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

Qualité de l'eau — Dosage des dioxines et furanes tétra- à octachlorés — Méthode par dilution d'isotopes HRGC/SMHR

Reference number
ISO 18073:2004(E)

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Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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.

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.

ISO 18073 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

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

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This standard 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 and to ensure compliance with any national regulatory conditions.

Attention is drawn to any relevant national safety regulations. The 2,3,7,8-chloro-substituted PCDDs/PCDFs are among the most toxic of chemicals. All work with PCDDs/PCDFs requires, therefore, the utmost care; the national safety measures which correspond to those for toxic substances shall be strictly adhered to.

1 Scope

This International Standard specifies a method for the determination of tetra- to octa-chlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in waters and waste waters (containing less than 1 % by mass solids) using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS).

This International Standard is applicable to the seventeen 2,3,7,8-substituted PCDDs/PCDFs specified in Table 1.

The detection limits and quantitation levels in this method are usually dependent on the level of interferences rather than instrumental limitations. The minimum levels (MLs) specified in Table 2 are the levels at which the PCDDs/PCDFs can be determined with no interferences present. The method detection limit (MDL) for 2,3,7,8-TCDD has been determined as 4,4 pg/l based on this method using a sample volume of 1 l. Lower detection limits may be achieved by using a larger sample volume.

This method is “performance based”. The analyst is permitted to modify the method to overcome interferences or lower the cost of measurements, provided that all performance criteria in this International Standard are met. The requirements for establishing method equivalency are given in 9.1.2.

2 Normative references

The following referenced documents are indispensable for the application 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 3696:1987, *Water for analytical laboratory use — Specification and test methods*

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

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

ISO 6879:1995, *Air Quality — Performance characteristics and related concepts for air quality measuring methods.*

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3 Terms, definitions and abbreviated terms

3.1 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 6879:1995 and the following apply.

3.1.1
analyte
PCDD or PCDF tested for by this method

Note See Table 1 for a list of compounds.

3.1.2
calibration standard
solution prepared from a secondary standard and/or stock solutions and used to calibrate the response of the instrument with respect to analyte concentration

3.1.3
calibration verification standard
midpoint calibration standard that is used to verify calibration

3.1.4
congener
any one of the 210 individual PCDDs/ PCDFs

3.1.5
internal standard
$^{13}C_{12}$-labelled 2,3,7,8-PCDD/PCDF analogue added to samples prior to extraction against which the concentrations of native PCDDs and PCDFs are calculated

3.1.6
keeper
high boiling-point solvent added to the sampling standard solution

3.1.7
method blank
aliquot of reagent water that is treated exactly as a sample through the complete analytical procedure including extraction, clean-up, identification and quantification including all the relevant reagents and materials

3.1.8
operational performance characteristics
influence of the physical and chemical environment and maintenance problems, for example; mains voltage, temperature, supply of certain substances, set-up time, period of unattended operation

[See ISO 6879:1995]

3.1.9
pattern
chromatographic print of any series of PCDD/PCDF isomers

3.1.10
PCDD/PCDF isomers
PCDDs or PCDFs with identical chemical compositions but different structures

3.1.11
profile
graphic representation of the sums of the isomer concentrations of the PCDDs and the PCDFs

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3.1.12
recovery standard
$^{13}C_{12}$-labelled 2,3,7,8-chloro-substituted PCDD/PCDF, added before injection into the GC

3.1.13
spiking
addition of $^{13}C_{12}$-labelled PCDD/PCDF standards

3.1.14
statistical performance characteristics
quantification, for measured values, of the possible deviations resulting from the random part of the measuring process, e.g. repeatability or instability

[See ISO 6879:1995]

3.2 Abbreviated terms

DCDPE decachlorodiphenyl ether

GC/MS gas chromatography/mass spectrometry

GPC gel-permeation chromatography

HpCDD heptachlorodibenzo-*p*-dioxin

HpCDF heptachlorodibenzofuran

HpCDPE heptachlorodiphenyl ether

HPLC high-performance liquid chromatography

HRGC high-resolution gas chromatography

HRMS high-resolution mass spectrometry

HxCDD hexachlorodibenzo-*p*-dioxin

HxCDF hexachlorodibenzofuran

HxCDPE hexachlorodiphenyl ether

MDL method detection limit

ML minimum level (see Table 2)

NCDPE nonachlorodiphenyl ether

OCDD octachlorodibenzo-*p*-dioxin

OCDF octachlorodibenzofuran

OCDPE octachlorodiphenyl ether

PCDD/PCDF polychlorinated dibenzo-*p*-dioxin/dibenzofuran

PeCDD pentachlorodibenzo-*p*-dioxin

PeCDF pentachlorodibenzofuran

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PTFE polytetrafluoroethylene

SIM selected ion monitoring

TCDD tetrachlorodibenzo-*p*-dioxin

T**C**DF tetrachlorodibenzofuran

TEF toxic equivalent factor

TEQ toxic equivalent

4 Principle

4.1 Spiking and extraction

Internal standards, analogues of the 2,3,7,8-substituted PCDDs/PCDFs labelled with a stable isotope (see Table 1) in a suitable solvent such as acetone, are spiked into a 1 l aqueous sample containing less than 1 % by mass solids. A minimum of one labelled standard per homologue group is used and the sample is extracted by one of two procedures as specified in 4.1 a) or 4.1 b).

a) Samples containing no visible particles are extracted with dichloromethane in a separatory funnel or by solid-phase extraction. The extract is concentrated for clean-up.

b) Samples containing visible particles are vacuum filtered through a glass-fibre filter. The filter is extracted in a Soxhlet extractor using toluene and the filtrate is extracted with dichloromethane in a separatory funnel. The dichloromethane extract is concentrated and combined with the Soxhlet extract prior to clean-up.

Other solvents and extraction techniques may be substituted, provided that all the performance criteria can be met.

4.2 Clean-up

After extraction, sample extracts are cleaned up to remove interfering components. Sample clean-ups may include back-extraction with acid and/or base, and gel-permeation, alumina, silica, Florisil[1)] or activated-carbon chromatography.

4.3 Concentration

After clean-up, the extract is concentrated to near dryness. Prior to injection, recovery standards are added to each extract, and an aliquot of the extract is injected into the gas chromatograph. The analytes are separated by the GC and detected by a high-resolution mass spectrometer. Two exact masses are monitored for each analyte.

Resolution equal to or greater than or 10 000 is recommended. High-resolution gas chromatography/high-resolution mass spectrometry at a resolution equal to or greater than 10 000 is, at present, required to achieve adequate sensitivity and selectivity, and to allow the use of all $^{13}C_{12}$-labelled standards. Resolution in the range of 6 000 to 10 000 should be acceptable if the absence of interferences has been documented. If a determination of PCDD/PCDF homologue group totals is required, then a resolution of 10 000 is necessary. At resolutions less than 10 000, some $^{13}C_{12}$-PCDFs interfere with native PCDDs of the same level of chlorination.

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1) Florisil is an example of a suitable product available commercially.This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

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Table 1 — PCDDs and PCDFs analyzed by this method

PCDDs/PCDFs	CAS registry	Labelled analogue	CAS registry
2,3,7,8-TCDD –	1746-01-6 –	$^{13}C_{12}$-2,3,7,8-TCDD $^{13}C_{12}$-1,2,3,4-TCDD	76523-40-5 –
Total TCDD	41903-57-5	–	–
2,3,7,8-TCDF	51207-31-9	$^{13}C_{12}$-2,3,7,8-TCDF	89059-46-1
Total TCDF	55722-27-5	–	–
1,2,3,7,8-PeCDD	40321-76-4	$^{13}C_{12}$-1,2,3,7,8-PeCDD	109719-79-1
Total PeCDD	36088-22-9	–	–
1,2,3,7,8-PeCDF	57117-41-6	$^{13}C_{12}$-1,2,3,7,8-PeCDF	109719-77-9
2,3,4,7,8-PeCDF	57117-31-4	$^{13}C_{12}$-2,3,4,7,8-PeCDF	116843-02-8
Total PeCDF	30402-15-4	–	–
1,2,3,4,7,8-HxCDD	39227-28-6	$^{13}C_{12}$-1 2,3,4,7,8-HxCDD	109719-80-4
1,2,3,6,7,8-HxCDD	57653-85-7	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	109719-81-5
1,2,3,7,8,9-HxCDD	19408-74-3	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	109719-82-6
Total HxCDD	34465-46-8	–	–
1,2,3,4,7,8-HxCDF	70648-26-9	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	114423-98-2
1,2,3,6,7,8-HxCDF	57117-44-9	$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	116843-03-9
1,2,3,7,8,9-HxCDF	72918-21-9	$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	116843-04-0
2,3,4,6,7,8-HxCDF	60851-34-5	$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	116843-05-1
Total HxCDF	55684-94-1	–	–
1,2,3,4,6,7,8-HpCDD	35822-46-9	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	109719-83-7
Total HpCDD	37871-00-4	–	–
1,2,3,4,6,7,8-HpCDF	67562-39-4	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	109719-84-8
1,2,3,4,7,8,9-HpCDF	55673-89-7	$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	109719-94-0
Total HpCDF	38998-75-3	–	–
OCDD	3268-87-9	$^{13}C_{12}$-OCDD	114423-97-1
OCDF	39001-02-0	$^{13}C_{12}$-OCDF	109719-78-0

4.4 Identification

An individual PCDD/PCDF is identified by comparing the GC retention time and the ion-abundance ratio of the two exact masses monitored with the respective retention time of an authentic standard and the theoretical or analytical ion-abundance ratio of the two exact masses.

The non-2,3,7,8-substituted isomers and congeners are identified by the agreement of their retention times and ion-abundance ratios with accepted values within predefined limits.

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4.5 Quantification

Quantitative analysis is performed using selected-ion monitoring (SIM) in one of three ways.

a) For the 2,3,7,8-substituted PCDDs/PCDFs for which labelled analogues have been added to the sample (4.1), the isotope-dilution technique is used to calibrate the GC/MS system and to determine the concentration of each compound.

b) For the 2,3,7,8-substituted PCDDs/PCDFs for which labelled analogues are not added to the samples prior to extraction and for the labelled internal standards themselves, the internal-standard technique is used to calibrate the GC/MS system and to determine the concentration of each compound.

c) For non-2,3,7,8-substituted isomers and for all isomers at a given level of chlorination (i.e. total TCDDs), concentrations are determined using response factors from the calibration of the PCDDs/PCDFs at the same level of chlorination.

4.6 Analytical quality

The quality of the analysis is assured through reproducible calibration and testing of the procedures for extraction, clean-up, and GC/MS operation.

5 Contamination and interferences

5.1 Where possible, purify reagents by extraction or solvent rinse.

Solvents, reagents, glassware, and other sample processing hardware can yield artefacts and/or elevated baselines causing misinterpretation of chromatograms. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.

5.2 Clean glassware such that the method blank requirements of this International Standard are met (9.4.3). An example of a cleaning procedure is given in 5.2 a) to 5.2 c).

a) Disassemble glassware with removable parts, particularly separatory funnels with fluoropolymer stopcocks, prior to washing with detergent. Rinse glassware with solvent and wash with a detergent solution as soon after use as is practical. Sonication of glassware in a detergent solution for approximately 30 s may aid in cleaning.

b) After washing with detergent, rinse glassware immediately, first with methanol, then with hot tap water. The tap water rinse shall be followed by another methanol rinse, then acetone, and then dichloromethane.

c) Immediately prior to use, pre-extract the Soxhlet apparatus with toluene for approximately 3 h. Shake separatory funnels with a dichloromethane/toluene mixture (80/20 by volume) for 2 min, drain, and then shake with pure dichloromethane for 2 min.

Proper cleaning of glassware is extremely important, because glassware not only can contaminate the samples but also can remove the analytes of interest by adsorption on the glass surface.

5.3 Demonstrate that all materials used in the analysis are free from interferences by running reference-matrix method blanks initially and with each sample batch (samples, up to a maximum of 20, processed through the extraction procedure on a given 12-h shift).

5.4 The reference matrix shall simulate, as closely as possible, the sample matrix under test. Ideally, the reference matrix shall not contain the PCDDs/PCDFs in detectable amounts, but shall contain potential interferents in the concentrations expected to be found in the samples to be analyzed.

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Table 2 — Suggested quantitation relationships for individual PCDDs/PCDFs

PCDDs/PCDFs	Reference for retention time and quantitation	Relative retention time[a]	Minimum level[a,b] in water pg/l	Minimum level[a,b] in extract pg/µl
2,3,7,8-TCDF	$^{13}C_{12}$-2,3,7,8-TCDF	0,999 to 1,003	10	0,5
2,3,7,8-TCDD	$^{13}C_{12}$-2,3,7,8-TCDD	0,999 to 1,002	10	0,5
1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,7,8-PeCDF	0,999 to 1,002	50	2,5
2,3,4,7,8-PeCDF	$^{13}C_{12}$-2,3,4,7,8-PeCDF	0,999 to 1,002	50	2,5
1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,7,8-PeCDD	0,999 to 1,002	50	2,5
$^{13}C_{12}$-2,3,7,8-TCDF	$^{13}C_{12}$-1,2,3,4-TCDD	0,923 to 1,103	50	2,5
$^{13}C_{12}$-2,3,7,8-TCDD	$^{13}C_{12}$-1,2,3,4-TCDD	0,976 to 1,043	50	2,5
$^{13}C_{12}$-1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,4-TCDD	1,000 to 1,425	50	2,5
$^{13}C_{12}$-2,3,4,7,8-PeCDF	$^{13}C_{12}$-1,2,3,4-TCDD	1,011 to 1,526	50	2,5
$^{13}C_{12}$-1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,4-TCDD	1,000 to 1,567	50	2,5
1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	0,999 to 1,001	50	2,5
1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	0,997 to 1,005	50	2,5
1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	0,999 to 1,001	50	2,5
2,3,4,6,7,8-HxCDF	$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	0,999 to 1,001	50	2,5
1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	0,999 to 1,001	50	2,5
1,2,3,6,7,8-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	0,998 to 1,004	50	2,5
1,2,3,7,8,9-HxCDD	See note[c]	1,000 to 1,019	50	2,5
1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	0,999 to 1,001	50	2,5
1,2,3,4,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	0,999 to 1,001	50	2,5
1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	0,999 to 1,001	50	2,5
OCDD	$^{13}C_{12}$-OCDD	0,999 to 1,001	100	5,0
OCDF	$^{13}C_{12}$-OCDD	0,999 to 1,008	100	5,0
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0,944 to 0,970	—	—
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0,949 to 0,975	—	—
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0,977 to 1,047	—	—
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0,959 to 1,021	—	—
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0,977 to 1,000	—	—
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	0,981 to 1,003	—	—
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1,043 to 1,085	—	—
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1,057 to 1,151	—	—
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1,086 to 1,110	—	—
$^{13}C_{12}$-OCDD	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	1,032 to 1,311	—	—

[a] Minimum levels and relative retention times are given for guidance only.

[b] The minimum level (ML) for each analyte is defined as the level for which the entire analytical system shall give a recognizable signal and acceptable calibration point. It is equivalent to the concentration of the lowest calibration standard, assuming that all method-specific sample masses, volumes and clean-up procedures have been used, i.e. based on 1 l of sample.

[c] The retention time reference for 1,2,3,7,8,9-HxCDD is $^{13}C_{12}$-1,2,3,6,7,8-HxCDD; 1,2,3,7,8,9-HxCDD is quantified using the averaged responses for $^{13}C_{12}$-1,2,3,4,7,8-HxCDD and $^{13}C_{12}$-1,2,3,6,7,8-HxCDD.

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Interfering compounds in the sample extracts can vary considerably from source to source, depending on the diversity of the site being sampled. These compounds can be present at concentrations several orders of magnitude higher than the target PCDDs/PCDFs. The most frequently encountered interferences are chlorinated biphenyls, methoxybiphenyls, hydroxydiphenyl ethers, benzylphenyl ethers, polynuclear aromatics, and pesticides. Because this International Standard applies to very low levels of PCDDs/PCDFs, the elimination of interferences is essential. The example clean-ups given in Clause 13 can be used to reduce or eliminate these interferences and thereby permit reliable determination of the PCDDs/PCDFs at the concentrations shown in Table 2.

5.5 When a reference matrix that simulates the sample matrix under test is not available, use reagent water (6.1) as a substitute.

5.6 Number each piece of reusable glassware to associate that glassware with the processing of a particular sample. This will assist the laboratory in tracking possible sources of contamination for individual samples, identifying glassware associated with highly contaminated samples that may require extra cleaning, and determining when glassware shall be discarded.

6 Reagents and standards

Use only reagents of recognized analytical grade, unless otherwise specified.

6.1 **Water**, complying with grade 3 as defined in ISO 3696.

6.2 pH adjustment and back-extraction procedures.

6.2.1 **Potassium hydroxide solution**.

Dissolve 20 g of potassium hydroxide, KOH, in 100 ml of water.

6.2.2 **Sulfuric acid**, H_2SO_4, ρ = 1,84 g/ml.

6.2.3 **Hydrochloric acid**, c(HCl) = 6 mol/l.

6.2.4 **Sodium chloride solution**.

Dissolve 5 g of sodium chloride, NaCl, in 100 ml of water.

6.3 Solution drying and evaporation procedures.

6.3.1 **Sodium sulfate**, Na_2SO_4, granular, anhydrous.

Rinse granular, anhydrous sodium sulfate with dichloromethane (20 ml/g), heat at 400 °C for at least 1 h, cool in a desiccator, and store in a pre-cleaned glass bottle with a hermetically sealing screw-cap.

If, during heating, the sodium sulfate develops a noticeable greyish cast (due to the presence of carbon in the crystal matrix), discard, as it is not suitable for use. Extraction (in lieu of simple rinsing) with dichloromethane and heating at a lower temperature can produce sodium sulfate that is suitable for use.

6.3.2 **Nitrogen**, N_2 99,999 %.

6.4 Extraction solvents

The extraction solvents, distilled in glass, of pesticide quality and certified to be free of interferences, include the following:

a) **Acetone**, C_3H_6O.

b) **Toluene**, C_7H_8.

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c) **Cyclohexane**, C_6H_{12}.

d) **Hexane**, C_6H_{14}.

e) **Methanol**, CH_3OH.

f) **Dichloromethane**, CH_2Cl_2.

g) **Nonane,** C_9H_{20}.

6.5 GPC calibration solution, dichloromethane containing 300 mg/ml of corn oil; 15 mg/ml of bis(2-ethylhexyl)phthalate, $C_{24}H_{38}O_4$; 1,4 mg/ml of pentachlorophenol, C_6Cl_5OH; 0,1 mg/ml of perylene, $C_{20}H_{12}$; and 0,5 mg/ml of sulfur, S.

6.6 Adsorbents for sample clean-up.

6.6.1 Silica

6.6.1.1 Activated silica, 75 to 140 µm.

Rinse silica with dichloromethane, heat at 180 °C for at least 1 h, cool in a desiccator, and store in a pre-cleaned glass bottle with a hermitically sealing screwcap.

6.6.1.2 Acid silica, 30 % mass fraction.

Thoroughly mix 44,0 g of concentrated sulfuric acid (6.2.2) with 100 g of activated silica in a clean container. Break up aggregates with a stirring rod until a uniform mixture is obtained. Store in a bottle with a fluoropolymer-lined screw-cap.

6.6.1.3 Basic silica.

Thoroughly mix 30 g of sodium hydroxide solution [c(NaOH) = 1 mol/l] with 100 g of activated silica in a clean container. Break up aggregates with a stirring rod until a uniform mixture is obtained. Store in a bottle with a fluoropolymer-lined screw cap.

6.6.1.4 Potassium silicate.

Dissolve 56 g of high-purity potassium hydroxide (KOH) in 300 ml of methanol in a 750 ml to 1 000 ml flat-bottom flask. Add 100 g of silica and a stirring bar, and stir on a hotplate at 60 °C to 70 °C for 1 h to 2 h. Decant the liquid and rinse the potassium silicate twice with 100-ml portions of methanol, followed by a single rinse with 100 ml of dichloromethane. Spread the potassium silicate on solvent-rinsed aluminium foil and dry for 2 h to 4 h in a hood. Activate the potassium silicate overnight at 200 °C to 250 °C.

6.6.2 Alumina

One of two types of alumina, acid or basic, may be used in the clean-up of sample extracts, provided that the laboratory meet the performance specifications for the recovery of internal standards in accordance with 9.3. The same type of alumina shall be used for all samples, including those used to demonstrate initial precision and recovery (9.2).

a) **Acid alumina**, activated by heating at 130 °C for at least 12 h.

b) **Basic alumina**, activated by heating at 600 °C for at least 24 h.

Store at 130 °C in a covered flask. Use within 5 d of activating.

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ICN Alumina Super I[2] or an equivalent may be used without activation.

6.6.3 Carbon

Thoroughly mix 9,0 g of Carbopak C[3] (Supelco 1-0258 or equivalent) and 41,0 g of Celite 545[4] (Supelco 2-0199 or equivalent). Activate the mixture at 130 °C for at least 6 h. Store in a desiccator.

NOTE Active carbon-impregnated silica[5] (Wako Pure Chemical Industries 019-11941) and active carbon-dispersed silica gel[6] (Kanto Chemical Co 01875-43) are examples of alternative materials.

6.6.4 Florisil column

Soxhlet-extract the Florisil[1] packing, 150 µm to 250 µm, in 500-g portions for 24 h using dichloromethane.

Insert a glass wool plug into the tapered end of a graduated serological pipette (7.5.2). Pack with 1,5 g (approximately 2 ml) of the prepared Florisil, then with approximately 1 ml of sodium sulfate (6.3.1) and top with a glass wool plug. Activate in an oven at 130 °C to 150 °C for at least 24 h, then cool for 30 min. Use within 90 min of cooling.

6.7 Reference matrices

Reference matrices are solutions, as similar to the matrix as possible, in which the PCDDs/PCDFs and interfering compounds are not detected by this method. When a reference matrix that simulates the sample matrix under test is not available, use reagent water (6.1) as a substitute.

6.8 Standard solutions

Purchase ready-made standard solutions having a certification as to their purity, concentration and authenticity; or prepare standards from materials of known purity and composition. Standard preparations (6.9 to 6.15) and Tables 2, 3, 4 and 5 give examples of standard concentrations that are acceptable. Other concentrations and spiking schemes may be used, provided the performance criteria of this International Standard can be met (see also 4.1).

When the purity of the chemical components of the standard solutions is equal to or greater than 98 % by mass, it is not necessary to correct the mass for the impurities during the computation of the concentration of the standard.

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2) ICN Alumina Super I is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

3) Carbopak C is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

4) Celite 545 is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

5) Active carbon-impregnated silica (019-11941) from Wako Pure Chemical Industries, is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

6) Active carbon-dispersed silica gel (01875-43) from Kanto Chemical Company, is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

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Table 3 — Suggested concentrations of PCDDs/PCDFs in stock and spiking solutions

PCDDs/PCDFs	Labelled-compound stock solution µg/l	Internal Standard spiking solution (6.10) µg/l	PAR stock solution (6.9) µg/l	PAR spiking solution (6.13) µg/l
2,3,7,8-TCDD	–	–	40	0,2
2,3,7,8-TCDF	–	–	40	0,2
1,2,3,7,8-PeCDD	–	–	200	1
1,2,3,7,8-PeCDF	–	–	200	1
2,3,4,7,8-PeCDF	–	–	200	1
1,2,3,4,7,8-HxCDD	–	–	200	1
1,2,3,6,7,8-HxCDD	–	–	200	1
1,2,3,7,8,9-HxCDD	–	–	200	1
1,2,3,4,7,8-HxCDF	–	–	200	1
1,2,3,6,7,8-HxCDF	–	–	200	1
1,2,3,7,8,9-HxCDF	–	–	200	1
2,3,4,6,7,8-HxCDF	–	–	200	1
1,2,3,4,6,7,8-HpCDD	–	–	200	1
1,2,3,4,6,7,8-HpCDF	–	–	200	1
1,2,3,4,7,8,9-HpCDF	–	–	200	1
OCDD	–	–	400	2
OCDF	–	–	400	2
$^{13}C_{12}$-2,3,7,8-TCDD	100	2	–	–
$^{13}C_{12}$-2,3,7,8-TCDF	100	2	–	–
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	2	–	–
$^{13}C_{12}$-1,2,3,7,8-PeCDF	100	2	–	–
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	2	–	–
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	2	–	–
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	100	2	–	–
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	2	–	–
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	2	–	–
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	2	–	–
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	100	2	–	–
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	2	–	–
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	2	–	–
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	2	–	–
$^{13}C_{12}$-OCDD	200	4	–	–

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Note the following precautions.

- Observe the safety precautions in the introductory warning note.
- Store standards, when they are not being used, in screw-cap vials with fluoropolymer-lined caps in the dark at room temperature.
- Check the mass of the standard-filled vials so that solvent loss by evaporation can be detected. If solvent loss has occurred, replace the solution.
- Check stock standard solutions for signs of degradation prior to the preparation of calibration or performance test standards.
- Analyze standard solutions used for quantitative purposes (6.9 to 6.13) periodically to assure stability, and assay against reference standards (see Note following) before further use.

NOTE Reference standards that can be used to determine the accuracy of calibration standards are available commercially.

6.9 Precision-and-recovery (PAR) stock solution

Using the solutions described in 6.8, prepare the PAR stock solution with the PCDDs/PCDFs at the concentrations shown in Table 3. When diluted, the solutions are referred to as the PAR standard solutions (6.13).

6.10 Internal standard spiking solution

Prepare the internal standard spiking solutions with the labelled PCDD/PCDF compounds in nonane at the concentrations shown in Table 3.

Dilute a sufficient volume of the internal standard spiking solution by a factor of 50 with acetone to prepare a diluted spiking solution. Each sample requires 1,0 ml of the diluted solution, but no more solution should be prepared than can be used in 1 d.

6.11 Recovery standard(s)

Prepare the recovery standard solution to contain $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD in nonane at the concentration shown in Table 4.

Table 4 — Suggested concentration of PCDDs/PCDFs in the recovery standard solution

Recovery standards	Concentration ng/ml
$^{13}C_{12}$-1,2,3,4-TCDD	200
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	200

6.12 Calibration standards

Combine the proper proportions of solutions specified in 6.9 to 6.11 in nonane to produce the five calibration solutions (CS1 through CS5) given in Table 5.

NOTE These solutions permit the relative response (of the labelled to the native compound) and the response factor to be measured as a function of concentration.

Use the CS3 standard for calibration verification (VER).

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Table 5 — Suggested calibration standard concentrations

Isomer	CS1 µg/l	CS2 µg/l	CS3[a](VER) µg/l	CS4 µg/l	CS5 µg/l
2,3,7,8-TCDF	0,5	2	10	40	200
2,3,7,8-TCDD	0,5	2	10	40	200
1,2,3,7,8-PeCDF	2,5	10	50	200	1 000
2,3,4,7,8-PeCDF	2,5	10	50	200	1 000
1,2,3,7,8-PeCDD	2,5	10	50	200	1 000
1,2,3,4,7,8-HxCDF	2,5	10	50	200	1 000
1,2,3,6,7,8-HxCDF	2,5	10	50	200	1 000
2,3,4,6,7,8-HxCDF	2,5	10	50	200	1 000
1,2,3,7,8,9-HxCDF	2,5	10	50	200	1 000
1,2,3,4,7,8-HxCDD	2,5	10	50	200	1 000
1,2,3,6,7,8-HxCDD	2,5	10	50	200	1 000
1,2,3,7,8,9-HxCDD	2,5	10	50	200	1 000
1,2,3,4,6,7,8-HpCDF	2,5	10	50	200	1 000
1,2,3,4,7,8,9-HpCDF	2,5	10	50	200	1 000
1,2,3,4,6,7,8-HpCDD	2,5	10	50	200	1 000
$^{13}C_{12}$2,3,7,8-TCDF	100	100	100	100	100
$^{13}C_{12}$2,3,7,8-TCDD	100	100	100	100	100
$^{13}C_{12}$1,2,3,4-TCDD	100	100	100	100	100
$^{13}C_{12}$ 1,2,3,7,8-PeCDF	100	100	100	100	100
$^{13}C_{12}$2,3,4,7,8-PeCDF	100	100	100	100	100
$^{13}C_{12}$1,2,3,7,8-PeCDD	100	100	100	100	100
$^{13}C_{12}$1,2,3,4,7,8-HxCDF	100	100	100	100	100
$^{13}C_{12}$1,2,3,6,7,8-HxCDF	100	100	100	100	100
$^{13}C_{12}$2,3,4,6,7,8-HxCDF	100	100	100	100	100
$^{13}C_{12}$1,2,3,7,8,9-HxCDF	100	100	100	100	100
$^{13}C_{12}$1,2,3,4,7,8-HxCDD	100	100	100	100	100
$^{13}C_{12}$1,2,3,6,7,8-HxCDD	100	100	100	100	100
$^{13}C_{12}$1,2,3,7,8,9-HxCDD	100	100	100	100	100
$^{13}C_{12}$1,2,3,4,6,7,8-HpCDF	100	100	100	100	100
$^{13}C_{12}$1,2,3,4,7,8,9-HpCDF	100	100	100	100	100
$^{13}C_{12}$1,2,3,4,6,7,8-HpCDD	100	100	100	100	100
$^{13}C_{12}$OCDD	200	200	200	200	200
OCDF	5,0	20	100	400	2 000
OCDD	5,0	20	100	400	2 000

[a] The CS3 standard is used for calibration verification (VER).

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6.13 Precision-and-recovery (PAR) spiking solution

Use this spiking solution to determine initial precision and recovery.

For each sample matrix, dilute 10 µl of the precision-and-recovery stock solution (6.9) to 2,0 ml with acetone.

6.14 GC-retention-time-window-defining solution and isomer specificity test standard

Use this standard solution to define the initial and final retention times for the dioxin and the furan isomers and to demonstrate the isomer specificity of the GC columns employed to analyze 2,3,7,8-TCDD and 2,3,7,8-TCDF. The standard shall contain at least the compounds listed in Table 6.

Table 6 — GC-retention-time-window-defining solution and isomer-specificity test standards

Type of test	CG column	Retention-time window		Isomer specificity test standard
		First compound	Last compound	
Definition of GC retention-time windows	DB5-MS[7])	1,3,6,8-TCDF	1,2,8,9-TCDF	—
		1,3,6,8-TCDD	1,2,8,9-TCDD	—
		1,3,4,6,8-PeCDF	1,2,3,8,9-PeCDF	—
		1,2,4,7,9-PeCDD	1,2,3,8,9-PeCDD	—
		1,2,3,4,6,8-HxCDF	1,2,3,4,8,9-HxCDF	—
		1,2,4,6,7,9-HxCDD	1,2,3,4,6,7-HxCDD	—
		1,2,3,4,6,7,8-HpCDF	1,2,3,4,7,8,9-HpCDF	—
		1,2,3,4,6,7,9-HpCDD	1,2,3,4,6,7,8-HpCDD	—
TCDD specificity test standard	DB5-MS	—	—	1,2,3,7+1,2,3,8-TCDD
		—	—	2,3,7,8-TCDD
		—	—	1,2,3,9-TCDD
TCDF isomer specificity test standard	DB225[8]	—	—	2,3,4,7-TCDF
		—	—	2,3,7,8-TCDF
		—	—	1,2,3,9-TCDF

6.15 Quality control check sample

Obtain a quality control (QC) check sample from a source independent of the calibration standards. Ideally, this sample shall be a certified reference material containing the PCDDs/PCDFs in known concentrations in a sample matrix similar to that of the actual samples under test.

7) DB5-MS is an example of a suitable product available commercially.This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

8) DB-225 is an example of a suitable product available commercially.This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

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7 Apparatus and materials

7.1 Water sample containers

Sample bottles, for aqueous samples (containing 1 % by mass solids or less), minimum volume of 1 l, made of amber glass, with a screw cap.

If amber bottles are not available, protect samples from light.

Bottle caps shall be lined with fluoropolymer.

Wash bottles in detergent water and rinse with solvent before use as in 5.2 b). Wash fluoropolymer liners in detergent water, rinse with reagent water and dry prior to use.

7.2 Equipment for sample preparation

7.2.1 Laboratory fume hood, of sufficient size to contain the sample preparation equipment listed below.

7.2.2 Glove box (optional).

7.2.3 Oven, capable of maintaining a constant temperature (± 5 °C) in the range 105 °C to 250 °C.

7.2.4 Desiccator.

7.2.5 Balances, two types: an analytical balance capable of weighing 0,1 mg and a top-loading balance capable of weighing 10 mg.

7.3 Extraction apparatus

7.3.1 pH meter, with combination glass electrode.

7.3.2 pH paper, wide range.

7.3.3 Graduated cylinder, 1 l capacity.

7.3.4 Liquid/liquid extraction-separatory funnels, 250 ml, 500 ml, and 2 000 ml, with glass or fluoropolymer stopcocks.

7.3.5 Soxhlet extraction unit, consisting of

a) **Soxhlet extractor**, 50-mm i.d., 200 ml capacity, with 500 ml round-bottom flask;

b) **thimble**, 43 mm × 123 mm to fit Soxhlet extractor;

c) **hemispherical heating mantle**, to fit 500 ml round-bottom flask.

7.3.6 Beakers, 400 ml to 500 ml.

7.3.7 Spatulas, made of stainless steel.

7.4 Filtration apparatus

7.1.4 Glass wool, Soxhlet-extracted with toluene for at least 3 h then dried in an oven at 105 °C.

7.4.2 Glass funnel, 125 ml to 250 ml.

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7.4.3 Glass-fibre filter paper, to fit glass funnel specified in 7.4.2.

7.4.4 Drying column, 15 mm to 20-mm i.d. quartz chromatographic column equipped with coarse-glass frit or glass-wool plug.

7.4.5 Buchner funnel.

7.4.6 Glass-fibre filter paper, to fit Buchner funnel specified in 7.4.5.

7.4.7 Filtration flasks, 1,5 l to 2,0 l, with side arm.

7.4.8 Pressure-filtration apparatus, consisting of side-arm filtration flasks, filter holders and a vacuum source.

7.5 Clean-up apparatus

7.5.1 Automated gel permeation chromatograph, consisting of

a) **column**, 600 mm to 700 mm long × 25-mm i.d., packed with 70 g of SX-3 Bio-beads[9)];

b) **syringe**, 10 ml, with Luer fitting;

c) **syringe filter-holde**r, stainless steel, with glass-fibre or fluoropolymer filters;

d) **UV detector**, 254 nm wavelength, with preparative or semi-preparative flow cell.

7.5.2 Disposable pipettes, either **Pasteur pipettes**, 150 mm long × 5-mm i.d., or **serological pipettes**, 10 ml with 6 mm i.d.

7.5.3 Glass liquid chromatographic columns of the following sizes:

a) 150 mm long × 8-mm i.d., with coarse glass frit or glass-wool plug and a 250 ml reservoir;

b) 200 mm long × 15-mm i.d., with coarse glass frit or glass-wool plug and a 250 ml reservoir;

c) 300 mm long × 25-mm i.d., with glass or fluoropolymer stopcock and a 300 ml reservoir.

7.5.4 Oven, capable of maintaining a constant temperature (± 5 °C) in the range of 105 °C to 700 °C for heating and storage of adsorbents.

7.5.5 Hotplate, capable of maintaining temperature of 65 °C ± 5 °C.

7.6 Concentration apparatus

7.6.1 Rotary evaporator, equipped with a variable-temperature water bath and the following:

a) **vacuum source for rotary evaporator**, equipped with a shut-off valve at the evaporator and a vacuum gauge;

b) **recirculating water pump and chiller**;

NOTE The use of tap water for cooling the evaporator wastes large volumes of water and can lead to inconsistent performance, as water temperatures and pressures vary.

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9) SX-3 Bio-beads is an example of a suitable product available commercially.This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

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c) **round-bottom flask**, 100 ml and 500 ml or larger, with ground-glass fitting compatible with the rotary evaporator.

7.6.2 Kuderna-Danish (K-D) concentrator, comprising the following:

a) **concentrator tube**, 10 ml, graduated with calibration verified, and a ground-glass stopper with a 19/22 joint to prevent evaporation of the extracts;

b) **evaporation flask**, 500 ml, with spring attachments to the concentrator tube;

c) **Snyder column**, three-ball macro and two-ball micro;

d) **boiling chips**, of the following types:

1) **glass or silicon carbide**, approximately 10/40 mesh, extracted with dichloromethane and heated at 450 °C for at least 1 h;

2) **fluoropolymer** (optional), extracted with dichloromethane.

7.6.3 Water bath, heated, with a concentric-ring cover, capable of maintaining a temperature to within ± 2 °C, installed in a fume hood.

7.6.4 Nitrogen blowdown apparatus, equipped with a water bath capable of maintaining a temperature in the range of 30 °C to 60 °C, installed in a fume hood.

7.6.5 Sample vials, of the following types:

a) amber glass, 2 ml to 5 ml, with fluoropolymer-lined screw-cap;

b) glass, 0,3 ml, conical, with fluoropolymer-lined screw or crimp cap.

7.7 Other equipment

7.7.1 Gas chromatograph, with a splitless or on-column injection port for a capillary column, temperature-programmed with isothermal hold.

The chromatograph shall meet all of the performance specifications in accordance with Clause 15.

7.7.2 GC capillary column, for PCDDs/PCDFs and for isomer specificity for 2,3,7,8-TCDD, of fused-silica, 60 m long x 0,3-mm i.d.; 5 % phenyl-, 94 % methyl-, 1 % vinyl-silicone-bonded phase, 0,25 µm thick.

7.7.3 GC capillary column, for isomer specificity for 2,3,7,8-TCDF, of fused-silica, 30 m long × 0,32-mm i.d.; bonded phase (DB-225[8])), 25 µm thick.

7.7.4 Mass spectrometer, 28 eV to 80 eV electron impact ionization, capable of repetitively monitoring on a selective basis at least 12 exact masses at high resolution (> 6 000) during a period of approximately 1 s.

The mass spectrometer shall meet all of the performance specifications in accordance with Clause 15.

7.7.5 GC/MS interface.

The mass spectrometer (MS) shall be interfaced to the GC in accordance with the manufacturer's instructions.

7.7.6 Data system, capable of collecting, recording, and storing MS data.

8 Sample collection, preservation, storage and holding times

8.1 Collect samples in amber glass containers in accordance with ISO 5667-1 and ISO 5667-2.

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8.2 Maintain aqueous samples in the dark at 0 °C to 4 °C from the time of collection until receipt at the laboratory. If residual chlorine is present in aqueous samples, add 80 mg of sodium thiosulfate per litre of sample. If sample pH is greater than 9, adjust to pH 7 to pH 9 with sulfuric acid.

There are no demonstrated maximum holding times associated with PCDDs/PCDFs in aqueous sample matrices. If stored in the dark at 0 °C to 4 °C and preserved in accordance with 8.1 and this subclause, aqueous samples may be stored for up to 1 year (if required).

8.3 Store sample extracts in the dark until analyzed. Sample extracts may be stored in the dark at < –10 °C for up to 1 year.

9 Quality assurance/quality control

9.1 General

9.1.1 Each laboratory that adheres to this International Standard shall operate a formal quality assurance program. The minimum requirements of this program consist of the following:

a) initial demonstration of laboratory capability;

b) analysis of samples spiked with internal standards to evaluate and document data quality;

c) analysis of standards and blanks as tests of continued performance.

Laboratory performance shall be compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

9.1.2 The analyst shall make an initial demonstration of the ability to generate data of acceptable accuracy and precision in accordance with this International Standard. This ability shall be established as specified in 9.2.

9.1.3 In recognition of advances that are occurring in analytical technology, and to allow the analyst to overcome sample matrix interferences, the analyst shall be permitted certain options to improve separations or lower the costs of measurements. These options include alternate extraction, concentration, clean-up procedures, and changes in columns and detectors. Alternate determinative techniques, such as the substitution of spectroscopic or immuno-assay techniques, and changes that degrade method performance, shall not be allowed. If an analytical technique other than the techniques specified in this method is used, that technique shall have a specificity equal to or better than the specificity of the techniques in this method for the analytes of interest.

9.1.3.1 Each time a modification is made to this method, the analyst shall repeat the procedure in 9.2. If the detection limit of the method is affected by the change, the laboratory shall demonstrate that the MDL is lower than one-third of any regulatory compliance level in force or one-third of the ML in this method, whichever is higher. If calibration is affected by the change, the analyst shall recalibrate the instrument in accordance with Clause 10.

9.1.3.2 The laboratory shall maintain records of modifications made to this method. These records shall include at least the following:

a) the names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and of the quality control officer who witnessed and verified the analyses and modifications;

b) a list of pollutant(s) measured, by name and CAS Registry number;

c) a narrative stating the reason(s) for the modifications;

d) results from all quality control (QC) tests comparing the modified method to this International Standard, including

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1) calibration (see 10.6 and 10.7),

2) calibration verification (see 15.3),

3) initial precision and recovery (see 9.2),

4) internal standard recovery (see 9.3),

5) analysis of blanks (see 9.4),

6) accuracy assessment (see 9.3.4);

e) information that will allow an independent reviewer to trace and validate individual determinations from the extraction procedure to the final result, including

1) sample numbers and other identifiers,

2) extraction dates,

3) analysis dates and times,

4) analysis sequence/run chronology,

5) sample mass or volume (see Clause 11),

6) final extract volume prior to injection,

7) injection volume [see 14 c)],

8) dilution factor, differentiating between dilution of 1 sample or extract [see 19 c)],

9) instrument and operating conditions,

10) column (dimensions, liquid phase, solid support, film thickness, etc.),

11) operating conditions (temperatures, temperature program, flow rates),

12) detector (type, operating conditions, etc.),

13) chromatograms, instrument output (peak height, area, or other signal), printer tapes, and other recordings of raw data,

14) quantitation reports, data system outputs, and other information to link the raw data to the results reported.

9.1.4 Analyses of method blanks shall demonstrate freedom from contamination (5.3). The procedures and criteria for analysis of a method blank are described in 9.4 and 15.5.

9.1.5 Spike all samples with internal standards to monitor method performance (9.3). When results of these spikes indicate atypical method performance for samples, dilute the samples (17.3.2) to bring method performance within acceptable limits.

9.1.6 The laboratory shall, on an ongoing basis, demonstrate through calibration verification (Clause 15) that the analytical system is under control.

9.1.7 The laboratory shall maintain records to define the quality of data that is generated.

NOTE Development of accuracy statements is described in 9.3.4.

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9.2 Initial precision and recovery (IPR)

To establish the ability to generate acceptable precision and recovery, the analyst shall perform the following operations.

a) Extract, concentrate and analyze four aliquots of reagent water (6.1) spiked with the diluted internal standard spiking solution (6.10) and the precision-and-recovery (PAR) spiking solution (6.13) according to the procedures in Clauses 12 to 19. For an alternative sample matrix, four aliquots of the alternative reference matrix (6.7) are used. All sample-processing steps that are to be used for processing samples, including preparation (see Clause 11), extraction (see Clause 12), and clean-up (Clause 13), shall be included in this test.

b) Using results of the set of four analyses, compute the average concentration ($\bar{c}$) of the extracts in nanograms per millilitre and the relative standard deviation of the concentration (s_{rel}) in nanograms per millilitre for each compound, by isotope dilution for PCDDs/PCDFs with a labelled analogue, and by internal standard for those without a labelled analogue.

c) For each PCDD/PCDF compound, compare s_{rel} and $\bar{c}$ with the respective limits for initial precision and recovery: < 20 % for s_{rel} and $\pm$ 20 % of test concentration for $\bar{c}$. If s_{rel} and $\bar{c}$ for all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, any individual s_{rel} exceeds the precision limit or any individual $\bar{c}$ falls outside the range for accuracy, system performance is unacceptable for that compound. Correct the problem and repeat the test.

9.3 Spiking

9.3.1 Spike all samples with the diluted internal standard spiking solution (6.10) to assess method performance on the sample matrix.

9.3.2 Analyze each sample in accordance with the procedures in Clauses 11 to 18.

9.3.3 Determine the percent recovery F_{rec} of the internal standards using the internal standard method (10.7).

9.3.4 If the percent recovery of the internal standard falls outside the range 50 % to 130 % for tetra- to hexa-chlorinated congeners or 40 % to 130 % for hepta- to octa-chlorinated congeners, then provided the sum of the contributions to the total TEQ in the sample from all of the congeners with recoveries not within these ranges does not exceed 10 %, the acceptable ranges shall be 30 % to 150 % for the tetra- to hexa-chlorinated congeners and 20 % to 150 % for the hepta- to octa-chlorinated congeners.

If the recovery of any compound falls outside these limits, method performance shall be considered to be unacceptable for that compound in that sample. To overcome such difficulties, water samples shall be diluted and reanalyzed.

9.3.5 Assessment of the recovery of internal standard.

After the analysis of five samples of a given matrix type for which the internal standards pass the tests specified in 9.3.1 to 9.3.4, compute the average percent recovery ($\bar{F}_{rec}$) and the standard deviation of the percent recovery (s_{rec}) only for the internal standards. Express the assessment as a percent-recovery interval from [$\bar{F}_{rec}$ −2(s_{rec})] to [$\bar{F}_{rec}$ +2(s_{rec})] for each matrix.

EXAMPLE If $\bar{F}_{rec}$ = 90 % and s_{rec} = 10 % for five analyses, the recovery interval is expressed as 70 % to 110 %.

Update the accuracy assessment for each internal standard in each matrix on a regular basis (e.g. after each 5 to 10 new measurements).

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9.4 Method blanks

9.4.1 Reference matrix method blanks are analysed to demonstrate freedom from contamination (5.3).

9.4.2 Prepare, extract, clean-up and concentrate a method blank with each sample batch (consisting of a maximum of 20 samples with the same matrix extracted during the same 12-h shift). The matrix for the method blank shall be similar to sample matrix for the batch, e.g. an aliquot of reagent water blank (6.1). Analyze the blank immediately prior to analysis of the samples to demonstrate freedom from contamination.

9.4.3 Analysis of samples shall be halted if the blank associated with the sample batch shows the following evidence of contamination:

a) any 2,3,7,8-substituted PCDD/PCDF is found in the blank at a concentration greater than the minimum level for samples as specified in Table 2; or greater than one-third of any regulatory compliance level in force, whichever is greater;

or

b) any potentially interfering compound is found in the blank above the minimum concentration for each level of chlorination specified in Table 2 (assuming a response factor of 1 relative to the $^{13}C_{12}$1,2,3,4-TCDD recovery standard for compounds not listed in Table 1).

Analysis of samples shall not resume until the blank associated with the sample batch shows no evidence of contamination above the specified levels. All samples shall be associated with an uncontaminated method blank before the results for those samples may be reported for regulatory compliance purposes.

9.5 Quality control check sample

Analyze the QC check sample (6.15) periodically to assure the accuracy of calibration standards and the overall reliability of the analytical process. This should be done at least quarterly.

9.6 Method precision

The apparatus used shall be calibrated properly and then shall be maintained in a calibrated state. Under these circumstances, the specifications contained in this International Standard can be met. The standards used for calibration (Clause 10), for calibration verification (15.3) and for initial precision and recovery (9.2) should be identical in order to obtain the most precise results.

A GC/MS instrument will provide the most reproducible results if dedicated to the settings and conditions required for the analyses of PCDDs/PCDFs by this method.

This subclause (9.6), as indicated by its title, is concerned only with the random variation of results given by the instrument. The same can be said for the calibration check (Clause 10) and the recovery of internal standards (9.3). The inclusion of standards from other sources may give an indication of systematic as well as random error; however the former may be better evaluated using other procedures, i.e. with quality control standards.

Depending on specific program requirements, field replicates may be collected to determine the precision of the sampling technique, and spiked samples may be required to determine the accuracy of the analysis when the internal standard method is used.

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10 Calibration

10.1 Operating conditions

Operating conditions should be such to as to approximate the relative retention times for the PCDDs/PCDFs in Table 2.

GC operating conditions are dependent on GC column type. Examples of suggested conditions taken from EN 1948-3[3] and JIS K 0312[4] are given in Table 7.

Optimize GC conditions for compound separation and sensitivity. Once optimized, use the same GC conditions for the analysis of all standards, blanks, IPR aliquots and samples.

Table 7 — Typical GC columns and temperature programs

Column type and characteristics	Temperature program	Injector temperature	Analytes
SP-2331 60 m, 0,32 mm id, 0,2 µm film thickness	100 °C (1,5 min), 20 °C/min to 180 °C, 3 °C/min to 260 °C, hold 25 min	260 °C	TCDD, TCDF and PeCDF homologue groups and 2,3,7,8-chloro-substituted isomers
SP-2331 60 m, 0,32 mm id, 0,2 µm film thickness	100 °C (1,5 min), 20 °C/min to 210 °C, 3 °C/min to 260 °C, hold 25 min	260 °C	PeCDD, HxCDD and HxCDF homologue groups and 2,3,7,8-chloro-substituted isomers
DB-17 30 m, 0,32 mm id, 0,15 µm film thickness	100 °C (1,5 min), 20 °C/min to 200 °C, 10 °C/min to 280 °C, hold 5 min	280 °C	HpCDD and HpCDF homologue groups and OCDD, OCDF and 2,3,7,8-chloro-substituted isomers
SP-2331 60 m, 0,25 mm id, 0,2 µm film thickness	100 °C (1 min), 20 °C/min to 200 °C, 2 °C/min to 260 °C	260 °C	TCDDs through HxCDD and TCDFs through HxCDF and 2,3,7,8-chloro-substituted isomers
HP-5 25 m, 0;20 mm id, 0;25 µm film thickness	100 °C (1 min), 20 °C/min to 200 °C, 5 °C/min to 300 °C	300 °C	HpCDD and HpCDF homologue groups and OCDD, OCDF and 2,3,7,8-chloro-substituted isomers
DB-17 30 m, 0,32 mm id, 0,25 µm film thickness	150 °C (3 min), 20 °C/min to 200 °C, 3 °C/min to 280 °C	150 °C to 300 °C (100 °C/min)	All homologue group totals and selected 2,3,7,8-chloro-substituted isomers
Ultra 2 60 m, 0,25 mm id, 0,10 µm film thickness	70 °C (1 min), 25 °C/min to 200 °C, 3 °C/min to 300 °C, hold 1 min	270 °C	Total PCDDs and PCDFs
RTX-2330 60 m, 0,25 mm id, 0,1 µm film thickness	70 °C (1 min), 25 °C/min to 200 °C, 3 °C/min to 275 °C, hold 4 min	270°C	Specific isomers of PCDD/PCDF
DB5-MS 60 m, 0,25 mm id, 0,25 µm film thickness	140 °C (0,6 min), 35 °C/min to 210 °C, 1,6 °C/min to 250 °C, 3,5 °C/min to 310 °C, hold 10 min	300 °C	Homologue group totals and selected 2,3,7,8-chloro-substituted isomers
RTX-2330	140 °C (1 min), 12 °C/min to 200 °C, 2,8 °C/min to 270 °C, 0,6 °C/min to 275 °C, hold 2 min	280 °C	Selected 2,3,7,8-chloro-substituted isomers

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10.2 Mass spectrometer (MS) resolution

Obtain a selected ion current profile of each analyte specified in Table 3 for the two exact masses chosen (examples are given in Table 8) at a resolving power > 6 000 by injecting an authentic standard of the PCDDs/PCDFs either singly or as part of a mixture in which there is no interference between closely eluting components.

Using a perfluorokerosene (PFK) molecular peak (or that of any other reference material), tune the instrument to meet the minimum required resolving power of 6 000, with a 10 % valley, at mass 304,982 4 (PFK) or at any other reference signal close to mass 304 (from TCDF). For each descriptor (examples are given in Table 8), monitor and record the resolution and exact masses of three to five reference peaks covering the mass range of the descriptor. The resolution shall be equal to or greater than 6 000 and the deviation between the exact mass and the theoretical mass (Table 8) for each exact mass monitored shall be less than 10×10^{-6}.

Overloading an instrument with PFK (or any other reference substance) may cause noise problems and contamination of the ion source necessitating increased frequency of source cleaning.

Table 8 — Example programme of mass values to be monitored

Descriptor	Exact mass[a]	Mass type	Elemental composition	Substance
1	292,982 5	Lock	C_7F_{11}	PFK
	303,901 6	M	$C_{12}H_4{}^{34}Cl_4O$	TCDF
	305,898 7	M+2	$C_{12}H_4{}^{35}Cl_3{}^{37}ClO$	TCDF
	315,941 9	M	$^{13}C_{12}H_4{}^{34}Cl_4O$	TCDF[b]
	317,938 9	M+2	$^{13}C_{12}H_4{}^{35}Cl_3{}^{37}ClO$	TCDF[b]
	319,896 5	M	$C_{12}H_4{}^{35}Cl_4O_2$	TCDD
	321,893 6	M+2	$C_{12}H_4{}^{35}Cl_3{}^{37}ClO_2$	TCDD
	330,979 2	QC	C_7F_{13}	PFK
	331,936 8	M	$^{13}C_{12}H_4{}^{35}Cl_4O_2$	TCDD[b]
	333,933 9	M+2	$^{13}C_{12}H_4{}^{35}Cl_3{}^{37}ClO_2$	TCDD[b]
	375,836 4	M+2	$C_{12}H_4{}^{35}Cl_5{}^{37}ClO$	HxCDPE
2	339,859 7	M+2	$C_{12}H_3{}^{35}Cl_4{}^{37}ClO$	PeCDF
	341,856 7	M+4	$C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O$	PeCDF
	351,900 0	M+2	$^{13}C_{12}H_3{}^{35}Cl_4{}^{37}ClO$	PeCDF[b]
	353,897 0	M+4	$^{13}C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O$	PeCDF[b]
	354,979 2	Lock	C_9F_{13}	PFK
	355,854 6	M+2	$C_{12}H_3{}^{35}Cl_4{}^{37}ClO_2$	PeCDD
	357,851 6	M+4	$C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O_2$	PeCDD
	367,894 9	M+2	$^{13}C_{12}H_3{}^{35}Cl_4{}^{37}ClO_2$	PeCDD[b]
	369,891 9	M+4	$^{13}C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O_2$	PeCDD[b]
	409,797 4	M+2	$C_{12}H_3{}^{35}Cl_6{}^{37}ClO$	HpCDPE

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Table 8 — (*continued*)

Descriptor	Exact mass[a]	Mass type	Elemental composition	Substance
3	373,820 8	M+2	$C_{12}H_2{}^{35}Cl_5{}^{37}ClO$	HxCDF
	375,817 8	M+4	$C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O$	HxCDF
	383,863 9	M	$^{13}C_{12}H_2{}^{35}Cl_6O$	HxCDF[b]
	385,861 0	M+2	$^{13}C_{12}H_2{}^{35}Cl_5{}^{37}ClO$	HxCDF[b]
	389,815 7	M+2	$C_{12}H_2{}^{35}Cl_5{}^{37}ClO_2$	HxCDD
	391,812 7	M+4	$C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O_2$	HxCDD
	392,976 0	Lock	C_9F_{15}	PFK
	401,855 9	M+2	$^{13}C_{12}H_2{}^{35}Cl_5{}^{37}ClO_2$	HxCDD[b]
	403,852 9	M+4	$^{13}C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O_2$	HxCDD[b]
	430,972 9	QC	C_9H_{17}	PFK
	445,755 5	M+4	$C_{12}H_2{}^{35}Cl_6{}^{37}Cl_2O$	OCDPE
4	407,781 8	M+2	$C_{12}H^{35}Cl_6{}^{37}ClO$	HpCDF
	409,778 9	M+4	$C_{12}H^{35}Cl_5{}^{37}Cl_2O$	HpCDF
	417,825 3	M	$^{13}C_{12}H^{35}Cl_7O$	HpCDF[b]
	419,822 0	M+2	$^{13}C_{12}H^{35}Cl_6{}^{37}ClO$	HpCDF[b]
	423,776 6	M+2	$C_{12}H^{35}Cl_6{}^{37}ClO_2$	HpCDD
	425,773 7	M+4	$C_{12}H_{35}Cl_5{}^{37}Cl_2O_2$	HpCDD
	430,972 9	Lock	C_9F_{17}	PFK
	435,816 9	M+2	$^{13}C_{12}H^{35}Cl_6{}^{37}ClO_2$	HpCDD[b]
	437,814 0	M+4	$^{13}C_{12}H^{35}Cl_5{}^{37}Cl_2O_2$	HpCDD[b]
	479,716 5	M+4	$C_{12}H^{35}Cl_7{}^{37}Cl_2O$	NCDPE
5	441,742 8	M+2	$C_{12}{}^{35}Cl_7{}^{37}ClO$	OCDF
	442,972 8	Lock	$C_{10}F_{17}$	PFK
	443,739 9	M+4	$C_{12}{}^{35}Cl_6{}^{37}Cl_2O$	OCDF
	457,737 7	M+2	$C_{12}{}^{35}Cl_7{}^{37}ClO_2$	OCDD
	459,734 8	M+4	$C_{12}{}^{35}Cl_6{}^{37}Cl_2O_2$	OCDD
	469,777 9	M+2	$^{13}C_{12}{}^{35}Cl_7{}^{37}ClO_2$	OCDD[b]
	471,775 0	M+4	$^{13}C_{12}{}^{35}Cl_6{}^{37}Cl_2O_2$	OCDD[b]
	513,677 5	M+4	$C_{12}{}^{35}Cl_8{}^{37}Cl_2O$	DCDPE

[a] These masses refer to PFK ions, but other reference compounds may be used.

[b] Labelled compound.

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10.3 QC limits and theoretical ion abundance ratios for standards

Choose an injection volume of, for example 1 µl, consistent with the capability of the HRGC/HRMS instrument. (Large volume injection may be used.)

Inject an aliquot of the CS1 calibration solution (Table 5) using the GC conditions in accordance with 10.1.

The SIM areas for each analyte should be measured and the ion-abundance ratios should be computed at the exact masses given in Table 8. Compare the computed ratio to the theoretical ratio given in Table 9.

Additional masses may be monitored for each descriptor, and the masses may be divided among more than the five descriptors listed in Table 8, provided that the laboratory is able to monitor the masses of all the PCDDs/PCDFs that may elute from the GC within a given retention-time window.

Table 9 — Theoretical ion ratios and QC limits

Number of chlorine atoms	Mass-forming ratio	Theoretical ratio	Lower QC limit[a]	Upper QC limit[a]
4	M/(M+2)	0,77	0,62	0,92
5	(M+2)/(M+4)	1,55	1,24	1,86
6	(M+2)/(M+4)	1,24	0,99	1,49
6[b]	M/(M+2)	0,51	0,41	0,61
7	(M+2)/(M+4)	1,05	0,84	1,26
7[c]	M/(M+2)	0,44	0,35	0,53
8	(M+2)/(M+4)	0,89	0,71	1,07

a Upper and lower QC limits are based on ±20 % of theoretical.

b Used for $^{13}C_{12}$HxCDF only.

c Used for $^{13}C_{12}$HpCDF only.

Operate the mass spectrometer in a mass-drift correction mode, using PFK or any other suitable reference compound to provide lock masses. Monitor each lock mass and make sure that it does not vary by more than ± 20 % throughout its respective retention time window.

The lock-mass for each group of masses is shown in Table 8. A variations in the intensity of the lock mass by more than 20 % indicates the presence of co-eluting interferences that may significantly reduce the sensitivity of the mass spectrometer. Re-injection of another aliquot of the sample extract will not resolve the problem. Additional clean-up of the extract may be required to remove the interferences.

All native PCDDs/PCDFs and internal standards in the CS1 standard shall be within the QC limits in Table 9 for their respective ion abundance ratios; otherwise, the mass spectrometer shall be adjusted and this test repeated until the mass ratios fall within the limits specified. If adjustment alters the resolution of the mass spectrometer, the resolution shall be verified (10.2) prior to repeat of the test.

Verify that the HRGC/HRMS instrument meets the minimum levels in Table 2. The peaks representing the PCDDs/PCDFs and the internal standards in the CS1 calibration standard shall have signal-to-noise ratios (S/N) equal to or greater than 10. Otherwise, adjust the mass spectrometer and repeat this test until the minimum levels in Table 2 are met.

10.4 Retention time

Analyze the window-defining mixtures (6.14) using the optimized temperature program specified in 10.1. Table 6 gives the elution order (first/last) of the window-defining compounds.

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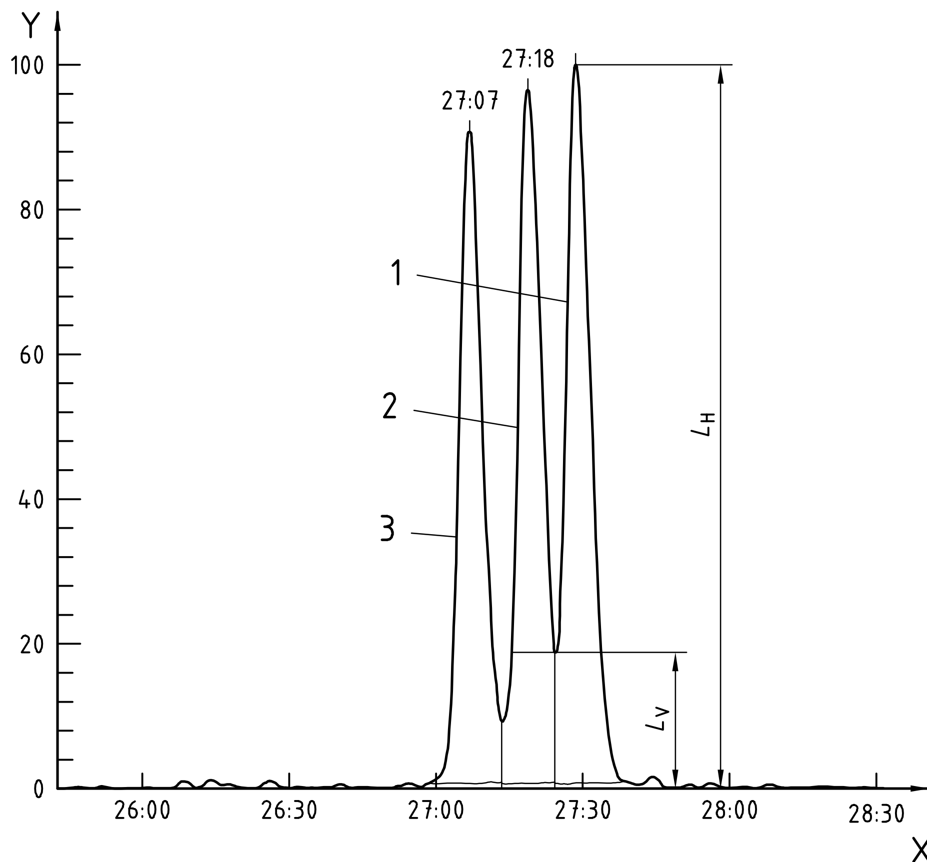
10.5 Isomer specificity

Analyze the isomer-specificity test standards (6.14) using the procedure specified in Clause 14 and the optimized conditions for sample analysis (10.1).

Compute the percent valley between the GC peaks of the 2,3,7,8-substituted isomers and those of the most closely eluting compounds by measuring the height above the baseline (distance L_V in Figures 1 and 2) of the valley between the peak of the 2,3,7,8-substituted isomer and that of the most closely eluting compound and dividing it by the peak height (distance L_H in Figures 1 and 2) of the 2,3,7,8-substituted isomer.

a) Verify that for 2,3,7,8-TCDD, the ratio of the of valley-height to peak-height on a DB5-MS column is less than 25 % (computed as $100 \times L_V/L_H$ in Figure 1). If this ratio is more than 25 %, adjust the analytical conditions and repeat the test or replace the GC column and recalibrate (10.2 to 10.8).

b) Verify that for 2,3,7,8-TCDF, the ratio of the of valley-height to peak-height on a DB225 column is les than 50 % (computed as $100 \times L_V/L_H$ in Figure 2). If this ratio is more than 50 %, adjust the analytical conditions and repeat the test or replace the GC column and recalibrate (10.2 to 10.8).



Key

1 2,3,7,8-TCDD

2 1,2,3,9-TCDD

3 1,2,3,7/1,2,3,8-TCDD

L_V valley height, in relative units (see 10.5)

L_H peak height, in relative units (see 10.5)

X retention time, in minutes

Y intensity of detector response, in percent

Figure 1 — Isomer-specific separation of 2,3,7,8-TCDD on a DB5-MS[7)] column

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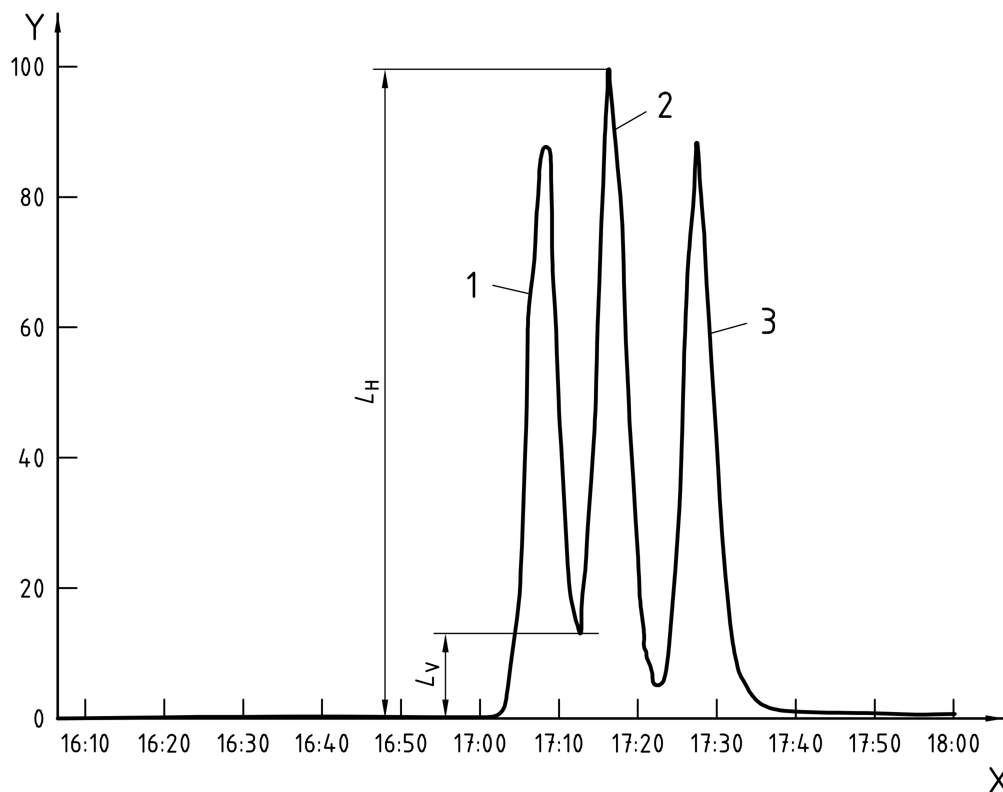

Key

1 2,3,4,7-TCDF

2 2,3,7,8-TCDF

3 1,2,3,9-TCDF

L_V valley height, in relative units (see 10.5)

L_H peak height, in relative units (see 10.5)

X retention time, in minutes

Y intensity of detector response, in percent

Figure 2 — Isomer-specific separation of 2,3,7,8-TCDF on a DB-225[8)] column

10.6 Calibration by isotope dilution

Isotope dilution calibration is used for the 2,3,7,8-substituted PCDDs/PCDFs for which labelled internal standard analogues are added to samples prior to extraction. The reference compound for each PCDD/PCDF compound is shown in Table 2.

Prepare a calibration curve encompassing the concentration range for each compound to be determined. Plot the relative response, R_{rel}, (of the labelled to the native compound) versus concentration in standard solutions or compute a linear regression equation. Determine the relative response in accordance with the procedures described below. Employ five calibration points.

Determine the response of each PCDD/PCDF relative to its labelled internal standard analogue, using the area responses of both the primary and secondary exact masses specified in Table 8, for each calibration standard, in accordance with Equation (1):

$$R_{rel} = \frac{(A_{1n} + A_{2n})\rho_L}{(A_{1L} + A_{2L})\rho_n} \quad (1)$$

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where

A_{1n} is the area of the primary mass for the native PCDD/PCDF;

A_{2n} is the area of the secondary mass for the native PCDD/PCDF;

A_{1L} is the area of the primary mass for the labelled internal standard analogue;

A_{2L} is the area of the secondary mass for the labelled internal standard analogue;

ρ_L is the concentration of the labelled internal standard analogue in the calibration standard (see Table 5);

ρ_n is the concentration of the native compound in the calibration standard (see Table 5).

To calibrate the analytical system by isotope dilution, inject a volume of the calibration standards CS1 to CS5 (6.12 and Table 5) identical to that chosen in 10.3, using the procedure in Clause 15 and the conditions in 11.1 and Table 2. Compute the relative response, R_{rel}, at each concentration.

If the relative response for any compound is constant (i.e. with a coefficient of variation of less than 20 %) over the five-point calibration range, an averaged relative response may be used for that compound; otherwise, the complete calibration curve for that compound shall be used over the five-point calibration range.

10.7 Calibration by internal standard

The internal standards method is used to analyze for 2,3,7,8-substituted PCDDs and PCDFs when no labelled internal standard analogues are added to the sample, for the non-2,3,7,8-substituted compounds, and for the internal standards for intra-laboratory statistics (9.3.4).

Calibration requires the determination of response factors, F_R, in accordance with Equation (2):

$$F_R = \frac{(A_{1s} + A_{2s})\rho_{is}}{(A_{1is} + A_{2is})\rho_s} \qquad (2)$$

where

A_{1s} is the area of the primary mass for the native and labelled PCDD/PCDF;

A_{2s} is the area of the secondary mass for the native and labelled PCDD/PCDF;

A_{1is} is the area of the primary mass for the internal standard;

A_{2is} is the area of the secondary mass for the internal standard;

ρ_{is} is the concentration, in micrograms per litre, of the internal standard (Table 5);

ρ_s is the concentration, in micrograms per litre, of the compound in the calibration standard (Table 5).

To calibrate the analytical system by internal standard, inject a volume of the calibration standards CS1 to CS5 (6.12 and Table 5) identical to that chosen in 10.3 using the procedure in Clause 14 and the conditions in 10.1 and Table 2. Compute the response factor, F_R, at each concentration.

If the response factor (F_R) for any compound is constant (with a coefficient of variation of less than 20 %) over the five-point calibration range, an averaged response factor may be used for that compound; otherwise, the complete calibration curve for that compound shall be used over the five-point range.

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10.8 Combined calibration

10.8.1 General

A single set of analyses is used to produce calibration curves for the isotope dilution and internal standard methods, based on the calibration solutions (6.12 and Table 5) containing the PCDDs/PCDFs and on the internal and the recovery standards. These curves are verified for each shift/batch (15.3) by analyzing the calibration-verification standard (VER, Table 5). Recalibration is required if any of the calibration-verification criteria (15.3) cannot be met.

10.8.2 Data storage

MS data shall be collected, recorded, and stored.

10.8.3 Data acquisition

The signal at each exact mass shall be collected repetitively throughout the monitoring period and stored on a mass-storage device.

10.8.4 Response factors and multipoint calibrations

The data system shall be used to record and maintain lists of response factors (response ratios for isotope dilution) and multi-point calibration curves. Computations of relative standard deviation (coefficient of variation) shall be used to test calibration linearity. Statistics on initial performance (9.2) should be computed and maintained, either on the instrument data system, or on a separate computer system.

11 Sample preparation

11.1 General

Sample preparation involves modifying the physical form of the sample so that the PCDDs/PCDFs can be extracted efficiently. In general, the samples shall be in a liquid form or in the form of finely divided solids in order for efficient extraction to take place.

For samples known or expected to contain high levels of the PCDDs/PCDFs, use the smallest sample size representative of the entire sample (17.3.2).

For all samples, process the blank and IPR aliquots through the same steps as the sample to check for contamination and losses in the preparation processes.

Because PCDDs/PCDFs may be bound to suspended particles, the preparation of aqueous samples is dependent on the solids content of the sample.

a) For samples that contain particulates, determine the mass fraction of solids in the sample using the procedure in 11.2.

b) Process aqueous samples with 10 g or less suspended solids per litre of sample in accordance with 11.3.

11.2 Determination of suspended solids

The mass fraction of suspended solids in the sample is determined as specified by 11.2 a) through 11.2 d).

a) Desiccate and weigh a glass-fibre filter paper (7.4.3) to three significant figures.

b) Filter (10,0 ± 0,02) ml of well mixed sample through the filter.

c) Dry the filter for a minimum of 12 h at (110 ± 5) °C and cool in a desiccator for a minimum of 1 h.

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d) Calculate the mass fraction, m_s, of suspended solids in the sample in accordance with Equation (3):

$$m_s = \frac{(m_{ds} - m_f) \times 100}{10} \quad (3)$$

where

10 is the mass, in grams, of the filtered sample;

m_{ds} is mass, in grams, of the dried sample;

m_f is mass, in grams, of the filter.

11.3 Preparation of aqueous samples containing 1 % or less by mass suspended solids

11.3.1 General

Prepare aqueous samples with no visible particles as specified in 11.3.2 and extract directly, using the separatory funnel technique specified in 12.1. Prepare aqueous samples containing visible particles and 1 % or less by mass suspended solids using the procedure specified in 11.3.2 and 11.3.3. After the filtration procedure, continue with Soxhlet extraction of the filter and particles (12.2) and separatory funnel extraction of the filtrate (12.1).

11.3.2 Preparation of samples and QC aliquots

Samples and QC aliquots are prepared as specified in 11.3.2 a) through 11.3.2 e).

a) Mark the original level of the sample on the sample bottle for reference. Weigh the sample plus bottle to ± 1 g.

b) Spike the sample with 1,0 ml of the diluted internal standard spiking solution (6.10). Cap the bottle and mix the sample by careful shaking. Allow the sample to equilibrate for 1 h to 2 h, with occasional shaking.

c) For each sample or sample batch (to a maximum of 20 samples to be extracted during the same 12-h shift), place one 1,0-l aliquot of reagent water in a clean sample bottle or flask.

d) Spike the reagent water aliquot with 1,0 ml of the diluted internal standard spiking solution (6.10). Use this as the method blank.

e) If the sample does not contain particles visible to the naked eye, proceed to 12.1 for extraction. If the sample contains particles visible to the naked eye, proceed to 11.3.3 for filtration of particles.

11.3.3 Filtration of particles

The filtration process is carried out as specified by 11.3.3 a) through 11.3.3 f).

a) Assemble a Buchner funnel (7.4.5) with a glass-fibre filter (7.4.6) on top of a clean filtration flask. Apply vacuum to the flask, and pour the entire contents of the sample bottle into the funnel, swirling the sample remaining in the bottle to suspend any particles.

b) Rinse the sample bottle twice with approximately 5 ml portions of reagent water to transfer any remaining particles onto the filter.

c) Rinse any particles off the sides of the Buchner funnel with two 5-ml portions of reagent water.

d) Weigh the empty sample bottle to ± 1 g. Determine the mass of the sample by difference. Save the bottle for further use.

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e) Extract the filtrate using the separatory funnel procedure specified in 12.1.

f) Extract the filter containing the particles using the Soxhlet procedure specified in 12.2.

12 Extraction and concentration

12.1 Extraction of samples with no visible particulates

Filtrates and aqueous samples with no visible particles are extracted in a separatory funnel as specified by 12.1 a) through 12.1 j).

a) Pour the spiked sample [11.3.2 b)] or filtrate [11.3.3 e)] into a 2-l separatory funnel.

b) Rinse the bottle or flask twice with 5 ml of reagent water and add these rinses to the separatory funnel.

c) Add 60 ml of dichloromethane to the empty sample bottle, seal, and shake 60 s to rinse. Other solvents may be used provided that all of the performance criteria of this International Standard can be met.

d) Transfer the solvent to the separatory funnel, and extract the sample by shaking the funnel for 2 min with periodic venting. Allow the organic layer to separate from the aqueous phase for a minimum of 10 min. If more than one third the volume of the solvent layer forms an emulsion, employ mechanical techniques to complete the phase separation.

e) The optimum technique to separate an emulsion depends upon the sample, but may include stirring, filtration through glass wool, use of phase separation paper, centrifugation, use of an ultrasonic bath with ice, addition of NaCl, or other physical methods. Alternatively, solid-phase or other extraction techniques may be used to prevent emulsion formation. Any alternative technique is acceptable so long as the requirements specified in Clause 9 are met.

f) Drain the dichloromethane extract through a solvent-rinsed glass funnel approximately one-half full of granular anhydrous sodium sulfate (6.3.1) supported on a clean glass-fibre filter into a solvent-rinsed concentration device (12.4).

g) Extract the water sample two more times with 60 ml portions of dichloromethane. Drain each portion through the sodium sulfate into the concentrator. After the third extraction, rinse the separatory funnel with at least 20 ml of dichloromethane, and drain this rinse through the sodium sulfate into the concentrator. Repeat this rinse at least twice. Set aside the funnel with sodium sulfate if the extract is to be combined with the extract from the particles.

h) Concentrate the extract using one of the macro-concentration procedures in 12.4.

i) If the extract is from a sample with no visible particulates [11.1 a)], adjust the final volume of the concentrated extract to approximately 10 ml with hexane, transfer to a 250 ml separatory funnel, and back-extract using the procedure in 12.3.

j) If the extract is from an aqueous filtrate [11.3.3 e)], set aside the concentration apparatus for the addition of the Soxhlet extract from the particles [12.2 k) 1)].

12.2 Soxhlet extraction of filters and/or disks

Filters and/or disks are Soxhlet-extracted as specified by 12.2 a) through 12.2 k).

a) Charge a clean extraction thimble [7.3.5 b)] with 5,0 g of silica (6.6.1.1).

Do not disturb the silica layer throughout the extraction process.

b) Place the thimble in a clean Soxhlet extractor [7.3.5 a)] and add 30 ml to 40 ml of toluene to the receiver and 200 ml to 250 ml of toluene to the flask.

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c) Pre-extract the glassware by heating the flask until the toluene is boiling. When the conditions are properly adjusted, 1 to 2 drops of toluene will fall per second from the condenser tip into the receiver. Extract the apparatus for at least 3 h.

d) After pre-extraction, cool and disassemble the apparatus and allow the thimble and the silica to dry, taking precautions to avoid contamination.

e) Load the filter and/or disk into the thimble.

f) Reassemble the pre-extracted Soxhlet apparatus, and add a fresh charge of toluene to the receiver and to the reflux flask. Apply power to the heating mantle to begin refluxing.

g) Adjust the reflux rate to match the rate of percolation through the sand and silica beds until water removal lessens the restriction to toluene flow. Frequently check the apparatus for foaming during the first 2 h of extraction. If foaming occurs, reduce the reflux rate until foaming subsides.

h) Extract the sample for a total of 16 h to 24 h.

i) Cool and disassemble the apparatus. Remove the distilling flask and add any toluene in the receiver to the extract in the flask.

j) Concentrate the extract using one of the macro-concentration procedures in 12.4.

k) For extracts from the particles in an aqueous sample containing less than 10 g of solids per litre of sample [see 11.3.3 f)]:

   1) concentrate the extract to approximately 5 ml using the rotary evaporator or heating mantle procedures in 12.4;

   2) quantitatively transfer the extract through the sodium sulfate [(12.1 g) through 12.1 j)] into the apparatus that was set aside [(see 12.1 j)] and reduce the volume to the previous level of the toluene;

   3) adjust to approximately 10 ml with hexane, transfer to a 250 ml separatory funnel, and proceed with the back-extraction (12.3).

12.3 Back-extraction with acid and base

The back-extraction with acid and base is carried out as specified by 12.3 a) through 12.3 f).

a) Partition the extract against 50 ml of sulfuric acid (6.2.2). Shake for 2 min with periodic venting into a hood. Remove and discard the aqueous layer. Repeat, to a maximum of four times, the acid washing until no colour is visible in the aqueous layer.

b) Partition the extract against 50 ml of sodium chloride solution (6.2.4) in the same way as in 12.3 a) Discard the aqueous layer. Continue with step 12.3 c) only if additional base washing is required; skip to step 12.3 e) if only acid washing is required.

c) Partition the extract against 50 ml of potassium hydroxide solution (6.2.1) in the same way as in 12.3 a). Repeat, to a maximum of four times, the base washing until no colour is visible in the aqueous layer. Minimize contact time between the extract and the base to prevent degradation of the PCDDs/PCDFs. Stronger potassium hydroxide solutions may be employed for back-extraction, provided that the laboratory meets the specifications for internal standard recovery and demonstrates acceptable performance using the procedure in 9.2

d) Repeat the partitioning against sodium chloride solution and discard the aqueous layer.

e) Pour each extract through a drying column containing a 7-cm to 10-cm layer of granular anhydrous sodium sulfate (6.3.1). Rinse the separatory funnel with 30 ml to 50 ml of solvent, and pour through the drying column. Collect each extract in a round-bottom flask.

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f) Re-concentrate the sample and QC aliquots in accordance with 12.4 and 12.5, and clean-up the samples and QC aliquots in accordance with Clause 13.

12.4 Macro-concentration

12.4.1 General

Concentrate extracts in toluene using a rotary evaporator or a heating mantle.

Concentrate extracts in dichloromethane or hexane using a rotary evaporator, heating mantle or Kuderna-Danish apparatus.

12.4.2 Rotary evaporation

Carry out the rotary evaporation as specified in 12.4.2 a) through 12.4.2 e).

a) Assemble the rotary evaporator in accordance with the manufacturer's instructions, and warm the water bath to 45 °C. Between samples, rinse three 2-ml to 3-ml aliquots of solvent down the feed tube into a waste beaker.

b) Attach a round-bottom flask containing the sample extract to the rotary evaporator. Slowly apply vacuum to the system, and begin rotating the sample flask.

c) Lower the flask into the water bath, and adjust the speed of rotation and the temperature as required to complete concentration in 15 min to 20 min. At the proper rate of concentration, the flow of solvent into the receiving flask will be steady, but no bumping or visible boiling of the extract will occur. If the rate of concentration is too fast, analyte loss may occur.

d) When the liquid in the concentration flask has reached a volume of approximately 2 ml, remove the flask from the water bath and stop the rotation. Slowly and carefully admit air into the system. Be sure not to open the valve so quickly that the sample is blown out of the flask.

e) Proceed to 12.5.1 for preparation for back-extraction or micro-concentration and solvent exchange.

12.4.3 Heating mantle

a) Carry out the evaporation using a heating mantle as specified in 12.4.3 a) through 12.4.3 c).

b) Add one or two clean boiling chips to a round-bottom flask, and attach a three-ball macro Snyder column [7.6.2 c)]. Pre-wet the column by adding approximately 1 ml of solvent through the top. Place the round-bottom flask in a heating mantle, and apply heat as required to complete the concentration in 15 min to 20 min. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood.

c) When the liquid has reached a volume of approximately 10 ml, remove the round-bottom flask from the heating mantle and allow the solvent to drain and cool for at least 10 min. Remove the Snyder column and rinse the glass joint into the receiver with two 5-ml portions of solvent.

d) Proceed to 12.5.1 for preparation for back-extraction or micro-concentration and solvent exchange.

12.4.4 Kuderna-Danish (K-D)

Concentrate the extracts in separate 500-ml K-D flasks equipped with 10-ml concentrator tubes. The K-D technique is used for solvents such as dichloromethane and hexane. Toluene is difficult to concentrate using the K-D technique unless a water bath fed by a steam generator is used.

a) Add one to two clean boiling chips to the receiver. Attach a three-ball macro Snyder column. Pre-wet the column by adding approximately 1 ml of solvent through the top. Place the K-D apparatus in a hot-water bath.

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b) Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 min to 20 min. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood.

c) When the liquid has reached a volume of approximately 1 ml, remove the K-D apparatus from the bath and allow the solvent to drain and cool for at least 10 min. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 ml to 2 ml of solvent. A 5-ml syringe is recommended for this operation.

d) Remove the three-ball Snyder column, add a fresh boiling chip, and attach a two-ball micro Snyder column to the concentrator tube. Pre-wet the column by adding approximately 0,5 ml of solvent through the top. Place the apparatus in the hot-water bath.

e) Adjust the vertical position and the water temperature as required to complete the concentration in 5 min 10 min. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood.

f) When the liquid reaches a volume of approximately 0,5 ml, remove the apparatus from the water bath and allow to drain and cool for at least 10 min.

g) Proceed to 12.5.1 for preparation for back-extraction or micro-concentration and solvent exchange.

12.5 Micro-concentration and solvent exchange

12.5.1 Sample preparation

12.5.1.1 For back-extraction (12.3), transfer the extract to a 250 ml separatory funnel. Rinse the concentration vessel with small portions of hexane, adjust the hexane volume in the separatory funnel to 10 ml to 20 ml, and proceed to back-extraction (12.3).

12.5.1.2 For clean-up procedures other than back-extraction, transfer the extract to a blowdown vial using two to three rinses of solvent. Proceed with micro-concentration and solvent exchange (12.5.2).

12.5.2 Analytical procedures

Extracts to be subjected to GPC clean-up are exchanged into dichloromethane. Extracts to be cleaned up using silica, alumina, carbon, and/or Florisil are exchanged into hexane.

The analytical procedures are carried out as specified in 12.5.2 a) to 12.5.2 g).

a) Transfer the vial containing the sample extract to a nitrogen blowdown device. Adjust the flow of nitrogen so that the surface of the solvent is just visibly disturbed. A large vortex in the solvent may cause analyte loss.

b) Lower the vial into a 45 °C water bath and continue concentrating.

c) If the extract is to be concentrated for injection into the GC/MS or the solvent is to be exchanged for extract clean-up, follow the specifications given in 12.5.2 d) to 12.5.2 g).

d) When the volume of the liquid is approximately 100 µl, add 2 ml to 3 ml of the desired solvent (dichloromethane for GPC, or hexane for the other clean-ups) and continue concentration until the volume of the sample is approximately 100 µl. Repeat the addition of solvent and concentrate once more.

e) If the extract is to be cleaned up by GPC, adjust the volume of the extract to 5,0 ml with dichloromethane. Proceed with GPC clean-up (13.2).

f) If the extract is to be cleaned up by column chromatography (alumina, silica, carbon, or Florisil), bring the final volume to 1,0 ml with hexane. Proceed with column clean-ups (13.3 to 13.6).

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g) If the extract is to be concentrated for injection into the GC/MS (Clause 14), quantitatively transfer the extract to a conical vial of suitable volume for final concentration, rinsing the larger vial with hexane and adding the rinse to the conical vial. Reduce the volume to approximately 100 µl. Add 10 µl of nonane to the vial, and evaporate the solvent to the level of the nonane. Seal the vial and label with the sample number. Store in the dark at room temperature until ready for GC/MS analysis. If GC/MS analysis is not performed the same day, store the vial in the dark at a suitable temperature to avoid losses by evaporation (see Clause 8).

13 Extract clean-up

13.1 General

13.1.1 The analyst may use any of the example procedures below or any suitable alternative procedures. Before using a clean-up procedure, the analyst shall demonstrate that the requirements of 9.2 can be met using the clean-up procedure. In addition, the isomer pattern and congener profile of a well-characterized sample extract containing all PCDDs and PCDFs (e.g. a fly-ash extract) shall not be altered by the applied clean-up procedure. Area response changes relative to the most abundant signal of each homologue series shall not exceed 20 %. Furthermore, differences between the sum responses of the PCDD/PCDF congener groups shall not exceed 20 %.

13.1.2 Gel-permeation chromatography (13.2) removes high-molecular-weight interferences that cause GC column performance to degrade. For this reason, it may be used for water extracts that are expected to contain high-molecular-weight organic compounds (e.g. polymeric materials, humic acids).

13.1.3 Acid, neutral, and basic silica (13.3), alumina (13.4), and Florisil (13.6) may be used to remove non-polar and polar interferences. Only alumina and Florisil shall be used to remove chlorodiphenyl ethers.

13.1.4 Carbon (13.5) may be used to remove non-polar interferences.

13.2 Gel-permeation chromatography (GPC)

13.2.1 Column packing

Gel-permeation columns are packed as specified in 13.2.1 a) to 13.2.1 d).

a) Place 70 g to 75 g of SX-3 Bio-beads[9)] in a 400 ml to 500 ml beaker.

b) Cover the beads with dichloromethane and allow to swell overnight (at least 12 h).

c) Transfer the swelled beads to the column and pump solvent through the column, from bottom to top, at 4,5 ml/min to 5,5 ml/min prior to connecting the column to the detector.

d) After purging the column with solvent for 1 h to 2 h, adjust the column head pressure to 7 Pa to 10 Pa and purge for 4 h to 5 h to remove air. Maintain a head pressure of 7 Pa to 10 Pa. Connect the column to the detector.

13.2.2 Column calibration

Gel-permeation columns are calibrated as specified in 13.2.2 a) to 13.2.1 e).

a) Load 5 ml of the calibration solution (6.5) into the sample loop.

b) Inject the calibration solution and record the signal from the detector. The elution order will be corn oil, bis(2-ethylhexyl) phthalate, pentachlorophenol, perylene, and sulfur.

c) Set the “dump time” to allow > 85 % removal of the corn oil and > 85 % collection of the phthalate.

d) Set the “collect time” to the valley between perylene and sulfur.

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e) Verify the calibration with the calibration solution after every 20 extracts. Calibration is verified if the recovery of the pentachlorophenol is greater than 85 %. If calibration is not verified, the system shall be recalibrated using the calibration solution, and the previous 20 samples shall be re-extracted and cleaned up using the calibrated GPC system.

13.2.3 GPC extract clean-up

It is a requirement of GPC that the column shall not be overloaded. If the extract is known or expected to contain more than 0,5 g, the extract shall be split into aliquots for GPC, and the aliquots shall be combined after elution from the column.

The column specified in this method is designed to handle a maximum of 0,5 g of high-molecular-weight material in a 5–ml extract. The mass of residue in the extract may be obtained gravimetrically by evaporating the solvent from a 50-µl aliquot.

The GPC extract clean-up is carried out as specified in 13.2.3 a) to 13.2.3 e).

a) Filter [7.5.1 c)] the extract or load through the filter-holder to remove the particles.

b) Load the 5,0-ml extract onto the column and elute the extract using the calibration data determined in 13.2.2. Collect the eluate in a clean 400 ml to 500 ml beaker.

c) Rinse the sample loading tube thoroughly with dichloromethane between extracts to prepare for the next sample.

d) If a particularly dirty extract is encountered, run a 5,0 ml dichloromethane blank through the system to check for carry-over.

a) Concentrate the eluate in accordance with 12.4 and 12.5 for further clean-up or injection into the GC/MS.

13.3 Silica clean-up

13.3.1 Clean-up of normal samples with silica is carried out as specified in 13.3.1 a) to 13.3.1 e).

a) Place a glass-wool plug in a 15-mm i.d. chromatography column [7.5.3 b)]. Pack the column bottom to top with the following: 1 g of silica (6.6.1.1), 4 g of basic silica (6.6.1.3), 1 g of silica, 8 g of acid silica (6.6.1.2), 2 g of silica and 4 g of granular anhydrous sodium sulfate (6.3.1). Tap the column to settle the adsorbents.

b) Pre-elute the column with 50 ml to 100 ml of hexane. Close the stopcock when the hexane is within 1 mm of the sodium sulfate. Discard the eluate. Check the column for channelling. If channelling is present, discard the column and prepare another.

c) Apply the concentrated extract to the column. Open the stopcock until the extract is within 1 mm of the sodium sulfate.

d) Rinse the receiver twice with 1-ml portions of hexane, and apply separately to the column. Elute the PCDDs/PCDFs with 100 ml hexane, and collect the eluate.

e) Concentrate the eluate in accordance with 12.4 and 12.5 for further clean-up or injection into the GC/MS.

13.3.2 Clean-up of highly concentrated samples.

For extracts of samples known to contain large quantities of other organic compounds, it may be advisable to increase the capacity of the silica column. This may be accomplished by increasing the strengths of the acid and basic silicas. The strength of the acid silica (6.6.1.2) may be increased to as much as 44 % mass fraction (7,9 g sulfuric acid per 10 g silica). The strength of the basic silica (6.6.1.3) may be increased to as much as 33 % mass fraction (50 ml of 1 mol/l NaOH per 100 g silica), or potassium silicate (6.6.1.4) may be used.

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The use of the more acidic silica (44 % mass fraction) may lead to a charring of the organic compounds in some extracts. The charred material may retain some of the analytes and lead to lower recoveries of PCDDs/PCDFs. Increasing the strengths of the acid and basic silica may also require different volumes of hexane than those specified in 13.3.1 a) to 13.3.1 e) to elute the analytes off the column.

After modification, the performance of the method shall be verified by the procedure in 9.2.

13.4 Alumina clean-up

Clean-up of samples with alumina is carried out as specified in 13.4 a) to 13.4 h).

a) Place a glass-wool plug in a 15-mm i.d. chromatography column [7.5.3 b)].

b) If using acid alumina, pack the column by adding 6 g of acid alumina [6.6.2 a)]. If using basic alumina, use 6 g of basic alumina [6.6.2 b)]. Tap the column to settle the adsorbents.

c) Pre-elute the column with 50 ml to 100 ml of hexane. Close the stopcock when the hexane is within 1 mm of the alumina.

d) Discard the eluate. Check the column for channelling. If channelling is present, discard the column and prepare another.

e) Apply the concentrated extract to the column. Open the stopcock until the extract is within 1 mm of the alumina.

f) Rinse the receiver twice with 1 ml portions of hexane and apply separately to the column. Elute the interfering compounds with 100 ml hexane and discard the eluate.

g) The choice of eluting solvents will depend on the choice of alumina (acid or basic) made in 13.4 b); follow the instructions given in 13.4 g) 1), 13.4 g) 2) and 13.4 h):

   1) if using acid alumina, elute the PCDDs/PCDFs from the column with 20 ml dichloromethane: hexane (20:80 volume fraction) and collect the eluate;

   2) if using basic alumina, elute the PCDDs/PCDFs from the column with 20 ml dichloromethane: hexane (50:50 volume fraction) and collect the eluate.

h) Concentrate the eluate in accordance with 12.4 and 12.5 for further clean-up or injection into the GC/MS.

13.5 Carbon column

Clean-up of samples with carbon is carried out as specified in 13.5 a) to 13.5 f).

a) Cut both ends from a 10 ml disposable serological pipette (7.5.2) to produce a 10-cm column. Fire-polish both ends and flare both ends if desired. Insert a glass-wool plug at one end, and pack the column with 0,55 g of Carbopak/Celite[3)] (6.6.3) to form an adsorbent bed approximately 2 cm long. Insert a glass-wool plug on top of the bed to hold the adsorbent in place.

b) Pre-elute the column with 5 ml of toluene followed by 2 ml of dichloromethane:methanol:toluene (15:4:1 volume fraction), 1 ml of dichloromethane: cyclohexane (1:1 volume fraction), and 5 ml of hexane. If the flow rate of eluate exceeds 0,5 ml/min, discard the column.

c) When the solvent is within 1 mm of the column packing, apply the sample extract to the column. Rinse the sample container twice with 1-ml portions of hexane and apply separately to the column. Apply 2 ml of hexane to complete the transfer.

d) Elute the interfering compounds with two 3-ml portions of hexane, 2 ml of dichloromethane: cyclohexane (1:1 volume fraction), and 2 ml of dichloromethane:methanol: toluene (15:4:1 volume fraction). Discard the eluate.

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e) Invert the column, and elute the PCDDs/PCDFs with 20 ml of toluene. If carbon particles are present in the eluate, filter through glass-fibre filter paper.

f) Concentrate the eluate in accordance with 12.4 and 12.5 for further clean-up or injection into GC/MS.

13.6 Florisil clean-up

Clean-up of samples with Florisil[1)] is carried out as specified in 13.6 a) to 13.6 d).

a) Pre-elute the activated Florisil column (6.6.4) with 10 ml of dichloromethane followed by 10 ml of hexane:dichloromethane (98:2 volume fraction) and discard the solvents.

b) When the solvent is within 1 mm of the packing, apply the sample extract (in hexane) to the column. Rinse the sample container twice with 1-ml portions of hexane and apply to the column.

c) Elute the interfering compounds with 20 ml of hexane: dichloromethane (98:2 volume fraction) and discard the eluate.

d) Elute the PCDDs/PCDFs with 35 ml of dichloromethane and collect the eluate. Concentrate the eluate in accordance with 12.4 to 12.5 for further clean-up or for injection into the GC/MS.

14 HRGC/HRMS analysis

HRGC/HRMS analysis is carried out as specified in 14 a) to 14 e).

a) Establish the operating conditions given in 10.1.

b) Add 10 µl of the appropriate recovery standard solution (6.11) to the sample. If an extract is to be reanalyzed and evaporation has occurred, do not add more recovery standard solution. Rather, bring the extract back to its previous volume (e.g. 19 µl; or 18 µl if 2–µl injections are used) with solvent.

c) Inject the appropriate volume of the concentrated extract containing the recovery standard using on-column or splitless injection. The volume injected shall be identical to the volume used for calibration (see Clause 10).

d) Start the GC-column initial isothermal hold upon injection. Start MS data collection after the solvent peak elutes. Stop data collection after the OCDD and OCDF have eluted.

e) Return the column to the initial temperature for analysis of the next extract or standard.

15 System and laboratory performance

15.1 General

At the beginning of each 12-h analytical shift, or per batch of samples (up to a maximum of 20), the GC/MS system performance and calibration shall be verified for all PCDD/PCDF internal standards. For these tests, analysis of the CS3 calibration verification standard (VER; 6.12 and Table 5) and the isomer specificity test standards (6.14 and Table 6) shall be used to verify all performance criteria. Adjustment and/or recalibration (see Clause 10) shall be performed until all performance criteria are met. Only after all performance criteria are met may samples, blanks, and IPRs be analyzed.

15.2 MS resolution

A static resolving power of at least 6 000 (10 % valley definition) shall be demonstrated at the appropriate mass before any analysis is performed. Corrective actions shall be implemented whenever the resolving power does not meet the requirement.

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15.3 Calibration verification

The calibration verification is carried out as specified in 15.3 a) to 15.3 c).

a) Inject the VER standard using the procedure in Clause 14.

   The mass-abundance ratios for all PCDDs/PCDFs shall be within 20 % of the theoretical shown in Table 9; otherwise, the mass spectrometer shall be adjusted until the mass-abundance ratios fall within the limits specified, and the verification test shall be repeated. If the adjustment alters the resolution of the mass spectrometer, resolution shall be verified (10.2) prior to repeat of the verification test.

   The peaks representing each PCDD/PCDF and internal standard in the VER standard shall have a signal-to-noise (S/N) ratio of at least 10; otherwise, the mass spectrometer shall be adjusted and the verification test repeated.

b) Compute the concentration of each PCDD/PCDF compound by isotope dilution (17.1) for those compounds that have labelled analogues (Table 2). Compute the concentration of the internal standards by the internal standard method (17.2). These concentrations are computed based on the calibration data in Clause 10.

c) For each compound, confirm that the result of the VER analysis is within 20 % of the nominal concentration shown in Table 5. If all compounds are within this limit, calibration has been verified and analysis of standards and sample extracts shall proceed. If, however, any compound falls outside its respective limit, the measurement system is not performing properly for that compound. In this event, prepare a fresh calibration standard or correct the problem causing the failure and repeat the resolution (15.2) and verification (15.3) tests, or recalibrate (see Clause 10).

15.4 GC resolution

To verify the GC resolution, inject the isomer specificity standards (6.14) on their respective columns.

The valley height between 2,3,7,8-TCDD and the other tetradioxin isomers at mass 319,896 5 shall not exceed 25 %, and that between 2,3,7,8-TCDF and the other tetrafuran isomers at mass 303,901 6 shall not exceed 50 %. (See Figures 1 and 2).

If the 2,3,7,8-isomers are not resolved, the GC is not performing properly. In this event, adjust the GC and repeat the verification test (15.3) or recalibrate (see Clause 10), or replace the GC column and either verify calibration or recalibrate.

15.5 Blank

The results of the analysis of the blank shall meet the specifications in 9.4.3 before sample analyses proceed.

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16 Qualitative determination

The qualitative determination is carried out as specified in 16 a) to 16 e)

a) The presence of a PCDD, a PCDF, or an internal standard in a standard, blank, or sample is confirmed when all of the criteria in 16 b) to 16 e) are met. If the criteria are not met, the PCDD or PCDF has not been identified and the results shall not be reported for regulatory compliance purposes. If interferences preclude identification, a new aliquot of sample shall be extracted, further cleaned up, and analyzed.

b) The signals for the two exact masses in Table 8 shall be present and shall peak within the same 2 s.

c) The signal-to-noise ratio (S/N) for the GC peak at each exact mass shall be equal to or greater than 3 for each CDD or CDF detected in a sample extract.

d) The ratio of the integrated areas of the two exact masses specified in Table 8 shall be within 20 % of the theoretical shown in Table 9, or within $\pm$ 10 % of the ratio in the midpoint (CS3) calibration or the calibration verification (VER), whichever is most recent.

e) The retention time of a native 2,3,7,8-congener (Cl_4 to Cl_6) shall be within a time window of 0 s to + 3 s based on the retention time of the respective $^{13}C_{12}$-labelled congener in the sample. For hepta- and octa-chloro-congeners, deviations of −2 s to + 3 s shall be acceptable. Alternatively, retention times relative to 1,2,3,7,8-PeCDF can be calculated. The difference shall be less than 0,25 % relative to the calibration standard.

   The retention time of peaks representing non-2,3,7,8-substituted PCDDs/PCDFs shall be within the retention time windows established in 10.4.

At present, no chromatographic column currently available is able to separate all 2,3,7,8-chloro-substituted congeners from all other non-2,3,7,8-chlorosubstituted congeners. Complete separation can be achieved by multi-analysis of the sample extract on columns of different polarities. However, in practice, the contribution of non-toxic congeners to the total TEQ amount from a single-column analysis may be of the same order as the precision of the test (10 % to 20 %). Single-column data may therefore be reported by this method; however in cases where a regulatory limit is exceeded by 20 % or less, a confirmatory analysis should be performed on a second column.

17 Quantitative determination

17.1 Concentration calculations for isotope dilution

17.1.1 By adding a known amount of an internal standard (labelled analogue) to every sample prior to extraction, correction for recovery of the PCDD/PCDF can be made because the PCDD/PCDF and its labelled analogue exhibit similar effects upon extraction, concentration, and gas chromatography. Relative response values, R_{rel}, shall be used in conjunction with the initial calibration data described in 10.6 to calculate concentrations directly, so long as internal-standard spiking levels are constant, in accordance with Equation (4):

$$\rho_{ex} = \frac{(A_{1n} + A_{2n})\rho_L}{(A_{1L} + A_{2L})R_{rel}} \quad (4)$$

where

A_{1n} is the area of the primary mass for the native PCDD/PCDF;

A_{2n} is the area of the secondary mass for the native PCDD/PCDF;

A_{1L} is the area of the primary mass for the labelled internal standard analogue;

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A_{2L} is the area of the secondary mass for the labelled internal standard analogue;

ρ_L is the concentration, in micrograms per litre, of the labelled internal standard analogue in the calibration standard (see Table 5);

ρ_{ex} is the concentration, in micrograms per litre, of the PCDD/PCDF in the extract;

R_{rel} is the relative response of the labelled to the native compound, as defined in 10.6.

17.1.2 If the labelled analogue of OCDF has not been added to the sample because of a potential interference, the OCDF shall be quantified against the labelled OCDD. As a result, the concentration of OCDF is corrected for the recovery of the labelled OCDD.

In instances where OCDD and OCDF behave differently during sample extraction, concentration, and clean-up procedures, the accuracy of the OCDF results may be reduced. However, given the low toxicity of this compound relative to the other dioxins and furans, the potential decrease in accuracy is not considered significant.

It is possible to achieve a good separation between OCDD and OCDF on certain GC columns currently in use. In this case, standards and spiking solutions may be prepared containing labelled OCDF. Native OCDF may then be quantified against the labelled OCDF.

17.1.3 Because some $^{13}C_{12}$-labelled standards are used as recovery standards (i.e. not added until after extraction of the sample), they shall not be used to quantify the respective native congener by strict isotope dilution procedures. Therefore, these native congeners shall be quantified using the response of the labelled analogue of the same homologue series.

17.1.4 Any peaks representing non-2,3,7,8-substituted PCDDs/PCDFs shall be quantified using an average of the response factors from all of the labelled 2,3,7,8-isomers at the same level of chlorination.

17.2 Internal standard recovery and concentration calculations

17.2.1 Calculate the concentrations of those native congeners referred to in 17.1.2 and 17.1.3 and of the labelled internal standards in the extract using the response factors determined from the initial calibration data (10.6) in accordance with Equation (5):

$$\rho_{ex} = \frac{(A_{1s} + A_{2s})\rho_{is}}{(A_{1is} + A_{2is})F_R} \quad (5)$$

where

A_{1s} is the area of the primary mass for the native and labelled PCDD/PCDF;

A_{2s} is the area of the secondary mass for the native and labelled PCDD/PCDF;

A_{1is} is the area of the primary mass for the internal standard;

A_{2is} is the area of the secondary mass for the internal standard;

ρ_{is} is the concentration, in micrograms per litre, of the internal standard (Table 5);

ρ_{ex} is the concentration, in micrograms per litre, of the PCDD/PCDF in the extract;

F_R is the response factor as defined in 10.7.

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17.2.2 Using the concentration in the extract determined above, calculate the percent recovery, R_{rec}, of the $^{13}C_{12}$-internal standards in accordance with Equation (6):

$$R_{rec} = \frac{\rho_{sam} \times V_{sam}}{\rho_{sp} \times V_{sp}} \times 100 = \frac{m_{rec}}{m_{sp}} \times 100 \quad (6)$$

where

R_{rec} is the percent of the spiked compound recovered;

m_{sp} is the mass, in micrograms, of compound added as a spike;

m_{rec} is the mass, in micrograms, of compound determined from analysis.

17.3 Concentration in the aqueous sample

17.3.1 Calculation of concentrations

The concentration, ρ_{aq}, of a PCDD/PCDF in the aqueous phase of the sample shall be calculated using the concentration of the compound in the extract and the volume of water extracted (11.3) in accordance with Equation (7):

$$\rho_{aq} = \frac{(\rho_{ex} \times V_{ex})}{V_{sam}} \times 1\,000 \quad (7)$$

where

ρ_{ex} is the concentration, in micrograms per litre, of the PCDD/PCDF in the extract;

ρ_{aq} concentration in the aqueous phase of the sample;

V_{ex} is the volume, in millilitres, of the extract;

V_{sam} is the volume, in litres, of the sample.

17.3.2 Treatment of results exceeding calibration range

If the SIM peak area at either quantification mass for any compound exceeds the calibration range of the system, extract a smaller sample aliquot.

Dilute 100 ml, 10 ml, etc., of sample to 1 l with reagent water and re-prepare, extract, clean-up, and analyze the diluted sample in accordance with Clauses 11 to 14.

If a smaller sample size is not representative of the entire sample, dilute the sample extract by a factor of 10, adjust the concentration of the recovery standard to 100 pg/µl in the extract, and analyze an aliquot of this diluted extract by the internal standard method.

17.4 Toxicity equivalents (TEQs)

17.4.1 The concept of toxic equivalents (TEQs) was devised in order to assess the toxicity of complex mixtures of PCDDs and PCDFs. A toxicity equivalent factor (TEF) is assigned to each individual dioxin and furan on the basis of its toxicity relative to 2,3,7,8-TCDD, the most potent dioxin, which has been assigned a value of 1,0. For example, animal and cell tests show that 2,3,7,8-TCDF is approximately one-tenth as toxic as 2,3,7,8-TCDD; consequently, its toxic equivalent factor is 0,1.

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17.4.2 Toxicity equivalent factors have been developed for those dioxins and furans that make the greatest contribution to the toxicity of a complex mixture. In general, these are compounds which have chlorine substitution in at least the 2,3,7,8 positions. Multiplication of the concentration of a particular PCDD or PCDF by its TEF, therefore, gives a 2,3,7,8-TCDD toxic equivalent (TEQ). The toxicity of any mixture, relative to 2,3,7,8-TCDD, is therefore the sum of the individual TEQs.

17.4.3 Of the 210 dioxins and furans, 17 contribute most to the toxicity of a complex mixture and are of most concern. This does not mean that the remaining 193 dioxins and furans are not toxic, but that they contribute comparatively little to the toxicity of a complex mixture.

Examples of toxic equivalent factors compiled by different organizations and authors are shown in Table 10. Use the set of factors appropriate for the respective circumstances.

Table 10 — Toxic equivalent factors accepted by different organizations

Compound	NATO/CCMS[a]	WHO/IPCS		Nordic	EPA[c]	Eadon 86	BGA/UBA	BUS
		1998[b]	1997					
2,3,7,8-TCDF	0,100	0,100	0,100	0,100	0,100	0,330	0,100	0,100
2,3,7,8-TCDD	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
1,2,3,7,8-PeCDF	0,050	0,050	0,050	0,010	0,100	0,330	0,100	0,100
2,3,4,7,8-PeCDF	0,500	0,500	0,500	0,500	0,100	0,330	0,100	0,100
1,2,3,7,8-PeCDD	0,500	0,500	1,000	0,500	0,500	1,000	0,100	0,100
1,2,3,4,7,8-HxCDF	0,100	0,100	0,100	0,100	0,010	0,021	0,100	0,100
1,2,3,6,7,8-HxCDF	0,100	0,100	0,100	0,100	0,010	0,021	0,100	0,100
2,3,4,6,7,8-HxCDF	0,100	0,100	0,100	0,100	0,010	0,021	0,100	0,100
1,2,3,7,8,9-HxCDF	0,100	0,100	0,100	0,100	0,010	0,021	0,100	0,100
1,2,3,4,7,8-HxCDD	0,100	0,100	0,100	0,100	0,040	—	0,100	0,100
1,2,3,6,7,8-HxCDD	0,100	0,100	0,100	0,100	0,040	—	0,100	0,100
1,2,3,7,8,9-HxCDD	0,100	0,100	0,100	0,100	0,040	—	0,100	0,100
1,2,3,4,6,7,8-HpCDF	0,010	0,010	0,010	0,010	0,010	—	0,010	0,100
1,2,3,4,7,8,9-HpCDF	0,010	0,010	0,010	0,010	0,010	—	0,010	0,100
1,2,3,4,6,7,8-HpCDD	0,010	0,010	0,010	0,010	0,001	—	0,010	0,010
OCDF	0,001	0,001	0,000 1	0,001	—	—	0,001	—
OCDD	0,001	0,001	0,000 1	0,001	—	—	0,001	—
Σ TCDDs	—	—	—	—	0,010	—	0,010	0,010
Σ PeCDDs	—	—	—	—	0,005	—	0,010	0,100
Σ HxCDDs	—	—	—	—	0,000 4	—	0,010	0,100
Σ HpCDDs	—	—	—	—	0,000 01	—	0,001	0,010
Σ TCDFs	—	—	—	—	0,001	—	0,010	0,100
Σ PeCDFs	—	—	—	—	0,001	—	0,010	0,100
Σ HxCDFs	—	—	—	—	0,0001	—	0,010	0,100
Σ HpCDFs	—	—	—	—	0,000 01	—	0,001	0,100

a NATO Committee on the Challenges of Modern Society.

b WHO International Program on Chemical Safety.

c Environmental Protection Agency.

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17.4.4 Calculate TEQs in accordance with Equation (8).

$$R_{\text{TEQ}} = \sum_{i=1}^{17} R_{\text{teq},i} = \sum_{i=1}^{17} \left(\rho_{\text{aq},i} \times R_{\text{tef},i} \right) \quad (8)$$

where

R_{TEQ} is the sum of the toxic equivalents for the individual compounds;

$R_{\text{teq},i}$ is the toxic equivalent for congener "i", equal to its concentration times its toxic equivalent factor; see Table 10.

18 Analysis of complex samples

18.1 General

When an extract cannot be concentrated to 10 µl after carrying out all of the clean-up procedures or when a sample overloads the GC column and/or mass spectrometer, a smaller aliquot of the sample (17.3.2) shall be analyzed. These are often samples which may contain high concentrations (> 10 µg/l) of the compounds of interest, interfering compounds, and/or polymeric materials.

When chromatographic peaks are detected at the retention time of any PCDD/PCDF in any of the mass channels being monitored for the chlorodiphenyl ethers (CDEs; Table 8), clean-up procedures shall be employed until these interferences are removed.

Alumina (13.4), carbon (13.5) and Florisil (13.6) are recommended for removal of chlorodiphenyl ethers.

18.2 Recovery of internal standards

18.2.1 Recoveries of the internal standards for most samples will be similar to those from reagent water.

18.2.2 If the extraction standard recovery range exceeds 50 % to 130 % for tetra- to hexa-chlorinated congeners or 40 % to 130 % for hepta- to octa-chlorinated congeners, then provided the sum of the contributions to the total TEQ in the sample from all of the congeners with recoveries not within these ranges does not exceed 10 %, the acceptable ranges shall be 30 % to 150 % for the tetra- to hexa-chlorinated congeners and 20 % to 150 % for the hepta- to octa-chlorinated congeners.

18.2.3 If the recovery of any of the internal standards is outside of these ranges, a diluted sample shall be analyzed (17.3.2).

18.2.4 If the recovery of any of the internal standards in the diluted sample is outside of normal range, the calibration verification standard (6.12) shall be analyzed and calibration verified (15.3).

18.2.5 If the calibration cannot be verified, a new calibration shall be performed and the original sample extract reanalyzed.

18.2.6 If the calibration is verified and the diluted sample does not meet the limits for internal standard recovery, this method is not suitable for the sample being analyzed and the result shall not be reported for regulatory compliance purposes. In this case, extraction and clean-up procedures other than those specified in this International Standard shall be employed to resolve the interference. If all clean-up procedures in this method have been employed and internal standard recovery remains outside of the normal range, extraction and/or clean-up procedures that are beyond this scope of this method shall be required to analyze these samples.

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19 Results and reporting

The test report shall contain as a minimum the following information:

a) identification of the test specimen;

b) reference to this International Standard (e.g. ISO 18073:2004);

c) results of the test, in picograms per litre, (calculated in accordance with Clause 17);

1) Report results, in picograms per litre, to two significant figures for the PCDDs/PCDFs found in all blanks and samples. Report recoveries, in percent, to two significant figures for the internal standards found in all standards, blanks, and samples.

2) Calculate detection limits as that amount of PCDD/PCDF which would give a signal-to-noise ratio of 3.

3) Report results below the minimum level as “not detected,” or as required by the regulatory authority. For blanks, report results that are above one third the ML.

4) Report TEQs calculated in accordance with 17.4. If the mass of a congener or congeners is below the detection limit, then two TEQ concentrations should be reported per sample:

i) with the mass of those congener(s) below the lower detection limit being taken as equal to the lower detection limit;

ii) with the mass of those congener(s) taken as zero.

5) Results for PCDDs/PCDFs in samples that have been diluted shall be reported at the lowest dilution level for which the peak areas of the quantification masses are within the calibration range.

6) For PCDDs/PCDFs having a labelled-analogue internal standard, results shall be reported at the lowest dilution level for which the area of the quantification mass is within the calibration range and the internal standard recovery is within the normal range for the method.

7) Additionally, if requested, the total concentration of all isomers at a particular level of chlorination (i.e. total TCDD, total TCDF, total PeCDD, etc.) may be reported by summing the concentrations of all isomers identified at that level of chlorination, including both 2,3,7,8-substituted and non-2,3,7,8-substituted homologues;

d) details of any deviation from this International Standard or from the international standards to which reference is made, and details of any operations regarded as optional.

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Annex A
(informative)

Application of HRGC/HRMS

A.1 Scope

In the case of screening methods or for the analysis of highly contaminated water samples (e.g. waters from waste treatment plants containing large amounts of solids or solvents), the use of low-resolution mass spectrometry may be possible, if it can be shown that interferences from other substances have been eliminated by a successful clean-up-procedure.

A.2 Principle

The procedure is similar to the procedure given in this International Standard, except that different (highly polar) coatings are used for the GC capillary columns (A.5) and that a low-resolution, instead of a high-resolution, mass spectrometer is used.

A.3 Interferences

In rare cases, mixed halogenated dibenzodioxins can overlap with the $^{13}C_{12}$-substituted standards. The masses $(M + 4)+$ and $(M + 6)+$ of the $^{13}C_{12}$-substituted standards overlap the masses $M+$ and $(M + 2)+$, respectively, of the PCDDs.

A.4 Apparatus

Low-resolution mass spectrometer, above 60-eV electron impact ionization, capable of monitoring a minimum of 12 exact masses at a resolution > 800 during a period of approximately 1 s.

A.5 HRGC/LRMS Analysis

A.5.1 Gas chromatography

Analyze the sample by capillary gas chromatography using a highly polar separation column. Examples of highly polar capillary columns (film thickness 0,20 µm to 0,35 µm) include the following:

— Silar 10 C[10)]

— Sil 88[11)]

— SP 2330[12)]

10) Silar 10 C is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

11) Sil 88 is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

12) SP 2330 is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

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— SP 2331[13)]

— DB DXN[14)]

A.5.2 Examination of the separation capacity

Ensure the capillary column meets the following minimum conditions:

— is made of glass or fused-silica with an inner diameter smaller than 0,5 mm and a length greater than 60 m;

— is coated with a highly polar separation phase (A.5.1) 0,2 µm thick;

— is of sufficient quality that the 2,3,7,8-TCDDs and the 2,3,7,8-TCDFs do not co-elute with other isomers (see also 15.4).

Using the appropriate standard solutions and samples with known concentrations of PCDDs/PCDFs, establish the separation capacity of the column. The GC and MS conditions used for the standard solutions should be identical with those used for the analysis of sample extracts.

A.5.3 Determination of SIM acquisition time windows

Using the appropriate standard mixtures, establish the time windows for the different chlorination levels of the PCDDs/PCDFs at the optimized GC conditions and optimize the width of the gas chromatographic peaks. Using the peak-widths, determine the cycle time for the single-mass acquisition for the scan procedure, ensuring that a minimum of 5 data points is collected across each GC peak.

A.5.4 Determination of the linear working range

Establish the linearity of the GC-MS for all native and $^{13}C_{12}$-labelled 2,3,7,8-substituted PCDDs/PCDFs and $^{13}C_{12}$-1,2,3,4-TCDDs over the range 1 pg to 1 000 pg for a specific column. For this purpose, inject a minimum of 5 solutions of different concentrations and use these measurements to determine the linear working range of the instrument.

A.6 Mass spectrometry

A.6.1 Determining the instrument-specific chlorine isotope ratios

Determine these ratios by from the mean ratio of the linearity check data. Determine the chlorine isotope ratios by measuring the peak areas at the peak maximum of the respective mass trace.

A.6.2 Identification

The identification of PCDDs and PCDSs is confirmed as follows.

a) Analyze all PCDDs and PCDFs with chlorine substitution in the 2,3,7,8 positions as single components.

b) If, as is generally the case, the concentrations are too small to allow the recording of complete mass spectra, proceed via single defined-mass registration, i.e. only the signals of selected ions of a specific mass are continuously acquired [selected-ion monitoring (SIM)].

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13) SP 2331 is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

14) DB DXN is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

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c) For this purpose, identify the 3 ions with the most intense signals produced by the isotopes ^{35}Cl and ^{37}Cl in the molecular ion cluster with a determinable mass ratio. Simultaneously determine the $^{13}C_{12}$ internal standards at the corresponding higher mass ratio (see Table A.1).

The acquisition of complete mass spectra is possible only when the instrument is sufficiently sensitive. Full-scan acquisition is preferable, as complete mass spectra give more information than SIM. Furthermore, full-scan acquisition will increase the confidence in identification, and, at the same time, interferences will be identified more easily.

Use the retention times defined by gas chromatography for identification of single compounds.

The use of silica capillary columns with a strongly polar separation phase is recommended. (If heptachlorodibenzofurans are poorly separated from the octochlorodibenzofurans, use a new column).

d) Determine the retention-time windows for ion acquisitions if all of the tetra- to octachlorodibenzodioxins and furans and the corresponding standards cannot be acquired simultaneously (see Figure A.1).

e) Accept the identification of PCDDs and PCDFs as correct if the ion ratios for a substance do not differ by more than 15 % from the ratios evaluated during calibration under identical conditions. Additionally, provided the concentration ranges are comparable, ensure that the relative retention times (RRTs) do not differ by more than ± 0,1 %. Ensure that the signal-to-noise ratio is $>$ 5:1.

f) Determine the RRTs of single components from the $^{13}C_{12}$-substituted internal PCDD and PCDF standards of the respective isomer.

g) Ensure that injection volume is not less than 1 µl.

A.6.3 GC operating conditions

For GC operating conditions, see Table 7.

Confirm that the components being analyzed do not contain interfering substances. If interfering substances are present, then repeat the clean-up stages.

Confirm that the PCDDs/PCDFs with chlorine substitution in the 2,3,7,8 positions do not elute at the same time as other isomers.

A.6.4 Chlorine isotope ratios

Tables A.1 to A.5 present the exact masses and the normalized intensities of various mass fragments for the tetra- to octa-substituted PCDDs/PCDFs.

Table A.1 — Mass-fragment ratios for tetrachlorodibenzodioxins and tetrachlorodibenzofurans

Mass fragments	Normalized intensity	Exact masses			
		Furan	$^{13}C_{12}$-furan	Dioxin	$^{13}C_{12}$-dioxin
M+ ($^{35}Cl_4$)	76 %	303,90	315,94	319,90	331,94
M+ ($^{35}Cl_3{}^{37}Cl$)	100 %	305,90	317,94	321,89	333,93
M+ ($^{35}Cl_2{}^{37}Cl_2$)	49 %	307,90	319,94	323,89	335,93
M+ ($^{35}Cl^{37}Cl_3$)	11 %	309,89	321,93	325,89	337,93

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Tableau A.2 — Mass-fragment ratios for pentachlorodibenzodioxin and pentachlorodibenzofuran

Mass fragments	Normalized intensity	Exact masses			
		Furan	$^{13}C_{12}$-furan	Dioxin	$^{13}C_{12}$-dioxin
M+ ($^{35}Cl_5$)	61 %	337,86	349,90	353,86	365,90
M+ ($^{35}Cl_4{}^{37}Cl$)	100 %	339,86	351,90	355,85	367,89
M+ ($^{35}Cl_3{}^{37}Cl_2$)	66 %	341,86	353,90	357,85	369,89
M+ ($^{35}Cl_2{}^{37}Cl_3$)	22 %	343,85	355,89	359,85	371,89

Table A.3— Mass-fragment ratios for hexachlorodibenzodioxin and hexachlorodibenzofuran

Mass fragments	Normalized intensity	Exact masses			
		Furan	$^{13}C_{12}$-furan	Dioxin	$^{13}C_{12}$-dioxin
M+ ($^{35}Cl_6$)	51 %	371,82	383,86	387,82	399,86
M+ ($^{35}Cl_5{}^{37}Cl$)	100 %	373,82	385,86	389,82	401,86
M+ ($^{35}Cl_4{}^{37}Cl_2$)	82 %	375,82	387,86	391,81	403,85
M+ ($^{35}Cl_3{}^{37}Cl_3$)	36 %	377,81	389,85	393,81	405,85
M+ ($^{35}Cl_2{}^{37}Cl_4$)	9 %	379,81	391,85	395,81	407,85

Table A.4 — Mass-fragment ratios for heptachlorodibenzodioxin and heptachlorodibenzofuran

Mass fragments	Normalized intensity	Exact masses			
		Furan	$^{13}C_{12}$-furan	Dioxin	$^{13}C_{12}$-dioxin
M+ ($^{35}Cl_7$)	44 %	405,78	417,82	421,78	433,82
M+ ($^{35}Cl_6{}^{37}Cl$)	100 %	407,78	419,82	423,78	435,82
M+ ($^{35}Cl_5{}^{37}Cl_2$)	98 %	409,78	421,82	425,77	437,81
M+ ($^{35}Cl_4{}^{37}Cl_3$)	53 %	411,78	423,82	427,77	439,81
M+ ($^{35}Cl_3{}^{37}Cl_4$)	18 %	413,77	425,81	429,77	441,81

Table A.5 — Mass-fragment ratios for octachlorodibenzodioxin and octachlorodibenzofuran

Mass fragments	Normalized intensity	Exact masses			
		Furan	$^{13}C_{12}$-furan	Dioxin	$^{13}C_{12}$-dioxin
M+ ($^{35}Cl_8$)	34 %	439,75	451,79	455,74	467,78
M+ ($^{35}Cl_7{}^{37}Cl$)	88 %	441,74	453,78	457,74	469,78
M+ ($^{35}Cl_6{}^{37}Cl_2$)	100 %	443,74	455,78	459,73	471,77
M+ ($^{35}Cl_5{}^{37}Cl_3$)	65 %	445,74	457,78	461,73	473,77
M+ ($^{35}Cl_4{}^{37}Cl_4$)	27 %	447,73	459,77	463,73	475,77

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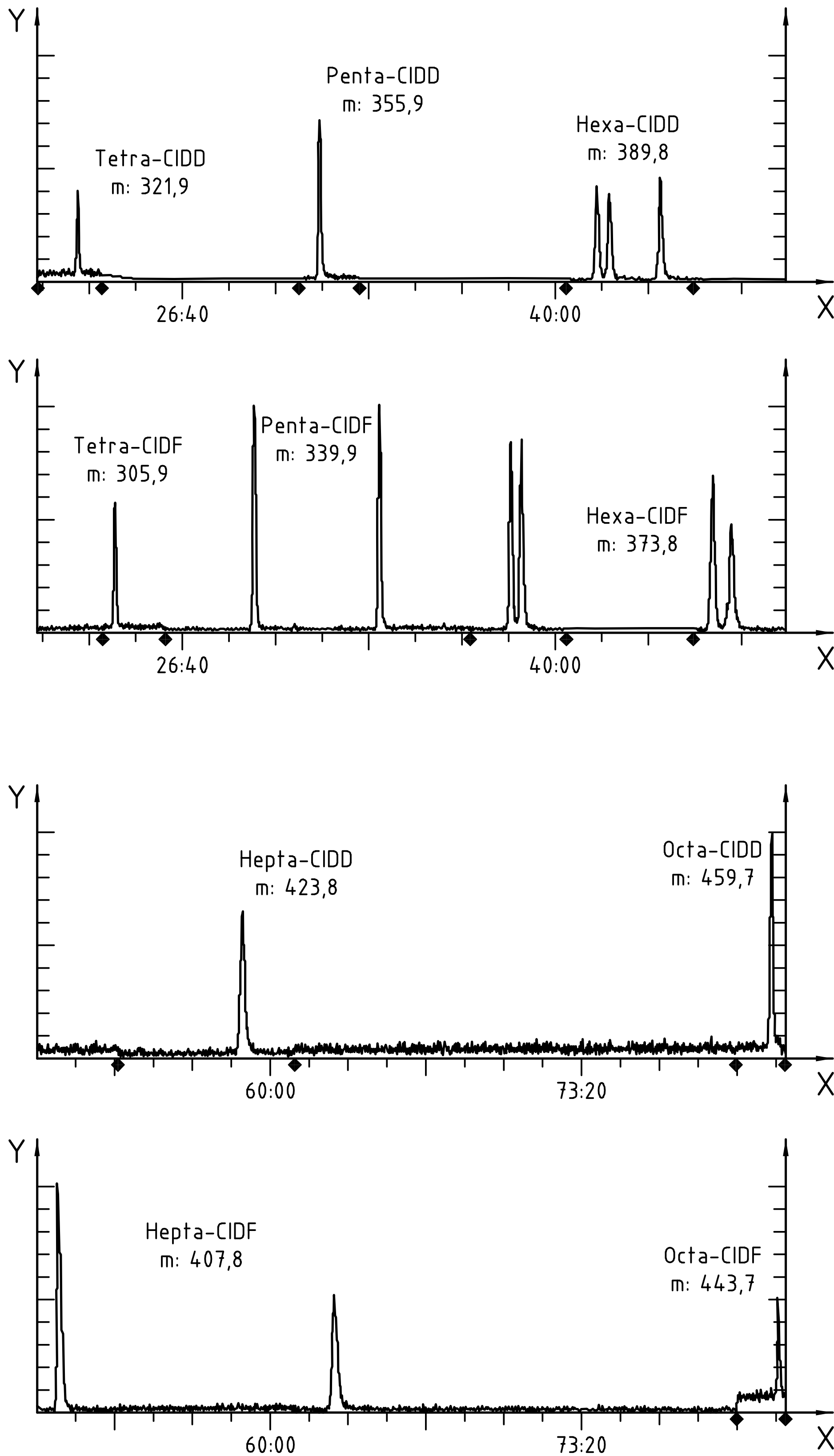

Key

X Retention time, in minutes

Y Spectrometer response, in millivolts

Figure A.1 — Typical chromatograms of a low-resolution mass spectrometer showing all PCDDs/PCDFs with chlorine substitution in the 2,3,7,8-positions [capillary column: DB DXN(EL1)[14)]]

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Annex B
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Interlaboratory trial data

B.1 Data from the interlaboratory studies

Tables B.1 to B.5 present a summary of the data from interlaboratory studies, after the outliers have been removed.

Table B.1 — Synthetic effluent[7] with low concentrations

Compound	l[a]	n[b]	P_{out}[c]	$\bar{X}_l$[d]	X_{kn}[e]	R_{rec}[f]	s_R[g]	$s_{R,P}$[h]	s_r[i]	$s_{r,P}$[j]
			%	pg/l	pg/l	%	pg/l	%	pg/l	%
2,3,7,8–TCDD	7	13	30,0	18,3	20	91,5	2,12	11,6	0,673	3,7
2,3,7,8–TCDF	8	15	20,0	20,2	20	101,0	3,68	18,2	1,19	5,9
1,2,3,7,8–PeCDD	7	14	30,0	95,3	100	95,3	15,4	16,2	5,10	5,4
1,2,3,7,8–PeCDF	7	14	30,0	94,9	100	94,9	8,28	8,7	8,28	8,7
2,3,4,7,8–PeCDF	9	18	10,0	93,7	100	93,7	17,3	18,4	14,2	15,1
1,2,3,4,7,8–HxCDD	9	18	10,0	94,3	100	94,3	16,2	17,2	12,3	13,1
1,2,3,6,7,8–HxCDD	10	20	0,0	93,8	100	93,8	21,3	22,8	13,2	14,1
1,2,3,7,8,9–HxCDD	8	16	20,0	93,7	100	93,7	12,1	12,9	9,78	10,5
1,2,3,4,7,8–HxCDF	8	16	20,0	95,6	100	95,6	13,6	14,2	9,86	10,3
1,2,3,6,7,8–HxCDF	8	16	20,0	100	100	100,0	8,36	8,4	8,36	8,4
1,2,3,7,8,9–HxCDF	8	16	20,0	99,3	100	99,3	10,0	10,1	8,61	8,7
2,3,4,6,7,8–HxCDF	8	16	20,0	99,5	100	99,5	11,7	11,8	8,57	8,6
1,2,3,4,6,7,8–HpCDD	8	16	20,0	104	100	104,0	8,09	7,8	4,76	4,6
1,2,3,4,6,7,8–HpCDF	8	16	20,0	97,2	100	97,2	11,5	11,8	11,5	11,8
1,2,3,4,7,8,9–HpCDF	10	20	0,0	93,1	100	93,1	20,9	22,5	15,3	16,4
OCDD	8	16	20,0	198	200	98,9	21,3	10,7	18,3	9,3
OCDF	8	16	20,0	187	200	93,5	26,5	14,2	23,1	12,4

[a] l is the number of laboratories;
[b] n is the number of values;
[c] P_{out} is the percentage of outliers;
[d] $\bar{X}_l$ is the mean of the means of the laboratory data;
[e] X_{kn} is the known value based on the spiking/standard concentration);
[f] R_{rec} is the percentage recovery of the spike;
[g] s_R is the reproducibility standard deviation;
[h] $s_{R,P}$ is the percentage reproducibility variation;
[i] s_r is the repeatability standard deviation;
[j] $s_{r,P}$ is the percentage repeatability variation.

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Table B.2 — Synthetic effluent[7] with high concentrations

Compound	l[a]	n	P_{out} %	$\bar{X}_l$ pg/l	X_{kn} pg/l	R_{rec} %	s_R pg/l	$s_{R,P}$ %	s_r pg/l	$s_{r,P}$ %
2,3,7,8–TCDD	7	14	22,2	95,0	100	95,0	14,2	14,9	4,36	4,6
2,3,7,8–TCDF	8	16	11,1	98,4	100	98,4	14,1	14,3	4,50	4,6
1,2,3,7,8–PeCDD	10	20	0,0	434	500	86,8	94,4	21,8	44,5	10,3
1,2,3,7,8–PeCDF	8	16	20,0	457	500	91,4	35,3	7,7	25,4	5,6
2,3,4,7,8–PeCDF	9	18	10,0	449	500	89,8	69,9	15,6	32,1	7,2
1,2,3,4,7,8–HxCDD	9	18	10,0	445	500	89,0	65,4	14,7	36,1	8,1
1,2,3,6,7,8–HxCDD	10	20	0,0	446	500	89,2	73,9	16,6	42,7	9,6
1,2,3,7,8,9–HxCDD	10	20	0,0	458	500	91,6	90,8	19,8	40,3	8,8
1,2,3,4,7,8–HxCDF	10	20	0,0	444	500	88,8	86,9	19,6	35,1	7,9
1,2,3,6,7,8–HxCDF	8	16	20,0	473	500	94,6	46,2	9,8	29,6	6,3
1,2,3,7,8,9–HxCDF	8	16	20,0	470	500	94,0	46,5	9,9	22,3	4,7
2,3,4,6,7,8–HxCDF	8	16	20,0	474	500	94,8	69,2	14,6	24,9	5,3
1,2,3,4,6,7,8–HpCDD	10	20	0,0	467	500	93,4	39,5	8,5	31,1	6,7
1,2,3,4,6,7,8–HpCDF	8	16	20,0	465	500	93,0	40,5	8,7	35,1	7,6
1,2,3,4,7,8,9–HpCDF	8	16	20,0	493	500	98,6	31,2	6,3	24,3	4,9
OCDD	10	20	0,0	910	1 000	91,0	84,7	9,3	59,2	6,5
OCDF	8	16	20,0	923	1 000	92,3	118	12,7	62,3	6,7

[a] For an explanation of the symbols, see Table B.1

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Table B.3 — Industrial waste water with low concentrations

Compound	l[a]	n	P_{out} %	$\bar{X}_l$ pg/l	X_{kn} pg/l	R_{rec} %	s_R pg/l	$s_{R.P}$ pg/l	s_r %	$s_{r,P}$ %
2,3,7,8–TCDD	8	16	20,0	18,0	20	90,0	2,11	11,8	0,505	2,8
2,3,7,8–TCDF	9	18	10,0	21,2	20	106,0	3,79	17,9	1,52	7,2
1,2,3,7,8–PeCDD	8	16	20,0	97,4	100	97,4	8,55	8,8	4,20	4,3
1,2,3,7,8–PeCDF	7	14	30,0	95,7	100	95,7	6,86	7,2	4,59	4,8
2,3,4,7,8–PeCDF	9	18	10,0	94,2	100	94,2	11,7	12,4	3,95	4,2
1,2,3,4,7,8–HxCDD	8	16	20,0	93,7	100	93,7	11,1	11,9	2,70	2,9
1,2,3,6,7,8–HxCDD	9	18	10,0	93,1	100	93,1	14,7	15,8	9,30	10,0
1,2,3,7,8,9–HxCDD	8	16	20,0	98,4	100	98,4	6,81	6,9	4,82	4,9
1,2,3,4,7,8–HxCDF	8	16	20,0	96,7	100	96,7	8,53	8,8	4,00	4,1
1,2,3,6,7,8–HxCDF	8	16	20,0	97,1	100	97,1	9,76	10,0	3,76	3,9
1,2,3,7,8,9–HxCDF	8	16	20,0	94,7	100	94,7	14,2	15,0	13,5	14,2
2,3,4,6,7,8–HxCDF	8	16	20,0	99,0	100	99,0	11,8	11,9	3,49	3,5
1,2,3,4,6,7,8–HpCDD	8	15	20,0	101	100	101,0	7,71	7,6	3,12	3,1
1,2,3,4,6,7,8–HpCDF	8	16	20,0	99,7	100	99,7	14,1	14,1	4,37	4,4
1,2,3,4,7,8,9–HpCDF	10	20	0,0	93,1	100	93,1	12,4	13,3	7,44	8,0
OCDD	9	18	10,0	194	200	96,8	24,3	12,6	20,8	10,7
OCDF	8	16	20,0	192	200	96,0	20,4	10,6	9,27	4,8

[a] For an explanation of the symbols, see Table B.1

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Table B.4 — Industrial waste water with high concentrations

Compound	l[a]	n	P_{out} %	$\bar{X}_l$ pg/l	X_{kn} pg/l	R_{rec} %	s_R pg/l	$s_{R,P}$ %	s_r pg/l	$s_{r,P}$ %
2,3,7,8–TCDD	9	17	10,0	99,0	100	99,0	12,6	12,7	5,16	5,2
2,3,7,8–TCDF	10	19	0,0	104	100	104,0	12,6	12,1	5,62	5,4
1,2,3,7,8–PeCDD	9	18	10,0	448	500	89,6	77,4	17,3	13,0	2,9
1,2,3,7,8–PeCDF	8	16	20,0	466	500	93,2	43,5	9,3	12,7	2,7
2,3,4,7,8–PeCDF	9	17	10,0	463	500	92,6	51,3	11,1	20,4	4,4
1,2,3,4,7,8–HxCDD	9	18	10,0	471	500	94,2	48,2	10,2	21,6	4,6
1,2,3,6,7,8–HxCDD	10	20	0,0	465	500	93,0	64,7	13,9	23,8	5,1
1,2,3,7,8,9–HxCDD	8	16	20,0	479	500	95,8	48,9	10,2	16,8	3,5
1,2,3,4,7,8–HxCDF	9	18	10,0	473	500	94,6	45,0	9,5	24,3	5,1
1,2,3,6,7,8–HxCDF	9	18	10,0	474	500	94,8	38,6	8,1	31,7	6,7
1,2,3,7,8,9–HxCDF	8	16	20,0	497	500	99,4	42,9	8,6	33,1	6,7
2,3,4,6,7,8–HxCDF	8	16	20,0	490	500	98,0	52,4	10,7	33,4	6,8
1,2,3,4,6,7,8–HpCDD	9	18	10,0	503	500	100,6	31,4	6,2	31,4	6,2
1,2,3,4,6,7,8–HpCDF	8	16	20,0	493	500	98,6	38,8	7,9	12,7	2,6
1,2,3,4,7,8,9–HpCDF	10	20	0,0	464	500	92,8	65,3	14,1	33,3	7,2
OCDD	10	20	0,0	928	1 000	92,8	82,5	8,9	50,7	5,5
OCDF	7	14	30,0	920	1 000	92,0	47,4	5,2	16,4	1,8

[a] For an explanation of the symbols, see Table B.1

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Table B.5 — Standard solution

Compound	l^{a}	n	P_{out} %	$\bar{X}_l$ pg/l	X_{kn} pg/l	R_{rec} %	s_R pg/l	$s_{R.P}$ %	s_r pg/l	$s_{r,P}$ %
2,3,7,8–TCDD	10	20	9,1	1,89	2	94,5	0,226	12,1	0,190	10,1
2,3,7,8–TCDF	10	20	9,1	2,07	2	103,5	0,217	10,5	0,099	4,8
1,2,3,7,8–PeCDD	10	20	9,1	9,85	10	98,5	0,983	10,0	0,277	2,8
1,2,3,7,8–PeCDF	9	18	18,2	10,0	10	100,0	0,813	8,2	0,514	5,2
2,3,4,7,8–PeCDF	9	18	18,2	9,86	10	98,6	1,04	10,6	0,399	4,1
1,2,3,4,7,8–HxCDD	10	20	9,1	9,90	10	99,0	1,13	11,4	0,393	4,0
1,2,3,6,7,8–HxCDD	10	20	9,1	10,0	10	100,0	1,42	14,2	0,665	6,7
1,2,3,7,8,9–HxCDD	10	20	9,1	9,80	10	98,0	1,33	13,5	0,609	6,2
1,2,3,4,7,8–HxCDF	10	20	9,1	9,81	10	98,1	0,669	6,8	0,209	2,1
1,2,3,6,7,8–HxCDF	10	20	9	9,62	10	96,2	0,783	8,1	0,409	4,3
1,2,3,7,8,9–HxCDF	9	18	18,2	10,1	10	101,0	0,810	8,1	0,612	6,1
2,3,4,6,7,8–HxCDF	10	20	9,1	9,56	10	95,6	1,66	17,4	0,424	4,4
1,2,3,4,6,7,8–HpCDD	10	20	9,1	10,4	10	104,0	0,864	8,3	0,763	7,4
1,2,3,4,6,7,8–HpCDF	10	20	9,1	10,0	10	100,0	0,990	9,9	0,424	4,2
1,2,3,4,7,8,9–HpCDF	10	20	9,1	9,72	10	97,2	1,12	11,6	0,493	5,1
OCDD	9	18	18,2	20,5	20	102,5	2,68	12,5	0,550	2,7
OCDF	9	18	18,2	19,9	20	99,5	1,88	9,5	0,551	2,8

[a] For an explanation of the symbols, see Table B.1

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Annex C
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Pollution prevention and waste management

C.1 Pollution prevention

C.1.1 The solvents used in this method pose little threat to the environment when managed properly. The solvent-evaporation techniques used in this method are amenable to solvent recovery, and it is recommended that the laboratory recover solvents wherever feasible.

C.1.2 Standards shall be prepared in volumes consistent with laboratory use to minimize disposal of standards.

C.2 Waste management

C.2.1 The laboratory shall comply with all national and local regulations governing waste management, particularly the hazardous-waste-identification rules and land-disposal restrictions, to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. The laboratory shall also comply with any sewage-discharge regulations and requirements of sewage-discharge permits.

C.2.2 Samples containing HCl at $pH < 2$ are hazardous and either shall be neutralized before being poured down a drain or shall be handled as hazardous waste.

C.2.3 Low-level waste such as absorbent paper, tissues, animal remains, and plastic gloves shall be burned in an appropriate incinerator. PCDDs/PCDFs in milligram and greater quantities shall be packaged securely and disposed of through commercial or governmental channels that are capable of handling extremely toxic wastes.

NOTE The PCDDs/PCDFs decompose above 800 °C.

C.2.4 Liquid or soluble waste shall be dissolved in methanol or ethanol and irradiated with ultraviolet light at a wavelength shorter than 290 nm for several days. Liquid wastes shall be analyzed and the solutions shall be disposed of when the PCDDs/PCDFs can no longer be detected.

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Annex D
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Additional extraction and clean up techniques

D.1 General

This annex describes additional extraction and clean-up techniques that can be used, but which were not part of the original method evaluation for this International Standard.

D.2 Solid-phase extraction

D.2.1 Apparatus and materials

The following equipment, in addition to that specified in Clause 7 of this International Standard, is necessary for performing the solid-phase extractions (SPEs).

D.2.1.1 Filtration apparatus, 1 l, including glass funnel, glass frit support, clamp, adapter, stopper, filtration flask, and vacuum tubing.

For wastewater samples, the apparatus shall accept 90-mm or 144-mm disks. For drinking water or other samples containing low solids, smaller disks may be used.

D.2.1.2 Vacuum source, capable of maintaining a vacuum of 85 kPa, equipped with shut-off valve and vacuum gauge.

D.2.1.3 Glass-fibre filter, 1 µm pore size, to fit the filtration apparatus in D.2.1.1.

D.2.1.4 Solid-phase extraction disk, containing octadecyl-bonded (C_{18}-bonded) silica, uniformly distributed in an inert matrix, to fit the filtration apparatus in D.2.1.1.

D.2.2 Solid-phase extraction (SPE) of samples containing less than 10 mg solids per litre of sample

D.2.2.1 Sample preparation

Add 5 ml of methanol (MeOH) to samples for SPE after spiking with internal standards as specified in 11.3.2.

D.2.2.2 Disk preparation

The SPE disks are prepared as specified in D.1.2.2 a) through D.1.2.2 d).

a) Place an SPE disk on the base of the filter holder and wet with toluene. While holding a glass fibre filter above the SPE disk with tweezers, wet the filter with toluene and lay the filter on the SPE disk, making sure that air is not trapped between the filter and disk. Clamp the filter and SPE disk between the 1-l glass reservoir and the vacuum filtration flask.

b) Rinse the sides of the filtration flask with approximately 15 ml of toluene using a squeeze bottle or syringe. Apply vacuum momentarily until a few drops appear at the drip tip. Release the vacuum and allow the filter/disk to soak for approximately 1 min. Apply vacuum and draw all of the toluene through the filter and disk. Repeat the wash step with approximately 15 ml of acetone and allow the filter and disk to air dry.

c) Re-wet the filter and disk with approximately 15 ml of methanol, allowing the filter and disk to soak for approximately 1 min. Pull the methanol through the filter and disk using the vacuum, but retain a layer of

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methanol approximately 1 mm thick on the filter. Do not allow the disk to go dry from this point until the end of the extraction.

d) Rinse the filter and disk with two 50-ml portions of reagent water by adding the water to the reservoir and pulling most of it through, leaving a layer of water on the surface of the filter.

D.2.2.3 Extraction

The extraction is carried out as specified in D.2.2.3 a) through D.2.2.3 e).

a) Pour the spiked sample [11.3.2 b)], blank [11.3.2 d)], or IPR aliquot into the reservoir and apply the vacuum to begin the extraction. Adjust the vacuum to complete the extraction in no less than 10 min.

b) For samples containing a high concentration of particles (suspended solids), filtration times may be 8 h or longer.

c) Before all of the sample has been pulled through the filter and disk, rinse the sample bottle with approximately 50 ml of reagent water to remove any solids, and pour into the reservoir. Pull through the filter and disk. Use additional reagent water rinses until all visible solids are removed.

d) Before all of the sample and rinses have been pulled through the filter/disk, rinse the sides of the reservoir with two 5-ml portions of reagent water.

e) Allow the filter and disk to dry, then remove the filter and disk and place in a glass Petri dish. Soxhlet-extract the filter and disk in accordance with 12.2.

D.3 Silver nitrate/silica column clean-up

D.3.1 General

Silver nitrate/silica may be used to remove sulfur compounds and some organohalogen compounds.

The following reagents and apparatus, in addition to those specified in Clauses 6 and 7 of this International Standard, are necessary for performing silver nitrate/silica column clean-up.

D.3.2 Silver nitrate/silica

This solution is used in the preparation of a chromatography column for the elimination of organosulfur and organohalogen compounds.

Dissolve 10 g of silver nitrate (AR grade or equivalent) in 40 ml water, add by portions 90 g silica (53 µm to 63 µm) and shake until the mixture is homogeneous. Let stand for 30 min. Transfer the mixture to a drying oven preheated to 70 °C and heat from 70 °C to 125 °C during 5 h. Activate at 125 °C for 5 h. Store the mixture in a brown glass bottle.

D.3.3 Silver nitrate/silica column

Fill about 5 mm of a chromatography column with sodium sulfate (6.3.1) and add 2 g of silver nitrate/silica (D.3.2). Top with a 5-mm layer of sodium sulfate. Rinse the column with 50 ml of hexane.

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D.3.4 Extraction procedure

The extraction procedure is carried out as specified in D.3.4 a) to D.3.4 c).

a) Add the extract (approximately 3 ml in hexane) to the column. Rinse the extract container 3 times with hexane and add to the column, ensuring that the column does not run dry.

b) Elute the dioxins/furans with 35 ml of hexane, collecting the eluate.

c) Concentrate the eluate in accordance with 12.4 and 12.5 for further clean-up or for injection into the GC/MS.

D.4 High performance liquid chromatography (HPLC)

D.4.1 General

HPLC may be used to provide specificity for the 2,3,7,8-substituted and other PCDD and PCDF isomers.

D.4.2 Apparatus and materials

The following apparatus, in addition to the apparatus and reagents specified in Clauses 6 and 7 of this International Standard, is necessary for performing silver nitrate/silica column clean-up.

High-performance liquid-chromatography (HPLC) equipment, consisting of the following:

a) **column oven and detector**, operated at 0,02 adsorbance units full-scale (AUFS) at 235 nm;

b) **injection valve**, with 50-µl sample loop;

c) **columns**, two 6,2-mm × 250-mm octadecylsilicate (ODS) columns in series, operated at 50 °C with 2,0 ml/min methanol isocratic effluent;

d) HPLC pump.

D.4.3 HPLC procedure

D.4.3.1 Column calibration

The column calibration is carried out as specified in D.4.3.1 a) through D.4.3.1 d).

a) Prepare a calibration standard containing the 2,3,7,8-substituted isomers and/or other isomers of interest at a concentration of approximately 500 pg/µl in dichloromethane.

b) Inject 30 µl of the calibration solution into the HPLC and record the signal from the detector. Collect the eluant for reuse. The elution order will be the tetra- through octa-isomers.

c) Establish the collection time for the tetra-isomers and for the other isomers of interest. Following calibration, flush the injection system with copious quantities of dichloromethane, including a minimum of five 50-µl injections while the detector is monitored, to ensure that residual PCDDs/PCDFs have been removed from the system.

d) Verify the calibration with the calibration solution after every 20 extracts. Calibration is verified if the recovery of the PCDDs/PCDFs from the calibration standard [D.4.3.1 a)] is 75 % to 125 % compared to the calibration [D.4.3.1 b)]. If calibration is not verified, recalibrate the system using the calibration solution, and re-extract and clean-up the previous 20 samples using the calibrated system.

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D.4.3.2 HPLC extract clean-up

The column specified in this method is designed to handle a maximum of 30 µl of extract.

It is a requirement that the HPLC column shall not be overloaded. If the extract cannot be concentrated to less than 30 µl, it shall be split into fractions and the fractions shall be combined after elution from the column.

The HPLC extract clean-up is carried out as specified in D.4.3.2 a) to D.4.3.2 d).

a) Rinse the sides of the vial twice with 30 µl of dichloromethane and reduce to 30 µl with the evaporation apparatus (see 12.5).

b) Inject the 30 µl extract into the HPLC. Elute the extract based on the calibration data determined in 13.6 a). Collect the fraction(s) in a clean 20-ml concentrator tube containing 5 ml of hexane: acetone (50 % volume fraction).

c) If an extract containing greater than 100 µg/l of total PCDDs or PCDFs is encountered, run a 30-µl dichloromethane blank through the system to check for carry-over.

d) Concentrate the eluate in accordance with 12.5 for injection into the GC/MS.

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