PD ISO/TS 28581:2012

BSI Standards Publication

Water quality — Determination of selected non-polar substances — Method using gas chromatography with mass spectrometric detection (GC-MS)

bsi.

...making excellence a habit.™

National foreword

This Published Document is the UK implementation of ISO/TS 28581:2012.

The UK participation in its preparation was entrusted to Technical Committee EH/3/2, Physical chemical and biochemical methods.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

ISBN 978 0 580 66221 8

ICS 13.060.50

Compliance with a British Standard cannot confer immunity from legal obligations.

This Published Document was published under the authority of the Standards Policy and Strategy Committee on 31 March 2012.

Amendments issued since publication

Date	Text affected

PD ISO/TS 28581:2012

TECHNICAL SPECIFICATION

ISO/TS 28581

First edition
2012-02-15

Water quality — Determination of selected non-polar substances — Method using gas chromatography with mass spectrometric detection (GC-MS)

Qualité de l'eau — Détermination de substances non polaires sélectionnées — Méthode par chromatographie en phase gazeuse avec détection par spectrométrie de masse (CG-SM)

Reference number
ISO/TS 28581:2012(E)

COPYRIGHT PROTECTED DOCUMENT

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

Published in Switzerland

Contents

Page

Foreword

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

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.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of document:

— an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;

— an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.

An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

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/TS 28581 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

Introduction

Non-polar substances occur in nearly all types of water. These substances are adsorbed on solids (sediments, suspended matter) as well as dissolved in the liquid phase.

A large group of non-polar substances are polycyclic aromatic hydrocarbons (PAH). Some PAH are known or suspected to cause cancer. Maximum acceptable levels have been set in a number of countries. For instance, the European Council Directive 98/83/EC[10] on the quality of water intended for human consumption set the maximum acceptable level for benzo[*a*]pyrene at 0,010 µg/l, and for the sum of four specified PAH (benzo[*b*] fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, indeno[1,2,3-*cd*]pyrene) at 0,100 µg/l.

There are further International Standards for the analytical determination of PAH in water and waste water.

ISO 6468 specifies methods for the determination of certain organochlorine insecticides, polychlorinated biphenyls and chlorobenzenes in drinking water, ground water, surface water and waste water.

ISO 17993[6] specifies methods for the determination of 15 PAH by high performance liquid chromatography in drinking water, ground water and surface water.

ISO 7981[2] specifies methods for the determination of 6 PAH by high performance thin layer chromatography or by high performance liquid chromatography in drinking water and ground water.

ISO 17858[5] specifies methods for the determination of dioxin-like polychlorinated biphenyls in waters and waste waters.

ISO 28540[9] specifies the determination of PAH using gas chromatography with mass spectrometric detection (GC-MS).

Water quality — Determination of selected non-polar substances — Method using gas chromatography with mass spectrometric detection (GC-MS)

WARNING — The use of this Technical Specification may involve hazardous materials, operations and equipment.

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

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

1 Scope

This Technical Specification specifies a method for the determination by gas chromatography with mass spectrometric detection (GC-MS) of polycyclic hydrocarbons and pesticide residues in drinking water and ground water at mass concentrations above 0,005 µg/l and surface water and waste water at mass concentrations above 0,01 µg/l (for each single compound).

This method can apply to non-polar substances other than polycylic aromatic hydrocarbons (PAH) and pesticide residues. However, it is necessary to verify the applicability of this method for these compounds.

NOTE 1 A potentially suitable method for this verification is specified in ISO/TS 13530.[3]

This Technical Specification can be used for samples containing up to 150 mg/l of suspended matter.

NOTE 2 Determination of PAH using GC-MS lies within the scope of ISO 28540.[9]

2 Normative references

The following document, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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

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

ISO 6468, *Water quality — Determination of certain organochlorine insecticides, polychlorinated biphenyls and chlorobenzenes — Gas-chromatographic method after liquid-liquid extraction*

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

3 Terms and definitions

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

3.1
analyte
substance to be determined

[SOURCE: ISO 15089:2000,[4] definition 3.2]

Note 1 to entry Substances covered by this specification are listed in Table 1.

3.2
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

[SOURCE: ISO 18073:2004,[7] definition 3.1.2]

3.3
diagnostic ion
selected fragment ion, molecular ion or other characteristic ion from the mass spectrum of the target compound with the highest possible specificity

[SOURCE: ISO 22892:2006,[8] definition 3.6]

3.4
injection standard
standard mixture added to a sample before injection into the GC-MS apparatus, to monitor variability of instrument response and to calculate internal standard recovery

3.5
internal standard
isotopically labelled standard or a non-polar substance added to samples prior to extraction, unlikely to be present in the sample, against which the concentrations of native substances are calculated

Note 1 to entry The substance is added to the sample before extraction and is used for quantification of the components to be measured. Recoveries of these standards are also calculated and used to check the performance of the procedure.

3.6
native compound
non-labelled compound

3.7
selected ion mode
SIM
selected ion recording
SIR
measuring the intensity of selected diagnostic ions only

[SOURCE: ISO 22892:2006,[8] definition 3.8, modified — the last two synonyms have been added.]

4 Principle

The non-polar substances determinable by the method specified in this Technical Specification are listed in Table 1.

The non-polar substances present in the aqueous sample are extracted from the water sample by liquid-liquid extraction with hexane. An internal standard mixture is added to the sample prior to extraction. The extract is concentrated by evaporation and the residue taken up in a solvent appropriate for clean-up or gas chromatography (GC).

Other volatile solvents can also be used if it is proven that there is equal or better recovery (recovery between 70 % and 110 %).

NOTE Other possible suitable solvents are: isohexane C_6H_{15} (CAS: 107-83-5); cyclohexane: C_6H_{12} (CAS: 110-82-7); pentane: C_5H_{12} (CAS: 109-66-0); petroleum ether: boiling range 40 °C to 60 °C.

The liquid-liquid extraction method shall not be used with samples containing more than 150 mg/l of suspended matter.

If necessary, extracts of surface water or waste water samples can be cleaned by column chromatography prior to analysis. Prior to injection, injection standards are added to each extract, and an aliquot of the extract is injected into the gas chromatograph.

The non-polar substances are separated on a suitable fused silica capillary column, coated with a film of cross-linked non-polar polysiloxane or slightly polar modified polysiloxane with an efficient separation. The column shall be suitable for separating critical and isomeric pairs of substances. Identification and quantification is performed by means of mass spectrometry (MS) using electron impact ionization (EI).

Table 1 — Non-polar substances determinable that can be determined by using this Technical Specification

Name	Molecular formula	Molar mass g/mol	CAS number
PAH			
Naphthalene	$C_{10}H_8$	128,17	91-20-3
Acenaphthylene	$C_{12}H_8$	152,20	208-96-8
Acenaphthene	$C_{12}H_{10}$	154,21	83-32-9
Fluorene	$C_{13}H_{10}$	166,22	86-73-7
Phenanthrene	$C_{14}H_{10}$	178,23	85-01-8
Anthracene	$C_{14}H_{10}$	178,23	120-12-7
Pyrene	$C_{16}H_{10}$	202,26	129-00-0
Fluoranthene	$C_{16}H_{10}$	202,26	206-44-0
Chrysene	$C_{18}H_{12}$	228,29	218-01-9
Benzo[*a*]anthracene	$C_{18}H_{12}$	228,29	56-55-3
Benzo[*b*]fluoranthene	$C_{20}H_{12}$	252,32	205-99-2
Benzo[*k*]fluoranthene	$C_{20}H_{12}$	252,32	207-08-9
Benzo[*a*]pyrene	$C_{20}H_{12}$	252,32	50-32-8
Dibenzo[*a,h*]anthracene	$C_{22}H_{14}$	278,35	053-70-3
Benzo[*ghi*]perylene	$C_{22}H_{12}$	276,34	191-24-2
Indeno[1,2,3-*cd*]pyrene	$C_{22}H_{12}$	276,34	193-39-5
PCB			
PCB-28: 2,4,4′-trichlorobiphenyl	$C_{12}H_7Cl_3$	257,54	7012-37-5
PCB-52: 2,2′,5,5′-tetrachlorobiphenyl	$C_{12}H_6Cl_4$	291,99	35693-99-3
PCB-101: 2,2′,4,5,5′-pentachlorobiphenyl	$C_{12}H_5Cl_5$	326,43	37680-73-2
PCB-118: 2,3′,4,4′,5-pentachlorobiphenyl	$C_{12}H_5Cl_5$	326,43	31508-00-6
PCB-138: 2,2′,3,4,4′,5′-hexachlorobiphenyl	$C_{12}H_4Cl_6$	360,88	35065-28-2
PCB-153: 2,2′,4,4′,5,5′-hexachlorobiphenyl	$C_{12}H_4Cl_6$	360,88	35065-27-1
PCB-180: 2,2′,3,4,4′,5,5′-heptachlorobiphenyl	$C_{12}H_3Cl_7$	395,33	35065-29-3
OCP			
Hexachlorobenzene (HCB)	C_6Cl_6	284,78	118-74-1
α-Hexachlorocyclohexane (α-HCH)	$C_6H_6Cl_6$	290,83	319-84-6
β-Hexachlorocyclohexane (β-HCH)	$C_6H_6Cl_6$	290,83	319-85-7

Table 1 *(continued)*

Name	Molecular formula	Molar mass g/mol	CAS number
γ-Hexachlorocyclohexane (γ-HCH)	$C_6H_6Cl_6$	290,83	58-89-9
δ-Hexachlorocyclohexane (δ-HCH)	$C_6H_6Cl_6$	290,83	319-86-8
ε-Hexachlorocyclohexane (ε-HCH)	$C_6H_6Cl_6$	290,83	6108-10-7
Aldrin	$C_{12}H_8Cl_6$	364,93	309-00-2
Dieldrin	$C_{12}H_8Cl_6O$	380,91	60-57-1
Endrin	$C_{12}H_8Cl_6O$	380,91	72-20-8
Heptachlor	$C_{10}H_5Cl_7$	373,32	76-44-8
Heptachlor epoxide (exo-, *cis*- or β-isomer)	$C_{10}H_5Cl_7O$	389,30	28044-83-9
Heptachlor epoxide (endo-, *trans*- or α-isomer)	$C_{10}H_5Cl_7O$	389,30	1024-57-3
α-Endosulfan	$C_9H_6Cl_6O_3S$	406,92	959-98-8
β-Endosulfan	$C_9H_6Cl_6O_3S$	406,92	33213-65-9
p,*p*′-DDE	$C_{14}H_8Cl_4$	318,02	72-55-9
o,*p*′-DDD	$C_{14}H_{10}Cl_4$	320,04	53-19-0
o,*p*′-DDT	$C_{14}H_9Cl_5$	354,49	784-02-6
p,*p*′-DDD	$C_{14}H_{10}Cl_4$	320,04	72-54-8
o,*p*′-DDE	$C_{14}H_8Cl_4$	318,02	3424-82-6
p,*p*′-DDT	$C_{14}H_9Cl_5$	354,49	50-29-3
Methoxychlor	$C_{16}H_{15}Cl_3O_2$	345,65	72-43-5
Chlorobenzenes			
1,2,4-Trichlorobenzene	$C_6H_3Cl_3$	181,45	120-82-1
1,2,3-Trichlorobenzene	$C_6H_3Cl_3$	181,45	87-61-6
1,3,5-Trichlorobenzene	$C_6H_3Cl_3$	181.45	108-70-3
1,2,3,4-Tetrachlorobenzene	$C_6H_2Cl_4$	215,89	634-66-2
1,2,3,5-Tetrachlorobenzene	$C_6H_2Cl_4$	215,89	634-90-2
1,2,4,5-Tetrachlorobenzene	$C_6H_2Cl_4$	215,89	95-94-3
Pentachlorobenzene	C_6HCl_5	250,34	608-93-5
Pentachloronitrobenzene	$C_6Cl_5NO_2$	295,34	82-68-8
Organophosphorus			
Azinphos-ethyl	$C_{12}H_{16}N_3O_3PS_2$	345,40	2642-71-9
Bromofenvinphos-ethyl	$C_{12}H_{14}BrCl_2O_4P$	404,02	33399-00-7
Chlorofenvinphos	$C_{12}H_{14}Cl_3O_4P$	359,57	470-90-6
Chloropyriphos-ethyl	$C_9H_{11}Cl_3NO_3PS$	350,59	2921-88-2
Chloropyriphos-methyl	$C_7H_7Cl_3NO_3PS$	322,53	5598-13-0
Heptenophos	$C_9H_{12}ClO_4P$	250,02	23560-59-0

5 Interferences

5.1 Interferences with sampling, extraction and concentration

Use sampling containers of materials that do not affect the analyte content during the contact time (preferably of stainless steel or glass). Avoid plastics and organic materials other than polytetrafluoroethene (PTFE) during sampling, sample storage or extraction. Care should be taken with the use of surfactants for cleaning sample containers because they may lead to the formation of emulsions during liquid-liquid extraction.

If automatic samplers are used, avoid the use of silicone or rubber material for the tubes. If these materials are present, ensure that the contact time is minimized. Rinse the sampling line with the water to be sampled before taking the test sample. Use ISO 5667-1 and ISO 5667-3 for guidance.

Keep the test samples away from direct sunlight and prolonged exposure to light. Store the samples in coloured containers. Clear glass bottles are also suitable, but then the samples shall be kept in a dark box.

During storage of the test samples, loss of components may occur due to adsorption on the walls of the containers. The extent of the losses may depend on the storage time.

Concentration of organic solvents can lead to loss of volatile components like naphthalene, chlorobenzenes and phosphorous containing pesticides.

5.2 Interferences with gas chromatography

Non-polar substances are separated on a suitable fused silica capillary column, coated with a film of cross-linked non-polar polysiloxane or slightly polar modified polysiloxane with an efficient separation. The column shall be suitable for the separation of benzo[*a*]pyrene and benzo[*e*]pyrene. Identification and quantification is performed by means of MS using electron-impact ionization (EI). Sufficient resolution (e.g. not less than $R = 0{,}8$) between the peaks of benzo[*b*]fluoranthene and benzo[*k*]fluoranthene as well as of benzo[*a*]pyrene and benzo[*e*]pyrene is to be set as a quality criterion for the capillary column. Benzo[*j*]fluoranthene cannot be separated from benzo[*k*]fluoranthene and benzo[*b*]fluoranthene. It is possible that triphenylene is not completely separated from benzo[*a*]anthracene and chrysene. If this occurs, state this fact in the test report.

NOTE Benzo[*j*]fluoranthene, benzo[*e*]pyrene and triphenylene are not part of the 16 target PAH analytes.

Chromatographic separation between the following pairs can be critical. Due to their molecular mass differences, quantification can be made by mass selective detection. When incomplete resolution is encountered, peak integration shall be checked and, when necessary, corrected.

— PCB 52 – PCB 73;

— PCB 101 – PCB 89/PCB 90;

— PCB 118 – PCB 106;

— PCB 138 – PCB 164/PCB 163.

Interferences between the following isomeric pairs of chlorobiphenyls can also be critical as they have the same mass and fragmentation pattern. Therefore, the resolution between the compounds should be $R > 0{,}8$.

PCB	**Ballschmitter No.**
— Trichloro	PCB 28 – PCB 31
— Tetrachloro	PCB 52 – PCB 43
— Pentachloro	PCB 101 – PCB 113
	PCB 118 – PCB 149
— Hexachloro	PCB 153/PCB 168 – PCB132
	PCB 138/PCB 164/PCB163 – PCB PCB160
— Heptachloro	PCB 180 – PCB 193

Adsorptions and disruption of selected parameters, for example 4,4′-DDT (*p*,*p*′-DDT); 2,4′-DDT (*o*,*p*′-DDT) and/or endrin, can occur in the injector.

5.3 Interferences with GC-MS

Substances that co-elute with the target components may interfere with the determination. These interferences may lead to incompletely resolved signals and may, depending on their magnitude, affect accuracy and precision of the analytical results. Non-symmetrical peaks and peaks that are broader than the corresponding peaks of the reference substance suggest interferences.

Chromatographic separation between dibenzo[*a,h*]anthracene and indeno[1,2,3-*cd*]pyrene is mostly critical. Due to their molecular mass differences, quantification can be made by mass selective detection. When incomplete resolution is encountered, peak integration shall be checked and, if necessary, the baseline corrected.

6 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade, “for residue analysis” or “for GC analysis”, where appropriate, and distilled or demineralized water or water of equivalent purity. Pay extra attention that each batch of solvents does not contain blank concentrations affecting the results.

6.1 Solids

6.1.1 Sodium sulfate, Na_2SO_4, anhydrous, precleaned by heating to 500 °C for 4 h or free of interfering compounds.

6.2 Solvents

6.2.1 Hexane, C_6H_{14}.

6.2.2 Acetonitrile, CH_3CN.

6.2.3 Acetone, C_3H_6O.

6.2.4 Decane, $C_{10}H_{22}$.

6.2.5 Isooctane, C_8H_{18}.

6.2.6 Dichloromethane, CH_2Cl_2.

6.3 Gases

6.3.1 Nitrogen, volume fraction 99,999 %, for evaporating the extracts.

6.4 Standards

6.4.1 Reference substances (see Table 2) and **internal standards**.

Choose internal standards with physical and chemical properties (such as extraction behaviour, retention time) that are similar to those of the compounds to be analysed.

Use an internal standard for every class of compounds for the GC-MS method to evaluate results. Use at least two internal standards per class of substance. Verify the stability of the internal standards regularly. Table 2 contains compounds that can be used. The internal standards are added to the sample to be extracted and are therefore dissolved in a water-soluble solvent.

NOTE ^{13}C isotopically labelled standards can also be used as internal standard.

Certified solutions of non-polar substances of certified purity are available from a limited number of suppliers, e.g. the Institute for Reference Materials and Measurements (IRMM)[1)], the National Institute of Science and Technology (NIST)[2)] or other commercial providers. Because of the dangerous nature of the substances used, commercially available, preferably certified, standard solutions should be used. Skin contact should be avoided.

Table 2 — Native and deuterated non-polar substances

Native reference substances	Labelled internal standard substances
PAH	**PAH**
Naphthalene	Naphthalene-d_8 (CAS No. 1146-65-2)
Acenaphthene	Acenaphthene-d_{10} (CAS No. 15067-26-2)
Acenaphthylene	Acenaphthylene-d_8 (CAS No. 93951-97-4)
Fluorene	Fluorene-d_{10} (CAS No. 81103-79-9)
Anthracene	Anthracene-d_{10} (CAS No. 1719-06-8)
Phenanthrene	Phenanthrene-d_{10} (CAS No. 1517-22-2)
Fluoranthene	Fluoranthene-d_{10} (CAS No. 93951-69-0)
Pyrene	Pyrene-d_{10} (CAS No. 1718-52-1)
Benzo[*a*]anthracene	Benzo[*a*]anthracene-d_{12} (CAS No. 1718-53-2)
Chrysene	Chrysene-d_{12} (CAS No. 1719-03-5)
Benzo[*b*]fluoranthene	Benzo[*b*]fluoranthene-d_{12} (CAS No. 93951-98-5)
Benzo[*j*]fluoranthene[a] (CAS No. 205-82-3)	
Triphenylene[a] (CAS No 217-59-4)	
Benzo[*k*]fluoranthene	Benzo[*k*]fluoranthene-d_{12} (CAS No. 93952-01-3)
Benzo[*a*]pyrene	Benzo[*a*]pyrene-d_{12} (CAS No. 63466-71-7)
Benzo[*e*]pyrene[a] (CAS No. 192-97-2)	d_{12} Available (CIL)[b]
Indeno[1,2,3-*cd*]pyrene	Indeno[1,2,3-*cd*]pyrene-d_{12} (CAS No. 203578-33-0)
Dibenzo[*a,h*]anthracene	Dibenzo[*a,h*]anthracene-d_{14} (CAS No. 13250-98-1)
Benzo[*ghi*]perylene	Benzo[*ghi*]perylene-d_{12} (CAS No. 93951-66-7)
PCB-28: 2,4,4′-trichlorobiphenyl	PCB-28: ^{13}C-2,4,4′-trichlorobiphenyl
PCB-52: 2,2′,5,5′-tetrachlorobiphenyl	PCB-52: ^{13}C-2,2′,5,5′-tetrachlorobiphenyl
PCB-101: 2,2′,4,5,5′-pentachlorobiphenyl	PCB-101: ^{13}C-2,2′,4,5,5′-pentachlorobiphenyl
PCB-118: 2,3′,4,4′,5-pentachlorobiphenyl	PCB-118: $^{13}C_{12}$- 2,3′,4,4′,5-pentachlorobiphenyl (CAS No. 104130-40-7)
PCB-138: 2,2′,3,4,4′,5′-hexachlorobiphenyl	PCB-138: ^{13}C-2,2′,3,4,4′,5′-hexachlorobiphenyl (CAS No. 35065-28-2)
PCB-153: 2,2′,4,4′,5,5′-hexachlorobiphenyl	PCB-153: ^{13}C-2,2′,4,4′,5,5′-hexachlorobiphenyl
PCB-180: 2,2′,3,4,4′,5,5′-heptachlorobiphenyl	PCB-180: ^{13}C-2,2′,3,4,4′,5,5′-heptachlorobiphenyl
OCP	**OCP**
α-Hexachlorocyclohexane (α-HCH)	(α-HCH) $^{13}C_6H_6Cl_6$ (CAS No. 222966-66-7)
β-Hexachlorocyclohexane (β-HCH)	^{13}C available (CIL)[b]
γ-Hexachlorocyclohexane (γ-HCH)	(γ-HCH) $^{13}C_6H_6Cl_6$ (CAS No.104215-85-2)
δ-Hexachlorocyclohexane (δ-HCH)	^{13}C Available (CIL)[b]
ε-Hexachlorocyclohexane (ε-HCH)	

1) Institute for Reference Materials and Measurements (IRMM), Geel, Belgium is an example of a suitable supplier. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this supplier.

2) National Institute of Science and Technology (NIST), Washington, DC, USA, is an example of a suitable supplier. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this supplier.

Table 2 *(continued)*

Native reference substances	Labelled internal standard substances
Aldrin	^{13}C available (CIL)[b]
Dieldrin	^{13}C available (CIL)[b]
Endrin	^{13}C available (CIL)[b]
Heptachlor	^{13}C available (CIL)[b]
Heptachlor epoxide (exo-, *cis*- or α-isomer)	^{13}C available (CIL)[b]
Heptachlor epoxide (endo-, *trans*- or β-isomer)	
α-Endosulfan	d_4 and ^{13}C available (CIL)[b]
β-Endosulfan	d_4 and ^{13}C available (CIL)[b]
p,*p*′-DDE	^{13}C available (CIL)[b]
o,*p*′-DDD	^{13}C available (CIL)[b]
o,*p*′-DDT	^{13}C available (CIL)[b]
p,*p*′-DDD	^{13}C available (CIL)[b]
o,*p*′-DDE	^{13}C available (CIL)[b]
p,*p*′-DDT	^{13}C available (CIL)[b]
Methoxychlor	^{13}C available (CIL)[b]
1,2,4-Trichlorobenzene	d_3 available (CIL)[b]
1,2,3-Trichlorobenzene	d_3 available (CIL)[b]
1,3,5-Trichlorobenzene	d_3 available (CIL)[b]
1,2,3,4-Tetrachlorobenzene	^{13}C available (CIL)[b]
1,2,3,5-Tetrachlorobenzene	
1,2,4,5-Tetrachlorobenzene	d_2 and $^{13}C_6$ available (CIL)[b]
Pentachlorobenzene	^{13}C available (CIL)[b]
Pentachloronitrobenzene	^{13}C available (CIL)[b]
Hexachlorobenzene (HCB)	HCB $^{13}C_6Cl_6$
Organophosphorus	**Organophosphorus**
Azinphos-ethyl	d_{10} available (Ehrenstorfer)
Bromofenvinphos-ethyl	
Chlorofenvinphos	d_{10} available (Ehrenstorfer)
Chloropyriphos-ethyl	d_{10} available (CIL, Ehrenstorfer)[b]
Chloropyriphos-methyl	d_6 available (Ehrenstorfer)
Heptenophos	

[a] Not part of the 16 target analytes, but only for checking whether resolution is sufficient.

[b] Cambridge Isotope Laboratory (CIL) and Dr. Ehrenstorfer are examples of suitable suppliers. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these suppliers.

The most commonly used internal standards are isotopically labelled substances. They are highly recommended. They are used for the evaluation of the results and quantification of the individual substances (Clauses 11 and 12).

6.4.2 Injection standard

Add an isotopically labelled non-polar substance to the final extract and to the calibration solutions (6.5.3) before GC-MS injection to check the recovery of the internal standards.

Prepare a stock solution of the injection standard in an appropriate solvent, e.g. acetonitrile (6.2.2) or hexane (6.2.1), with a mass concentration, $\rho \approx 10$ µg/ml.

6.5 Solutions

6.5.1 Single substance stock solutions

Prepare solutions of the single native substances and internal standards (see Table 2) in an appropriate solvent, e.g. acetonitrile (6.2.2) or hexane (6.2.1), with mass concentration, $\rho \approx 200$ µg/ml.

These solutions can be used for confirmation and identification of single components in the chromatogram.

6.5.2 Multiple substance stock solution

Dilute a sufficient volume, e.g. 5 ml, of the single substance stock solutions (6.5.1) in a volumetric flask (e.g. 100 ml) with an appropriate solvent, e.g. acetonitrile (6.2.2) or hexane (6.2.1), to prepare a solution with a mass concentration, $\rho \approx 10$ µg/ml.

Alternatively, commercially available (certified) combined/mixed solutions containing one or a few of the reference substances (see Table 2) at an appropriate mass concentration of the respective individual substance, e.g. 10 µg/ml in an appropriate solvent, e.g. acetonitrile (6.2.2) or hexane (6.2.1), may be used.

Solutions 6.4.2, 6.5.1 and 6.5.2 are stable for at least 1 year when stored in the dark at room temperature and protected from evaporation. The stability of the standard solution shall be checked regularly. For that purpose, independent solutions for quality control shall be available within a laboratory.

6.5.3 Calibration solutions

Prepare at least five calibration solutions (CS1 to CS5) by appropriate dilution of the multiple substance stock solution (6.5.2), using hexane (6.2.1) or acetonitrile (6.2.2) as solvent. Add to each solution the same amount of the stock solution of the injection standard to a final concentration, $\rho \approx 100$ ng/ml.

It is recommended that the solvent for the calibration solutions be the same as the solution of the final extract.

Transfer, for example, 50 µl of the multiple stock solution into a 5 ml one-mark volumetric flask and make up to the mark with an appropriate solvent. A volume of 1 µl of this reference solution contains 100 pg of the individual substances concerned ($\rho \approx 100$ ng/ml).

The mass concentration of the non-polar substances in the multiple substance stock solution shall be checked by comparison with an independent, preferably certified, standard solution. All individual substances shall agree within $\pm$10 %.

These solutions shall be used for the calibration of the GC system [mixture in hexane (6.2.1)] as well as for the investigation of recovery rates [mixture in acetone (6.2.3)].

Store the solutions at (3 $\pm$ 2) °C in the dark. These solutions are stable for at least 1 month.

7 Apparatus

7.1 General requirements

Standard laboratory glassware and stirring bars cleaned to eliminate all interferences.

NOTE All glassware and stirring bars can be cleaned, for example by rinsing with detergent and hot water and drying for about 15 min to 30 min at about 120 °C. After cooling, the glassware can be rinsed with acetone and sealed and stored in a clean environment.

Do not re-use glassware and stirring bars that have been in contact with waste-water samples or samples with high concentrations for drinking water analysis. This applies especially for PAH, PCB and HCH.

7.2 Coloured glass bottles, narrow-necked, flat-bottomed, 1 000 ml, with aluminium-lined cap.

7.3 Magnetic stirrer, with stirring bars (size approximately 2 cm), glass or PTFE-coated, kept under the solvent used for extraction.

7.4 Separating funnel, nominal capacity 1 000 ml, with PTFE stopcock and glass stopper.

7.5 Conical flask, nominal capacity 250 ml, with glass stopper.

7.6 Equipment for concentrating the eluates by evaporation, e.g. a rotary evaporator, regulatable for constant vacuum and with a temperature-controlled water bath, or stripping equipment using nitrogen gas.

7.7 Vacuum device for solid-phase extraction, e.g. vacubox, extraction box.

7.8 Microlitre syringes, e.g. 500 µl and 1 000 µl.

7.9 Reduction flask, 100 ml (e.g. as shown in Figure B.3).

7.10 Centrifuge with rotor, with centrifuge tubes (e.g. as shown in Figure B.2) with tapered bottom, 50 ml.

7.11 Shaking apparatus, with adjustable rotational speed.

7.12 Glass autosampler vials, capacity e.g. 2 ml, with inert cap and PTFE-coated septum.

7.13 Glass vials, e.g. centrifuge tubes, graduated (scale division 0,1 ml), nominal capacity 10 ml, with glass stoppers.

7.14 Gas chromatograph, with MS detector (EI).

7.15 High resolution, low-bleeding capillary column for GC (see Annex A).

7.16 Microfilter, with solvent-resistant hydrophilic membrane, pore size 0,45 µm.

7.17 Pasteur pipettes

7.18 Glass cartridges, filled with at least 0,5 g silica (see 7.19).

NOTE These cartridges are commercially available.

7.19 Silica, average particle size approximately 40 µm, heated at 450 °C for 3 h and stored in a desiccator to ensure maximum activity.

NOTE Pre-packed silica cartridges are commercially available.

7.20 Molecular sieve beads, pore diameter 0,4 nm.

7.21 Glass wool

8 Sampling

Collect the sample in a coloured glass bottle with a volume of 1 000 ml (7.2).

When sampling drinking water from a mains tap, collect the sample before the tap is sterilized by flame treatment for bacteriological sampling.

Fill the bottle to the shoulder (approximately 950 ml). Determine the volume of the sample to be extracted by weighing, before extraction and after emptying, with an accuracy of ±5 g. Store the sample at (3 ± 2) °C and protect it from light until the extraction is carried out (see also ISO 5667-3).

Ensure that the extraction is carried out within the maximum preservation time, as specified in ISO 5667-3, to avoid losses.

It is generally recommended that the extraction be carried out as soon as practicable to minimize potential adherence to glass which could be an issue.

9 Procedure

9.1 General considerations

The liquid-liquid extraction method shall not be used with samples containing more that 150 mg/l of suspended matter.

NOTE Volatile solvents other than hexane can be used if it is proven that there is equal or better recovery (recovery between 70 % and 110 %).

9.2 Extraction

9.2.1 Sample preparation and extraction

Add a precisely defined amount of the internal standard (e.g a volume containing 50 ng), dissolved in a water-soluble solution (6.4.2). Add 25 ml of hexane (6.2.1) and a stirring bar, then close the flask with a PTFE cap liner or close the conical flask (7.5) with a ground stopper. Thoroughly mix the sample using the magnetic stirrer (7.3) at maximum setting for 60 min. Transfer the sample to a separating funnel and allow the phases to separate for at least 5 min. If an emulsion is formed during the extraction process, collect it in a centrifuge tube and centrifuge (7.10), e.g. for 10 min at about 3 000 r/min. Remove the separated water with a Pasteur pipette. Transfer the extract to a conical flask (7.5) and dry it according to 9.2.2.

For waste and surface waters, repeat the extraction procedure twice. Transfer the sample from the separating funnel back into the sample container, add 25 ml of hexane (6.2.1), and proceed as described above.

The extraction procedure can also be carried out in a separating funnel (7.4) using a shaking apparatus (7.11) and a micro-separator (see Annex B). Rinse the bottle thoroughly with extraction solvent to extract any adsorbed components.

NOTE 1 Other volatile solvents can also be used if it is proven that there is equal or better recovery (recovery between 70 % and 110 %).

NOTE 2 For the extraction of waste water and other water samples with expected high concentrations of PAH, only 10 ml to 100 ml of the homogenous sample can be transferred to a 250 ml conical flask (7.5) with a pipette and diluted with water to 200 ml. After adding 25 ml of hexane (6.2.1), proceed as described above.

9.2.2 Drying of the extract

Transfer the hexane layer obtained according to 9.2.1 into a 100 ml conical flask. Rinse the funnel or centrifuge tube with 5 ml of hexane and add it to the total extract.

Dry the extract with approximately 1,0 g sodium sulfate (6.1.1) for at least 15 min, swirl the vessel frequently. The extract can also be dried by filtering through anhydrous sodium sulfate.

Decant the dry extract into a reduction flask (7.9). Rinse the conical flask twice with 5 ml of hexane and decant this also into the reduction flask.

9.2.3 Enrichment

Evaporate the dried hexane extract obtained according to 9.2.2 until it fills only the tapered tip of the reduction flask (approximately 2 ml), with, for example, a rotary evaporator, at a temperature of 30 °C, slowly lowering the pressure to 20 kPa.

Do not evaporate the extracts to dryness, as losses of 2-ring or 3-ring compounds, for example, and 1,2,4-trichlorobenzene can occur. Adding a few drops of decane (6.2.4) or isooctane (6.2.5) reduces the loss of the most volatile compounds.

Dissolve the extract into a known volume, e.g. 2 ml. Be sure that any residues that may be deposited on the glass wall are dissolved by shaking the extract using the shaking apparatus.

Clean the extracts of waste-water samples and other samples of unknown origin by silica clean-up according to 9.2.4, if the chromatogram shows interferences that hamper the quantification.

Transfer the enriched sample, if necessary after filtration through a filter (7.16), into a glass sample vial. Keep the sample in a cool and dark place until the analysis is carried out.

Proceed as described in 9.4.

NOTE Alternative enrichment methods can also be used. If a large volume injection is used or if higher concentrations of the target compounds are expected, a lower enrichment factor can be used.

9.2.4 Clean-up

Applying the procedure described in 9.2 can lead to co-extraction of relatively polar and/or other undesired substances, which can interfere by the appearance of unknown peaks overlapping the target compounds. When the target compounds are PAH, the silica clean-up procedure described in Annex C can be used. Use the clean-up procedures for other non-polar substances specified in ISO 6468.

9.3 Gas chromatography

Operate the gas chromatograph according to the manufacturer's instructions.

Select a capillary GC column and chromatographic conditions that will lead to efficient separation (see Annex A).

When using an injection standard, add a precisely known amount of the injection standard (6.4.2) to the sample extract, mix thoroughly and inject immediately into the GC.

9.4 Blank measurement

Perform blank determinations at least once per batch using water prior to and during a series of analyses. This water should be free of detectable target compounds. Blank measurements shall include all steps of the analytical procedure from the arrival of the sample in the laboratory to the evaluation of the gas chromatogram. If blank values are unusually high (over 50 % of the lowest reporting level), every step in the procedure shall be checked in order to find the reason for these high blanks. Ensure that blanks are reduced as much as possible by various procedures, e.g. elimination of contamination of the sample by ambient air and solvents and checks of analytical instrumentation.

If sample concentrations are close to the limit of detection, however, blank values higher than 50 % of the lowest reported value can be tolerated. If this occurs, it is recommended that the sample be concentrated and retested to provide confirmation.

9.5 Mass spectrometric conditions

Adjust the mass spectrometer in accordance with the manufacturer's instructions. Chromatograms are recorded in full scan (50 amu to 420 amu) or selected ion monitoring/recording mode (SIM/SIR).

Adjust the scan rate of the mass spectrometer to a velocity allowing one GC peak to be described by at least seven data points.

Diagnostic ions with relative intensities with reference to ISO 22892[8] are listed in Table 4.

NOTE Each of the non-polar target analytes can be quantified by using the deuterated internal standard stated above.

10 Calibration

10.1 General

A calibration curve encompassing the concentration range is prepared for each compound to be determined. The relative response [R_{rel} or F_R depending on whether labelled internal standards or other native (non-labelled) standards were used] versus concentration in standard solutions is plotted or computed using a regression function. Relative response is determined according to the procedures described below. At least five calibration points are employed. Also consult ISO 8466-1.

10.2 Calibration by labelled internal standards

Labelled internal standard calibration is used for the non-polar substances for which labelled compounds are added to samples.

Prepare a calibration curve encompassing the concentration range for each compound to be determined. Plot the relative response, R_{rel} (labelled to native), versus concentration in standard solutions or compute using a linear regression. Determine the relative response factor for each non-polar substance according to the procedures described below. Employ at least five calibration points.

Determine the response of each non-polar substance relative to its labelled analogue using Equation (1):

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

where

A_{1n} is the area of diagnostic ion 1 for the non-polar substance;

A_{1L} is the area of diagnostic ion 1 for the labelled compound;

ρ_L is the concentration of the labelled compound in the calibration standard, in micrograms per litre;

ρ_n is the concentration of the native compound in the calibration standard, in micrograms per litre.

NOTE 1 If the relative response for any compound is constant (coefficient of variation of less than 20 %) over the five-point calibration range, an averaged relative response can be used for that compound. Otherwise, the complete calibration curve for that compound can be used over the five-point calibration range.

NOTE 2 It is also possible to use only one mass for calibration and quantification.

10.3 Calibration by internal standard

The internal standard method is applied to determination of other non-polar substances for which no labelled standards have been added to the sample.

Calibration requires the determination of response factors, F_R, defined by Equation (2):

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

where

A_{1s} is the area of diagnostic ion 1 for the non-polar substance;

A_{1is} is the area of diagnostic ion 1 for the internal standard;

ρ_{is} is the concentration of the internal standard, in micrograms per litre;

ρ_s is the concentration of the compound in the calibration standard, in micrograms per litre.

NOTE If the response factor, F_R, for any compound is constant (coefficient of variation of less than 20 %) over the five-point calibration range, an averaged response factor can be used for that compound. Otherwise, the complete calibration curve for that compound over the five-point range can be used.

For the daily check of the calibration (recalibration), inject at least two calibration standards, e.g. concentrations of (20 ± 10) % and (80 ± 10) % of the established linear range. Compare the calculated response factor with those obtained in the previous batch of samples. They should not differ more than 20 %.

11 Measurement of samples

Equilibrate the measuring system before measuring samples and adjust the mass spectrometer according to the manufacturer's instructions.

The following measurement conditions shall apply.

Ionization method:	electron impact
Mass range of the spectrum:	50 amu to 420 amu, at least 10 amu above the highest mass of the substances to be determined
Cycle duration:	<2 s so that five spectra can be taken per substance peak

If only single masses are registered in order to increase sensitivity, register the base peak and at least two more ions, with the same cycle duration as above.

12 Identification

The quantification of a single substance requires a secure and non-ambiguous identification. Components that have less fragmentation therefore require additional criteria for identification.

When taking whole spectra, the sample spectrum and the reference spectrum taken under the same working conditions should be identical. The reference spectrum should be produced by each laboratory using their equipment and should be stored in a reference spectrum database. These spectra are to be used for identification purposes by MS.

The deviation of the non-basic peak (not 100 % mass peak) should be less than 10 %.

If there is a shift of retention time, confirmation of identity can be done by spiking. It is possible that the use of isotopically labelled standards is the best way to confirm the identity.

A single substance is identified, if:

— the retention time of a substance in the total ion current chromatogram of the sample is congruent with that of a reference standard in the latest acquired reference solution performed under identical chromatographic conditions (limit: ±1 %, maximum ±6 s);

— the relative intensity of the diagnostic ions recorded in the mass spectrum of the sample acquired under identical conditions does not differ by more than $\pm(0{,}1I + 10)$ % from those relative intensities of the reference substance, where I is the relative intensity recorded from the characteristic ions in the mass spectrum of the reference solution.

See Table 3.

Table 3 — Degree of confirmation of the identity

Technique	Degree of identification	Operating principle	Additional criterion
MS	Possible	Single mass monitoring (SIM/SIR)	Compliance of the mass ratio with that of the standard compound within given limits
	Confirmed	Acquisition of total spectra (scan)	Compliance of the spectrum with that of the standard compound within given limits

Critical peak couples can lead to incorrect automatic assignment. In such cases, a manual check is essential. Critical peak pairs are: phenanthrene and anthracene; benzo[*a*]anthracene and chrysene; benzo[*b*]fluoranthene and benzo[*k*]fluoranthene; benzo[*a*]pyrene and benzo[*e*]pyrene; and PCB28 and PCB31.

Overlapping compounds with similar masses can be identified reliably only if the minimum between both peaks is at least 25 % of the base peak; otherwise they are reported as a sum.

When using single masses, all three mass signals should be present. The signal-to-noise ratio for the smallest peak of a mass should be over 3.

The ratio of the three masses in the spectrum should be determined from the peak heights at the peak maximum. The two peaks corresponding to the masses below 100 % that are determined should, in relation to the 100 % mass, be within 10 % of the value determined under the same conditions with the reference material.

The mass spectrum of the sample should include all ions that have a relative intensity of 10 % in the reference spectrum. The ratio of the intensities of the different ions in the sample spectrum and the reference spectrum should be within 20 %, tested on the three most important ions.

Single mass registration should be noted in the report.

For detection by MS, use the peak area of the base peak of substance *i*, after checking the identity by comparing the spectra, or, with the SIM technique, the isotope and/or fragment ratios. If using an internal standard, the reference is always the signal of the most intensive mass (main ion), after this signal has been checked for purity.

Table 4 — Recommended characteristic masses of non-polar substances, as specified in ISO 22892[8]

Compound	Diagnostic ion 1[a] *m*/*z*	Diagnostic ion 2[a] *m*/*z*	Diagnostic ion 3[a] *m*/*z*
PAH			
Naphthalene	128 (100)	102 (11)	—
Acenaphthylene	152 (100)	150 (3)	76 (10)
Acenaphthene	153 (100)	154 (70)	76 (10)
Fluorene	165 (100)	166 (81)	139 (4)
Phenanthrene	178 (100)	152 (9)	76[b] (3)
Anthracene	178 (100)	152 (12)	76 (6)
Fluoranthene	202 (100)	200 (31)	100[b] (3)
Pyrene	202 (100)	200 (2)	101[b] (4)
Benzo[*a*]anthracene	228 (100)	226 (3)	114[b] (2)
Chrysene	228 (100)	226 (6)	113[b] (4)
Benzo[*b*]fluoranthene	252 (100)	250 (22)	126 (5)
Benzo[*k*]fluoranthen	252 (100)	250 (22)	126 (5)
Benzo[*a*]pyrene	252 (100)	250 (18)	113 (11)
Indeno[1,2,3-*cd*]pyrene	276 (100)	138 (12)	274[b] * (4)
Dibenzo[*a,h*]anthracene	278 (100)	139 (9)	276[b] * (5)

Table 4 *(continued)*

Compound	Diagnostic ion 1[a] *m/z*	Diagnostic ion 2[a] *m/z*	Diagnostic ion 3[a] *m/z*
Benzo[*ghi*]perylene	276 (100)	138 (12)	274 (4)
PCB			
PCB 28	186 (100)	258 (74)	186 (82)
$^{13}C_{12}$-PCB 28	268	270	—
PCB 52	292 (100)	294 (49)	220 (95)
$^{13}C_{12}$-PCB 52	304	306	—
PCB101	326 (100)	328 (65)	256 (62)
$^{13}C_{12}$-PCB101	338	340	—
PCB 118	326 (100)	328 (62)	254 (57)
$^{13}C_{12}$-PCB 118	338	340	—
PCB 138	290 (100)	358 (42)	360 (94)
$^{13}C_{12}$-PCB 138	372	374	—
PCB 153	360 (100)	362 (92)	290 (73)
$^{13}C_{12}$-PCB 153	372	374	—
PCB 180	394 (100)	396 (96)	324 (84)
$^{13}C_{12}$-PCB 180	406	408	—
OCP			
Hexachlorobenzene (HCB)	284 (100)	142 (22)	249 (24)
α-Hexachlorocyclohexane (α-HCH)	181 (100)	219 (33)	109 (29)
β-Hexachlorocyclohexane (β-HCH)	181 (97)	219 (54)	109 (49)
γ-Hexachlorocyclohexane (γ-HCH)	181 (97)	219 (34)	109 (33)
δ-Hexachlorocyclohexane	109 (100)	219 (96)	183 (90)
ε-Hexachlorocyclohexane	109 (88)	219 (100)	183 (90)
Aldrin	66 (100)	263 (78)	293 (41)
Dieldrin	79 (100)	263 (70)	277 (18)
Endrin	81 (100)	263 (70)	277 (18)
Heptachlor	100 (100)	65 (65)	272 (89)
Heptachlor epoxide (*cis*-isomer)	253 (100)	183 (90)	289 (85)
Heptachlor epoxide (*trans*-isomer)	353 (100)	81 (67)	263 (26)
α-Endosulfan	195 (100)	159 (93)	265 (55)
β-Endosulfan	195 (100)	241 (80)	159 (56)
p,p′-DDE	246 (100)	318 (37)	176 (36)
o,p′-DDD	235 (100)	165 (66)	199 (29)
o,p′-DDT	235 (100)	165 (67)	199 (27)
p,p′-DDD	235 (100)	165 (66)	199 (20)
o,p′-DDE	246 (100)	318 (37)	176 (27)
p,p′-DDT	235 (100)	165 (68)	199 (20)
Methoxychlor	227 (100)	228 (18)	274 (5)
Chlorobenzenes			
1,2,4-Trichlorobenzene	180 (100)	182 (97)	145 (45)
1,2,3-Trichlorobenzene	180 (100)	182 (92)	145 (38)
1,3,5-Trichlorobenzene	180 (100)	182 (95)	145 (32)
1,2,3,4-Tetrachlorobenzene	216 (100)	214 (74)	108 (24)

Table 4 *(continued)*

Compound	Diagnostic ion 1[a] *m/z*	Diagnostic ion 2[a] *m/z*	Diagnostic ion 3[a] *m/z*
1,2,3,5-Tetrachlorobenzene	216 (100)	214 (78)	108 (14)
1,2,4,5-Tetrachlorobenzene	216 (100)	214 (79)	108 (12)
Pentachlorobenzene	250 (100)	252 (63)	215 (25)
Pentachloronitrobenzene	237 (100)	295 (83)	142 (50)
Organophosphorus			
Azinphos-ethyl	132 (100)	160 (80)	77 (68)
Bromofenvinphos	267	269	323
Chlorofenvinphos	267 (100)	323 (59)	81 (58)
Chloropyriphos-ethyl	97 (100)	197 (90)	314 (47)
Chloropyriphos-methyl	286 (100)	125 (95)	288 (78)
Heptenophos	124 (100)	89 (77)	215 (13)

[a] In brackets: relative intensity of the fragment ion.

[b] Often missing fragments.

13 Calculation

13.1 Quantification by internal standards

Compute the concentrations of those non-polar substances for which an internal standard is added using the response factors determined from the initial calibration data (10.3) and Equation (3):

$$m_{\mathrm{ex}} = \frac{A_{1\mathrm{s}} m_{\mathrm{is}}}{A_{1\mathrm{is}} F_{\mathrm{R}}} \tag{3}$$

where

m_{ex} is the amount of the non-polar substance in the extract, in nanograms;

$A_{1\mathrm{s}}$ is the area of diagnostic ion 1 for the non-polar substance;

m_{is} is the amount of the internal standard, in nanograms;

$A_{1\mathrm{is}}$ is the area of diagnostic ion 1 for the internal standard;

F_{R} is the response factor as defined in 10.3.

Determine the response factor of the internal standards relative to the injection standard using the area response of the diagnostic ion. Using the amount in the extract determined using Equation (3), compute the percentage recovery, η, of the internal standards using Equation (4):

$$\eta = \frac{m_{\mathrm{ex}}}{m_{\mathrm{spk}}} \times 100 \tag{4}$$

where

m_{ex} is the amount found, in nanograms;

$m_{\mathrm{spk}}$is the amount spiked, in nanograms.

For compounds for which no internal standard has been added, the recovery is determined as follows.

Add, for example, 2 ml of reference solution prepared according to 6.4.1 to 1 000 ml water and proceed as specified in Clause 9.

Determine the recovery rates for surface water samples by the method of standard additions.

Determine the mean recovery, $\overline{\eta}$, of analyte i using Equations (5) and (6):

$$\eta_{i,N} = \frac{\rho_{i,N_f}}{\rho_{i,N_e}} \tag{5}$$

$$\overline{\eta_i} = \frac{\sum_{N=1}^{n} \eta_{i,N}}{n} \tag{6}$$

where

$\eta_{i,N}$ is the recovery of analyte i at concentration level N;

ρ_{i,N_f} is the mass concentration of analyte i found at concentration level N, calculated with the calibration function, in micrograms per litre;

ρ_{i,N_e} is the mass concentration of analyte i given at concentration level N, in micrograms per litre;

$\overline{\eta}_i$ is the mean recovery;

n is the number of concentration levels.

13.2 Quantification by labelled internal standards

By adding a known amount of a labelled compound to every sample prior to extraction, correction for recovery can be made because the non-polar substance and its labelled analogue exhibit similar effects upon extraction, concentration, and GC. Use relative response, R_{rel}, values in conjunction with the initial calibration data described in 10.2 to determine concentrations directly, as long as labelled compound spiking levels are constant, using Equation (7):

$$m_{\text{ex}} = \frac{A_{1\text{n}} m_{\text{L}}}{A_{1\text{L}} R_{\text{rel}}} \tag{7}$$

where

m_{ex} is the amount of the non-polar substance in the extract, in nanograms;

$A_{1\text{n}}$ is the area of diagnostic ion 1 for the native compound;

$A_{1\text{L}}$ is the area of diagnostic ion 1 for the labelled compound;

m_{L} is the amount of the labelled compound in the calibration standard, in nanograms;

R_{rel} is the relative response as defined in 10.2.

Determine the recovery rates for surface-water and waste-water samples by the method of standard additions.

Determine the mean recovery, $\overline{\eta}_i$, of analyte i using Equations (8) and (9):

$$\eta_{i,N} = \frac{\rho_{i,N_f}}{\rho_{i,N_e}} \tag{8}$$

$$\overline{\eta_i} = \frac{\sum_{N=1}^{n} \eta_{i,N}}{n} \tag{9}$$

where

$\eta_{i,N}$ is the recovery of analyte i at concentration level N;

ρ_{i,N_f} is the mass concentration of analyte i found at concentration level N, calculated with the calibration function, in micrograms per litre;

ρ_{i,N_e} is the mass concentration of analyte i given at concentration level N, in micrograms per litre;

$\overline{\eta_i}$ is the mean recovery;

n is the number of concentration levels.

13.3 Recovery of internal standards

Recoveries of the internal standards for most samples are similar to those from reagent water. The recovery limits are between 70 % and 110 %.

If the internal standard recovery is outside these ranges, a diluted sample shall be analysed.

If the recovery of any of the internal standards in the diluted sample is outside the normal range, the calibration solution CS3 (6.5.3) shall be analysed and the calibration verified. For each compound, confirm that the result of the verification analysis is within 20 % of the nominal concentration. 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 tuning of the MS (9.5) and verification test, or recalibrate (10.2).

13.4 Concentration in the sample

Compute the concentration of a non-polar substance in the aqueous phase of the sample using the concentration of the compound in the extract and the volume of water extracted, as follows:

$$\rho = \frac{m_{\text{ex}}}{V_{\text{s}} \times 1\,000} \tag{10}$$

where

ρ is the concentration in aqueous phase, in micrograms per litre;

m_{ex} is the amount of the compound in the extract, in nanograms;

V_{s} is the sample volume, in litres.

14 Expression of results

Report the mass concentration of non-polar substance, in micrograms per litre, to not more than two significant figures. Concentrations $<$ 0,01 µg/l are rounded up to the nearest 0,001 µg/l. Rounding examples are given in Table 5.

Table 5 — Examples of rounding measurement results

Measured value µg/l	Reported result µg/l
13,54	14
1,354	1,4
0,135 4	0,14
0,013 5	0,014
0,008 6	0,009

15 Test report

The test report shall contain at least the following information:

a) the test method used, together with a reference to this Technical Specification (ISO/TS 28581:2012);

b) the data required for identification of the sample examined;

c) relevant information about sampling and sample preservation;

d) the concentration of each of the non-polar substances, expressed according to Clause 14;

e) if used, a note on single mass registration during MS analysis;

f) all operations not described in this Technical Specification which could have affected the results.

Annex A
(informative)

Examples of GC-MS conditions

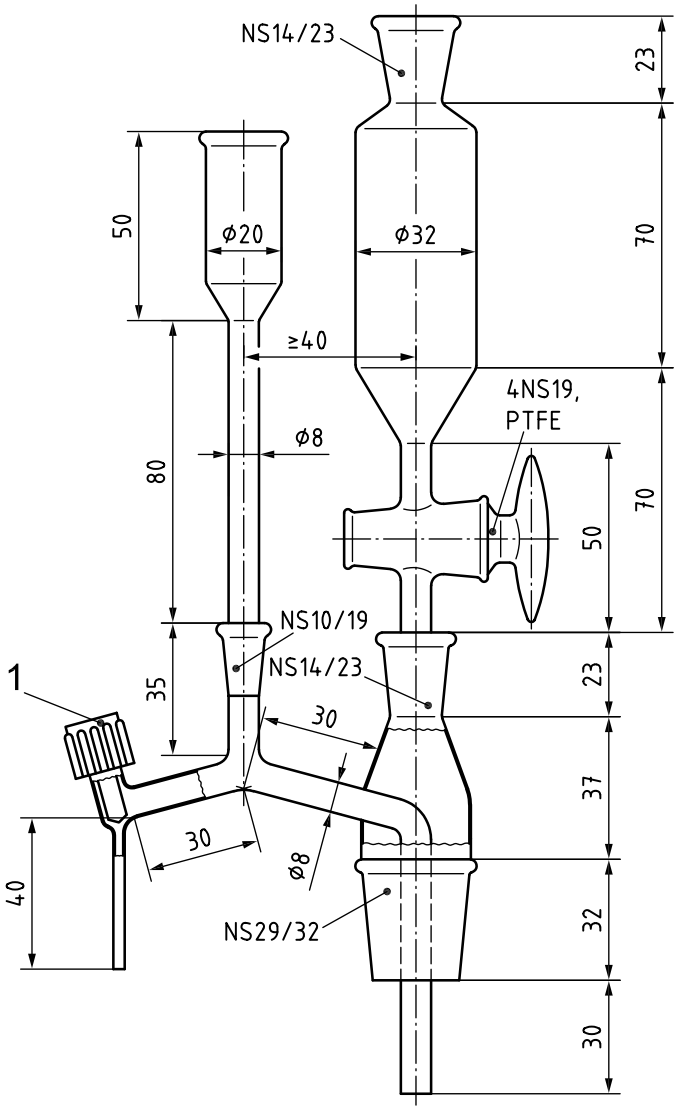
Table A.1 — Examples of chromatographic conditions

Column	Dimensions	Temperature programme
95 % dimethylpolysiloxane 5 % diphenylpolysiloxane	Length: 30 m	40 °C, 8 min isothermal
	Inner diameter: 0,25 mm	5 °C/min to 310 °C
	Film thickness: 25 µm	15 min isothermal
86 % dimethylpolysiloxane 14 % cyanopropylene-polysiloxane	Length: 30 m	40 °C, 6 min isothermal
	Inner diameter: 0,25 mm	5 °C/min to 220 °C
	Film thickness: 1,0 µm	4 min isothermal

Annex B
(informative)

Examples for the construction of special apparatus

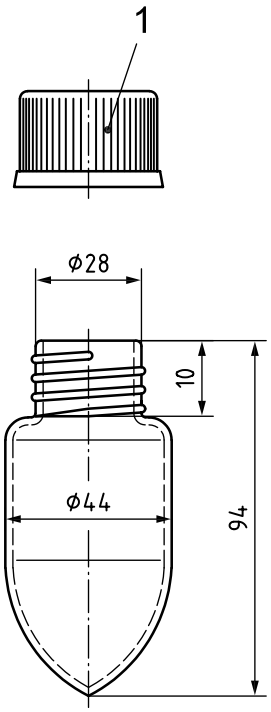
Dimensions in millimetres



Key
1 PTFE screw cock

Figure B.1 — Microseparator

Dimensions in millimetres

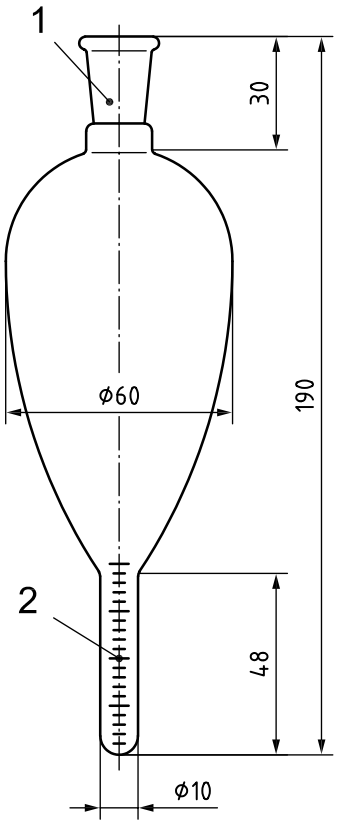


Key

1 PTFE screw cap

Figure B.2 — Centrifuge tube with tapered bottom and screw cap

Dimensions in millimetres



Key

1 ISO 383:[1] 14/23

2 total graduated volume, 2 ml; graduations of 0,1 ml

Figure B.3 — Reduction flask

Annex C
(informative)

Silica clean-up

For clean-up of the extract, use columns [Pasteur pipettes (7.17) with a glass wool plug] or cartridges (7.18) containing at least 0,5 g of silica (7.19). Clean the silica in the column or in the cartridge by rinsing with five bed volumes of a mixture of dichloromethane (6.2.6)/hexane (6.2.1) (1+1), followed by conditioning with the same volume of hexane (6.2.1).

NOTE 1 The clean-up is not possible for solutions that contain acetone (6.2.3).

Dry the solvents used for cleaning the extract by applying a molecular sieve (7.20). The silica should have its maximum activity.

Concentrate the enriched extract (9.2.3) by blowing with a gentle stream of nitrogen (6.3.1) so that a volume of 500 µl remains.

Transfer the concentrated extract using a Pasteur pipette (7.17) on to the hexane-covered silica and allow it to soak almost completely into the silica. Collect the eluate in a glass vial (7.13).

Rinse the reduction flask with 500 µl of hexane (6.2.1), add this solution to the column and allow it to soak almost completely into the silica.

Elute the PAH with a mixture of dichloromethane (6.2.6)/hexane (6.2.1) (1+1).

NOTE 2 Commercially available cartridges containing 0,5 g of silica require a volume of at least 3 ml of the mixture of dichloromethane (6.2.6)/hexane (6.2.1) (1+1) for the elution of the PAH.

Add a few drops of decane (6.2.4) or isooctane (6.2.5) to the eluate, homogenize by shaking, and concentrate (see 9.2.3) to between 200 µl and 250 µl, e.g. first with a rotary evaporator (7.6) to about 2 ml, then by a stream of nitrogen (6.3.1).

Fill the extract up to a known volume (e.g. 2 ml) with the same solvent that has been used for the preparation of the calibration solutions (6.5.3).

Proceed as described in 9.4. Use an aliquot for the GC-MS determination.

Bibliography

[1] ISO 383, *Laboratory glassware — Interchangeable conical ground joints*

[2] ISO 7981 (all parts), *Water quality — Determination of polycyclic aromatic hydrocarbons (PAH)*

[3] ISO/TS 13530:2009, *Water quality — Guidance on analytical quality control for chemical and physicochemical water analysis*

[4] ISO 15089:2000, *Water quality — Guidelines for selective immunoassays for the determination of plant treatment and pesticide agents*

[5] ISO 17858:2007, *Water quality — Determination of dioxin-like polychlorinated biphenyls — Method using gas chromatography/mass spectrometry*

[6] ISO 17993:2002, *Water quality — Determination of 15 polycyclic aromatic hydrocarbons (PAH) in water by HPLC with fluorescence detection after liquid-liquid extraction*

[7] ISO 18073:2004, *Water quality — Determination of tetra- to octa-chlorinated dioxins and furans — Method using isotope dilution HRGC/HRMS*

[8] ISO 22892:2006, *Soil quality — Guidelines for the identification of target compounds by gas chromatography and mass spectrometry*

[9] ISO 28540:2011, *Water quality — Determination of 16 polycyclic aromatic hydrocarbons (PAH) in water — Method using gas chromatography with mass spectrometric detection (GC-MS)*

[10] Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. *Off. J.* 1998-12-05, **L330**, pp. 32–54

ICS 13.060.50

Price based on 25 pages

This page deliberately left blank

British Standards Institution (BSI)

BSI is the national body responsible for preparing British Standards and other standards-related publications, information and services.

BSI is incorporated by Royal Charter. British Standards and other standardization products are published by BSI Standards Limited.

About us

We bring together business, industry, government, consumers, innovators and others to shape their combined experience and expertise into standards -based solutions.

The knowledge embodied in our standards has been carefully assembled in a dependable format and refined through our open consultation process. Organizations of all sizes and across all sectors choose standards to help them achieve their goals.

Information on standards

We can provide you with the knowledge that your organization needs to succeed. Find out more about British Standards by visiting our website at bsigroup.com/standards or contacting our Customer Services team or Knowledge Centre.

Buying standards

You can buy and download PDF versions of BSI publications, including British and adopted European and international standards, through our website at bsigroup.com/shop, where hard copies can also be purchased.

If you need international and foreign standards from other Standards Development Organizations, hard copies can be ordered from our Customer Services team.

Subscriptions

Our range of subscription services are designed to make using standards easier for you. For further information on our subscription products go to bsigroup.com/subscriptions.

With **British Standards Online (BSOL)** you'll have instant access to over 55,000 British and adopted European and international standards from your desktop. It's available 24/7 and is refreshed daily so you'll always be up to date.

You can keep in touch with standards developments and receive substantial discounts on the purchase price of standards, both in single copy and subscription format, by becoming a **BSI Subscribing Member**.

PLUS is an updating service exclusive to BSI Subscribing Members. You will automatically receive the latest hard copy of your standards when they're revised or replaced.

To find out more about becoming a BSI Subscribing Member and the benefits of membership, please visit bsigroup.com/shop.

With a **Multi-User Network Licence (MUNL)** you are able to host standards publications on your intranet. Licences can cover as few or as many users as you wish. With updates supplied as soon as they're available, you can be sure your documentation is current. For further information, email bsmusales@bsigroup.com.

BSI Group Headquarters

389 Chiswick High Road London W4 4AL UK

Revisions

Our British Standards and other publications are updated by amendment or revision.

We continually improve the quality of our products and services to benefit your business. If you find an inaccuracy or ambiguity within a British Standard or other BSI publication please inform the Knowledge Centre.

Copyright

Useful Contacts:

Customer Services
Tel: +44 845 086 9001
Email (orders): orders@bsigroup.com
Email (enquiries): cservices@bsigroup.com

Subscriptions
Tel: +44 845 086 9001
Email: subscriptions@bsigroup.com

Knowledge Centre
Tel: +44 20 8996 7004
Email: knowledgecentre@bsigroup.com

Copyright & Licensing
Tel: +44 20 8996 7070
Email: copyright@bsigroup.com

bsi.

...making excellence a habit.™