RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2008; **22**: 916–924
Published online in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/rcm.3443

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Identification and trace level determination of brominated flame retardants by liquid chromatography/quadrupole linear ion trap mass spectrometry

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Received 30 July 2007; Revised 12 January 2008; Accepted 16 January 2008

We describe the development of instrumental methodology for the simultaneous determination of hexabromocyclododecane (HBCD) diastereoisomers and tetrabromobisphenol A (TBBPA) and its derivatives by liquid chromatography/quadrupole linear ion trap mass spectrometry (LC-QqLIT-MS). Two different experiments were developed, optimized and compared. The first is based on a selected reaction monitoring (SRM) method in which the two most abundant transitions were selected for each analyte, as well as for the internal standards. In the second, the ion trap was used for the storage and subsequent fragmentation of precursor ions, obtaining an enhanced product ion (EPI) experiment. Both methods were validated by measuring quality parameters such as linearity, sensitivity, reproducibility and repeatability. Limits of detection (LODs) were in the range of 0.1–1.8 pg and 0.01–0.5 pg for SRM and EPI experiments, respectively, being lower than those published for the LC/QqQ-MS methods. Thus, LC-QqLIT-MS, used for quantification and confirmation, proved to be a powerful and very sensitive analytical tool.

Brominated flame retardants (BFRs) are a structurally diverse group of chemicals that are used in a variety of commercial applications to prevent fires, by reducing the flammability of combustible materials such as plastics and synthetics polymers.[1] BFRs include polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBBs), hexabromocyclododecane (HBCD) and tetrabromobisphenol A (TBBPA). The Bromine Science Enviromental Forum (BSEF) estimated the usage of selected BFRs in different areas of the world in 2001, with TBBPA being the most used (59%) and showing an increased use of HBCD.[2]

Tetrabromobisphenol A is the primary flame retardant used in electronics circuits and is preferred over other BFRs because it can be covalently bound to the polymer in the manufacturing process.[3] It is not, however, frequently analyzed in environmental laboratories, perhaps because of its lower bioaccumulation potential, with concentrations lower than PBDEs and HBCD in the environment. However, TBBPA, being a phenolic compound, may have a greater adverse effect on humans and wildlife.[4] Due to its low solubility in water (0.72 mg/L) and high log K_{ow} (4.5), TBBPA is likely to be associated with suspended particulate matter once released in the water column and ultimately buried in sediments.[5,6] There are indications of the potential toxicity of TBBPA as an endocrine disruptor and immunotoxic compound[7,8] and other studies showed that is toxic to aquatic life.[9,10] Biodegradation studies have shown that TBBPA can be partly degraded to less brominated analogues under both aerobic and anaerobic conditions in soil and river sediment.[7,11] This degradation is dependent on type, temperature, humidity and composition of the environmental matrix;[6] for example, Ronen and Abeliovich[10] reported a half life of 5 days for TBBPA in anaerobic sediment.

Hexabromocyclododecane, a brominated cyclic hydrocarbon, is the principal flame retardant in polystyrene foams and is used as thermal insulation in the building industry.[12] Technical 1,2,5,6,9,10-HBCD is produced industrially by addition of bromine to *cis-trans-trans*-1,5,9-cyclododecatriene. This process leads theoretically to a mixture of 16 stereoisomers, six pairs of enantiomers and four mesoforms. The product is usually a mixture of the three diastereoisomers α-, β- and γ-, but some authors have also found δ- and ε-diastereoisomers.[13] Normally, the γ-isomer is the most abundant in commercial mixtures (ranging between 75 and 89%), followed by the α- and then the β-isomer (10–13% and 1–12%, respectively).[14] The physicochemical properties of HBCD are similar to those of PBDEs and other persistent organic pollutants; in fact the log K_{ow} of HBCD is 5.6 and that

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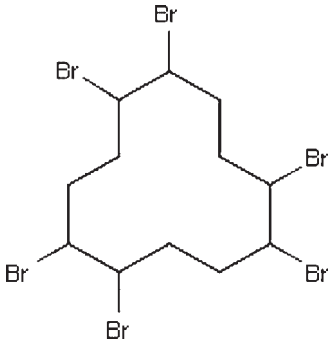
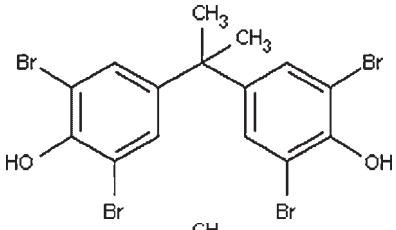
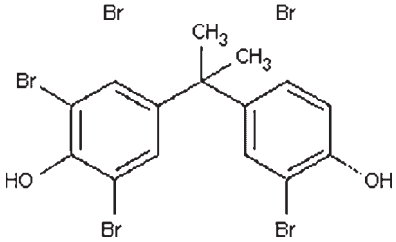
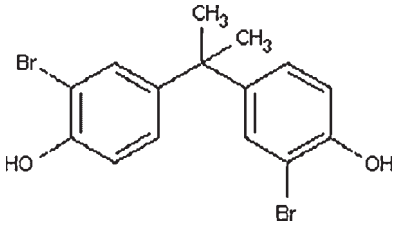
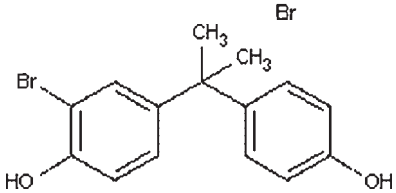
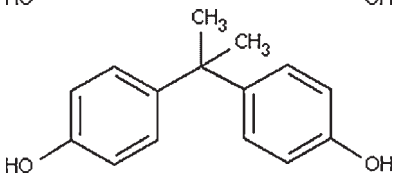
Contract/grant sponsor: European Union (AQUATERRA); contract/grant number: 505428.
Contract/grant sponsor: Spanish Ministry of Education and Science; contract/grant number: CTM2005-25168-E, CTM2005-07402-C02-02/TECNO, CTM2005-25150-E/TECNO and CEMAGUA (CGL2007-64551/HID).

places it in the optimum range for bioaccumulation.[12] HBCD is not covalently bonded to the material into which it is impregnated, leading to the risk of migration out of the product during use or disposal.[15] The dissimilarities in the structures of the α-, β- and γ-isomers might lead to differences in polarity, dipole moment and in solubility in water; for example, the solubilities of α-, β- and γ-HBCD in water are 48.8, 14.7 and 2.1 μg/L, respectively. Therefore, these different properties may explain the differences observed in their environmental behavior.[16]

The first methods for the analysis of HBCD were mainly based on gas chromatography (GC), but GC has limitations because it only provides information about the total HBCD isomers due to coelution of the diastereoisomers and interconversion at temperatures over 160°C.[15,17] Liquid chromatography/mass spectrometry (LC/MS) and tandem mass spectrometry (LC/MS/MS) are currently the best methods for measuring HBCD diastereoisomers separately in environmental samples.[18,19] However, GC/MS has the advantage of higher sensitivity.[20] In the case of TBBPA, the advantage of LC/MS over GC/MS is that the derivatization step is not necessary.[21] In order to improve sensitivity and specificity of LC/MS methods, in the field of environmental analysis, use has been made of MS/MS, with triple quadrupole instruments (QqQ), and, more recently, with LC/hybrid MS techniques such as are provided by

Table 1. Structure and physicochemical properties of the selected analytes

Compound	Acronym	Chemical structure	log K_{ow}	MW
Hexabromocyclododecane	HBCD		5.6	641.7
Tetrabromobisphenol A	TBBPA		4.5	543.9
Tribromobisphenol A	Tri-BBPA		2.1*	465.0
Dibromobisphenol A	Di-BBPA		2.1*	386.1
Monobromobisphenol A	Mono-BBPA		3.7*	307.2
Bisphenol A	BPA		3.3	228.3

*Estimated, not experimental (IALOG P).

DOI: 10.1002/rcm

quadrupole time-of-flight (QqTOF) and quadrupole linear ion trap (QqLIT) instruments.[22]

The objective of the present study was to develop a simultaneous method for the analysis of HBCD diastereoisomers (α, β and γ) and TBBPA and its derivatives (bisphenol A, monobromobisphenol A, dibromobisphenol A and tribromobisphenol A) (Table 1), based on an LC/QqLIT-MS method. The main advantage of QqLIT over QqQ is that the final quadrupole (Q3) can be operated in two different modes. The first mode is based on selected reaction monitoring (SRM), where the trap operates like a third quadrupole, similar to QqQ instruments; and the second uses the enhanced product ion (EPI) mode, where the ion trap is used for storage and subsequent fragmentation of precursor ions.[23,24] In this work, both modes were tested and compared.

EXPERIMENTAL

Standards and reagents

α-, β- and γ-HBCD were obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA) and were of minimum 97% purity; TBBPA, ^{13}C-TBBPA, d_{18}-α- and d_{18}-γ-HBCD were obtained from Wellington Laboratories Inc. (Guelph, Ontario, Canada) and were of minimum 98% purity; BPA and d_{16}-BPA were from Aldrich (Milwaukee, WI, USA) and were of 99% purity. Mono-BBPA, di-BBPA and tri-BBPA were a kind gift from Dr Göran Marsh (Department of Environmental Chemistry, Stockholm University, Sweden).[25] Ammonium acetate was of minimum 98% purity from Aldrich. Solvents for organic trace analysis were purchased from Merck (Darmstadt, Germany). All solvents and other reagents were of analytical grade.

Individual stock standard solutions were prepared on a weight basis in methanol and stored at -20°C. A mixture of all selected analyte standards was prepared by appropriate dilution of individual stock solutions. Further dilutions of this mixture were prepared in methanol before each analytical run and were used as working standard solutions. Stock solutions of internal standards were also prepared in methanol and were stored at -20°C. A mixture of these standards, used for internal standard calibration, was also prepared by diluting the individual stock solutions in methanol.

LC/QqLIT-MS analysis

The LC system used was an Agilent HP 1100 binary pump (Agilent Technologies, Palo Alto, CA, USA) with a Symmetry C_{18} column (2.1×150 mm, 5 μm) preceded by a C_{18} guard column (2.1×10 mm) supplied by Waters (Milford, MA, USA). Experiments were carried out in negative ion (NI) mode using H_2O/methanol (3:1 v/v) as eluent A and methanol as eluent B, at a flow rate of 0.25 mL/min. The injection volume was set at 4 μL. The elution program started at an initial composition of 100% A and was ramped to 10% A in 17 min and the initial conditions were reached again in 3 min and maintaned for an additional 15 min.

Mass spectrometric analysis was performed with an hybrid triple quadrupole/linear ion trap Applied Biosystem MSD Sciex 4000QTRAP^{TM} (Applied Biosystems, Foster City, CA, USA) instrument equipped with an electrospray ionization (ESI) Turbospray interface. All data were acquired and processed using Analyst 1.4.2. software (Applied Biosystems). For target quantitative analyses, data acquisition was performed in SRM mode, recording the transitions between the precursor ion and the two most abundant product ions. The MS/MS conditions were optimized to afford the highest relative signal intensity. Some parameters were set at default values: curtain gas pressure (CUR) at 50 psi, collision gas pressure (CAD) at 4.5×10^{-5} Torr, temperature of the turbo gas in the TurboIonSpray source (TEM) at 700°C, and the ion source gas 1 (GS1) and ion source gas 2 (GS2) pressures both at 50 psi.

Other parameters such as the declustering potential (DP), collision energy (CE), collision cell exit potential (CXP), ion spray voltage (IS) and entrance potential (EP) were optimized. The DP was modified between 55 and 130 V for TBBPA and related compounds, and between 50 and 80eV for HBCD diastereoisomers. The CE, CXP, IS and EP optimization was carried out by modifying their values between 10 and 110 eV, 1 and 40 V, 4500 and 5500 V, and 5 and 30 V, respectively.

For the enhanced product ion scan (EPI) method the same detection conditions as used in the SRM method were applied. This experiment was performed in information-dependent acquisition (IDA) mode which combines a survey scan with an EPI carried out at three different collision energies, 10, 20 and 40 eV, at a scan rate of 4000 m/z units/s and a linear ion trap fill time of 20 ms.

Method characterization

The method characterization was carried out for the above two experiments, the first one working in SRM mode and the second in EPI mode. Quality control was carried out by repeated injections of solvent blanks (methanol) and standards. Identification of selected analytes was based on the following restrictive criteria: (i) the retention time for all monitored transitions for a given analyte should maximize simultaneously $\pm$1 s, with a signal-to-noise ratio $\geq$3 for each; and (ii) the ratio between the two monitored transitions should be within 15% of the theoretical value (calculated upon standards). Quantification was carried out by an isotopic dilution technique, based on the addition of labeled standards: d_{18}-α-HBCD for α- and β-HBCD quantification, d_{18}-γ-HBCD for γ-HBCD, d_{16}-BPA for BPA and ^{13}C-TBBPA for TBBPA, tri-BBPA, di-BBPA and mono-BBPA. The linearity of the method was checked using calibration curves made from standard solutions at six concentration levels (5, 50, 100, 500, 1000, 2500 and 5000 pg/μL), and by triplicate injections. The precision of the method was determined by repeated intra-day and inter-day analysis with five successive injections of a standard solution in one day and on five successive days, respectively. The instrumental limit of detection (LOD), defined as 3 times the noise level, and the limit of quantification (LOQ), defined as 10 times the noise level, were calculated with the first SRM transition for both experiments.

DOI: 10.1002/rcm

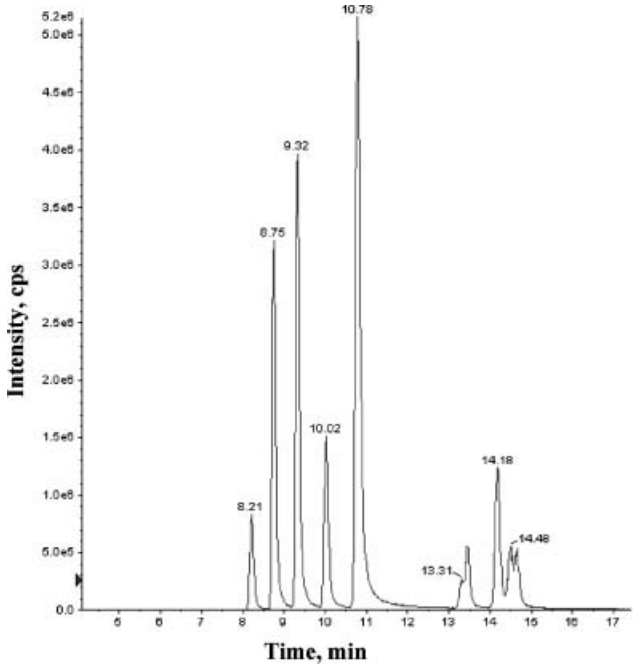


Figure 1. Total ion chromatogram (TIC) obtained for a standard mixture of 2500 pg/μL.

RESULTS AND DISCUSSION

Two different LC/QqLIT-MS assays were developed for the selective identification and confirmation of HBCD diastereoisomers, TBBPA and related compounds. The first is based on a SRM experiment, and the second used an EPI experiment.

Chromatographic separation

Various mobile phase compositions have been reported for BPA, TBBPA or HBCD analysis by LC/MS. A series of preliminary experiments was performed, testing different mobile phases consisting of methanol, water and acetonitrile, and using or not additives such as ammonium acetate. We found that the optimal separation of selected compounds was achieved using methanol as the mobile phase, with higher responses of target compounds than were obtained using acetonitrile. It was also observed that the use of additives gives a similar response for HBCD diastereoisomers, but that the sensitivity of TBBPA and related compounds decreased considerably. Therefore, using methanol and water as the mobile phase was concluded to be more advantageous for this quantitative analysis. A representative chromatogram of a 2500 pg/μL standard mixture of selected analytes and internal standards is illustrated in Fig. 1 (see also Table 2).

SRM experiment optimization

SRM transitions and other compound-dependent parameters (DP, CE and CXP) were optimized by infusing via a syringe pump a standard mixture, containing all of the compounds at 500 pg/μL. The optimum values are summarized in Table 2. An example of the analysis of a mixture of selected analytes using the developed SRM method is shown in Fig. 2. In all cases the $[M–H]^-$ ions were selected as the precursor ions in the NI mode. The product ions observed for each target compound were in good agreement with those previously reported. For example, the detection of HBCD isomers was based on the signal from the *m/z* 639 → 79 transition, with the $[M–H]^-$ to *m/z* 81 being used as the confirmation transition. The same transitions were selected by Budakowsky and Tomy.[19] For TBBPA, Saint-Louis and Pelletier[5] found an abundant $[M–H]^-$ ion at *m/z* 543 with two product ions at *m/z* 528 and 448 corresponding to [M–H–methyl] and $[M–H–methyl–Br]^-$. Chu *et al.*[6] found that the sensitivity using the *m/z* 543 → 81 $[Br]^-$ transition was about 10 times higher than that using *m/z* 543 → 528. The *m/z* 543 → 79 and *m/z* 543 → 81 transitions were selected in our study.

The MS/MS conditions were optimized to afford the highest relative intensity (Table 2). The optimized DP values were the same for the precursor and product ions, with values between 65 and 120 V for TBBPA and related compounds, and between 60 and 80 V for the HBCD diastereoisomers (Fig. 3(a)). Values between 30 and 98 eV,

Table 2. MS/MS parameters for the analysis of selected compounds by SRM in negative ion mode

Compound	Rt (min)	Precursor ion (*m/z*)	DP-CE-CXP (V-eV-V)	SRM1 (*m/z*)	DP-CE-CXP (V-eV-V)	SRM2 (*m/z*)	Ratio (Mean ± SD)*
BPA	8.3	227 $[M–H]^-$	80-30-1	227 > 133	80-24-29	227 > 211	0.36 ± 0.01
d_{16}-BPA	8.4	241 $[M–H]^-$	95-38-7	241 > 142	95-28-19	241 > 223	
Mono-BBPA	8.8	305 $[M–H]^-$	65-58-9	305 > 79	65-34-5	305 > 133	137 ± 85
Di-BBPA	9.4	385 $[M–H]^-$	75-44-13	385 > 79	75-44-7	385 > 81	1.1 ± 0.04
Tri-BBPA	10.0	461 $[M–H]^-$	120-74-1	461 > 79	120-52-15	461 > 338	4.1 ± 0.20
^{13}C-TBBPA	10.5	555 $[M–H]^-$	120-98-3	555 > 79	120-98-5	555 > 81	
TBBPA	10.9	543 $[M–H]^-$	105-84-1	543 > 79	105-92-1	543 > 81	0.96 ± 0.05
α-HBCD	13.3	639 $[M–H]^-$	60-46-1	639 > 79	60-38-3	639 > 81	2.0 ± 0.20
β-HBCD	14.4	639 $[M–H]^-$	60-46-1	639 > 79	60-38-3	639 > 81	2.1 ± 0.20
γ-HBCD	14.9	639 $[M–H]^-$	60-46-1	639 > 79	60-38-3	639 > 81	1.9 ± 0.21
d_{18}-α-HBCD	13.1	656 $[M–H]^-$	80-46-1	656 > 79	80-50-1	656 > 81	
d_{18}-γ-HBCD	14.7	656 $[M–H]^-$	80-46-1	656 > 79	80-50-1	656 > 81	

Rt: retention time; DP: declustering potential; CE: collision energy; CXP: collision cell exit potential; SRM1: first transition, used as quantitative transition; SRM2: second transition, used as confirmative transition; Ratio = SRM 1 abundance/SRM 2 abundance; SD: standard deviation; *calculated upon the standard calibration curves.

DOI: 10.1002/rcm

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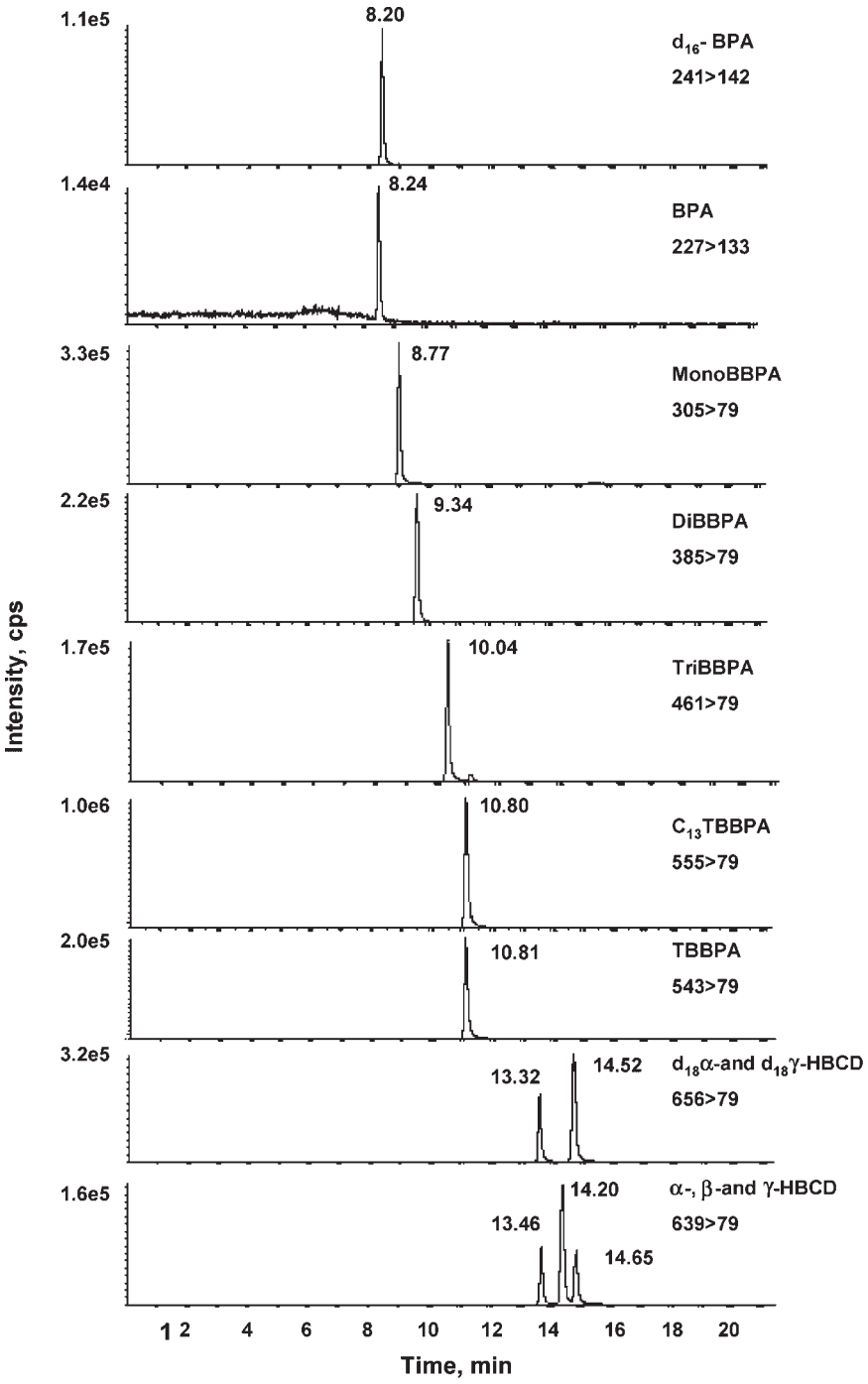

Figure 2. SRM chromatogram obtained with the LC/ESI(-)-MS/MS method for a standard solution (100 pg/μL).

and between 1 and 29 V, were found for CE and CXP optimization, respectively (Fig. 3(b)). Finally, the IS and EP were set at, respectively, 4500 V and 10 V for all the selected analytes.

EPI experiment optimization

In addition to all the capabilities of a triple quadrupole, the fact that the apparatus can be operated with Q3 configured as the final analysis quadrupole in a conventional tandem triple quadrupole mass spectrometer or as a linear ion trap mass spectrometer allows comparison of the performance characteristics of the two operational modes. The linear ion trap (LIT) offers some extra scan possibilities, because it can be filled with ions during a specified time, resulting in more sensitive scan modes: enhanced MS (EMS), enhanced product ion (EPI) and MS/MS/MS. Using the QqLIT configuration and with the EPI mode, the selection of the precursor ion is performed in Q1 using radio-frequency (RF)/direct current (DC) isolation at any resolution. Collision-induced dissociation (CID) occurs in collision cell q2, and product ions are trapped in Q3 operated in the LIT mode. RF/DC isolation has a significant advantage over isolation waveform where, for the isolation of fragile ions, elimination of the precursor ion can be observed.

In addition, the QTRAP™ instrument allows us to use IDA, a procedure for maximizing the amount of information

DOI: 10.1002/rcm

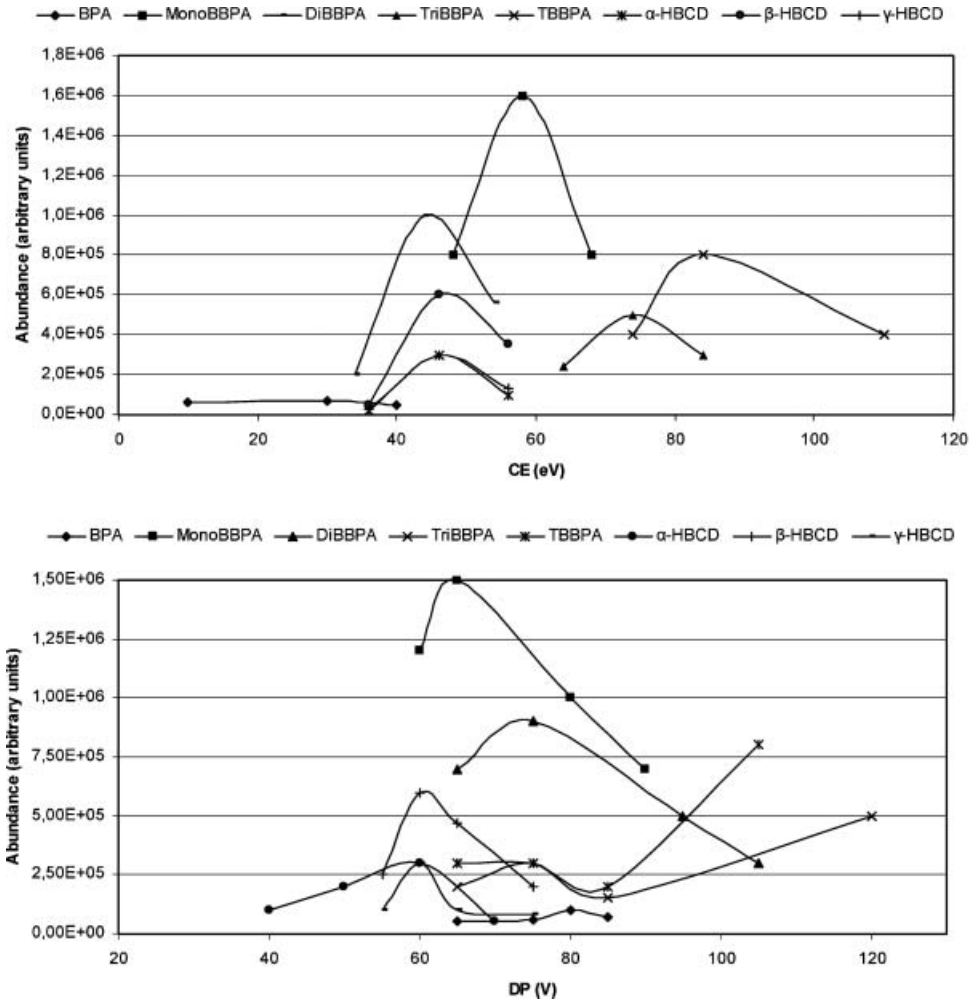

Figure 3. Variation of the abundance for selected analytes vs. (a) DP and (b) CE.

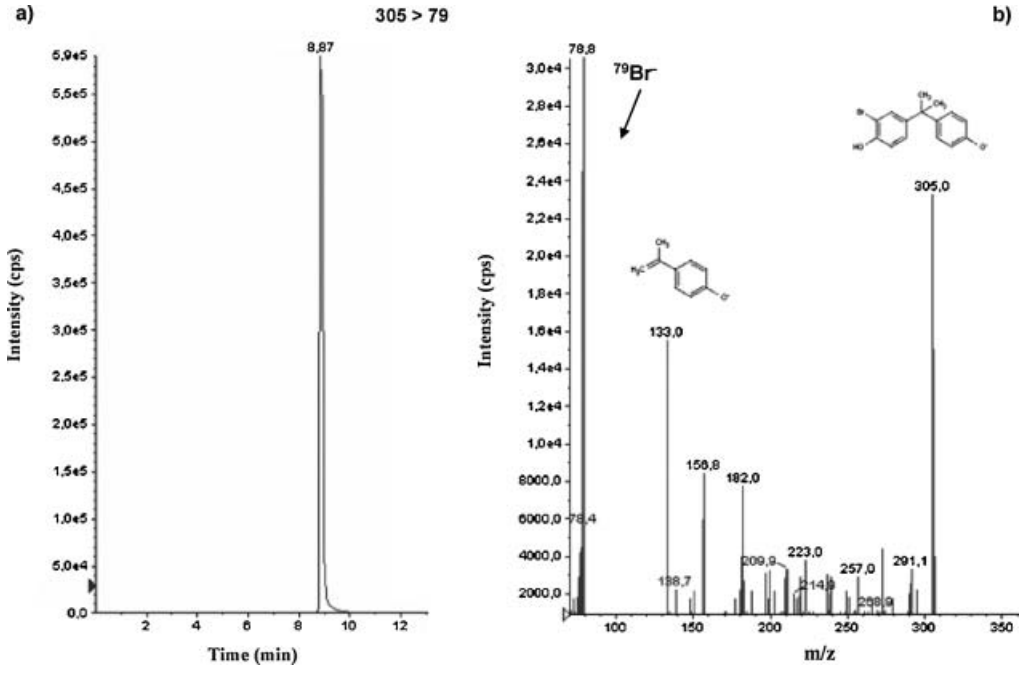

Figure 4. IDA experiment for the determination of monobromobisphenol A in a standard solution (100 pg/μL): (a) SRM transition and (b) EPI at a CE of 40 eV.

DOI: 10.1002/rcm

Table 3. Quality parameters for SRM and EPI methods (RSD: relative standard deviation)

	SRM					EPI			
Compound	R^2	LOD (pg)	LOQ (pg)	Repeatability (% RSD, n = 5)	Reproducibility (% RSD, n = 5)	R^2	LOD (pg)	LOQ (pg)	Repeatability (% RSD, n = 5)
Bisphenol A	0.999	1.8	6.0	5.0	1.9	0.995	0.5	1.6	2.0
Monobromobisphenol A	0.998	0.3	0.9	4.7	4.4	0.999	0.1	0.2	7.0
Dibromobisphenol A	0.999	0.2	0.7	5.3	8.9	0.998	0.03	0.1	5.6
Tribromobisphenol A	0.998	0.4	1.3	4.8	4.6	0.992	0.1	0.2	4.1
Tetrabromobisphenol A	0.998	0.1	0.2	3.1	8.1	0.996	0.01	0.03	3.5
α-Hexabromocyclododecane	0.995	0.5	1.7	5.7	12	0.992	0.2	0.6	5.8
β-Hexabromocyclododecane	0.991	0.5	1.8	5.6	11	0.995	0.1	0.3	5.5
γ-Hexabromocyclododecane	0.998	1.2	4.0	5.3	6.5	0.991	0.3	1.1	4.9

that can be exploited in a single LC/MS/MS run because it combines two or more different scans modes in a sequential fashion with an EPI.

In our QqLIT experiment, the same parameter values that were optimized for the SRM method were applied (Table 2). Moreover, the fill time of the LIT was optimized, showing the best result at 20 ms. Finally, the collision energy was set at 10, 20 and 40 eV. As an example, a representative chromatogram obtained for the determination of mono-BBPA at 100 pg/μL by the EPI method is shown in Fig. 4. The same precursor ion product transition selected for the SRM experiment was also used for the EPI method, corresponding to formation of the $[Br]^-$ ion from the $[M–H]^-$ ion.

Method characterization

Quality assurance of the developed methods was evaluated by measuring its linearity, sensitivity, reproducibility and repeatability. Characterization data for the two proposed methods are presented in Table 3. Calibration curves were generated using linear regression analysis over the established concentration range (5–5000 pg/μL). Both methods (SRM and EPI) gave good fits, with R^2 always higher than 0.99. The variability (n = 5) of the SRM experiment was acceptable, with values below 6% and below 12% for run-to-run and day-to-day, respectively. For the EPI experiment, similar values were obtained.

For the SRM experiment, the LOD and LOQ were between 0.05 and 1.8 pg and between 0.2 and 6.0 pg, respectively. For the EPI experiment, the LODs were between 0.01 and 0.5 pg. Very little published information could be found on validation data for HBCD and TBBPA determination. Morris *et al.*,[17] who used a single quadrupole, found that the LOQ for HBCD was 150 pg on-column. Gómara *et al.*,[26] who used LC/ion trap MS/MS for HBCD determinations, obtained LODs between 30 and 86 pg. On the other hand, Budakowsky and Tomy[19] obtained, using a triple quadrupole mass spectrometer, LODs of between 4 and 6 pg injected for HBCD; this was also higher than our results. For TBBPA and related compounds, there is no information about the quality control of the published methods although all the published values were higher than our results. It should be pointed out that a more realistic comparison would be with LOD values obtained using the new generation of QqQ instruments, but unfortunately these data are not available.

If we compare our LODs for the SRM and EPI modes, we observe that the EPI method gives slightly better sensitivity. The same situation was observed for other applications such as the analysis of reserpine[27] and the characterization of drug metabolites.[28]

The applicability of the LC/QqLIT-MS method working in the EPI mode was tested with extracts of real samples including different matrices such as sediment and biota. Sample preparation of extracts was carried out using a selective pressurized liquid extraction (SPLE) method in which the extraction and purification steps were carried out simultaneously.[29] As an example, Fig. 5 shows the determination of BPA in a sediment sample working with the developed IDA experiment. We can observe the BPA transition from the $[M–H]^-$ ion at *m/z* 227 to 133. Moreover, the full scans obtained at different CEs gave useful information, because the fragmentation varies with the energy applied. In the case of BPA, working at low CE (10 and 20 eV) basically only the $[M–H]^-$ ion was detected. However, when the CE was increased to 40 eV, more fragmentation was observed with product ions at *m/z* 133 and 211.

CONCLUSIONS

A simultaneous method for the analysis of HBCD diastereoisomers, TBBPA and related compounds was developed and characterized. Through the use of a methanol/water LC gradient, it is possible to completely separate TBBPA, tri-BBPA, di-BBPA, mono-BBPA, BPA, α-, β- and γ-HBCD diastereoisomers in a short time (less than 15 min).

Two different MS/MS methods were developed. The first was based on a SRM experiment and the second on an EPI experiment. Both methods are more sensitive than other published studies with LODs below 1 pg. The developed methods display excellent detection limits in SRM mode (0.1–1.8 pg), but slightly better results are obtained in EPI mode (0.01–0.5 pg). Moreover, the selectivity was enhanced by working with IDA, because we can obtain the complete spectrum for the fragmentation of the $[M–H]^-$ ion, opening the possibility for the identification of potential product ions of selected precursor ions. Thus, we propose for the unequivocal analysis and quantification of brominated flame retardants in environmental samples at the ultratrace level the use of the IDA experiment working in the EPI mode.

DOI: 10.1002/rcm

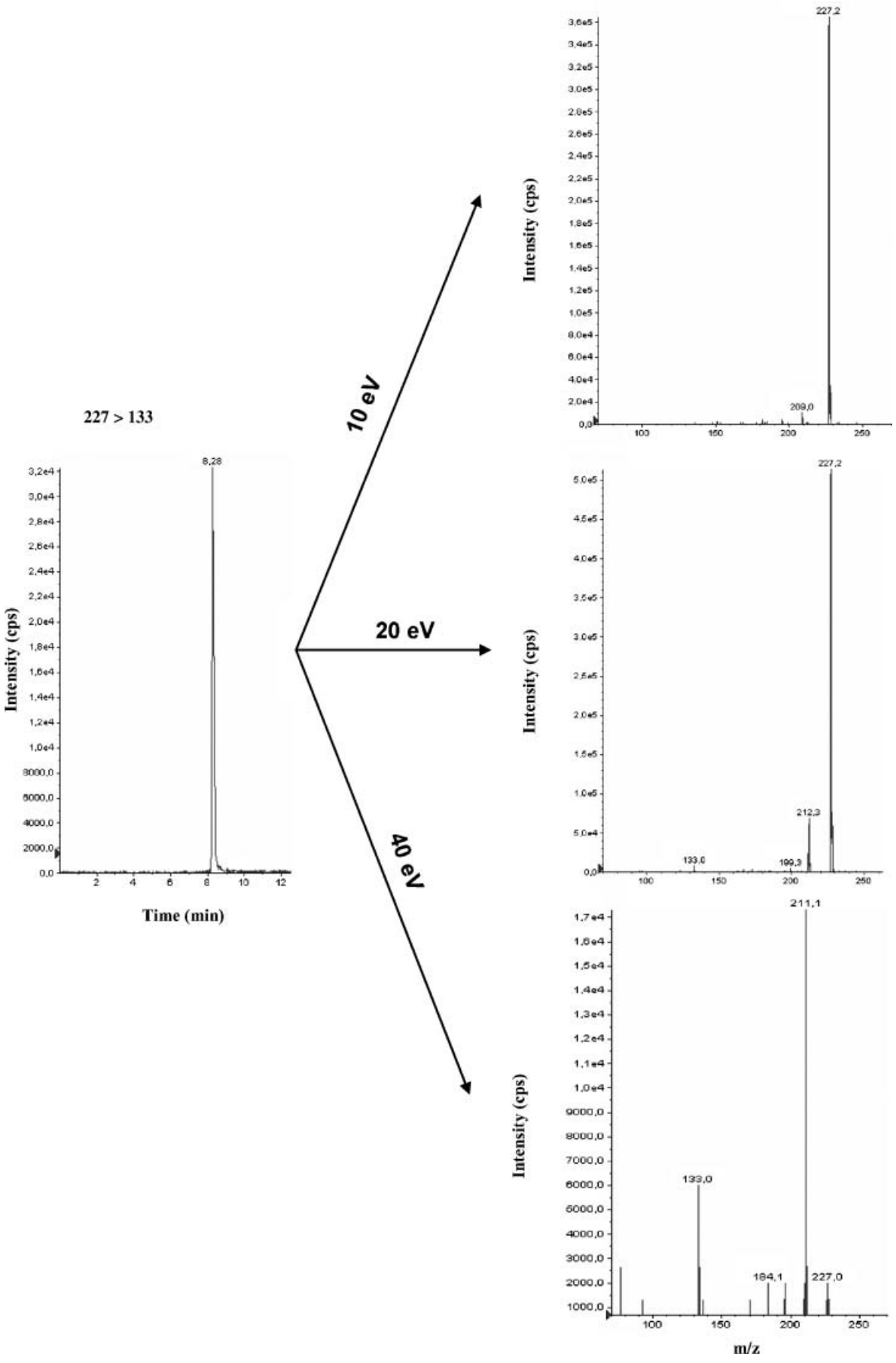

Figure 5. Determination of BPA in a sediment sample by IDA experiment, showing the selected transition and scans obtained at the different CEs applied.

DOI: 10.1002/rcm

Acknowledgements

This work was funded by the European Union: Integrated modelling of the river-sediment-soil-groundwater system; advanced tools for the management of catchment areas and river basins in the context of global change (AQUATERRA, Project number 505428), and by Spanish Ministry of Education and Science (Projects numbers: CTM2005-25168-E, CTM2005-07402-C02-02/TECNO, CTM2005-25150-E/TECNO and CEMAGUA (CGL2007-64551/HID)). This works reflects only the authors' views and the European Community is not liable for any use that may be made of the information contained therein. We thank Dr Göran Marsh (Department of Environmental Chemistry, Stockholm University, Sweden) for mono-BBPA, di-BBPA and tri-BBPA standards. Waters Corporation (USA) is acknowledged for providing the HPLC column. Technical assistance provided by D. Fanjul, R. Chaler and M. Comesaña (IIQAB-CSIC) is also gratefully acknowledged.

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DOI: 10.1002/rcm