NON-TARGET GC/EI-MS SCREENING OF POLYHALOGENATED COMPOUNDS

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Introduction

Polyhalogenated organic compounds are distributed throughout the environment. This generic term includes not only different classes of polybrominated and polychlorinated anthropogenic compounds but also a range of naturally produced polyhalogenated compounds [1, 2]. The determination of polyhalogenated compounds is usually carried out by means of gas chromatography (GC) coupled to mass spectrometry (MS). Two different ionization methods, GC/EI-MS as well as GC/ECNI-MS, are frequently used and operated in the selected ion monitoring (SIM) mode. GC/ECNI-MS is usually more sensitive and enables detection of all brominated compounds by screening of *m/z* 79 and *m/z* 81, the characteristic bromide ion isotopes [3, 4]. However, full scan GC/ECNI-MS analysis often suffers from low abundant or even non-detectable molecular ions and fragment ions in the high-mass range. Thus, the identification of compounds without authentic reference standards is difficult. In contrast, GC/EI-MS provides fragment ions in the high-mass range and therefore more molecular information. However, its lower sensitivity compared to GC/ECNI-MS does not allow for the detection of persistent organic pollutants (POPs) in the picogram-range. Furthermore, background noise from the sample matrix mostly in the low-mass range disturbs the chromatograms and mass spectra. To achieve the required low limits for the detection for POPs, quadrupole instruments must be operated in the SIM mode. Such GC/EI-MS-SIM methods are designed to detect carefully selected POPs while unknown compounds that do not form the SIM-ions are excluded. To compensate the negative properties of a single quadrupole-system in the scan mode we developed an alternative GC/MS SIM method. The method included an identification scheme for unknown polyhalogenated compounds. This method was based on the fact that the retention times of polyhalogenated compounds correlate with their mass on nonpolar GC-columns. Thus a method was developed with time windows covering a certain mass range of polyhalogenated compounds. This non-target screening method was tested with passive water sampler extracts known to contain halogenated natural products (HNPs) and different unknown compounds [5].

Material and methods

Chemicals: *Iso*-octane (SupraSolv, for gas chromatography) was from Merck (Darmstadt, Germany). *n*-Hexane (HPLC grade) was purchased from Fisher Scientific (Leicestershire, UK). The sources of polyhalogenated reference standards have been reported elsewhere [6]. The standard mixture for method development contained the following in elution order on a HP-5ms column: 2,4,6-tribromoanisole (TBA), 2,4,6-tribromophenol (TBP), allyl-2,4,6-tribromophenyl ether (ATE), 2-bromoallyl-2,4,6-tribromophenyl ether (BATE), (1*R*,2*S*,4*R*,5*R*,1'*E*)-2-bromo-1-bromomethyl-1,4-dichloro-5-(2'-chloroethenyl)-5-methylcyclohexane (MHC-1), 2,3,3',4,4',5,5'-heptachloro-1'-methyl-1,2'-bipyrrole (Q1), 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE), 2,2'-diMeO-BB 80 (BC-1), 2'-MeO-BDE 68 (BC-2), 6-MeO-BDE 47 (BC-3), DBP-Br_4Cl_2 (BC-10), 2',6-diMeO-BDE 68 (BC-11), 2,7-dibromo-4a-bromo-methyl-1,1-dimethyl-2,3,4,4a,9,9a-hexahydro-1*H*-xanthene (TriBHD), and 2,5,7-tribromo-4a-bromomethyl-1,1-dimethyl-2,3,4,4a,9,9a-hexahydro-1*H*-xanthene (TetraBHD).

Passive Water Sampler Samples: Experimental details for the passive water sampling method can be found in Vetter *et al*. [5]. In short, semipermeable membrane devices were deployed at a range of sites along the Great Barrier Reef at a depth of about 1 m for 1-2 months. After retrieval, each sampler was stored at -17 °C until analysis. Samples from the same collection site were partly combined and analyzed.

Gas chromatography coupled to electron ionization mass spectrometry (GC/EI-MS): GC/EI-MS analysis was performed on an HP 5890 Series II Plus GC coupled with a HP 5972 mass selective detector (MSD). An HP-5ms column (30m x 25 mm i.d. x 0.25 µm film thickness, J&W Scientific, Agilent) was used with the following oven program: after 2 min isothermal at 60 °C, the temperature was raised at 10 °C/min to 300 °C which was held for 14 min. Injections were performed in splitless mode at 250 °C. Helium 5.0 (Sauerstoffwerke, Friedrichshafen, Germany) was used as carrier gas with a constant flow rate of 1.2 mL/min. In full scan mode, the mass ranges *m/z* 200-700 and *m/z* 300-600 were measured, respectively. In SIM mode, the following method was used: measuring 15 masses per run (30 ms dwell time) in eight runs (Table 1).

Table 1: GC/EI-MS SIM schedule for non-target screening of polyhalogenated compounds (* individual u were recorded in SIM mode)

Run #	Segment 1 * (10.0-17.5 min) (m/z)	Segment 2 * (17.5-22.5 min) (m/z)	Segment 3 * (22.5-40.0 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
∑ 1-8	300-412	350-462	450-562

Results and discussion

Development of a non-target GC/EI-MS method

In first order, polyhalogenated compounds elute with increasing molecular weights from DB-5 columns. The mass overlap is usually < 100 u (Figure 1).

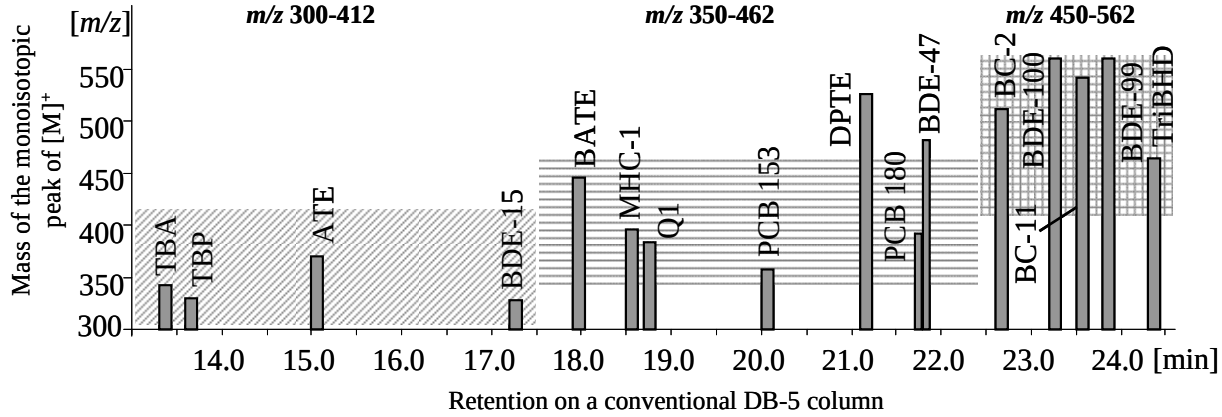

Figure 1: Retention times of different polyhalogenated compounds, divided into three time intervals and mass ranges: 10-17.5 min (*m/z* 300-412), 17.5-22.5 min (*m/z* 350-462) and 22.5-40 min (*m/z* 450-562).

Based upon this observation, a standard GC/MS run was divided into three time windows that each corresponded with a mass range of 112 u (Table 1). For SIM measurements each time window was split in eight sequences of 15 u, which were recorded in eight subsequent runs. Each SIM run consisted of three time segments (Table 1) so that the entire mass range could be analyzed in SIM mode after only eight GC runs. The method largely eliminated matrix interferences such as aliphatic compounds because only masses > 300 Da, typically for polyhalogenated compounds, were monitored. We noticed that only a few potentially interesting compounds fell out of this scheme (e.g. BDE-47 and DPTE, Figure 1). Likewise, compounds with high polarity are supposed to be retained longer on the column and thus will not be detected in the SIM window. However, these compounds may be identified by their well-known fragment ions. For instance the $[M-2Br]^+$ fragment for DPTE (*m/z* 368) was used as identification ion.

Proof of the developed method during analyses of passive water sampler extracts

The efficiency of the method was studied by analyses of known and unknown organohalogen compounds in passive water sampler extracts from the Great Barrier Reef, Australia. Previous screening by GC/ECNI-MS allowed the detection of a range of known and unknown polybrominated compounds. However, most GC/ECNI-MS spectra showed only the bromide ion isotopes.

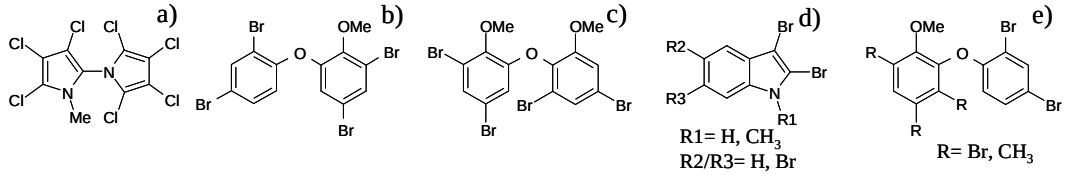

Figure 2: Structures of halogenated natural products determined in this study. a) Q1, b) BC-2, c) BC-11, d) brominated indoles (#1, #5, #6 and 8#) and e) MeO-Me-tetrabromo diphenyl ether (#11)

The GC/EI-MS full scan chromatogram (*m/z* 200-700) of the passive sampler extract was overloaded by a large amount of low-mass compounds that co-eluted between 18 and 22 min, which even made the identification of HNPs known to be present in the sample virtually impossible (Figure 3a). Narrowing the mass range to *m/z* 300-600 partly sorted out the matrix interferences but the sensitivity was still too low for the direct detection of any polyhalogenated compounds (Figure 3b).

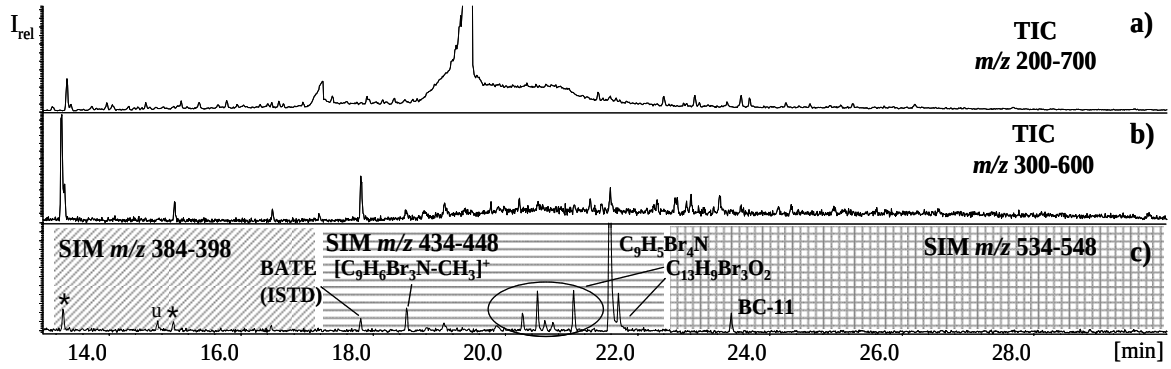

Figure 3: GC/EI-MS full scan (a and b) and SIM (divided into three time segments, c) chromatograms of a passive water sampler extract from Low Island, Great Barrier Reef, Australia (*artifact, [u] still unknown) and proposed formula of detected compounds.

This situation was improved when the novel SIM-method was applied. Almost every SIM-segment contained several peaks that could be traced back to polyhalogenated compounds. Using the new method 35 polyhalogenated compounds (including the natural products Q1, BC-2 and BC-11 and analogues of these compounds by means of the isotopic pattern) were identified in the sample. Eight compounds including Q1, BC-2 and BC-11 (Figure 2a-c) could be identified by comparison with reference standards (excerpt in Table 2). A number of compounds showed ion clusters with odd masses. This usually indicates the presence of an odd number of nitrogen or originates from fragment ions instead of $[M]^+$. For clarification a conventional SIM run was programmed to monitor the potential $[M+Cl]^+$ and $[M+Br]^+$, and $[M-Cl]^+$ and $[M-Br]^+$ ions. Compounds # 1, 5, 6 and 8 (Table 2) gave no response for $[M+Br]^+$ and $[M+Cl]^+$ which supported the suggestion that these compounds were tribromo- or tetrabrominated and had one (or three) nitrogen. A search of the literature pointed to naturally produced bromoindoles with known structures. Such naturally produced compounds were isolated from red algae (*Laurencia sp.*) and previously studied in common oyster from the western Atlantic coast (Figure 2d) [7, 8, 9]. Compound #5 and 8 differ from compound #1 and 6 in the detection of an additional high mass fragment, $[M-CH_3]^+$ (Figure 3d)). These *N*-methylated tri- and tetrabromo indoles were described in the literature as well [8, 9]. However, none of them were described before in Australian samples.

Table 2: Polyhalogenated compounds identified with non-target screening of water passive sampler extracts from Low Island (excerpt of 11 detected compounds out of 38) and Fitzroy Island (2nd sample), Great Barrier Reef, Australia.

#	SIM run #	Time segment	t_R	monoisotopic mass (*m/z*)	most abundant mass (*m/z*)	isotopic pattern	fragment	2nd sample	"proposed" formula
1	4	1	16.93	351	353	Br_3	$[M]^+$	-	"$C_8H_4Br_3N$" [7]
2	1	1	17.10	308	308	Br	$[M]^+$	-	"$C_{16}H_{21}BrO$"
3	3	2	18.61	384	386	Cl_7	$[M]^+$	X	$C_9H_3N_2Cl_7$ (Q1) [10]
4	7	2		435	439	Br_4	$[M\text{-}CH_3]^+$	-	undetermined
	8	2	18.63	450	454	Br_4	$[M]^+$	-	
5	2	2		365	367	Br_3	$[M]^+$	X	"$C_9H_6Br_3N$"
	1	2	18.81	350	354	Br_3	$[M\text{-}CH_3]^+$		[8, 9]
6	6	2	19.20	429	433	Br_4	$[M]^+$	X	"$C_8H_3Br_4N$" [8]
7	7	2	20.63	434	436	Br_3	$[M]^+$	X	$C_{13}H_9Br_3O_2$ [11]
8	7	2		443	447	Br_4	$[M]^+$	-	"$C_9H_5Br_4N$"
	6	2		428	432	Br_4	$[M\text{-}CH_3]^+$	-	[8, 9]
	2	2	21.69	364	368	Br_3	$[M\text{-}Br]^+$	-	
9	5	3	22.70	512	516	Br_4	$[M]^+$	X	$C_{13}H_8Br_4O_2$ (BC-2) [12]
10	7	3	23.55	542	546	Br_4	$[M]^+$	X	$C_{14}H_{10}Br_4O_3$ (BC-11)
11	6	3	25.17	526	530	Br_4	$[M]^+$	X	"$C_{14}H_{10}Br_4O_2$" [13]

Compound 11 showed a tetrabromo pattern with the monoisotopic peak *m/z* 526 (SIM run 6, time segment 3, Table 2). In the same SIM-segment we detected two additional tetrabrominated isomers in a narrow retention time range (24.8 min – 25.7 min). Since the isomeric 2,2'-diMeO-BB 80 (BC-1) eluted approximately 2 min earlier we assumed another class of polyhalogenated compounds. Unger *et al.* recently described a bioaccumulated compound with the same isotopic mass in whale blubber from the northern North Atlantic Ocean [13]. Comparison of the GC retention time range and the observed mass spectrum with the ones mentioned in the literature resulted in good agreement. Importantly, none of these compounds had been detected during GC/EI-MS full scan analyses. GC/ECNI-MS analyses resulted for almost all compounds in complete fragmentation to the bromide ion isotopes. In a similar way we identified five congeners of tribromophenoxyanisoles, one compound was tentatively identified as a tribromoaniline and the mass spectral data of a monobrominated compound fitted with the backbone of polybrominated hexahydroxanthene derivatives [14] (Table 2, #2).

Acknowledgements
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