# Guidance Document on the Determination of Organobromine Contaminants

## ANNEX A

Version 1.0

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## Example of Methodology for the Determination of Polybrominated Diphenylethers (PBDEs) in Food and Feed

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**Disclaimer**

This annex refers to a number of products (analytical standards and equipment) as examples of items that may be used in the described procedures. Alternative sources of these or similar products may be available, and the annex does not endorse or recommend any particular product for use in the described procedures.

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# 1. Description and Scope

The methodology described in this annex is an example of the procedures that may be used for the determination of polybrominated diphenylethers (PBDEs) in food and animal feed samples. It is based on the use of internal standardisation using $^{13}C$-labelled surrogates that are commercially available and analyte separation using gas chromatography (GC) followed by measurement using high resolution mass spectrometry (GC-HRMS) or tandem mass spectrometry (GC-MS/MS). The targeted analytes are PBDE congeners which may be reported as individual and summed concentrations as described in the main guidance document. The limit of quantification (LOQ) that will be achieved by using this methodology will depend on the successful purification of the examined matrices and the sensitivity of the measurement technique used. Under routine conditions it is expected to be below 0.01 µg/kg for each congener with a targeted LOQ of 0.001 µg/kg, except for BDE-209 (0.01 µg/kg).

In order to allow flexibility of use and incorporation of individual laboratory practices, the methodology for sample extraction, purification and measurement by different GC-MS techniques is presented in modular form. Laboratories may choose modules based on available equipment. The description of analytical standards, quantitation and reporting format, described in other sections of this annex is however, common, notwithstanding which modules are chosen.

**Module 1:** **Description of extraction procedures** (page 7)

- Module 1A: Cold Extraction
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**Module 2:** **Description of purification procedures** (page 12)

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- Module 4A: Measurement by GC-HRMS
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Assuming competence in trace analysis and GC-MS, the first steps for a laboratory wishing to set up PBDE determination would be procurement of the required standards and the use of these to establish the GC-MS conditions required to measure PBDEs. Modules may then be chosen to complement the equipment available in individual laboratories. The modules are given as examples of working methodology that will allow users to meet the method performance parameters given in the main guidance document. As these are performance based, the procedures described here may be adapted to suit the prevailing circumstances/ equipment in individual laboratories.

## 2. Analytical Standards that are used for PBDE determination

The analytical standards that would be required for determination of the PBDEs listed in the guidance document are commercially available from current suppliers of persistent organic pollutant (POP) standards, e.g. LGC Standards, Wellington Laboratories, Cambridge Isotope Laboratories, Inc. Restek Corporation, etc. An example of a full set of standards that could be used for routine determination is listed in **Table 1** below. Note that some standard mixtures, e.g. native PBDEs, may be available as a solution that contains additional congeners that are not listed in the guidance document. These congeners may be helpful as indicators of wider PBDE contamination in unknown samples. Mixed standard solutions may be prepared in nonane, heptane or another suitable solvent in a concentration range of 1 pg/µl – 500 pg/µl or as commercially available.

**Table 1:** Example of a set of analytical standards (commercially available native, isotopically labelled internal (IS) and recovery standards (RS)) for the determination of PBDEs

| Standard type | PBDE standard description |
|---|---|
| **Native standard** | 2,4,4′-tribromodiphenyl ether (BDE-28) |
| | 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) |
| | 2,2′,4,5′-tetrabromodiphenyl ether (BDE-49) |
| | 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99) |
| | 2,2′,4,4′,6-pentabromodiphenyl ether (BDE-100) |
| | 2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE-153) |
| | 2,2′,4,4′,5,6′-hexabromodiphenyl ether (BDE-154) |
| | 2,2′,3,4,4′,5′,6-heptabromodiphenyl ether (BDE-183) |
| | 2,2′,3,3′,4,4′,5,5′,6,6′-decabromodiphenyl ether (BDE-209) |
| **$^{13}$C labelled Internal Standard** | 2,4,4′-tribromodiphenyl ether (BDE-28) |
| | 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) |
| | 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99) |
| | 2,2′,4,4′,6-pentabromodiphenyl ether (BDE-100) |
| | 2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE-153) |
| | 2,2′,4,4′,5,6′-hexabromodiphenyl ether (BDE-154) |
| | 2,2′,3,4,4′,5′,6-heptabromodiphenyl ether (BDE-183) |
| | 2,2′,3,3′,4,4′,5,5′,6,6′-decabromodiphenyl ether (BDE-209) |
| **$^{13}$C labelled Recovery Standard[1]** | 3,3',4,4'-tetrabromodiphenylether (BDE-77) for tri-hepta-BDE |
| | 3,3',4,5'-tetrabromodiphenylether (BDE-79) for tri-hepta-BDE |
| | 2,2',3,4,4',6-hexabromodiphenyl ether (BDE-139) for tri-hepta-BDE |
| | 2,2',3,4,4',5,5'-heptabromodiphenyl ether (BDE-180) for hepta-deca-BDE |
| | 2,3,3',4,4',5,6-heptabromodiphenyl ether (BDE-190) for hepta-deca-BDE |
| | 2,3,3',4,4',5,5',6-octabromodiphenyl ether (BDE-205) for hepta-deca-BDE |
| | 2,2',3,3',4,4',5,5',6-nonabromodiphenyl ether (BDE-206) for deca-BDE |

[1] It is recommended that any two of the listed recovery standards are used – one each corresponding to early and late eluting congeners

## 3. General Advice on Standards and Reagents

Recommendations for reliable analysis with respect to analytical standards, reagents and other conditions used during sample extraction, purification and analysis are given below.

- Individual congener standards or mixtures should be checked for impurities before use, with respect to other PBDE congeners and also other halogenated POPs when applying multi-POPs-methods; the resulting chromatograms should be clear of interferences, otherwise the standards may need to be purified before use.
- Reagents of recognized analytical grade and purity (both in terms of PBDEs and other contamination) should be used.
- Purity of the reagents and reference materials (e.g. standard solutions) should be verified and all possible contamination sources should be checked (all equipment used e.g. freeze dryer etc.) by performing a procedural blank test under the same conditions as used in the method; the resulting chromatograms should be clear of interferences at the retention time of compounds of interest, otherwise the source of contamination needs to be identified and further steps to contain the blank problem should be taken.
- Check on the stability of standards. E.g. it may be practical to prepare separate standards of BDE-209, for both native and internal standard working solutions and calibration solutions rather than mixing it with the other PBDE congener standards due to adsorption and/or degradation in the standard solution, in the injector and on column.
- The volume of standard solutions should be checked by weighing prior to use and afterwards.
- Addition of internal standards (IS): either at the very beginning (before sample pre-treatment and extraction) or to the extracted fat (if fat content is above 10%, as is done for PCDD/F and PCB analysis) to compensate for analyte losses during the whole sample preparation procedure and matrix effects/absorption effects/variability of injection volume during measurement.
- Addition of the recovery standards (RS) should be the final step before GC-MS measurement.

## 4. Preparation of sample material

Use a representative sample for analysis. For advice on representative sampling, guidelines such as those described in COMMISSION REGULATION (EU) 2017/644 and COMMISSION REGULATION (EC) No 152/2009 for PCDD/Fs and PCBs, EN ISO 6497 and EN ISO 6498 may be helpful [1, 2, 3, 4].

Representative samples of foods with a high moisture content and animal feed materials such as grasses and silages and liquid feed may be initially blended, homogenised and then freeze-dried (air-drying may be adequate for feed). After drying, the lyophilised or air dried material may be re-homogenised to yield a dry and representative sample.

Some laboratories may prefer to use a drying agent such as anhydrous $Na_2SO_4$ or polyacrylate when using small aliquots for analysis.

Representative samples of dry or low moisture products such as, bread, nuts, cereals and cereal products, mixed feeds, and hay should be ground carefully so that the material can pass through a 1 mm mesh sieve. Post-grinding, the material should be homogenised to yield a representative ground sample.

High lipid content foods/feed such as butter, fish oils and vegetable oils, animal fats etc. may be homogenised by blending when warm to ensure a representative sample and used without further treatment.

For feed samples the determination of the moisture content (optional) may be useful if the calculation of analyte results based on 12% moisture content is required.

# 5. Independent Modules on sample extraction, purification and measurement

## 5.1 General aspects

The equipment used for sample preparation should not allow degradation of the extracted PBDEs (e.g. through photo-degradation). Protection from direct sunlight or other sources of ultraviolet-radiation is recommended using amber glassware/aluminium foil to cover glassware. The equipment (e.g. glassware, rotary evaporators etc.) should also be free from any PBDE contamination and therefore should be carefully rinsed with e.g. dichloromethane, heptane, cyclohexane or acetone or mixtures of these and heated at minimum 400°C for 4 to 8 hours before use.

Depending on the equipment available, either manual or automated procedures may be used. Each module (1, 2, 3 and 4) describes a part of the whole PBDE determination method. For sample preparation, each extraction method described in Module 1 can be combined with any clean-up method described in Module 2. Module 3 (section 5.4) describes a combined manual extraction and purification method. Each combination out of Module 1 and 2 and Module 3 independently can be combined with any determination method describe in Module 4. All modular procedures described here are given as examples and could be adapted or substituted with alternative methods to match the equipment and expertise available in the laboratory.

## 5.2 Module 1 – Extraction

The procedures described in Module 1 are extraction methods for the isolation of PBDEs from the sample of food or animal feed. The extraction efficiency is affected by the properties of the chosen extraction solvent, the particle size of the raw material, the solvent-to-solid ratio as well as the extraction temperature and the extraction duration/repetitions. The extraction procedures described are divided into cold, hot and hot pressurized extraction and can each be applied to effectively extract PBDEs from any kind of food or feed sample matrix. Before extraction the preparation recommendations for different types of samples should be followed as describe in chapter 4.

Note: For those laboratories that are also required to determine PCDD/Fs and PCBs, all extraction procedure described in Module 1 may be used for multi-POP-analysis. See also standard EN 16215:2020 [5]. (Section 11.2 automated sample clean-up 11.3 manual clean-up)

For all procedures, representative sample aliquots are internally standardised and extracted using mixed organic solvents or pressurised fluids, either at ambient temperature or higher temperatures depending on the equipment used.

### 5.2.1 Module 1A – Cold Extraction

#### Principle

Cold solvent extraction is an easy and relatively inexpensive procedure to isolate the analytes of interest from the raw material. The optimum solvent for this purpose penetrates into the solid or wet matrix, the analytes dissolves in the solvent and diffuse out of the matrix. The solvent with lower density separates at the top of the extraction mixture. The supernatant, containing the analytes of interest, can be collected. Multiple different mechanical techniques e.g. shaking, stirring, open column extraction after mixing with drying agent or Ultra Turrax® extraction of the fresh sample (according to [6]) are known and may be used if they allow the complete extraction of PBDE congeners.

#### Extraction procedure

For high-performance dispersion extraction (using e.g. Ultra Turrax®), isopropanol and cyclohexane are added to the homogenized sample. Immiscible liquid/liquid mixtures or raw powders can be homogenized, dispersed and extracted by high speed stirring. The extraction efficiency is enhanced due to the high shear forces. After addition of water, the sample emulsion is extracted once again. The organic upper phase is collected and the extraction procedure is repeated twice.

### 5.2.2 Module 1B – Hot Extraction

#### Principle

Continuous hot extraction procedures that operate at temperatures that are close to the boiling point of the used extraction solvent or solvent mixture at atmospheric pressure are an efficient means of extracting PBDE congeners from the matrix. The high temperature increases solubility and diffusion and therefore enhances the extraction efficiency of the analyte of interest. The extraction is at its most efficient when equilibrium of the solute is reached inside and outside the solid material.

#### Extraction procedures - manual

Before extraction the sample should be freeze-dried or mixed with a drying agent (e.g. polyacrylate or sodium sulphate). Depending on the composition of the sample, a mixture of cyclohexane and toluene (1/1, v/v) or ethanol and toluene (7/3, v/v) is used for e.g. Twisselmann extraction **(Figure 1).** The extraction procedure at the boiling point of the solvent mixtures is set to six hours. Permanent hot extraction of the sample takes place with solvent vapour and condensed solvent. A further cleaning of the raw extract with *tert*-butyl methyl ether for fat determination may be necessary.

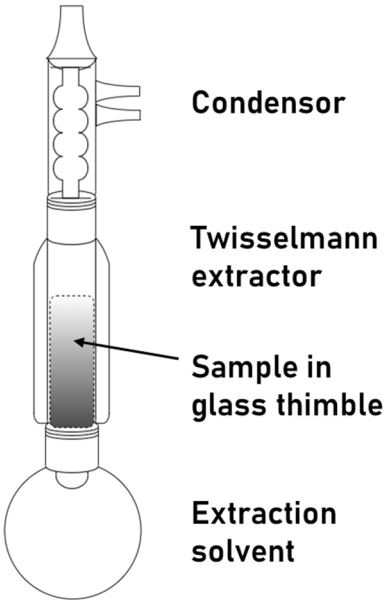


**Figure 1:** Twisselmann hot extractor with condenser, glass thimble and 500 – 1000 ml round bottom flask

### Extraction procedures – automated

A procedure for Soxhlet extraction as described in the standard EN 16215:2020 [5] for the extraction of PCDD/Fs and PCBs may be used for the extraction of PBDEs.

The semi-automated Soxtec™ hot extraction can be applied for various groups of POPs. Before extraction the sample aliquot should be freeze dried or mixed with a drying agent. Samples are continuously extracted by an organic solvent mixture heated close to the boiling point at atmospheric pressure. Method parameters are summarized in **Table 2**.

**Table 2:** Example of a semi-automatic hot extraction instrumental method

| Instrumentation | FOSS Soxtec™ 2055 |
|---|---|
| Thimble volume | 65 mL |
| Typical sample aliquot | 5-10 g |
| Drying agent (if applicable) | sodium sulphate |
| Solvent, volume | DCM/hexane (1/1, v/v), 75 mL |
| Temperature | 130 °C |
| Programme | 1. immersion of the sample in refluxing solvent for 30 min;<br>2. rinsing of the thimble for 15 min;<br>3. careful solvent elimination for 15 min[2] |
| Sample capacity | 6 parallel samples |

[2] Complete solvent evaporation by flushing of a gentle stream of nitrogen in the extraction cup

## Extraction procedures – Microwave extraction

### Principle

Microwave-assisted extraction (MAE) is an automated extraction technique that allows the reduction of the extraction time and solvent consumption. During MAE, microwaves pass through the solvent and are absorbed, converting into thermal energy. Microwaves rapidly heat the solvent mixture, by direct interaction of microwave energy with the free water molecule present in the sample. This results in a drastic increase in the internal temperature (superheating), causing the liquid vaporization within the cells, which may rupture the cell wall facilitating the release of fat and compounds of interest into the solvent.

### Extraction procedure – Monowave 400 (Anton Paar)

Before extraction the sample should be freeze-dried or mixed with a drying agent (e.g. polyacrylate or sodium sulphate). The extraction solvents are similar to these used with Soxhlet or Twisselmann hot extraction or hot pressurized extraction. The stir bar allows constant mixing of the sample and the hot solvents during the extraction procedure **(Figure 2).** Example of extraction conditions are summarized in **Table 3**.

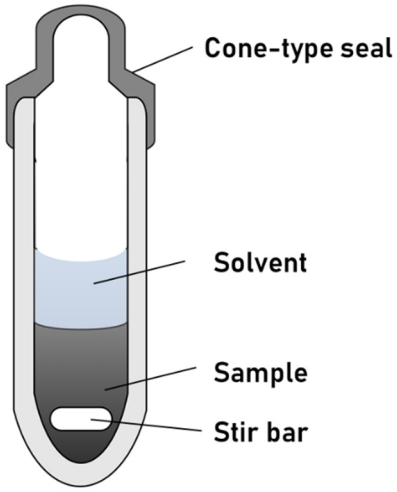


**Figure 2:** Microwave extraction principle (Anton Paar)

**Table 3:** Example of Monowave 400 MAE extraction

| Instrumentation | Monowave 400 |
|---|---|
| Solvents | toluene/acetone (70/30, v/v) |
| Solvent volume | 20 mL |
| Weight of sample | 5 g |
| Temperature | 120 °C |
| Static time | 10 min |
| Cooling temperature | 55°C |
| Stirrer speed | 600 rpm |

### 5.2.3 Module 1C – Hot Pressurized Extraction

#### Principle

The hot pressurized extraction process uses high temperature and high pressure, which decreases the extraction time and the quantity of solvent used, and shows better repeatability compared to other methods that use less extreme conditions. The high temperature increases solubility and diffusion and therefore enhances the extraction efficiency of the analyte of interest. The high pressure is used to keep the solvent in a liquid state as the temperature is increased above its boiling point, resulting in a higher solubility and higher diffusion rate of lipid solutes into the solvent, and a higher penetration of the solvent into the matrix compared to other less drastic methods.

#### Extraction procedure – ASE (ThermoFisher Scientific)

A procedure for automated pressurized fluid extraction as described in standard EN 16215:2020 [5] for the extraction of PCDD/Fs and PCBs may be used for the extraction of PBDEs.

Pressurized liquid extraction requires freeze-drying or mixing of the sample with drying agent. The extraction solvents are similar to those used with Soxhlet or Twisselmann hot extraction. Suitable method conditions are summarized in **Table 4**.

#### Extraction procedure – SpeedExtractor (Büchi)

Pressurized liquid extraction can be carried out after freeze-drying or mixing of the sample with drying agent. The extraction solvents are similar to these used with Soxhlet or Twisselmann hot extraction. Suitable method conditions are summarized in **Table 4.**

**Table 4:** Examples of two hot pressurized extraction devices

| | Dionex ASE 200 Food | Dionex ASE 200 Feed | SpeedExtractor E-914 |
|---|---|---|---|
| Solvents | toluene/cyclohexane (50/50, v/v) | toluene/ethanol (80/20, v/v) | toluene/cyclohexane or toluene/ethanol |
| Cell volume | **33 ml** | **33 ml** | **120 ml** |
| Sample weight | 10 – 17 g | 10 – 17 g | 10 – 30 g |
| Drying agent | 5 – 10 g Hydromatrix<br>10 g Sodium acrylate/vinyl alcohol copolymer | 5 – 10 g Hydromatrix<br>10 g Sodium acrylate/vinyl alcohol copolymer | 20 – 50 g Hydromatrix<br>30 – 50 g Sodium acrylate/vinyl alcohol copolymer |
| Pressure | 10 MPa | 10 MPa | 10 MPa |
| Temperature | 120 °C | 100 °C | 100/120 °C |
| Static cycles | 2 | 2 | 2 |
| Heat time | 6 min | 5 min | 5 min |
| Static time | 5 min | 10 min | 5 min |
| $N_2$ Purge time | 60 sec | 20 sec | 120 sec |

## 5.3 Module 2 – Extract Purification

The procedures described in this module are manual, semi-automated or fully automated clean-up methods for the isolation of PBDEs from other components and co-extractives present in a sample of food or animal feed. After extraction of the analytes of interest with an apolar solvent and evaporation to dryness, the extracted fat fraction is dissolved and an analyte specific purification procedure is carried out using firstly, a multi-layer silica column followed by a second clean-up step, using an alumina or Florisil® column.

**Note:** For those laboratories that are also required to determine PCDD/Fs and PCBs, all clean-up procedure described in Module 2 may be combined for in-tandem fractionation of planar PCDD/Fs and DL-PCBs and NDL-PCBs, by connecting the outflow of the multi layered silica column directly to an activated carbon column, (alternatively, the concentrated outflow of the multi-layer column may be fractionated using a separate carbon column method). The fractions – mono-ortho substituted PCBs and NDL-PCBs (combined fraction) and planar PCDD/Fs and non-ortho substituted PCBs (combined fraction) may then be further purified on activated basic alumina. This procedure is described in detail in standard EN 16215:2020 [5], Section 11.3.4.

### 5.3.1 Module 2A – Semi-Automated Purification

#### Principle

The extracts derived from either the manual or automated techniques described above require purification before they can be analysed and quantified. Manual or automated purification procedures use broadly similar principles of chromatographic separation of the PBDEs from residual lipid and other co-extracted interferences, by employing adsorbents such as alumina, silica, Florisil® or techniques such as GPC. These internally standardised procedures are carried out using equipment that is commercially available.

The semi-automated clean-up is based on established PCDD/Fs and PCBs analysis.

#### Procedure

For plant and feed matrices with low fat content, a high capacity multi-layer silica column clean-up step should be integrated to oxidize interfering high-molecular substances (e.g. carotenoids). Waxes might be separated using gel permeation chromatography (GPC).

#### High capacity multi-layer silica column clean-up (optional)

Preparation of adsorbents:

- Silica 60, 70-230 mesh: Baking over night at 600 °C
- Deactivation with water: Addition of 5 % of water
- Silica-sulphuric acid mixture: Addition of 32 % of conc. sulphuric acid (96 %)
- Silica-sodium hydroxide mixture: Addition of 23 % of 1 M NaOH solution
- Shaking of mixtures for several hours and equilibration over night

Preparation of chromatographic column:
Column: Length 30 cm, inner diameter 40 mm

Packing of column (bottom to top):

- Glass wool plug
- Deactivated silica (5 g)
- Silica-sodium hydroxide mixture (10 g)
- Deactivated silica (5 g)
- Silica-sulphuric acid mixture (30 g)
- Deactivated silica (5 g)
- Silica-sodium hydroxide mixture (35 g)

Condition of prepacked column and elution of analytes of interest:

- Re-dissolving of dried extraction residue: 50 ml of cyclohexane/toluene (1/1, v/v) using ultrasonic bath
- Conditioning: 150 ml of cyclohexane/toluene (1/1, v/v)
- Elution: 150 ml of cyclohexane/toluene (1/1, v/v) in 500 ml round bottom flask
- Concentration of eluate to dryness using rotary evaporator and nitrogen

**Gel-permeation chromatography (GPC)**

The GPC system consists of an HPLC-system with one HPLC solvent pump, an autosampler, an injector, a chromatographic column and a fraction collector. The chromatographic column has a length of 58 cm, an inner diameter 25.4 mm and adjustable plunger can be used. As adsorbent 50 g Bio-Beads S-X3 (dissolved in ethyl acetate/ cyclohexane (1/1, v/v) before filling of column) are used. The solvent used is a mixture of ethyl acetate and cyclohexane (1/1, v/v). Firstly 100 ml are eluted to waste and the following 80 ml (per injection at flow rate of 5 ml/min) are eluted in 500 ml round bottom flasks. Depending on the amount of fat to be separated several consecutive injections of aliquots of the extract might be necessary.

**Multi-layer silica column**

Preparation of adsorbents: (described under *High capacity multi-layer silica column clean-up*)

Preparation of chromatographic column:
Column: Length 10 cm, inner diameter 10 mm, with reservoir

Packing of column (bottom to top):

- Glasswool plug
- Deactivated silica (ca. 0.3 g)
- Silica-sodium hydroxide mixture (ca. 1 g)
- Deactivated silica (ca. 0.3 g)
- Silica-sulphuric acid mixture (ca. 1 g)
- Deactivated silica (ca. 0.3 g)

Condition of prepacked column and elution of analytes of interest:

- Re-dissolving of concentrated extract (of GPC or large multi-layer silica column) in 2 ml of n-heptane
- Conditioning: 40 ml of n-heptane
- Elution: 20 ml of n-heptane in 25 ml round bottom flask with appendix

### 5.3.2 Module 2B– Automated Purification

This module describes procedures for the automated clean-up of PBDEs from other components and co-extractives present in an extracted sample of food or animal feed. The procedures can be applied to extracts obtained using either the manual or automated extraction procedure as described earlier. This internally standardised procedure is carried out using equipment that is commercially available. Descriptions of automated procedures using Miura™ and LC-Tech™ equipment are given below but other automated procedures may also be used.

**Principle**

Although differing in the detail and order of procedures used, the principle is essentially the same as that described in Module 2A. After manual or automated extraction (section 5.2) of PBDEs the purification procedure is carried out by using automated adsorption chromatography with different kinds of packed clean-up columns (silica, sulphuric acid, carbon and alumina/Florisil®)

**Procedure**

For plant and feed matrices with low fat content, a high capacity multi-layer silica column clean-up step should be integrated to oxidize interfering high-molecular substances (e.g. carotenoids). Waxes might be separated using gel permeation chromatography (GPC).

**Clean up procedure – Miura™ GO-2HT (Miura Co. Ltd)**

This procedure is carried out using the sample extract obtained from any of the extraction modules, and provides purified extracts for PBDEs (as well as PCDD/Fs and PCBs). For the analysis of PBDEs only, the carbon column stage may be omitted using a dummy column or a specific adapter.

The packed columns are provided ready-to-use by the supplier. The sample extract (or the equivalent of 2 to 3 g of the extracted fat) is dissolved in 9.5/0.5 ml of n-hexane/ethylacetate (v/v) and manually injected on the silver nitrate silica column (column 1). The use of columns containing silver nitrate results in lower recoveries when lower amounts of fat are used. In such a case the elution volume may be increased with lower fat amounts. After clean-up on the sulphuric acid column (column 2) which is used for hydrolysing fats and other co-extracted material and washing with 140 ml n-hexane, the PBDEs can be collected in Fraction 1 after reverse elution of the alumina column with 1.2 mL toluene (**Figure 3**).

(In a multi-POP-method mono- and di-ortho-PCBs also elute in Fraction 1. Non-ortho-PCBs and PCDD/Fs may be collected in Fraction 2 after reverse elution of the carbon column with 1.5 mL toluene). The conditioning, washing and elution steps are chosen according to the operating instruction from Miura and were slightly modified to optimise purification (**Table 5**).

Fraction 1 is concentrated to 3 ml followed by addition of 10 µl of dodecane (optional). This solution is then carefully evaporated to dryness under a gentle stream of nitrogen and finally reconstituted with 90 µl toluene containing the recovery (syringe) standard.

**Table 5:** Programme for the Miura method. Fraction 1: ndl-PCBs, mono-ortho-PCBs, PBDEs. Fraction 2: non-ortho-PCBs, PCDD/Fs

| Step | Solvent | Volume [ml] | Flow [ml/min] | Time [min] | Analytes |
|---|---|---|---|---|---|
| Purification | | | | | |
| Load. Hex | Hexane | 130 | 2.5 | 3120 | |
| Fractioning | | | | | |
| Fraction 1 | Toluene | 3.4 | 0.4 | 510 | **PBDEs** (ndl-PCBs, mono-ortho-PCBs) |
| Fraction 2 | Toluene | 4 | 0.25 | 960 | non-ortho-PCBs, PCDD/Fs |

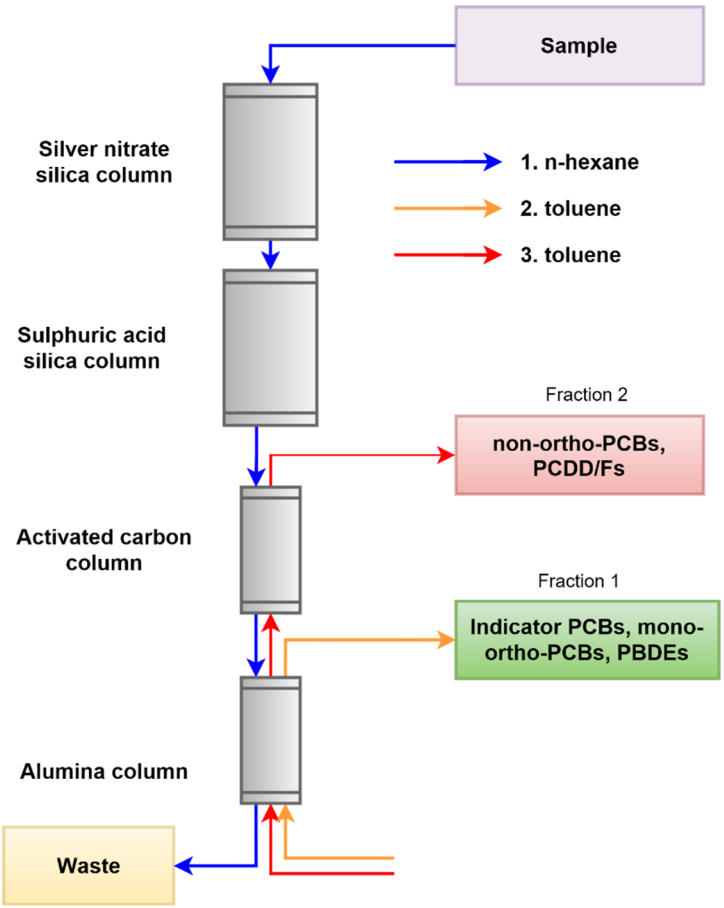


**Figure 3:** Schematic representation of automated purification using Miura equipment

### Clean up procedure – DEXTech™ Plus/Pure (LCTech GmbH)

This procedure is carried out using the sample extract obtained from any of the extraction modules, and provides purified extracts for PBDEs (as well as for PCDD/Fs and PCBs). For the analysis of PBDEs only, the carbon column stage may be omitted.

The sample (fat) extract is dissolved in 1 ml of acetone and 5 ml of cyclohexane. If there is incomplete dissolution, up to 1 ml of toluene can be added. As this solubilised extract will be injected into the purification system, the final volume should not exceed 10 ml for a 15 ml sample loop.

Different sample types can be purified for the determination of PBDEs (as well as PCDD/Fs and PCBs) using the steps described in **Table 6**, and as seen in the **Figure 4** below. This is a default method using a multi-layer silica sulphuric column (column 1) together with an alumina column (column 2) and a carbon column (column 3). The packed columns are provided ready-to-use by the supplier.

**Table 6:** Method parameters for fractionation of: Fraction 1: ndl-PCBs, mono-ortho-PCBs, PBDEs. Fraction 2: non-orthos-PCBs, PCDD/Fs using the DexTech system

| Step | Solvent | Volume [ml] | Flow [ml/min] | Time [min] | Analytes |
|---|---|---|---|---|---|
| Purification | | | | | |
| Load | Hexane | 182 | 7 | 26 | |
| Fractioning | | | | | |
| Fraction 1 | DCM/Hexane | 36 | 3 | 12 | **PBDEs** (ndl-PCBs, mono-ortho-PCBs) |
| Fraction 2 | Toluene | 10 | 1 | 10 | non-ortho-PCBs, PCDD/Fs |
| $N_2$ | | | | 3 | |
| Rinse | | 1 | | | |

The system starts with a column conditioning step, then injects the samples automatically and collects the fractions for each sample. Using this method, the samples are cleaned-up with an acidic silica-, an alumina- and a carbon-column. There are different kinds of acidic silica-columns available. The Universal and Standard-columns are used for fat samples with a maximum amount of fat of 5 g. The SMART-column is used for fat samples with a maximum amount of 1.5 g fat. Depending on the used acidic silica-column, the time taken by the procedure, as well as the amount of solvent consumed, will vary.

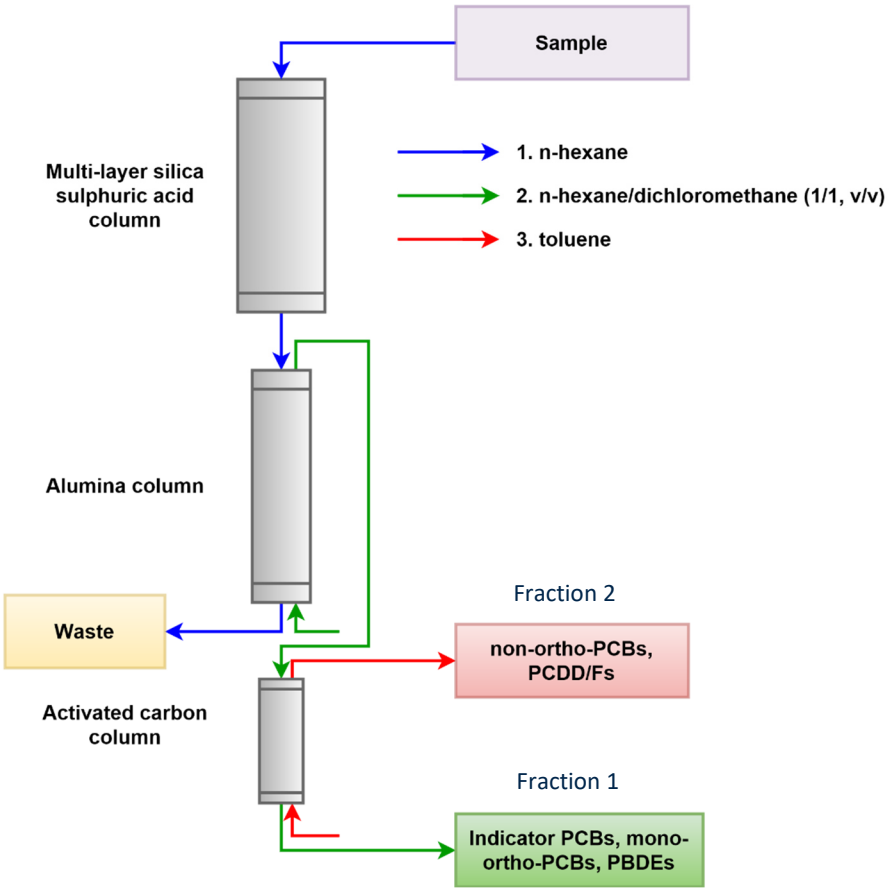


**Figure 4**: Schematic representation of automated purification using DexTech equipment

During purification, the sample extract is loaded first onto the acidic silica column after which it is transferred onto the alumina column. The mono-ortho-PCBs, ndl-PCBs and PBDEs, the non-ortho-PCBs and the PCDD/Fs are flushed from the alumina-column by a mixture of n-hexane and dichloromethane (50:50 %) onto the carbon-column. The mono-ortho-PCBs and ndl-PCBs together with the PBDEs flow directly through the carbon column and are collected as Fraction 1. The non-ortho-PCBs and the PCDD/Fs are retained on top of the carbon-column. They may be collected in a back-elution step with toluene as Fraction 2 (**Figure 4**).

Fraction 1 is concentrated to 3 ml followed by addition of 10 µl of dodecane. This solution is then carefully evaporated to dryness under a gentle stream of nitrogen and finally reconstituted with 90 µl toluene containing the recovery (syringe) standard.

**Note:** Automated purification of extracts may also be carried out using other commercially available systems, e.g. PowerPrep™ (Fluid Management Systems, Inc). The procedure described in detail (for PCDD/Fs and PCBs) in standard EN16215:2020 [5] Section 11.2, may also be used for PBDE analytes. This would require addition of the PBDE IS and collection of fraction A.

## 5.4 Module 3 – Combined manual Extraction and Purification

The procedure described in this module is a manual method for the isolation and purification of PBDEs from other components and co-extractives present in a sample of food or animal feed. This internally standardised procedure combines extraction and preliminary purification in a single stage.

### Principle

Aliquots of the test material are fortified with the internal standard, stabilised, comprehensively ground and dispersed in solution. The solution is passed through a multi-layer silica column where the PBDEs are isolated from the matrix using cold solvent extraction with simultaneous preliminary purification through acid and base hydrolysis on the multi layered silica. The eluate is concentrated and further purified using an activated basic alumina column. The PBDE containing fraction from this column is reduced to the final extract volume with the introduction of the keeper solvent and the internal sensitivity standard.

**Note:** For those laboratories that are also required to determine polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs), this procedure may be combined for in-tandem fractionation of planar PCDD/Fs and dioxin-like and non-dioxin-like PCBs (DL-PCBS and NDL-PCBs), by connecting the outflow of the multi layered silica column directly to an activated carbon column, (alternatively, the concentrated outflow of the multi-layer column may be fractionated using a separate carbon column method). The fractions – mono-ortho substituted PCBs and NDL-PCBs (combined fraction) and planar PCDD/Fs and non-ortho substituted PCBs (combined fraction) may then be further purified on activated basic alumina. This procedure is described in detail in the CEN method - EN16215:2020 [5], section 11.3.4.

### Procedure

Accurately weigh a sub-sample of the prepared sample material into a sturdy 500 ml glass bottle. The weight will depend on the sensitivity on the measurement process, but for most samples, typically 5 - 10g of dry material (or 2-3 g lipid equivalent) should be used to ensure a representative sample size.

Using a pipette, fortify the weighed sample with the required volume of internal standard solution, by spiking small droplets of the solution over the cross-section of the sample surface. Typical volumes of a few hundred microliters containing approximately 1 ng of each congener are suggested, but the standard amount may be varied depending on the level of expected contamination. Allow the fortified sample to infuse and stabilise for an hour before extraction.

Add 75 g of acid modified silica gel (prepared by roller-mixing sulphuric acid and [63 µm to 200 µm mesh] silica in a ratio of 1:1.5 for min. 6 h.) to the fortified sample and blend (e.g. using an Ultra-Turrax$^{TM}$) with 200 mL hexane (200 mL) for 1 min, allow to stand for 5 min and repeat. After allowing to stand for a further 5-10 min, transfer the produced slurry to a multi-layered silica column that is prepared as follows:

Wash a glass column (70 mm x 600 mm with PTFE coated tap at the bottom end) with dichloromethane and plug the lower end with silanised glass wool. Add two glass fibre discs over the wool and pack from the bottom with 10g anhydrous $Na_2SO_4$, 50 g base modified silica gel (prepared by mixing/shaking 5 M potassium hydroxide in methanol:silica in the ratio of 3:1,

until free flowing), 25 g acid modified silica gel and topped with 30 g of anhydrous $Na_2SO_4$ (**Figure 5**). Tap the column gently to allow the layers to settle.

Quantitatively transfer the slurry to the top of the prepared column along with the rinsing of the bottle - 3 times with 10 to 30 mL n-hexane. Ensure that there are no air-locks or channelling in the column by adjusting the tap at the bottom. Raise the level of the column to allow the sample to drain through into a 1 L bottle. Elute the column with 400 ml of dichloromethane/hexane (40:60 ratio) into the 1 litre bottle. This may be gravity assisted by raising the height of the column or by applying head pressure of nitrogen (approximately 0.1 to 0.2 bar).

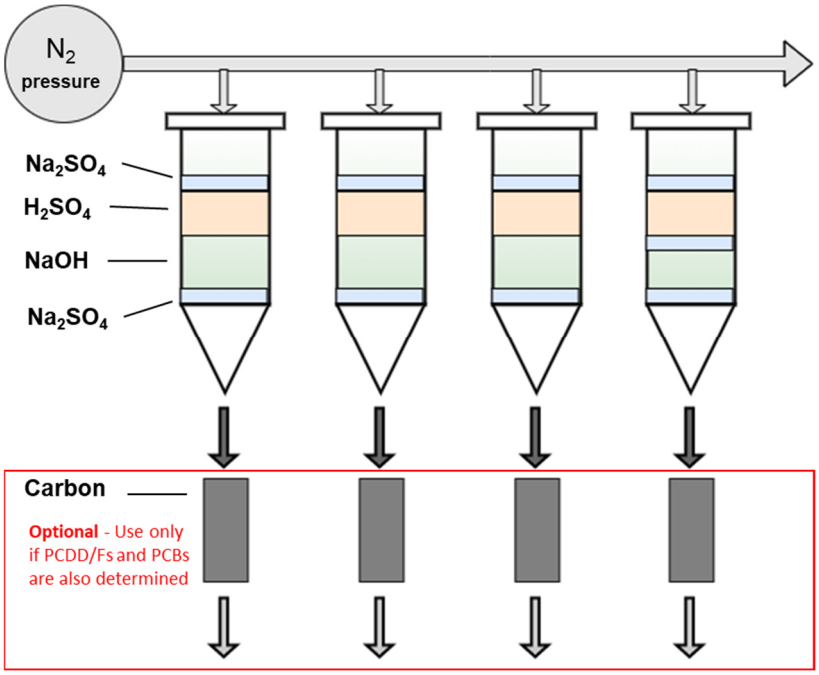


**Figure 5:** Multi-layer column after sample has eluted, showing relative position of layers.

Concentrate the total volume of the eluate and solvent exchange to hexane (~0.5 mL). Transfer this extract to a vial and add 5 drops of 37N sulphuric acid and mix by rotary shaking. Allow to stand for 15 min to allow the aqueous acid and organic layers to separate. Discard the bottom aqueous layer and repeat the acid treatment. Then repeat the process a third time but use water instead of the acid.

Prepare two mini-columns (6 mm x 100 mm – Pasteur pipettes may be used), by plugging the ends with glass wool. The first column is packed with base modified silica gel (~3.5 cm) at the bottom, acid modified silica gel (~3.5 cm) above, and topped with a 1 cm layer of anhydrous $Na_2SO_4$. The second column is packed with activated alumina (~7 cm of WB5-Basic alumina, muffled at 450 °C for min 24 h). Position the columns in series so that the outflow of the mixed acid/base silica column elutes directly into the alumina column. A solvent reservoir may be added above the columns in order to regulate the flow of eluting solvent (**Figure 6**). Condition the columns by washing through with 20 mL of a dichloromethane: hexane (30:70) solvent mix, followed by 10 ml of hexane.

Transfer the acid treated sample extract to the top of the mixed acid/base column together with hexane rinsing of the vial. Elute the columns (in series) with 10 mL of hexane to waste. Dispose of the mixed silica column and elute the alumina column with 20 mL of dichloromethane/ hexane, (30/70). Concentrate the eluate to ~ 100 µL. Then add the PBDE internal sensitivity standard contained in 50 µL of nonane as keeper solvent and gently concentrate to a final volume of approximately 50 µL.

**Note:** During measurement, the response of PBDE congeners reduces with increasing levels of bromination. It is therefore recommended that two internal sensitivity standards are used – one each for early and late eluting congeners.

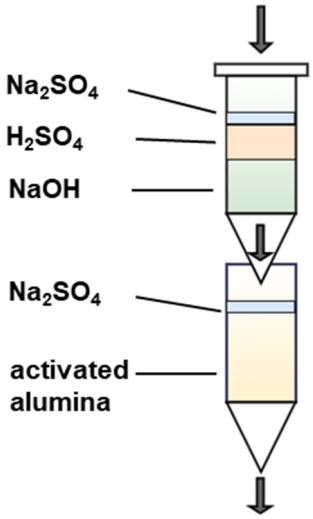


**Figure 6:** Schematic of mini-column setup for final purification

## 5.5 Module 4 – Measurement of Extracted PBDEs

The procedures described in this module assumes competence in the practice of gas chromatography (GC) with capillary columns and High Resolution Mass Spectrometry (HRMS). The equipment required for this module is a combined GC-HRMS system consisting of:

- A gas chromatograph equipped with a splitless injector as a minimum or preferably with a large volume injector such as a programmable temperature vaporizer (PTV); a capillary GC column and a heated transfer line interfaced to the mass spectrometer.
- A high resolution mass spectrometer (HRMS) using electron ionisation (EI) with magnetic and electrostatic sectors, high gain detection and acquisition capacity and integrated data handling capability.
- Alternatively a tandem mass spectrometer (MS/MS) using electron ionisation (EI) or atmospheric pressure chemical ionisation (APCI) with triple quadrupole (QqQ) or ion trap (IT) detectors
- The combined GC-MS system may be automated by the use of an autosampler.

**General considerations for GC separation**

An important, but undesirable side-effect noticed during GC separation is the absorption/breakdown of thermolabile PBDEs (mostly observed for PBDEs with >6 bromines and especially for decaBDE) in the injector or on column. To prevent this from happening, a clean injector, preferably with a liner without any filling material (such as glass wool) should be used.

Examples for liners with good practical experiences are:

- PTV Liner, Single Baffle, Deactivated (e.g Agilent, Restek)
- Splitless liner, splitless, single taper, deactivated (e.g Agilent, Restek)

Examples for GC column types that provide adequate chromatographic separation of PBDE congener are:

- Rtx-1614 (Restek)
- DB-5MS (Agilent J&W)

**General considerations for MS detection**

Inject a medium concentration range PBDE calibration standard into the GC-MS system and ensure that all the ions corresponding to the required analytes from tri-BDE to deca-BDE are recorded with the required sensitivity. (Use the Table on Typical Performance characteristics for PBDE, given in the guidance document, together with method parameters such as sample weight used, to verify that the measurement sensitivity is adequate). Sensitivity is dependent on the condition (cleanliness) of the GC injector and column and transfer line as well as the MS tuning and cleanliness of the source. If required, take appropriate action, e.g. cleaning of the injector and insert, retuning and recalibrating the MS, cleaning the ion source or changing/cutting/modifying the analytical column (if peak broadening is observed).

When the performance of the GC-MS system has been verified, the extracts can be analysed according to good QC practice. Typically the sequence of injections should commence and end with the set of PBDE calibration standards. The batch of samples including a procedural blank and reference material may be run in between these standards separated by solvent blanks to ensure that there is no carryover from the calibration standards.

### 5.5.1 Module 4A –Gas Chromatography-High Resolution Mass Spectrometry (GC-EI-HRMS) – Sector field instrument

**Principle**

Sample extracts containing PBDEs are introduced into the GC where they are volatilised and separated into congeners on the GC column, based on volatility and chromatographic affinity. The separated congeners are transferred sequentially into the MS where they are ionised (using electron ionisation - EI), separated into discrete mass groups by the combined action of magnetic and electrostatic forces and recorded after magnification by a detection system. The

acquired signals can then be processed to yield quantitative data, either on the system or remotely by networked access.

**Procedure - GC**

The GC should be fitted with a capillary column that will allow adequate response and separation of PBDE congeners. Ensure that the injector insert in the GC is scrupulously clean (this is critical for the determination – if in doubt, install a clean new insert). In some cases a 1-2 m long guard capillary (column without phase) placed between the injector and GC column, may help to reduce contamination of the main GC column with matrix components and provide better peak shapes and responses.

Equilibrate the GC with helium carrier gas under the above conditions. Flow rate: 1 mL $min^{-1}$

The following volatilisation and temperature programmes may be used as initial guidance on conditions for PTV and GC oven programme for a 30m RTX-1614 GC column:

**PTV programme:** 70 $^0$C (held for 3 min) ramped at 12 $^0$C/second to 310 $^0$C (held for 3 min), then increased at 12 $^0$C/second to 350 $^0$C (held for 35 min). Injection volume – 5 to 10 µL depending on sensitivity requirements.

**GC oven programme:** 60 $^0$C (held for 4 min) ramped at 12 $^0$C/minutes to 150 $^0$C (held for 1 min), then increased at 20 $^0$C/minutes to 230 $^0$C (held for 1 min), further increased at 1 $^0$C/minute to 250$^0$C and finally ramped at 20 $^0$C/minutes to 330 $^0$C (held for 8.5 min).

**GC columns:** 30 x 0.25 mm, 0.10 µm phase thickness Rtx-1614. This column allows separation of all 9 PBDE analytes in a single run. Other column lengths (e.g. 15 m) could also be used if adequate analyte peak separation can be demonstrated. Alternatively the GC separation may be carried out in two separate runs using a 60 x 0.25mm, 0.25 µm phase DB5-MS for PBDE congeners from BDE-28 to BDE-183 and a 15 x 0.25mm, 0.10 µm phase DB5-MS for BDE-209.

GC to MS transfer line temperature: 280 °C

**Procedure - MS**

Introduce a MS calibrant such as high-boiling perfluorokerosene (PFK or any other suitable calibrant that covers the relevant mass range) into the stabilised source and tune the MS to a minimum required resolving power of 10 000 (10 % valley) at a mass that is within the mass range of the PBDE ions, e.g. - m/z 554.9659. Verify the resolution at different masses within the range (m/z 405.8026 – m/z 815.3872). Calibrate and record the resolution and mass deviation for the full set of ion mass groups corresponding to the required PBDEs from tri-BDE to deca-BDE (**Table 7**).

**Table 7:** m/z molecular ions used for monitoring unlabelled and $^{13}C$ labelled PBDE congeners

| | $M^+$ / M-2$Br^+$ Unlabelled | $M^+$ / M-2$Br^+$ $^{13}C$-labelled |
|---|---|---|
| Di-BDE | 325.8936 | 337.9339 |
| | 327.8916 | 339.9319 |
| Tri-BDE | 405.8021 | 417.8424 |
| | 407.8001 | 419.8403 |
| Tetra-BDE | 483.7126 | 495.7529 |
| | 485.7106 | 497.7508 |
| Penta-BDE | 403.7864 | 415.8267 |
| | 405.7845 | 417.8247 |
| Hexa-BDE | 483.6950 | 495.7352 |
| | 485.6930 | 497.7332 |
| Hepta-BDE | 561.6055 | 573.6457 |
| | 563.6035 | 575.6437 |
| Octa-BDE | 639.5156 | 641.5140 |
| | 651.5562 | 653.5542 |
| Nona-BDE | 719.4245 | 721.4225 |
| | 731.4647 | 733.4626 |
| Deca-BDE | 797.3350 | 799.3330 |
| | 809.3752 | 811.3732 |
| Alternatively for Deca-BDE **(M-2Br)$^{2+}$** | 398.6672 | 404.6873 |
| | 399.6662 | 405.6863 |

For a satisfactory calibration, the resolution should be greater than or equal to 10 000, the deviation between the exact m/z and the theoretical m/z for each exact m/z monitored should be less than 5 ppm. Set up an MS monitoring programme to include a minimum of two masses per homologue group (two each for native and $^{13}C$ labelled) and using the appropriate PBDE standards, adjust the time windows for each (or combined, where overlaps occur) homologue group. Typical MS conditions are given in **Table 8** an example chromatogram is given in **Figure 7.**

**Table 8:** Typical EI-HRMS conditions used for PBDE measurement.

| Parameter | Specification |
|---|---|
| System used | GC-HRMS |
| Ionisation mode | EI + |
| Acquisition mode | Selected ions |
| Acquisition requirement | At least 10 ions at ≥10 000 within ~1s |
| Source Ionisation energy | 20 eV to 70 eV |
| Acceleration voltage | 5000 / 7000 v |
| Electron Trap current | 450 - 600 µA |

### Example Chromatogram HRMS

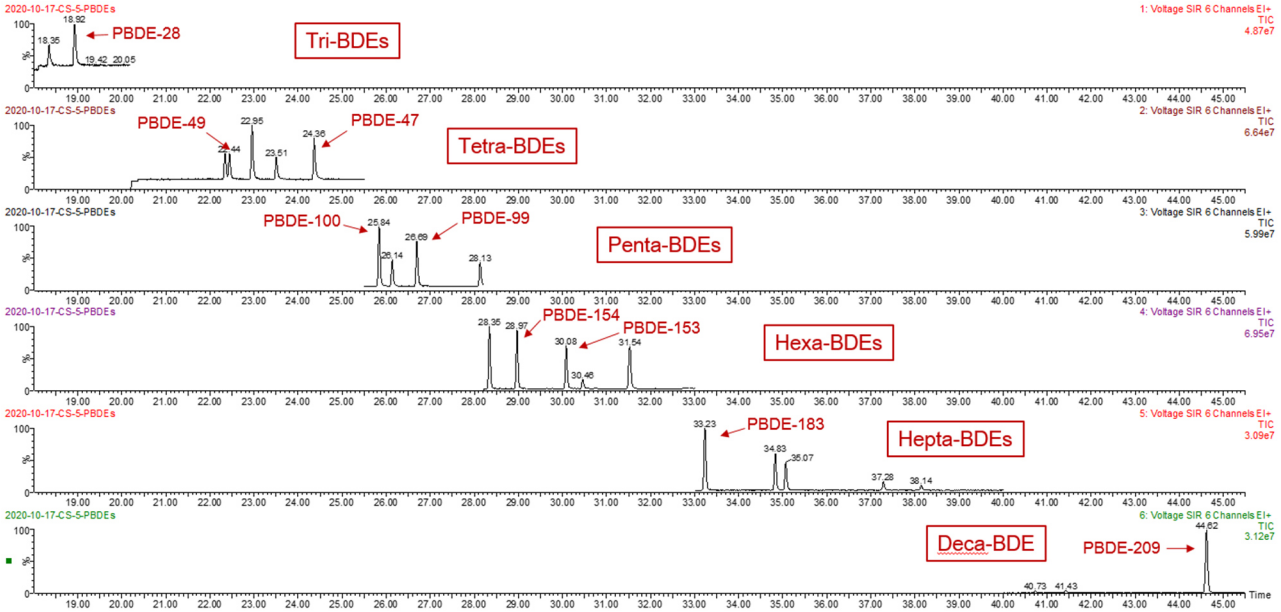


**Figure 7:** TIC for PBDE mixture. Single run for all selected PBDEs. Instrument: Autospec; Column – Rtx-1614 30 x 0.25 x 0.1. Target PBDEs are indicated.

## 5.5.2 Module 4B –Gas Chromatograph-Tandem Mass Spectrometry (GC-MS/MS)

### GC-EI-MS/MS

### Principle

As an alternative to HRMS detection, GC-EI-MS/MS may also effectively be used for routine analysis of PBDEs. GC equipment requirements remain the same as for GC-HRMS. Selective determination could be ensured by using triple quadrupole (QqQ) or ion trap (IT) detectors operated in selected reaction monitoring (SRM) mode which support fast MRM scan speed.

### Procedure – GC

The same GC conditions as used for GC-HRMS (in section 5.5.1) may be used for GC-MS/MS.

### Procedure - MS

The GC-MS/MS system should be adjusted and calibrated with the appropriate calibrant (e.g. heptacosa) according to the guidelines provided by manufacturer and adequate ion transmission through the ion optics should be verified within the mass range of the selected MS/MS transitions (see **Table 9**).

**Table 9:** SRM transitions of unlabelled and 13C labelled PBDE congeners (TSQ Quantum XLS Ultra; ThermoFisher Scientific™)

| Homologue group | Quantifier SRM | Qualifier SRM | Collision energy, eV |
|---|---|---|---|
| Native tri-BDEs | 405.8 > 245.9 | 407.8 > 247.9 | 20 |
| $^{13}C_{12}$-labeled tri-BDEs | 417.8 > 257.9 | 419.8 > 259.9 | 20 |
| Native tetra-BDEs | 485.7 > 325.8 | 487.7 > 327.8 | 20 |
| $^{13}C_{12}$-labeled tetra-BDEs | 497.7 > 337.8 | 499.7 > 339.8 | 20 |
| Native penta-BDEs | 563.7 > 403.8 | 565.7 > 405.8 | 20 |
| $^{13}C_{12}$-labeled penta-BDEs | 575.7 > 415.8 | 577.7 > 417.8 | 25 |
| Native hexa-BDEs | 643.5 > 483.7 | 645.5 > 485.7 | 25 |
| $^{13}C_{12}$-labeled hexa-BDEs | 655.5 > 495.7 | 657.5 > 497.5 | 25 |
| Native hepta-BDEs | 721.4 > 561.8 | 723.4 > 563.8 | 25 |
| $^{13}C_{12}$-labeled hepta-BDEs | 733.4 > 573.8 | 735.4 > 575.8 | 30 |
| Native deca-BDE | 801.4 > 641.5 | 803.4 > 643.5 | 35 |
| $^{13}C_{12}$-labeled deca-BDE | 813.4 > 653.5 | 815.4 > 655.5 | 35 |

Establish an MS method including at least two specific SRM transitions for each homologue group for both native compounds and $^{13}C$ labelled surrogates. Specific time windows may be adjusted by the analysis of selected congeners and where possible include only one homologue group per window. In some cases where overlapping of homologue groups occurs (e.g. penta- and hexa-BDEs), combine SRM transitions in one window. Example of MS conditions are given in **Table 10**.

**Table 10:** EI-MS/MS conditions used for PBDE measurement (TSQ Quantum XLS Ultra; ThermoFisher Scientific™).

| Parameter | Specification |
|---|---|
| System used | GC(EI)-QqQ |
| Ionisation mode | $EI^+$ |
| Acquisition mode | SRM |
| Source Ionisation energy | ~ 70 eV |
| Emission current | ~ 50 µA |
| Resolution (Q1, Q3) | 0.7 Da |
| Collision gas | Argon, 1.3 mTorr |

### GC-APCI-MS/MS

#### Principle

GC- Atmospheric Pressure Chemical Ionization – (APCI)-MS/MS operates on similar principles as GC-HRMS or GC-EI-MS/MS, for the determination of PBDEs. Using soft ionization at atmospheric pressure, chemical ionization results in quasi molecular ions and specific fragments detected by a triple quadrupole (QqQ) detector.

#### Procedure - GC

Similar GC conditions as used for GC-HRMS (in section 5.5.1) may be used for GC-APCI-MS/MS. A more specific alternative set of conditions are given below:

**PTV programme** (Gerstel CIS 4): 70 $^{0}$C (held for 0.13 min) ramped at 720 $^{0}$C/min to 325 $^{0}$C (held for 10 min). Injection volume – 5 µL in cyclohexane. Vent pressure / flow / end time: 36 kPa, 90 ml/min, 0.03 min; Injector liner: Deactivated baffled liner, 2mm ID

**GC oven programme:** 70 $^{0}$C (held for 1 min) ramped at 20 $^{0}$C/min to 310 $^{0}$C (held for 5 min)

**GC column:** 30 x 0.25 mm, 0.10 µm film RTX-1614

**Carrier gas:** Helium; Flow rate: 3.0 ml/min to 7.0 ml/min (ramped)

Heated transfer line: 280 °C

#### Procedure - MS

The GC-APCI-MS/MS system should be adjusted and calibrated with the appropriate calibrant (e.g. FC-71) according to the guidelines provided by the manufacturer and the adequate ion transmission through the ion optics should be verified within the mass range of selected MRM transitions.

Establish an MS method including at least two specific MRM transitions for each homologue group for both native compounds and $^{13}$C labelled surrogates. Specific time windows may be adjusted by the analysis of selected congeners. Where possible, include only one homologue group per window. In some cases where overlapping of homologue groups occurs (e.g. penta- and hexa-BDEs), combine MRM transitions in one window. Example of MS conditions are given in **Table 11**. Exemplarily m/z transitions are obtained with APCI-MS/MS are summarized in **Table 12** at a collision energy (CE) of 25V.

**Table 11:** Typical APCI-MS/MS conditions used for PBDE measurement (Xevo TQ-XS; waters™).

| Parameter | MS settings |
|---|---|
| System used | GC(APCI)-QqQ |
| Ionisation mode | APCI under dry source conditions (charge transfer) |
| Source | Dry $N_2$ |
| Source temperature | 150 °C |
| Corona current | 1.0 µA |
| Sampling cone voltage | 40 V |
| Cone gas flow | 240 L/h |
| Auxiliary gas flow | 300 L/h |
| GC make-up flow | 300 ml/min |
| Acquisition mode | MRM |
| Precursor Ion | $M^{+\cdot}$ |
| Product Ion | $M\text{-}Br_2^{+\cdot}$ |
| Collision energy | 25 V |
| Collision gas flow | 0.17 mL/min (Ar) |
| Cycle time | ca. 0.1 – 0.25 s |
| Dwell time | 20 - 59 ms |
| Resolution (Q1, Q3) | 0.7 Da |

**Table 12:** APCI-MS/MS conditions used for PBDE measurement (Xevo TQ-XS; waters™)

| Homologue group | Quantifier MRM | Qualifier MRM |
|---|---|---|
| Native di-BDEs | 325.9 > 168.1 | 327.9 > 168.1 |
| $^{13}C_{12}$-labeled tri-BDEs | 337.9 > 180.1 | 339.9 > 180.1 |
| Native tri-BDEs | 405.8 > 245.9 | 407.8 > 247.9 |
| $^{13}C_{12}$-labeled tri-BDEs | 417.8 > 258.0 | 419.8 > 260.0 |
| Native tetra-BDEs | 483.7 > 323.8 | 485.7 > 325.9 |
| $^{13}C_{12}$-labeled tetra-BDEs | 495.7 > 335.9 | 497.7 > 337.9 |
| Native penta-BDEs | 561.6 > 403.8 | 563.6 > 405.8 |
| $^{13}C_{12}$-labeled penta-BDEs | 575.7 > 415.8 | 577.7 > 417.8 |
| Native hexa-BDEs | 641.5 > 481.7 | 643.5 > 483.7 |
| $^{13}C_{12}$-labeled hexa-BDEs | 653.5 > 493.7 | 655.5 > 495.5 |
| Native hepta-BDEs | 721.4 > 561.8 | 723.4 > 563.8 |
| $^{13}C_{12}$-labeled hepta-BDEs | 733.4 > 573.8 | 735.4 > 575.8 |
| Native octa-BDE | 799.4 > 639.5 | 801.4 > 641.5 |
| $^{13}C_{12}$-labeled octa-BDE | 811.4 > 651.5 | 813.4 > 653.5 |
| Native nona-BDE | 879.3 > 719.4 | 881.3 > 721.4 |
| $^{13}C_{12}$-labeled nona-BDE | 891.3 > 731.5 | 893.3 > 733.5 |
| Native deca-BDE | 957.2 > 797.3 | 959.2 > 799.3 |
| $^{13}C_{12}$-labeled deca-BDE | 969.2 > 809.4 | 971.2 > 811.4 |

## 6. Quantitation and Presentation of results

Quantitation is based on internal standardisation (isotope dilution principles for those analytes with surrogate labelled internal standards), which provides a measure of the analyte content that is automatically corrected for any extraction, purification or measurement losses. The use of the labelled internal standards also allows for calculation of the recovery of the analytical process when measured against the analytical sensitivity standard(s). For analytes without surrogate labelled internal standards e.g. BDE-49, another closely eluting labelled internal standard from the same mass trace may be used.

PBDE are identified from the individual ion chromatograms based on retention time of the native and corresponding labelled standard, exact mass or transition and mass ratio. These parameters should be established before quantitation. The retention times of all PBDEs of interest should be checked and, if necessary, modified in the processing method that is usually provided with the instrument software. Use the modified method to integrate all ion chromatograms that were produced during the analytical sequence. It is strongly recommended that the output is manually checked for correct integration for each individual compound.

Prepare a calibration curve encompassing the concentration range to be determined and verify the linearity of the PBDE standard calibration for each compound. If the relative response for any PBDE congener is constant (less than 20 % coefficient of variation) over the calibration range, an averaged relative response factor (RRF) may be used for that congener. The RRF for a native PBDE congener (i) in the analytical standard may be determined from the formula below.

$$\mathrm{RRF}_i = \left(\frac{c_{iN}}{S_{iN}}\right) \div \left(\frac{c_{iIS}}{S_{iIS}}\right) \quad (1)$$

The amount $m_i$ in [µg] of the congener (i) in the sample, may be determined form the derived formula:

$$m_i = \frac{m_{IS}}{S_{iIS}} \times \frac{1}{\mathrm{RRF}_i} \times S_{iN} \quad (2)$$

$m_{IS}$: amount (in µg) of the internal standard added to the test sample aliquot
$c_{iN}$: concentration of a native PBDE congener$_{(i)}$
$c_{iIS}$: concentration of the corresponding labelled PBDE congener$_{(i)}$
$S_{iN}$: response (sum of two m/z's) of the native PBDE congener$_{(i)}$
$S_{iIS}$: response (sum of two m/z's) of the corresponding labelled PBDE congener$_{(i)}$

The concentration of congener$_{(i)}$ in the sample may then be determined by dividing the determined amount in the sample in [µg] by the weight $w_i$ in [kg] of the aliquot taken for analysis.

$$c_i = \frac{m_i}{w_i} \quad (3)$$

The quantitation software on most GC-MS systems allows the above calculation process to be automated, so that a collated list of the PBDE concentration values for the measured sample extract is obtained.

For quality control purposes, the analytical recovery of an internal standard can be calculated using equation 4 below:

$$R_i[\%] = \left(\frac{S_{iIS}}{m_{IS}}\right) \div \left(\frac{S_{RS}}{m_{RS}}\right) \times \frac{1}{RRF_{iLS}} \times 100 \quad (4)$$

$m_{IS}$: amount (in ng) of the internal standard$_{(i)}$ added to the test sample aliquot
$m_{RS}$: amount (in ng) of the internal sensitivity standard added to the test sample aliquot
$S_{iIS}$: response (sum of two m/z's) of the internal standard$_{(i)}$
$S_{RS}$: response (sum of two m/z's) of the recovery standard
$RRF_{(iIS)}$: relative response factor of the internal standard$_{(i)}$

During calibration, the coefficient of variation for any $^{13}C$ labelled PBDE should be less than 30 %.

# 7. Reporting of Results – Format

An example of a reporting format for PBDEs concentrations in food and feed samples is given in **Table 13**.

The conventional units for reporting PBDE concentrations are µg $kg^{-1}$ (µg/kg) wet weight (w.w.). The concept of lower bound and upper bound sums are described in section 1.4 in the main guidance document which also provides more information on reporting in section 2.8.

**Table 13:** Example of a reporting format for PBDE samples

| | |
|---|---|
| **Sample ID** | |
| **Location** | |
| **Sample Type** | |
| **Year of sampling** | |
| **Routine or Incident related** | |

| **PBDE congener** | **Concentration [µg/kg w.w.]** | Concentration [ng/g fat] | MU [%] | Recovery [%] | **LOQ [µg/kg w.w.]** |
|---|---|---|---|---|---|
| **BDE - 28** | | | | | |
| **BDE - 47** | | | | | |
| **BDE - 49** | | | | | |
| **BDE - 99** | | | | | |
| **BDE - 100** | | | | | |
| **BDE - 153** | | | | | |
| **BDE - 154** | | | | | |
| **BDE - 183** | | | | | |
| **BDE - 209** | | | | | |
| | | | | | |
| **Sum of 8 (without BDE-209)** | | | | | |
| **Lower bound** | | | | | |
| **Upper bound** | | | | | |
| | | | | | |
| **Sum of 9 (including BDE-209)** | | | | | |
| **Lower bound** | | | | | |
| **Upper bound** | | | | | |
| | | | | | |
| Other measured congeners | | | | | |
| BDE-xx | | | | | |
| BDE-xxx | | | | | |
| BDE-xxx | | | | | |
| Etc. | | | | | |

| | |
|---|---|
| % Lipid content (food) | |
| % Moisture content (feed) | |
| Extraction method used | |
| Purification method used | |
| Quantitation method used | |
| Other relevant information | |

Note: Fields in grey font are optional

## 8. References

[1] Commission Regulation (EU) 2017/644 of 5 April 2017 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EU) No 589/2014 (OJ L92, 6.4.2017)

[2] European Commission, 2009. Commission Regulation (EC) No. 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. Off J Eur Union. L 54:1–130.

[3] International Standardization Organization (ISO), 2020. EN ISO 6497:2002 - Animal feeding stuffs — Sampling. Last review and confirmation in 2020.

[4] International Standardization Organization (ISO), 2017. EN ISO 6498:2012 - Animal feeding stuffs — Guidelines for sample preparation. Last review and confirmation in 2017.

[5] European Committee for Standardization (CEN), 2020. CEN Standard – EN16215:2020 - Animal feeding stuffs: Methods of sampling and analysis - Determination of dioxins and dioxin-like PCBs and of indicator PCBs by GC/HRMS.

[6] Smedes, F. 1999. Determination of total lipid using non-chlorinated solvents. Analyst, 124, 1711–1718