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Probing new approaches using atmospheric pressure photo ionization for the analysis of brominated flame retardants and their related degradation products by liquid chromatography–mass spectrometry

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Abstract

Atmospheric pressure photo ionisation has been evaluated for the analysis of brominated flame retardants and their related degradation products by LC–MS. Degradation mixtures obtained from the photochemical degradation of tetrabromobisphenol A and decabromodiphenylether were used as model systems for the assessment of the developed methodology. Negative ion mode gave best results for TBBPA and its degradation compounds. $[M-H]^-$ ions were formed without the need of using a doping agent. MS and MS/MS experiments allowed the structural identification of new TBBPA "polymeric" degradation compounds formed by attachment of TBBPA moieties and/or their respective cleavage products. In the case of polybromodiphenylethers, the positive mode provided $M^{\bullet+}$ ions and gave better results for congeners ranging from mono- to pentabromodiphenylethers whereas for higher bromination degrees, the negative ion mode (providing $[M-Br+O]^-$ ions) was best suited. Under both positive and negative ionisation modes, the use of toluene as doping agent gave better results. Liquid chromatography–mass spectrometry by means of atmospheric pressure photo-ionisation was applied to the analysis of aromatic brominated flame retardants and their degradation products. This methodology proved to be particularly useful, for the characterisation and structural identification of some compounds which are not amenable to GC–MS, especially in the case of apolar "polymeric" degradation products of tetrabromobisphenol A investigated in this work.

Keywords: Brominated flame retardants; Tetrabromobisphenol A; Polybromodiphenylethers; LC–MS; APPI

1. Introduction

Brominated flame retardants (BFRs) are widely used as additives in the production of polymers and plastics in order to slow down or inhibit the early steps of catching fire. Thus, this group of chemicals is present in many consumer goods such as televisions, computers and other products containing printed circuit boards, and they are also found in plastics and foams used in the car industry [1]. These chemicals are widely released into the environment and are considered as "new" environmental contaminants. Due to their physico-chemical properties, many of them are described as persistent organic pollutants (POPs). BFRs are mainly represented by tetrabromobisphenol A (TBBPA, Fig. 1), polybrominated diphenylethers (PBDEs, Fig. 1), which are aromatic compounds, and the cycloaliphatic compound hexabromocyclododecane (HBCD). The worldwide production of BFRs is greater than 200 ktonnes/year [2], among which the above mentioned compounds account for ca. 70%. Since the beginning of their use 25 years ago, their occurrence has been reported in almost all environmental compartments (sediments, air, water) as well as in animals and humans [3]. Some BFRs are suspected to be carcinogenic and recently, some polybrominated diphenyl ether (PBDEs) congeners as well as tetrabromobisphenol A (TBBPA) have been shown to act as endocrine disrupters [4,5]. Although their impact on the environment and their potential risk for

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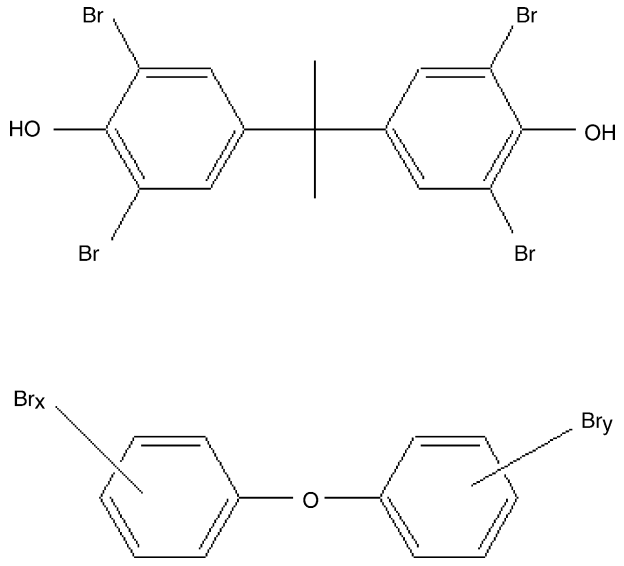

Fig. 1. Chemical structures of TBBPA and PBDEs.

animals is a present time concern for the scientific community, little is known about the fate of these compounds in the environment as well as in living organisms.

Since the end of the 1990s, several methods have been developed for the analysis of BFRs. As mentioned by Covaci et al. in a recent review article [6], the analysis of BFRs is usually achieved using gas chromatography coupled to electron capture or mass spectrometric detection [7–11], on the basis of analytical methodologies previously developed for chlorinated POPs, such as polychlorinated biphenyls (PCBs). Similarly to those compounds, PBDEs present 209 possible congeners with different degree of bromination. Although the composition of technical mixtures used in the industry is less complex than for PCBs, highly brominated PBDEs (i.e. octa-, nona- and deca-BDEs) have been shown to undergo degradation into analogues with a lower degree of bromination [12,13]. For this reason, many different congeners may be released into the environment, even from simple technical mixtures such as DeBDE (97–98% decabromodiphenylether [14]). Powerful analytical methods have been proposed for the analysis of PBDEs by GC–MS [6,11,15]. However, the analysis of highly brominated congeners (particularly DeBDE) is known to be difficult due to thermal degradation problems [6]. For this compound short columns are generally used, which is detrimental for the separation of the other congeners. Another problem concerns the relative lack of sensitivity observed for highly brominated PBDEs when analysed using electron ionisation mass spectrometry. Therefore, electron capture negative ionisation (ECNI) based on the monitoring of the *m/z* 79 and 81 ions for the bromine trace is now the most widely used method for the analysis of PBDEs [6]. Concerning the gas chromatographic analysis of other BFRs, HBCD is also known to be affected by thermal degradation, and in the case of TBBPA, a derivatisation step is needed. For this reason, electrospray ionisation mass spectrometry coupled to liquid chromatography has been successfully used in recent works conducted on TBBPA [16] and HBCD [17,18]. However, as far as we know, LC–MS based methodologies have not been investigated for the analysis of PBDEs yet.

Atmospheric pressure ionisation techniques were one of the main causes of the tremendous development of LC–MS techniques over the last 20 years. Nowadays, electrospray [19,20] (ESI) and atmospheric pressure chemical ionisation (APCI) [21,22] are the most used ionisation techniques for LC–MS. However, there are still some compounds for which neither ESI nor APCI produce any ion current. Recently, atmospheric pressure photo ionisation (APPI) [23] appeared as a complementary ionisation technique for LC–MS, allowing the ionisation of a wide range of compounds, and broadening the applicability of LC–MS. The present paper reports a new original approach for the analysis of aromatic BFRs by LC–MS, using APPI as the ionisation technique, with the main objective to provide a powerful methodology for the study of some of BFRs metabolites and degradation products which are not easily amenable to GC–MS analysis. Indeed, gas chromatography may be less suited in the context of degradation or metabolism studies, due to its limitations for the analysis of compounds such as some hydroxylated derivatives, and even more inappropriate for glucuronic acid or glutathione conjugates, all of which are encountered as usual xenobiotic metabolites. In this work, efforts were focused on aromatic BFRs (i.e. TBBPA and PBDEs), which represent more than 95% of the estimated world market BFR demand [24]. Thus, this work excluded HBCD for which a powerful LC–MS methodology using electrospray ionisation has been recently reported [17].

First, various atmospheric pressure ionisation techniques such as ESI, APCI and APPI have been assessed under both positive and negative ionisation conditions, for TBBPA and different PBDE congeners. Although good results could be obtained with negative ESI for TBBPA, ESI and APCI were found to be not well suited for the analysis of some of its degradation products. For these compounds as well as for PBDEs, APPI gave interesting results. The various ionic species formed under both positive and negative APPI conditions were investigated as well as the main fragment ions obtained in MS/MS experiments achieved into an ion trap device. The application of LC–APPI-MS to the analysis of TBBPA degradation compounds and its potential for the analysis of PBDE mixtures are presented.

2. Experimental

2.1. Reagents and chemicals

Analytical grade acetic acid, HPLC grade acetonitrile and toluene were purchased from Scharlau (Barcelona, Spain). Reference PBDEs were purchased from Wellington Laboratories (Guelph, Canada). Tetrabromobisphenol A

was from Fluka (St. Quentin Fallavier, France) and octabromodiphenylether from Promochem (Molsheim, France). Monobromodiphenylethers and decabromodiphenylether were from Aldrich (St. Quentin Fallavier, France). For LC–MS experiments, a home synthesised mixture of PBDEs ranging from penta- to hepta-congeners was prepared by partial bromination of diphenylether according to previously published procedures [25]. The photo degradation mixture of TBBPA was obtained by exposure of a 0.5 mg/ml solution of TBBPA in acetonitrile to solar light during five days. After that period, the solution was kept at −20 °C in the dark until analysis.

2.2. LC–MS

LC–MS analyses were carried out on a quadrupole ion trap mass spectrometer (Finnigan LCQ DecaXP, Thermo Electron, Les Ulis, France) fitted with the Finnigan APCI/APPI dual ionisation source. This source generates 10 eV photons by means of a Krypton discharge lamp. For infusion preliminary studies, samples (typically 1 ng/μl in MeOH–H_2O (50–50, v/v)) were introduced into the ionisation source at a flow rate of 10 μl/min. Typical ionisation source operating conditions were as follows: heated nebulizer temperature, 450 °C; heated transfer capillary temperature, 250 °C; heated transfer capillary voltage, −20 V, tube lens offset, −35 V. All other parameters for MS and MS/MS experiments were adjusted for each compound in order to get maximum sensitivity and structural information for the compound of interest. All analyses were achieved under automatic gain control conditions using helium as damping as well as collision gas for MS/MS experiments.

For LC–MS, a TSP 4000 (Thermo Electron, Les Ulis, France) pump fitted with a Rheodyne injector was used. UV detection was achieved with a UV 1000 detector from Thermo Separation Products (Les Ulis, France). Reversed phase columns consisted in a Nucleodur (Interchim, Montluçon, France) 100-C8 column (250 mm × 4 mm, 5 μm) for TBBPA and its related degradation compounds; or an Ultrabase (SFCC, Eragny, France) RP18 column (250 mm × 2 mm, 5 μm) for PBDEs. The following gradient elution was used for TBBPA: 100% A from 0 to 4 min, 100–40% A from 4 to 6 min, 60% B from 6 to 30 min, then 60–100% B from 30 to 50 min, and finally 100% B from 50 to 64 min; with A: H_2O/ACN/CH_3COOH (95:5:0.1) and B: ACN. The flow rate was 1 ml/min and a post column splitting was made in order to let 0.1 ml/min entering the ionisation source. For the LC–MS analysis of PBDEs, an isocratic elution system consisting in a H_2O/MeOH (1:99) mixture was used at a flow rate of 0.2 ml/min. A post column addition of toluene (5 μl/min, i.e. 2.5%) as the doping agent was made.

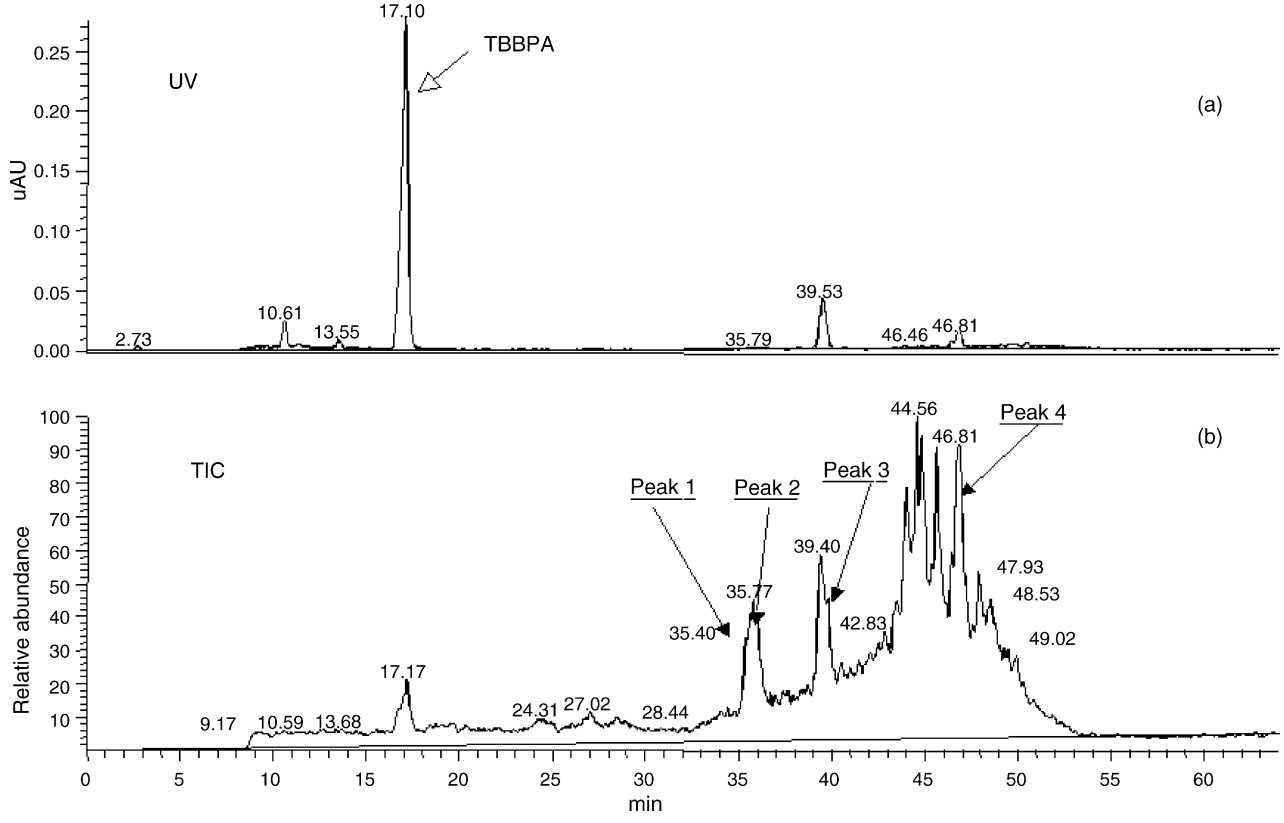

Fig. 2. Chromatogram of a TBBPA photodegradation mixture analysed by LC with (a) UV and (b) NI-APPI-MS detection.

3. Results and discussion

3.1. Tetrabromobisphenol A

3.1.1. LC–APPI-MS analysis of a model TBBPA degradation mixture

TBBPA can be considered as the most polar molecule among major BFRs. The use of electrospray ionisation for the analysis of this compound has already been mentioned as an interesting alternative to GC–MS/MS mainly by providing simpler determination procedures due to the absence of derivatisation. In terms of sensitivity, LC–ESI-MS/MS can be competitive with published GC–EI-MS/MS techniques with limits of detection in the ppt range [16]. Electrospray ionisation has also been successfully used for the structural identification of metabolites produced in vitro from incubations of TBBPA and its non brominated analogue bisphenol A (BPA) with rat and human subcellular liver fractions [26,27]. This ionisation technique proved to be well suited for the analysis of polar metabolites resulting from the cleavage of the C–C bond between the central carbon atom and the aromatic rings of the molecules, as well as for sulfate or glucuronide conjugates produced by phase II metabolism enzymes. In this work, we have submitted a solution of TPPBA to solar radiation in order to induce its photolytic degradation. Fig. 2 shows the separation of a crude degradation mixture of TBBPA after five days, by liquid chromatography coupled to UV (254 nm, Fig. 2a) and to mass spectrometric detection after APPI ionisation (Fig. 2b). The UV trace shows the occurrence of several polar degradation compounds eluted between 3 and 15 min. Some of the main polar decomposition products were, respectively, identified as 2,6-dibromophenol; 3-hydroxy-4-isopropylene-2,6-dibromophenol and tribromophenol. Traces of debrominated TBBPA (i.e. tribromo-, dibromo- and monobromobisphenol A) were also detected. This shows that the decomposition of TBBPA involved processes leading to the breakage of the central carbon bonds as well as possible subsequent bromine migrations. On the other hand, additional degradation compounds were detected after the elution time of unchanged TBBPA. For the mass spectrometric analysis of these apolar compounds, electrospray ionisation was almost inefficient. The use of APPI proved to constitute an interesting alternative. First, the influence of the flow rate on the overall detection sensitivity of TBBPA photo-degradation compounds by APPI was investigated (data not shown). A flow rate of 0.1 ml/min gave the best results in terms of sensitivity using post-column infusion experiments with TBBPA. This result was consistent with previously published works in which APPI optimal flow rates were in the 0.1–0.2 ml/min range, depending on the configuration of the ionisation source that was used [28–31]. As indicated in Fig. 2b, the ionisation of TBBPA degradation compounds by means of APPI was very efficient, especially for apolar compounds eluted at retention times greater than 30 min. According to the gradient elution used in this work, this corresponded to acetonitrile contents greater than 65%. The susceptibility of APPI towards the mobile phase solvent composition has already been mentioned as a potential drawback of this technique [30,31], and is clearly illustrated in Fig. 2b. Relatively weak signals are observed for early eluting peaks (i.e. for water rich mobile phases). On the other hand, at the end of the gradient elution (i.e. close to 100% acetonitrile in the mobile phase) small peaks observed on the UV trace (Fig. 2a) gave very intense signals with APPI-MS (Fig. 2b). This particular feature could constitute a limitation for the quantitative analysis of mixtures of BFR degradation compounds or metabolites.

3.1.2. Molecular mass determination of new TBBPA apolar degradation compounds

The structure of these unusual TBBPA apolar degradation compounds has been further investigated by means of MS/MS experiments conducted on the molecular species produced by APPI. Under the negative ionisation conditions used in this work, $[M-H]^-$ ions were observed as the molecular species. Selected MS and MS/MS spectra of apolar TBBPA degradation compounds chosen as representative examples are presented in Fig. 3. The MS spectra displayed very characteristic isotopic patterns allowing for the attribution of the number of bromine atoms contained in the analysed structure, as shown in Fig. 3 (a–d), respectively, for peaks 1 (35.4 min), 2 (35.8 min), 3 (39.4 min), and 4 (46.8 min). According to their isotopic clusters and molecular ion mass, peaks 1 and 2 could be identified as hexabrominated molecules, peak 3 as an heptabrominated species whereas peak 4 appeared to bear 10 bromine atoms. The first two compounds were detected as their $[M-H]^-$ ions. Surprisingly, peak 3 appeared as a $[M-2H+Na]^-$ adduct ion. In the case of peak 4, the two pseudo-molecular ionic species coexisted (see Fig. 3d). The mechanism of formation of negative ions in atmospheric pressure photo ionisation has already been discussed by Traldi and coworkers [32] who concluded that negative ions were likely produced via slow electrons emitted not only by the positive APPI ionisation process but also by the metallic surfaces of the ionisation source. Considering the ionisation energy of Fe (7.7 eV) and the energy of the photons from the Kr lamp (ca. 10 eV), these electrons are emitted in the range of ca. 2 eV, which is suitable for both associative and dissociative resonance capture processes. According to these authors, the molecular ionic species formed from TBBPA was produced trough a $H^\bullet$ loss resulting from a dissociative electron capture process. These authors also observed the formation of a $[M-Br+O]^-$ anion from TBBPA, which was not the case in our work. This may be due to different geometries of the ionisation sources used in our respective studies (i.e. the source used by Traldi and coworkers [32] displayed a Kr lamp located in front of the stainless steel surfaces of the ion entrance device whereas our source presents a lamp orthogonal to both the nebulizer and the ion entrance device). This difference may minimise the electron stripping from stainless steel surfaces in our configuration, and induce the emission of photo electrons ranging

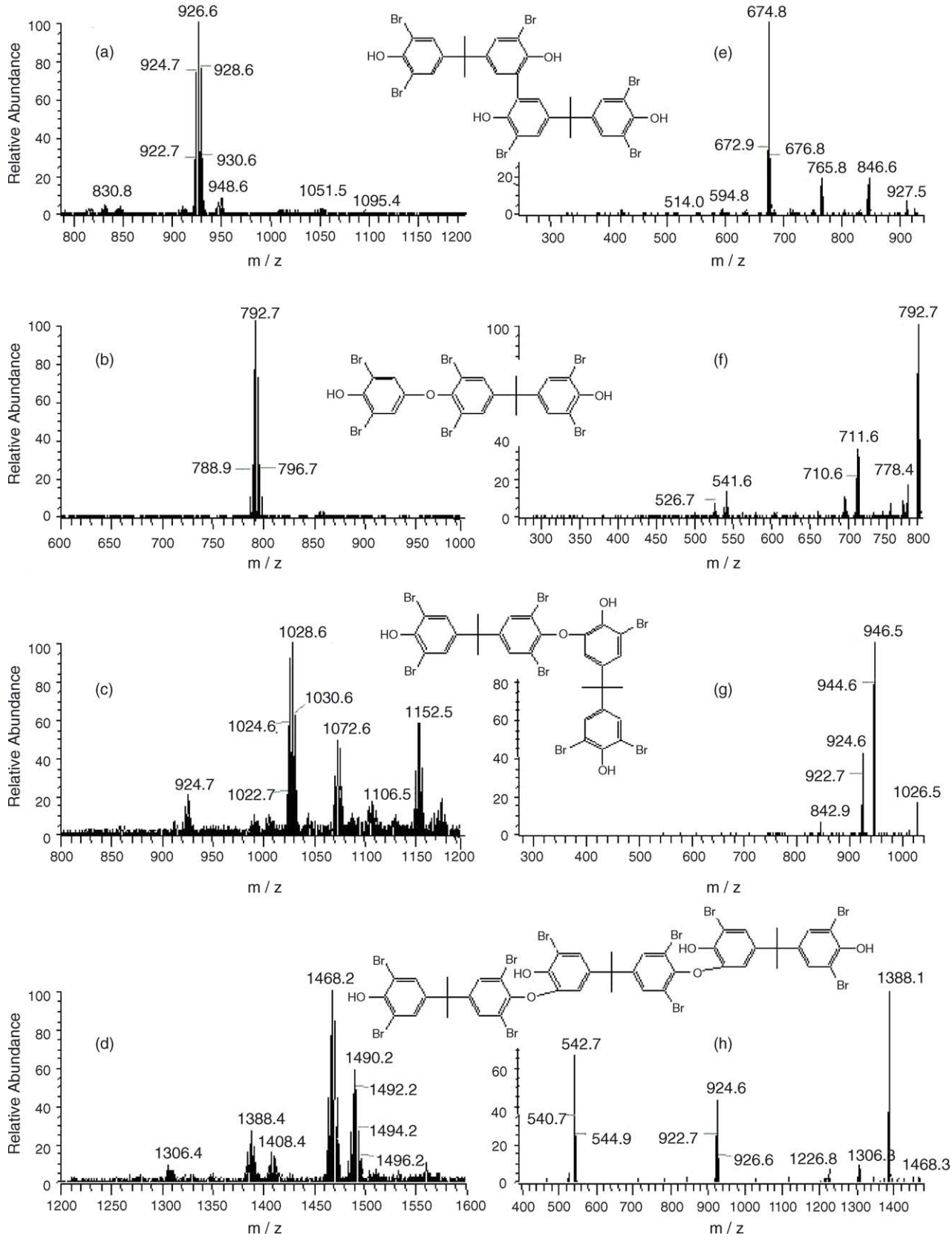

Fig. 3. Mass spectra obtained from the LC–MS analysis of peaks 1–4 using NI-APPI ionisation. Photoionisation mass spectra of (a) peak 1, (b) peak 2, (c) peak 3, (d) peak 4, and MS/MS spectra of (e) *m/z* 927, (f) *m/z* 793, (g) *m/z* 1027 and (h) *m/z* 1468 selected ions from peaks 1 to 4, respectively. Insets represent the proposed structure for each compound.

in different energy levels. Therefore, depending on the source configuration, certain resonance capture processes may have been favoured or hindered with respect to the others.

3.1.3. MS/MS structural investigation of the TBBPA apolar degradation compounds

Fig. 3e–h show the MS/MS spectra generated from the most abundant ion of the molecular ion cluster for each of the above mentioned compounds. The MS/MS spectrum presented in Fig. 3e, is the result of the collisional excitation of the *m/z* 927 parent ion from peak 1 (Figs. 2 and 3a) in an ion trap device. This spectrum displayed diagnostic fragment ions at *m/z* 846/848, 765/767, and 675. Another fragment ion of weaker intensity was observed at *m/z* 912, corresponding to the elimination of a methyl radical from the selected parent ion. The first cluster of fragment ions present from *m/z* 845 to *m/z* 848, arised from the loss of either a bromine atom ($^{79}Br^{\bullet}$ or $^{81}Br^{\bullet}$) or a neutral bromhydric acid ($H^{79}Br$ or $H^{81}Br$) moiety. A second cluster was observed from *m/z* 763 to *m/z* 769, corresponding to the consecutive elimination of either $Br^{\bullet}$ or HBr from the previous ions. The formation of the *m/z* 675 fragment ion was consistent with the breakage of the C–C bond between the central carbon and the aromatic ring on one of the tetrabromobisphenol A moieties. Based on these data, 2,2′-dihydroxy-3,3′-dibromo-5,5′-di(3,5-dibromo-4-hydroxycumyl)-biphenyl could be proposed as a structure for peak 1 (see inset in Fig. 3e) although isomeric structures (TBBPA units linkage positions) were also possible. Fig. 3f represents the MS/MS spectrum of the *m/z* 793 parent ion from peak 2 (Figs. 2 and 3b). The main fragment ions were observed at *m/z* 778, 711–714 and 543. The latter one corresponded to the deprotonated form of TBBPA as shown by characteristic fragment ions generated in a subsequent MS^3 experiment carried out from the *m/z* 543 fragment ion (data not shown). The other characteristic fragment ions arose from the elimination of radical species such as $CH_3^{\bullet}$ (*m/z* 778), $^{79}Br^{\bullet}$ or $^{81}Br^{\bullet}$ (*m/z* 712/714), as well as neutral $H^{79}Br$ and $H^{81}Br$ (*m/z* 711/713) from the molecular ion. From these data and other complementary data generated by NMR [27], peak 2 could be identified as 2,6-dibromo-4-(3′,5′-dibromo-4′-hydroxycumyl)-1-(3″,5″-dibromo-4″-hydroxy-phenoxy)-benzene. Fig. 3g displays the MS/MS spectrum of the main apolar compound according to the UV trace, i.e. peak 3 (Figs. 2 and 3c). The decomposition of the *m/z* 1027 $[M - 2H + Na]^-$ parent ion did not give useful structural information. The only observed fragmentation pathways involved the elimination of either HBr (*m/z* 947/945) or NaBr (*m/z* 925/923) from the selected parent species. The observed elimination of NaBr confirmed the nature of the molecular species as a sodium adduct. The reason why this particular compound appeared exclusively in the form of a sodium adduct could not be explained. Nevertheless, these data allowed us to propose a reasonable structure for peak 3 (see inset in Fig. 3g). The last selected MS/MS spectrum is presented in Fig. 3h for peak 4 (Figs. 2 and 3d). The decomposition of the *m/z* 1468 parent ion led to the formation of main fragment ions at *m/z* 1386/1388, 925 and 543. As observed for the products discussed above, the elimination of Br and HBr was the first decomposition process occurring from the selected molecular species. Consecutive eliminations led to the fragment ion clusters centred on the *m/z* 1388, 1306 and 1207 ratios. This compound also underwent a specific cleavage of the ether bond which led to the complementary daughter ions at *m/z* 925 and 543, representing one and two TBBPA moieties, respectively. This kind of compound constituted by three TBBPA units may be formed considering a double nucleophilic substitution of a bromine atom of TBBPA by another TBBPA molecule. This process could generate several positional isomeric structures among which one is proposed in Fig. 3h.

As indicated in Fig. 2, the LC–APPI-MS analysis of TBBPA submitted to photochemical degradation in acetonitrile allowed to evidence the formation of numerous degradation products. These compounds could be splitted into two main groups. The first one is constituted by compounds that are more water soluble than TBBPA, which are eluted before TBBPA in our reversed phase chromatographic run. The dehalogenation of TBBPA into BPA was very weakly observed in the present study, whereas it has been described as the main anaerobic biodegradation process of TBBPA [33,34]. However, this degradation process was reported to occur under reductive conditions, which is not the case in our study.

The second group of TBBPA degradation products concerned compounds which were less water soluble than TBBPA itself. Their occurrence could be clearly evidenced by LC–APPI-MS. These compounds resulted from the combination of several TBBPA and/or 2,6-dibromophenol units, and were eluted as a group of poorly resolved peaks between 35 and 55 min. This shows that a great number of apolar degradation compounds are formed after five days of irradiation of a solution of TBBPA in acetonitrile by solar UV light. APPI was the only LC–MS technique allowing the efficient ionisation and detection of these apolar degradation compounds of TBBPA. This paper is the first report on the occurrence of such compounds in the photochemical degradation of TBBPA. In 2003, Eriksson et al. [35] studied the photochemical degradation of TBBPA in water. The degradation of TBBPA under aerobic and anaerobic conditions has also been studied [33,34]. 2,6-Dibromophenol was shown to be one of the polar photo-degradation products of TBBPA but none of the apolar compounds we identified was reported in these studies. Although the possible role of acetonitrile in the formation of these apolar compounds has to be considered, structurally related compounds were recently identified by our group during the investigation of the in vitro metabolism of TBBPA [27].

Besides, the formation of apolar metabolites eluted after the parent compounds has also been reported in previous studies conducted with BPA, the non brominated analogue of TBBPA [26]. In this case, polymeric structures such as BPA dimers were characterised [26]. These compounds accounted for 1–2% of the total amount of BPA put in incubation. In

similar incubations carried out with TBBPA, amounts close to 20% were found to be associated with apolar metabolites [27]. The presence of bromine atoms clearly influences the ability of bisphenols to undergo oxidation, either by chemical or biochemical pathways.

3.2. Preliminary data on the LC–APPI-MS analysis of PBDEs

3.2.1. Ion species produced by APPI from various PBDE congeners

In the course of metabolism studies undertaken on BFRs, our aim was to probe an LC–MS methodology which could be applied to the identification of PBDEs as well as their metabolites which were expected to be hardly amenable to GC–MS analysis. However, neither ESI nor APCI could give any ion current whatever the ion mode that was used (positive or negative). On the other hand, APPI gave interesting results, as shown in Fig. 4, representing the MS spectra obtained from BDE85 and BDE100, two isomeric penta-BDEs, under positive and negative ionisation conditions, respectively. $M^{\bullet+}$ radical cations were produced under PI-APPI conditions (see Fig. 4a and b), according to the positive APPI ionisation mechanism of such compounds having ionisation energies lower than 10 eV. On the other hand, $[M - Br + O]^-$ ions were formed under NI-APPI (Fig. 4c and d), meaning that a dissociative resonance capture occurred, leading to $[M - Br]^-$ ions, which immediately reacted with oxygen molecules present in the atmospheric pressure ionisation source, as previously reported [32].

Different PBDE congeners ranging from di- to deca-BDE were analysed (direct injection) under both positive and negative APPI conditions using methanol–water–toluene (98:2:1, v/v/v)) as the solvent system. Signal-to-noise ratios obtained from these experiments are reported in Table 1. From these results, positive ion mode appeared to be well suited for PBDE congeners ranging from di- to penta-BDEs, whereas lower S/N ratios were observed for hexa- and hepta-BDEs and no signal was obtained for octa- and deca-BDE. On the other hand, the negative ion mode gave no signal for di- and tri-BDEs, low S/N ratios for tetra-BDEs, and worked better for congeners ranging from penta- to deca-BDE. Indeed, for penta-BDEs, similar performances were obtained with both positive and negative ionisation modes. On the other hand, in the case of octa- and deca-BDE, the negative APPI ionisation mode was the only way to get signal under the conditions we used. In general, these results are in good agreement with the high electron affinity of bromine, i.e. the more brominated the molecule, the easier the electron capture process.

Table 1
Signal-to-noise ratios obtained from different PBDE congeners analysed under PI and NI-APPI conditions (calculated from the $M^{\bullet+}$ or $[M - Br + O]^-$ isotopic clusters from 2 ng injections)

Number of Br atoms	BDE#	S/N PI-APPI	S/N NI-APPI
2	7	9	–
	15	9	–
3	17	22	–
4	47	18	4
	54	8	6
	77	25	6
5	85	20	14
	100	26	20
6	138	7	34
7	183	6	16
	190	6	8
8	201	–	4
10	209	–	4

3.2.2. MS/MS analysis of APPI produced ions from various PBDE congeners

The MS/MS spectra obtained by resonant excitation of APPI produced $M^{\bullet+}$ ions from BDE85 and BDE100 are presented in Fig. 5a and b. The loss of $Br^{\bullet}$ and Br_2 represented the only decomposition processes observed for BDE85 as well as BDE100. The $M^{\bullet+}$ ions of other congeners ranging from di- to hepta-BDEs (i.e. BDE7, BDE15, BDE17, BDE47, BDE54, BDE77, BDE138, BDE183 and BDE190) behaved similarly towards collisional excitation into the ion trap, although some slight differences could be observed in the relative fragmentation yields (data not shown). The PI-APPI ionisation process appeared to be much softer than EI and the $M^{\bullet+}$ ions are always by far the most intense ions produced by interaction of PBDEs with photons in the gas phase (see Fig. 4), whereas $[M - Br_2]^{\bullet+}$ fragments are formed using EI, especially in the case of *ortho*-substituted bromines [11,36,38]. However, the decomposition of the APPI produced $M^{\bullet+}$ ions using MS/MS also led to $[M - Br_2]^{\bullet+}$ as the most intense and almost unique fragment ions, and the presence of *ortho*-bromines also favoured their formation [37]. Until now, the production of negative ions from PBDEs has exclusively been achieved by means of electron capture negative ionisation (ECNI), i.e. via the capture of a thermal electron by electrophilic molecules such as PBDEs in a high pressure chemical ionisation source. This soft ionisation technique was described to produce Br^- ions as the most intense signals to monitor, except for BDE209 (deca-BDE) for which the *m/z* 487 formed by cleavage of the ethereal bond has been mentioned as the most predominant ion [6,15,38,39]. The formation of $[M - Br + O]^-$ ions has never been mentioned under ECNI conditions, showing that thermal electrons used in ECNI are not in the same range of energy as those produced by the negative APPI process.

The MS/MS spectra generated from the NI-APPI produced $[M - Br + O]^-$ ions suffered from the absence of one of the bromine atoms in the parent ion selected for MS/MS. This feature hindered part of the initially available structural information. This is illustrated by the MS/MS spectra of BDE85 and BDE100 given in Fig. 5c and d. The decomposition of these compounds mainly involved the elimination of HBr and Br_2. The same trend was observed

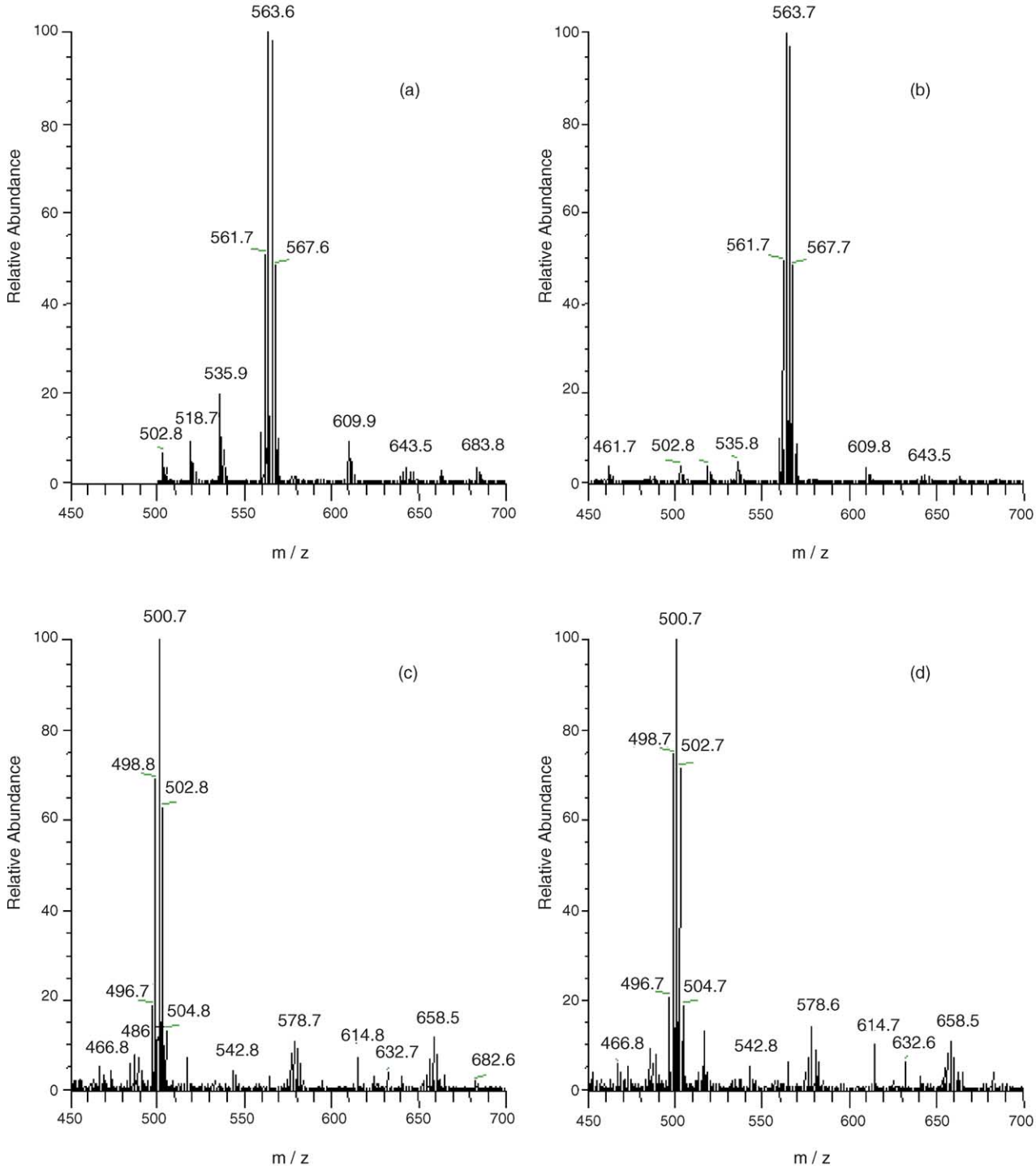

Fig. 4. Photoionisation mass spectra of the two isomeric penta-BDEs BDE85 and BDE100 obtained in (a and b) the positive and (c and d) the negative ion mode, respectively. Spectra were obtained from 5 ng PBDEs.

for the other analogues studied from penta- to hepta-BDEs (data not shown). The cleavage of the ethereal bond with charge retention on the oxygenated part of the molecule was also observed, leading to the formation of a $C_6H_2O_2Br_2$ radical anion species at *m*/*z* 266. This process is favoured for BDE100 compared to BDE85, although the $[M - Br + O]^-$ ion was found to be more stable in the case of BDE100. The position of the missing bromine atom in the $[M - Br + O]^-$ ions studied in MS/MS as well as their decomposition mechanisms are now under investigation [37].

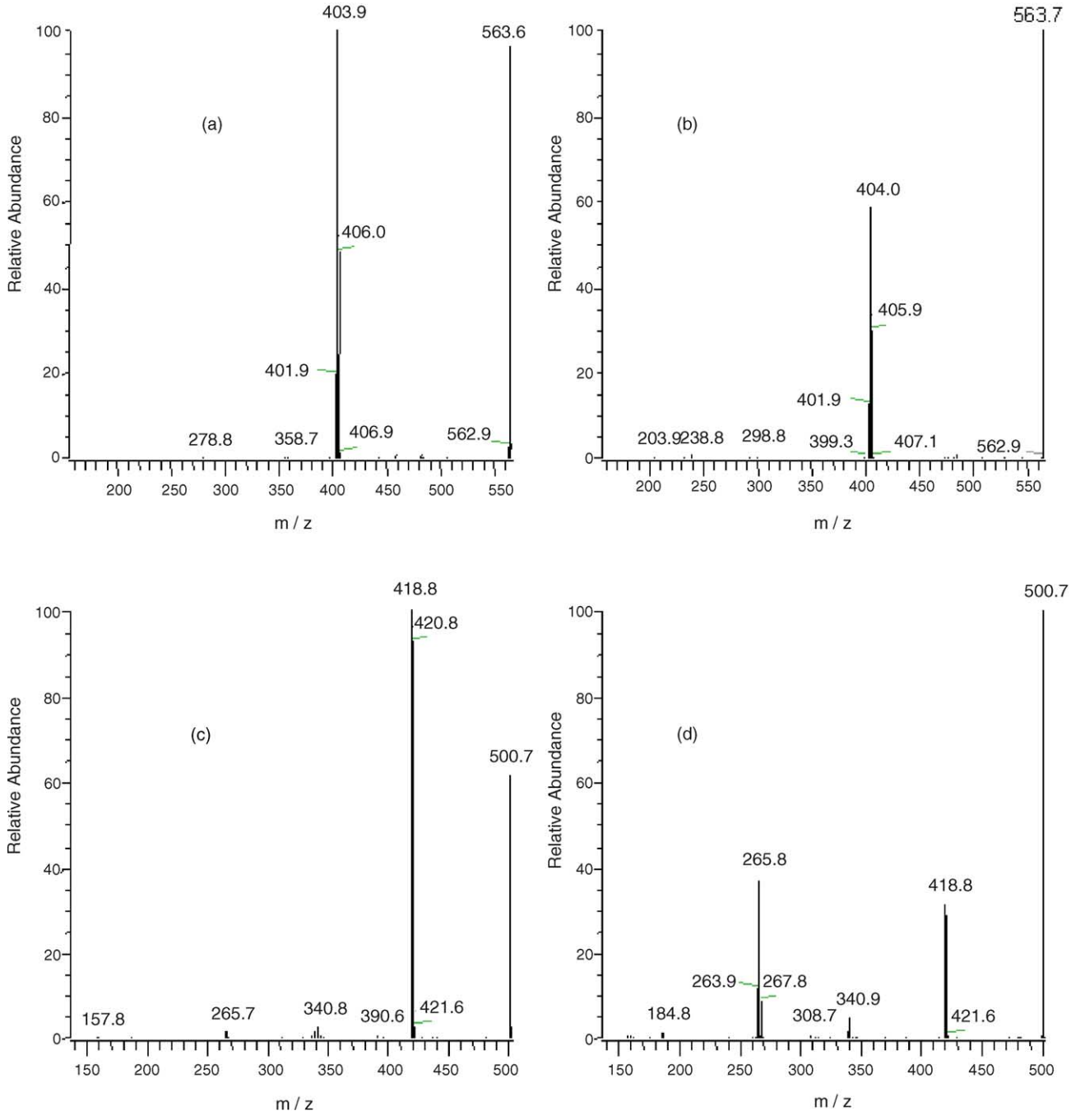

Fig. 5. MS/MS spectra generated from (a and b) the PI-APPI produced *m*/*z* 564 ion and (c and d) the NI-APPI produced *m*/*z* 501 parent ions selected as the most intense ions of the molecular isotopic clusters from BDE85 and BDE100, respectively. Spectra were obtained from 5 ng PBDEs.

3.2.3. LC–APPI-MS analysis of a model PBDE synthetic mixture

Our first concern was the development of a methodology for the analysis of degradation compounds and/or metabolites of deca-BDE, which have been reported to be mainly tetra- to nona-BDE congeners [11,15,38–41]. Therefore, the negative ion mode was chosen for the continuation of our work since it gave a better sensitivity for the detection of congeners bearing more than five bromine atoms. An application of the developed methodology for the analysis of PBDEs using NI-APPI-MS is presented in Fig. 6, displaying the reconstructed ion chromatograms obtained from the injection of a home-made synthetic mixture of PBDEs ranging from penta- to hepta-congeners. Selecting the most abundant ion of their isotopic clusters, penta-, hexa- and hepta-BDEs can be best detected at *m*/*z* 501, 581 and 659, respectively. Although it does not appear on the spectra presented in Fig. 4, the elimination of HBr was observed as a weak process (ca.

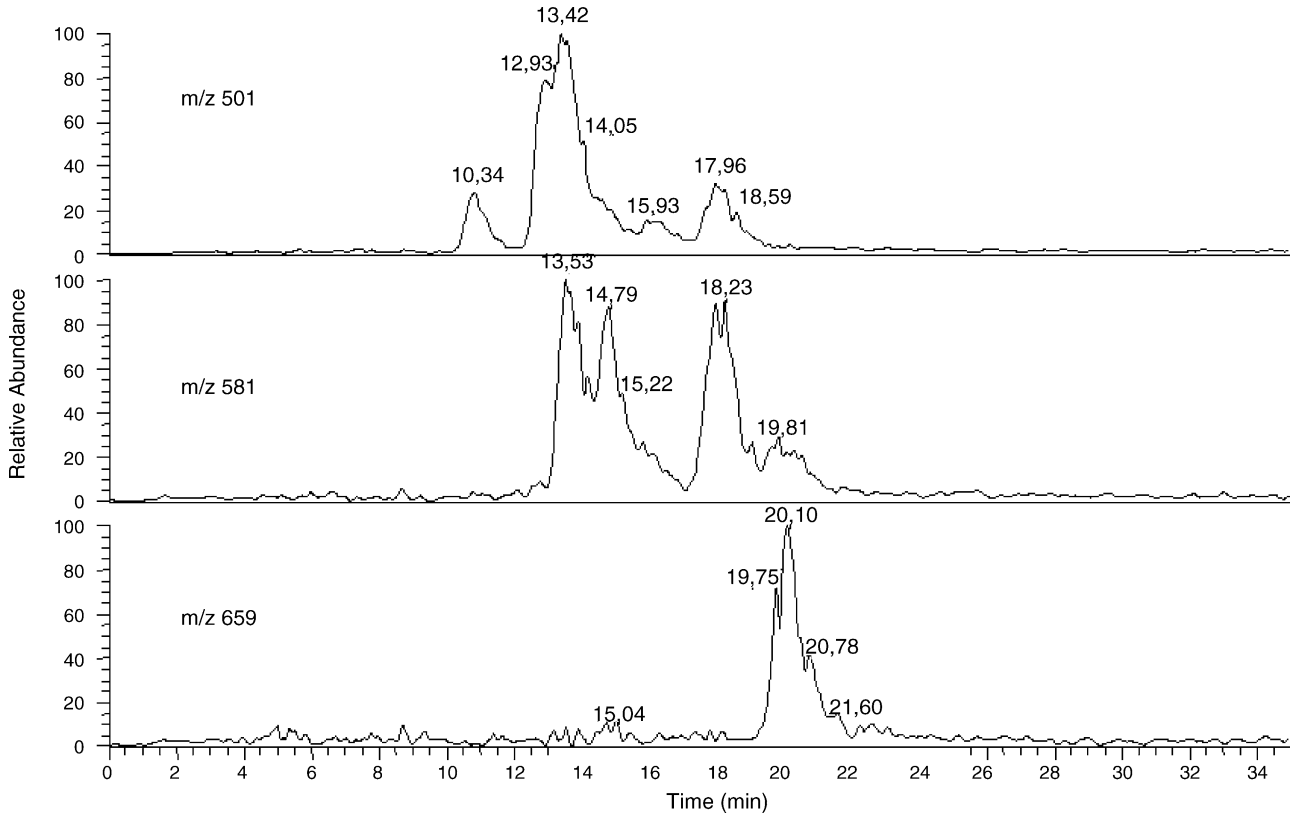

Fig. 6. Extracted ion chromatograms of penta- to hepta-BDEs obtained from the LC–APPI-MS analysis of a PBDE mixture (50 ng mixture injected): (a) penta-BDEs at *m*/*z* 501, (b) hexa-BDEs at *m*/*z* 581 and (c) hepta-BDEs at *m*/*z* 659.

10% relative abundance) on the MS spectra of PBDEs. This implied that *m*/*z* 581 and *m*/*z* 501 ions were present on the MS spectra of the hepta- and hexa-BDEs, respectively. Consequently, since several peaks appeared at the same retention time on Fig. 6, minor interfering signal may be present from hepta-BDEs on the hexa-BDEs trace, or from hexa-BDEs on the penta-BDEs trace.

However, although the resolving power of liquid chromatography is much lower than that of gas chromatography, these preliminary data indicated that LC–APPI-MS could provide an interesting alternative for the separation and characterisation of isomeric PBDE congeners (Fig. 6) as well as for their differentiation (Fig. 5). As far as we know, this work represents the first report on the analysis of PBDEs using an LC–MS methodology. Since hydroxylated derivatives have already been described as metabolites of PBDEs [5,42], this methodology may be useful for the identification of phase II PBDE metabolites such as glucuronic acid or glutathione conjugates for which the use of LC–MS may be necessary.

The same methodology has also been assessed for the analysis of deca-BDE (BDE209). Fig. 7 represents the mass fragmentogram obtained from the injection of 50 ng of commercial deca-BDE, together with the corresponding MS and MS/MS spectra presented as inserts. Deca-BDE was eluted as a quite large peak at 36.6 min. As observed for the other PBDE congeners using negative APPI, deca-BDE was characterised by a $[M - Br + O]^-$ ion cluster (*m*/*z* 886–904). Contrary to less brominated analogues, fragment ions were present on the MS spectrum of deca-BDE, resulting from the elimination of Br_2 ($[M - Br + O–Br_2]^-$, *m*/*z* 729–743 cluster) and from the cleavage of the ether bond of deca-BDE ($[C_6Br_5O]^-$, *m*/*z* 483–493 isotopic cluster). The MS/MS spectrum of the selected *m*/*z* 896 parent ion exhibited two main diagnostic ion clusters at *m*/*z* 734/736/738 (elimination of Br_2) and *m*/*z* 469–475. This latter was consistent with a molecular formula of $[C_6Br_5]^-$, meaning that the cleavage of the ether bond occurred with charge retention on the non oxygenated part of the molecule. This orientation was different from that observed on the MS spectrum, suggesting that different fragmentation processes are involved depending on the energy deposition mode (i.e. in-source photon induced process or collisional excitation into the ion trap). This preliminary result showed that liquid chromatography coupled to negative atmospheric pressure photo ionisation could be used as a potential alternative for the analysis of deca-BDE, which is known to be present in the environment at high levels, and is difficult to analyse using GC–MS.

In this preliminary study, elevated amounts were injected in order to get high signal levels and to assess separation as well as ionisation capabilities of our methodology. In terms of sensitivity, although no systematic optimisation study has been undertaken, the limits of detection were estimated in the

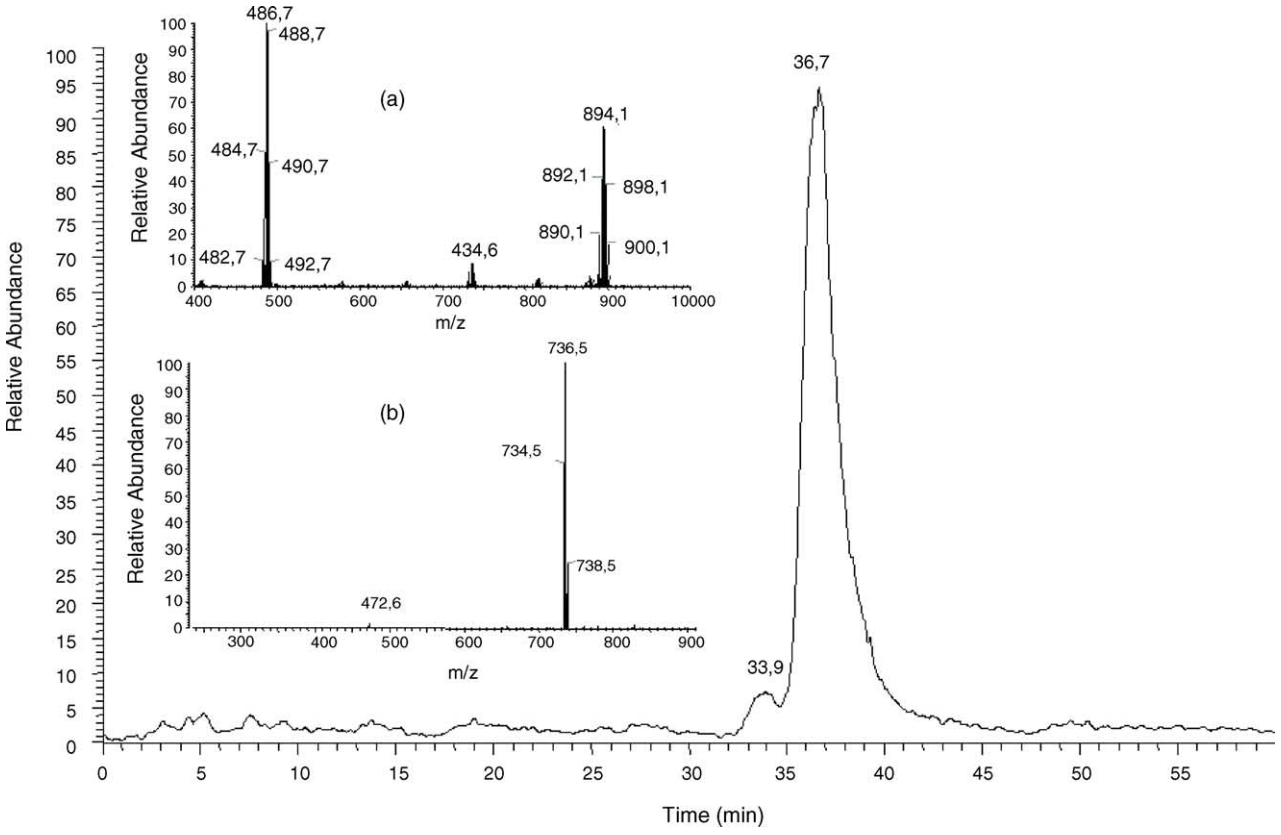

Fig. 7. Extracted ion chromatogram obtained from the LC–APPI-MS (MS/MS) analysis of deca-BDE (50 ng injected). Insets represent the NI-APPI (a) MS and (b) MS/MS spectra of deca-BDE.

range of 200–1500 pg of PBDEs injected onto the LC column, depending on the nature and the bromination degree of the congener. This preliminary estimation needs to be improved for the detection of PBDEs in in vivo samples, and work is now in progress in order to reach better sensitivity levels. Nevertheless, taking into account the higher volumes which can be injected in LC in comparison to GC, the performance of our method is not so far from those reached with GC in terms of concentration of the injected solution.

4. Conclusion

APPI proved to constitute an efficient tool for the mass spectrometric analysis of aromatic BFRs using atmospheric pressure ionisation coupled to liquid chromatography. As stated in the text, this recent ionisation technique involves complex ionisation chemistry which is not fully understood yet. Indeed, different molecular species are observed depending upon the ionisation mode used (positive versus negative) and the nature of the compound to be analysed. Although the developed methodology was primarily intended to be used for the qualitative analysis of BFR degradation compounds and/or their metabolites, a potential drawback was found for quantitative analyses, related to the APPI susceptibility with regard to the solvent composition during a gradient elution. This feature may render difficult the simultaneous determination of several compounds without the use of several internal standards. Nevertheless, the proposed methodology is applicable for the LC–MS analysis of BFR degradation compounds and/or metabolites which are not amenable to GC–MS and for which ESI and APCI are inefficient. This methodology opens a way to the use of LC–MS/MS based methods for the identification of apolar BFR biotransformation compounds which can be formed in vitro [27] and may be used for checking the occurrence of such metabolites in vivo. Work is now in progress to improve both the MS detection sensitivity and the chromatographic separation selectivity (isomers) via the use of (i) normal phase HPLC systems for which APPI was reported to be applicable [43] and (ii) more selective stationary phases.

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