets) for the mass ion fragment combination *m/z* 411 and m/z 449	Regression coefficient with associated standard deviation (in brackets) for the mass ion fragment *m/z* 411
	0,029 4; $s(b_2)$ = 0,001 34	−0,186 8; $s(b_2)$ = 0,135 11	
Standard deviation of the predicted concentration	0,015 2	0,095 8	0,101 9
Correlation coefficient	0,996	0,825	0,769

The calibration should be checked with independent quality control mixtures of known concentrations of the sum of SCCPs. Any variation in the expected values should not exceed specified levels. Typical examples are shown in Annex A.

Verify the limit of quantification and the limit of detection according to, for example, ISO/TS 13530. Use the graphical presentation of the goodness of fit (see Annex E) as a start-estimate of the concentration of the limits of quantification and detection. Verify these measures in suitable matrix.

9.5.3 Identification and quantification with mass fragment combinations

Values based on mass ion fragment combinations *m/z* 375 and *m/z* 423 are used to quantify the concentration, as this combination produces most precise results.

Identification criteria:

a) the chromatographic hump should be situated in the *m/z* specific retention time range of the different SCCP-standard solutions (see Table 4);

b) the shape of the chromatographic hump should resemble the SCCP-standard patterns (see Annex D and Annex H);

c) the calculated result based on *m/z* 411 (simple linear regression) or on *m/z* 411 and *m/z* 449 (negative results are a hint to MCCP-abundance) should not differ more than ±70 % of the result based on *m/z* 375 and *m/z* 423. Higher deviations can be observed with single SCCP-mixtures containing lower chlorine.

9.5.4 Calculation of the results

Calculate the results according to Formula (3) using the regression coefficients determined by the calibration (see 9.5.2).

$$\rho_{\sum \mathrm{SCCPs}} = \left(b_0 + b_1 \frac{A_1}{A_{\mathrm{IS}}} + b_2 \frac{A_2}{A_{\mathrm{IS}}} \right) \frac{\rho_{\mathrm{IS,s}}}{\rho_{\mathrm{IS,cal}}} \tag{3}$$

where

$\rho_{\Sigma \mathrm{SCCPs}}$ is the concentration of the sum of SCCPs in the water sample, in micrograms per litre, µg/l;

$\rho_{\mathrm{IS,s}}$ is the concentration of the internal standard in the water sample, in micrograms per litre, µg/l;

$\rho_{\mathrm{IS,cal}}$ is the concentration of the internal standard in the calibration solutions, in micrograms per litre, µg/ml;

b_0, b_1, b_2 are the regression coefficients, in micrograms per millilitre, known from Formula (1) or Formula (2);

A_1, A_2 are the peak areas of the analyte, e.g. m/z 375, m/z 423;

A_{IS} is the peak area of the internal standard, e.g. m/z 460.

9.5.5 Quality checks for internal standardization

Perform a linearity check according to ISO 8466-1 with the solutions, see e.g. Table 3.

Determine recovery rates of the internal standard after optimizing the clean-up procedure. Adjust, differing from 9.3, the final volume to 1 ml. Then calculate the recovery rates of the internal standards from Formula (4):

$$R = \frac{A_{IS,s}}{A_{IS,cal}} \times 100 [\%] \quad (4)$$

where

$A_{IS,cal}$ is the average peak area of the internal standard in the calibration samples;

$A_{IS,s}$ is the peak area of the internal standard in a water sample.

The minimum recovery rate of the internal standard in real samples is 25 %.

10 Expression of results

The analysis results obtained when applying this document are subject to a measurement uncertainty that is to be considered in the interpretation of the results.

Report the results as the sum of SCCPs (with chlorine content between 50 % and 67 %), in micrograms per litre, to two significant figures.

11 Test report

The test report shall contain at least the following information:

a) the test method used, together with a reference to this document, i.e. ISO 12010:2019;

b) identity of the sample;

c) expression of the results according to Clause 10;

d) any deviations from this procedure as well as the selection of alternative quantification mass fragments.

Annex A
(normative)

Independent quality control check solutions

Use commercially available solutions, e.g. 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 A.1, first two columns). Alternatively, use commercially available ready mixed solutions of independent quality check solutions (see Table A.1, last two columns). See also 6.2.

Table A.1 — Solutions for independent quality check, ng/ml

Commercially available standard solutions			**Independent quality check solutions** (Synthetic mixed standard solutions, which resemble technical mixtures composition, ng/ml)	
n-alkane chain length	Chlorine content % of the individual C-number mixtures as certified	Mean number of chlorine atoms in the molecules (calculated)	**Hordalub 80 –s1** Chlorine content[a] 55,8 %	**Hordalub 80 –s2** Chlorine content[a] 55,7 %
C_{10}	44,82	3,22		
C_{10}	50,18	3,97	500	
C_{10}	55,00	4,79	500	1 000
C_{10}	60,09	5,86		
C_{10}	65,02	7,16		
C_{11}	45,50	3,63		
C_{11}	50,21	4,37	500	500
C_{11}	55,20	5,31	2 000	2 500
C_{11}	60,53	6,55	1 900	1 400
C_{11}	65,25	7,94		
C_{12}	45,32	3,93		
C_{12}	50,18	4,76	500	500
C_{12}	55,00	5,74	2 500	2 500
C_{12}	65,08	8,59	200	200
C_{12}	69,98	10,62		
C_{13}	44,90	4,19		
C_{13}	50,23	5,16		
C_{13}	55,03	6,22	1 000	1 000
C_{13}	59,98	7,56	400	400
C_{13}	65,18	9,34		
Sum of SCCP (ng/ml)			**10 000**	**10 000**

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

See Table 2.

Table A.2 — Typical results of the independent quality check standards

SCCP-mix	Assigned concentration µg/ml	Measured concentration µg/ml
Hordalub 80-s1	0,6	0,557
Hordalub 80-s1	1	1,065
Hordalub 80-s2	0,6	0,626
Hordalub 80-s2	1	1,010

Annex B
(informative)

Explanation of the calibration of the sum of SCCPs with multiple linear regression

B.1 Common calibration with linear regression and inverse calibration

Linear regression is used, usually, with one independent variable (concentration, ρ) and one dependent variable (response, y).

The common calibration function is:

$$y = b_0 + b_1\rho \tag{B.1}$$

where

ρ is the concentration of the analyte;

b_0, b_1 are the regression coefficients;

y is the peak area or response of the analyte.

It is also possible to use the inverse function, i.e.

$$\rho = b_0 + b_1 y \tag{B.2}$$

The concentration is now a function of the response of the analyte. The difference between Formulae (B.1) and (B.2) is that linear regression now minimizes the error squares in the concentration ρ-axis and not, as before, in peak area axis y. This difference is not relevant or significant.

The type of calibration function [expressed in Formula (B.2)] can be graphically expressed in two dimensions. This two-dimensional expression line can also be expressed three dimensionally (see Figure B.1).

The goodness of fit can be expressed by the root mean square error of prediction (RMSEP), given by Formula (B.3):

$$\mathrm{RMSEP} = \sqrt{\frac{\sum_i (\rho_i - \bar{\rho}_i)^2}{v}} \tag{B.3}$$

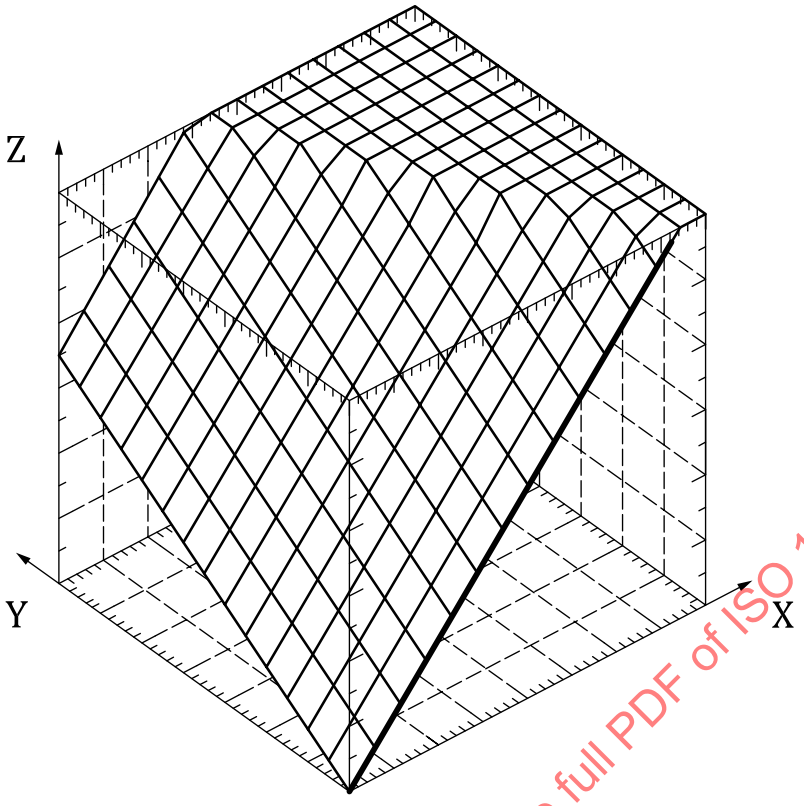
where

$\bar{\rho}_i$ is the predicted concentration of the analyte;

ρ_i is the true concentration of the analyte in the calibration sample;

v is the degrees of freedom.

The RMSEP reflects the deviation between known concentration values and calculated concentration values.



Key

X y_1 = response 1
Y y_2 = response 2
Z ρ = concentration

Figure B.1 — Common (inverse) calibration function in a three dimensional space

B.2 Multiple linear regression calibration

Compared to the common inverse linear regression with only one independent variable [see Formula (B.1)], the inverse multiple linear regression attempts to model the relationship between two or more explanatory variables such as peak areas of certain m/z values and the concentration of the analyte. In the case of this document the calibration is performed with two variables. The concentration is now dependent on two different responses, y_1 and y_2. See Formula (B.4).

$$\rho = b_0 + b_1 y_1 + b_2 y_2 \tag{B.4}$$

where

ρ is the concentration;

b_0, b_1, b_2 are the regression coefficients, determined by the software algorithm;

y_1, y_2 are different peak areas of the analyte.

The goodness of fit can also be expressed by the RMSEP (root mean square error of prediction), given by Formula (B.5):

$$\mathrm{RMSEP} = \sqrt{\frac{\sum_i \left(\rho_i - \bar{\rho}_i\right)^2}{v}} \tag{B.5}$$

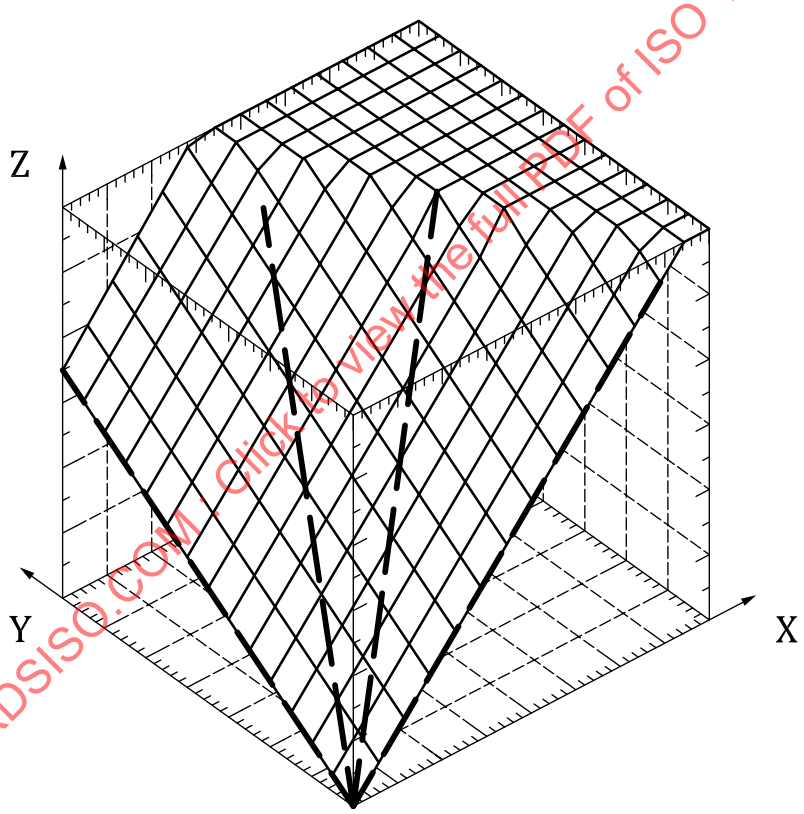
where

$\bar{\rho}$ is the predicted concentration of the analyte;

ρ_i is the true concentration of the analyte in the calibration sample;

v is the degree of freedom.

The calibration function is now expressed not by a line but by an area, demonstrated here by an area and four lines in it (see Figure B.2).



Key

X y_1 = response 1

Y y_2 = response 2

Z ρ = concentration

Figure B.2 — Calibration function with two variables y1, y2 — The calibration area

A two-dimensional presentation of the goodness of fit can be given as a recovery of predicted concentrations against true concentrations, see also Annex E.

B.3 Multiple regression calibration to analyse the sum of SCCPs

The determination of the sum of SCCPs by ECNI-MS is demanding because very different response factors are given depending on the chlorine content of the compounds (Reference [3]). A calibration by multiple regression is a way to use the information of the peak areas of two mass fragments.

SCCP congeners with smaller chlorine content contribute to smaller mass fragments, e.g. *m/z* 375. In addition, SCCP congeners with higher chlorine content contribute to heavier mass fragments, e.g. *m/z* 423. By a weighted sum of the two selected mass fragments, a description of the sum of SCCPs is possible. See References [5] and [9] for the selection procedure and the validation experiments.

A typical regression area by the selected mass fragments *m/z* 375 and *m/z* 423 is shown in Figure B.3. The calibration solutions described in Table 3 were used. A broad variety of SCCP-mixtures with different C-chain numbers and different chlorine contents is demonstrated within the calibration area.

$$\rho_{\sum \mathrm{SCCPs}} = b_0 + b_1 \frac{A_1}{A_{\mathrm{IS}}} + b_2 \frac{A_2}{A_{\mathrm{IS}}} \tag{B.6}$$

where

$\rho_{\Sigma \mathrm{SCCPs}}$ is the concentration of the sum of SCCPs, in micrograms per litre, µg/l;

b_0, b_1, b_2 are the regression coefficients, in micrograms per litre, µg/l;

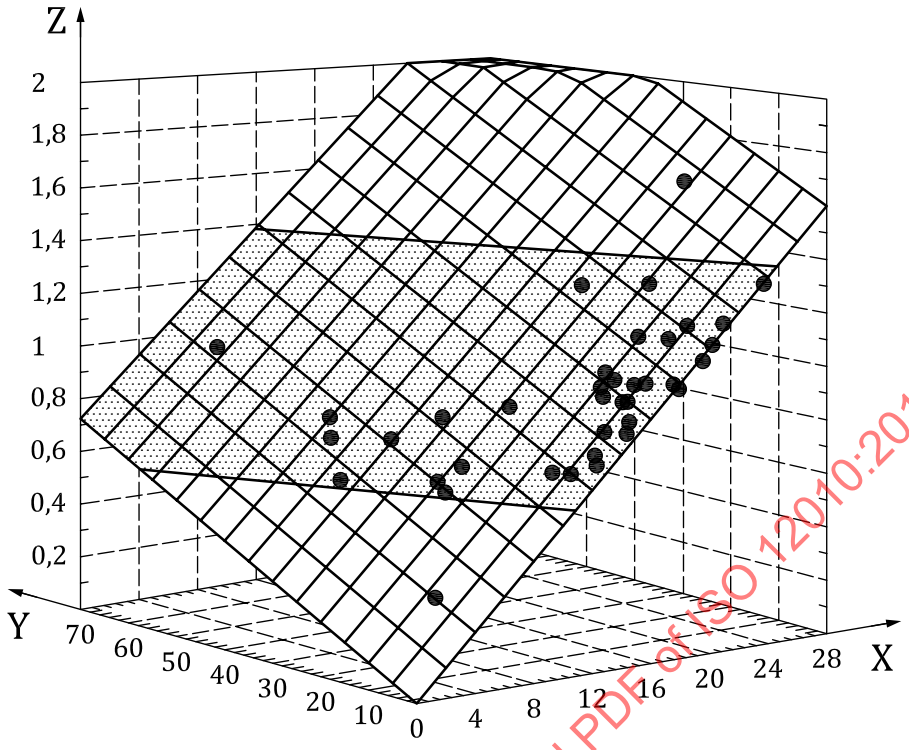
A_1, A_2 are the peak areas of the analyte, e.g. *m/z* 375, *m/z* 423;

A_{IS} is the peak area of the internal standard, e.g. *m/z* 460.

With the help of, for example, Excel[4], the calculation with the LINEST[5] function is easily possible. An example of a working sheet is given in http://standards.iso.org/iso/12010/ed-2/en.

4) Excel is the trade name of a product supplied by Microsoft. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

5) RGP in the German version of Excel.



Key

X A_1 = relative peak area m/z 375

Y A_2 = relative peak area m/z 423

Z $\rho_{\Sigma SCCPs}$ = sum of SCCPs, µg/ml

Figure B.3 — Concentration — Response area of the sum of SCCPs

In Figure B.3, data of 39 different individual mixtures in the range of 50 % to 67 % chlorine and varying C10, C11, C12 and C13 contents are presented. The calibration area is fitted by multiple linear regression of the relative responses of m/z 375 and m/z 423, grey area (1 ± 0,4) µg/ml, the assigned value is 1 µg/ml. For details concerning the individual mixtures and the belonging results, see Reference [4].

B.4 Mass spectrometric interpretation of the selected mass ions

In ECNI, mass spectrometry of SCCP's the degree of fragmentation is relatively low when compared with techniques like electron impact and positive chemical ionization (Reference [6]). In ECNI-MS, the predominant m/z values are $[M\text{-}Cl]^-$, $[M\text{-}HCl]^-$ and $[M+Cl]^-$ (see Reference [10]). This fact was tested with the adjustments of the GC-ECNI-MS equipment used in this examination. Confirmation was performed by a measured spectra of 1,2,5,5,6,9,10-heptachlorodecane. No molecule ion was detected, the major ions and their associated chlorine isotopes are m/z 345 $[M\text{-}Cl]^-$, m/z 344 $[M\text{-}HCl]^-$ and m/z 415 $[M+Cl]^-$ along with smaller amounts of m/z 309 $[M\text{-}Cl^-HCl]^-$ or $[M\text{-}HCl\text{-}Cl]^-$, m/z 308 $[M\text{-}HCl\text{-}HCl]^-$, and m/z 272 $[M\text{-}HCl\text{-}HCl\text{-}HCl]^-$.

After confirming this fragmentation pathway in the ECNI-MS apparatus used, some possible fragments belonging to the selected m/z values for quantification are the following.

The proposed elemental compositions of the selected m/z value 375 are:

M-Cl $[C_{12}H_{19}{}^{35}Cl_5{}^{37}Cl]^-$, M+Cl $[C_{12}H_{21}{}^{35}Cl_6]^-$,

And for the *m/z* value 423:

M+Cl $[C_{13}H_{22}{}^{35}Cl_7]^-$,

M+Cl $[C_{10}H_{15}{}^{35}Cl_4{}^{37}Cl_4]^-$,

M-Cl $[C_{10}H_{13}{}^{35}Cl_3{}^{37}Cl_5]^-$,

M-Cl $[C_{13}H_{20}{}^{35}Cl_6{}^{37}Cl]^-$,

M-Cl-HCl $[C_{10}H_{11}{}^{35}Cl_2{}^{37}Cl_6]^-$,

M-Cl-HCl $[C_{13}H_{18}{}^{35}Cl_5{}^{37}Cl_2]^-$,

M-HCl $[{}^{12}C_{12}{}^{13}C_1H_{19}{}^{35}Cl_6{}^{37}Cl]^-$,

M-HCl $[{}^{12}C_9{}^{13}C_1H_{12}{}^{35}Cl_3{}^{37}Cl_5]^-$.

It is difficult to explain why some particular *m/z* values, for example 375 and 423, are suitable for quantification whereas others are not. However, a multitude of single compounds belong to a retention range of nearly 1 min in a very fast GC heating rate (70 °C/min) and a relatively short column (15 m) as shown in the selected ion chromatograms of the standard mixtures (see Annex D).

The selection for quantification was carried out using a specific empirical approach, see Reference [4].

Annex C
(informative)

Typical GC-MS conditions

C.1 Example 1

Injection:	Pressure-pulse splitless	344,7 kPa (50 psi); 1 min splitless time
Injector temperature:	275 °C	
Injection volume:	2 µl	
Transfer line temperature:	280 °C	
Ion source (ECNI)	150 °C	CH_4 (99,995 %); 5 ml/min
Quadrupole	106 °C	
Resolution power	Low resolution power, 0,9 u	
Flow rate:	1,3 ml/min constant	
Carrier gas:	Helium 99,999 %	
Capillary column:	length:	15 m
	film thickness:	0,1 µm
	inner diameter:	0,25 mm
Column material:	medium polar, e.g. methylphenyl silicone phase DB5 MS[a]	
Temperature programme:	100 °C 2 min → 70 °C/min to 280 °C → 2 min 280 °C → 70 °C/min to 320 °C 7 min	

[a] The chromatographic columns are given only as an information for the convenience of users of this document and do not constitute an endorsement by ISO of this product.

C.2 Example 2

Injection:	Pulse splitless	90 kPa; 1,5 min splitless time
Injector temperature:	300 °C	
Injection volume:	5 µl	
Transfer line temperature:	280 °C	
Ion source (ECNI)	150 °C	CH_4 (99,995 %); 5 ml/min
Quadrupole	106 °C	
Resolution power	Low resolution power. 0,9 u	

Flow rate:	1,8 ml/min constant	
Carrier gas:	Helium 99,999 %	
Capillary column:	length:	15 m
	film thickness:	0,25 µm
	inner diameter:	0,25 mm
Column material:	HB5 MS[a]	
Temperature programme:	80 °C 2 min → 40 °C/min to 300 °C → 3 min 300 °C → 70 °C/min to 320 °C 7 min	

[a] The chromatographic columns are given only as an information for the convenience of users of this document and do not constitute an endorsement by ISO of this product.

C.3 Example 3

Injection:	Pulse splitless	150 kPa (21,8 psi); 1,25 min splitless time
Injector temperature:	275 °C	
Injection volume:	2 µl	
Transfer line temperature:	280 °C	
Ion source (ECNI)	150 °C	CH_4 (99,995 %); 5 ml/min
Quadrupole	150 °C	
Resolution power	Low resolution power, 0,9 u	
Flow rate:	1,6 ml/min constant	
Carrier gas:	Helium 99,999 %	
Capillary column:	length:	15 m
	film thickness:	0,1 µm
	inner diameter:	0,25 mm
Column material:	DB5-MS[a]	
Temperature programme:	120 °C 2 min → 50 °C/min to 325 °C → 9 min 325 °C	

[a] The chromatographic columns are given only as an information for the convenience of users of this document and do not constitute an endorsement by ISO of this product.

C.4 Example 4

Injection:	splitless
Injector temperature:	260 °C
Injection volume:	2 µl

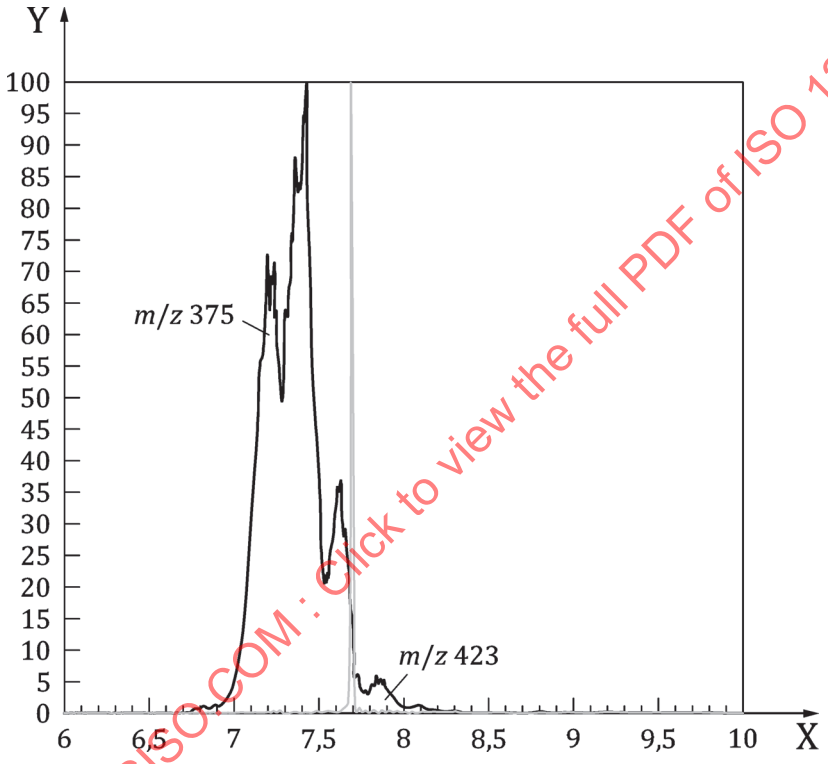
Transfer line temperature:	320 °C	
Ion source (NCI)	160 °C	CH_4 (99,995 %); 1,7 ml/min
Quadrupole	—	
Resolution power	0,8 u	
Flow rate:	1,5 ml/min constant	
Carrier gas:	Helium 99,999 %	
Capillary column:	length:	15 m
	film thickness:	0,10 µm
	inner diameter:	0,25 mm
Column material:	slightly polar, e.g. 5 % diphenyl- / 95 % dimethylpolysiloxane (DB5-MS)[a]	
Temperature programme:	60 °C, 2 min → 45 °C/min to 340 °C, 10 min	

[a] The chromatographic columns are given only as an information for the convenience of users of this document and do not constitute an endorsement by ISO of this product.

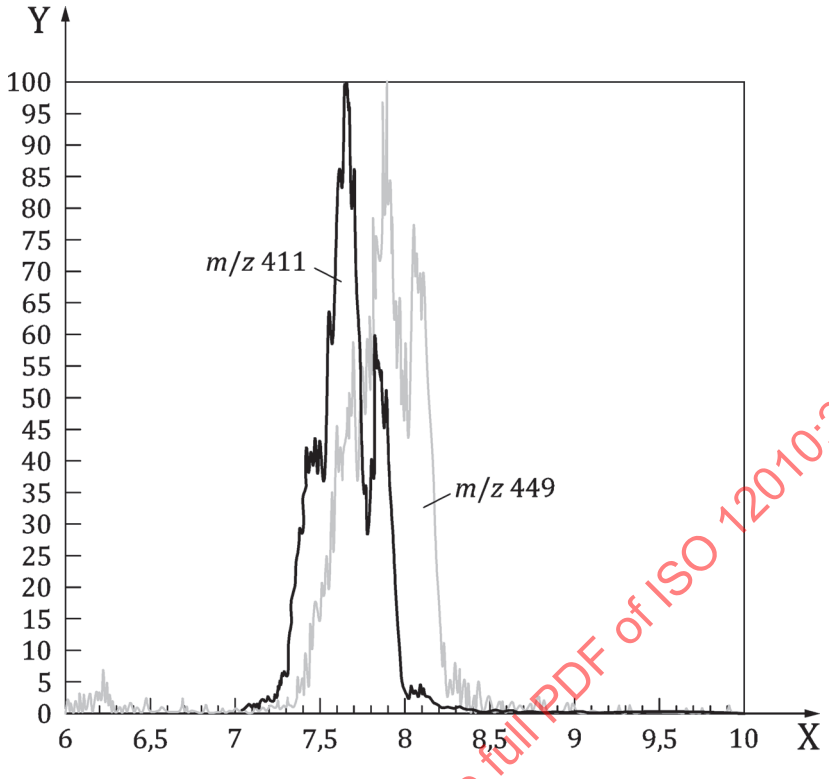
Annex D
(informative)

Typical chromatograms of standard solutions and quality control check solutions 1 µg/ml

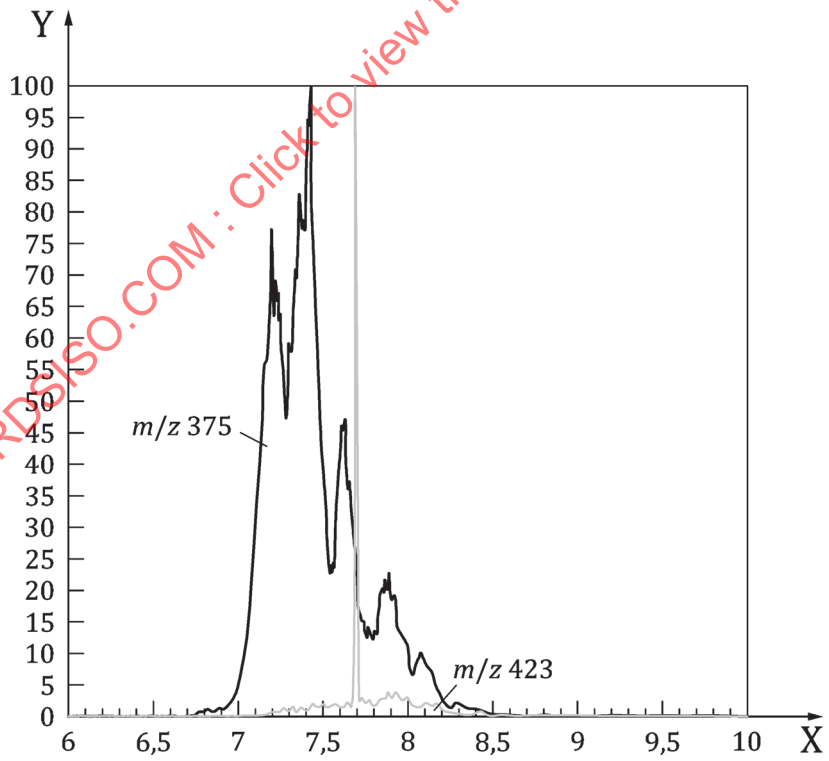
See Figure D.1 for example chromatograms of standard solutions and quality check control solutions of SCCP. Each first chromatogram shows the plot of *m/z* 375 and *m/z* 423 (*m/z* values for quantification), each second chromatograms the plot of *m/z* 411 and *m/z* 449 (*m/z* values for identification).



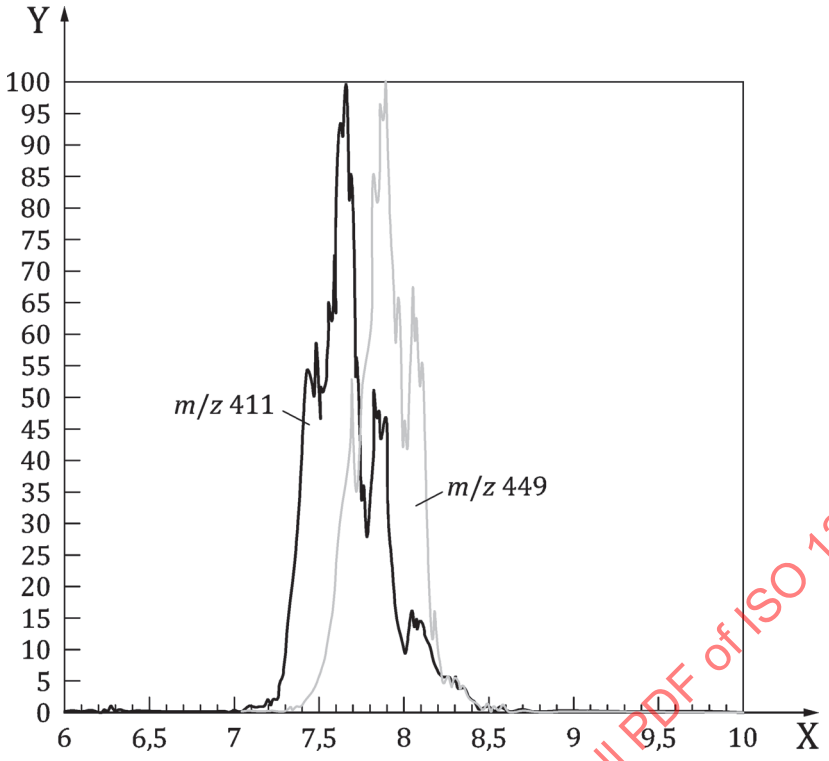
a) "Lake Ontario water", 1 µg/ml, *m/z* 375 and *m/z* 423



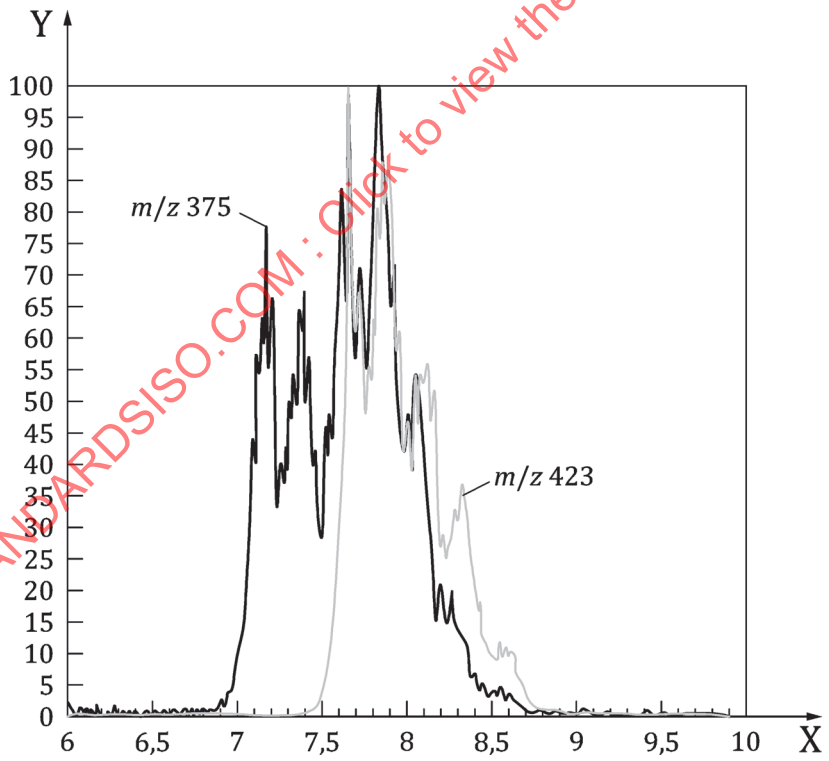
b) "Lake Ontario water", 1 µg/ml, *m/z* 411 and *m/z* 449



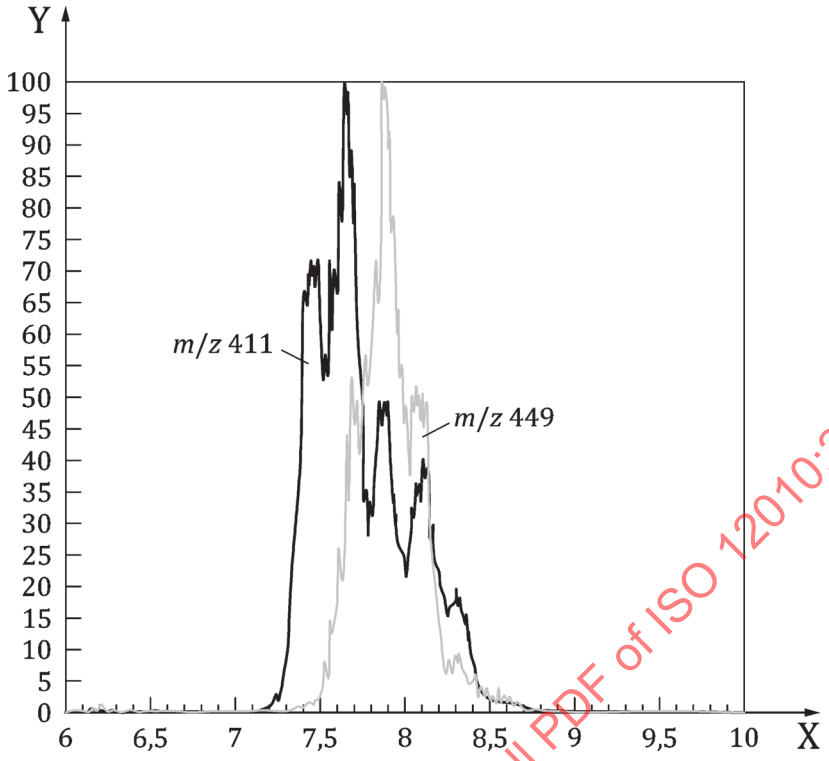
c) "Perch", 1 µg/ml, *m/z* 375 and *m/z* 423



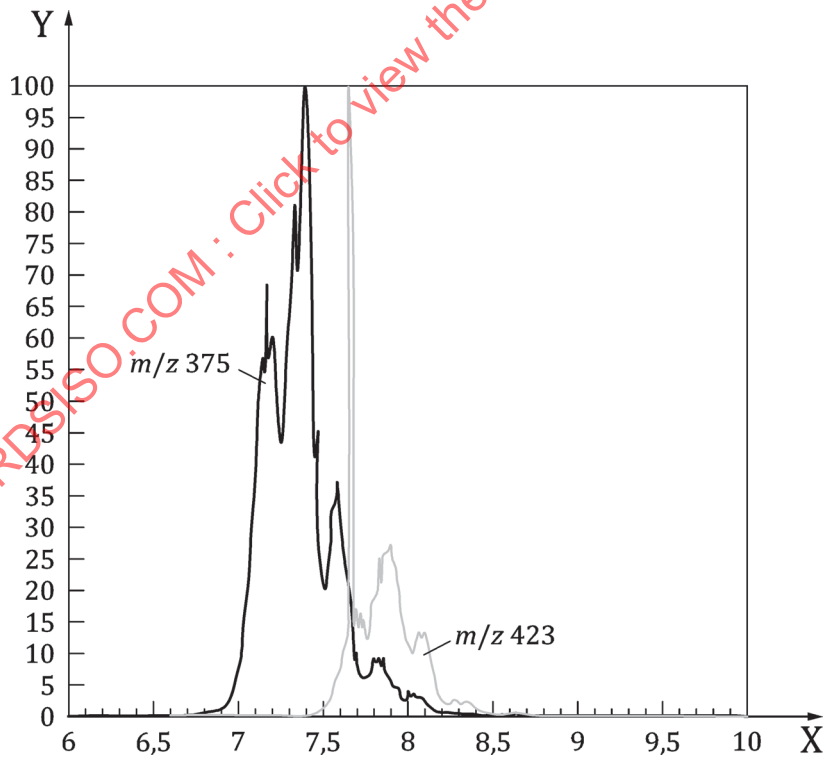
d) "Perch", 1 µg/ml, *m/z* 411 and *m/z* 449



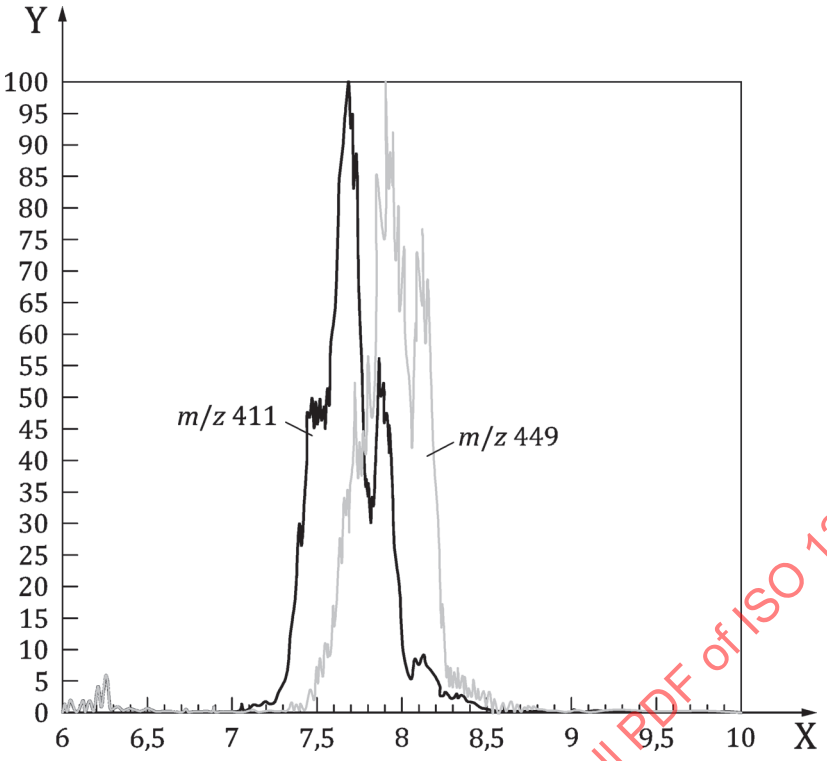
e) "Sediment Drevnice", 1 µg/ml, *m/z* 375 and *m/z* 423



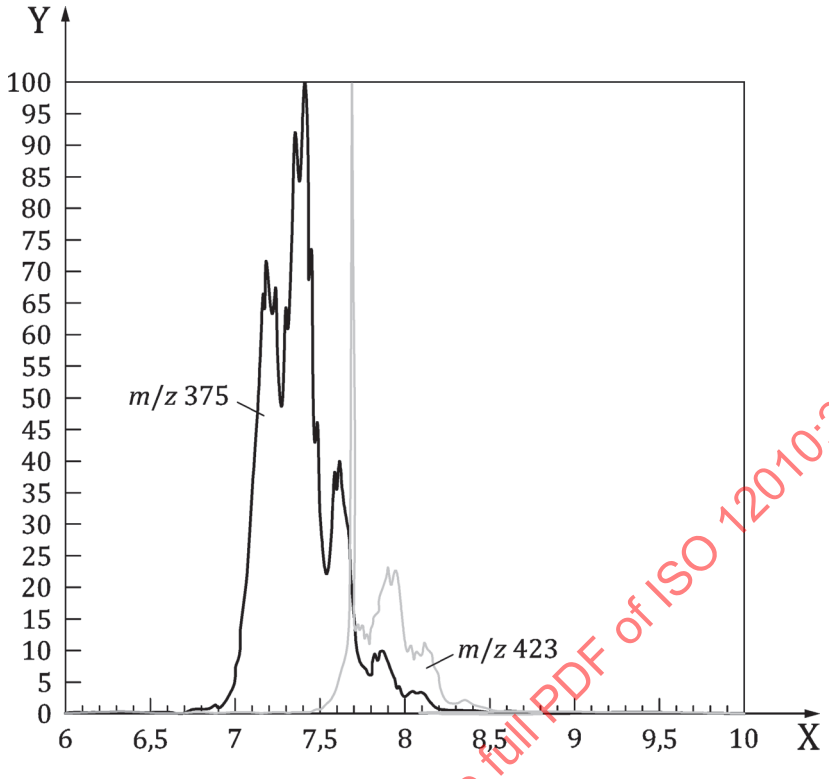
f) "Sediment Drevnice", 1 µg/ml, *m/z* 411 and *m/z* 449



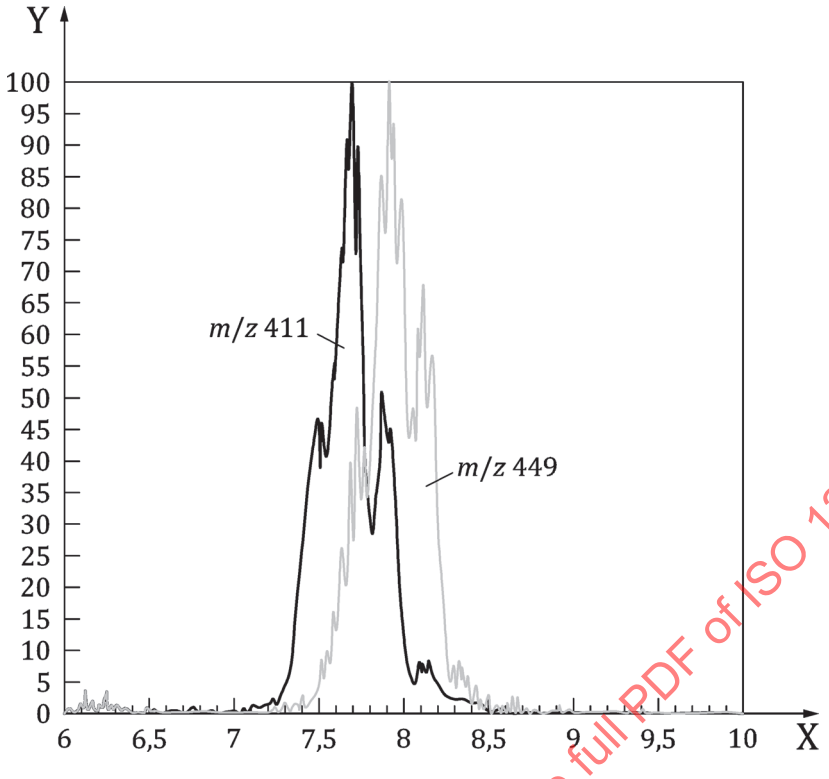
g) Hordalub 80 -s1, 1 µg/ml, *m/z* 375 and *m/z* 423



h) Hordalub 80 -s1, 1 µg/ml, *m/z* 411 and *m/z* 449



i) Hordalub 80 -s2, 1 µg/ml, *m/z* 375 and *m/z* 423



j) Hordalub 80 –s2, 1 µg/ml, *m/z* 411 and *m/z* 449

Key

X time, min

Y relative abundance or response

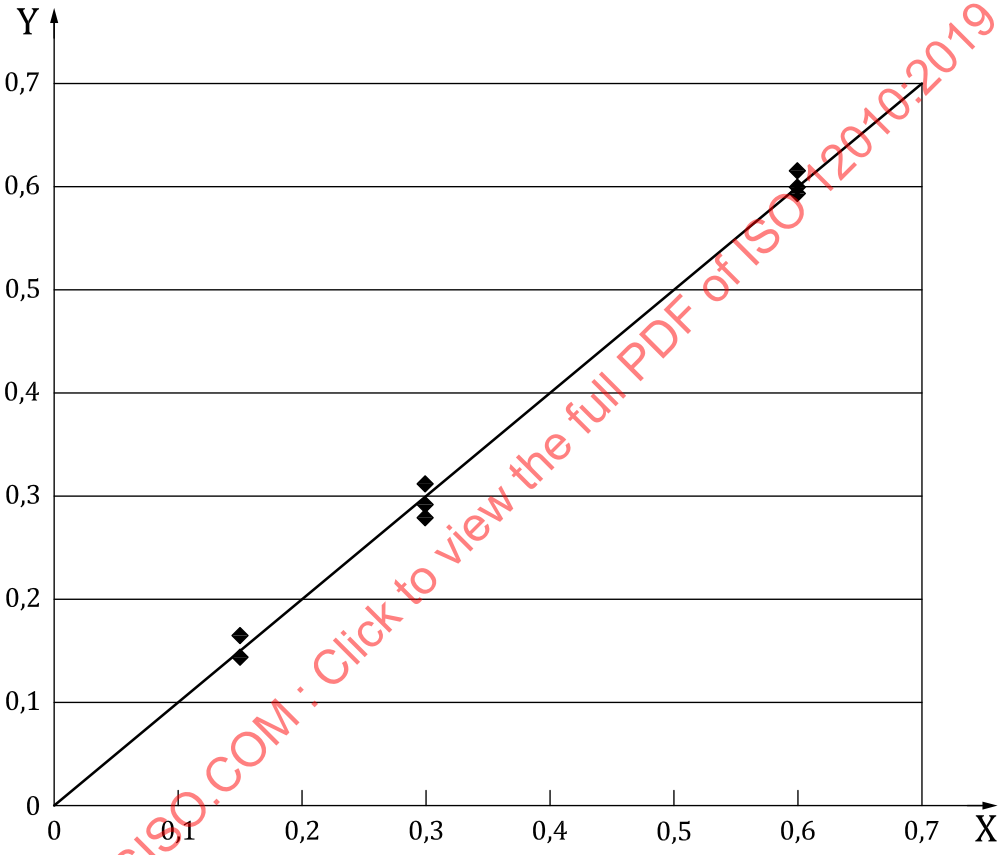
NOTE The single sharp peak of the mass trace *m/z* 423 is an interference from the internal standard 1,1,1,3,11,13,13,13-octachlorotridecane. The peak area of this interference is low compared to the area of SCCP's even at the LOD and can therefore be tolerated.

Figure D.1 — Typical chromatograms of standard solutions and quality control check solutions

Annex E
(informative)

Presentation of goodness of fit

An example for a presentation of goodness of fit (calculated and target concentrations) with the calibration working solutions in 6.4, Table 3 up to 0,6 µg/ml after basic calibration is given in Figure E.1.



Key

X ρ_{targ} target concentration, µg/ml
Y ρ_{calc} calculated concentration, µg/ml

Figure E.1 — Example for a recovery with the calibration working solution (6.4, Table 3)

Annex F
(normative)

Alternative clean-up with column chromatography

F.1 Reagents

In addition to Clause 6, the following reagents are used.

F.1.1 Activated magnesium silicate, $MgO/3{,}75\ SiO_2/(x)\ H_2O$, for column chromatography).

Activated magnesium silicate is used in the clean-up procedure to separate organohalogenic compounds like polychlorinated biphenyls and naphthalenes.

Use activated magnesium silicate with the following characteristics: particle size 0,15 mm to 0,25 mm, of which 80 % > 0,15 mm; surface area, determined according to the BET (Brunauer–Emmett–Teller) method), 170 m^2/g to 300 m^2/g; pH 9 to pH 10. Activate the magnesium silicate by heating, for example, 200 g in a shallow dish at 140 °C for at least 4 h. Allow the activated magnesium silicate to cool to room temperature in a desiccator. Activated magnesium silicate can be stored in a closed bottle at room temperature for up to one month.

F.2 Extract clean-up procedure

Before using the clean-up procedure with real samples, the analyst shall demonstrate that the fraction collected contains more than 80 % of the SCCP by performing recovery tests according to ISO/TS 13530. This shall apply to internal standard substances as well as the calibration working solutions.

Place a glass-wool plug in a 10 mm ID chromatographic column (7.5). Pack the column bottom to top in the sequence with the following.

Add 2,5 g copper powder (6.5.1), then activate it by rinsing the column with 10 ml hydrochloric acid (6.5.2). Proceed to wash the copper powder with 25 ml deionized water followed by 20 ml propanone (6.1). Finally, rinse the column three times with 2 ml of *n*-heptane (6.1).

Add 3 g of activated magnesium silicate and 1 g granular anhydrous sodium sulfate (6.8) to the column.

Gently tap the column to allow the adsorbents to settle.

Condition the prepared column with 10 ml of *n*-heptane (6.1). Do not allow the meniscus of the solvent to go below the level of the sodium sulfate, at any time. Discard the eluate, check the column for channelling. If a channelling effect is observed, discard the column and prepare another.

Add the concentrated extract (see 9.1 or 9.2) to the conditioned column, elute the column and discard the first eluate of approximately 1 ml. Do not allow the meniscus of the extract to go below the level of the sodium sulfate.

The following elution should be tested and optimized by each laboratory undertaking SCCP determinations. SCCPs are expected to elute in step d of Table F.1.

Table F.1 — Elutions to be tested and optimized

Step	Volume of elution solvent		Elution solvent composition, parts by volume	Eluate
a	5 ×	2 ml	*n*-heptane/propanone (6.1) (98 + 2) **98 % *n*-heptane and 2 % volume fraction propanone**	Discard
b	1 ×	2 ml	*n*-heptane/propanone (6.1) (85 + 15) **85 % volume fraction *n*-heptane and 15 % volume fraction propanone**	Discard
c	1 ×	2 ml	*n*-heptane/propanone (6.1) (85 + 15) **85 % volume fraction *n*-heptane and 15 % volume fraction propanone**	Collect and concentrate to 0,2 ml only for optimization, discard, if < 10 % of the internal standard peak area is obtained
d	2 ×	2 ml	*n*-heptane/propanone (6.1) (50 + 50) **50 % volume fraction *n*-heptane and 50 % volume fraction propanone**	Collect and concentrate to 0,2 ml
e	1 ×	1 ml	*n*-heptane/propanone (6.1) (50 + 50) **50 % volume fraction *n*-heptane and 50 % volume fraction propanone**	Collect and concentrate to 0,2 ml only for optimization, discard, if < 10 % of the internal standard peak area is obtained

Concentrate the eluate of step d (or alternative step according to the optimization) to e.g. 0,2 ml ± 0,1 ml and transfer to a sample vial (7.4) for injection into the GC-MS line.

F.3 Interferences with chlorinated chemicals (technical products)

Non-specific matrix interferences, as well as interferences from other environmental situations are dealt with using the given clean-up procedure. Following the entire procedure, including the concentration factor of approximately 5 000, the following pollutants have been tested and found not to cause interferences below the following concentrations (see Table F.2).

Table F.2 — Interferences with chlorinated chemicals (technical products)

Potential interfering compounds	Highest concentration level which causes no interferences higher that the limit of detection
Aroclor 1262[a]	0,5 µg/l
Aroclor 1242	0,5 µg/l
Aroclor 1221	1 µg/l
Camphechlor (toxaphene)	0,2 µg/l
Halowax 1014	1 µg/l
Halowax 1051	1 µg/l
Technical chlordane	0,5 µg/l

[a] Aroclor 1262, Aroclor 1242, Aroclor 1221, Halowax 1014 and Halowax 1052 are examples of suitable products available commercially. These examples are given only as information for the convenience of users of this document and do not constitute an endorsement by ISO of these products.

Table F.2 *(continued)*

Potential interfering compounds	Highest concentration level which causes no interferences higher that the limit of detection
MCCP (medium-chain chlorinated *n*-alkanes) 42 %	0,2 µg/l
MCCP (medium-chain chlorinated *n*-alkanes) 52 %	**0,2 µg/l**
MCCP (medium-chain chlorinated *n*-alkanes) 57 %	**0,2 µg/l**

[a] Aroclor 1262, Aroclor 1242, Aroclor 1221, Halowax 1014 and Halowax 1052 are examples of suitable products available commercially. These examples are given only as information for the convenience of users of this document and do not constitute an endorsement by ISO of these products.

If the clean-up procedure is repeated, interferences can be further reduced.