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FISHING HALOGENATED ENVIRONMENTAL CONTAMINANTS IN BIOTA BASED ON ISOTOPIC PATTERN AND MASS DEFECT PROVIDED BY HRMS PROFILING

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1. Introduction

Bioaccumulation of Persistent Organic Pollutants (POPs) is predictably associated with the compounds characterised by an ability to accumulate in the fatty tissues of living organisms. POPs are typically halogenated organic compounds. Untargeted methods are required to seek unknown degradation products or unknown/emerging exposure substances. Last generations of high resolution mass spectrometers (HRMS, e.g. Orbitrap or TOF) open the way to untargeted full scan footprints for addressing this issue [1]. However, large datasets arise from such couplings. Thus, extracting useful information stands as a central question, requiring the development of fit-for-purpose intelligent data processing, analysis and visualisation tools, supported by appropriate biocomputering solutions. In our work, we discuss the proof of concept of a new methodology capable of seeking unknown organohalogenated compounds in environmental sentinels. Our strategy relies on an automated peak picking step based upon the centWave function (xcms package, R environment), a VBA script developed for pairing features according to the exact mass difference between Cl and Br isotopes to filtering potential organohalogenated clusters among full scan HRMS datasets. H/Cl-scale mass defect (MD) plots were used to visualize the datasets before and after filtering. The filtering script was successfully applied to an LC-HRMS untargeted profiling dataset generated from an eel sample.

2. Materials and methods

2.1. Sample preparation and LC-ESI(-)-HRMS data acquisition
Fat from an eel muscle sample (1 g) was partitioned 4 times between hexane and concentrated sulphuric acid, reconstituted into a methanol/water mixture and analysed with a Hypersil Gold analytical column (100 mm × 2.1 mm, 1.9 µm) coupled to an Orbitrap Q-Exactive mass spectrometer fitted with a heated electrospray source (Thermo Fisher Scientific). Mobile phases consisted of 20 mM ammonium acetate in water (A) and methanol/acetonitrile 1:1 (v/v) (B) and ramped from 95:5 to 0:100 (A/B) over 11.6 min. HRMS data were acquired in the negative and full scan mode over the m/z range 250-900 at a chosen resolving power of 70,000 FWHM at m/z 200.

2.2. Peak integration, isotopic clusters pairing, data representation and interpretation
After conversion to the open format mzXML using the msConvert software (ProteoWizard), the dataset was processed by the xcms package (R environment) using the centWave peak detection algorithm [2] to extract chromatographic features. A data treatment tool (Visual Basic for Applications language) based upon the MD was developed in order to pair features from a same isotopic pattern (chlorinated and/or brominated) generated from an Excel spreadsheet. An iteration loop selects features from the most intense to the less intense. If the selected feature (M) is not yet paired to a more intense feature, it searches, among the less intense features, the ones compatible with a Cl or Br isotopic contribution at M+2 and with a ^{13}C contribution at M+1, pending a match on the RT and the exact mass with 1 s and 5 ppm tolerances by default, respectively. If an M+2 is paired, the macro searches for M+4 and M-2 from Cl or Br isotope contributions, as well as for M+3 from ^{13}C contribution, and so on up to M+12 and M+12 (Figure 1). For each sample, the H/Cl-scale MD plot with all features was drawn. Display options allow narrowing the features selection to a RT range, a minimum area and/or paired categories. Then, paired isotopic clusters are manually investigated (cluster profile, elemental composition, structural hypotheses).

3. Results and discussion

The centWave function resulted in 9,789 non redundant features in the 4-22 min RT range, paired in 6,895 groups. Among them 4,681 were not paired, 1,635 were paired with an M+1 only (assumed to be a ^{13}C contribution and 579, to be further investigated, were suspected to contain at least one atom of Cl or Br. Figure 2 illustrates the efficiency of the filtering script. The 10 clusters showing the largest spans,

from M-6 to M+6 or M-7, all belong to hexabromocyclododecane isomers (α, β, γ), as native form, internal standards ($^{13}C_{12}$), external standard ($^{2}H_{18}$) or related adducts, with an accuracy found to be –6.3 ± 0.8 ppm. The most striking feature in is the array of large clusters coloured in red, with apparent horizontal and vertical series characterized by [$-^{35}Cl/+^{37}Cl$] and [$-^{12}C/+^{13}C$] vectors, respectively. The orientation of the clusters was suggesting the presence of several chlorine atoms. Some clusters from this series showed coeluted clusters with a shift vector of [$-C_2H_4O_2$], guiding the conclusion to acetate adducts of 121 isomer mixtures of chlorinated paraffins (CPs, $CnH_{2n+2-x}Cl_x$, with n # [10;35] and x # [5;10]) with relevant mass accuracy. Short and Medium Chain CPs identification was ultimately confirmed with analytical standards. Additionally to HBCDDs and CPs, other isotopic clusters were investigated, among them 2,4,6 tribromophenol, endosulfane sulfate and β-hexachlorocyclohexane acetate adduct identifications were confirmed by analytical standards.

4. Conclusions

We addressed the question of seeking organohalogenated compounds in complex matrices, particularly unknowns, by developing a VBA script for pairing features according to the exact mass difference between Cl and Br isotopes in order to filter potential organohalogenated clusters among full scan HRMS datasets. This macro appeared highly efficient for revealing such clusters prior to realistic manual investigations. Much effort should be devoted to automation of replicate steps, e.g. selection of a cluster, display of corresponding EIC, comparison of isotopic pattern with the hypothesis on number of Cl and Br atoms, monoisotopic peak m/z and elemental composition hypotheses. Additionally, a dereplication user database shall help in rapidly qualifying known organohalogen compounds before focusing on unknowns.

5. References

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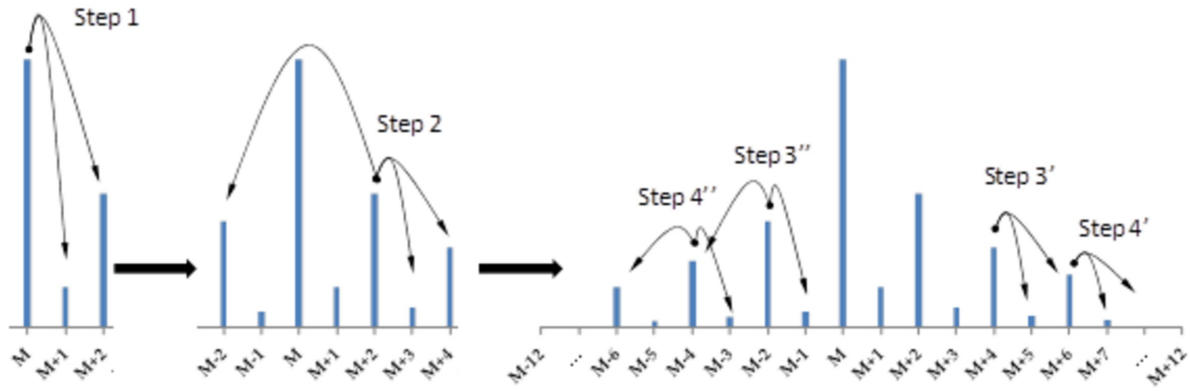

Figure 1: Schematic representation of the pairing process. M ± even numbers features successively searched from the substitution of ^{35}Cl by ^{37}Cl or ^{79}Br by ^{81}Br; M ± odd numbers features searched from the substitution of ^{12}C by ^{13}C from previously found even feature.

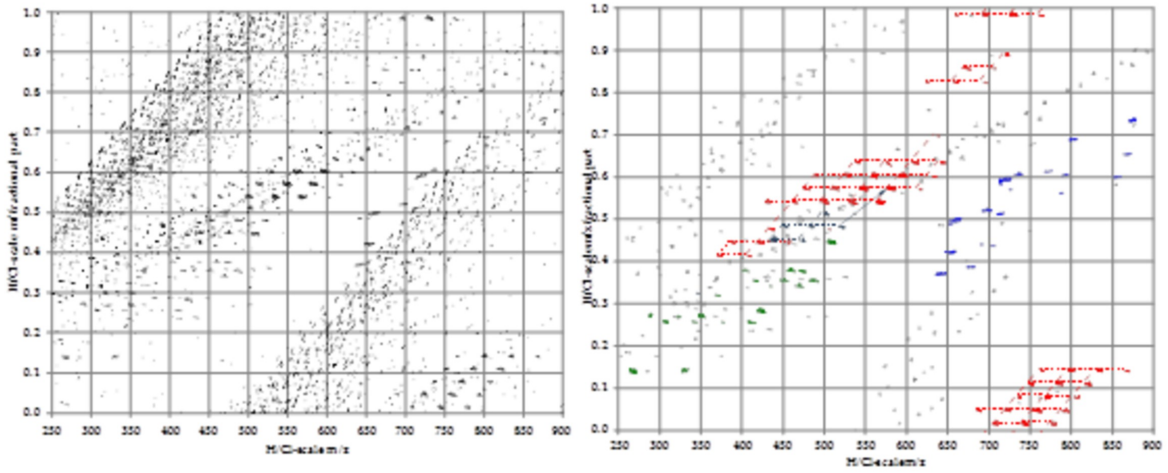

Figure 2: H/C1-scale MD plot obtained for the 9,789 features (left) or restricted to the >M+2 paired clusters (n=1,994, right). Blue: $[M–H]^-$ clusters of HBCDD isomers and related adducts; Red: acetate adducts of CPs and theoretical grid; Purple: $[M–H]^-$ clusters of CPs, theoretical grid and $C_2H_4O_2$ vector; Green: Selection of investigated isotopic clusters.