DRAFT – HORIZONTAL STANDARD

Soil and Sludge. Determination of selected polybrominated diphenylethers (PBDEs) by gas chromatography-mass spectrometry (GC-MS).

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Foreword

The European project HORIZONTAL is focused on the standardisation of analytical methods for the analyses of various inorganic and organic contaminants in soil, sludge and bio waste. During the preparation of the project, several desk studies have been started to elaborate the possibility of horizontal standardisation on specific subjects. One of these subjects was the horizontal standardisation of selected Polybrominated diphenylethers (PBDEs).

This document has been developed in the framework of the project “Horizontal-ORG” based on the results of a desk study. Numerous test methods published in literature and available ISO standard have been reviewed, compiled and described in this study.

This standard is applicable for the determination of selected PBDEs in soil and sludge.

Introduction

Brominated flame retardants (BFRs) are a chemically diverse class of compounds used in a variety of commercial applications, such as in plastics, textiles, electronic circuitry and other materials to prevent fires. The most used BFRs are polybrominated diphenylethers (PBDEs), hexabromocyclododecane (HBCD) and tetrabromobisphenol A (TBBPA). As the properties of PBDEs make them efficient flame retardants, their demand is increasing rapidly and, therefore, so is their production. PBDE exhibit the typical characteristics of persistent organic pollutants (POPs). PBDEs are resistant towards acids and bases as well as heat and light and also to reducing or oxidising compounds, so are, therefore, persistent in the environment. However, they are likely to be more susceptible to environmental degradation than PCBs due to the C-Br bond being weaker than the C-Cl bond. Similar to dioxins and PCBs, PBDEs are of environmental concern because of their high lipophilicity and high resistance to degradation process. They have been shown to accumulate in the food chain and can already be found in samples from all over the world.

There are a total of 209 theoretically PBDE congeners (in a similar manner to PCBs). However, commercially produced PBDE mixtures contain a limited number of congeners and are less complex than the corresponding technical PCB mixtures. PBDEs are typically produced at three different degrees of bromination, i.e., Penta-BDE, Octa-BDE and Deca-BDE. Penta-BDE formulation consists of 41-42% tetra-BDEs (mainly BDE-47) and 44-45% penta-BDEs (predominantly BDE-99 and BDE-100), whereas Deca-BDE formulation consists mainly of BDE-209 (97-98%), with a small amount of nona-BDES (0.3-3%). On the other hand, hepta-BDE-183 is often taken as indicative of the presence of the Octa-BDE formulations.

1 Scope

This European Standard specifies a method for the determination of selected polybrominated diphenylethers (PBDE) (Table 1) in sediment and sludge using gas chromatography/mass spectrometry (GC-MS) in the electron impact (EI) or negative ion chemical ionisation (NCI) mode. The risk of misinterpretation of interfering substances is smaller with EI due to its higher specifity, but its sensitivity is ten times lower than that obtained with NCI. It is possible to analyse as well other brominated diphenylethers according to this European Standard but its applicability shall be verified in each case.

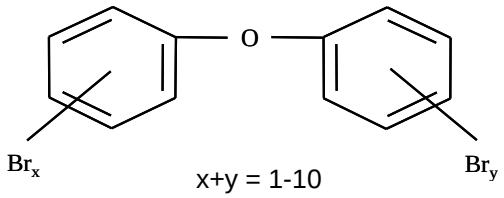

Figure 1

Table 1 — PBDE congeners determined by this method

No	Congener	Formula	Abbreviation[a]	Molar mass g/mol
1	2,2',4,4'-Tetrabromodiphenylether	$C_{12}H_6Br_4O$	BDE-47	485,7950
2	2,2',4,4',5-Pentabromodiphenylether	$C_{12}H_5Br_5O$	BDE-99	564,6911
3	2,2',4,4',6-Pentabromodiphenylether	$C_{12}H_5Br_5O$	BDE-100	564,6911
4	2,2',4,4',5,6'-Hexabromodiphenylether	$C_{12}H_4Br_6O$	BDE-154	643,5872
5	2,2',4,4',5,5'-Hexabromodiphenylether	$C_{12}H_4Br_6O$	BDE-153	643,5872
6	2,2',3,4,4',5',6-Heptabromodiphenylether	$C_{12}H_3Br_7O$	BDE-183	722,4832
7	2,2',3,3',4,4',5',6-Octabromodiphenylether	$C_{12}H_2Br_8O$	BDE-196	801,3793
8	2,2',3,3',4,4',6,6'-Octabromodiphenylether	$C_{12}H_2Br_8O$	BDE-197	801,3793
9	2,2',3,4,4',5,5',6-Octabromodiphenylether	$C_{12}H_2Br_8O$	BDE-203	801,3793
10	Decabromodiphenylether	$C_{12}Br_{10}O$	BDE-209	959,1714
[a] Numbering analog to IUPAC nomenclature for PCB				

2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references, the latest edition of the publication referred to applies (including amendments).

ISO 14507 Soil quality – Guidance for sample pre-treatment for the determination of organic contaminants in soil.

ISO/DIS 16720 Soil quality – Pretreatment of samples by freeze drying for subsequent analysis.

ISO/FDIS 22032 Water quality – Determination of selected polybrominated diphenylethers in sediment and sewage sludge – Method using extraction and gas chromatography/mass spectrometry.

3 Definitions

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

Spiking

Addition of $^{13}C_{12}$-labelled PBDE standards or any internal standards

Analytical blank

A blank sample covering the complete analytical procedure including extraction, cleanup, identification and quantification including all the relevant reagents and materials

Internal standard

Standard added before extraction and used for calculation of results

Congener

Any one of the 209 individual PBDEs

Limit of detection (LOD)

The limit of detection is expressed as the mean sample blank value plus three times the standard deviation (3s) of the blank (EUROCHEM Guide)

Limit of quantification (LOQ)

The limit of quantification is expressed as the mean sample blank value plus, either, five, six or ten times the standard deviation of the blank (EUROCHEM Guide)

4 Symbols and abbreviations

HBCD

Hexabromocyclododecane

MeTBBPA

Dimethyl tetrabromobisphenol A

PBB

Polybrominated biphenyl

PBDE

Polybrominated diphenylether

TBBPA

Tetrabromobisphenol A

5 Principle

This Standard is based on the use of gas chromatography/mass spectrometry in the electron impact (EI) or negative ion chemical ionisation (NCI) mode, to enable the separation, detection and quantification of selected PBDEs in soil and sludge. When NCI mode is used, determination of the concentration in the sample was based on an internal standard calibration. However, when EI mode is applied, the isotope dilution technique is used.

Soil and sludge is sampled according to EN yyyy: (Horizontal standard module(s) for sampling of sludge, soil and waste). Internal standards are added prior to extraction by an organic solvent. Extraction

procedures are normally based on soxhlet or equivalent extraction methods of dried preferable freezedried samples. Sample cleanup is usually carried out by column liquid chromatographic techniques using different adsorbents. This step allowed the removal of sample matrix components, which may overload the separation method and disturb the quantification. Furthermore, an enrichment of the analytes in the final sample extract is achieved.

The deca-BDE-209 congener has a number of additional analytical difficulties. This compound is not stable at high temperatures in the injector and on the GC column. Moreover, the compound is sensitive to degradation by UV light and strongly adsorbs to any kind of surfaces. Thus, special attention must be paid on the sample preparation and instrumental determination for this compound.

6 Reagents and standards

Only use reagents with negligibly low concentrations of brominated diphenylethers compared with the concentration to be determined and verify by blank determinations. To prevent degradation, store standards in the dark at temperatures recommended by the manufacturer (calibration solutions preferably approximately at –18 °C).

6.1 Solvents for extraction, cleanup and preparation of stock solutions

A variety of solvents may be used depending on the particular sample matrix to be analysed and the availability of commercial standard solution. E.g. toluene (C_7H_8), or acetone (propanone, C_3H_6O), or a mixture of acetone (propanone, C_3H_6O) and hexane (C_6H_{14}), or heptane (C_7H_{16}), or iso-octane (2,2,4-trimethylpentane C_8H_{18}), or nonane (C_9H_{20}), or dichloromethane (CH_2Cl_2). Solvents used for extraction and cleanup have to be of pesticide grade or equivalent quality and checked for blanks. Adsorbents like alumina oxide, silica gel and others used for cleanup have to be of p.a. quality or better and pre-cleaned and activated if necessary.

6.2 Reference substances

See Table 1. Solutions of reference substances are commercially available.

6.3 Internal standard substances

Solutions of reference substances for use as internal standards for electron impact ionisation (Table 2, substances 1 to 8) and for negative ion chemical ionisation (Table 2, substances 9, 10 and 11) are commercially available. For electron impact ionisation, use at least one mass labelled PBDE congener for each degree of bromination.

Table 2 — Examples of internal standards

No	Name	Formula	Abbreviation	Molar mass (g/mol)
	Internal standards for GC-MS with electron impact ionisation			
1	2,2',4,4' –Tetrabromo[$^{13}C_{12}$]diphenylether	$^{13}C_{12}H_6Br_4O$	^{13}C-BDE-47	497,7035
2	2,2',4,4',5 – Pentabromo[$^{13}C_{12}$]diphenylether	$^{13}C_{12}H_5Br_5O$	^{13}C-BDE-99	576,5995
3	2,2',4,4',6 – Pentabromo[$^{13}C_{12}$]diphenylether	$^{13}C_{12}H_5Br_5O$	^{13}C-BDE-100	576,5995
4	2,2',4,4',5,6' – Hexabromo[$^{13}C_{12}$]diphenylether	$^{13}C_{12}H_4Br_6O$	^{13}C-BDE-154	655,4955
5	2,2',4,4',5,5' – Hexabromo[$^{13}C_{12}$]diphenylether	$^{13}C_{12}H_4Br_6O$	^{13}C-BDE-153	655,4955
6	2,2',3,4,4',5',6 –Heptabromo[$^{13}C_{12}$]diphenylether	$^{13}C_{12}H_3Br_7O$	^{13}C-BDE-183	734,3916
7	2,2',3,3',4,4',6,6' –Octabromo[$^{13}C_{12}$]diphenylether	$^{13}C_{12}H_2Br_8O$	^{13}C-BDE-197	813.2878
8	Decabromo[$^{13}C_{12}$]diphenylether	$^{13}C_{12}Br_{10}O$	^{13}C-BDE-209	971,0797
	Internal standards for GC-MS with negative ion chemical ionisation			
9	3,3',4,4' –Tetrabromodiphenylether	$C_{12}H_6Br_4O$	BDE-77	485,7950

10	2,2',3,4,4',5,6 –Heptabromodiphenylether	$C_{12}H_3Br_7O$	BDE-181	722,4832
11	Decabromo[$^{13}C_{12}$]diphenylether	$^{13}C_{12}Br_{10}O$	^{13}C-BDE-209	971,0797

NOTE: Internal standards for GC-MS with negative ion chemical ionisation: Check for interferences when non-labelled PBDE is used as an internal standard. Other BDE congeners are suitable as internal standards, e.g. BDE-140. Internal standards for GC-MS with electron impact ionisation: other compounds, such as fluorinated polybrominated diphenyl ethers, are also commercially available and are also suitable as internal standards [1].

6.4 Calibration solutions for multicomponent-multilevel calibration

Prepare calibration solutions with concentrations according to the detection capacity of the mass spectrometer. Combine the multicomponent stock solutions of reference substances and internal standards to produce the solutions e.g. shown in Table 6 by appropriate dilution with the appropriate solvent e.g. toluene, or nonane, or iso-octane.

In order to avoid potential photodegradation, store the solutions in the dark. Check the concentrations of calibration solutions before use.

Use one of the calibration solutions to optimise the GC-MS system and to determine the retention times.

7 Apparatus

The apparatus and materials listed below are meant as minimum requirements for "conventional" sample treatment with soxhlet extraction and column chromatographic cleanup. Additional apparatus and materials may be necessary due to different methods of sample extraction and cleanup methods.

In order to avoid photodegradation under influence of direct sunlight, use amber glassware (or cover normal glassware with e.g. aluminium foil).

Clean all glassware by heating at 420 °C for several hours and rinsing with toluene prior use. Volumetric apparatus will require recalibration prior to use if heated.

7.1 Equipment for sample preparation

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

Freeze drying apparatus

Deep freezer

Mortar and pestle, or a grinding mill

Drying ovens, capable of maintaining temperatures in the ranges of 100 °C to 450 °C for baking and storage of cleanup materials, for baking of glassware and for dry residue determination of samples.

Sieve shaker with appropriate sieve meshes (aperture size), e.g. 2 mm.

Desiccator

Balances, consisting of an analytical type capable of weighing 0.1 mg and a top-loading type capable of weighing 10 mg.

7.2 Extraction apparatus

Soxhlet extractor consisting of: round bottom flasks e.g. 250 ml, Soxhlet extractors and Soxhlet thimbles e.g. 27 mm x 100 mm, vertical condensers e.g. 300 mm, heating apparatus.

7.3 Cleanup apparatus

Disposable pipettes, either disposable Pasteur pipettes, or disposable serological pipettes.

Glass columns for chromatographic cleanup

7.4 Concentration apparatus

Rotary evaporator, equipped with a variable temperature water bath and:

a) vacuum source for rotary evaporator equipped with shutoff valve at the evaporator and vacuum gauge;

b) recirculating water pump and chiller (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);

c) round-bottom flak, 100 mL and 500 mL or larger, with ground-glass fitting compatible with the rotary evaporator

Other evaporation devices such as turboevaporator, can also be used.

Nitrogen blowdown apparatus, equipped with bath controlled in the range of 30ºC to 60ºC, installed in a fume hood.

Amber glass with fluoropolymer-lined screw-cap.

7.5 Other equipment

Gas chromatograph, with either a splitless injection port or an on-column injection port coupled to a mass spectrometer (GC-MS) with electron impact or chemical ionisation and appropriate reactant gas (e.g. CH_4).

Fused silica column with non-polar low bleed separating phase; e.g. inner diameter < 0,25 mm, length 15 m, film thickness of 0,1 µm.

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

8 Sampling and sample pre-treatment

8.1 Sampling

Sampling should be carried out in accordance with EN yyyy: (Horizontal standard module(s) for sampling of sludge, soil and waste).

In addition to this standard the following requirements apply. Samples should be stored in suitable containers with an appropriate closure material such as PTFE. Samples to be frozen may be stored in aluminium containers pre-cleaned by heating to 450ºC for minimum 4 hours or by rinsing with a non-chlorinated solvent.

Store and transport in the dark at approximately 4 °C. The sample pre-treatment should take place within 24 hours of sampling. Alternatively, samples may be frozen (-18ºC) directly after sampling and kept frozen for a maximum of one month before sample pre-treatment.

8.2 Sample pre-treatment

Drying and homogenization should be carried out according to EN wwww: (Horizontal standard module(s) for pre-treatment of solid materials).

Store the ground material in a desiccator or a tightly closed glass container.

9 Extraction and Cleanup

There are a variety of possible techniques for extraction and cleanup. Different combinations of those techniques result in a number of procedures, which can be applied to the analysis of PBDEs. Usually, many

other compounds interfering with the determination of PBDES are co-extracted with the analytes Thus, extensive cleanup procedures have to be applied prior the extract can be subjected to GC-MS analysis.

9.1 Extraction

Sample amount used for extraction may vary (up to 10 g) depending on the expected level of contamination.

Internal standards listed in Table 2 shall be added before extraction.

The extraction procedure is carried out using Soxhlet extraction. Various solvents produce similar extraction efficiencies after a 16 h Soxhlet extraction. However, extraction of deca-BDE-209 requires specific attention and, sometimes, longer extraction times than other PBDE congeners. The use of toluene as an extraction solvent for extraction of BDE-209 is recommended.

Soxhlet extraction is described as reference procedure, but other techniques, e.g. accelerated solvent extraction or pressurized liquid extraction, offering shorter extraction times may also be used after testing their comparability to Soxhlet extraction.

After the extraction is complete, concentrate the extract nearly to dryness using a suitable evaporation device.

9.2 Cleanup

Depending on the different sample matrices encountered, a variety of sample extract cleanup procedures may be suitable. Examples of cleanup procedures are given in Annex A.

In general, methods are based on cleanup by adsorption column chromatography. Depending on the matrix in which the PBDEs are analysed additional cleanup steps to remove matrix constituents such as lipids, sulphur etc. including gel permeation chromatography (GPC), sulphuric acid and copper treatment may be necessary.

9.3 Final concentration of cleaned sample extract

To achieve sufficient detection limits, the cleaned sample extract shall be concentrated to a volume in the order of 25 µL to 500 µL before quantification.

Depending on the method to be used for solvent volume reduction the following precautions have to be taken into consideration:

a) Rotary evaporators: Losses might be substantial when reducing solvent volumes below 10 mL..

b) Counter gas flow evaporators (e.g. TurboVap®): Volumes should not be reduced to less than 1 mL.

c) Nitrogen flow: An excessive flow of nitrogen which disturbs the solvent surface should be avoided. The vial shape has also some influence on possible losses. V-shaped vials or vial inserts shall be used for volume reductions below around 200 µL.

It should be avoided that the extracts would be evaporated until dryness, because deca-BDE-209 may not completely re-dissolve after that step. Thus, during concentration, use toluene as a keeper.

9.4 Blank determination

Analyse as a blank, a clean Soxhlet thimble in exactly the same way as the sample, but replacing the sample by the appropriate amount of baked sand. The concentration of PBDE in the blank should be negligible, compared with the concentrations of PBDEs to be determined.

10 GC-MS analysis

The configuration of the GC system and its operational conditions may have a significant influence on the determination of PBDEs, in particular, on the response and peak shape of deca-BDE-209. The injection technique, type of retention gap, press-fit connector, column brand, stationary phase and column length significantly affect the yield of PBDEs from the chromatographic system, as well as the precision of the determination [2]. Especially for deca-BDE-209 the response can decrease to zero by selecting non-optimal GC-MS conditions.

10.1 Gas chromatographic analysis

Gas chromatographic separation has to be carried out in such a way, that sufficient separation of all PBDE congeners is achieved.

Different gas chromatographic stationary phases could be applied for BDE determinations (Table 3), but especial attention must be paid on the potential co-elutions with other PBDE congeners, as well as with other brominated flame retardants, such as hexabromocyclododecane (HBCD), dimethyl tetrabromobisphenol A (MeTBBPA) and polybrominated biphenyls (PBBs), which are also present in many environmental samples [3].

Especially for the analysis of deca-BDE-209, minimise the exposure of the samples to high temperatures for long periods of times during the injection and separation stages, because of the thermal degradation of BDE-209 at temperatures higher than 300 °C. Optimise the chromatographic separation step, paying special attention to the peak height of BDE-209. It is recommended to use pressure pulse injection or a short splitless time. On-column injection may be also a suitable alternative, however this injection technique is sensitive to contamination.

Table 3 – Co-elution of selected BDE congeners on seven different GC columns [4]

Compound	DB-1 (30mx0.25mmx0.25µm)	DB-5 (30mx0.25mmx0.25µm)	HT-5 (30mx0.25mmx0.10µm)	DB-17 (30mx0.25mmx0.25µm)	DB-XLB (30mx0.25mmx0.25µm)	HT-8 (25mx0.22mmx0.25µm)	CP-Sil 19 (17mx0.15mmx0.30µm)
BDE-47	-	-	-	-	-	-	-
BDE-99	-	-	BDE-116	BDE-127	-	-	-
BDE-100	-	-	BDE-109	BDE-101	-	BDE-109, BDE-120	-
BDE-153	-	-	HBCD	BDE-168	-	-	-
BDE-154	MeTBBPA, BB-153	MeTBBPA, BB-153	-	BDE-105	-	BDE-126	BB-153
BDE-183	BB-169	BB-169	-	-	-	-	-

NOTE: DB-1 (100% methylpolysiloxane), DB-5 (5% phenyl-methylpolysiloxane), DB-17 (50% phenyl-methylpolysiloxane) and DB-XLB (Proprietary) from J&W Scientific, Folsom, CA, USA. HT-5 (5% phenyl-methylpolysiloxane (carborane)) and HT-8 (8% phenyl-methylpolysiloxane (carborane)) from SGE International, Ringwood, Australia. CP-Sil 19 (14% cyanopropyl-methylpolysiloxane) from Chrompack, Middelburg, The Netherlands.

The following conditions can be used as a starting point for optimizing a method:

a) From tetra- to hepta-BDEs

Chromatographic column: HP-5MS (30 m × 0.25 mm i.d., 0.25 µm film thickness) containing 5% phenyl methyl siloxane.

Oven temperature program: from 110ºC (hold for 1 min) to 180ºC (hold for 1 min.) at 8ºC/min., then from 180ºC to 240ºC (hold for 5 min) at 2ºC/min., and then from 240ºC to 265ºC (hold for 6 min) at 2ºC/min.

Injector: splitless injection mode during 1 min. Injector temperature = 275ºC.

Carrier gas: Helium at 6 psi.

MS-Interface temperature: 250ºC

b) From octa- to deca-BDEs

Chromatographic column: DB-5MS (15 m × 0.25 mm i.d., 0.10 µm film thickness) containing 5% phenyl methyl siloxane.

Oven temperature program: from 140ºC (hold for 2 min.) to 325ºC (hold for 10 min.) at 10ºC/min.

Injector: splitless injection mode during 1 min. Injector temperature = 275ºC.

Carrier gas: Helium at 6 psi.

MS-Interface temperature: 270ºC

10.2 Mass spectrometric detection

The mass spectrometer is used in the MID-Mode (Multiple Ion Detection). The ion source temperature should be between 200ºC and 250ºC depending on type of instrument.

For identification and quantification the masses given in the following tables 4 and 5 have to be recorded in MID mode.

Table 4 — Ions for negative ion chemical ionisation detection

Compound	Ions for quantification	Ions for qualification
BDE-47	79	81
BDE-99	79	81
BDE-100	79	81
BDE-154	79	81
BDE-153	79	81
BDE-183	79	81
BDE-196	79	81
BDE-197	486.7	484.7
BDE-203	79	81
BDE-209	486,7[a]	484,7
Internal standards		
BDE-77	79	81
BDE-181	79	81
^{13}C-BDE-209	494,7	496,7

[a] Do not use the ion 488,7 because of overlapping with a fragment ion from ^{13}C-BDE-209.

Table 5 — Ions for electron impact ionisation detection

Compound	Ion	Monitored Ions (*m/z*)
BDE-47	$[M+2]^+$; $[M+4]^+$	483,7; 485,7
BDE-99	$[M+4]^+$; $[M+6]^+$	563,6; 565,6
BDE-100	$[M+4]^+$; $[M+6]^+$	563,6; 565,6
BDE-153	$[M-2Br+2]^+$; $[M-2Br+4]^+$	481.7; 483.7
BDE-154	$[M-2Br+2]^+$; $[M-2Br+4]^+$	481.7; 483.7
BDE-183	$[M-2Br+4]^+$; $[M-2Br+6]^+$	561.6; 563,6
BDE-196	$[M-2Br+4]^+$; $[M-2Br+6]^+$	639.5; 641.5
BDE-197	$[M-2Br+4]^+$; $[M-2Br+6]^+$	639.5; 641.5
BDE-203	$[M-2Br+4]^+$; $[M-2Br+6]^+$	639.5; 641.5
BDE-209	$[M-2Br+6]^+$; $[M-2Br+8]^+$	797,4; 799,4
Internal standards		
^{13}C-BDE-47	$[M+2]^+$; $[M+4]^+$	495,7; 497,7
^{13}C-BDE-99	$[M+4]^+$; $[M+6]^+$	575,6; 577,6
^{13}C-BDE-100	$[M+4]^+$; $[M+6]^+$	575,6; 577,6
^{13}C-BDE-153	$[M-2Br+4]^+$; $[M-2Br+6]^+$	495.7; 497,7
^{13}C-BDE-154	$[M-2Br+4]^+$; $[M-2Br+6]^+$	495.7; 497,7
^{13}C-BDE-183	$[M-2Br+4]^+$; $[M-2Br+6]^+$	573,6; 575.6
^{13}C-BDE-197	$[M-2Br+4]^+$; $[M-2Br+6]^+$	651.5; 653.5
^{13}C-BDE-209	$[M-2Br+6]^+$; $[M-2Br+8]^+$	809,4; 811,4

Check that the ions used are free from interferences caused by matrix components.

10.3 Minimum requirements for identification of PBDEs

Consider an analyte to be identified, if

— the retention time of the analyte in the mass chromatogram of the sample is the same as the retention time of the reference substance in the mass chromatogram of the calibration standard solution measured under identical experimental conditions (the deviation shall be below 1%, and not exceeding 12 s).

— the ratio of the two monitored mass is within ± 10 % of the theoretical bromine isotope ratio.

— the signal-to-noise ratio of the raw data shall be at least 3:1 for the signal used for identification. The base line noise shall be measured in front of the signal of the native congener within a signal-free window corresponding to 10 times the signal width at half height.

11 Calibration of the GC-MS

11.1 General

Modern mass spectrometric detection provides linear correlations between the concentrations of single substances and the corresponding responses over several decades of concentration. This facilitates an effective means of calibration.

11.2 Evaluation of the range of the linear relationship

The calibration must be carried out with at least five calibration solutions. These solutions contain all native PBDE congeners of interest in different precisely defined amounts and all internal standards in the same concentrations as expected in the spiked sample solutions.

See Table 6 as an example of concentrations for evaluating a linear range over several decades of concentration. The linear relationship should be assured with the concentration and response relationships. Plot, for example, the ratio values $\frac{y_i}{y_{is,i}}$ (peak areas, peaks heights or integration units) for each substance i on the ordinate and the associated ratio of mass concentrations $\frac{r_i}{r_{is,i}}$ on the abscissa.

Establish the linear function of values $\frac{y_i}{y_{is,i}}$ and $\frac{r_i}{r_{is,i}}$ of the measured series using the following Equation (1):

$$\frac{y_i}{y_{is,i}} = a_i \frac{r_i}{r_{is,i}} + b_i \qquad (1)$$

where

y_i is the measured response of substance i; the unit depends on the evaluation; e.g. area value;

r_i is the mass concentration of substance i, in the working standard solution, in nanograms per millilitre, ng/ml;

a_i is the slope of the calibration function of substance i, the unit depends on the evaluation, e.g. area value x millilitres per nanograms, ml/ng.

b_i is the ordinate intercept of the calibration curve. The unit depends on the evaluation, e.g. area value;

$y_{is,i}$ is the measured response of the internal standard for the substance i, the unit depends on the evaluation, for example, area value;

$r_{is,i}$ is the mass concentration of the internal standard, for the substance i, in nanograms per millilitre, ng/ml.

Calibration frequency depends on the stability of the instrument. Daily calibration checks shall be run. In addition a full calibration shall be repeated after major changes such as:

- use of new or repaired equipment
- replacement of GC columns
- after cleaning of the separation and detection systems
- if the deviation of an injected calibration standard exceeds 20%

The obtained calibration curves are used to calculate the concentration of each congener of interest. The concentrations of all congeners of interest in the samples shall be within the linear range of the method.

11.3 Calibration with internal standards

The use of an internal standard for the determination of the concentration minimises both possible errors made during injection and by sample losses during sample pre-treatment steps, furthermore differences in the final sample extract volumes and changes in recoveries caused by matrix effects.

See Table 6 for typical concentrations of reference compounds and internal standards in solutions for evaluating the linear range and for a listing of which internal standard to use for each PBDE compound. Adjust the concentrations according to the sensitivity of the equipment used and the range of determinations required.

Table 6 — Example concentrations in solutions for evaluating the linear range

Compound	Solution 1	Solution 2	Solution 3	Solution 4	Solution 5	Solution 6	Solution 7
	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL
BDE-47	5	12,5	25	50	100	150	250
BDE-99	5	12,5	25	50	100	150	250
BDE-100	5	12,5	25	50	100	150	250
BDE-154	5	12,5	25	50	100	150	250
BDE-153	5	12,5	25	50	100	150	250
BDE-183	5	12,5	25	50	100	150	250
BDE-196	25	50	100	200	500	700	1000
BDE-197	25	50	100	200	500	700	1000
BDE-203	25	50	100	200	500	700	1000
BDE-209	25	50	100	200	500	700	1000
Internal standards for NCI							
BDE-77 (Internal standard for BDE-47, BDE-99 and BDE-100)	100	100	100	100	100	100	100
BDE-181 (Internal standard for BDE-153, BDE-154 and BDE-183)	100	100	100	100	100	100	100
^{13}C-BDE-209 (Internal standard for BDE-196, BDE-197, BDE-203 and BDE-209)	500	500	500	500	500	500	500
Internal standards for EI							
^{13}C-BDE-47 (Internal standard for BDE-47)	200	200	200	200	200	200	200
^{13}C-BDE-99 (Internal standard for BDE-99 and BDE-100)	200	200	200	200	200	200	200
^{13}C-BDE-153 (Internal standard for BDE-154 and BDE-153)	200	200	200	200	200	200	200
^{13}C-BDE-183 (Internal standard for BDE-183)	400	400	400	400	400	400	400

^{13}C-BDE-197 (Internal standard for BDE-196, BDE-197 and BDE-203)	500	500	500	500	500	500	500
^{13}C-BDE-209 (Internal standard for BDE-209)	500	500	500	500	500	500	500

11.4 Quantification with the internal standard

Add a known amount of the internal standard to the sample prior to extraction. The mass concentration $r_{is,i}$ in the final volume of extract shall be the same for calibration and sample measurement. Use the same solvent composition for the working standard solutions and the extracts.

Pre-treat and analyse the samples as described in 9.1 to 9.3. Inject identical volumes of the sample extracts as injected as calibration solutions.

Calculate the mass concentration $r_{i,sample}$ of the substance using Equation (2).

$$r_{i,\text{sample}} = \frac{\frac{y_{i,\text{sample}}}{y_{is,i,\text{sample}}} - b_i}{a_i} \cdot \frac{m_{is,i}}{m_{\text{sample}}} = \frac{r_{i,\text{sample extract}}}{r_{is,i,\text{sample extract}}} \cdot \frac{m_{is,i}}{m_{\text{sample}}} \quad (2)$$

where

$y_{i,\text{sample}}$ is the measured response, e.g. peak area, of the substance i in the sample extract;

$y_{is,i,\text{sample}}$ is the measured response, e.g. peak area, of the internal standard, for substance i, of the sample;

$r_{i,\text{sample extract}}$ is the mass concentration of the substance i in the sample extract, in nanograms per millilitre, ng/ml;

$r_{is,i,\text{sample extract}}$ is the mass concentration of the internal standard in the sample extract, for substance i, in nanograms per millilitre, ng/ml;

$r_{i,\text{sample}}$ is the mass concentration of the substance i in the solid sample in micrograms per kilogram, µg/kg.

$m_{is,i\ \text{sample}}$ is the mass of the added internal standard substance, in micrograms, µg;

m_{sample} the sample mass in kilogram, in kg;

a_i see Equation (1);

b_i see Equation (1).

11.5 Results and reporting

Report results to two significant figures for the PBDE congeners.

12 Test report

The test report shall contain the following information:

a) a reference to this European Standard including its date of publication;

b) sampling report including precise identification of the sample;

c) sample storage and pre-treatment report;

d) short description of the method used for extraction and sample cleanup;

e) analytical results containing the levels of the individual PBDE congeners;

f) any deviation from this standard, and any facts which may have influenced the result.

Annex A
(informative)

Examples of operation of extraction and cleanup methods

A.1 Example A

A.1.1 General

This method is applicable for the determination of selected PBDE congeners in dry soil samples.

Sample volumes used for analysis have to be adapted in such a way that the expected amount of analyte lies between detection limit and upper end of calibration range. Samples exceeding the upper limit of the calibration range have to be repeated with smaller amounts of sample.

A.1.1.1 Chemicals

Alumina (neutral)

Cooper

Dichloromethane

Hexane

Hydromatrix

A.1.2 Procedure

A.1.2.1 Spiking of the sample

Weigh an exact amount of 1 g of the freeze-dried and grounded soil sample. Spike with internal standards. Spiked samples are kept over night to equilibrate.

A.1.2.2 Simultaneous extraction and cleanup

Pressurized liquid extraction system is used. Neutral alumina is selected as sorbent in the extraction cell. A 22 mL extraction cell is loaded by inserting two cellulose filters into the cell outlet, followed by 6 g of neutral alumina previously activated (activation at 150ºC overnight). Spiked soil samples were ground with neutral alumina (2 g) and cooper (2 g). The mixture is loaded into the extraction cell on top of neutral alumina. The dead volume is filled with Hydromatrix, and the cell is sealed with the top cell cap. The extraction cell is filled with hexane:dichloromethane (1:1) mixture until the pressure reach 1500 psi, and heated to 100ºC. After an oven heat-up time of 5 min. under these conditions, two static extractions of 10 min. at constant pressure and temperature are developed. After this static period, fresh solvent is introduced to flush the lines and cell, and the extract is collected in the vial. The flush volume amounted to 100% of the extraction cell. The extraction is cycled twice. The volume of the resulting extract is about 35 mL.

A.1.2.3 Preparation of sample solution for measurement

The concentrated extract is quantitatively transferred to a vial by applying a gentle N_2-stream. Adjust the endvolume to 50-250 µL. The vial should be labelled with sample ID and type of analyte. The sample can be stored in the dark at room temperature until measurement. For longer period of time the sample has to be stored in a refrigerator at app 4ºC.

A.2 Example B

A.2.1 General

This method is applicable for the determination of selected PBDE congeners in dry soil samples.

Sample volumes used for analysis have to be adapted in such a way that the expected amount of analyte lies between detection limit and upper end of calibration range. Samples exceeding the upper limit of the calibration range have to be repeated with smaller amounts of sample.

A.2.1.1 Chemicals

Alumina (neutral)

Cooper

Dichloromethane

Hexane

A.2.2 Procedure

A.2.2.1 Spiking of the sample

Weigh an exact amount of 1 g of the freeze-dried and grounded soil sample. Spike with internal standards. Spiked samples are kept over night to equilibrate.

A.2.2.2 Extraction

Soxhlet extraction is accomplished in cellulose thimbles containing 1 g of soil. 2 g of copper are added to soil to remove sulfur interference. Extraction is done using 100 mL of a mixture of hexane:dichloromethane (1:1) for 24 h. After extraction, the extracts and the rinses of the Soxhlet are combined, concentrated to a few mL by rotary evaporation and then subjected to the cleanup procedure.

A.2.2.3 Cleanup

Five grams alumina SPE cartridges are used. SPE cartridges are conditioned with 20 mL hexane. The sample volume loaded is ~ 1 mL, and the elution step is performed with 30 mL hexane:dichloromethane (1:2).

A.2.2.4 Preparation of sample solution for measurement

The concentrated extract is quantitatively transferred to a vial by applying a gentle N_2-stream. Adjust the endvolume to 50-250 µL. The vial should be labelled with sample ID and type of analyte. The sample can be stored in the dark at room temperature until measurement. For longer period of time the sample has to be stored in a refrigerator at app 4ºC.

A.3 Example C

A.3.1 General

This method is applicable for the determination of selected PBDE congeners in dry sludge samples.

Sample volumes used for analysis have to be adapted in such a way that the expected amount of analyte lies between detection limit and upper end of calibration range. Samples exceeding the upper limit of the calibration range have to be repeated with smaller amounts of sample.

A.3.1.1 Chemicals

Alumina (neutral)

Concentrated sulphuric acid

Cooper

Dichloromethane

Hexane

Silica

A.3.2 Procedure

A.3.2.1 Spiking of the sample

Weigh an exact amount of 1 g of the freeze-dried and grounded sludge sample. Spike with internal standards. Spiked samples are kept over night to equilibrate.

A.3.2.2 Extraction

Soxhlet extraction i accomplished in cellulose thimbles containing 1 g of soil. 2 g of copper were added to soil to remove sulfur interference. Extraction is done using 100 mL of a mixture of hexane:dichloromethane (1:1) for 24 h. After extraction, the extracts and the rinses of the Soxhlet are combined, concentrated to a few mL by rotary evaporation and then subjected to the cleanup procedure.

A.3.2.3 Cleanup

The volumes Soxhlet extracts are reduced to 10 ml and treated with concentrated sulfuric acid (2 × 10 mL) in centrifugations tubes. The organic layers are combined and further cleaned up on a column containing activated silica impregnated with concentrated sulfuric acid (1 g, 2:1, w/w) by elution with 15 ml of dichloromethane:hexane (1:1). Subsequently the extracts are purified on five grams of alumina SPE cartridges. SPE cartridges are conditioned with 20 mL hexane. The sample volume loaded is ~ 1 mL, and the elution step is performed with 30 mL hexane:dichloromethane (1:2).

A.3.2.4 Preparation of sample solution for measurement

The concentrated extract is quantitatively transferred to a vial by applying a gentle N_2-stream. Adjust the endvolume to 50-250 µL. The vial should be labelled with sample ID and type of analyte. The sample can be stored in the dark at room temperature until measurement. For longer period of time the sample has to be stored in a refrigerator at app 4ºC.

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