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Liquid chromatography/multi-stage mass spectrometry of bisphenol A and its halogenated derivatives

Héctor Gallart-Ayala, Encarnación Moyano and Maria T. Galceran*
Department of Analytical Chemistry, University of Barcelona, Martí i Franquès 1-11, 08028 Barcelona, Spain

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We report a liquid chromatography/tandem mass spectrometry (LC/MS/MS) method for analyzing bisphenol A (BPA) and its halogenated derivatives. Since only tetrachlorobisphenol A and tetrabromobisphenol A (TBBPA) are commercially available, mono-, di- and trichlorobisphenol A were synthesized and purified in order to be used as analytical standards. This family of compounds was studied using electrospray ionization and an ion trap mass analyzer in order to characterize the new compounds and to propose fragmentation pathways. Multi-stage mass spectrometry was used to confirm the genealogical relationship between the ions. Some product ions were traced from MS/MS to MS^4 and the labelled compounds BPA-d_{16} and TBBPA-$^{13}C_{12}$ were used to assign some product ion structures. In general, the deprotonated molecule $[M–H]^-$ loses a methyl and/or a halogen group during both MS/MS and MS^3, while the neutral loss of CO was also observed in MS^3 spectra. We selected the most intense and characteristic MS/MS transitions for LC/MS/MS analysis. LC separation was performed in a reversed-phase column; methanol/water (no additives) was used as the mobile phase in gradient elution mode; and BPA-d_{16} was chosen as the internal standard. Solid-phase extraction (SPE) was used to pre-concentrate and to clean up water samples. The SPE LC/MS/MS method allows BPA and its halogenated derivatives to be detected at a few parts-per-billion (ppb) in surface water. Copyright © 2007 John Wiley & Sons, Ltd.

Bisphenol A (BPA) is widely used in the production of epoxy resins and polycarbonate plastics, which are employed as coatings especially for food-contact surfaces in cans, for electric and electronic equipment, and for digital supports such as CDs and DVDs. Hydrolysis of polycarbonate plastics and epoxy resins results in BPA monomer leaching into the environment. BPA has therefore been found in sediments (0.6–5.0 ng g^{-1}),[1,2] sewage sludge (25–325 μg g^{-1}),[3] and environmental water samples (20–1300 ng L^{-1}).[4–11] Some halogenated derivatives of BPA – such as tetrabromobisphenol A (TBBPA) and tetrachlorobisphenol A (TeCBPA) – are commonly used as flame retardants in polymers and due to their extensive use they are also found in the environment. For instance, TBBPA has been detected in sediments (2–300 ng/g),[8,12,13] sewage sludge (65–100 ng g^{-1}),[13] and air (14–150 ng m^{-3} in indoor air).[14,15] During water disinfection and blenched paper recycling, BPA can become chlorinated and its derivatives (monochloro-, dichloro-, trichloro- and tetrachlorobisphenol A) can be released into the environment. Final effluents from paper recycling plants have been found to contain 0.2–2.0 μg L^{-1} of these compounds.[16,17]

BPA is acutely toxic to aquatic organisms,[19–22] and, at low doses (below 5 mg/kg/day),[18–20] it has been reported to disrupt endocrine function. Toxicity studies of the chlorinated derivatives suggest that their estrogenic activity is stronger than that of BPA,[16,23–25] and TeCBPA also shows thyroid hormonal activity.[26] Furthermore, *in vitro* toxicity experiments indicate that TBBPA is an immunotoxic compound[27] that disrupts endocrine function. At present there are no restrictions on any of these compounds, although TBBPA is currently being evaluated in the EU, US and Asian-Pacific countries.[28] Nevertheless, as more than 1000 tons/year of TBBPA is produced, it is now considered a high volume substance under REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) and it will have to be registered within 3 years of REACH coming into force in the EU.

BPA is usually detected by using liquid chromatography (LC) with different detection systems, such as spectrophotometry (UV),[11,29,30] mass spectrometry (MS),[2,4,31] or fluorescence,[30,32] although some methods based on gas chromatography coupled to mass spectrometry (GC/MS) have also been used.[2,3,5–7] For TBBPA, both LC and GC coupled to MS are used.[12,14,15,33] Chlorinated derivatives of BPA are generally analyzed by GC/MS after derivatization of the phenolic groups with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA)[25,26] or *N*-(*tert*-butyldimethylsilyl)-*N*- methyltrifluoroacetamide (MTBSTFA).[34,35] Since there are no published LC/MS and LC/MS/MS methods for the simultaneous detection of BPA and its halogenated derivatives (MCBPA, DCBPA,

*Correspondence to: M. T. Galceran, Department of Analytical Chemistry, University of Barcelona, Martí i Franquès 1-11, 08028 Barcelona, Spain.
E-mail: mtgalceran@ub.edu
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TCBPA, TeCBPA and TBBPA), the aim of the present work is to develop a sensitive and selective LC/MS/MS method for their detection in water samples. Furthermore, we apply multi-stage ion trap mass spectrometry (MS^n) to study the fragmentation pathways of this family of compounds.

EXPERIMENTAL

Chemicals

Bisphenol A, 2,2-bis(4-hydroxyphenyl)propane (BPA) (Table 1), bisphenol A-d_{16} (BPA-d_{16}), tetrachlorobisphenol A, 2,2-bis(3,5-dichloro-4-hydroxyphenyl)propane (TeCBPA), tetrabromobisphenol A, 2,2-bis(3,5-dibromo-4-hydroxyphenyl)propane (TBBPA) were obtained from Sigma-Aldrich (Steinheim, Germany), and tetrabromobisphenol A (Ring-$^{13}C_{12}$) (TBBPA-$^{13}C_{12}$) was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). HPLC-gradient grade methanol (MeOH), acetonitrile (ACN), ethanol (EtOH), dichloromethane (DCM) and water as well as hydrochloric acid (25%) and sodium hydroxide for analysis were purchased from Merck (Darmstadt, Germany), while sodium hypochlorite solution (10% chlorine) and anhydrous sodium sulfate were obtained from Flucka (Buchs, Germany). $CDCl_3$ purchased from Sigma-Aldrich was used as the solvent for NMR. Stock standard solutions of individual compounds and BPA-d_{16} (10 mg L^{-1}) were prepared in MeOH and stored at 4°C. Mobile phases were filtered using a 0.45 μm nylon filter (Whatman, Clifton, NJ, USA). Bond Elute C_{18} (500 mg) cartridges purchased from Varian (Harbor City, CA, USA) were used for solid-phase extraction (SPE).

Nitrogen (99.8% pure) supplied by a Claind N_2 FLO nitrogen generator (Lenno, Italy) was used in the atmospheric pressure ionization (API) source. Helium of high purity purchased from Air Liquide (Madrid, Spain) was used as a damper gas for the ion trap.

Synthesis of chlorinated derivatives of bisphenol A

Chlorinated derivatives of BPA (MCBPA, DCBPA and TCBPA) (Table 1) were synthesized and purified to be used as standards. The synthesis was based on the chlorination of BPA by aromatic electrophilic substitution since the hydroxyl group activates the *ortho* position.

Sodium hypochlorite solution (75 mL, >10% chlorine available) and hydrochloric acid (25%) were mixed to generate a chlorine gas current that was bubbled, at room temperature, into a stirred solution of BPA (0.5 g) previously prepared in EtOH/H_2O (20:80 v/v) and adjusted to pH 8 with sodium hydroxide (0.1 M). After the chlorination step, the solution was heated to 60°C to eliminate the residual chlorine and centrifuged (45 min, 2000 rpm). The aqueous solution was decanted and extracted with DCM (5 × 20 mL). The solid residue was dissolved in the DCM extract and this organic solution was dried using anhydrous sodium sulfate and then evaporated to dryness. The residue was reconstituted in 30 mL of ACN/water (50:50). A reversed-phase C_{18} semi-preparative column (eluent: ACN/water, 50:50) was used to purify the extract. BPA and its chlorinated derivatives were separated at base line resolution and multiple injections (500 μL) were performed to collect combined individual fractions of each compound. Each combined fraction was evaporated using a Turbo Vap II concentration workstation (Zymark Corp., Hopkinton, MA, USA) (30 min at 8 psi and 25°C) to eliminate the ACN. The aqueous extract was re-extracted with DCM (5 × 20 mL). Finally, the solvent was evaporated to leave a crystalline product. The purity (>99%) of these compounds was established by ^{1}H NMR and confirmed by LC/MS.

Monochlorobisphenol A (MCBPA) ^{1}H NMR ($CDCl_3$); δ: 1.60 ppm (6H, s, 2 × CH_3), 4.98 ppm (1H, s, OH), 5.45 ppm (1H, s, OH), 6.74 ppm (2H, dt, $^3J_{ortho} = 8.8$ Hz, $^4J_{meta} = 2.6$ Hz, 2 × ArH), 6.89 ppm (1H, d, $^3J_{ortho} = 8.4$ Hz, ArH), 7.00 ppm (1H, dd, $^3J_{ortho} = 8.4$ Hz, $^4J_{meta} = 2.4$ Hz, ArH), 7.07 ppm (2H, dt, $^3J_{ortho} = 9.2$ Hz, $^4J_{meta} = 2.6$ Hz, 2 × ArH), 7.17 ppm (1H, d, $^4J_{meta} = 2.4$ Hz, ArH).

Dichlorobisphenol A (DCBPA) ^{1}H NMR ($CDCl_3$); δ: 1.60 ppm (6H, s, 2 × CH_3), 5.47 ppm (2H, s, 2 × OH), 6.91 ppm (2H, d, $^3J_{ortho} = 8.4$ Hz, 2 × ArH), 7.00 ppm (2H, dd, $^3J_{ortho} = 8.6$ Hz, $^4J_{meta} = 2.2$ Hz, 2 × ArH), 7.16 ppm (2H, d, $^4J_{meta} = 2.00$ Hz, 2 × ArH). Only 3,3′-dichlorobisphenol A was obtained.

Trichlorobisphenol A (TCBPA) ^{1}H NMR ($CDCl_3$); δ: 1.60 ppm (6H, s, 2 × CH_3), 5.51 ppm (1H, s, OH), 5.77 ppm (1H, s, OH), 6.93 ppm (1H, d, $^3J_{ortho} = 8.4$ Hz, ArH), 6.98 ppm (1H, dd, $^3J_{ortho} = 8.6$ Hz, $^4J_{meta} = 2.2$ Hz, ArH), 7.15 ppm (1H, d, $^4J_{meta} = 2.4$ Hz, ArH), 7.08 ppm (2H, s, 2 × ArH).

These spectra match the published[17] spectra for these compounds perfectly and no signal from impurities was found in the synthesized compounds.

Instrumentation

^{1}H NMR

^{1}H NMR spectra of the synthesized compounds dissolved in deuterated chloroform were obtained using a Varian Model Mercury 400 NMR spectrometer (400 MHz; Varian, Palo Alto, CA, USA).

Liquid chromatography

A liquid chromatograph (Alliance 2695 separations module; Waters, Milford, MA, USA) equipped with a low-pressure quaternary solvent pumping system and an autosampler was used. The chromatographic separation was carried out in a SunFire™ C_{18} column, 150 × 2.1 mm i.d., 3.5 μm particle size (Waters), using MeOH/water as the mobile phase, a gradient elution program, a flow rate of 200 μL min^{-1} and an injection volume of 10 μL. Gradient 1: started at 65% MeOH and rose to 85% MeOH in 5 min; this percentage was maintained for 9 min. Finally, the mobile phase was returned to the initial conditions over 5 min and equilibrated for a further 10 min. Gradient 2: started at 50% MeOH for 1.5 min followed by a linear gradient up to 65% MeOH in 0.5 min and a second linear gradient step up to 85% MeOH in 5 min; this percentage was maintained for 8 min. Finally, the mobile phase was returned to the initial conditions over 5 min and this was followed by a 10 min equilibration step.

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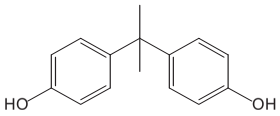
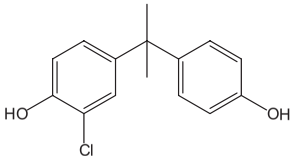
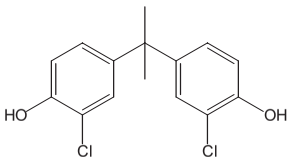
Table 1. Optimized CID conditions in MS^2 and MS^3 and main product ions obtained for BPA and its halogenated compounds and the corresponding labelled compounds

	MS		MS/MS				MS^3			
Compound	*m/z* (% Rel.Ab.)	Assignment	NCE (%)	AQ	*m/z* (% Rel.Ab.)	Assignment	NCE (%)	AQ	*m/z* (% Rel.Ab.)	Assignment
Bisphenol A (BPA) 	227 (100)	$[M–H]^-$	44	0.35	227 (23)	$[M–H]^-$				
					212 (100)	$[M–H–CH_3]^{-\bullet}$	43	0.35	212 (10)	$[M–H–CH_3]^{-\bullet}$
									211 (100)	$[M–H–CH_3–H]^-$
					211 (35)	$[M–H–CH_4]^-$			196 (21)	$[M–H–CH_3–CH_4]^{-\bullet}$
									117 (34)	$[M–H–CH_3–C_6H_7O]^-$
									93 (16)	$[M–H–CH_3–C_8H_7O]^-$
					133 (85)	$[M–H–C_6H_6O]^-$	44	0.35	133 (100)	$[M–H–C_6H_6O]^-$
									93 (22)	$[M–H–C_6H_6O–C_3H_4]^-$
					93 (20)	$[M–H–C_9H_{10}O]^-$				
Monochlorobisphenol A (MCBPA) 	261 (100) 263 (34)	$[M–H]^-$	44	0.45	261 (8)	$[M–H]^-$				
					246 (100)	$[M–H–CH_3]^{-\bullet}$	44	0.45	246 (11)	$[M–H–CH_3]^{-\bullet}$
									210 (100)	$[M–H–CH_3–HCl]^{-\bullet}$
									182 (4)	$[M–H–CH_3–CHOCl]^{-\bullet}$
					225 (4)	$[M–H–HCl]^-$				
					210 (68)	$[M–H–CH_4Cl]^{-\bullet}$	32	0.45	210 (20)	$[M–H–CH_4Cl]^{-\bullet}$
									209 (10)	$[M–H–CH_4Cl–H]^-$
									195 (6)	$[M–H–CH_4Cl–CH_3]^-$
									182 (100)	$[M–H–CH_4Cl–CO]^{-\bullet}$
					195 (<5)	$[M–H–C_2H_7Cl]^-$				
					182 (7)	$[M–H–C_2H_4OCl]^{-\bullet}$				
					167 (<5)	$[M–H–C_6H_6O]^-$				
					133 (<5)	$[M–H–C_6H_5OCl]^-$				
Dichlorobisphenol A (DCBPA) 	295 (100) 297 (66) 299 (11)	$[M–H]^-$	44	0.45	295 (5)	$[M–H]^-$				
					280 (26)	$[M–H–CH_3]^{-\bullet}$	28	0.45	280 (10)	$[M–H–CH_3]^{-\bullet}$
									244 (100)	$[M–H–CH_3–HCl]^{-\bullet}$
					259 (6)	$[M–H–HCl]^-$	39	0.45	259 (60)	$[M–H–HCl]^-$
									244 (100)	$[M–H–HCl–CH_3]^{-\bullet}$
									216 (64)	$[M–H–HCl–C_2H_3O]^{-\bullet}$
									208 (24)	$[M–H–HCl–CH_4Cl]^{-\bullet}$
					244 (100)	$[M–H–CH_4Cl]^{-\bullet}$	34	0.45	244 (15)	$[M–H–CH_4Cl]^{-\bullet}$
									229 (11)	$[M–H–CH_4Cl–CH_3]^-$
									216 (100)	$[M–H–CH_4Cl–CO]^{-\bullet}$
					216 (5)	$[M–H–C_2H_4OCl]^{-\bullet}$				
					167 (<5)	$[M–H–C_6H_5OCl]^-$				

(*Continues*)

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Table 1. (Continued)

Compound	MS		MS/MS				MS^3			
	m/z (% Rel.Ab.)	Assignment	NCE (%)	AQ	*m/z* (% Rel.Ab.)	Assignment	NCE (%)	AQ	*m/z* (% Rel.Ab.)	Assignment
Trichlorobisphenol A (TCBPA)	329 (100) 331 (99) 333 (32) 335 (3)	$[M–H]^-$	44	0.45	329 (7) 314 (29) 293 (5) 278 (100) 250 (6) 201 (<5)	$[M–H]^-$ $[M–H–CH_3]^{-\bullet}$ $[M–H–HCl]^-$ $[M–H–CH_4Cl]^{-\bullet}$ $[M–H–C_2H_4OCl]^{-\bullet}$ $[M–H–C_6H_5OCl]^-$	28 38 32 31	0.45 0.45 0.45 0.45	314 (11) 278 (100) 293 (100) 278 (94) 250 (51) 278 (19) 263 (<5) 250 (100) 250 (100) 249 (46) 214 (16)	$[M–H–CH_3]^{-\bullet}$ $[M–H–CH_3–HCl]^{-\bullet}$ $[M–H–HCl]^-$ $[M–H–HCl–CH_3]^{-\bullet}$ $[M–H–HCl–C_2H_3O]^{-\bullet}$ $[M–H–CH_4Cl]^{-\bullet}$ $[M–H–CH_4Cl–CH_3]^-$ $[M–H–CH_4Cl–CO]^{-\bullet}$ $[M–C_2H_4OCl]^{-\bullet}$ $[M–C_2H_4OCl–H]^-$ $[M–C_2H_4OCl–HCl]^{-\bullet}$
Tetrachlorobisphenol A (TeCBPA)	363 (76) 365 (100) 367 (47) 369 (10)	$[M–H]^-$	46	0.45	363 (32) 348 (20) 327 (<5) 312 (100) 284 (<5) 201 (<5)	$[M–H]^-$ $[M–H–CH_3]^{-\bullet}$ $[M–H–HCl]^-$ $[M–H–CH_4Cl]^{-\bullet}$ $[M–H–C_2H_4OCl]^{-\bullet}$ $[M–H–C_6H_4OCl_2]^-$	30 46 35 38	0.45 0.45 0.45 0.45	348 (19) 312 (100) 327 (36) 312 (49) 284 (100) 312 (21) 297 (<5) 284 (100) 284 (30) 248 (100)	$[M–H–CH_3]^{-\bullet}$ $[M–H–CH_3–HCl]^{-\bullet}$ $[M–H–HCl]^-$ $[M–H–HCl–CH_3]^{-\bullet}$ $[M–H–HCl–C_2H_3O]^{-\bullet}$ $[M–H–CH_4Cl]^{-\bullet}$ $[M–H–CH_4Cl–CH_3]^-$ $[M–H–CH_4Cl–CO]^{-\bullet}$ $[M–H–C_2H_4OCl]^{-\bullet}$ $[M–H–C_2H_4OCl–HCl]^{-\bullet}$
Tetrabromobisphenol A (TBBPA)	539 (18) 541 (70) 543 (100) 545 (63) 547 (15)	$[M–H]^-$	40	0.45	539 (20) 524 (65) 459 (15) 444 (100) 416 (<5) 289 (<5)	$[M–H]^-$ $[M–H–CH_3]^{-\bullet}$ $[M–H–HBr]^-$ $[M–H–CH_4Br]^{-\bullet}$ $[M–H–C_2H_4OBr]^{-\bullet}$ $[M–H–C_6H_4OBr_2]^-$	27 34	0.45 0.45	524 (32) 444 (100) 444 (90) 429 (30) 416 (100)	$[M–H–CH_3]^{-\bullet}$ $[M–H–CH_3–HBr]^{-\bullet}$ $[M–H–CH_4Br]^{-\bullet}$ $[M–H–CH_4Br–CH_3]^-$ $[M–H–CH_4Br–CO]^{-\bullet}$

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Semi-preparative liquid chromatography

A Waters 600E liquid chromatograph equipped with a Rheodyne injection valve (Cotati, CA, USA) with a 500 μL loop and a model 432 fixed wavelength detector (Kontro Instruments, Fantoly, Italy) was used to purify the chlorinated BPA derivatives. A SunFire™ Prep C_{18} column, 100 × 10 mm i.d., 5 μm particle size (Waters), was used for the chromatographic separation at a flow rate of 3 mL min^{-1}, injecting 500 μL onto the column. An ACN/water gradient elution was used for the separation of BPA and its halogenated derivatives. The elution program started with 40% ACN and proceeded with a linear gradient up to 60% ACN over 10 min. This was followed by a 10 min isocratic step. Finally, the mobile phase was returned to the initial conditions over 5 min, and the column was equilibrated for 5 min. A model II fraction collector from Waters was used to collect peak fractions. Data were processed using Chromatography Software from Borwin (Vienna, VA, USA).

Mass spectrometry

The liquid chromatographic system (Alliance 2695) was coupled to a Classic LCQ instrument (ThermoFinningan, San Jose, CA, USA) equipped with an ion trap mass analyzer and both a coaxial pneumatically assisted electrospray ionization (ESI) and an atmospheric pressure chemical ionization (APCI) source. Data acquisition was performed in the negative ion mode and the Xcalibur software version 1.4 (ThermoFinnigan) was used to control the LC/MS system and to process data.

The ESI working conditions were: sheath gas and auxiliary gas (N_2) flow rates 74 a.u. (arbitrary units) and 52 a.u., respectively; capillary heater temperature 280°C; electrospray needle voltage −4.0 kV; and tube lens offset voltage −13.0 V. The APCI working parameters were: sheath gas flow rate 35 a.u. and no auxiliary gas; vaporizer temperature 275°C; capillary heater temperature 200°C; corona discharge voltage −4.0 kV; spray current 4.5 μA; and tube lens offset voltage −10.0 V. Product ion spectra from the multi-stage mass spectrometry (MS^n) experiments were acquired (m/z 50–600) using profile mode. The $[M–H]^-$ ion was used as the precursor ion for tandem mass spectrometry experiments under the following working conditions: isolation width of 1.5 m/z units; 5 μscans; maximum injection time 200 ms; and activation time 30 ms. The trapping radio-frequency voltage (AQ) was set at a value between 0.35 and 0.45 and the normalized collision energy (NCE%, amplitude of the voltage applied to the end-cap electrode) was from 38 to 44%. Table 1 gives the AQ and NCE% values selected for each analyte.

A 10 mg L^{-1} stock standard solution of each compound prepared in MeOH was infused at a flow rate of 10 μL min^{-1} using the syringe pump integrated in the LCQ instrument. It was mixed with the mobile phase (200 μL min^{-1}, MeOH/H_2O (75:25 v/v)) by means a Valco zero dead volume tee piece (Supelco, Alcobendas, Spain) to optimize the source working conditions and to carry out the MS^n experiments. Quantitative analysis was carried out by the standard addition method and using BPA-d_{16} as the internal standard.

Sample treatment

Wastewater samples from a paper recycling plant were collected in 1-L glass bottles and 1 mL of ascorbic acid (0.1 M) was added in order to avoid chlorination during storage (4°C) due to the residual chlorine. Off-line SPE using reversed-phase C_{18} cartridges (Bond Elute, 500 mg; Varian) was used as a clean-up and pre-concentration step before LC/MS/MS analysis. First, the C_{18} cartridge was conditioned using 10 mL of MeOH and 6 mL of water, then 50 mL of a water sample was loaded and the cartridge was washed with 10 mL of water and 10 mL of MeOH/water (20:80 v/v). Finally it was dried and the analytes were eluted using 5 mL of MeOH. The collected fraction was evaporated to dryness, the extract was reconstituted with 500 μL of an internal standard MeOH solution (BPA-d_{16} at 145 μg L^{-1}) and 10 μL of this extract was injected into the LC/MS/MS system. A Supelco Visisprep and a Supelco Visidry SPE vacuum manifold (Supelco, Gland, Switzerland) were used for SPE and solvent evaporation.

RESULTS AND DISCUSSION

Chlorinated derivatives of BPA were synthesized and purified to provide analytical standards of mono-, di- and trichloro-BPA since these are not commercially available. ^{1}H NMR allowed us to identify the DCBPA isomer synthesized, since two isomers, 3,3′-DCBPA and 3,5-DCBPA, can be obtained. The analysis of the aromatic range of the ^{1}H NMR spectrum (Fig. 1) revealed signals associated with three different types of aromatic protons and the multiplicity of these signals agreed with those of the 3,3′-DCBPA isomer. The doublet (d) at 6.91 ppm (2H, d, $^3J_{ortho} = 8.4$ Hz, 2 × ArH) corresponded to ortho-protons, the doublet doublet (dd) at 7.00 ppm (2H, dd, $^3J_{ortho} = 8.6$ Hz, $^4J_{meta} = 2.2$ Hz, 2 × ArH) could be related to meta-protons and the doublet at 7.16 ppm (2H, d, $^4J_{meta} = 2.00$ Hz, 2 × ArH) was due to meta-protons in the *ortho* position with respect to the chlorine atoms. Moreover, no signals that could be associated with the presence of the other isomer were present.

Mass spectrometry fragmentation studies

Electrospray ionization (ESI) in negative ion mode was used for the fragmentation studies. This ionization technique provided a full scan single MS spectrum where the base peak was the isotopic cluster corresponding to the deprotonation of one hydroxyl group $[M–H]^-$ and no additional fragments or adducts were observed.

Multi-stage mass spectrometry (MS^n) was used to study the fragmentation of BPA and its halogenated derivatives (MCBPA, DCBPA, TCBPA, TeCBPA and TBBPA). Labelled compounds (BPA-d_{16} and TBBPA-$^{13}C_{12}$) were also fragmented to help interpret the MS^n spectra. The lightest ion in the isotopic cluster (lowest m/z value) was used as the precursor ion for tandem mass spectrometry experiments. For further MS^n experiments the most abundant product ions or the most characteristic ones were used as precursor ions. Table 1 summarizes the collision-induced dissociation (CID) working conditions, the main product ions and their

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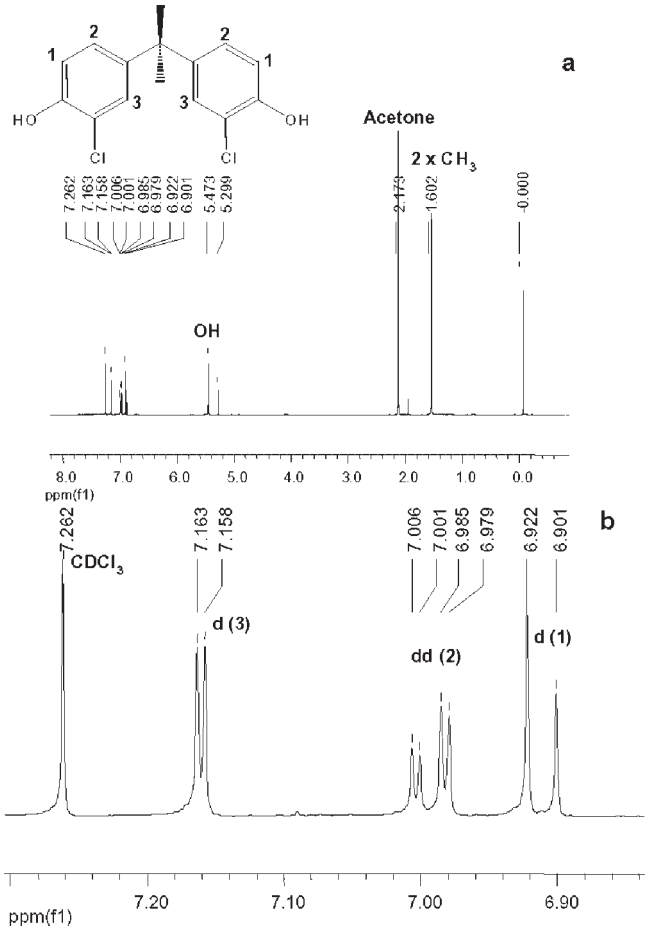

Figure 1. ^{1}H NMR spectra of DCBPA: (a) complete spectrum and (b) aromatic range enlargement.

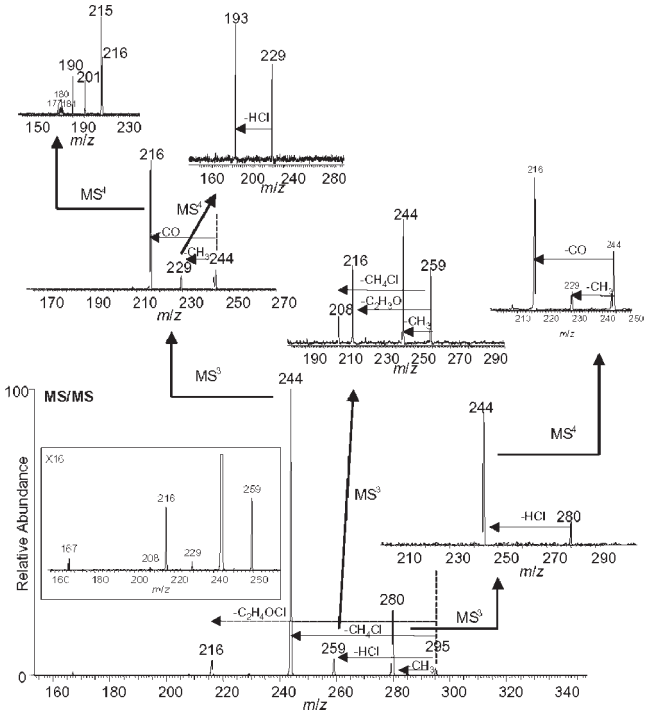

Figure 2. MS^2 spectra of DCBPA and higher-order mass spectra of some of the most important product ions. Working conditions as detailed in the Experimental section; CID conditions as given in Table 1.

assignment in MS/MS and MS^3 for BPA and its halogenated compounds. Figure 2 shows the MS/MS spectrum of the selected precursor ion of DCBPA and MS^n spectra of some of the most abundant product ions.

For BPA and MCBPA the MS/MS spectra show as base peaks the product ion resulting from the loss of a methyl group $[M–H–CH_3]^{-\bullet}$ (*m/z* 212 and 246, respectively). BPA also produced an abundant product ion resulting from the cleavage of the hydroxybenzyl group $[M–H–C_6H_5OH]^-$ (*m/z* 133, 85%) while for MCBPA this ion had a low relative abundance, <5%. Although the $[M–H–CH_3]^{-\bullet}$ ion is also present in the MS/MS spectra of the other halogenated derivatives of BPA, for these compounds the base peak is an ion resulting from the combined loss of a methyl group and HCl or HBr, $[M–H–CH_4X]^{-\bullet}$ (X = Cl, Br). Moreover, for chlorinated derivatives, the $[M–H–HCl]^-$ product ions had relative abundances of less than 6%, while for TBBPA the $[M–H–HBr]^-$ ion was slightly more abundant, 15%. In the MS/MS fragmentation of BPA, a product ion was observed at *m/z* 93, probably due to the cleavage of the hydroxyphenyl propyl bond $[M–H–C_9H_{10}O]^-$. This product ion also appeared in the MS^3 spectra, and it was generated along two different pathways: (i) the consecutive losses of $CH_3^{\bullet}$ (*m/z* 212) and the hydroxyphenylethyl (C_8H_7O) group, and (ii) the loss of the hydroxyphenyl (C_6H_6O) group (*m/z* 133) followed by the loss of the propyl group.

Halogenated derivatives of BPA showed a $[M–H–CH_4X–28]^{-\bullet}$ MS^3 product ion. To investigate this fragmentation the MS^n spectra of TBBPA-$^{13}C_{12}$ (^{13}C in the aromatic rings) were studied. Figure 3 shows the MS/MS spectrum of the most abundant ion in the isotopic cluster ($^{79}Br_2$ $^{81}Br_2$, *m/z* 555) and higher order fragmentation spectra of the main product ions. The MS^3 spectra obtained from precursor ions

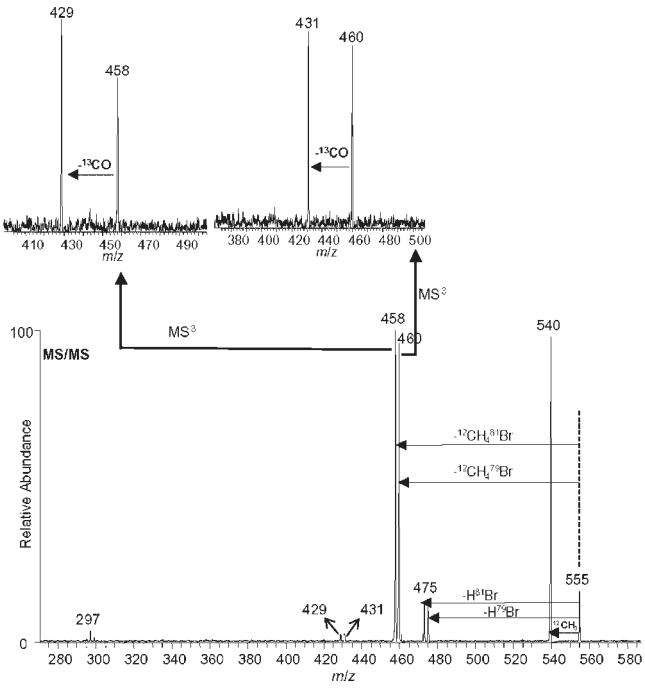

Figure 3. MS^2 spectra of TBBPA-$^{13}C_{12}$ (ring) and higher-order mass spectra of some of the most important product ions.

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at *m/z* 460 and 458 show a product ion formed by loss of 29 Da, indicating that a ^{13}C atom is involved in this fragmentation step; this is due to the neutral loss of a CO group, a characteristic fragmentation of phenolic compounds.

These fragmentation study results lead us to propose a common fragmentation pathway for this family of compounds (Fig. 4). First a $CH_3^{\bullet}$ and/or HCl or HBr are lost, followed by the loss of HCl, HBr or $CH_3^{\bullet}$ depending on the first fragmentation step. Later a neutral loss of CO takes place, and finally in the MS^4 or MS^5 spectra the cleavage of the other methyl and the other halogen group occurred. Moreover, the elimination of one aromatic ring in MS/MS is another important fragmentation that provides information about the number of halogen substitutions in each phenolic group.

We also observed in the MS/MS spectra of BPA and BPA-d_{16} (used as internal standard, IS) ions at *m/z* values – *m/z* 244 and 255, respectively – higher than the ions for the deprotonated molecules. These ions could be obtained from an ion-molecule reaction[36] between the ion originating from the loss of the methyl group and MeOH from the mobile phase. As an example, Fig. 5 shows the MS/MS spectra of BPA-d_{16} where an ion is observed at *m/z* 255. Moreover, the single MS spectrum of this compound showed an ion at *m/z* 241 $[(M-D_2+H_2)-H]^-$ instead of at *m/z* 242 $[M-D]^-$,[37] due to a deuterium/hydrogen (D/H) exchange in the hydroxyl groups. D/H exchange was also observed in the MS^3 spectrum of the adduct ion at *m/z* 255 that shows the loss of one $H^{\bullet}$ (from the hydroxyl group) to *m/z* 254 and the loss of MeOH (*m/z* 222) or MeOD (*m/z* 221).

For quantitative proposes, transitions between the most abundant ion of the molecular cluster and the most abundant product ion were used.

Liquid chromatography/mass spectrometry

When analyzing this family of compounds by LC/MS, it was observed that mobile phase composition had an important effect on sensitivity, especially in the response of BPA. Different elution gradients were tested in order to optimize the separation. The best conditions were obtained using an MeOH/water gradient from 65 to 85% MeOH (gradient 1). In order to promote deprotonation of BPA and its halogenated derivatives, a post-column addition using different bases (ammonia, triethylamine and dimethylamine, 100 mM at a flow rate of 100 $\mu L\,min^{-1}$) was tested. The addition of basic additives to the mobile phase produced important signal suppression – especially for BPA – in agreement with Benijts *et al*.[38] Therefore, no mobile phase additives were used.

When coupling liquid chromatography to mass spectrometry we tested both atmospheric pressure ionization sources: electrospray (ESI) and atmospheric pressure chemical ionization (APCI), in negative ion mode. The ESI source

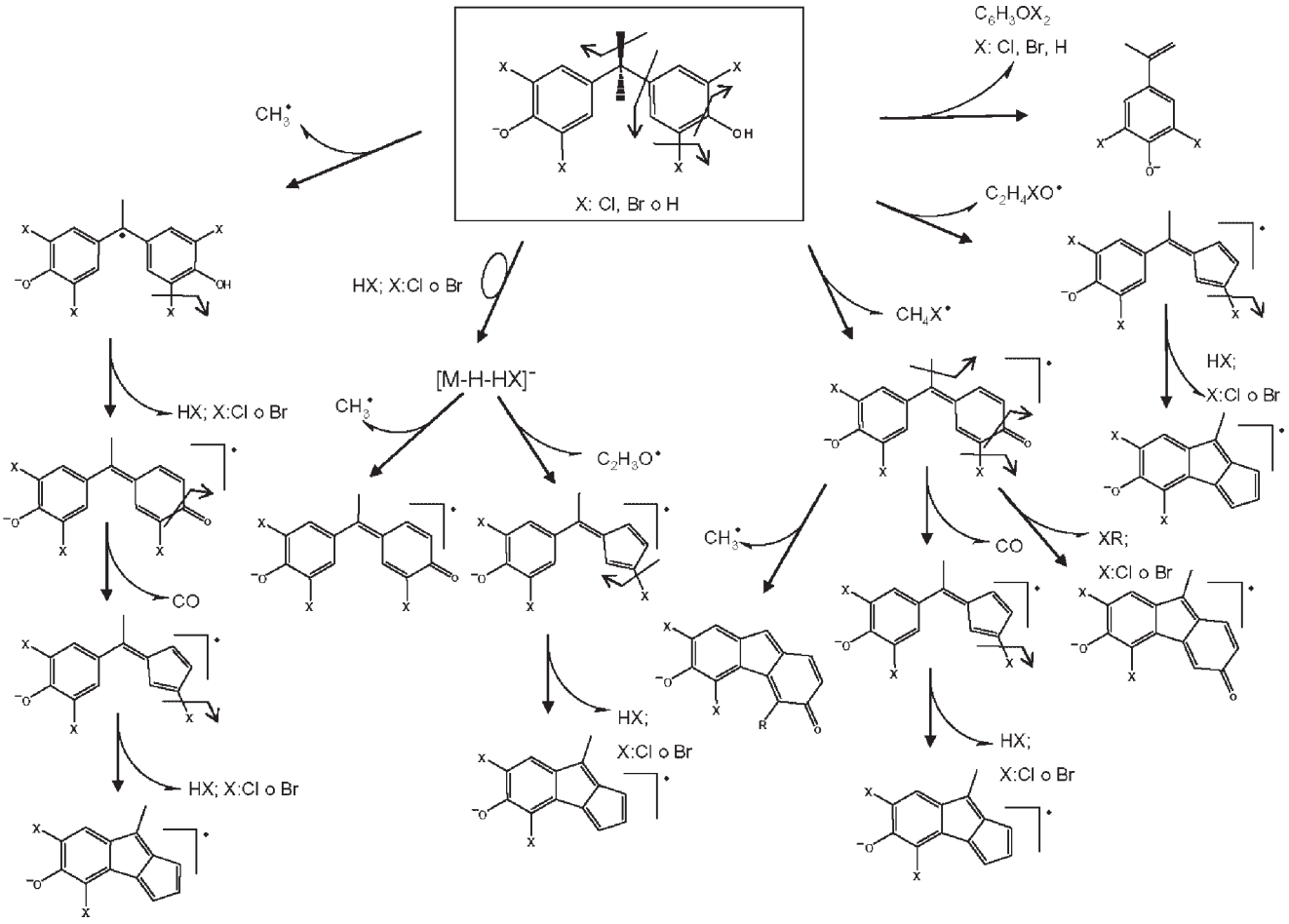

Figure 4. Fragmentation pathway of BPA and its halogenated derivatives.

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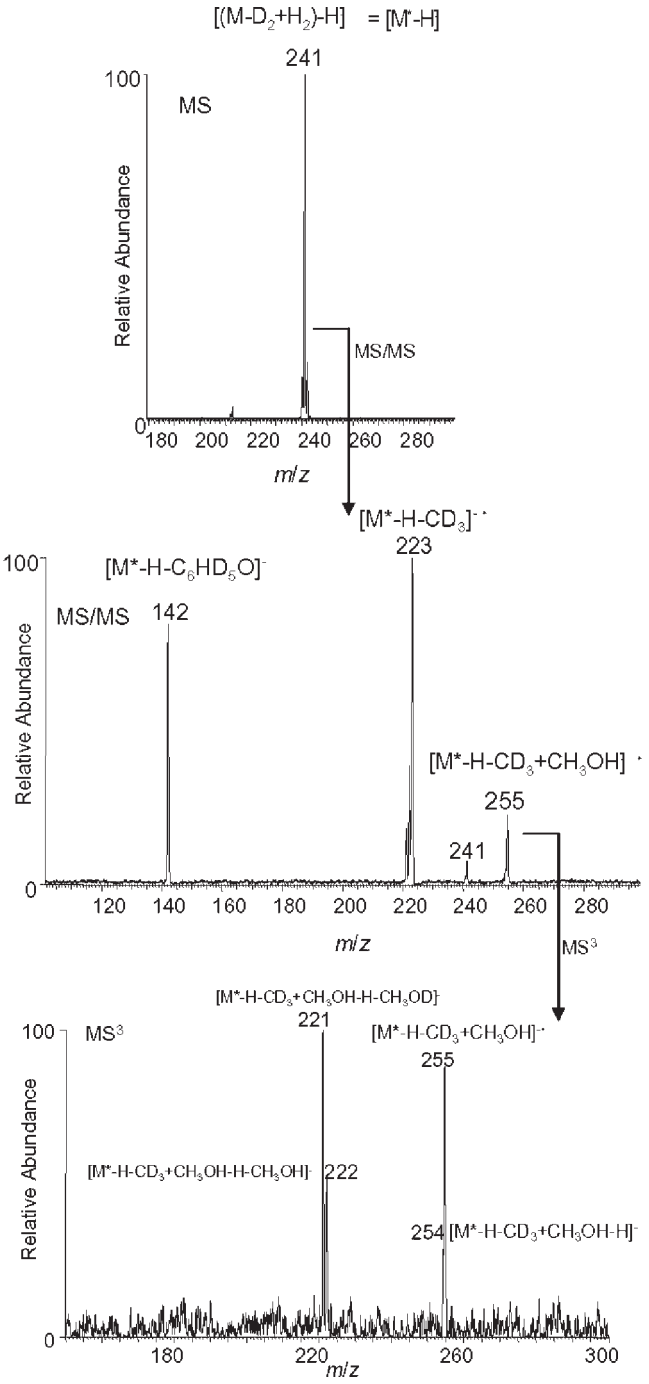

Figure 5. MS, MS/MS and MS^3 spectra of BPA-d_{16}.

provided better responses than the APCI source; at least six times higher (Table 2), since fragmentation of the deprotonated molecule by loss of $CH_3^{\bullet}$ and/or HX occurred in APCI. This fragmentation occurred even under mild temperature conditions and an important increase was observed at high vaporizer and heated capillary temperatures. Moreover, this fragmentation in the APCI source was more important for the highly halogenated compounds: TeCBPA and TBBPA. Therefore, we chose ESI in negative ion mode (which provides soft ionization without in-source fragmentation) for the determination of BPA and its halogenated derivatives in water samples by LC/MS/MS. Under these conditions, detection limits (signal-to-noise (S/N) ratio 3:1) for halogenated derivatives were 10–30 times lower than those for BPA, due to the lower fragmentation efficiency for this compound. In contrast, in single MS (Table 2) the limits of detection (LODs) for BPA were only slightly higher than for the halogenated derivatives compounds.

Water sample analysis

In a preliminary study, surface water samples free from BPA and its halogenated derivatives were spiked (200 $\mu g\,L^{-1}$), pre-concentrated by SPE, and analyzed by LC/MS/MS. The responses for BPA and MCBPA were much lower than those obtained when we analyzed water (HPLC-gradient) spiked at the same level. This can be explained by a matrix ion suppression effect, since both BPA and MCBPA eluted at the beginning of the chromatogram where matrix components also eluted. To avoid ion suppression and thereby enhance the signal for BPA and MCBPA, the gradient elution program was modified: we decreased the methanol percentage of the initial conditions to 50% and included an isocratic step (gradient 2). Under these conditions responses were enhanced. Figure 6 shows the LC/MS chromatograms obtained for mountain river water (Garona, Vall d'Aran, Spain) spiked with BPA and halogenated derivatives at 200 $\mu g\,L^{-1}$ analyzed using both gradient elution programs. At the lower methanol percentage in the mobile phase, the isocratic step increased retention times, reducing the matrix effect and producing similar responses to those obtained with the spiked water (HPLC-gradient). Under these new separation conditions, recovery values and LODs of the off-line SPE-LC/MS/MS method were estimated. We obtained recovery values of more than 85% for all the compounds. Method limits of detection (MLODs), based on a S/N ratio of 3:1, were determined using different aliquots of

Table 2. LC/MS instrumental LODs using ESI and APCI and MLODs for BPA and its halogenated compounds using a river water sample by LC/MS/MS

	Standard solution (LODs)			Water sample (MLODs)	
Compound	LC/APCI-MS (pg inject)	LC/ESI-MS (pg inject)	LC/ESI-MS/MS (pg inject)	SPE *off-line* LC/ESI-MS/MS ($\mu g\,L^{-1}$)	SPE *off-line* LC/ESI-MS/MS (pg inject)
BPA	260	44	182	0.38	324
MCBPA	218	28	20	0.23	197
DCBPA	166	26	10	0.062	53
TCBPA	128	20	6	0.067	57
TeCBPA	186	28	10	0.016	14
TBBPA	232	34	20	0.02	18

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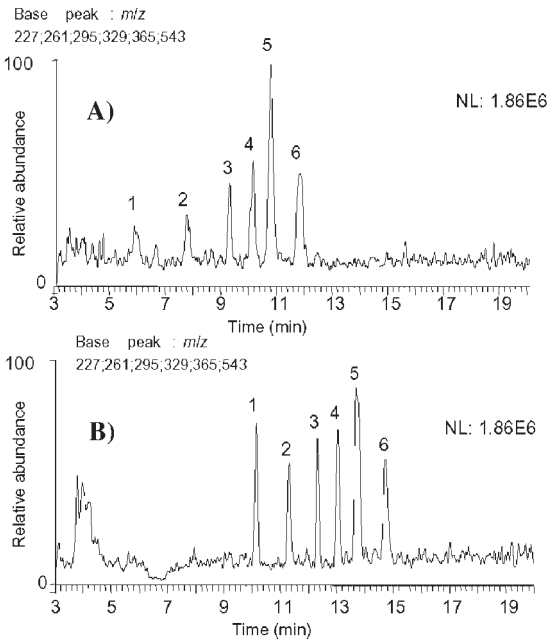

Figure 6. Full scan LC/MS chromatogram of BPA and its halogenated derivatives using two gradient elution programs: (A) gradient 1 and (B) gradient 2. Peak numbers: 1. BPA, 2. MCBPA, 3. DCBPA, 4. TCBPA, 5. TeCBPA, 6. TBBPA.

a mountain river water sample spiked at low concentrations (0.05–0.5 $\mu g\,L^{-1}$). LODs ranging from 16 to 230 $ng\,L^{-1}$ (Table 2) for the halogenated derivatives and of 380 $ng\,L^{-1}$ for BPA were obtained. However, MLODs 2 to 10 times higher than the instrumental LODs were obtained for the first four compounds eluted in the chromatogram (BPA, MCBPA, DCBPA and TCBPA). This could be explained by a residual matrix effect.

To assess the applicability of the optimized off-line SPE-LC/MS/MS method, a water sample from a paper recycling plant was analyzed. Figure 7 shows the LC/MS/MS chromatograms and the mass spectra of the chromatographic peaks obtained for this water sample. Some chlorinated derivatives of BPA (MCBPA, DCBPA, TCBPA and TeCBPA) were identified. A standard addition method (3 zero levels and 4 addition levels) was used for quantification and the concentrations for the identified chlorinated compounds ranged from 464 to 810 $ng\,L^{-1}$ with acceptable relative standard deviations (%RSD from 5 to 16). These concentrations agree with those reported by other authors when analyzing water samples from paper recycling plants in Japan.[22]

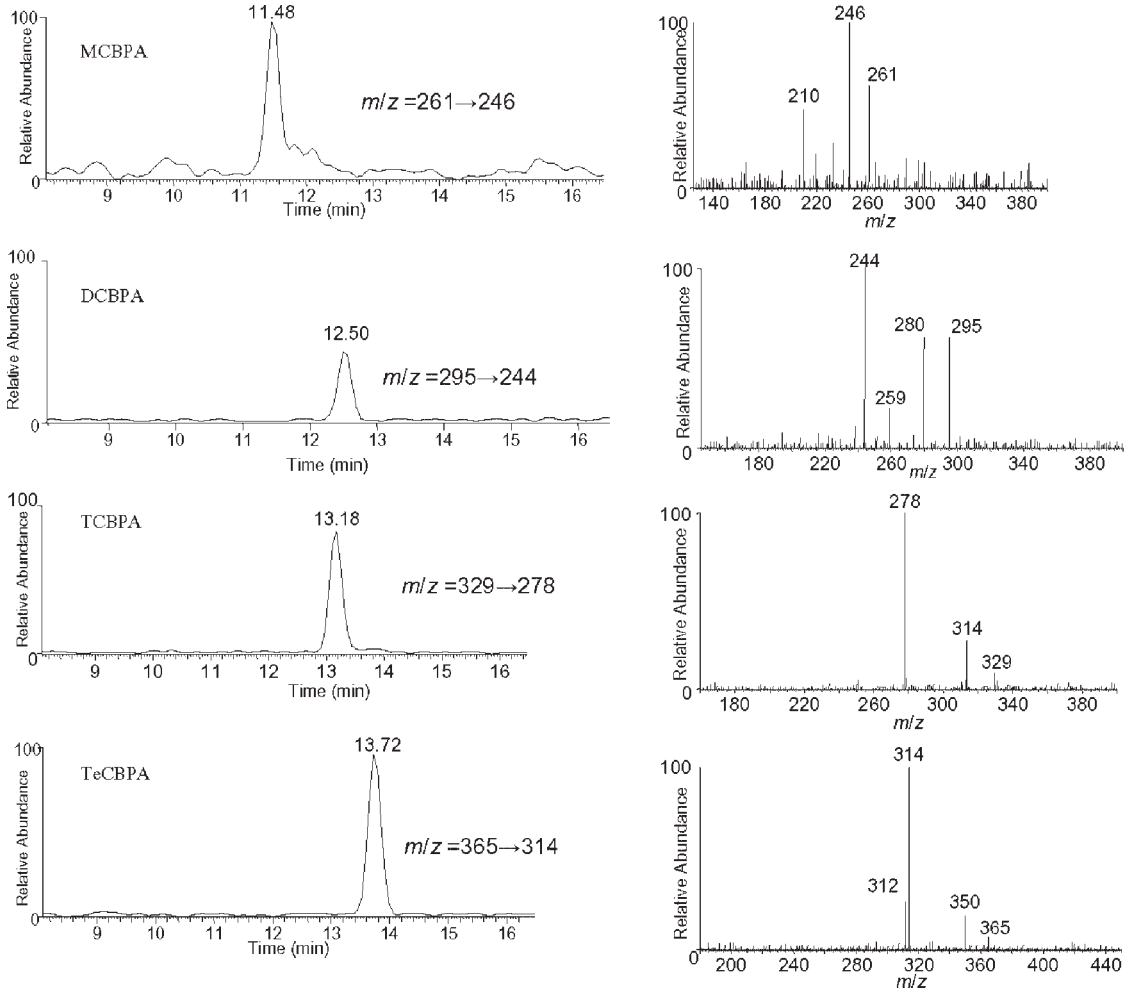

Figure 7. LC/MS/MS chromatograms and product ion scan spectra obtained for the effluent from a paper recycling plant contained: MCBPA, 0.63 $\mu g\,L^{-1}$ (%RSD, 16); DCBPA, 0.76 $\mu g\,L^{-1}$ (%RSD, 7); TCBPA, 0.81 $\mu g\,L^{-1}$ (%RSD, 5); and TeCBPA, 0.46 $\mu g\,L^{-1}$ (%RSD, 15).

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CONCLUSIONS

In the present study, several halogenated derivatives of BPA (MCBPA, DCBPA and TCBPA) were synthesized to be used as analytical standards for their analysis in water samples. We used multi-stage mass spectrometry in an ion trap mass analyzer to establish, for the first time, the fragmentation pathways of BPA and its halogenated derivatives. Generally, the fragmentation behavior of this family of compounds starts with the loss of $CH_3^{\bullet}$ and/or HCl or HBr and is followed by the neutral loss of CO. For BPA, ion-molecule reactions in the ion trap occurred between the radical anion $[M–H–CH_3]^{-\bullet}$ and MeOH. We have also developed a selective and sensitive off-line SPE-LC/MS/MS method for the simultaneous determination of BPA and its halogenated derivatives in water samples, with LODs in the low ppb range. This method has been applied to the analysis of water from a paper recycling plant.

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