

# Method Development for the Identification of Novel Brominated Flame Retardants Using a Q Exactive HRAM Mass Spectrometer

Aristide P. Ganci<sup>1</sup>, Tabiwang N. Arrey<sup>2</sup>, Thomas Moehring<sup>2</sup>, and Stuart Harrad<sup>1</sup>

<sup>1</sup>University of Birmingham, School of Geography, Earth and Environmental Sciences, Birmingham, United Kingdom

<sup>2</sup>Thermo Fisher Scientific, Bremen, Germany

## OVERVIEW

**Purpose:** Identification of legacy and novel brominated flame retardants in dust samples on Thermo Scientific™ 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].

## MATERIALS AND 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.

### 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 at m/z 200
- m/z 300–1000

### 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/C). 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.

## 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 m<sup>2</sup> of carpet was vacuumed for 2 min and in case of bare floors 4 m<sup>2</sup> 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.

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

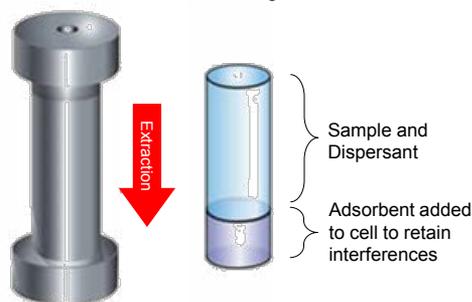


Figure 2. Thermo Scientific Dionex ASE 350 and Thermo Scientific Rocket Evaporator system



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



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

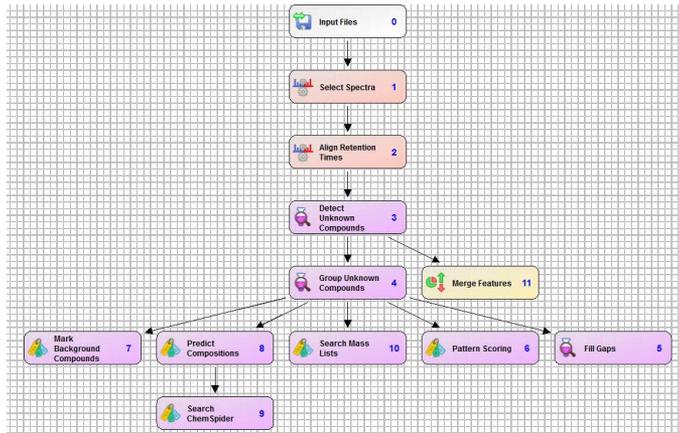
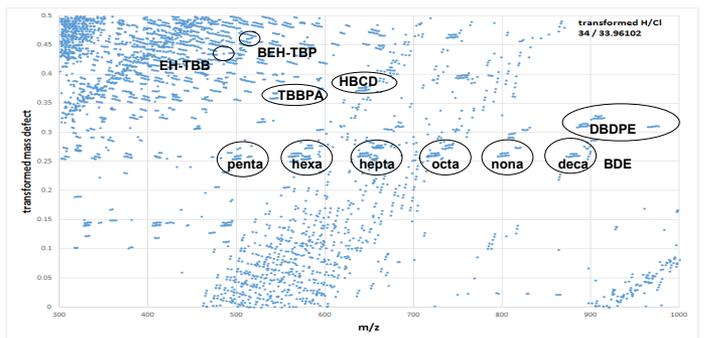


Figure 5. Mass defect plot of dust sample transformed with H/C1 scale 34 / 33.96102 scale



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-ethylhexyltetra bromobenzate (EH-TBB), decabromodiphenyl ethane (DBDPE), bis(2-ethylhexyl)tetra bromophthalate (BEH-TBP), tetrabromobisphenol A (TBBP-A) and hexabromocyclododecane (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	$\Delta$ m/z (ppm)	Time (min)
EH-TBB	C <sub>15</sub> H <sub>18</sub> Br <sub>4</sub> O <sub>2</sub>	[M-Br+O] <sup>-</sup> 484.87706	0.08	10.24
BEH-TBP	C <sub>24</sub> H <sub>34</sub> Br <sub>4</sub> O <sub>4</sub>	[M-C <sub>8</sub> H <sub>17</sub> +H-Br] <sup>-</sup> 512.87402	0.98	11.16
DBDPE	C <sub>14</sub> H <sub>4</sub> Br <sub>10</sub>	[M-Br+O] <sup>-</sup> 906.28362	0.82	12.06
TBBP-A	C <sub>15</sub> H <sub>12</sub> Br <sub>4</sub> O <sub>2</sub>	[M-H] <sup>-</sup> 542.74571	0.09	8.30
HBCD	C <sub>12</sub> H <sub>18</sub> Br <sub>6</sub>	[M-H] <sup>-</sup> 640.63746	0.23	9.02

Compounds: (2-ethylhexyl)tetra bromobenzate (EH-TBB), bis(2-ethylhexyl)tetra bromophthalate (BEH-TBP), decabromodiphenyl ethane (DBDPE), tetrabromobisphenol A (TBBP-A), hexabromocyclododecane (HBCD)

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



## 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.

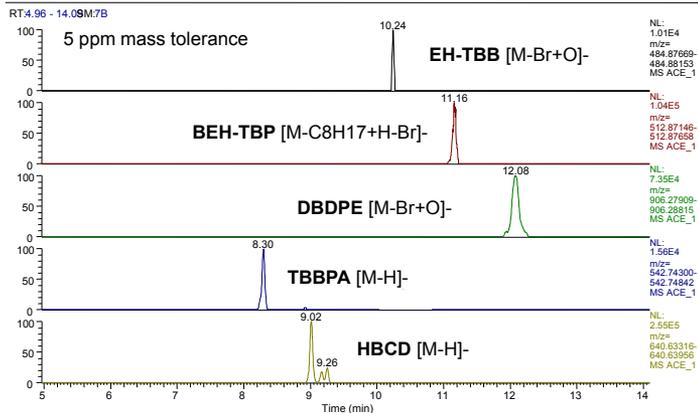
### Mass defect plot

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 (SF1)>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 6. XIC of selected (N)BFRs in dust sample



## CONCLUSIONS

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.

## REFERENCES

- Covaci, A., et al., Novel brominated flame retardants: a review of their analysis, environmental fate and behaviour. *Environment International*, 2011. 37(2): p. 532-56.
- Zhou, S.N., et al., Development of liquid chromatography atmospheric pressure chemical ionization tandem mass spectrometry for analysis of halogenated flame retardants in wastewater. *Analytical and Bioanalytical Chemistry*, 2010. 396(3): p. 1311-20.
- Jobst, K.J., et al., The use of mass defect plots for the identification of (novel) halogenated contaminants in the environment. *Analytical and Bioanalytical Chemistry*, 2013. 405(10): p. 3289-97.
- Abdallah, M.A., S. Harrad, and A. Covaci, Hexabromocyclododecanes and Tetrabromobisphenol-A in Indoor Air and Dust in Birmingham, UK - Implications for Human Exposure. *Environmental Science & Technology*, 2008. 42: p. 6855-6861.

## ACKNOWLEDGEMENTS

The research leading to these results has received funding from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme FP7/2007-2013/ under REA grant agreement n° 606857.

[www.thermofisher.com](http://www.thermofisher.com)

©2016 Thermo Fisher Scientific Inc. All rights reserved. Florisil is a trademark of U. S. Silica Company, ChemSpider is a trademark of the Royal Society of Chemistry. Microsoft and Excel are registered trademarks of Microsoft Corporation ISO is a trademark of the International Standards Organization. All other trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.



Thermo Fisher Scientific,  
Sunnyvale, CA USA is  
ISO 9001 Certified.

**Africa** +43 1 333 50 34 0  
**Australia** +61 3 9757 4300  
**Austria** +43 810 282 206  
**Belgium** +32 53 73 42 41  
**Brazil** +55 11 2730 3006  
**Canada** +1 800 530 8447  
**China** 800 810 5118 (free call domestic)  
 400 650 5118

**Denmark** +45 70 23 62 60  
**Europe-Other** +43 1 333 50 34 0  
**Finland** +358 10 3292 200  
**France** +33 1 60 92 48 00  
**Germany** +49 6103 408 1014  
**India** +91 22 6742 9494  
**Italy** +39 02 950 591

**Japan** +81 6 6885 1213  
**Korea** +82 2 3420 8600  
**Latin America** +1 561 688 8700  
**Middle East** +43 1 333 50 34 0  
**Netherlands** +31 76 579 55 55  
**New Zealand** +64 9 980 6700  
**Norway** +46 8 556 468 00

**Russia/CIS** +43 1 333 50 34 0  
**Singapore** +65 6289 1190  
**Sweden** +46 8 556 468 00  
**Switzerland** +41 61 716 77 00  
**Taiwan** +886 2 8751 6655  
**UK/Ireland** +44 1442 233555  
**USA** +1 800 532 4752  
 PN64759-EN 0616S

**Thermo**  
SCIENTIFIC

A Thermo Fisher Scientific Brand