LEVERAGING Cl-H MASS DEFECT PLOTS FOR THE IDENTIFICATION OF HALOGENATED ORGANIC CONTAMINANTS

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Introduction

Targeted analytical methods are often very robust, sensitive and selective; yet, a conundrum for many researchers doing complex environmental analysis is "What else is in my sample". Time-of-flight mass spectrometry (TOFMS) is unsurpassed for non-target analysis because full range mass spectra are acquired simultaneously with minimal mass bias at acquisition rates suitable for narrow gas chromatographic peaks. This provides a number of advantages including the possibility of deconvolving chromatographic interferences using modern software, further enhancing the ability to isolate and identify a greater number of compounds.

Analysts are continuously building a tool box for compound discovery which may include multivariate statistical analysis, high resolution mass spectrometry, and soft or selective ionization techniques. Mass defect plots are another tool to add to the analyst arsenal. Mass defect is the difference between the nominal and exact masses of a compound or its fragments[1]. Halogenated compounds have characteristic mass defects that make them readily distinguishable from most other compound classes. Two fairly recent papers have highlighted the utility of Cl-H mass defect for the identification of halogenated environmental contaminants[2,3]. Mass defect (Cl-H) can be calculated according to the following equations, where the IUPAC mass is the observed mass and the scaling factor for chlorine substituted for hydrogen equals 34/33.960479:

$$\textit{Cl-H Scaled Mass} = \textit{IUPAC Mass} \times \textit{Scaling Factor}$$

$$\textit{Cl-H Mass Defect} = \textit{Cl-H Scaled Mass} - \textit{Nominal Cl-H Scaled Mass}$$

In this study we used non-target analysis in the form of Cl-H mass defect plots, to identify halogenated contaminants in eels (*Anguillid rostrata*) from Lake Ontario, Canada. This study was meant to serve as a proof-of-concept for the identification of unknown compounds in complex matrices.

Materials and methods

A total of 10 large freshwater eels were collected from eastern Lake Ontario, Canada in 2008. Details of the sample collection and biological characteristics of the eels have been described peviously[4]. Whole fish tissues were homogenized and mixed with anhydrous sodium sulfate, spiked with $^{13}C_{12}$- 2,2',3,3',4,4',-heptachlorobiphenyl ($^{13}C_{12}$-PCB-170), and extracted using dichloromethane. Bulk lipids were removed by gel permeation chromatography eluted with a mixture of 1:1 hexane:dichloromethane (v/v). Sample extracts were pooled for instrumental analysis on a Leco Pegasus GC-HRT, high resolution TOFMS. Extracts were injected (1 µL) using an Agilent 7693 autosampler attached to a 7890 GC fitted with a multi-mode inlet operated in solvent vent mode. A Restek Rxi-guard column (5 m x 0.25 mm) with a Rxi-5MS (30 m x 0.25 mm x 0.25 µm) was used for chromatographic separation. The oven program was initial 90°C (held 2.4 min) then ramped to 320 °C at 8.5°C/min (hold 15 min). The HRT was operated in EI mode with filament energy of 36 eV, a mass range from m/z 35 to 850, and an acquisition rate of 6 spectra/s. Data were processed using Chromatof-HRT, which included peak finding with mass spectral deconvolution.

Results and discussion

The total ion chromatogram of a pooled eel sample from Lake Ontario, Canada (Figure 1a), illustrates that the pooled fish extract was extremely complex. The combined mass spectrum of the entire chromatographic run (Figure 1b), shows that the main components were siloxanes, possibly due in part to contamination from laboratory equipment, and hydrocarbons, most likely from fish tissues not removed during GPC.

Peaks were detected in the sample extract using Leco's Chromatof-HRT software and more than 900 unique peaks were identified. As a tool to simplify the data and reduce the number of peaks to a manageable number, a Cl-H mass defect plot was generated (Figure 2a) for the combined mass spectrum shown in Figure 1b. A large portion of the Cl-H mass defect plot is inaccessible for halogenated compounds, so several steps were taken to isolate potential halogenated compounds visually for further investigation (Figure 2b). First, only masses with an IUPAC mass defect less than 0.01 were considered, and a Cl-H mass defect filter was applied between 0.05 and 0.40. An isotope filter was also applied which required at least two masses to occur within 1.9965 ± 0.0005 Da or 1.9974 ± 0.0005 Da, corresponding to the mass difference between ^{37}Cl -^{35}Cl and ^{81}Br -^{79}Br, respectively. The result shown in Figure 2b is an illustration that highlights a number of compounds and compound classes identified by their accurate mass molecular and fragment ions.

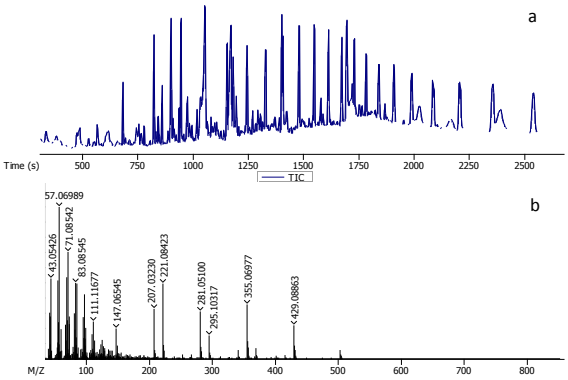


Figure 1: (a) Total ion chromatogram and (b) combined mass spectrum of a pooled eel sample from Lake Ontario, Canada. The combined mass spectrum was generated by expanding the caliper over the entire chromatographic run, which was dominated by ions corresponding to siloxanes and hydrocarbons. More than 900 peaks were identified using high resolution deconvolution in the Chromatof-HRT software.

The workflow for compound identification shown in Figure 3, demonstrates the steps necessary to tentatively identify a compound: starting with selecting points on the Cl-H mass defect plot through to spectral library database matching (i.e. NIST) with accurate mass chemical formula determination. For peaks that did not have a reference spectral match, accurate mass data were used to calculate likely chemical formulae, which in turn were used to search online databases such as ChemSpider for tentative structural identification. It is worth noting, that although the generation of a mass defect plot disregards chromatography, this dimension of information is not lost. Plotting an extracted ion chromatogram for a point on the mass defect plot regains chromatographically resolved isomers or compounds that share similar mass fragments.

In the present study, a number of legacy contaminants such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polychlorinated diphenyl ethers (PCDEs), dieldrin, mirex, hexachlorobenzene (HCB) and other pesticides, as well as a number of previously unknown compounds were identified in the pooled sample. The detection of fenclorim (Figure 2b), a herbicide safener commonly used with pretilachlor on rice, was unexpected. Many breakdown products and metabolites were also detected such as DDD, DDE, and methoxy nonachlorodiphenyl ether. A tribromo analogue of Dechlorane indicated by the fragments $C_8H_5Br_3^{\bullet+}$ and $C_7H_3Br_3Cl^+$, first reported by Jobst et al. [3] in Lake Ontario lake trout, was detected similarly in Lake Ontario eels; accurate mass of the molecular ion cluster and verification with an authentic standard confirmed the identification. This study shows that Cl-H mass defect plots are a useful tool for filtering through complex data for the identification of halogenated contaminants. This technique functions as a screening tool for the identification of unknowns, and in the future, may be used as a form of fingerprinting to compare samples.

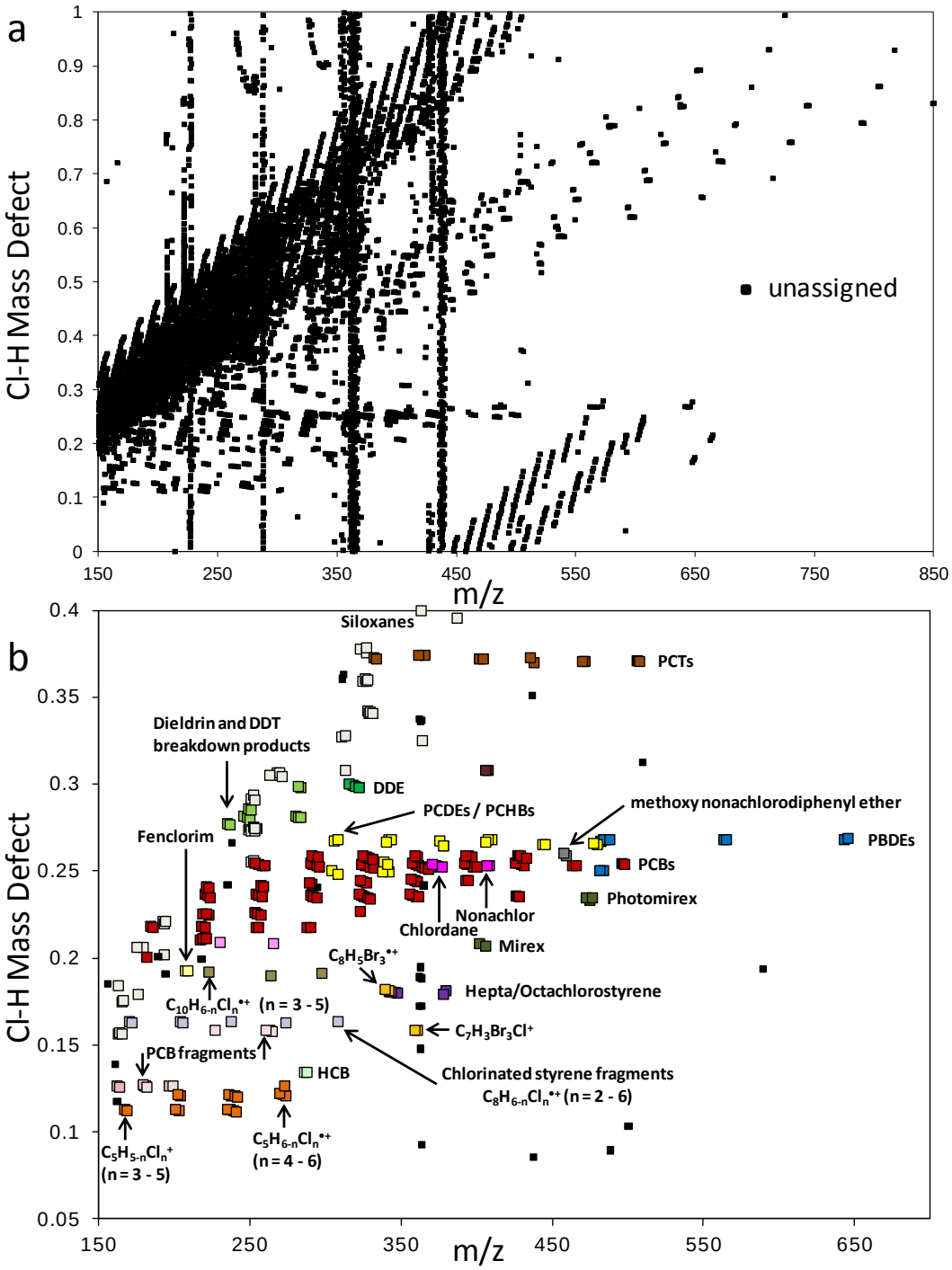
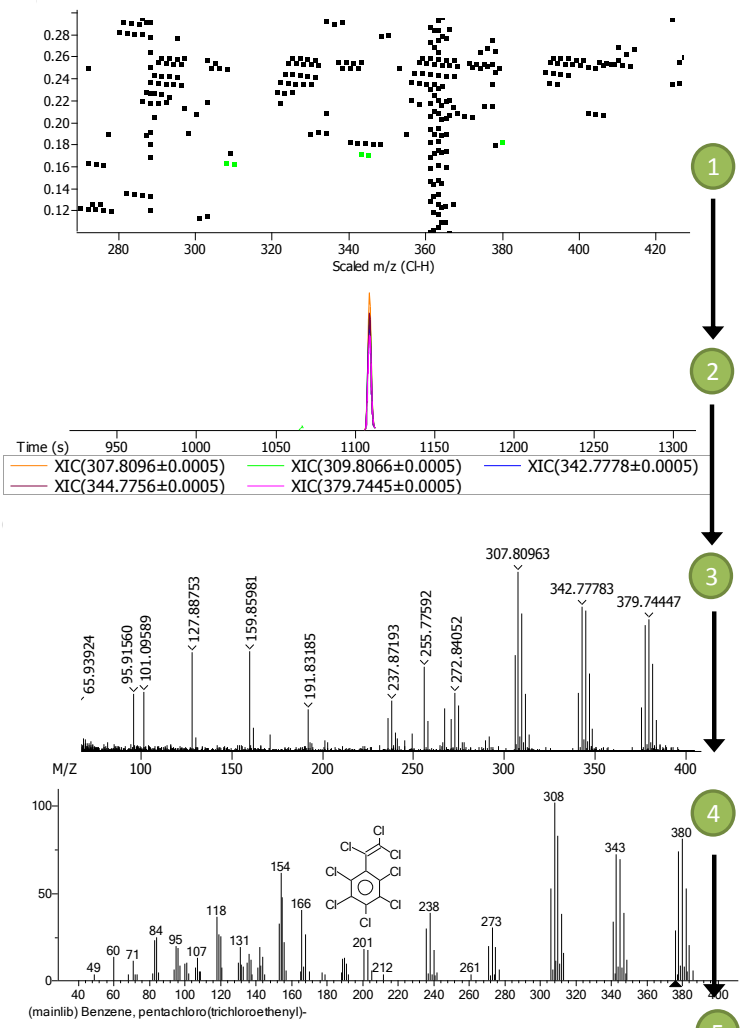


Figure 2: (a) Cl-H mass defect plot of the raw mass spectral data for a pooled Lake Ontario eel sample. (b) A zoomed-in view of the Cl-H mass defect plot highlighting the region containing halogenated species. The coloured points represent m/z values with elemental compositions including Cl and/or Br calculated with a mass accuracy < 2 ppm. The masses displayed in *b* were filtered from *a* by mass defect, and also required at least two masses to occur within 1.9965 ± 0.0005 Da or 1.9974 ± 0.0005 Da, corresponding to the mass difference between ^{37}Cl -^{35}Cl and ^{81}Br -^{79}Br, respectively.



Mass	Formula	Mass Accuracy (ppm)	Mass Defect (Da)	SMD (Cl-H)	Scaled Mass (Cl-H)	RDBE
375.7508	C8Cl8	1.288	-0.2492	0.18195	376.182	5
377.7475	C8Cl7[37]Cl	0.434	-0.2525	0.18098	378.181	5
379.7445	C8Cl6[37]Cl2	0.224	-0.2555	0.18024	380.1802	5
381.7408	C8Cl5[37]Cl3	-1.524	-0.2592	0.17891	382.1789	5
383.7384	C8Cl4[37]Cl4	-0.151	-0.2616	0.17878	384.1788	5

Figure 3: Workflow for the identification of compounds using Cl-H mass defect plot. (1) Select and display masses of interest on chromatogram; (2) select peak; (3) deconvoluted mass spectrum; (4) compare to NIST or other library database; (5) verify correct chemical formula with accurate mass data.

References:

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