GC×GC–ToF MS FOR THE ANALYSIS OF ANTHROPOGENIC AND NATURALLY PRODUCED ORGANOBROMINATED COMPOUNDS

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Introduction

Comprehensive two-dimensional gas chromatography (GC×GC) has gradually been introduced to solve some separation problems found during the analysis with one-dimensional GC[1]. The use of high speed time-of-flight mass spectrometry (HS-ToF MS) as a detector for GC×GC (GC×GC–ToF MS) adds an additional dimension to the analysis allowing unambiguous determination based on nominal mass. This is especially relevant for environmental applications dealing with the determination of individual isomers belonging to complex families of micropollutants, e.g. polychlorinated biphenyls (PCBs)[2], polybrominated diphenyl ethers (PBDEs)[3] and other closely related persistent organic pollutants (POPs)[4,5]. Many studies have proved that one-dimensional GC systems, especially when combined with MS detectors, provide enough resolution to allow unambiguous determination of relevant congeners within complex families, such as PCBs[6], PBDEs[7] and PCDD/Fs[8]. However, in these studies, either an exhaustive clean-up and fractionation of the extracts or several GC runs[9] are typically required, especially when different classes of analytes have to be determined in the same extracts. In addition, most studies are target-orientated and information concerning the possible presence of other relevant known and especially unknown micropollutants is lost. In this context, GC×GC and, especially GC×GC–ToF MS, are powerful analytical tools that simultaneously contribute to simplify sample treatment and allow simultaneous determination of trace compounds from different analytes classes, while preserving complete information regarding non-targeted compounds. The feasibility of the technique to provide structured chromatograms is an additional feature that efficiently contribute to the tentative identification of analytes and families of analytes for which standards are not available and so to the (virtual) identification of unknown compounds[1, 10].

Up to now, the number of studies in which the simultaneous screening of relevant organobromines (OB) have been done are scarce in the literature[9, 11-12]. These studies have covered a wide range of OB families, including new brominated flame retardants (NBFRs), some methoxylated brominated biphenyl congeners (MeO-PBBs), other methoxylated phenoxyanisoles (mono and di-MeO-PBDEs) and several organohalogenated methyl and dimethyl bipyrroles (MBP and DBPs). These databases provide a useful tool for tentative identification and/or confirmation of non-targeted compounds when standards are not available.

The present work focuses on the separation and detection via GC×GC–ToF MS of a number of environmentally relevant OB families, including PBDEs, environmentally relevant MeO-PBDEs, and other naturally-produced organohalogens (HNPs), such as the polybrominated hexahydroxanthene derivates (PBHDs), 2,4,6-tribromoanisole (TBA) and a mixed halogenated monoterpene compound (MHC-1), in 26 bluefin tuna muscle samples (*Thunnus thynnus*). Special attention was paid to solve analyte co-elutions previously observed in the one-dimensional GC analysis[13]. In addition, tentative identification of other NBFRs as well as elucidation of some non-identified OBs detected in the samples was carried out.

Materials and methods

Sample collection and preparation. Bluefin tuna was collected during 2003 in the Mediterranean Sea and preserved at -20ºC until analysis. Sample preparation was carried out by using an automat Soxhlet extracted and further purification with multisilica-filled columns. Detailed information about the sample preparation methodology was previously reported[13].

Chemicals. The 26 OBs included in the present study were selected because of their toxicity, environmentally relevance and occurrence in aquatic environmental samples. The 9 PBDEs and 13 MeO-PBDEs were purchased from Wellington Laboratories (Guelph, ON, Canada) and Accustandard (New Haven, CT). TBA was obtained from Dr. Ehrenstorfer (Augsburg, Germany). PBHD isomers together with MHC-1 were kindly supplied by Prof. Dr. W. Vetter (University of Hohenheim, Germany). A PCB stock solution containing 23 PCBs (Dr. Ehrenstorfer) and two PBDE mix standards (BDE-MXA and BDE-MXB, Wellington Laboratories) were used for method development.

GC×GC–ToF MS. Determination of selected OBs was performed by using a GC×GC system (Agilent GC 6890) coupled to ToF MS (Pegasus IV, Leco Corporation). Once the chromatographic conditions were optimised, samples were injected in the hot splitless mode (1µL, 300ºC, splitless time 2.0 min) in the HT-

8×BPX-50 column arrangement (30 m × 0.25 mm i.d. × 0.25µm film thickness and 1.6 m × 0.1 mm × 0.1 µm, respectively) (SGE, Melbourne, Australia). The final oven temperature programs were set as follows: from 80 ºC (2.5 min) to 190 ºC (50 min) at a rate of 15ºC/min and then to 300 ºC (20 min) at 3 ºC/min in the main oven, and from 110 ºC (2.5 min), then heated to 210 ºC at a rate of 15 ºC/min and raising 300 ºC at 3 ºC/min (20 min) in the secondary oven. Helium was used as carrier gas (constant pressure, 31 psi). A nitrogen quad-jet dual-stage cryogenic modulator was used for analyte focusing and re-injection in the secondary oven. Modulation conditions were as follows: two 0.60 s hot pulses with a 1.90 s cold pulse between stages. The transfer line temperature was set at 295 ºC.

The ion source temperature was set at 250 ºC and detector voltage fixed at 1800 eV. A scan acquisition range from 50 to 750 *m/z* was used during method development. In order to achieve enough sensitivity for analyte detection in real samples, data acquisition rate was set at 50 Hz. ChromaToF software was used for acquiring data raw. This software allowed automated baseline correction and peak area determination. A user library for all OBs was created from the corresponding standards.

Results and discussion

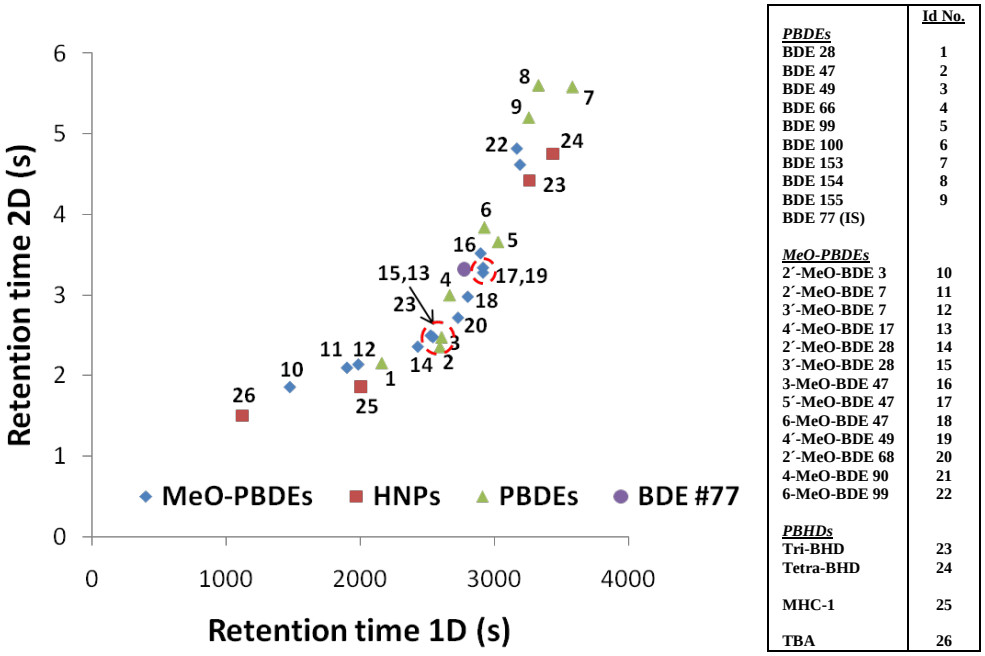
GC×GC optimization. Previous studies carried out in our group for the screening of different families of POPs in environmental samples using GC×GC–µECD concluded that the HT-8×BPX-50 column setup provided an adequate separation between PBDEs and other aromatic organohalogenated pollutants, such as PCBs, PCDD/Fs, toxaphenes and polychlorinated terphenyls among others[4]. With this column combination, compounds containing bromines are more strongly retained in the second dimension than those containing chlorine atoms, a very interesting feature for the purpose of this study.

During method optimization, parameters affecting the GC×GC efficiency were carefully investigated. The most relevant aspects to be highlighted here are (i) the use of oven temperatures above 300 ºC resulted in an undesirable column bleeding; (ii) the application of pulse pressures during injection did not significantly improve the efficiency of the injection; (iii) low transfer line temperature resulted in a not satisfactory transfer of the targeted compounds from the second dimension oven to MS. Increasing the temperature resulted in a satisfactory transfer. However, column bleeding was again observed at temperatures above 295 ºC; (iv) A 0.6 s of hot pulse in a 6 s modulation period provided a reliable reinjection and separation of the target co-eluting analytes into the second dimension and, simultaneously, minimised wrap-around.

GC×GC–TOF MS separation of selected OBs. Resolution of critical pairs. The BDE mix standard solutions, two different MeO-PBDE stocks containing 13 congeners each, a mixture including the tri-BHD and tetra-BHD isomers and the individual MHC-1 and TBA standards were separately injected onto the GC×GC–ToF MS system under optimised conditions. The respective retention times of the compounds were exported to Microsoft Office Excel to reconstruct the overlapped bidimensional contour plot shown in Figure 1.

All 26 OBs included in the study were satisfactorily separated from each other in a single chromatographic run. Besides, as previously mentioned, wrap-around was not apparent, which can be considered a convenient feature of the optimised method for further application to real-life samples. Interestingly, some critical analyte pairs that typically co-elute in one-dimensional GC-based approaches[13] were satisfactorily separated on GC×GC. In this study a successful separation among critical pair 4´-MeO-BDE 17 and 3´-MeO-BDE 28 and from BDE 49 was achieved (Figure 1). Similarly, BDE 100 was separated from 4´-MeO-BDE 49 and 5´-MeO-BDE 47. The optimised GC×GC–ToF MS methodology allowed reliable separation both among the different families of the investigated OBs and among these and other close-related organohalogenated pollutants in only 54 min. The method was considered feasible for the analysis of these micropollutants in complex biological samples.

Application to real-life tuna samples. The optimised GC×GC method was applied to the identification of OBs in 26 samples of tuna muscle. Samples were grouped according to gender, age and origin (i.e., farmed or wild) to compare the respective OB profiles. Considering age as classification factor, similar accumulation patterns were



	Id No.
PBDEs	
BDE 28	1
BDE 47	2
BDE 49	3
BDE 66	4
BDE 99	5
BDE 100	6
BDE 153	7
BDE 154	8
BDE 155	9
BDE 77 (IS)	
MeO-PBDEs	
2´-MeO-BDE 3	10
2´-MeO-BDE 7	11
3´-MeO-BDE 7	12
4´-MeO-BDE 17	13
2´-MeO-BDE 28	14
3´-MeO-BDE 28	15
3-MeO-BDE 47	16
5´-MeO-BDE 47	17
6-MeO-BDE 47	18
4´-MeO-BDE 49	19
2´-MeO-BDE 68	20
4-MeO-BDE 90	21
6-MeO-BDE 99	22
PBHDs	
Tri-BHD	23
Tetra-BHD	24
MHC-1	25
TBA	26

Figure 1: Reconstructed chromatogram of the 26 OBs under finally GC×GC–ToF MS conditions

found for PBDEs and MeO-PBDEs in all investigated wild tunas. Not unexpectedly, the highest PBDEs and MeO-PBDEs levels were detected in adult tuna (i.e., more than 4 years old), which can be explained by the high persistence exhibited by most of the organohalogenated pollutants in biota. However, levels measured for HNPs look to be similar irrespective of age.

Due to the feasibility for group-type identification provided by GC×GC, a number of isomers belonging to the several investigated OB families were detected in the samples with highest levels of OBs (in general, those with higher fat content). The roof tile structure observed for PBDEs (Figure 2.A) allow the tentative identification of some additional tetra- and penta-BDE isomers on the base of previously reported data concerning the elution order of these pollutants on different stationary GC phases[14,15]. Such roof tile structure, in combination with MS information, also allowed the identification of new tri- and tetra-BHD isomers in tuna muscle (red figures in Figure 2.B). To the best of our knowledge, this is the first time that other tri- and tetra-BHD isomers other than 2,7-dibromo-4a-bromomethyl and 2,5,7-tribromo-4a-bromomethyl BHD derivates (indicated with a yellow circle in Figure 2.B) have been identified in real-life biological samples. The good agreement observed between the mass spectra obtained for these isomers and those registered for the two available standards as well as the mass spectra described in the literature for these analytes further supported this finding.

Regarding gender, MeO-PBDEs and other HNPs were found to accumulate dissimilarly in females and males. A particularly interestingly behaviour was observed for the pair 4´-MeO-BDE 49 and 5´-MeO-BDE 57. Only 7 out of the 26 investigated tunas displayed measurable levels of these congeners. Five out these 7 tunas were females. In addition, the 4´-MeOBDE 49 congener was either the only one detected or the one found at higher concentration. This particular trend could be associated to a special metabolism pathway for males.

Finally, and in agreement with other studies, differences in the investigated samples were also observed depending on their wild or farmed origin. Different profiles of MeO-PBDEs and HNPs were observed in wild and farmed tunas, with much higher levels in the case of wild individuals. This is probably a result of the extended interaction between tunas and the HNP producers, because of their higher mobility and wider feeding area.

Elucidation of new emergent OBs. Additional experiments were carried out to investigate the presence of other anthropogenic and naturally-produced brominated compounds in the investigated tuna samples. These emergent OBs were investigated on the basis of their retention time, their chromatographic behavior, by comparison of the

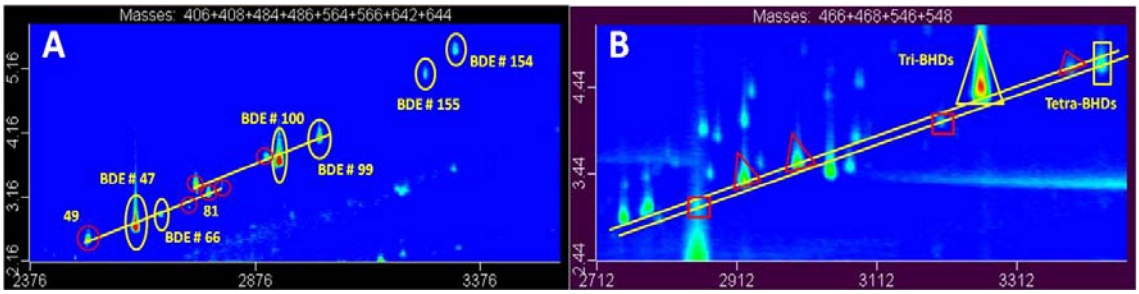


Figure 2: Elucidation of new emergent OBs: (A) tentative identification of PBDE isomers after selecting specific masses (B) roof tile detection for PBHD isomers

registered mass spectra with those reported in the literature (when available)[11, 16-17] and by mass spectrum interpretation.

Among eight selected NBFRs, only hexabromobenzene (HBB) was found at measurable levels in the studied samples. Several roof tile structured groups were detected when looking for other NBFRs. Mass spectrum interpretation allowed the identification of these compounds as PBBs. The extraction of the PBB homologue characteristic masses confirmed the presence of mono- to nona-BB congeners. Two extra HNPs, a dibromophenol isomer (DBP) and the 2,4-dibromoanisole (DBA) were also detected in the samples. Additionally, two families of natural brominated diMeO-derivatives were detected. On the basis of previous structural information reported, two di-MeO-PBBs and two-diMeO-PBDEs were found in the investigated samples. No data concerning the identification of two of these analytes, one belonging to each category, has been published in the specialised literature.

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