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

Carlos Sales[a], Tania Portolés[a], Juan Vicente Sancho[a], Esteban Abad[b], Manuela Ábalos[b], Jordi Sauló[b], Heidelore Fiedler[c], Belén Gómara[d], Joaquim Beltrán[a*]

[a] Research Institute for Pesticides and Water, University Jaume I, E-12071 Castellón, Spain.

[b] Laboratory of Dioxins, IDAEA, CSIC, E-08034 Barcelona, Spain.

[c] UNEP/DTIE Chemicals Branch, 11-13 Chemin des Anémones, CH-1219 Châtelaine (GE), Switzerland, and MTM Research Centre, Örebro University, SE-70182 Örebro, Sweden

[d] Institute of General Organic Chemistry, CSIC, E-28006 Madrid, Spain

*Tel: 34-964-387360, Fax: 34-964-387368, E-mail: beltranj@uji.es

Abstract

A fast method for the screening and quantification of hexabromocyclododecane (sum of all isomers) by gas chromatography using a triple quadrupole mass spectrometer with atmospheric pressure chemical ionization (GC-APCI-QqQ) is proposed. This novel procedure makes use of the soft atmospheric pressure chemical ionization source, which results in less fragmentation of the analyte than by conventional electron impact (EI) and chemical ionization (CI) sources, favoring the formation of the $[M-Br]^+$ ion and thus, enhancing sensitivity and selectivity. Detection was based in the consecutive loses of HBr from the $[M-Br]^+$ ion to form the specific $[M-H_5Br_6]^+$ and $[M-H_4Br_5]^+$ ions, which were selected as quantitation (Q) and qualification (q) transitions, respectively. Parameters affecting ionization and MS/MS detection were studied. Method performance was also evaluated; calibration curves were found linear from 1 pg/µL to 100 pg/µL for the total HBCD concentration; instrumental detection limit was estimated to be 0.10 pg/µL; repeatability and reproducibility, expressed as relative standard deviation, were better than 7% in both cases. The application to different real samples (polyurethane foam disks (PUFs), food, and marine samples) pointed out a rapid way to identify and allow quantification of this compound together with a number of polybrominated diphenyl ethers (BDE congeners 28, 47, 66, 85, 99, 100, 153, 154, 183, 184, 191, 196, 197, and 209) and two other novel brominated flame retardants, *i.e.*, decabromodiphenyl ethane (DBDPE) and 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), due to their presence in the same fraction when performing the usual sample treatment.

Keywords

Introduction

Hexabromocyclododecane (HBCD) is a highly lipophilic brominated flame retardant (BFR) most commonly used in expanded polystyrene (EPS) and extruded polystyrene (XPS) foams produced for the building and construction industry to meet fire safety standards (approximately 96% of total production). HBCD also has minor uses as a flame retardant in textile back coatings and high-impact polystyrene (HIPS) used in electronics housings. Its use has increased concurrently to restrictions on polybrominated diphenyl ethers (PBDEs) [1]. HBCD may be released to air, water, soil, and sediment during manufacture, processing, improper handling, improper storage or containment, product usage, and disposal of HBCD-containing products and materials. Because of its toxicity, persistence and tendency for bioaccumulation and biomagnification in food chains, HBCD is classified by REACH as a substance of very high concern. At its sixth meeting in 2013, the Conference of the Parties of the Stockholm Convention adopted the listing of HBCD to Annex A to the Stockholm Convention (with specific exemptions; decision SC-6/13) [2]. On 26 November 2014, one year after the official communication to Parties, the amendment to the Convention listing HBCD in Annex A to the Stockholm Convention entered into force for most parties. Technical HBCD is predominantly comprised of three 1,2,5,6,9,10-HBCD diastereomers, 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 capable to differentiate between the individual isomers, as the diastereomers interconvert at temperatures above 160 ºC.

Nevertheless, the availability of a highly sensitive and selective screening method for HBCD by GC-MS could be interesting as this compound elutes in the same fraction as PBDEs when applying conventional sample treatment for POPs analysis as used in the majority of routine laboratories. Thus, only in those cases where the concentration of total HBCD is above established limits in the GC-MS screening, quantitative analysis of individual isomers would be carried out using the isomer selective LC methods [5, 6]. Additionally, HBCD debromination in negative ion chemical ionization (NICI) [7–9] could yield overlapping signals with some PBDE congeners when monitoring ions at m/z 79 and 81 for quantitation. The determination of HBCD by GC-HRMS in positive EI mode has demonstrated greater selectivity, as the different fragmentation allows to select

higher, more specific ions for quantification. However, the high fragmentation on EI sources compromises sensitivity, leading to limits of detection (LODs) of approximately 0.04 ng on column [10].

In this scenario, the availability of a softer ionization source in GC could render higher-mass precursor ions, more specific, avoiding the potential interferences. The new soft atmospheric pressure chemical ionization (APCI) source has already been satisfactorily applied for GC-amenable compounds such as pesticides, polycyclic aromatic hydrocarbons (PAHs), PBDEs and, very recently dioxins/furans (PCDDs/PCDFs) [11, 12]. The aim of this work is to study the potential of GC coupled to a triple quadrupole mass spectrometer using APCI source (GC-APCI-QqQ) for the determination of total HBCD in complex matrices, such as polyurethane foam (PUF) disks used for passive air sampling, and marine samples (dolphin, fish, prawn, squid, and zooplankton) and two Standard Reference Materials (SRMs 2974a and 1954; *i.e.*, mussel tissue and human milk).

Materials and methods

Chemicals and reagents

HBCD standard (γ-1,2,5,6,9,10-Hexabromocyclododecane) as well as isotopically labeled HBCD (γ-1,2,5,6,9,10-hexabromo[$^{13}C_{12}$]cyclododecane) was purchased from Wellington Laboratories (Guelph, ONT, Canada) with a purity higher than 98% as 50 ng/μL solution in toluene.

All reagents used for the sample treatment were of trace analysis grade. Sulphuric acid (95%−97%) and silica gel were supplied by J.T. Baker (Deventer, The Netherlands) (for the analysis of PUF disks) and by Merck Co. (Darmstadt, Germany) (for the rest of samples). Basic alumina, EcoChrom™ MP Alumina B – Super I was purchased from MP Biomedicals Germany GmbH. Sodium hydroxide was from Carlo Erba (Milan, Italy). Acetone, n-hexane, dichloromethane, toluene and granular anhydrous sodium sulfate were obtained from J.T. Baker (Deventer, The Netherlands).

Samples

Samples analyzed in this work consisted of several PUF disks (140 mm ø, 13.5 mm thickness and approximately 5.6 g weight) used for passive air sampling (after three months exposure) in four different countries (Mali, Kenya, Fiji and Uruguay), a blank (non-exposed PUF) was also analyzed.

Extracts from two different NIST standard reference materials, SRM 2974a (mussel tissue) and SRM 1954 (human milk); and 11 sample extracts coming from the marine field (one dolphin, four different fish species, two prawns, two squids and two zooplankton) previously analyzed for PBDE [13] determination, were also used in this work to investigate the presence of HBCD.

Sample Treatment

Analytical procedure for PUFs was based on that optimized at Laboratory of Dioxins (IDAEA, CSIC, Barcelona, Spain). It consisted in a Soxhlet extraction with toluene for 24 h, previously cutting the disk in several pieces and adding a known amount of $^{13}C_{12}$-HBCD. Subsequent clean-up was carried out by a multilayer acid/base silica column eluted with n-hexane. Then, the concentrated extract was added onto a basic alumina column and HBCD was collected in the n-hexane:dichloromethane (80:20) fraction.

Marine samples extraction and purification procedures are previously described in the literature [14, 15]. Briefly, extraction involved matrix solid-phase dispersion (MSPD) of the samples. Among 6-200 g of fresh sample homogenized with 4:1 (w/w) silica gel/anhydrous sodium sulfate powder, and spiked with $^{13}C_{12}$-BDEs 47, 99, and 153 was ground to a fine powder, loaded onto a column, and extracted with 400 mL of 1:1 (v/v) acetone/n-hexane mixture. For the clean-up of the extracts two multilayer columns filled with neutral silica, silica modified with sulfuric acid (44%, w/w), and silica modified with KOH (56%, w/w) were employed using n-hexane as elution solvent. When required, the final extract containing the target compounds was subjected to further fractionation on Supelclean™ ENVI™-Carb SPE cartridges (Supelco, Palo Alto, USA), as described elsewhere [16] to separate *ortho*-substituted PCBs plus PBDEs from PCDD/PCDF and non-*ortho*-substituted PCBs. HBCD remains in the PBDEs fraction [17].

GC-(APCI)QqQ

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 positive mode. A fused silica DB-1HT capillary column, 15 m x 0.25 mm i.d. and a film thickness of 0.1 μm (J&W Scientific, Folson, CA, USA) was used. The oven temperature was programmed as follows: 140 ℃ (1 min); 20 ℃/min to 285 ℃; 40 ℃/min to 350 ℃ (1 min). Pulsed splitless injections (100 psi) of 1 μL sample extract were carried out at 280 ℃. Helium was used as carrier gas at 4 mL/min. To establish the Selected Reaction Monitoring (SRM) conditions, automatic dwell time (35 ms to 58 ms) was applied in order to obtain 15 points per peak. The interface temperature was set to 350 ℃ using N_2 as auxiliary gas at 250 L/h, as make-up gas at 300 mL/min and as cone gas at 170 L/h. 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. Final MS conditions selected to determine HBCD included a cone voltage of 20 V, collision energy of 20 eV and the transitions 560.4→129.0; 560.4→157.1 and 560.4→237 for native HBCD and 572.4→169 for the labeled HBCD.

Results and discussion

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

The typical HBCD determination by GC-MS is performed either using NICI [7–9] or EI coupled to HRMS [10, 18]. When using NICI, the molecular ion is not observed, being the $[M-HBr]^{-\bullet}$ ion the one with the highest mass (**Fig. 1A**). The fragmentation in negative mode does only permit to monitor either the ion at *m/z* 160 ($[M-H_2Br_6]^{-\bullet}$) or the bromide ion, which is preferred due to its higher sensitivity. However, the use of these non-specific ions is an impediment for the use of isotopically labeled HBCD standards [19]. On the other hand, EI source generates a spectrum with a high fragmentation pattern for HBCD (**Fig. 1B**). The $[M]^{+\bullet}$ ion is completely fragmented in the source being again non-specific ions at *m/z* 67 and 79 the most intense peaks of the spectrum. The ions with the highest mass correspond to the loss of one Br atom, followed by the subsequent losses of HBr. Under these conditions literature shows that the limit of detection is high (around 2 ng/g lipid weight (lw)) and not enough to detect HBCD in several expected positive samples [18]. **Table 1** enlists the majority of attempts

performed so far in order to detect and quantify HBCD, indicating the systems used and the achieved performance in each case in terms of limit of detection (LOD) and limit of quantification (LOQ). The results evidence the need of an improvement of the analytical performance in samples with low HBCD concentrations (lower than 2 ng/g) as foods and environmental samples are expected to be low contaminated, as a result of the HBCD use in the past [4, 18]

At this point APCI source was explored, revealing its softer ionization and the much lower in-source fragmentation of the HBCD in comparison with NICI and EI. Although $[M]^{+\bullet}$ was absent in the APCI spectrum, the loss of one Br atom, followed by subsequent losses of HBr were observed. Nevertheless, the relative abundances of these ions were significantly higher to those observed in EI, being the ion corresponding to the loss of $[H_3Br_4]^{\cdot}$ the base peak of the APCI spectrum (**Fig. 1C**). The abundant presence (50% of the base peak) of the ion corresponding to the loss of one single bromine atom ($[M-Br]^+$) was selected for the required application. It was considered a good candidate as precursor ion for MS/MS experiments due to its high *m/z* value and specificity. During these experiments cone voltage values between 5 and 50 V were tested in order to select the optimum value pursuing a low in-source fragmentation and maximum response. Finally, 20 V was selected as optimum.

Fragmentation of HBCD in the collision cell

In order to study the fragmentation of HBCD in the collision cell, two ions from the isotopic pattern corresponding to $[M-Br]^{+\bullet}$ (M+4 and M+6) were selected in the first quadrupole and fragmentation was performed using collision energies between 10 eV and 30 eV. A collision energy of 20 eV was selected as optimum for all the aforementioned transitions (**Fig. 2**). Accordingly, the selected transitions tested were 560.4→129.0; 560.4→157.1 and 560.4→237 corresponding to the fragmentation of the precursor ion $[M+4-Br]^+$ and 562.6→129.0, 562.6→157.1 and 562.6→236.9 taking $[M+6-Br]^+$ ion as precursor. The transition 560.4→157.1 demonstrated the highest sensitivity and was hence chosen as Q transition for further experiments. The transition 572.4→169 was selected for monitoring the $^{13}C_{12}$-labeled standard as its fragmentation pattern was comparable to the native HBCD.

Chromatographic optimization

Chromatographic conditions for the determination of HBCD were taken from a previous work [13] as the main goal of the developed method was to detect and/or quantitate the total amount of HBCD in the same sample extracts prepared for PBDEs determination in a single injection. Taking into account the literature regarding HBCD determination by GC (**Table 1)** two columns, a HP-5MS 30 m x 0.25 mm, 0.25 µm and a DB-1HT 15 m x 0.25 mm, 0.10 µm, were tested in order to determine the suitability of both stationary phases for the determination of HBCD. The results showed poor linearity and reproducibility for the HP-5MS column when compared to the DB-1HT column. An important decay of the signal with the increasing number of runs was observed in the HP-5MS column. This column-dependency and the fact that signal to noise (S/N) ratios were 5 times lower when using the 5MS column confirmed the DB-1HT column as the proper one. This kind of comparison between columns has been previously performed but at concentration levels of ng/µL, finding that the DB-1HT column provided sharper signals and better separation from egg matrix peaks, making the DB-1HT column more suitable for HBCD analysis [20]. It is worth to mention that the film thickness was different and that could also influence the column performance in the case of HBCD.

Analytical parameters

In order to test the reliability of the method, repeatability of response was studied by ten repeated injections of HBCD standards at 1 pg/µL level. The relative standard deviation was clearly lower than 10%. Linearity of the relative response of the HBCD (to its corresponding $^{13}C_{12}$ isotopically labeled standard) was studied by analyzing standard solutions, in triplicate, in the range of 1 pg/µL–100 pg/µL. The correlation coefficient (r^2) was higher than 0.999, with residuals lower than 2%. Sensitivity of the method can be derived from **Fig. 3A** (1 pg/µL standard solution in nonane). LOD was determined to be around 100 fg injected in pulsed splitless mode. With the purpose of evaluating the specificity provided by the use of the selected HBCD transitions, a CS4 (50 pg/µL) PBDEs mixture standard was injected monitoring the HBCD transitions through the whole chromatogram. **Fig. 3B** shows the result, in which only two signals appeared in the whole chromatogram; one "interferent" eluting 2.5 minutes later but one eluting at the same time as HBCD, showing a completely different q/Q ratio. The signal produced by the 50 pg/µL PBDEs mixture in the Q transition was around 1% of the signal

for the 1 pg/µL HBCD standard (see **Fig. 3**). Consequently, possible interferences coming from PBDEs which would led in false positives when determining HBCD are discarded and only over quantifications around 1% of the area of the native HBCD could appear in regular analysis, which is more than acceptable.

Conversely, the possible interferences of HBCD in PBDEs determination were also studied, by monitoring the transitions of PBDEs in an injection of the most concentrated HBCD standard available (CS5, 100 pg/µL). Degradation of HCBD to lower brominated analogues at injection port temperatures above 240 ℃ has been reported [21, 22]. These degradation products are an important PBDE quantitation issue when acquiring the bromide ion as quantitation ion. This situation is much less an issue when using the GC-APCI-QqQ method. **Fig. 4A** shows that the signal generated by thermal degradation product(s) originated from 100 pg of HBCD in the transitions of BDE 47 is only of about 1% of the corresponding 1 pg BDE 47 signal (**Fig. 4B**). This would have practically no negative effect on the quantification of this PBDE and constitutes another advantage of the APCI source and its associated low fragmentation, which allows to minimize the mutual interferences among brominated compounds.

Analysis of real samples

The usability/suitability of this new procedure was tested against real/naturally contaminated samples by analyzing PUF extracts from a UNEP project having been exposed for three months in Suva (Fiji), Montevideo (Uruguay), Nairobi (Kenya) and Bamako (Mali). Quantification of the samples was carried out by using calibration curve with standard in solvent, using relative responses to internal labeled $^{13}C_{12}$ γ-HBCD standard added as surrogate to the sample.

Total HBCD concentrations obtained using the GC-APCI-QqQ method ranged from 190 pg/µL to 400 pg/µL (in the extract) which correspond to 4 ng/PUF and 8 ng/PUF, respectively. In **Fig. 5A**, we can observe the detection and identification of HBCD in one of the PUF extracts (3.68 ng/PUF) by the presence of its 4 SRM transitions at expected retention time and the q/Q ratios within stablished tolerances.

Finally, the 11 marine samples and the two reference materials were re-injected into the system under optimized conditions for HBCD. HBCD was detected in ten out of the 13 samples analyzed. **Fig. 5** shows the chromatograms for a fish (B) and the mussel tissue reference (C) sample, respectively.

Conclusions

The use of the atmospheric pressure chemical ionization source has been proved to render better sensitivity and specificity than commonly used EI/CI sources, thanks to the acquisition of several transitions coming from a selective [M-Br]$^{+\bullet}$ precursor ion and leading to specific HBCD product ions instead of the common bromide ion at *m/z* 79/81. This fact allowed to decrease the LOD for total HBCD down to 100 fg/μL, which implies a significant advance when compared to traditional previous methodologies, based in GC or LC coupled to different sources and analyzers (**Table 1**) resulting in a limit of detection around 100 times lower in most of the cases. Additional specificity has been found for the simultaneous determination of both PBDEs and HBCD, as the fact of monitoring different transitions for each compound instead of the common bromide atom has shown to minimize their mutual interferences.

Although LC-MS/MS methods allow isomer specific determination at levels of few ppb, the developed methodology has demonstrated to be able to detect and quantify total concentration of HBCD in PBDE extracts without additional treatment or analysis. It could be effectively applied as a screening methodology to select positive samples to be processed by LC-MS/MS if individual isomer information is required, saving time and budget analysis.

Conflict of interest

The   authors   declare   that   they   have   no   conflict   of   interest.

FIGURE CAPTIONS

Fig. 1 (A) NICI, (B) EI, and (C) APCI spectra of γ-HBCD.

Fig. 2 ScanWave product ion spectrum at 20 eV of 560.4 (down) and 562.6 (up).

Fig. 3 (A) GC-APCI-QqQ chromatogram for 1 pg HBCD standard. (B) GC-APCI-QqQ chromatogram for 50 pg PBDE standard mixture acquired with the transitions of HBCD. S/N: signal-to-noise ratio; Q: Quantification transition; q: qualification transition.

Fig. 4 (A) GC-APCI-QqQ chromatogram for 1 pg PBDE standard solution. (B) GC-APCI-QqQ chromatogram for 100 pg of γ-HBCD acquired with the transitions of BDE 47. Q: Quantification transition; q: qualification transition.

Fig. 5 (A) GC-APCI-QqQ chromatogram for a PUF extract. (B) GC-APCI-QqQ chromatogram for a fish extract. (C) GC-APCI-QqQ chromatogram for a mussel tissue reference material extract. Q: Quantification transition; q: qualification transition.

References

1.   Lankova D, Kockovska M, Lacina O, Kalachova K, Pulkrabova J, Hajslova J (2013) Rapid and simple method for determination of hexabromocyclododecanes and other LC-MS-MS-amenable brominated flame retardants in fish. Anal Bional Chem 405:7829. doi: 10.1007/s00216-013-7076-x

2.   UNEP (2013) List. hexabromocyclododecane, Decis. SC-6/13. http://chm.pops.int/Implementation/NewPOPs/DecisionsRecommendations/tabid/671/Default.aspx. Accessed 30 Jul 2015

3.   Eljarrat E, Gorga M, Gasser M, Díaz-Ferrero J, Barceló D (2014) Dietary exposure assessment of spanish citizens to hexabromocyclododecane through the diet. J Agric Food Chem 62:2462–2468. doi: 10.1021/jf405007x

4.   Haug LS, Thomsen C, Liane VH, Becher G (2008) Comparison of GC and LC determinations of hexabromocyclododecane in biological samples - Results from two interlaboratory comparison studies. Chemosphere 71:1087–1092. doi: 10.1016/j.chemosphere.2007.10.044

5.   Han Q, Song H, Gao S, Zeng X, Yu Z, Yu Y, Sheng G, Fu J (2014) Determination of ten hexabromocyclododecane diastereoisomers using two coupled reversed-phase columns and liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 28:1473–1478. doi: 10.1002/rcm.6922

6.   Janák K, Covaci A, Voorspoels S, Becher G (2005) Hexabromocyclododecane in Marine Species from the Western Scheldt. Environ Sci Technol 39:1987–1994. doi: 10.1021/es0484909

7.   Covaci A, Roosens L, Dirtu AC, Waegeneers N, Van Overmeire I, Neels H, Goeyens L (2009) Brominated flame retardants in Belgian home-produced eggs: Levels and contamination sources. Sci Total Environ 407:4387–4396. doi: 10.1016/j.scitotenv.2008.09.057

8.   Eljarrat E, De La Cal A, Raldua D, Duran C, Barcelo D (2004) Occurrence and Bioavailability of Polybrominated Diphenyl Ethers and Hexabromocyclododecane in Sediment and Fish from the Cinca River, a Tributary of the Ebro River (Spain). Environ Sci Technol 38:2603–2608. doi: 10.1021/es0301424

9.   Sellström U, Kierkegaard A, de Wit C, Jansson B (1998) Polybrominated diphenyl ethers and hexabromocyclododecane in sediment and fish from a swedish river. Anal Environ Chem 17:1065–1072. doi: 10.1002/etc.5620170612

10.   Gerecke AC, Kohler M, Zennegg M, Schmid P, Heeb N V (2003) Detection of α -isomer dominated HBCD (hexabromocyclododecane) in Swiss fish at levels comparable to PBDEs (polybrominated diphenyl ethers). Organohalogen Compd 61:155–158.

11.   Cherta L, Portolés T, Beltran J, Pitarch E, Mol JGJ, Hernández F (2013) Application of gas chromatography-(triple quadrupole) mass spectrometry with atmospheric pressure chemical ionization for the determination of multiclass pesticides in fruits and vegetables. J Chromatogr A 1314:224–240. doi: 10.1016/j.chroma.2013.09.029

12. Portolés T, Mol JGJ, Sancho J V, Hernández F (2012) Advantages of Atmospheric Pressure Chemical Ionization in Gas Chromatography Tandem Mass Spectrometry: Pyrethroid Insecticides as a Case Study. Anal. Chem 84: 9802–9810. doi: 10.1021/ac301699c

13. Portolés T, Sales C, Gómara B, Sancho JV, Beltrán J, Herrero L, González MJ, Hernández F (2015) Novel analytical approach for brominated flame retardants based on the use of gas chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry with emphasis in highly brominated congeners. Anal. Chem 87: 9892–9899. doi: 10.1021/acs.analchem.5b02378

14. Gómara B, Lebrón-Aguilar R, Quintanilla-López JE, González MJ (2007) Development of a new method for the enantiomer specific determination of HBCD using an ion trap mass spectrometer. Anal Chim Acta 605:53–60. doi: 10.1016/j.aca.2007.10.019

15. Gómara B, Herrero L, González MJ (2006) Survey of polybrominated diphenyl ether levels in Spanish commercial foodstuffs. Environ Sci Technol 40:7541–7547. doi: 10.1021/es061130w

16. Concejero M, Ramos L, Jiménez B, Gómara B, Abad E, Rivera J, González MJ (2001) Suitability of several carbon sorbents for the fractionation of various sub-groups of toxic polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans. J Chromatogr A 917:227–237. doi: 10.1016/S0021-9673(01)00667-7

17. Stapleton HM, Dodder NG, Kucklick JR, Reddy CM, Schantz MM, Becker PR, Gulland F, Porter BJ, Wise S (2006) Determination of HBCD, PBDEs and MeO-BDEs in California sea lions (Zalophus californianus) stranded between 1993 and 2003. Mar Pollut Bull 52:522–31. doi: 10.1016/j.marpolbul.2005.09.045

18. Shaw SD, Berger ML, Brenner D, Kannan K, Lohmann N, Päpke O (2009) Bioaccumulation of polybrominated diphenyl ethers and hexabromocyclododecane in the northwest Atlantic marine food web. Sci Total Environ 407:3323–3329. doi: 10.1016/j.scitotenv.2009.02.018

19. Covaci A, Gerecke AC, Law RJ, Voorspoels S, Kohler M, Heeb NV, Leslie H, Allchin CR, Boer JDE (2006) Hexabromocyclododecanes (HBCDs) in the Environment and Humans : A Review. Environ. Sci. Technol. 40: 3679–3688. doi: 10.1021/es0602492 40:3679–3688.

20. Hiebl J, Vetter W (2007) Detection of hexabromocyclododecane and its metabolite pentabromocyclododecene in chicken egg and fish from the official food control. J Agric Food Chem 55:3319–3324. doi: 10.1021/jf063428b

21. Covaci A, Voorspoels S, D'Silva K, Huwe J, Harrad S (2008) Chapter 15 Brominated Flame Retardants as Food Contaminants. Compr Anal Chem 51:507–570. doi: 10.1016/S0166-526X(08)00015-9

22. Voorspoels S (2006) Environmental distribution of brominated flame retardants in Belgium. doi: ISBN 90-5728-060-4

Table 1. Techniques and conditions previously used for the determination of HBCD and their performance in terms of LOD and LOQ.

| Technique	Column	Gradient	Ionic species and *m/z*	LOD	LOQ	Reference
GC-HRMS (EI)	DB-1 (10 m × 0.28 mm × 0.1 μm)	--	[M - Br]+ ;560.7289; 562.7269	--	30 - 50 pg on column	
LC-MS (APCI-)	C18-RP (125 mm x 4 mm)	methanol/water (80:20); 1 ml/min	[M - H]−; 638.6; 640.6; 642.6	--	500 - 1000 pg on column a	[10]
LC-MS/MS (ESI-)	C30 YMC Carotenoid S-5 (4.6 x 250 mm)	water/methanol (20:80); to 100% methanol over 35 min; 0.5 mL/min	[M-H]−→Br−;640.8 →78.8	5 - 25 pg on column a	300 pg/g (ww)	[17]
LC-MS/MS (ESI-)	NUCLEODEX β-PM (200 mm × 4 mm, 5 μm)	water/methanol (50:50); 500 μL/min	[M+Cl]− →[M−H]−;676.6 → 640.6	1.5 - 4.3 pg/μL	5 - 14 pg/μL b	[14]
GC-MS (NICI)	HP-5MS (30 m × 0.25 mm x 0.25 μm)	110 °C (1 min); 8 °C/min to 180 °C (1 min); 2 °C/min to 240 °C (5 min); 2 °C/min to 265 °C (6 min)	Br−; 79	--	2000 pg/g dry wt.	[8]
LC-MS/MS (ESI-)	Symmetry C18 (2.1 mm × 150 mm, 5 μm) preceded by C18 guard column (2.1 × 10 mm)	H2O:MeOH (3:1 v/v); 0.25 mL/min; 8 min to MeOH 100%; 17 min to MeOH 97.5%; 3 min to initial cond (15 min).	[M-H]− →Br−;638.7 → 78.9, 638.7 → 80.9	30 to 60 pg/g	110 to 200 pg/g a	[3]
GC-MS (NICI)	DB-5MS (30 m × 0.25 mm x 0.25 μm)	80°C (2 min); 25°C /min to 240°C; 4°C/min to 315°C (50 min)	Br−; 79, 81	50 to 100 ng/g	--	[9]
LC-MS/MS (ESI-)	Symmetry C18 (2.1 × 150 mm, 5 μm) a NUCLEODEX β-PM (200 mm × 4 mm, 5 μm) b	water/ methanol/acetonitrile (60:30:10); 250 μL/min; 5 min to methanol/acetonitrile (50:50) (6 min) water/ methanol/acetonitrile (40:30:30) (0.5 min); 500 μL/ min; 8 min to methanol/acetonitrile (30:70) (14 min)	[M-H]− →Br−; 640.6 → 79	0.5 to 5 pg on column	15 to 75 pg/g (ww)	[6]
LC/LC-MS/MS (ESI-)	Eclipse Plus-C18 RP (250 mm × 4.6 mm, 5 μm) coupled to a Zorbax Eclipse XDB-C8 RP (150 mm × 4.6 mm, 5 μm)	MeOH (A)/ACN (B)/H2O (C); 0.5 mL/min. A/B/C (3:87:10) (25 min), 1 min to 100% B (9 min), 3 min to initial cond. (10 min)	[M-H]− →Br−; 640.7 → 78.8	0.4 to 0.8 pg on column	1 to 2.5 pg/μL a	[5]
GC-MS (NICI)	DB-5 (15 m× 0.25 mm× 0.10 μm)	90 °C (1.5 min); 15 °C/min to 295 °C (15 min)	Br−; 79, 81	--	100 pg/g (dry wt) 0.4 ng/g (lw)	[7]
GC-HRMS (EI)	DB-5 (30 m x 0.25 mm x 0.1 μm)	--	Isotope Ratio and RT identification	--	2400 pg/g (lw)	[18]
GC-MS (EI)	DB-5 (30 m × 0.25 mm x 0.25 μm)	--	full scan	200000 pg/g	--	[20]
	DB-1 (30 m × 0.25 mm x 0.25 μm)	--	full scan	200000 pg/g	--	

a Diastereoisomers

b Enantiomers
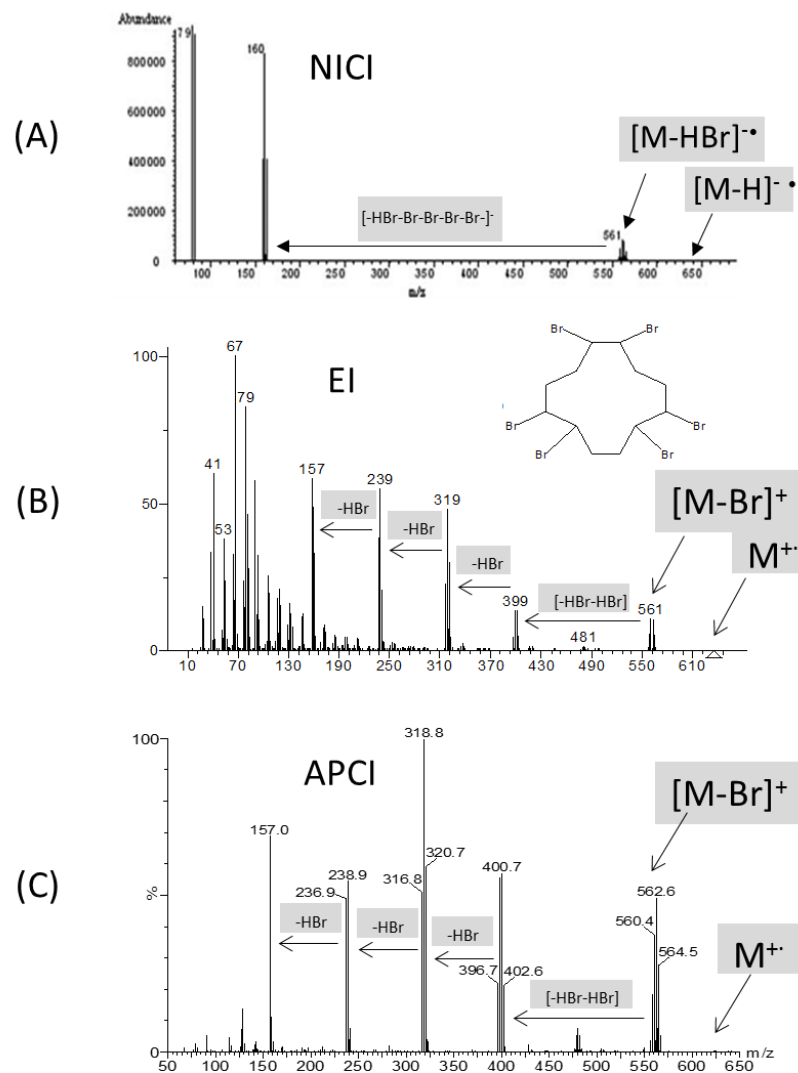
Fig. 1
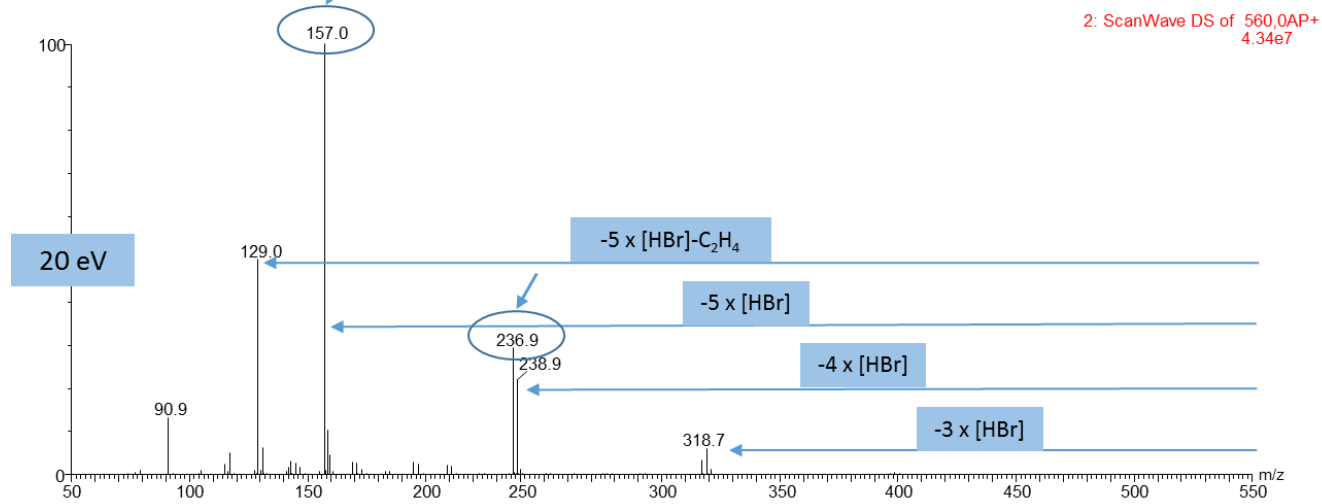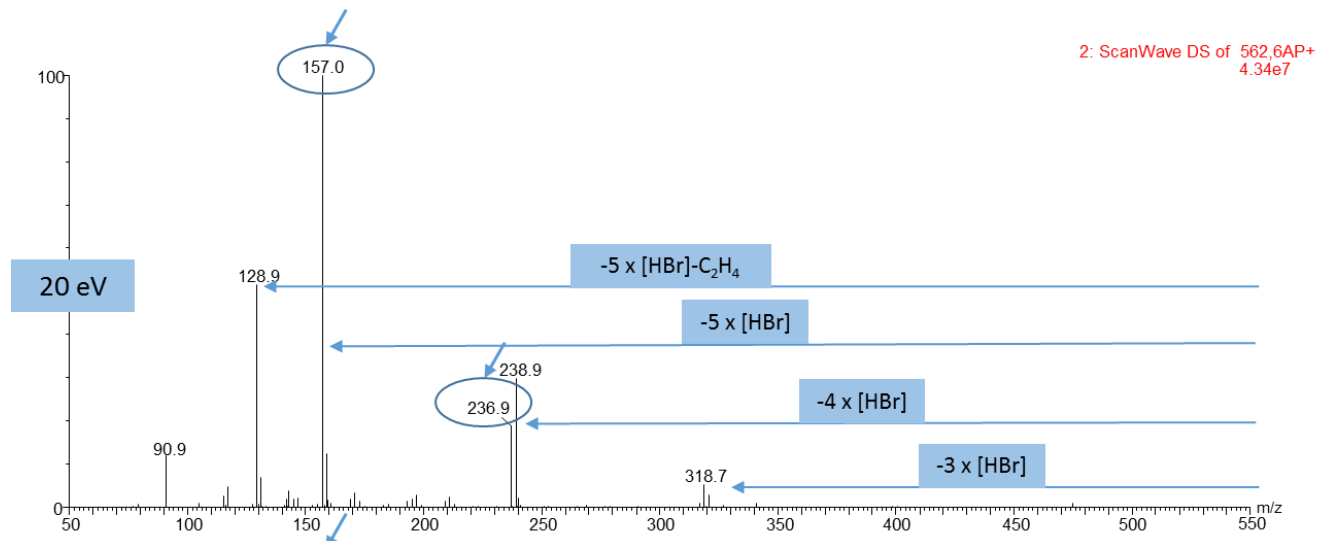
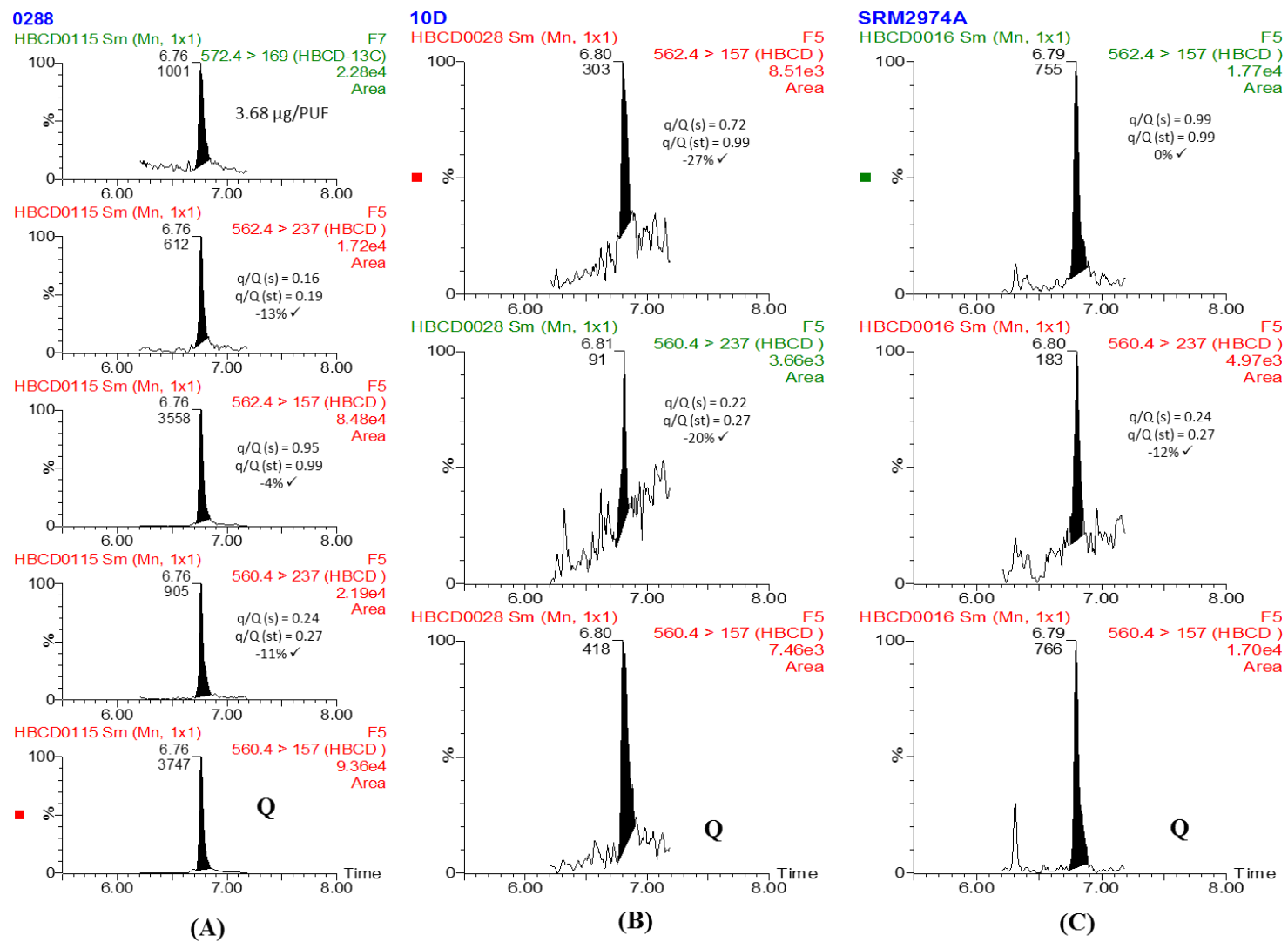Fig. 2
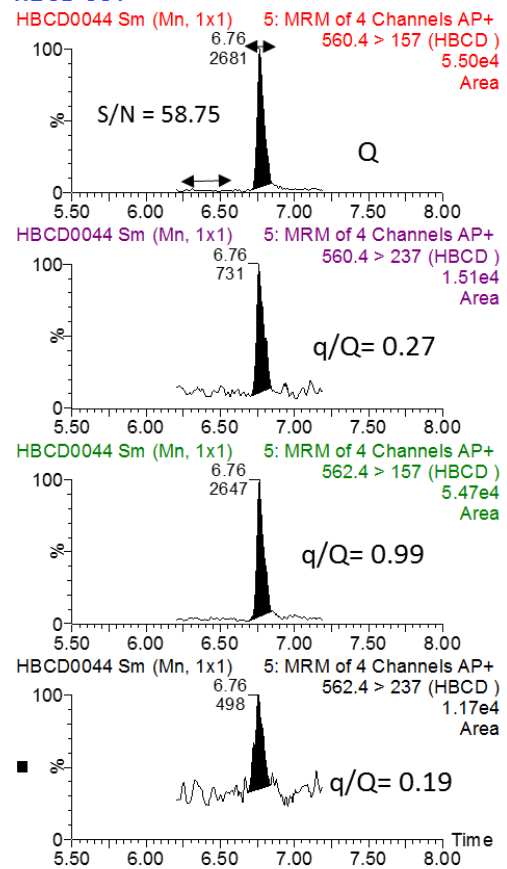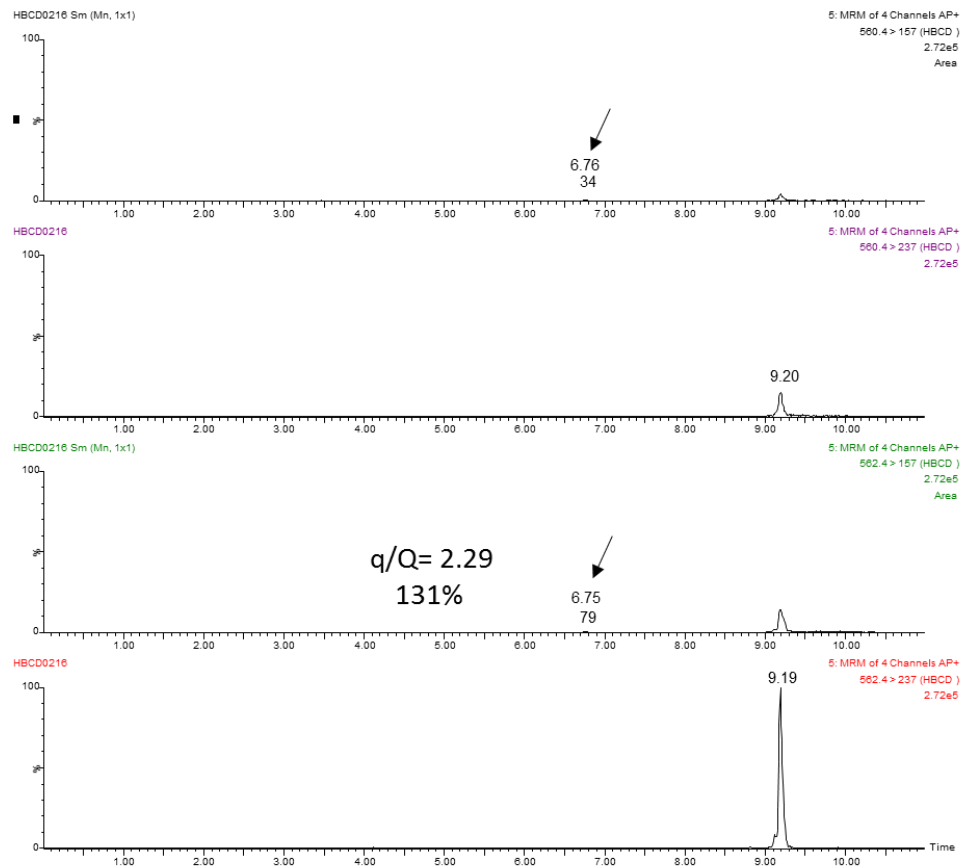
Fig. 3
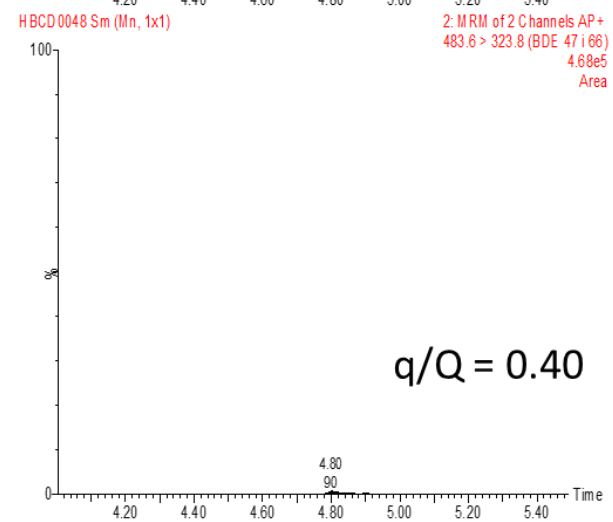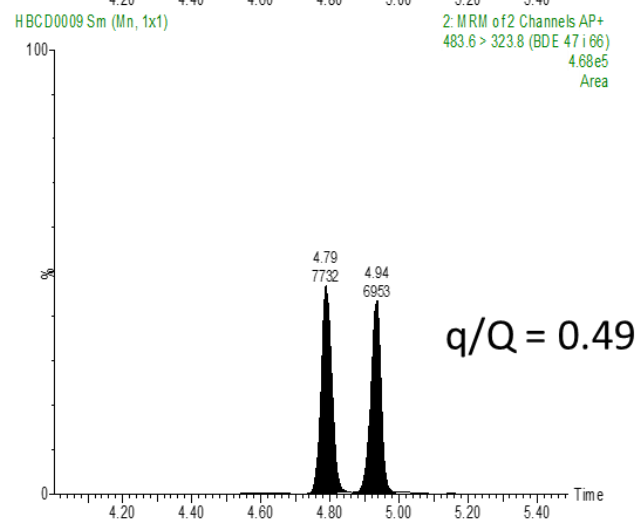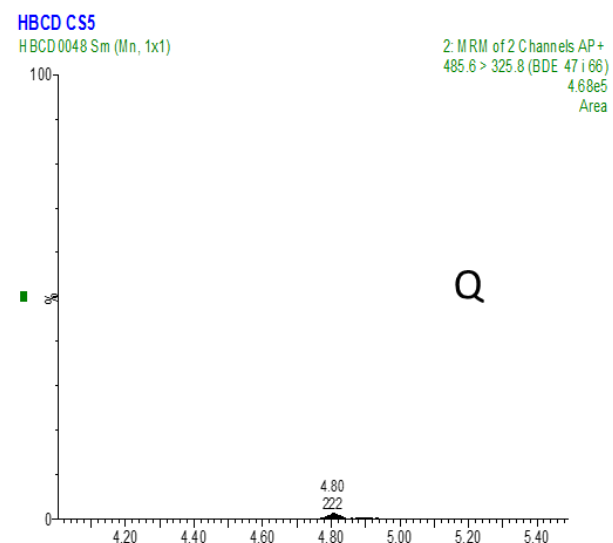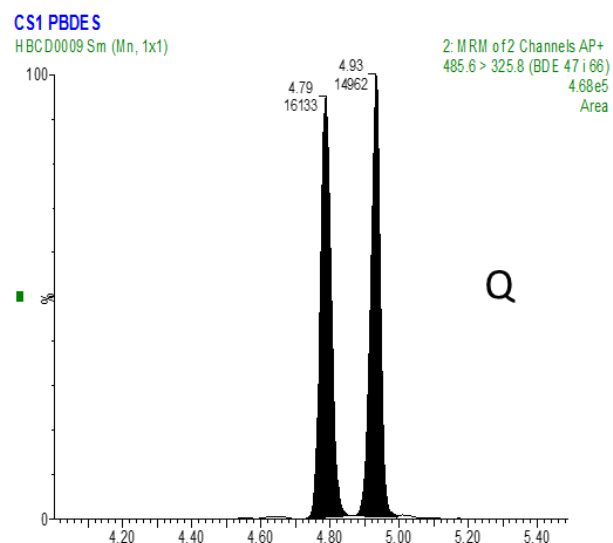
Fig. 4

Fig. 5

Carlos Sales is a PhD student in Sciences at the Research Institute of Pesticides and Water, University Jaume I (Castellón). His research is focused on the use of gas chromatography coupled to mass spectrometry techniques (GC-MS) with special emphasis on the use of the novel atmospheric pressure chemical ionization source for the identification, confirmation and quantification of persistent organic pollutants and volatile organic compounds in complex environmental and food samples. His interests also include the study of the potential of GC-MS-based metabolomics in food and biological fields.

Tania Portolés is member of the Research Institute of Pesticides and Water, University Jaume I (Castellón). Her research is focused on the use of chromatography/mass spectrometry techniques (GC-MS) in last generation instruments, with special emphasis on high-resolution (HRMS) for the identification, confirmation and quantification of organic contaminants in environment, food and biological fields. Special emphasis has been given in the last years to the research performed with the new atmospheric pressure chemical ionization source, in which her work has been pioneer at international level. She is also a teacher in Chemistry degree and in the Master in Applied Chromatographic Techniques.

Dr. Juan Vicente Sancho is Full Professor of Analytical Chemistry at University Jaume I in Castellón (Spain). He is involved in liquid chromatography coupled to tandem mass spectrometry, using both low and high resolution instruments, in different application fields, mainly environmental analysis and food safety, and recently also in doping analysis or food metabolomics. Recently, he is also involved in gas chromatography using atmospheric pressure interfaces.

Dr. Esteban Abad is a Scientist Staff, Head of the Laboratory of Dioxins of the Department of Environmental Chemistry at the Institute of Environmental Assessment and Water Research (IDÆA) of the Spanish Council for Scientific Research (CSIC) in Barcelona. His field of expertise focuses on the analysis of dioxins and other POPs by using HRGC/HRMS for biotic and non biotic samples. He is co-author of more than 80 articles included in the SCI and participates in several International Committees aiming to elaborate harmonized analytical procedures and his contribution in several research projects related the presence of POP in the environment, industrial processes, food and feed and human tissues.

Manuela Ábalos is the laboratory manager of the Laboratory of Dioxins of the Institute of Environmental Assessment and Water Research (IDÆA) of the Spanish Council for Scientific Research (CSIC). In 2004, she got her PhD in Chemistry at the University of Barcelona. Since 2002, she has been involved in the study of persistent organic pollutants (POPs) including dioxin-like compounds, in the field of feed and food as well as in projects related to environmental matrices. She has published more than 40 papers in international journals related to the development of methods and analysis of different groups of environmental organic pollutants.

Jordi Sauló got Engineer Degree at Polytechnic University of Catalonia. He is working at the Laboratory of Dioxin of the Institute of Environmental Assessment and Water Research (IDÆA) of the Spanish Council for Scientific Research (CSIC) since 1994. He has large experience in mass spectrometry instrumentation, including magnetic sector analyzers, QqQ, Orbitrap and related instrumentation such as automated clean up systems. He is also co-authoring large number of papers and related documents. It is also notable his participation in national and international research projects as well as national and international congresses.

Heidelore Fiedler is a professor in chemistry at Örebro University (Sweden), School of Science and Technology, MTM Research Centre since July 2015. Earlier professional positions include Senior Scientific Affairs Officer at the Chemicals Branch of the United Nations Environment Programme (UNEP) in Geneva, Switzerland and research positions at the University of Bayreuth and the Bavarian Institute of Waste Research in Germany. Her expertise is focused on persistent organic pollutants; e.g., in the identification and quantification of sources and releases of dioxins/furans, chemical analysis and monitoring and risk assessment and management.

Belén Gómara is a Tenured Scientist at the Department of Instrumental Analysis and Environmental Chemistry of the General Organic Chemistry Institute of the Spanish National Research Council. Her main research interests are focus on the development of innovative analytical methodologies for the determination of endocrine disrupting chemicals such as persistent organic pollutants and emerging contaminants associated with both the environment and the food packaging and preservation. And their application to real-life samples such as food, environmental samples and biota, including humans.

 Joaquin Beltran is Assistant Professor in the Physical and Analytical Chemistry department and member of the Research Institute of Pesticides and Water of the University Jaume I. His research is focused on the use of chromatography/mass spectrometry techniques (GC-MS) in last generation instruments, with special emphasis on high-resolution (HRMS) and hyphenated techniques for the determination of organic compounds in in environmental, food and biological fields. He has been coordinating the Master in Applied Chromatographic Techniques, as well as teaching the GC-MS module.