Method development for the identification of novel brominated flame retardants using a Q Exactive HRAM mass spectrometer

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Overview

Purpose: Identification of legacy and novel brominated flame retardants in dust samples on quadrupole Orbitrap benchtop instrumentation.

Methods: Identification and screening of brominated substances of interest was conducted in dust samples.

Results: Dust might be a relevant source of brominated flame retardants and therefore interesting for human exposure. HRAM instrumentation aids in the identification of these compounds.

Introduction

Due to legislative restrictions on manufacture and use of some brominated flame retardants (BFRs), several new chemicals (NBFRs) have been developed. To explore their presence in different environmental compartments and ultimately understand their environmental fate, analytical methods for targeted analysis are required [1]. Classically these compounds are determined by GC-based instrumental methods. In recent years, LC-based methods coupled to low resolution mass spectrometers have also been developed [2]. Advances in high resolution mass spectrometry facilitate accurate measurements and identification of unknowns, including degradation and transformation products. Moreover, bromine isotopic pattern analysis as well as the use of mass defect plots and filters, helps identify relevant substances, with such techniques starting to be more commonly used in environmental science [3].

Methods

Sample Preparation

0.5 g of dust was weighed, spiked with mass-labeled TBBP-A and mixed with diatomaceous earth as dispersant. Extraction was conducted using a Thermo Scientific™ Dionex™ ASE™ 350 accelerated solvent extractor and in-cell cleanup (Figure 1). Chemical adsorbents silica and Florisil™ were employed to reduce possible matrix interferences. Extraction solvents included hexane, dichloromethane and acetone. Samples were evaporated to dryness using a Thermo Scientific Rocket™ Evaporator system and then reconstituted in 2 mL of methanol:toluene (1:1). The extraction instrumentation is shown in Figure 2.

FIGURE 1. Schematic of selective extraction using Thermo Scientific ASE extraction cells
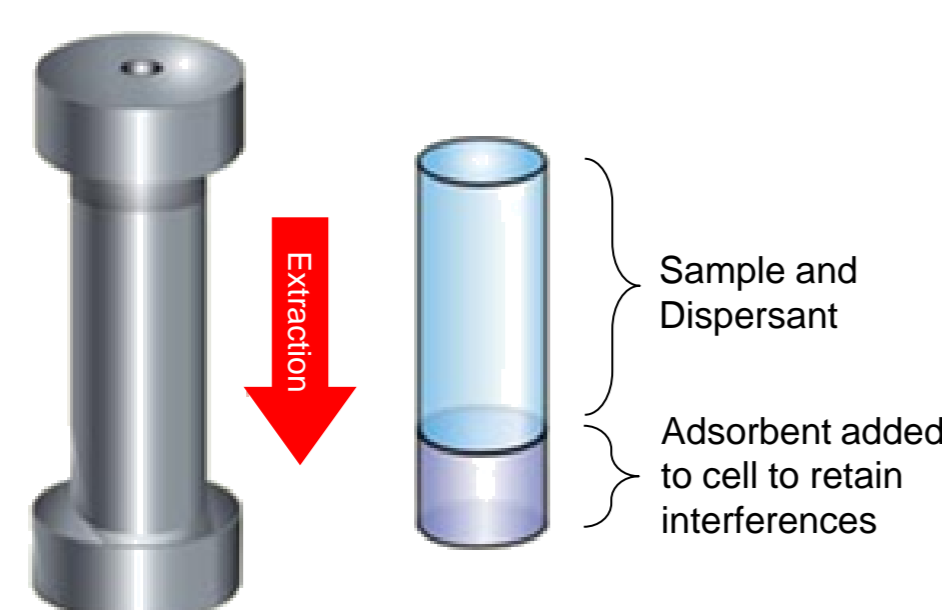

Sample and Dispersant

Adsorbent added to cell to retain interferences

FIGURE 2. Thermo Scientific Dionex ASE 350 and Thermo Scientific Rocket Evaporator system

Liquid Chromatography

Final extracts were separated on a Thermo Scientific Accucore™ RP-MS 100x2.1mm, 2.6µm column on a Thermo Scientific Accela™ HPLC system (Accela 1250 Pump and Open autosampler – Figure 3), using a 16 minutes gradient elution program with water (mobile phase A) and methanol (mobile phase B) at a flow rate of 400 µl/min. The gradient started at 20% phase B, was ramped to 40% B in 4 minutes, then further to 100% B in 5 minutes and was held for 3 minutes at 100% B. Initial conditions were restored within 0.1 minutes, with a final equilibration of 4 minutes.

Mass Spectrometry

Mass spectrometric analysis was run on a Thermo Scientific Q Exactive™ hybrid quadrupole-Orbitrap™ mass spectrometer (Figure 3) with an APCI source, using optimized conditions.

MS-parameter for screening purposes
Full Scan
- APCI negative
- R = 70,000
- *m/z* 300–1000

FIGURE 3. Q Exactive with Thermo Scientific Accela 1250 Pump and Open autosampler


Data Analysis

Raw data files were processed using both Thermo Scientific Xcalibur™ and Thermo Scientific Compound Discoverer software. The workflow used for screening of environmental unknowns is shown in Figure 4. It includes the detection of unknowns compounds, elemental composition predictions, hides background from blanks, performs a ChemSpider library search and scores the compounds based on selected bromine isotope patterns.

In addition, mass defect plots were created using Microsoft™ Excel™ to visualize the presence of brominated compounds. These chemicals have a unique negative mass defect, which readily distinguishes them from other molecules in a complex mass spectrum. One mass scale applicable to environmental analytical chemistry is defined by the substitution of a hydrogen atom by a chlorine atom (H/Cl). In analogy to the Kendrick mass calculation, the conversion used here is based on the multiplication of each peak of a chosen mass spectrum by 34/33.96102 and plotting the nominal mass vs. the transformed mass defect.

FIGURE 4. Workflow of the environmental unknown screening using Thermo Scientific Compound Discoverer
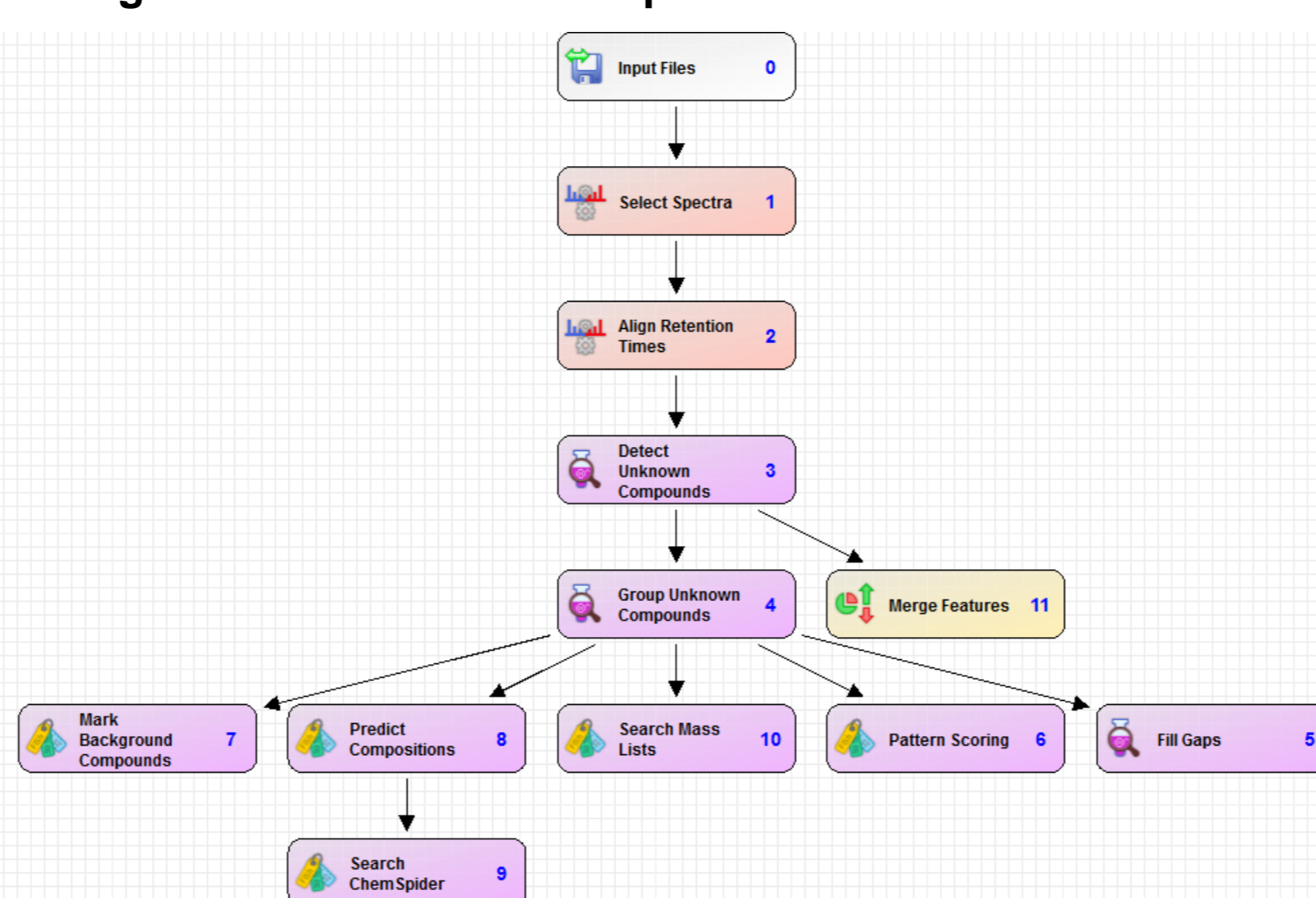
Sampling

Sampling strategy

All dust samples were taken in offices, laboratories and instrument assembly rooms of the Thermo Scientific Bremen factory site. The samples were collected according to an established protocol [4]. Shortly, in offices 1 m² of carpet was vacuumed for 2 min and in case of bare floors 4 m² was vacuumed for 4 min. Samples were collected using nylon sample socks (25 µm pore size) that were mounted in the furniture attachment tube of the vacuum cleaner. Prior to analysis samples were stored in sealed bags at 4°C.

Results

Method

The use of different extraction solvent mixtures was investigated to obtain the optimal extraction results. A mixture of hexane:dichloromethane (3:1) showed better extraction results compared to hexane:acetone (3:1) – compare Figure 7 (TBBP-A). Other chemical adsorbents, such as copper, alumina or activated carbon might need to be included in the in-cell cleanup depending on the complexity of the matrix. Dust samples collected in the instrument assembly rooms showed to contain more brominated compounds, compared to dust taken in offices and laboratories. This is most probably due to the increased presence of electronic equipment containing flame retardants in these areas.

Mass defect plot

To obtain an initial estimate of the number of brominated compounds within the dust samples, mass defect plots were calculated and plotted as shown in Figure 5. Isotopic pattern of compounds can be recognized. As an example polybrominated diphenyl ethers (PBDEs) – penta through deca - are plotted all in one band and separated by 79 Da increments, i.e. corresponding to the mass of bromine minus a hydrogen atom.

Screening

The suspect screening results using Compound Discoverer showed a large fraction of brominated flame retardants in the samples, both legacy BFRs, as well as NBFRs. For a suspect the following confirming criteria were selected: spectral similarity score (SFit>80%) and accurate mass deviation < 5 ppm. Confirmation was conducted through the measurement of reference standards where available. However additional MS/MS measurements might be needed. The use of high-resolution accurate mass (HRAM) instrumentation facilitates identification of compounds and unknowns by means of selectivity, elemental compositions and isotopic pattern scoring. The use of relevant databases, such as ChemSpider, can help identify compounds.

FIGURE 5. Mass defect plot of dust sample transformed with H/Br scale 79 / 77.91051 scale
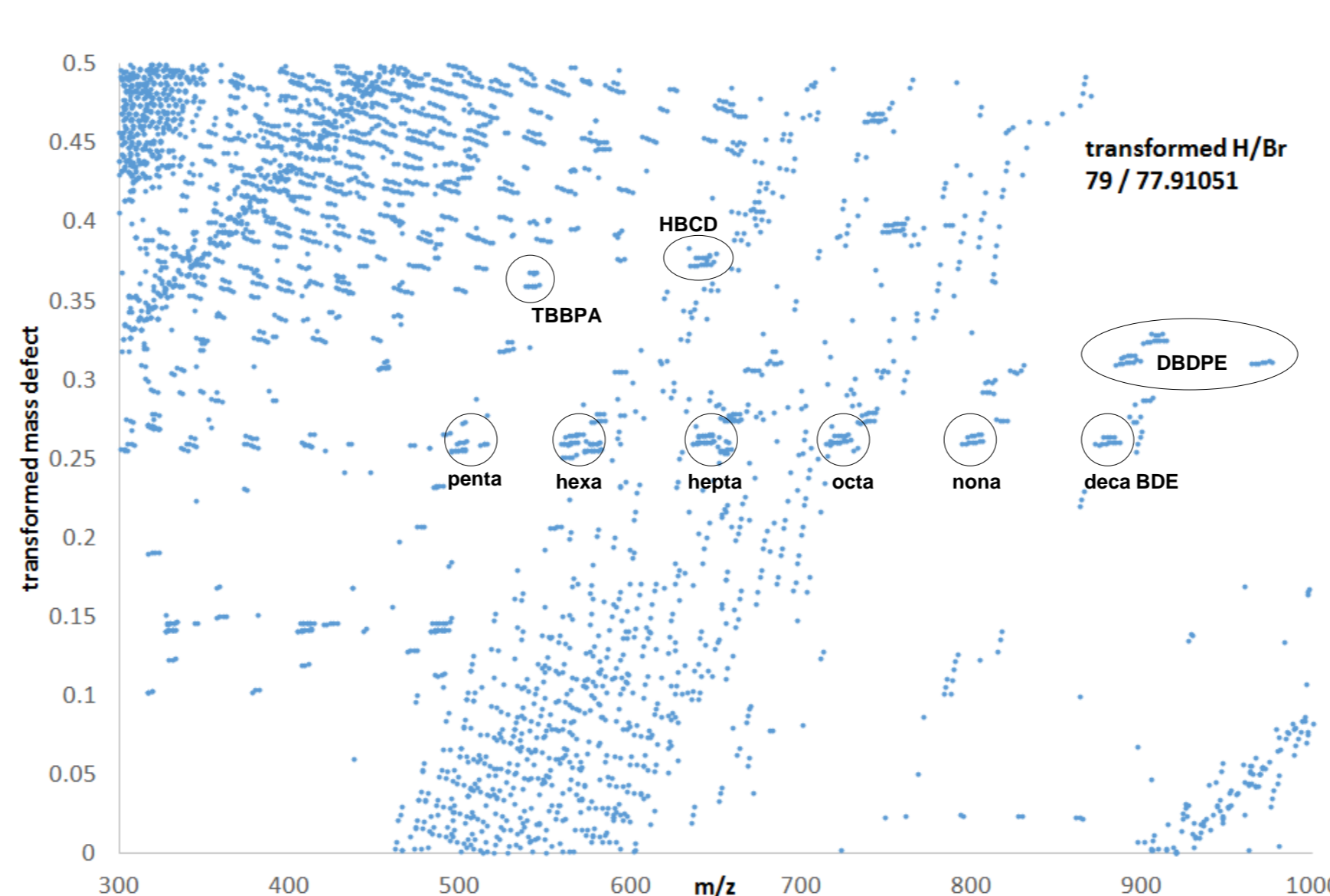

Selected compounds are shown in Table 1 and Figure 6. The formation of different quasi-molecular ions in APCI for these compounds has been reported in literature. Here, the most intense ions are shown. Compounds detected in the screening include: 2-ethylhexyltetrabromobenzoate (EH-TBB), decabromodiphenyl ethane (DBDPE), bis(2-ethylhexyl)tetrabromophtalate (BEH-TBP), tetrabrombisphenol A (TBBP-A) and hexabromcyclododecane (HBCD), in addition to the afore mentioned PBDEs.

TABLE 1. Selected (N)BFRs in dust sample

Suspect compound	Formula	quasi-molecular ion and m/z		Δ m/z (ppm)	Time (min)
EH-TBB	C15H18Br4O2	[M-Br+O]-	484.87706	0.08	10.24
BEH-TBP	C24H34Br4O4	[M-C8H17+H-Br]-	512.87402	0.98	11.16
DBDPE	C14H4Br10	[M-Br+O]-	906.28362	0.82	12.06
TBBP-A	C15H12Br4O2	[M-H]-	542.74571	0.09	8.30
HBCD	C12H18Br6	[M-H]-	640.63746	0.23	9.02

FIGURE 6. XIC of selected (N)BFRs in dust sample
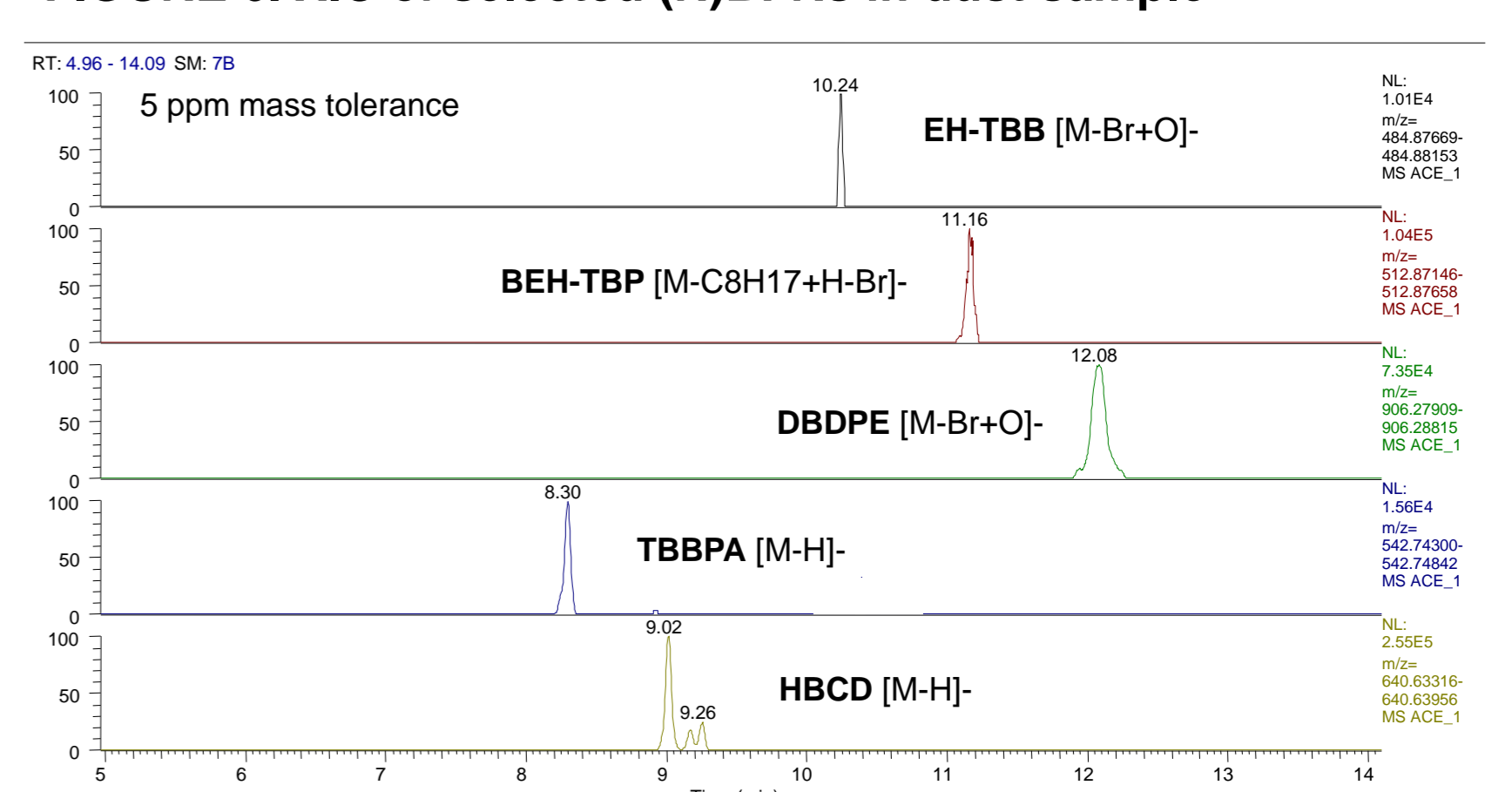
In addition, Compound Discoverer allows for unknown screening with unbiased peak detection within defined elemental composition ranges. Hereby additional halogenated compounds of interest that have not come to attention yet can be identified, as well as possible transformation products identified.

FIGURE 7. Results for HBCD (top) and TBBPA (bottom) in Compound Discoverer

Conclusion

Several legacy BFRs and NBFRs were identified in dust samples. This indicates the relevance of dust containing chemicals as an entry pathway to human and environmental exposure. Further studies to elucidate the environmental fate, especially of the novel compounds, have to be conducted.

Mass defect plots are a useful instrument to greatly simplify any chosen mass spectrum. Compound Discoverer is a powerful tool for unknown screening and structural identification of compounds in environmental sciences.

Acknowledgment

References

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