Screening halogenated contaminants in the marine environment based on high resolution mass spectrometry profiling

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Introduction

Polyhalogenated chemicals constitute a wide sub group of organic environmental contaminants and many of them are subjected to restrictions or bans due to the risks for the environmental and human health. Monitoring environmental contaminants is a major scientific activity supporting risk assessments. Highly specific and sensitive targeted analytical methods involving chromatography and mass spectrometry couplings are available for a wide range of known substances. Most of the time, however, only the pieces of information required to maximize the specificity and sensitivity for the targeted substance(s) are acquired. With the broad variety of polyhalogenated compounds, unknown substances (*e.g.* unidentified substances, transformation products) are likely to occur in the environment as additional potential contaminants. Investigating unknowns proactively and efficiently is challenging, targeted available methods being unfitted for this purpose.

Based on two specific physical-chemical properties of bromine and chlorine, we previously developed an innovative analytical strategy using high-resolution mass spectrometry (HRMS) data fingerprints to seek unknown polyhalogenated substances in complex matrices[1]. The first property takes advantage of the discriminant mass defect (MD) engendered by heavy heteroatoms such as bromine and chlorine compared to usual atoms composing organic substances, typically C, H, O, N. An elegant way to visualize and interpret the signals is to plot the *m/z* fractional part versus *m/z* (MD plot). In 2010, Taguchi et al. [1] suggested a mass-scale based on an exact mass of 34.00000 for the substitution of a hydrogen by a chlorine, $-H/+^{35}Cl$,. Thus, polychlorinated homologues series (as well as polybrominated series) exhibit the same fractional part and are aligned horizontally on an H/Cl-scale MD plot. The second property involves characteristic isotopic pattern, due to the presence of two natural and stable isotopes with specific abundances for both halogens. Isotopic patterns allow determining the number of halogen atoms in the ions, which decrease the number of potential chemical formula.

Acquiring data in HRMS full scan of complex samples leads however to a huge number of signals. In order to support and facilitate the data processing step, bioinformatics tools were developed under the open source programming R environment. It involves two main steps (i) the automated integration of chromatograms using *xcms* package and (ii) a script, which pairs signals according to retention time and MD between C, Cl and Br isotopes.

In the present work, we applied the approach to two selected samples, a sediment and mussels from the river Seine mouth (France). Sediment is known to act as a reservoir for environmental contaminants, hydrophobic ones binding strongly with it. Mussels are filter feeder organisms that accumulate contaminants present in the water, often considered as a sentinel species for the marine environment.

Materials and methods

Sample preparation

Sediment sample was collected in 2002 and mussel samples in 2017, both from the river Seine mouth (France) a highly industrialized and urbanized area. Lipophilic compounds were extracted from 1 g freeze-dried matter by

Pressurized Liquid Extraction (ASE, Dionex Corp., CA, USA) pending two consecutive extractions, using dichloromethane, at 100 °C and 100 bar. The organic extracts were evaporated until 3 mL with a rotary evaporator. The extracts were transferred into hexane (5 mL) by evaporating dichloromethane under a gentle stream of nitrogen. Activated copper was added to the sediment extract to remove sulfur-containing compounds. The mussel extract was treated by successive liquid-liquid partitioning with concentrated sulfuric acid (2 × 3 mL) to remove lipids. The organic layer was neutralized with 2 × 5 mL ultrapure water and dried with anhydrous sodium sulfate. The purified extracts were spiked with 2.5 ng of $^{2}H_{18}$-β-HBCDD in toluene, evaporated to dryness under a gentle stream of nitrogen, reconstituted in a MeOH/H_2O 1:1 mixture (v/v, 50 µL) and were centrifuged prior to analysis.

LC-HRMS data acquisition

Extracts were analyzed with an UltiMate 3000 UHPLC pumping system coupled to an Orbitrap Q-Exactive mass spectrometer fitted with a heated Electrospray source (Thermo Fischer Scientific, San José, CA, USA). Instrument control and data processing were carried out by Chromeleon Xpress and Xcalibur softwares (Thermo Fischer Scientific). Chromatographic separation was achieved using reversed phase chromatography on a Hypersil Gold analytical column (100 mm × 2.1 mm, 1.9 µm) (Thermo Fischer Scientific) kept at 45 °C. Mobile phases consisted of 10 mM ammonium acetate in water (A) and in acetonitrile (B). The gradient began with (A/B) 95:5 (v/v) for 2 min, then ramped linearly to 50:50 over 10 min and to 0:100 over 2 min to be maintained for 5 min, and returned to 95:5 over 2 min. The flow rate was set at 0.4 mL min^{-1} and the sample injection volume to 10 µL. HRMS data were acquired in negative mode in full scan mode over the *m/z* range 120-1000 at a resolving power of 140,000 full width half maximum at *m/z* 200.

Post-acquisition data treatment

The open access msConvert software (ProteoWizard) was used to convert raw data (*.raw*) to the open format *.mzXML*. Datasets were then processed by the *xcms* package using centWave peak detection algorithm to extract chromatographic features. Peak picking parameters were as follows: method, "centWave"; ppm, 5; snthresh, 10; prefilterstep, 5; prefilter level, 10000; peakwidth, 5-60; noise, 0; mzdiff, 0.001. A table report in *.csv* file format was created where each features was defined by an exact mass (*m/z*), a retention time (min) and an intensity (area). Features were paired according to a method developed by Cariou et al. [1]. Paired isotopic clusters were manually investigated (pattern, extracted ion chromatograms) allowing to deduce the number of halogen atoms. Elemental composition assignments were performed *via* Xcalibur, considering usual elements (C, H, O, N, P, S, Cl and Br). Then, structural hypotheses were suggested.

Results and discussion

Sediment

The centWave function resulted in the detection of 11,940 features, among them 775 paired clusters were suspected to exhibit at least one chlorine or bromine atom. A total of 183 paired clusters were manually investigated (Figure 1a). It resulted in 111 elemental composition hypotheses (61%) and 77 chemical structure propositions (40%). External standard mass deviation was 0.80 mmu. Particular attention was paid to two remarkable series.

- On Figure 1b, series potentially corresponding to hydroxylated polychlorinated biphenyls (OH-PCBs, 3 occurrences), hydroxylated polychlorinated diphenyl ethers (OH-PCDEs, 6 occurrences) and hydroxylated and methoxylated polybrominated diphenyl ethers (OH-PBDEs, 2 occurrences; MeO-PBDE, 1 occurrence) are presented in orange, blue and purple, respectively. Some of these compounds were suggested as potential

biotransformation products of the related parent compounds

- Three clusters exhibiting the same H/Cl-scale fractional part and being separated by the characteristic vector -H/+Br were suspected to form a series of homologous polybrominated compounds (Figure 1b, in red). The chemical formula suggested was $C_{16}H_{12-x}Br_xN_2$, with $x \in [4–6]$ (Figure 2). Extracted ion chromatograms show a logical increasing in retention time with bromine increments as well as possible presence of isomers for Br_4- and Br_5-containing compounds.

Mussel

The centWave function resulted in the detection of 6,416 features, among which 385 paired clusters were identified. A total of 34 clusters were manually investigated. It resulted in 28 elemental composition hypotheses (82%) and 4 chemical structure propositions (12%). External standard deviation was 0.23 mmu. Particular attention was paid to two remarkable series.

- A set of three series of homologous polybrominated compounds with a general formula of $C_{18}H_{14-x}Br_xO_3$, with $x \in [5–7]$ was suggested. Only the pentabrominated cluster appeared with several isomers. The formula may match the tetradecabromodiphenoxybenzene ($C_{18}Br_{14}O_2$) pending debromation and hydroxylation. Further investigation is needed to confirm such hypothesis.
- A group of 11 clusters, possibly mixed halogenated compounds (–Cl/+Br, –H/+Cl and –H/+Br vectors), appeared to form 3 series, penta- to heptahalogenated, with chemical formulas being $C_8Br_xCl_yO$ (4 clusters), $C_8HBr_xCl_y$ (4 clusters) and a $C_8H_2Br_xCl_yO$ (2 clusters), with $x \in [1–4]$ and $y \in [2–6]$.

Conclusion and perspectives

The analytical strategy succeeded in highlighting - in both biotic and abiotic samples - numerous polyhalogenated compounds representative of different class of contaminants, confirming the relevance of the proposed strategy to offer global detection of emerging contaminants. Such approach fully answers current risk assessment expectations. Further experiments, including MS^2, derivatization and/or standard injections, will be necessary to gain structural information. Complementary analytical approaches such as gas chromatography and/or Atmospheric Pressure Chemical Ionization could also allow investigating different sample fractions, thus increasing the range of compounds properties. Finally, a homemade friendly-user application is being developed to manage efficiently the complete post-acquisition data treatment processing.

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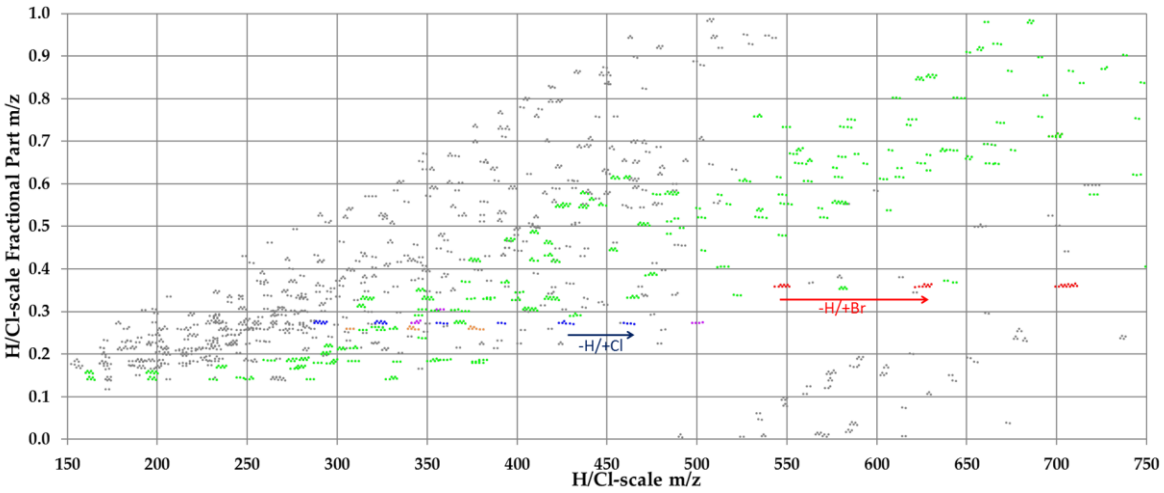

Figure 1: *H/Cl-scale MD plots obtained for sediment). Colors: investigated paired clusters.*

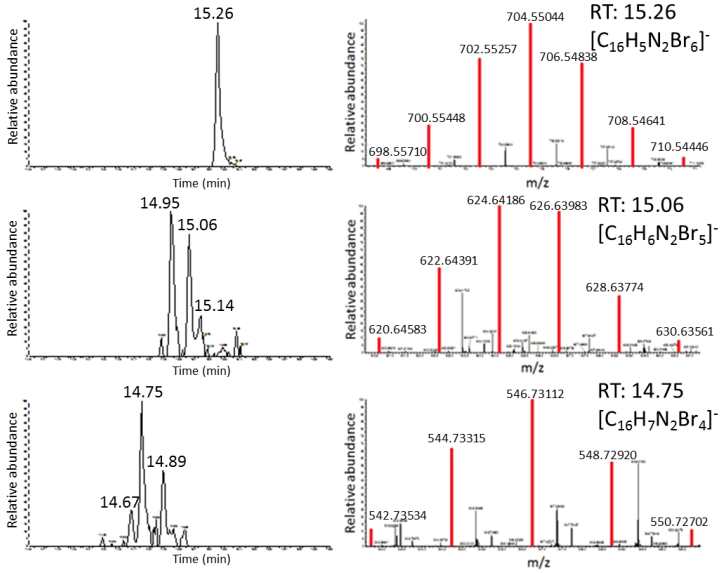

Figure 2: *Extracted ion chromatograms of base peaks (± 20 ppm) and mass spectrums (bromine isotopic contributions in red) of selected polybrominated series observed in the sediment sample.*