NOVEL GC/ECNI-MS-SIM NONTARGET SCREENING METHOD FOR POLYHALOGENATED COMPOUNDS IN ENVIRONMENTAL SAMPLES

Vetter W*, Hauler C

University of Hohenheim, Institute of Food Chemistry, Garbenstr. 28, D-70593 Stuttgart, Germany

Introduction

Polyhalogenated organic compounds are serious environmental contaminants, and their analysis in different matrices is a high priority task in environmental and food chemistry. Polyhalogenated compounds are composed of different classes of classic (e.g. chloropesticides and PCBs) and emerging (e.g. brominated flame retardants) anthropogenic contaminants along with various halogenated natural products.[1,2] While a range of methods has been developed for the quantification of known organohalogens, the detection and identification of "unknowns" is often difficult due to the lack of reference standards and limited information available from standard GC/MS measurements. Routine analysis of polyhalogenated compounds can be performed by GC/EI-MS as well as by GC/ECNI-MS, both of which are usually operated in the selected ion monitoring (SIM) mode. GC/ECNI-MS is usually more sensitive and enables detection of virtually all polybrominated compounds by the screening of *m/z* 79 and *m/z* 81, i.e. the characteristic bromide ion isotopes.[3,4] However, GC/ECNI-MS full scan analyses of polybrominated compounds often suffer from low abundant or even non-detectable ions in the high mass range. Since the molecular ion provides the most relevant structural information, several peaks detected in chromatograms cannot be identified. To overcome this drawback, we recently presented a nontarget GC/EI-MS-SIM method that provided a thorough picture of the organohalogen compound spectrum in environmental samples.[5] This method was based on the fact that the molecular mass of polyhalogenated compounds is usually directly linked with the GC retention time. Likewise, only compounds with a molecular mass >300 Da were studied. Based on these criteria, the retention time range was divided into three mass ranges of 112 u (segment A: *m/z* 300-412; segment B: *m/z* 350-462; segment C: *m/z* 450-562) which was screened in eight GC runs consisting of 15 consecutive SIM ions.[5] By this measure, 38 compounds not-detectable in the full scan mode could be traced back to at least the class of compounds.[5]

In the present study, we explored if this SIM strategy could also be applied to the GC/ECNI-MS mode. The working assumption of the study was the following: owing to the much better sensitivity of GC/ECNI-MS (i.e ~ two orders of magnitude) we assumed that the positive detection of traces of the molecular ion in GC/ECNI-MS-SIM would provide a better sensitivity than GC/EI-MS-SIM. This novel method was studied with a dolphin sample from Australia.

Materials and methods

Samples, chemicals and methods. A blubber sample extract was prepared from an adult male Indopacific humpback dolphin (*Sousa chinensis*) found dead in Gladstone Harbour (Queensland, Australia). The chemicals, standards, and the clean-up procedure (ASE, GPC, silica clean-up) were reported elsewhere.[6] In brief, the lyophilized sample (0.91 g) and the internal standard α-PDHCH were ASE-extracted, followed by gel permeation chromatography and silica gel clean up. Finally, the bulk of the PCBs and related compounds were separated from the chloropesticides and organobromine compounds on 8 g activated silica.[6] Only the fraction which targets the organobromine and the chloropesticides was analyzed.

GC/ECNI-MS and GC/EI-MS parameters. Analyses were performed with a 7890/5975C GC/MS system (Agilent Technologies, Waldbronn, Germany). The transfer line and the ion source temperature were set at 300 °C and 150 °C. In GC/ECNI-MS mode, methane 5.5 was used as the reagent gas at a flow rate of 2 mL/min. Sample solutions (1 µL) were injected by means of an Agilent 7673 GC/SFC automatic injector operated in pulsed splitless mode. An HP-5MS column (30 m length, 0.25 mm i.d., 0.25 µm film thickness) was installed in the GC. The flow of the carrier gas (helium 5.0) was set at 1.2 mL/min. The GC oven program started at 50 °C (hold time for 2 min), then at 10 °C/min to 300 °C (hold time 14 min).

In the full scan mode, we recorded *m/z* 50-800 throughout the run or *m/z* 300-412 (8.0-18.3 min), *m/z* 350-462 (18.3-23.3 min) and *m/z* 450-562 (23.3-40.0 min). Different SIM methods were used but the most relevant is shown in **Table 1**. With this method the border between segment A and segment B was set shortly before the elution of BATE and the switching to segment C was programmed shortly before the elution of BC-2.

Table 1: Run scheme (time and mass ranges) for the GC/MS-SIM nontarget analysis by GC/ECNI-MS and GC/EI-MS

GC run	Segment A (8.0-18.3 min) *m/z*	Segment B (18.3-23.3 min) *m/z*	Segment C (23.3-40.0 min) *m/z*
1	300-314	350-364	450-464
2	314-328	364-378	464-478
3	328-342	378-392	478-492
4	342-356	392-406	492-506
5	356-370	406-420	506-520
6	370-384	420-434	520-534
7	384-398	434-448	534-548
8	398-412	448-462	548-562
∑	300-412	350-462	450-562

Results and discussion

The classic SIM method (*m/z* 79/81, $[Br]^-$) enabled the detection of ~130 polybrominated compounds in the sample (**Figure 1a**). A total of 24 also formed $[Br_2]^-$ (*m/z* 158/160) albeit in most occasions only at very low abundance, and 31 peaks gave response to *m/z* 159/161 ($[HBr_2]^-$) (**Figure 1c**). Comparison of the full scan and Br^- ion trace (*m/z* 79) demonstrated that most intensity was concentrated on Br^- while high mass ions were often of low abundance only (**Figure 1d-f**). Using the GC/ECNI-MS full scan mode, only a few of major compounds such as the MeO-BDEs BC-2 and BC-3 and the dimethoxylated diMeO-BB-80 (BC-1) as well as the anthropogenic flame retardant BDE 47 could be identified. Typically, the abundance of M^- was negligible compared to *m/z* 79/81 and the identification of the compounds proved to be difficult. Accordingly, more than 100 of the polybrominated compounds remained unknown. In addition, the precision of the isotope pattern determination in the full scan mode was frequently insufficient and the data obtained proved to be equivocal.

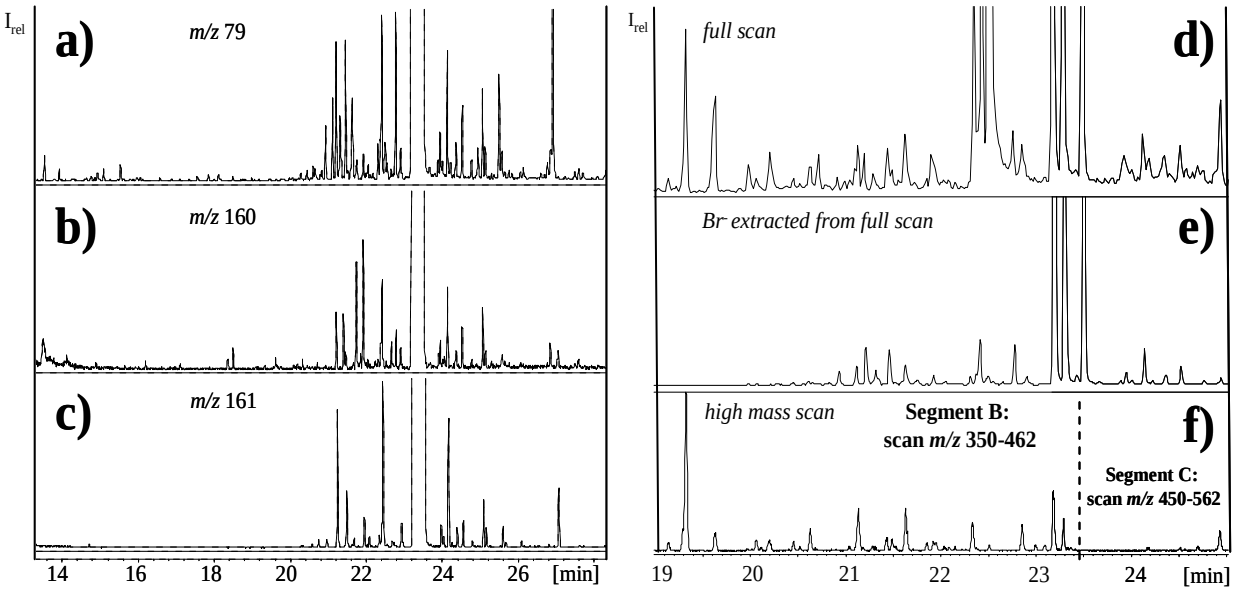

Figure 1: GC/NCI-MS-SIM chromatograms (excerpt) of the blubber extract of an Indopacific humpback dolphin (*Sousa chinensis*) with SIM ion traces (a) *m/z* 79 (b) *m/z* 160 and (c) *m/z* 161 as well as (d) *full scan* chromatogram (m/z 50-550), (e) *m/z* 79 extracted from the full scan and (f) high mass range of segment 2 (*m/z* 300-412) and segment 3 (*m/z* 450-562)

Accodingly, monitoring of the high-mass full scan mode allowed the unequivocal detection of a huge number of polyhalogenated compounds. Thus, we switched to the GC/ECNI-MS-SIM non-target method initially developed for GC/EI-MS measurements according to **Table 1**. Compared to the full scan run (**Figure 2a**), the GC/ECNI-MS-SIM-nontarget runs resulted in clearly structured chromatograms (**Figure 2b-e**). Virtually all individual runs contained a large number of peaks (**Figure 2**). However, co-elutions were scarcely observed and the evaluation was simplified because the response was mainly restricted to M^- or specific high-mass fragment ions. In several runs, many peaks were detected (e.g. *m/z* 378-392 and *m/z* 392-406), while other runs delivered virtually no peaks (e.g. *m/z* 492-506; *m/z* 520-534) (**Figure 2b-e**). All together segment B contained more peaks than segment A and C (**Figure 1d-f** and **Figure 2b-e**). The evaluation was based on the following mode: First, a table was constructed which contained all essential mass spectra (mass of the molecular ion, mass of the most abundant isotope peak and isotope pattern) in the GC runs. In the case of odd-mass ions we checked for $[M+nBr(\pm H)]^-$ and $[M+nCl(\pm H)]^-$. Although the overall sensitivity was reduced compared to *m/z* 79 (most polybrominated compounds show predominance for *m/z* 79/81 which was not recorded with the current method), the peaks recorded in the SIM runs contained structure-relevant information, i.e. typically the molecular ion and and its isotope pattern. The isotope patterns were of high quality, especially when the peaks were not too small.

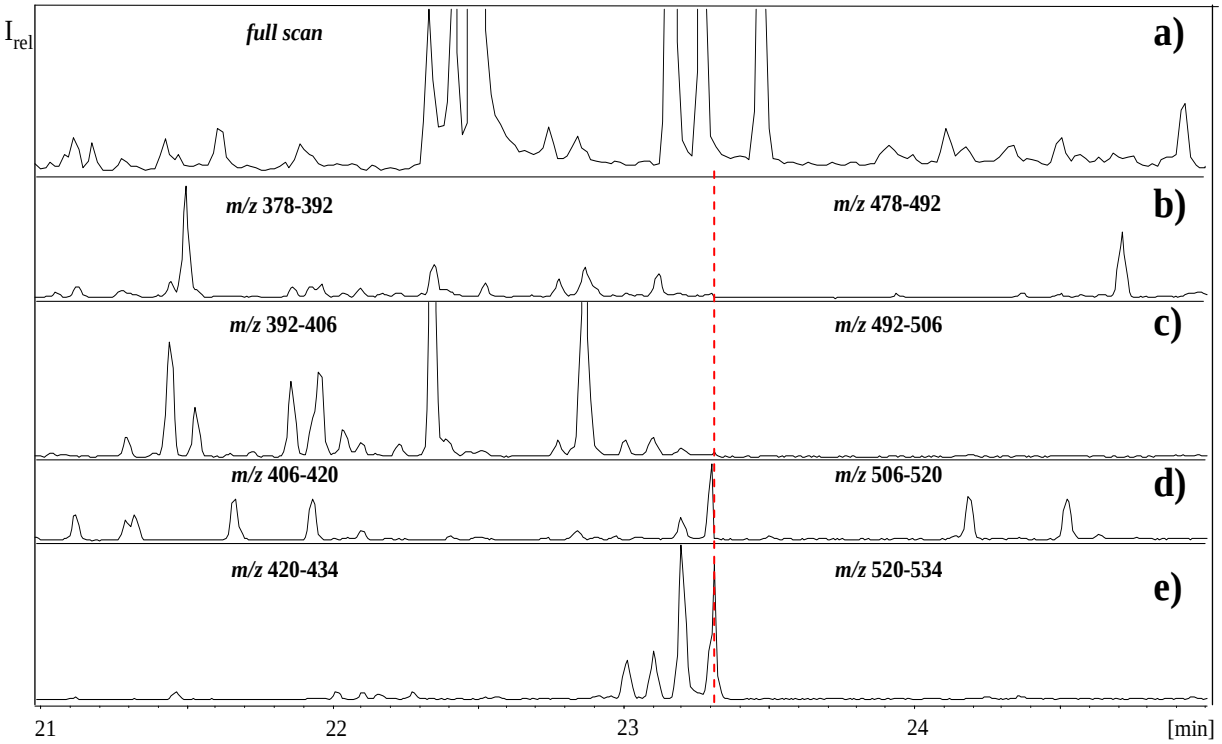

Figure 2: GC/ECNI-MS chromatograms (excerpt) of the blubber extract of an Indopacific humpback dolphin (*Sousa chinensis*). (a) full scan chromatogram and GC/ECNI-MS-SIM nontarget analysis with (b) run #3, (c) run #4, d) run #5 and (e) run #6. SIM-chromatograms are shown in the same Y-axis scaling.

GC/ECNI-MS-SIM-*nontarget* method delivered 420 peaks from polyhalogenated compounds which is more than the double obtained with GC/EI-MS-SIM and GC/ECNI-MS scan (**Table 2**). Due to the huge number of compounds, the samples were initially checked for known compounds (PBDEs, chloropesticides, PCBs, PBDEs, HNPs). The first two peaks identified were 2,4,6-tribromoanisole (#7) and 2,4,6-tribromophenol (#8). The sample contained at least ten chlordane compounds. Further chloropesticides were present in form of toxaphene (at least five congeners), dieldrin and DDT. In addition, at least 26 PCB congeners (note that the bulk of the PCBs was separated by adsorption chromatography prior to the GC/MS analysis) while BDE 47 and one further PBDE congener was detected. Special emphasis was directed to the polyhalogenated alkaloids.

Table 2: Summary of the key-findings in an Indopacific humpback dolphin (*Sousa chinensis*) by means of the novel GC/ECNI-MS-SIM nontarget method compared to alternative nontarget methods

	GC/ECNI-MS-SIM nontarget	GC/EI-MS-SIM nontarget	ECNI-MS full scan
Polyhalog. compounds	420	150	130
incl. PMBPs	4	1	4
PDBPs	18	2	2
Chlordane / PCBs	10 / 26	not calculated	- / -
Further HNPs	8	not calculated	not calculated

Polyhalogenated alkaloids. The GC/ECNI-MS-SIM nontarget runs enabled the detection of heptachloro-1´-methyl-1,2´-bipyrrole (Q1 or Cl_7-MBP) and three $BrCl_6$-MBP congeners while higher-brominated MBPs were not detected. The MBP pattern was similar to the one previously detected in marine mammals from Australia. The novel GC/ECNI-MS-SIM method also allowed detecting several hexahalogenated 1,1´-dimethyl-2,2´-bipyrroles (DBPs). Initially, run #7 (segment C) indicated the presence of three Br_4Cl_2-DBPs (**Figure 3a**). The ion traces extracted from the SIM chromatograms showed a full match and unequivocally verified the finding.

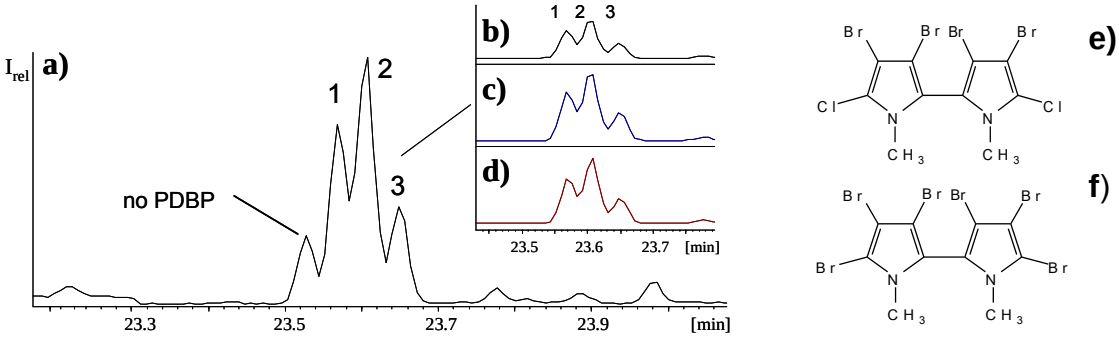

Figure 3: (a) GC/ECNI-MS-SIM nontarget chromatogram (segment C, run #7) with the detection of BC-10 (1) and two novel Br_4Cl_2-DBPs in the blubber of *Sousa chinensis*; extracted ion traces (b) *m/z* 540, (c) *m/z* 542, (d) *m/z* 544 and structures of (e) 5,5´-dichloro-1,1´-dimethyl-3,3´,4,4´-tetrabromo-2,2´-bipyrrole (BC-10; Br_4Cl_2-DBP isomer no. 1) and 1,1´-dimethyl-3,3´,4,4´,5,5´-hexabromo-2,2´-bipyrrole (Br_6-DBP)

Before this study, 5,5´-dichloro-1,1´-dimethyl-3,3´,4,4´-tetrabromo-2,2´-bipyrrole (**Figure 3e**) remained the only Br_4Cl_2-DBP isomer identified since its discovery in 1999.[7] Theoretically, there are nine Br_4Cl_2-DBP isomers.[8] To our surprise, the novel Br_4Cl_2-DBP isomers were neither detected in the GC/ECNI-MS full scan run nor in the GC/EI-MS-SIM nontarget mode. A more detailed study indicated that only BC-10 showed an abundant Br^-. Accordingly the small Br^- peaks of the novel Br_4Cl_2-DBP isomers disappeared under the major peak of BC-10. Detailed screening of other nontarget runs for further DBPs was based on the fact that Br→Cl exchange decreases the mass by 44 u. Screening for the corresponding congeners proved the presence of Br_6-DBP (**Figure 3f**), two Br_5Cl-DBPs, at least four Br_3Cl_3-DBPs, four Br_2Cl_4 -DBPs, three $BrCl_5$-DBPs as well as Cl_6-DBP. Fourteen of the detected 18 DBPs have not been described before in the scientific literature.
These exemplary results demonstrated the strength of the novel nontarget method. Still, >100 compounds in the dolphin blubber remained unidentified and this clearly indicates that targeted analysis only provide limited insights into the polyhalogenated compounds in the environment.

References

1. Vetter, W (2006); *Rev Environ Contam Toxicol* 188: 1–57.
2. Covaci A, Voorspoels S, Ramos L, Neels H, Blust R (2007); *J Chromatogr A* 1153: 145–171.
3. Buser H-R (1986); *Anal Chem* 58: 2913–2919.
4. Vetter W (2001); *Anal Chem* 73: 4951-4957.
5. Rosenfelder N, Van Zee NJ, Mueller JF, Gaus C, Vetter W (2010); *Anal Chem* 82: 9835-9842.
6. Vetter W, Stoll E, Garson MJ, Fahey SJ, Gaus C, Mueller JF (2002); *Environ Toxicol Chem* 21: 2014-2019.
7. Tittlemier SA, Simon M, Jarman WM, Elliott JE, Norstrom RJ (1999); *Environ Sci Technol* 33: 26-33.
8. Vetter W (2012) *The Alkaloids* 71: 211-276 (DOI: 10.1016/B978-0-12-398282-7.00003-5).