POTENTIAL OF GAS CHROMATOGRAPHY-ATMOSPHERIC PRESSURE CHEMICAL IONIZATION-TANDEM MASS SPECTROMETRY FOR SCREENING OF HEXABROMOCYCLODODECANE

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Introduction

Hexabromocyclododecane (HBCD) is a highly lipophilic brominated flame retardant (BFR) applied in extruded and high-impact polystyrene foams. Its use has increased concurrently to restrictions on polybrominated diphenyl ethers (PBDEs) [1]. Because of their toxicity, persistence and tendency for bioaccumulation, HBCD is classified by REACH as a substance of very high concern and currently assessed for inclusion in the Stockholm Convention [2]. Commercial HBCD is composed of a mixture of isomers, mainly γ-HBCD (70%), α-HBCD (16%) and β-HBCD (13%) [3]. Both GC-MS and LC-MS are commonly used for the determination of HBCD, obtaining similar results for total HBCD concentrations [4]. However, GC-MS is not suitable if information about levels of individual isomers is required, as diastereomers interconvert at temperatures above 160ºC.
Nevertheless, the availability of a high sensitive and selective screening method by GC-MS could be interesting as HBCD are also found in the same fractions as PBDEs when applying conventional sample treatment for POPs analysis in majority of labs. However, the selectivity attainable under these circumstances can be compromised by the use of negative chemical ionization as HBCD debromination could yield overlapping signals with some PBDE congeners.
In this scenario, the availability of a softer ionization source in GC could render higher-mass precursor ions, more specific, avoiding the aforementioned interferences. In this study, GC coupled to triple quadrupole mass spectrometer with the new and soft atmospheric pressure chemical ionization source (GC-(APCI)MS/MS) has been explored for the determination of HBCD in complex matrices, as polyurethane foam disk (PUFs) used for passive air sampling. Favoring the formation of a highly abundant molecular ion in the source has been the main goal because of the specificity added when choosing the molecular ion (or protonated molecule) as precursor ion in tandem MS experiments. SRM transitions from different precursor to product ions have been evaluated at different collision energies in order to select the most intense and, if possible, to avoid interferents coming from other BFRs. Analytical parameters of the method such as linearity, repeatability and LODs have been studied.
This new soft ionization APCI source has been satisfactorily applied before for GC-amenable compounds such as pesticides, PAHs, PCBs and, very recently, dioxins and dioxin-like PCBs [5,6].

Materials and methods

Samples analyzed in this work consisted in several PUFs, previously cleaned up with water (ultrasonic bath) and by Soxhlet extraction with acetone for 24 h. Air dried clean PUFs were used for passive air sampling (three months exposure) in four different countries (Mali, Kenya, Fiji and Uruguay) including a field blank (non-exposed PUF).
HBCD standard (γ-1,2,5,6,9,10-Hexabromocyclododecane) as well as isotopically labelled HBCD (γ-1,2,5,6,9,10-Hexabromo[$^{13}C_{12}$]cyclododecane were purchased form Wellington laboratories with a purity higher than 98% as 50 μg/mL solutions in toluene.
Extraction procedure for PUFs was based on that in house validated at Laboratory of Dioxins (IDAEA, CSIC, Barcelona, Spain). It consisted in a Soxhlet extraction with toluene for 24 h. Subsequent clean-up was carried out by silica/alumina solid liquid adsorption chromatography. Data were acquired using a GC system (Agilent 7890A, Palo Alto, CA, USA) equipped with an autosampler (Agilent 7693) and coupled to a triple quadrupole (QqQ) mass spectrometer (Xevo TQ-S, Waters Corporation, Manchester, UK), operating in APCI mode. A fused

silica DB-5MS capillary column with a length of 30 m x 0.25 mm i.d. and a film thickness of 0.25 µm (J&W Scientific, Folson, CA, USA) was used. The oven temperature was programmed as follows: 140ºC (1 min); 10ºC/min to 310ºC (1 min). Splitless injections (50 psi) of 1 µL sample extract were carried out at 280 ºC. Helium was used as carrier gas at 2 mL/min. In the SRM method, automatic dwell time (44 ms) was applied in order to obtain 15 points per peak. The interface temperature was set to 310 ºC using N2 as auxiliary gas at 250 L/hr, a make-up gas at 300 mL/min and cone gas at 170 L/hr. The APCI corona discharge pin was operated at 1.6 µA. The ionization process occurred within an enclosed ion volume, which enabled control over the protonation/charge transfer processes. Targetlynx (a module of MassLynx) was used to handle and process the acquired data.

Results and discussion

Ionization and in-source fragmentation of HBCD under EI and APCI

The widely used EI ionization source generated a highly fragmented EI spectrum for HBCD (**Figure 1A**). The ion $[M]^{+\bullet}$ was completely fragmented in the source being the non-specific *m/z* ions 67 and 79 the most abundant peaks of the spectrum. The *m/z* ion with higher mass corresponded to the loss of one Br atom, followed by the subsequent losses of HBr. Thus, the *m/z* ion 316.9537 ($C_{12}H_{15}Br_2$) was normally selected for the SIM acquisition with GC-(EI)HRMS instruments. Under these conditions the sensitivity of the determination is low (500 pg on-column) and cannot reach the expected concentration levels.

Alternatively, ionization of HBCD was studied under the "soft" APCI source. The ionization and the in-source fragmentation behavior of the HBCD under APCI were softer than in EI. $[M]^{+\bullet}$ was also absent in the APCI spectrum and the loss of one Br atom, followed by the subsequent losses of HBr were also observed. However, the relative abundances of these ions were different to those observed in EI (**Figure 1B**) being the *m/z* ion corresponding to the loss of $[H_3Br_4]^-$ the base peak of the spectrum. More important for the required application was the abundant presence (50% of the base peak) of the *m/z* ion corresponding to the loss of one bromine atom ($[M\text{-}Br]^{+\bullet}$) which is a good candidate as precursor ion for MS/MS experiments due to its high mass and specificity. Cone voltage values between 5 and 50 V were tested in order to select the optimum value.

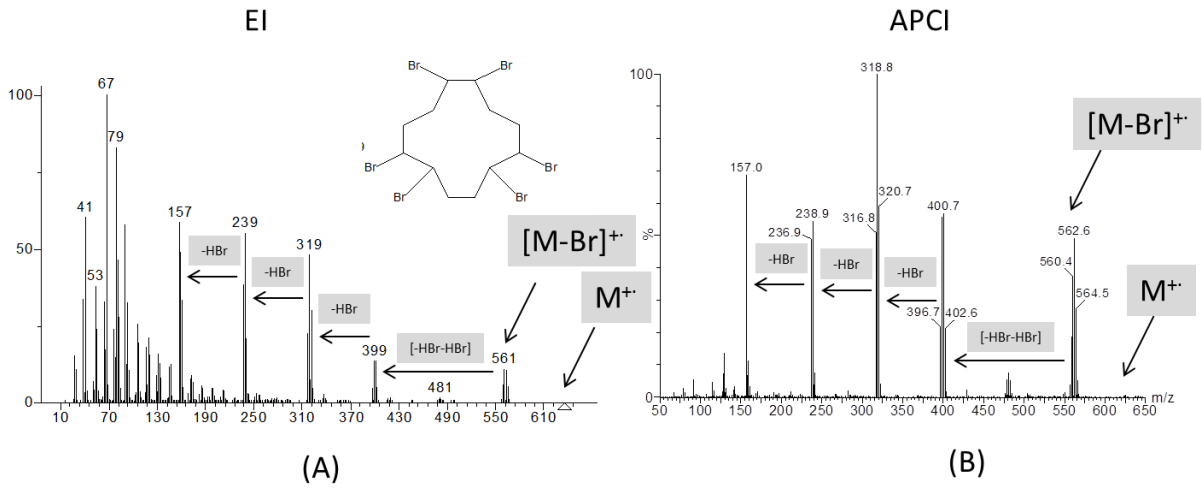

Figure 1. EI (A) and APCI (B) spectrum of HBCDD.

Fragmentation of HBCD in the collision cell

Fragmentation of the HBCD in the collision cell was studied. Two *m/z* ions from the isotopic cluster corresponding to $[M\text{-}Br]^{+\bullet}$ (M+4 and M+6) were selected in the first quadrupole and fragmentation was performed in the collision cell at collision energies in the range of 10-30 eV. As an example, Figure 2 shows ScanWave daughter scan spectrum at 10 eV where the main product ions are observed.

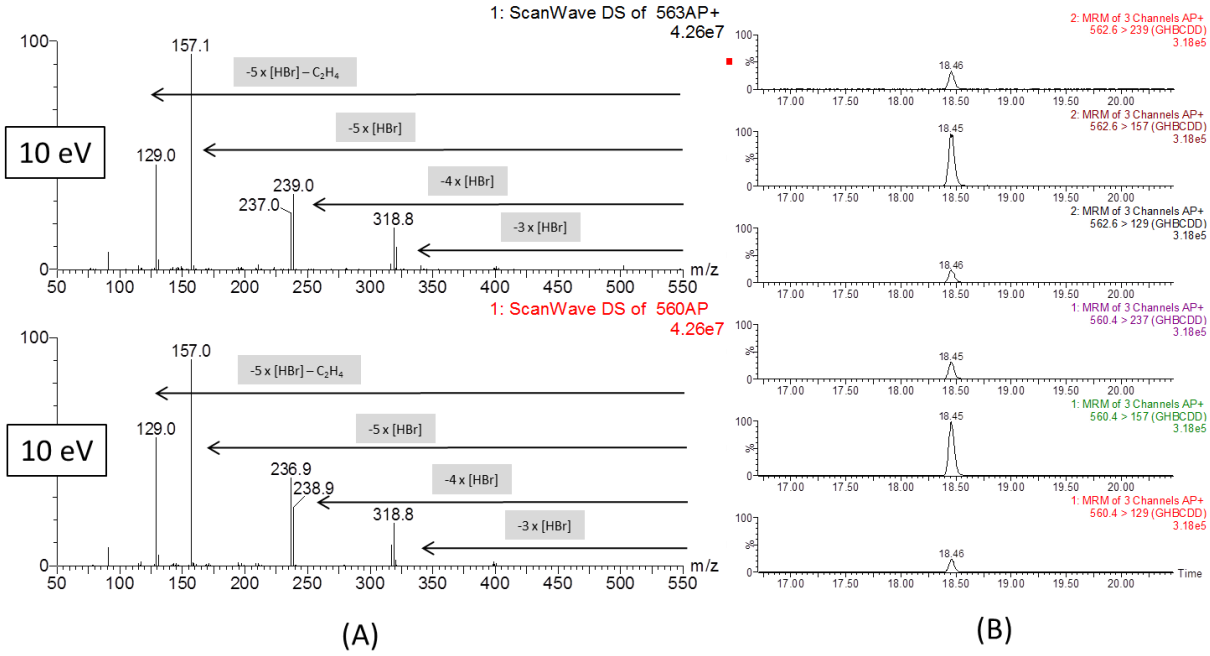

Figure 2. (A) ScanWave daughter scan spectrum at 10 eV for precursor ions m/z 560.4 (down) and m/z 562.6 (up). (B) GC-APCI-MS/MS chromatograms for HBCD at 10 ng/ml (10 pg on-column).

Analytical parameters

Sensitivity of the HBCD was evaluated under the conditions optimized by GC-APCI-MSMS. The repeatability of response (n=10 at LOQ) was also studied.

Linearity of the relative response of the HBCD (to its corresponding C_{13} isotopically labeled HBCD) was established by analyzing standard solutions, in triplicate, in the range of 1 – 100 ng mL^{-1}. The correlation coefficient (r) was higher than 0.99, with residuals lower than 20%. LOD was estimated to be around 100 fg. Sensitivity of the method can be appreciated in **Figure 3A** (1 ng mL^{-1} standard in nonane).

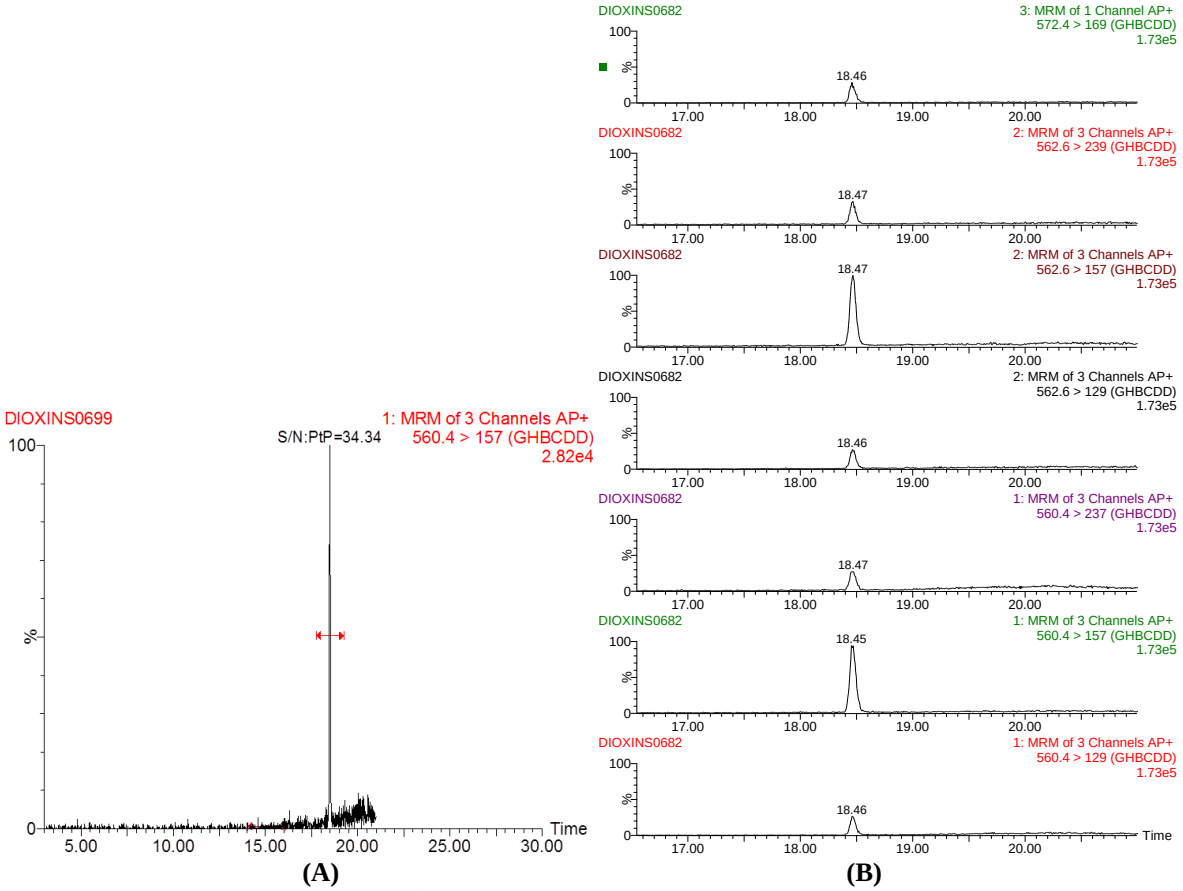

Figure 3. (A) GC-APCI-MS/MS chromatogram for HBCD at 1 ng/ml. S/N: signal-to-noise ratio. (B) GC-APCI-MS/MS chromatograms for a PUF extract were HBCD were detected and confirmed using up to six SRM transitions. Upper chromatogram corresponds to labeled HBCD

Analysis of real samples

The developed method was finally applied to the analysis of PUF extracts, obtained as described above. The concentrations obtained using the developed methodology were 208.3 and 776 pg/µl (in the extract). In Figure 3B, we can observe the detection and confirmation of HBCD in one of the PUF extracts, as well as the labeled $^{13}C_{12}$ HBCD (572.4>169)

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