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Water quality — Determination of dioxin-like polychlorinated biphenyls — Method using gas chromatography/mass spectrometry

Qualité de l'eau — Dosage des biphényls polychlorés de type dioxine — Méthode par chromatographie en phase gazeuse/spectrométrie de masse

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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 17858 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

Introduction

When using this International Standard, it may be necessary in some cases to determine whether and to what extent particular problems will require the specification of minor additional conditions.

Water quality — Determination of dioxin-like polychlorinated biphenyls — Method using gas chromatography/mass spectrometry

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This International 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 non-*ortho* and mono-*ortho* PCBs are co-planar and are among the most toxic of chemicals. All work with dioxin-like PCBs requires therefore the utmost care; the national safety measures which correspond to those for toxic substances shall be strictly adhered to.

IMPORTANT — It is absolutely essential that tests conducted according to this International Standard be carried out by suitably trained staff.

1 Scope

This International Standard specifies a method for the determination of dioxin-like tetra- to hepta-chlorinated biphenyls (PCBs) in waters and wastewaters (containing less than 1 % suspended solids) using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). The method is optimized for dioxin-like PCBs, but can include other co-planar compounds such as polychlorinated dioxins and furans (PCDDs/PCDFs) and polychlorinated naphthalenes (PCNs). This method can be used to determine dioxin-like PCBs in other matrices (e.g. biota, sediments, air); however, additional clean-up steps and techniques can be required for samples with high organic loadings.

This method is applicable to the twelve non- and mono-*ortho* PCBs designated by the World Health Organization, as well as to other PCBs and co-planar compounds.

The detection limits and quantification levels in this method are dependent on the level of interferences as well as instrumental limitations. The minimum levels (ML) in Table 2 are the levels at which the dioxin-like PCBs can typically be determined with no interferences present.

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 method are met. The requirements for establishing method equivalency are given in 9.2.

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, *Water for analytical laboratory use — Specification and test methods*

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

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

3 Terms, definitions and abbreviated terms

3.1 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1.1
analyte
dioxin-like polychlorinated biphenyl tested for by this method

See Table 1.

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
VER
midpoint calibration standard that is used to verify calibration

3.1.4
certified reference material
CRM
quality control sample used to determine accuracy and precision of method

3.1.5
congener
member of the same kind, class or group

EXAMPLE Any one of the 209 individual PCBs.

3.1.6
critical pair
pair of isomers that must be separated to a predefined degree (e.g. 25 % valley) to ensure chromatographic separation meets minimum quality criteria

3.1.7
dioxin-like isomer
PCB with identical chemical composition but different structure

3.1.8
homologue group
complete group of isomers

EXAMPLE Tetrachlorobiphenyls.

3.1.9
isotope dilution
method using labelled (usually $^{13}C_{12}$) internal standards to correct for losses during sample preparation and analysis

3.1.10
keeper solvent
high boiling point solvent added to the sampling standard solution

3.1.11
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

3.1.12
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

3.1.13
pattern
chromatographic fingerprint of any series of PCB isomers

3.1.14
profile
graphic representation of the sums of the isomer concentrations of the PCBs

3.1.15
spiking
addition of $^{13}C_{12}$-labelled PCB standards of which the recovery is calculated and used to correct values of native analytes of interest

3.1.16
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

3.1.17
toxic equivalent factor
TEF
relative toxicity to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)

3.1.18
toxic equivalent quantity
TEQ
sum of toxic equivalents of each individual congener

3.2 Abbreviated terms

CRM	certified reference material
GC/MS	gas chromatography/mass spectrometry
GPC	gel permeation chromatography
HPLC	high-performance liquid chromatography
HRGC	high-resolution gas chromatography
HRMS	high-resolution mass spectrometry
IPR	initial precision and recovery
LRMS	low-resolution mass spectrometry
MDL	method detection limit
ML	minimum level (see Table 2)
PAR	precision and recovery
PCB	polychlorinated biphenyl

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

PCN polychlorinated naphthalene

PFK perfluorokerosene

SIM selected ion monitoring

SPE solid-phase extraction

TEF toxic equivalent factor

TEQ toxic equivalent quantity

VER calibration verification standard

4 Principle

4.1 Spiking and extraction

Stable isotopically labelled analogues of dioxin-like PCBs (diluted in a suitable solvent such as acetone) are spiked into a 1 litre aqueous sample (a sample containing less than 1 % suspended solids). A minimum of one labelled standard per homologue group is used and the sample is extracted by one of three procedures noted in 4.1 a), 4.1 b) and 4.1 c). If the sample contains more than 1 % solid material, the solid portion can be analysed directly after filtration or drying and the aqueous portion can be discarded.

a) Samples containing no visible particles are extracted with dichloromethane [6.4 f)] 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 [6.4 f)] in a separatory funnel. The dichloromethane extract is concentrated and combined with the Soxhlet extract prior to clean-up.

c) The sample is vacuum filtered through a glass-fibre filter on top of a solid-phase extraction (SPE) disk. The filter and disk are eluted with suitable solvent mixtures or extracted in a Soxhlet or pressure filtration extractor, and the extract is concentrated for 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 to remove interfering components. Sample clean-up procedures can include washes with acid and/or base, gel permeation, alumina, silica, Florisil[1)] and activated carbon chromatography. High-performance liquid chromatography (HPLC) can be used for further isolation of other specific co-planar compounds if required. Due to the large number of potential interfering compounds, sample extracts shall be fractioned or analysed on at least two distinct GC column phases to ensure unique identification and accurate quantification of each dioxin-like PCB congener.

4.3 Concentration

After clean-up, the extract(s) 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 GC and detected by a high-resolution mass spectrometer. Two exact masses are monitored for each analyte.

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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.

Resolution greater than or equal to 10 000 is recommended. High-resolution gas chromatography/high-resolution mass spectrometry at a resolution greater than or equal to 10 000 is at present required to achieve adequate sensitivity and selectivity, and to allow the use of all $^{13}C_{12}$-labelled standards. If the sample extract is being analysed for multi-component analyte groups (PCDD/Fs, PCBs, PCNs), a resolution of 10 000 is necessary. At resolutions less than 10 000, some $^{13}C_{12}$ PCDFs and PCBs interfere with native PCDDs of the same level of chlorination. Resolutions less than 10 000 can be used for specific analyte groups (PCBs, PCNs) where the matrix and potential interferences are well characterized.

4.4 Identification

An individual dioxin-like PCB is identified by comparing the GC retention time and ion abundance ratio of two exact masses monitored (see Table 7) with the corresponding retention time of an authentic internal standard and the theoretical or acquired ion-abundance ratio of the two exact masses. The isomers and congeners for which there are no labelled analogues are identified when retention times or relative retention times and ion-abundance ratios agree within predefined limits. Masses of those PCBs with a degree of chlorination higher than three (e.g. PentaCB 110 for TetraCB 77) shall be monitored to ensure there is no contribution to the mass of interest.

4.5 Quantification

Quantitative analysis is performed using selected ion monitoring (SIM) areas, in one of two ways.

a) For the dioxin-like PCBs for which labelled analogues have been added to the sample (4.1), the GC/MS system is calibrated, and the concentration of each compound is determined using the isotope dilution technique.

b) For the dioxin-like PCBs for which labelled analogues are not added, the GC/MS system is calibrated for each compound using an isomer or congener with the most similar structure and the concentration of each compound is determined using the internal standard technique.

4.6 Analytical quality

The quality of the analysis is assured through reproducible calibration and testing of the extraction, clean-up, and GC/MS systems. Interferences, biases and limitations should be determined and identified for each target analyte through intercalibration (interlaboratory) studies, certified reference materials (CRM) and spiked matrix samples (SMS). A series of quality control (QC) samples (CRM, SMS) should be analysed with each set of samples and monitored through control charting or other quality review procedures.

5 Contamination and interferences

5.1 Where possible, monitor or clean reagents by extraction or solvent rinse.

Solvents, reagents, labware, and other sample processing hardware can yield artefacts and/or elevated baselines causing misinterpretation of chromatograms. (Example chromatograms showing typical retention times of native and labelled PCBs are given in Annex A.) Specific selection of reagents and purification of solvents by distillation in all-glass systems can be required. Many reagents, solvents and labware contain background levels of dioxin-like compounds, e.g. PCB118 and PCB105.

5.2 Clean labware such that the method blank requirements given in 9.5.3 are met. An example of a cleaning procedure is given below in a) to c).

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

b) After detergent washing, rinse labware immediately with hot tap water. The tap water rinse shall be followed by an acetone rinse, then a dichloromethane [6.4 f)] rinse/soak. For known contaminated labware, use toluene as a final rinse/soak.

c) Soxhlet apparatus should be cycled with toluene for at least 20 cycles. Shake separatory funnels with dichloromethane [6.4 f)] and/or toluene for 2 min, drain, and then shake with pure dichloromethane [6.4 f)] for 2 min.

Proper cleaning of labware is extremely important because labware can contaminate the samples but can also remove the analytes of interest by surface adsorption if the surface is activated during the cleaning procedure. Glassware can be checked for contamination by analysing solvent rinses.

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 started through the extraction process on a given 12-h shift, to a maximum of 20 samples); see 9.5, 15.5.

5.4 The reference matrix shall simulate, as closely as possible, the sample matrix under test. Ideally, the reference matrix shall not contain dioxin-like compounds in detectable amounts, but shall contain potential interferants in the concentrations expected to be found in the samples to be analysed.

Interferences co-extracted from samples can vary considerably from source to source, depending on the diversity of the site being sampled. Interfering compounds, including PCBs of higher degrees of chlorination can be present at concentrations several orders of magnitude higher than the dioxin-like PCBs being analysed. The most frequently encountered interferences are dibenzo-*p*-dioxins, dibenzofurans, diphenyl ethers, methoxy biphenyls, hydroxydiphenyl ethers, benzylphenyl ethers, aromatic sulfur compounds, polynuclear aromatics, and pesticides. Because very low levels of dioxin-like PCBs are measured by this method, 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 dioxin-like PCBs at the levels shown in Table 2.

5.5 When a clean reference matrix that simulates the sample matrix under test is not available, use reagent water (6.7) or a matrix that most closely resembles the sample.

5.6 Number each piece of reusable labware or minimally identify each set of specific type of labware (e.g. Soxhlet extractors, round-bottom flasks) to associate that specific labware with the processing of a particular sample or set of samples. This will assist the laboratory in tracking possible sources of contamination for individual samples, identifying labware associated with highly contaminated samples that may require extra cleaning, and determining when labware 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 reagents**.

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 **1 mol/l sulfuric acid**.

Dilute with care 56 ml of concentrated sulfuric acid (6.2.2) to 1 litre of water (6.1).

6.2.4 **Sodium chloride solution**.

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

6.2.5 **Sodium thiosulfate**, $Na_2S_2O_3$.

6.3 Solution drying and evaporation reagents.

6.3.1 Sodium sulfate, Na_2SO_4, granular, anhydrous.

Bake at 300 °C for at least 24 h, cool in a desiccator, and store in a precleaned glass bottle with a screw cap that prevents moisture from entering.

If, after heating, the sodium sulfate develops a noticeable greyish cast (due to the presence of carbon in the crystal matrix), discard that batch of reagent as it is not suitable for use. Rinse with about 20 ml of dichloromethane [6.4 f)] per gram of Na_2SO_4 or extract with dichloromethane [6.4 f)] if background contamination is detected.

6.3.2 Prepurified nitrogen, N_2 99,999 %.

6.4 Solvents for extraction and clean-up.

The extraction and clean-up solvents, distilled in glass, of pesticide quality and free of interferences, include the following:

a) **Acetone**, C_3H_6O.

b) **Toluene**, C_7H_8.

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

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

e) **Methanol**, CH_3OH.

f) **Dichloromethane**, CH_2Cl_2.

g) **Diethyl ether**, $C_4H_{10}O$.

h) **Ethanol**, C_2H_6O.

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

6.5 GPC calibration solution.

Dissolve 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, in dichloromethane [6.4 f)]. Store in glass and keep refrigerated. Prepare fresh monthly.

6.6 Adsorbents for sample clean-up.

6.6.1 Silica, 70 µm to 230 µm.

6.6.1.1 Activated silica, baked at 180 °C for a minimum of 1 h, cooled in a desiccator, and stored in a precleaned glass bottle with a screw cap that prevents moisture from entering. Prepare fresh every two weeks.

6.6.1.2 Acid silica, 30 % mass fraction.

Thoroughly mix 44,0 g of 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. 22 % acid silica and 44 % acid silica are prepared in a similar manner by adding 29 g and 80 g of sulfuric acid, respectively, to 100 g of activated silica. Prepare fresh every two weeks.

6.6.1.3 Basic silica.

Thoroughly mix 30 g of 1 mol/l 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. Prepare fresh every two weeks.