

# Identification and Quantification of Polybrominated Diphenyl Ethers (PBDEs) in Environmental Samples using Gas Chromatography Coupled to Orbitrap Mass Spectrometry

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## ABSTRACT

This work demonstrates the use of high resolution accurate mass Thermo Scientific™ GC-Orbitrap™ technology for the targeted analysis of 27 PBDE native congeners in air, ash, sediment, and sludge samples using a sensitive, fast, and robust high throughput method. Sediment, filter dust, air, and sludge samples were assessed for their PBDE content. In the sludge samples analysed, the predominant PBDE congeners identified were BDE-209, 47 and 99, whereas in the filter dust samples BDE-47, 119, 99, 53 and 183 were prevalent. This preliminary results offers excellent selectivity, and sensitivity for the analysis of PBDEs in complex samples. Moreover, the use of high resolution to achieve selectivity in difficult matrices, and the mass accuracy obtained allows for unambiguous identification, and elemental composition confirmation of chemical contaminants.

## INTRODUCTION

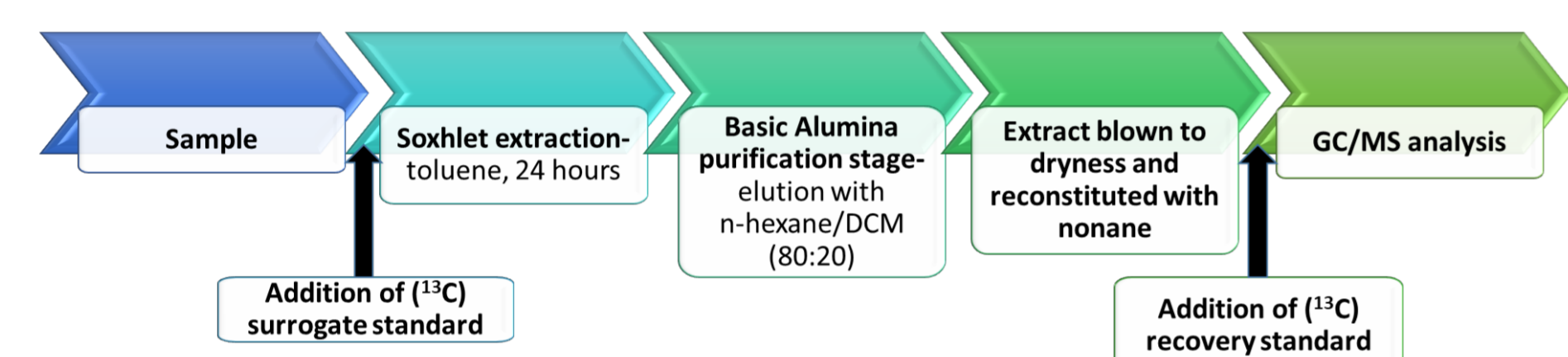
Polybrominated diphenyl ethers (PBDEs) are a group of organobrominated contaminants that inhibit or suppress combustion in organic material. They have been widely used since the 1970s as flame retardants in a broad range of commercial and household products including textiles, building materials electronics, furnishings, motor vehicles, and plastics.<sup>1</sup> Most PBDEs resist degradation, and bioaccumulate in environment, and food chains, and can be transported through air, and water over long distances.<sup>2</sup> Many PBDEs have been identified as toxic, with links to cancer, and endocrine disruption<sup>3</sup> and the use of certain PBDEs have been banned.<sup>4</sup> There are many analytical challenges to consider when developing a GC-MS method for the analysis of PBDEs, especially related to the active nature of high molecular mass PBDEs (e.g. BDE-209), and to the large number of compounds, resolution, and peak symmetry requirements (e.g. chromatographic separation of BDE 49 and BDE 71 can be challenging in complex environmental samples).

## MATERIALS AND METHODS

### Sample Preparation

The following environmental samples were provided by the Dioxins Laboratory, IDAEA-CSIC, Barcelona, Spain: three sediment samples (including two samples previously used in an inter-laboratory study, and one sample previously used in a QA/QC study), three sludge samples (from a waste water treatment plant), three filter dust samples (previously used in a QA/QC study), and one air sample (previously used in an inter-laboratory study). Samples (2 g), were Soxhlet extracted with toluene for 24 hours, followed by a basic alumina purification stage (6 g), activated overnight at 300 °C, and elution with 50 mL n-hexane/DCM (80:20). The extracts were then blown to dryness and reconstituted with 20 µL nonane prior to GC-MS analysis. A mass-labelled (13C) PBDE surrogate standard was added prior to extraction and a mass-labelled (13C) PBDE recovery standard was added prior to injection, as illustrated in the PBDE analytical workflow (Figure 1).

**Figure 1. PBDE analytical workflow, including sample extraction, extract purification, and concentration stages required prior to GC-MS analysis**



### Test Methods

Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS system coupled with a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph was used in all experiments. Liquid sample injections were performed with a Thermo Scientific™ TriPlus™ RSH™ autosampler, using the Thermo Scientific™ Instant Connect Programmed Temperature Vaporizing (PTV) injector for the TRACE 1300 GC system. Compound separation was achieved on a Thermo Scientific™ TG-PBDE™ 15 m × 0.25 mm I.D. × 0.10 µm film capillary column (P/N 26061-0350). The mass spectrometer was tuned and calibrated in <1.5 min using FC43 (CAS 311-89-7) to achieve mass accuracy of <0.5 ppm. The system was operated in electron ionization mode (EI) using full-scan, and 60,000 mass resolution (full width at half maxima, measured at m/z 200). Additional details of instrument parameters are shown in Table 1 and Table 2.

### Data Analysis

Data were acquired and processed using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS), version 7.2. Chromeleon CDS allows the analyst to build acquisition, processing, and reporting methods for high-throughput analysis, with easy data reviewing and data reporting.

**Table 1. GC and injector conditions.**

TRACE 1310 GC Parameters	
Injection Volume (µL):	1.0
Liner:	PTV baffled liner (Siltek) (P/N: 453T2120)
Inlet (°C):	40
Carrier Gas, (mL/min):	He, 1.5
Inlet Module and Mode:	PTV, Large Volume mode
Column:	Thermo Scientific™ TG-PBDE™ 15 m x 0.25 mm I.D. x 0.10 µm film capillary column (P/N 26061-0350)
Transfer delay (min):	0.2
Injection time (min):	0.1

PTV Parameters:	Rate (°C/sec)	Temperature (°C)	Time (min)	Flow (mL/min)
Injection	-	40	0.10	-
Transfer	2.5	330	5.00	-
Cleaning	14.5	330	5.00	50

Oven Temperature Program:	RT (min)	Rate (°C/min)	Target Temperature (°C)	Hold Time (min)
Initial	0	-	100	2.00
Final	2.00	30	340	3.00
Run Time	13	-	-	-

**Table 2. Mass spectrometer conditions.**

Exactive GC Mass Spectrometer Parameters	
Transfer line (°C):	300
Ionization type:	EI
Ion source (°C):	250
Electron energy (eV):	35
Acquisition Modes:	Targeted SIM
Mass range (Da):	68 - 1000
Mass resolution (FWHM):	60,000 (FWHM at m/z 200)

Calibration standards (BDE-CSV-G), containing 27 native PBDE congeners at five concentration levels, and 16 (13C labelled) PBDE internal standards, were acquired from Wellington Laboratories, Inc. (Ontario, Canada). Targeted screening experiments were developed for the PBDE congeners considered.<sup>5</sup>

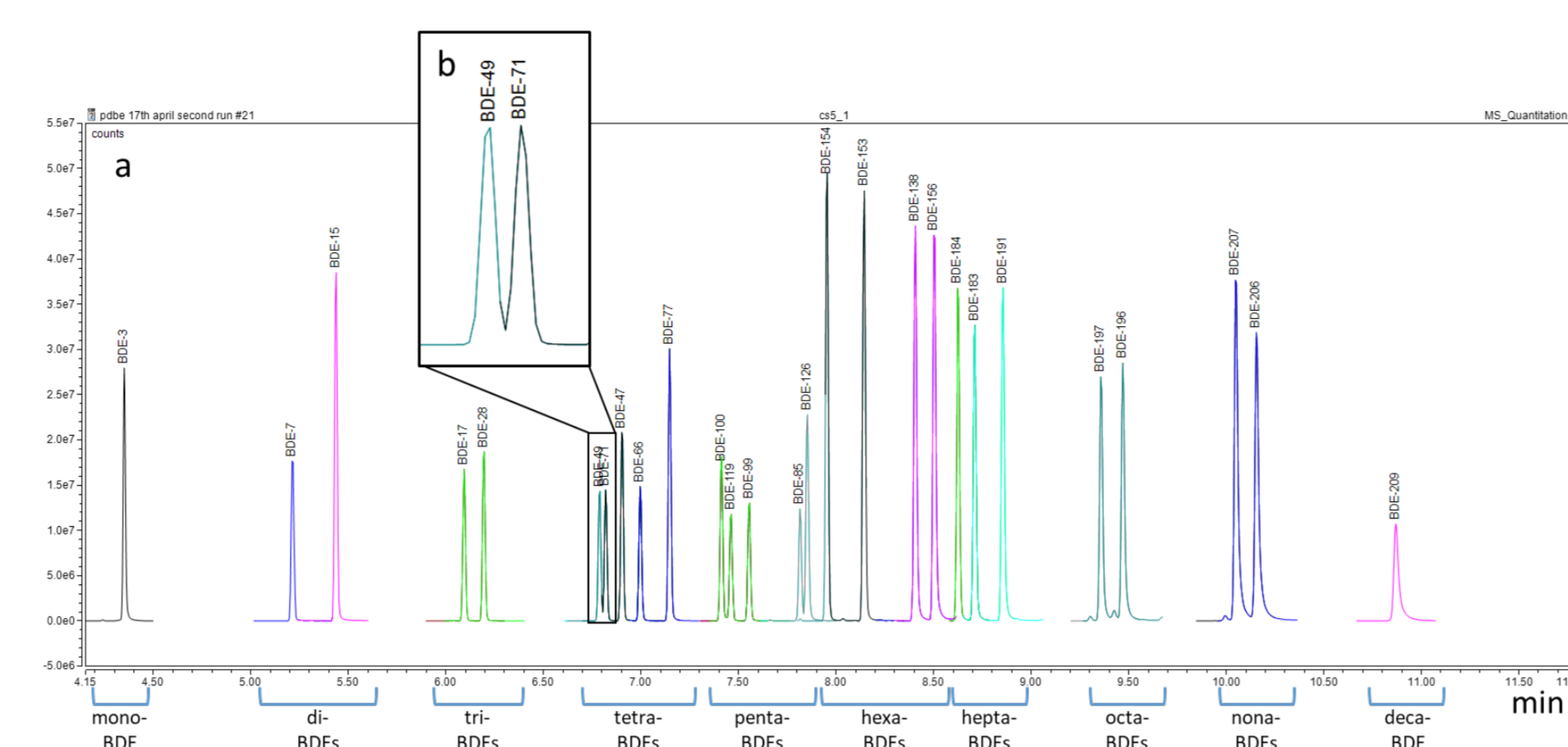
## RESULTS

The objective of this study was to evaluate the utility of Orbitrap-based GC-MS technology for the quantification of PBDEs to increase sample throughput and laboratory productivity. Various analytical parameters, including chromatographic resolution, instrument sensitivity, instrument detection limit, mass accuracy, peak area repeatability, and linearity were assessed.

### Chromatography

Good chromatographic separation, in under 13 minutes, was obtained. Extracted ion chromatograms (EICs) for 27 native PBDE congeners in a mixed solvent standard are shown in Figure 2a, with the excellent chromatographic resolution of the critical pair (BDE-49 and BDE-71) highlighted (Figure 2b).

**Figure 2. (a) Overlaid extracted ion chromatogram (EIC ±5 ppm extraction window) chromatograms for the 27 native PBDE congeners in a solvent standards at 400 pg on column for mono- to penta-PBDEs, 800 pg on column for hexa- to octa-PBDEs, and 2000 pg on column for nona- to deca-PBDEs, and (b) separation of critical pair (BDE-49 and BDE-71).**



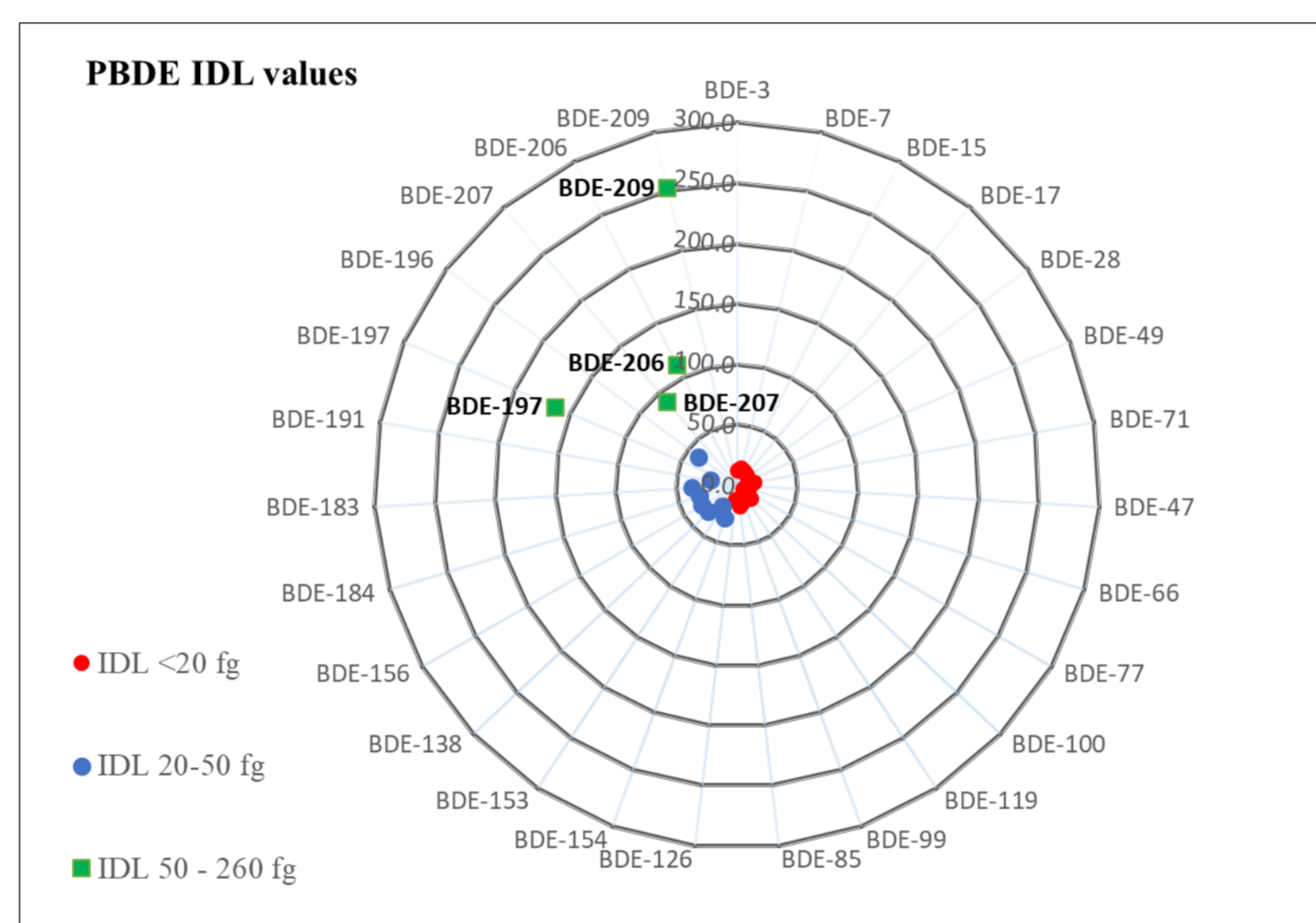
### Sensitivity

All BPDEs were detected in the lowest calibration standard, 1.0 ng/mL for mono- to penta-PBDEs, 2.0 ng/mL for hexa- to octa-PBDEs, and 5 ng/mL for nona- to deca-PBDEs.

### Estimation of Instrument Detection Limit (IDL)

System sensitivity, defined as instrumental detection limit (IDL) were determined experimentally for each compound by performing n=12 replicate injections of the lowest serially diluted solvent standard. Calculations of IDLs were made using one-tailed student t-test at the 99% confidence interval for the corresponding degrees of freedom, and taking into account the concentration on column for each PBDE congener and absolute peak area %RSD (Figure 3).

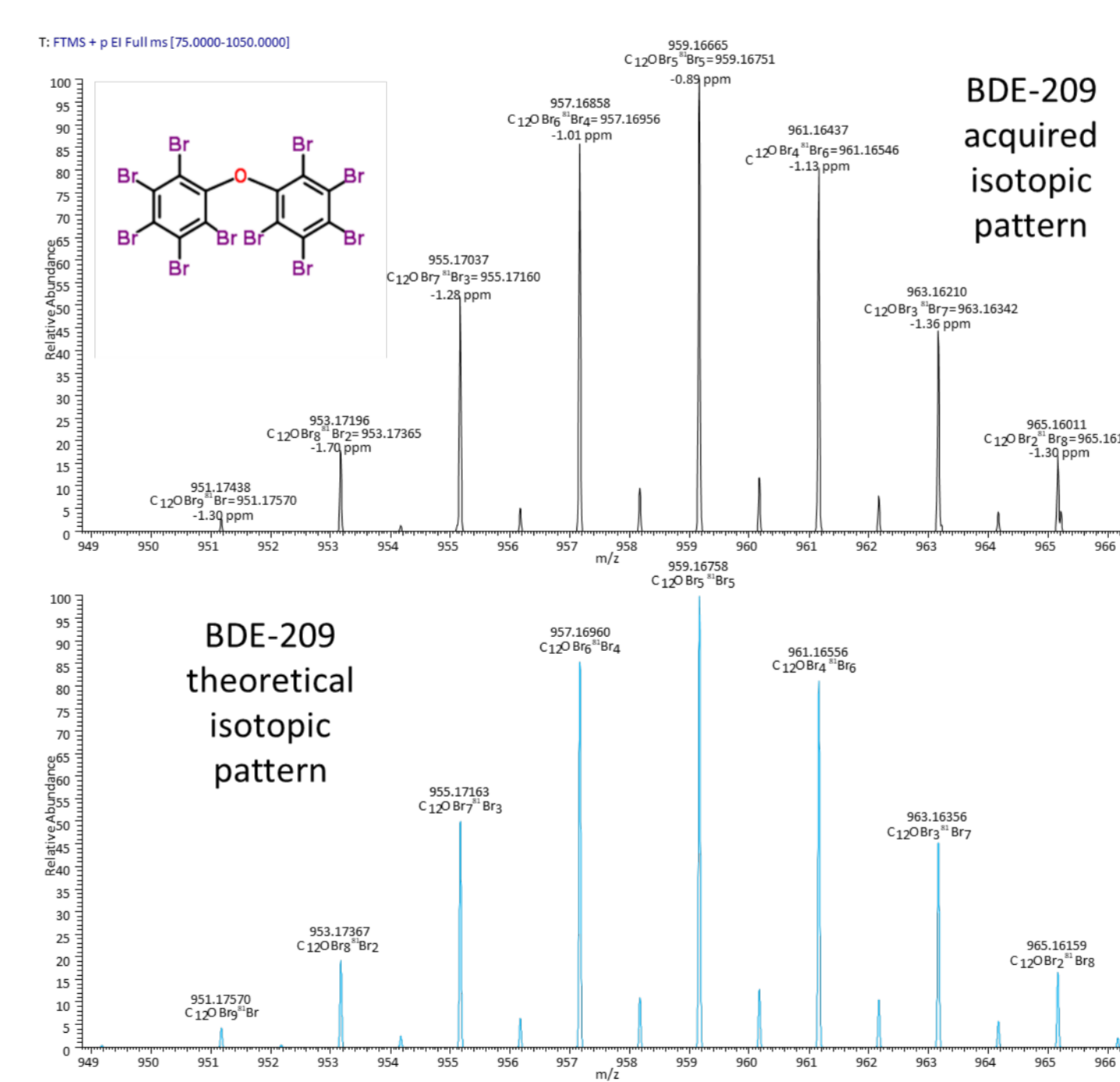
**Figure 3. Calculated IDL values for 27 native PBDE congeners, statistically calculated from the results of 12 replicate injections of the lowest serial diluted bottom mixed standard.**



### Mass accuracy

Maintaining mass accuracy and spectral fidelity is critical for correct compound identification in complex environmental samples. Figure 4, illustrates the mass accuracy and the isotopic pattern match achieved for BDE-209 with mass accuracy of <1.7 ppm consistently achieved for each ion in the isotopic cluster.

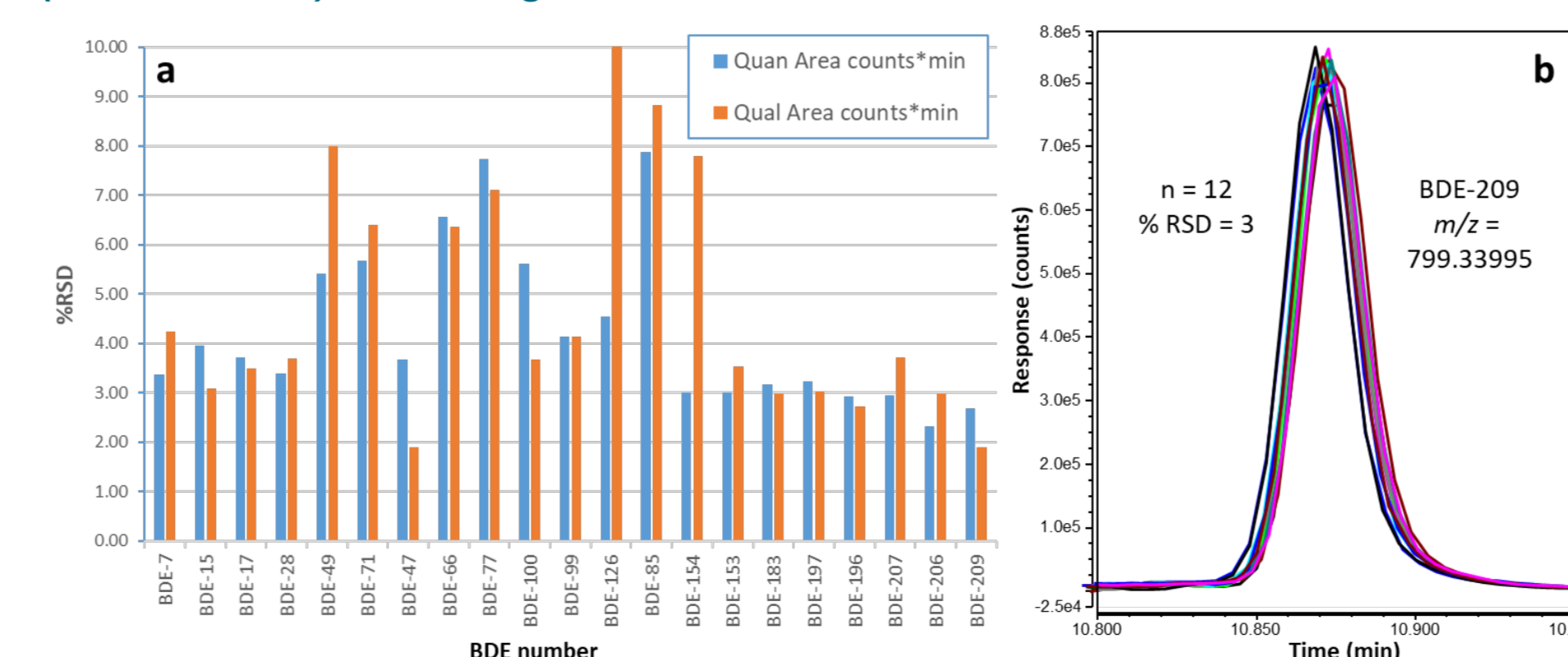
**Figure 4. Comparison of mass spectra for BDE-209, acquired isotopic pattern (upper) versus the theoretical isotopic pattern (lower). Consistent <1.5 ppm mass accuracy obtained for each of the ion in the cluster. Annotated are the measured mass, the elemental composition and the theoretical mass as well as the mass accuracy (ppm).**



### Peak area repeatability in matrix

In order to have confidence in routine PBDE quantification results achieved, stability or responses in matrix is critical. Repeatability of PBDE responses in matrix were assessed by carrying out n=12 repeat injections of a filter dust sample extract. Excellent repeatability was obtained as shown in Figure 5a, with RSD% for quantification and qualifier peak area counts between 2 and 10% for all identified congeners, and Figure 5b, overlaid EIC (m/z 799.33995) chromatograms for BDE-209.

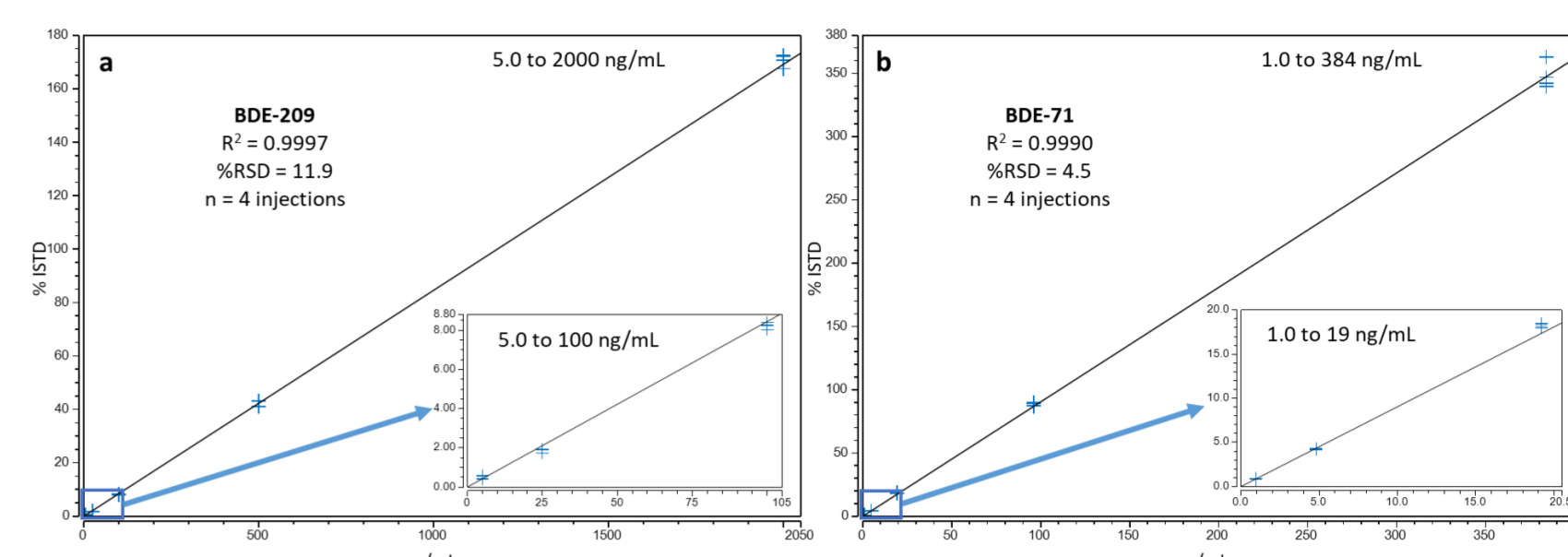
**Figure 5. Replicate injections (n=12) of a filter dust sample, a) quantification and qualification area counts %RSD values for identified congeners, b) overlaid EIC (m/z 799.33995) chromatograms for BDE-209.**



### Linearity of response

To assess compounds linearity, five calibration levels (pg on column 1 to 400 for mono- to penta-PBDEs, 2 to 800 for hexa- to octa-PBDEs, and 5 to 2000 for nona- to deca-PBDEs) were quantified using isotopic dilution for all the congeners considered. For all BDEs excellent linearity were obtained with R<sup>2</sup> values >0.998 and residual values %RSD <13%.<sup>5</sup> Example calibration responses for PCB-209 and PCB-71 are shown in Figure 6 were both the coefficient of determination (R<sup>2</sup>) and the residual %RSD are annotated.

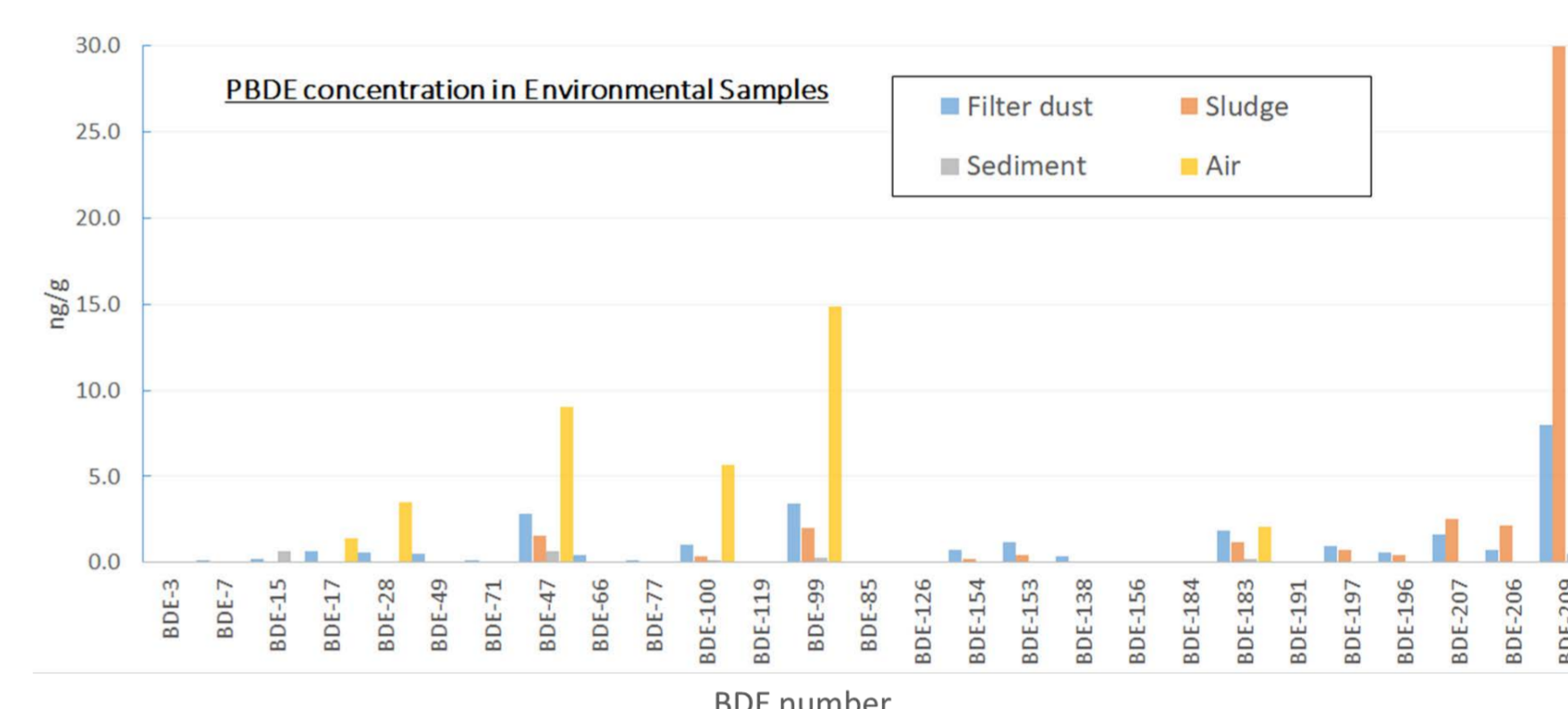
**Figure 6. Calibration curves for (a) BDE-209 and (b) BDE-71, illustrating the linearity obtained. The inset calibration curves exemplify the maintained linearity for the lowest 3 calibration points.**



### Sample analysis

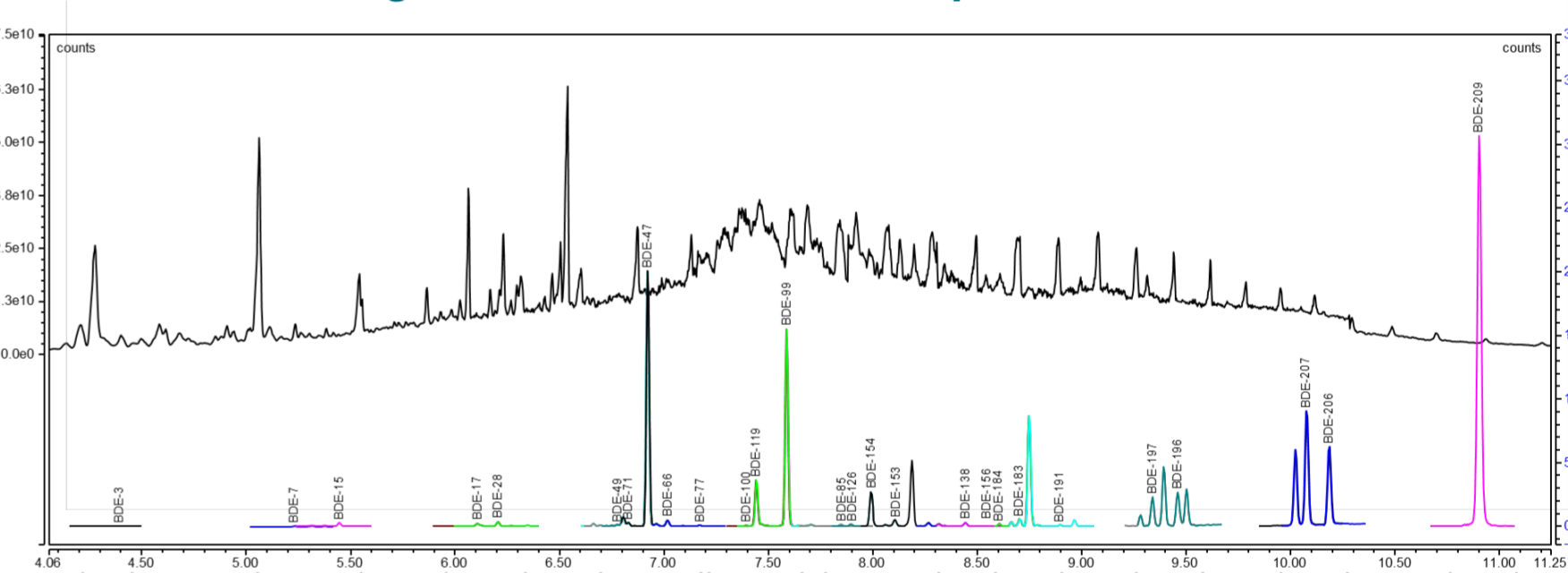
Samples of sludge, sediment, filter dust and air were prepared and analyzed as detailed, concentrations of the PBDEs identified are illustrated in Figure 7. The samples analyzed were extracted and quantified using isotopic dilution, using the mass-labelled PBDE surrogate standards, added to the sample prior to extraction as internal standards, and the mass-labelled PBDE recovery standard added to the extract prior to analysis as a syringe recovery standard.

**Figure 7. Calculated concentration of PBDEs, extracted and quantified from filter dust, sludge, sediment and air samples. Illustrating the predominant PBDE congeners identified in the analyzed sludge samples as BDE-209, 206, 207 and 99, filter dust samples as BDE-209, 47 and 99, air samples as BDE-99, 47 and 100 and sediment samples as BDE-15, 47 and 99.**



Examples of the complexity of extracted samples analyzed is shown as a total ion chromatogram (TIC) versus overlaid EICs for a sludge sample (Figure 8), where the predominant PBDE congeners identified were BDE-209, 207, 206, 99, 47 and 183. TIC and PBDE EICs signal intensities (Y-axis) were normalized to simplify the visual comparison. These results achieved demonstration excellent selectivity, and sensitivity for the analysis of PBDEs even in the most complex samples. Moreover, the routine high resolution offers excellent selectivity in difficult matrices, and the mass accuracy obtained allows for unambiguous identification, and elemental composition confirmation of chemical contaminants.

**Figure 8. Sludge sample chromatograms: (upper) TIC full scan; (lower) EICs for the native PBDE congeners identified in the sample.**



## CONCLUSIONS

- The results of this study demonstrate that the Exactive GC Orbitrap GC-MS coupled with a TRACE 1310 GC system provides an excellent solution for routine quantification of PBDEs in complex environmental samples.
- The predominant PBDE congeners identified, confirmed, and quantified in the samples were BDE-209, 206, 207, and 99 in sludge, BDE-209, 47, and 99 in filter dust, BDE-99, 47, and 100 in air, and BDE-15, 47, and 99 in sediment.
- Using a TG-PBDE 15 m capillary column, good chromatographic separation in <11 minutes for all the PBDE congeners was achieved, with excellent chromatographic resolution of the critical pair (BDE-49 and BDE-71).
- Outstanding peak area repeatability of PBDE responses in matrix with RSD% for quantification and qualifier peak area counts between 2% and 10% for all identified congeners, an important analytical parameter for routine GC-MS workflows.
- Compound linearity was demonstrated with R<sup>2</sup> >0.995 and residual values RSD% <13%, over five calibration levels.
- All PBDEs were detected in the lowest calibration standard, 1.0 ng/mL for mono- to penta-PBDEs, 2.0 ng/mL for hexa- to octa-PBDEs, and 5 ng/mL for nona- to deca-PBDEs. Instrumental detection limits between 6 and 250 fg on column were achieved for the PBDEs targeted.
- Chromeleon CDS software offers an ideal solution for the targeted isotopic dilution quantification of PBDEs in environmental samples with user-friendly data processing and reporting features.

## REFERENCES

- Alaee, M.; Arias, P.; Sjodin, A.; Bergman, A. An overview of commercially used brominated flame retardants, their applications, their use pattern in different countries/regions, and possible modes of release. *Environ Int.*, **2003**, *29*, 683–699.
- Hites, R. A. Polybrominated Diphenyl Ethers in the Environment and in People: A Meta-Analysis of Concentrations. *Environ. Sci. Technology*, **2004**, *38*, 945–956.
- Costa, L.G.; Giordano, G.; Tagliareri, S.; Caglieri, A.; Mutti, A. Polybrominated diphenyl ether (PBDE) flame retardants: environmental contamination, human body burden and potential adverse health effects. *Acta Bio-medica*, **2008**, *79*(3), 172–183.
- Guidance for the inventory of polybrominated diphenyl ethers (PBDEs) listed under the Stockholm Convention on POPs. [Online] <http://chem.pops.int/Implementation/NationalImplementationPlans/Guidance/GuidancefortheinventoryofPBDEs/tabid/3171/Default.aspx> (accessed May 8, 2018).
- Cooper J.; Parera J.; A Esteban.; R Law.; C Cojocariu. Overcoming analytical challenges for polybrominated diphenyl ethers (PBDEs) analysis in environmental samples using gas chromatography – Orbitrap mass spectrometry. Thermo Scientific Application Note 10644, September 2018.

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